8th World Congress on
Trauma, Shock, Inflammation and Sepsis

in conjunction with
23rd SIS-Europe Congress on Surgical Infections
and the
2nd Interdisciplinary Summit on Inflammation

TSIS 2010

March 9th–13th 2010, Munich, Germany

Book of Abstracts
Editorial

On behalf of the Scientific Program Committee we welcome all congress delegates to Munich/Germany for the 8th World Congress on Trauma, Shock, Inflammation and Sepsis – TSIS 2010 – from Tuesday, March 9th until Saturday, March 13th, 2010. TSIS 2010 is held in conjunction with the 23rd SIS-Europe Congress on Surgical Infections and the 2nd Interdisciplinary Summit on Inflammation (ISOI). ISOI, organized in cooperation with the International Association of Inflammatory Societies (ISIS), is convening prominent basic and clinical scientists to present a most up-to-date exchange on the current and future perspectives of this most complex disease entity.

During the recent years our understanding, predominantly of innate immune response mechanisms, and thus in parallel also the insight into the pathogenesis at the cellular and molecular level of chronic and acute inflammatory diseases has been revolutionized. And yet, notwithstanding these developments, we have to accept that still in many facets of inflammatory morbidity major disparities exist, between the novel advances of immunobiologic science and its conversion into clinically relevant progress of patient treatment.

We need to pursue new avenues for the assessment, monitoring and treatment of inflammatory diseases. Thus, the illumination of numerous unsolved but critically important issues in acute sterile (major tissue injury) and non-sterile (sepsis) inflammation, as well as of chronic inflammatory diseases like autoimmunity induced cell dysfunction, autoinflammation, atherosclerosis, metabolic syndrome, represents the major focus of the ISOI at TSIS 2010.

Better patient care, in my conviction, will only occur if we pursue keen, close-mesh monitoring of the clinical diagnosis via compelling assessment of meaningful biomarkers together with the synchronous use of molecular imaging.

This novel in-vivo technology will allow us to spot non-invasively intraorgan defects and otherwise undetectable tissue inflammation, which should then enable us to define the therapeutic targets unerringly.

The Scientific Program of TSIS 2010 will also comprise a wide spectrum of information related to emerging therapeutics strategies such as extracorporeal immunotherapeutic procedures, the use of compounds to harness defective innate immune responses, as well as the application of stem cells/progenitor cell therapies for multiple organ reprogramming and regeneration in critically ill patients with multiple organ dysfunction (MODS). Further on, novel concepts and targets of vaccination technology for treatment and prevention of acute septic disease as well as for chronic inflammatory morbidities will be discussed.

Special emphasis is also given at TSIS 2010 to the discussion of the problem of antibiotic resistance, ‘superbugs’ and the need for shrewd approaches to develop new antibiotics.

The scientific program of the 23rd SIS-Europe Congress on Surgical Infection is highlighted with the presentation of extensive information on the management of severe intraabdominal infections, intestinal failure and complicated surgical site infections.

The TSIS 2010 special conference on the Interdisciplinary Management of Obesity is presenting a comprehensive overview on novel insights into the biology of adipose tissue, a driving force of systemic inflammation, as well as on the therapeutic approaches towards the unhappy ‘triad’ of insulin resistance, obesity and hyperglycemia, specifically focussing on the role of metabolic and bariatric surgery.

TSIS 2010 – a worldwide unique medical conference with special emphasis on translational research and the biomedical sciences – will again present a unique platform of discussion between scientists from basic and clinical research from academic institutions and industry alike.

The most complex conference program comprises close to 780 science reports, distributed in 90 symposia and free communication sessions. The Scientific Committee greatly appreciates the fact that the International Association of Inflammation Societies (IAIS) together with numerous distinguished international Scientific Societies and Institutions have contributed to the program composition.

The Scientific Committee of TSIS 2010 is grateful to all its endorsing scientific partners for their most supportive cooperation and for taking the congress under their auspices.

Further on, I do express my profound gratitude to so many loyal friends and colleagues that have been giving their strong and unflagging support to bring this huge program to life.

I do thank the Editors, the Editorial Board and the Publisher of INFLAMMATION RESEARCH for their interest and efforts in the preparation of this issue.

For the readers of INFLAMMATION RESEARCH, who will not be able to attend the congress, this volume will present to you cutting-edge information on a wide spectrum of basic research and innovative patient care in systemic inflammatory disease, as it will be presented during TSIS 2010.

Prof. Dr. Eugen Faist, MD, FACS
Congress Chairman
Supplement

8th World Congress on Trauma, Shock, Inflammation and Sepsis - TSIS 2010
in conjunction with
23rd SIS-Europe Congress on Surgical Infections
and the
2nd Interdisciplinary Summit on Inflammation
Tuesday, March 9th until Saturday, March 13th 2010
Munich, Germany

Edited by:
Eugen Faist, MD, FACS

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For all enquiries after the congress please contact the Congress Secretariat:
Prof. Dr. Eugen Faist (eugen.faist@med.uni-muenchen.de)
Silvia Marth (silvia.marth@med.uni-muenchen.de)
Ludwig-Maximilians-University Munich - Campus Grosshadern
Department of Surgery
Marchioninistrasse 15
81377 Munich, Germany
Phone +49-89-7095-5461/2461
Fax +49-89-7095-2460
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Late Breaking Abstracts

A 417–A 426

Please note: If no abstracts have been received for some sessions, the session titles are therefore not included in this supplement.
Sevoflurane versus propofol in protection of the endothelial glycocalyx against ischemia–reperfusion injury
Thorsten Annecke, Daniel Chappell, Congcong Chen, Ulrich Welsch, Peter Conzen, Bernhard Becker

Objective: Aortic surgery is often followed by severe ischemia–reperfusion injury leading to endothelial dysfunction and multiple organ failure. Healthy vascular endothelium is coated by an endothelial glycocalyx, destroyed by ischemia–reperfusion. During shedding, components of the glycocalyx such as heparan sulfate and syndecan-1 are released into the circulation. We compared the impact of commonly used anesthetics sevoflurane and propofol on integrity of the glycocalyx following abdominal visceral and lower body ischemia–reperfusion. Effects of sevoflurane on myocardial ischemia–reperfusion induced coronary glycocalyx shedding were also investigated ex vivo.

Methods: Pigs were randomized to receive either propofol or sevoflurane (n = 9 each). Ischemia of 90 min, followed by 300 min of reperfusion was induced by a balloon-catheter in the thoracic aorta. Five animals each without aortic occlusion served as time controls. Serum heparan sulfate concentrations were measured as markers of glycocalyx disruption and immune histochemical staining of the glycocalyx was performed. Additional experiments were performed in an isolated model of myocardial ischemia–reperfusion injury: Isolated guinea pig hearts underwent 20 min stopped-flow (37°C) ischemia and reperfusion, either with or without 1 MAC Sevoflurane. Coronary release of glycocalyx constituents syndecan-1 and heparan sulfate was measured, and additional hearts were perfusion fixed to visualize the endothelial glycocalyx.

Results: Severe declamping shock occurred in animals after lower body ischemia. Initially heparan sulfate concentrations increased comparably in both experimental groups, but became stable in sevoflurane anesthetized animals, while increasing further with propofol (p < 0.05). Light microscopy revealed heparan sulfates as components of the vascular lining. An impressive shedding was visualized after ischemia. In isolated guinea pig hearts after stopped-flow ischemia, sevoflurane attenuated the significant increases in heparan sulfate and syndecan release during postischemic reperfusion. Electron microscopy also revealed that degradation of the glycocalyx was attenuated by sevoflurane.

Conclusions: Sevoflurane seems to be superior to propofol in protecting the endothelial glycocalyx after lower body ischemia–reperfusion injury in vivo. Protection by sevoflurane was also evident after global myocardial ischemia ex vivo.

Corresponding Author: Thorsten Annecke, MD, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Clinic of Anaesthesiology, Nussbaumstr. 20, 80336 Munich, Germany, thorsten.annecke@med.uni-muenchen.de

Phosphorylation of vasodilator-stimulated phosphoprotein (VASP) influences transendothelial movement of platelets and affects myocardial ischemia–reperfusion injury
David Koehler, Marion Faigle, Stephanie Zug, Rainer Lehmann, Sean P. Colgan, Peter Rosenberger

Objective: Myocardial ischemia–reperfusion (IR) injury is significantly influenced by the activation status of platelets and the presence of neutrophils. In addition, the translocation of platelets into areas of inflammation is facilitated by neutrophils. VASP, a central cytoskeletal protein, controls the activation status of platelets through phosphorylation and influences the migration of neutrophils. We investigated whether VASP and phosphorylation of VASP influences the migration of platelets across endothelial cells and affects the extent of myocardial tissue damage during IR injury.

Materials and methods: Movement of platelets and migration of neutrophils was studied in vitro, VASP phosphorylated and the impact on thrombocyte and PMN movement determined. Approval from the Regierungspräsidium Tübingen was obtained. WT and VASP-/− mice were used in a model of myocardial IR injury. Infarct size was determined by calculating the percentage of myocardial infarction compared to the area at risk (AAR) using a double staining technique with Evan’s blue and triphenyltetrazolium chloride (TTC). Presence of platelets and neutrophils in the infarct area was determined using immunohistological staining. Bone marrow chimeric animals were studied. Platelet transfer from VASP-/− to WT animals and vice versa was used to evaluate the role of platelet-specific VASP during myocardial IR. Additionally, thrombocytes and neutrophils (PMN) were depleted. Phosphorylation of VASP was performed using atrial natriuretic peptide (ANP) and prostaglandin E1 (PGE1) and impact on myocardial IR injury was evaluated.

Results: Movement of platelets across endothelial cells was dependent on PMNs, and was reduced when VASP was phosphorylated with PGE1 or ANP. In-vivo, myocardial IR injury was significantly reduced in VASP-/− mice. Studies using chimeric animals identified platelet-derived VASP to be associated with increased myocardial IR injury and to be responsible for platelet movement into the affected myocardial areas. Platelet separation and cross-over injection reduced myocardial IR injury when VASP-/− platelets where transferred to WT animals. VASP phosphorylation was associated with significantly reduced IR injury in WT mice. Tropinin measurements and histological costaining studies determined results.

Conclusion: Taken together, these studies identified platelet-derived VASP as a crucial protein in cardioprotection and myocardial ischemia.

Corresponding Author: David Koehler, PhD, University Hospital Tuebingen, Department of Anaesthesiology and Intensive Care Medicine, Wilhelmstr. 56, 72074 Tuebingen, Germany, david.koehler@medizin.uni-tuebingen.de
A 3

Tourniquet-applied upper limb orthopaedic surgery results in changes to various inflammatory parameters

Stephan Hughes, Beverly Hendricks, David Edwards, Kirsty Maclean, Jim Middleton

Objectives: In the United Kingdom orthopaedic problems impose an enormous social and economic burden on our society. In this pilot study we hypothesised that tourniquet-applied upper limb orthopaedic surgery results in changes to various inflammatory markers.

Materials and methods: Patients scheduled for elective tourniquet-applied upper limb orthopaedic surgery were recruited. Three venous blood samples were collected from the surgical arm at the ante-cubital fossa, at baseline (pre-operatively), 5 and 15 min reperfusion (post-operatively). The mean time of ischaemia during surgery was 32 ± 4.16 min (n = 10). Neutrophil and monocyte leukocyte subpopulations were isolated by density gradient centrifugation techniques. Cell surface expression of CD62L, CD11b and the intracellular production of hydrogen peroxide (H2O2) was measured via flow cytometry. Highly sensitive C-reactive protein (CRP) was measured using a clinical chemistry analyser. Plasma concentrations of protein C and von Willebrand factor (vWF) were measured using enzyme-linked fluorescent assays.

Results: Following surgery, there was a decrease in neutrophil CD62L expression (p = 0.001); an increase in CD11b expression and in the intracellular production of H2O2 by neutrophils and monocytes (p < 0.05). These parameters were measured to investigate leukocyte adhesion and activation. Following surgery, CRP concentrations increased (p < 0.001), which was measured as a marker of non-specific inflammation. Protein C was measured to assess coagulation activity and following surgery its concentration decreased (p = 0.004). vWF was measured as a marker of endothelial activation, and although these changes were not significant (p = 0.232), a trend of increasing vWF concentration was observed following surgery.

Conclusions: This study reveals that during brief periods of ischaemia and reperfusion in surgical subjects, neutrophils and monocytes are rapidly activated and produce potent reactive oxygen intermediates. This was associated with evidence of increased inflammation and coagulation activity, with a trend toward endothelial activation. We report that biological markers such as CD11b, protein C and H2O2 may provide alternative ways of assessing adhesion, coagulation, activation and oxidative stress peri-operatively. It is proposed that by allowing orthopaedic surgeons access to these laboratory markers, an accurate assessment of the extent of inflammation due to the surgery per se may be made.

Corresponding Author: Stephen Hughes, PhD, Chester University, Biological Sciences, Parkgate Road, Chester CH1 4BJ, UK, stephen.hughes@chester.ac.uk

A 4

The presence of hospital pathogens shifts the lethality of intestinal ischemia–reperfusion

David Fink, Kathleen Romanowski, Trissa Babrowski, Vesta Valuckaite, Olga Zaborina, John Alverdy

Objective: Intestinal ischemia–reperfusion (IR) injury can lead to multiple organ failure and death by mechanisms that involve activation of immune mediated cellular damage. Yet most animal models of intestinal IR do not account for the fact that the intestinal tract of critically ill humans, in contradistinction to laboratory mice, are densely colonized by highly opportunistic hospital pathogens, which themselves can affect the function of both local and systemic immune elements. Our lab has been working with the human opportunistic pathogen *Pseudomonas aeruginosa* and has shown that it can sense and respond to discrete physiologic signals released by host tissues during injury. Here we hypothesized that the mere presence of intestinal *P. aeruginosa* would shift the lethality curve of intestinal ischemia–reperfusion injury.

Materials and methods: 7–9-week-old male C57/BL6 mice were housed under standard conditions. Intestinal IR was created by occluding the superior mesenteric artery for either 5 or 15 min, and 200 mL of *P. aeruginosa* (1.0 × 10^9 CFU total) was injected directly into the terminal ileum during IR. Mice were observed for mortality for 48 h. Virulence activation of *P. aeruginosa* in response to IR was assessed by photon camera imaging of constitutive bioluminescent strain XEN41 and PA-I lectin reporter strain PAO1/lecA::lux.

Results: Multiple dose response curves at varying durations of ischemia and concentration of bacteria for both IR alone, instillation of intestinal *P. aeruginosa* alone, and IR+ intestinal *P. aeruginosa* demonstrated that intestinal *P. aeruginosa* shifted the lethality of IR significantly.

Table

| 48 h mortality | 15 min IIR alone 50% (n = 20) | Sham surgery + *P. aeruginosa* 8.3% (n = 24) | 15 min IIR + *P. aeruginosa* 100% (n = 15) | 5 min IIR alone 9.5% (n = 21) | Sham surgery + *P. aeruginosa* 4.8% (n = 21) | 5 min IIR + *P. aeruginosa* 36.4% (n = 11) |

![Photon camera imaging confirmed that in vivo virulence activation of *P. aeruginosa* developed in response to IR injury. At 12 h post-ischemia, *P. aeruginosa* virulence expression was increased 2.68-fold (95% interval 2.2–25.4) in strains present in the intestine and 15.4-fold (95% interval 2.2–25.4) in strains present in the mesenteric lymph nodes.

Conclusion: The presence of hospital pathogens such as *P. aeruginosa* within the intestinal tract during IR increases mortality in part as a result of their ability to enhance virulence expression as they respond to local microenvironmental “cues” that are unique to the intestine of an injured host.

Corresponding Author: David Fink, MD, University of Chicago, Department of Surgery, 5841 South Maryland Avenue, MC 5029, Chicago, IL 60637, USA, dfink@uchicago.edu

A 5

Prolonged human intestinal ischemia–reperfusion results in paneth cell apoptosis: new insight into ischemia–reperfusion induced bacterial translocation

Joep Grootjans, Caroline Hodin, Jacco-Juri de Haan, Kaatje Lenaerts, Cornelis Dejong, Wim Buurman

Objective: Human intestinal ischemia–reperfusion (I-IR) is a frequent phenomenon associated with excessive inflammation. Bacterial translocation is a well-known factor accounting for these vigorous inflammatory responses. We aimed to investigate the role of Paneth cells (PCs), important cells of the intestinal immune barrier, on bacterial translocation during human I-IR.

Material and methods: PC viability was studied using a unique human I-IR model. In 30 patients, 6 cm of healthy jejunum, to be removed for surgical reasons, was exposed to 30, 45, or 60 min of ischemia...
followed by 30 and 120 min of reperfusion (n = 10 for each ischemic period). Tissue was collected at all time points. Double staining for lysozyme (PCs) and M30 (apoptosis) was performed to assess PC apoptosis. PC apoptosis was quantified by two independent observers. To investigate the consequences of PC apoptosis during I-IR, we studied bacterial translocation after selective PC-ablation in a rat intestinal hypoperfusion (IH) model. Male Sprague-Dawley rats were subjected to IH by withdrawal of 30–40% of their circulating volume (n = 12). In 6 rats, PCs were selectively ablated by administering the zinc-chelator dithizone (0.1 mg/g BW i.v.) prior to IH. Paneth cell ablation was confirmed with Western blot and immunohistochemistry for lysozyme, and bacterial translocation to mesenteric lymph nodes (MLN) was assessed. Bonferroni’s multiple comparison (after significant one-way ANOVA) or Mann–Whitney U test was used when appropriate.

Results: Double stainings revealed that 45 and 60 min of ischemia with 30 min of reperfusion resulted in PC apoptosis (p < 0.01 and p < 0.001, respectively), whereas other crypt cells were hardly affected. The number of apoptotic PCs correlated significantly with ischemia-time (r² = 0.51, p < 0.0001). Apoptotic PCs were shed into the intestinal lumen, resulting in decreased PC numbers at 120 min of reperfusion (p < 0.01). Using a rat IH model with selective PC ablation we found that absence of PCs during IH led to significantly more bacterial penetration to MLN (p < 0.01) compared to IH alone, which was independent from physical barrier integrity damage.

Conclusions: We describe for the first time the occurrence of PC-apoptosis during human I-IR, a newly discovered phenomenon. The importance of this finding was studied in a rat model, which revealed that absence of PCs during intestinal ischemia led to increased bacterial translocation.

Corresponding Author: Joep Grootjans, MD, Maastricht University Medical Center, Department of Surgery, Universiteitsingel 50, 6211 BL Maastricht, The Netherlands, j.grootjans@ah.unimaas.nl

A 6 Urokinase-type plasminogen activator promotes paracellular transmigration of neutrophils to postischemic tissue

Christoph Reichel, Christoph Reichel, Max Lenchenberger, Wolfgang Schmalix, Sandip Kanse, Fritz Krombach

Objective: This study aimed to investigate the role of urokinase-type plasminogen activator (uPA) for the regulation of each single step of the leukocyte recruitment process as well as of microvascular leakage during ischemia–reperfusion (I/R).

Materials and methods: Using in vivo transillumination microscopy, leukocyte rolling, firm adherence, and transmigration were analyzed in the cremaster muscle of wild-type (WT), uPA−/−, and uPA receptor (uPAR)−/− mice as well as of WT mice receiving the uPA antagonist WX-340. Leukocyte transmigration routes as well as microvascular leakage were assessed by in vivo fluorescence microscopy. Phenotyping of transmigrated leukocytes was performed by immunohistochemistry. Activation of neutrophils was determined by flow cytometry. As a measure of postischemic tissue injury, serum AST and ALT levels were analyzed upon hepatic I/R. All substances were administered immediately at the end of ischemia–reperfusion.

Results: Following IR we observed a significant increase of leukocyte adhesion in the intestinal submucosal venules and a reduced capillary perfusion in the muscular intestinal wall layers. NECA improved leukocyte activation and improved capillary perfusion significantly. Administration of the adenine A2B receptor antagonist completely reversed the NECA effect, whereas A1 receptor inhibition only partially abolished the action of NECA.

Conclusions: The data supports the hypothesis, that adenosine signalling is involved in intestinal I/R injury. More important than adenosine A1 receptors are the adenosine A2B receptors since A2B receptor inhibition by MRS1754 completely reversed the effect of the adenosine receptor agonist NECA.

Corresponding Author: Christoph Reichel, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Walter Brendel Centre of Experimental Medicine, Marchioninistr. 15, 81377 Munich, Germany, christoph.reichel@med.uni-muenchen.de

A 7 The adenosine receptor agonist NECA improves microcirculation following intestinal ischemia and reperfusion

Katrin Zimmermann, Thomas Krieg, Mariette Soltow, Dragan Pavlovic, Juan Zhou, Christian Lehmann

Objectives: Gut ischemia and reperfusion (IR), e.g. in small bowel transplantation or following resuscitation, may result in severe impairment of the intestinal microcirculation. Potential sequelaes are mucosal damage, loss of barrier function, bacterial translocation, systemic inflammation, multiple organ failure and death. We hypothesized a protective role for extracellular adenosine signalling in intestinal IR injury. Using intravital microscopy we investigated the effects of the adenosine receptor agonist NECA (5'-N-ethyl carbamoyladenosine) on leukocyte-endothelial interactions and capillary perfusion in the intestinal microcirculation following intestinal IR.

Materials and methods: Six groups of animals (n = 44) were studied: control, NECA, IR (30 min of intestinal ischemia, 2 h of reperfusion), IR + NECA, IR + NECA + MRS1754 (adenosine A2B receptor antagonist), IR + NECA + DPCPX (adenosine A1 receptor antagonist). All substances were administered immediately at the end of ischemia. Intravital microscopy was performed after 2 h of reperfusion.

Results: Following IR we observed a significant increase of leukocyte adhesion in the intestinal submucosal venules and a reduced capillary perfusion in the muscular intestinal wall layers. NECA reduced leukocyte activation and improved capillary perfusion significantly. Administration of the adenosine A2B receptor antagonist completely reversed the NECA effect, whereas A1 receptor inhibition only partially abolished the action of NECA.

Conclusions: The data supports the hypothesis, that adenosine signalling is involved in intestinal I/R injury. More important than adenosine A1 receptors are the adenosine A2B receptors since A2B receptor inhibition by MRS1754 completely reversed the effect of the adenosine receptor agonist NECA.

Corresponding Author: Christian Lehmann, Prof. MD, PhD, Ernst Moritz Arndt University Greifswald, Department of Anesthesia, Friedrich-Loeffler-Str. 23b, 17475 Greifswald, Germany, christian.lehmann@uni-greifswald.de
**A 8**

**Interferon regulatory factor 1 regulates the balance between apoptosis and autophagy in endotoxemia**

*John Evankovich, Pinhua Pan, Jon Cardinal, Xingping Tan, David Geller, Allan Tsung*

Objectives: The pathophysiology of sepsis is complex, and unfortunately poorly understood. Sepsis induced apoptosis has been extensively studied and is known to contribute to end organ injury and ultimately death. Recent evidence suggests that the evolutionarily conserved process of autophagy is also activated in sepsis and is believed to primarily play a protective role in the progression of the disease. In addition, apoptosis and autophagy share a number of common signaling pathways and mediators, and therefore may be linked to one another. However, how the balance between these two cellular processes affects sepsis induced outcomes has not been described. In this study, we show that the transcription factor interferon regulatory factor-1 (IRF-1) is pivotal in regulating the balance between apoptosis and autophagy in sepsis.

Material and methods: Male IRF1-KO and matched C57BL/6 mice were injected i.p. with a lethal dose of LPS (35 mg/kg). Control mice received injections of sterilized PBS. 96 h survival rates were assessed. Tissue was analyzed by western blot, immunofluorescent staining, flow cytometry, TUNEL staining, RT-PCR, and transmission electron microscopy. In vitro, murine RAW 264.7 cells were transfected with pEGFP-LC3 and analyzed by immunofluorescent microscopy.

Results: In vivo, IRF-1 knockout mice were protected from endotoxin induced mortality. This protection was associated with less end organ apoptosis and increased autophagy compared to IRF-1 wildtype mice. In vitro, LPS/IFNγ stimulation induced greater autophagy in IRF-1 knockout macrophages compared to IRF-1 wildtype cells as measured by LC3 activation and autophagic vesicle formation. Conversely, in response to the same stimuli, IRF-1 knockout macrophages experienced less apoptosis compared to IRF-1 wildtype cells as measured by caspase-3 cleavage and chromatin condensation. Janus associated kinase inhibition blocked LPS induced IRF-1 activation in immune cells and recapitulated the effects seen in IRF-1 knockout cells with diminished apoptosis and elevated autophagy activation.

Conclusions: IRF-1 plays a significant role in the pathogenesis of sepsis by impacting the balance between apoptosis and autophagy, and therefore, may serve as a target to improve sepsis related outcomes.

**Corresponding Author:** John Evankovich, University of Pittsburgh School of Medicine, Department of Surgery, 200 Lothrop Street, Pittsburgh, PA 15213, USA, evankovich.john@medstudent.pitt.edu

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**A 9**

**Thiopental protects human SK-N-SH neuroblastoma cells from apoptosis by inducing a heat shock response**

*Martin Roesslein, Christian Froehlich, Verena Miltenberger, Torsten Loop*

Objective: Thiopental (T) has been demonstrated to have neuroprotective effects. However, the underlying molecular mechanism of this phenomenon is unknown. T has also been shown to inhibit nuclear factor (NF)-κB, a central regulator of the inflammatory response. Other inhibitors of NF-κB induce a heat shock response (HSR), a highly conserved cellular defense system, which is characterized by the expression of several heat shock proteins (HSP) following the DNA-binding of the activated heat shock factor (HSF)-1 to an heat shock element (HSE). We hypothesized that T is able to inhibit the degradation of pro-caspase-3 and caspase-3-substrate Poly-(ADP-Ribose)-Polymerase (PARP) as well as the activity of caspase-3, a pivotal enzyme for the execution of apoptosis by means of inducing a HSR.

Methods: Human SK-N-SH cells were incubated with T (100, 300, 500 μM) for up to 4 h before the addition of pro-apoptotic staurosporine (S [2 μM, 4 h]). Additionally, cells were pre-exposed to the HSP synthesis inhibitor KNK437 (100 μM, 24 h) or transfected with small interfering RNA (siRNA) targeting hsp70 before incubation with T. Activity of caspase-3 was assessed by fluorogenic caspase activity assay, DNA-binding activity of HSF-1 was analyzed by Electrophoretic Mobility Shift Assay, mRNA-expression of hsp-27, -32, -70 and -90 by Northern Blot, Western Blot was used to determine phosphorylation of HSF-1 and Hsp70 protein expression as well as degradation of pro-caspase-3 and PARP.

Results: T resulted in a time and dose dependent induction of a HSR identified by the phosphorylation of HSF-1, the induction of HSF-1/ HSE binding, and the expression of several HSP on mRNA (hsp27, hsp70, hsp90) as well as protein level (Hsp70). Pre-incubation with T significantly attenuated the S-induced increase in caspase-like activity (0.55 ± 0.11 relative fluorescent units [RFU] [S] vs. 0.21 ± 0.10 RFU [T100 μM + S] and 0.04 ± 0.03 [T300 μM + S]; n = 6, mean ± SD, p < 0.001, ANOVA), as well as degradation of pro-caspase-3 and PARP. Pre-exposure with KNK437 before T-incubation significantly offset the T-mediated effect (0.52 ± 0.12 RFU [KNK + T100 μM + S] and 0.21 ± 0.08 RFU [KNK + T300 μM + S]; n = 6, mean ± SD, p < 0.001, ANOVA). However, hsp70 knockdown by transfection with hsp70-siRNA did not sustain the T-mediated attenuation in caspase-like activity.

Conclusion: Thiopental mediates cytoprotection via the induction of a HSR in a human neuronal cell line.

**Corresponding Author:** Martin Roesslein, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Anesthesiology and Critical Care, Marchioninistr. 15, 81377 Munich, Germany, martin.roesslein@med.uni-muenchen.de

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**A 10**

**The protective role of autophagy and heme-oxygenase 1 against sepsis-induced apoptosis**

*Evie Carchman, Brian Zuckerbraun*

Introduction: The response to infection/sepsis is not only aimed to control infection, but also to prevent tissue injury and restore homeostasis. Studies illustrate that the initial organ dysfunction in sepsis involves minimal loss of structural integrity or cell death, allowing for the eventual recovery of function. We hypothesize that autophagic signaling, a process of cellular autodigestion, is an adaptive response to sepsis that protects against cell death. Furthermore, that autophagic signaling is regulated by heme oxygenase (HO), a known important anti-inflammatory/apoptotic enzyme.

Objective: Demonstrate that the induction of autophagic signaling in sepsis is regulated by HO signaling and is associated with decreased cell death.

Materials and methods: Cecal ligation and perforation (CLP) or laparotomy and bowel manipulation without CLP was performed on C57BL/6 mice. To inhibit HO mice were treated with tin protoporphyrin (SnPP) or lapatinib for up to 4 h prior to CLP. Autophagy was determined by Western blotting for autophagic proteins (LC3 and Atg 5/12) and immunohistochemistry and electron microscopy. Apoptosis was determined by staining for activated caspase-3 or TUNEL. In vitro studies were performed utilizing primary mouse hepatocytes treated with lipopolysaccharide (LPS) (100 ng/mL). Autophagy was
inhibited pharmacologically using 3 methyl-adenine (3-MA; 2 mM) or siRNA for VPS34, an upstream activator of autophagic signaling.

Results: CLP increased autophagic signaling in both liver and kidney tissues as demonstrated by increased expression and punctate staining for autophagic proteins. Additionally, autophagosomes were visualized by electron microscopy. Inhibition of HO activity using SnPP decreased autophagic signaling, and conversely increased apoptosis. Furthermore, inhibition of HO activity led to increased tissue injury and decreased survival. In vitro data shows a similar response. Hepatocytes demonstrate increased autophagic signaling in response to LPS. This is inhibited by SnPP, as well as by 3-MA or knockdown of VPS34. Moreover, inhibition of autophagy was associated with increased apoptosis.

Conclusions: Experimental sepsis in vivo or LPS in vitro increases autophagic signaling, which is dependent, at least in part, on heme oxygenase signaling. This induction of autophagy is important in the prevention of cell death and permanent loss of organ function. Autophagic signaling may prove to be an important adaptive signaling pathway in sepsis.

Corresponding Author: Evie Carchman, MD, University of Pittsburgh Medical Center, Department of Surgery, 200 Lothrop Street, Pittsburgh, PA 15213, USA, carchmaneh@upmc.edu

A 11 Genetic polymorphisms of TP53, FAS ligand (FASLG) and promoter (FASP) may clinically progress coronary artery disease after prior coronary artery bypass grafting

Sandra Eifert, Martin Angele, Carine Koutang, Bruno Reichart, Dorothea Nagel, Peter Lohse

Introduction: Progression of coronary artery disease (CAD) after primary coronary artery bypass grafting is frequent and may lead to recurrent symptoms. Various data indicate that apoptosis is main event occurring during development and progression of atherosclerotic plaque. Plaque vascular smooth muscle cells (VSMCs) are more sensitive than regular VSMCs tp TP53-mediated apoptosis.

Methods: We investigated EDTA blood of 198 patients (18% females, age 59.2 ± 8.4 years) who had primary CABG more than 5 years ago. CAD progression was defined as clinical endpoints: reoperation (n = 88, 46%), reintervention (n = 58, 30%), or angina at follow-up (n = 79, 46%). Apoptotic gene polymorphisms (toll-like receptor 2 (TLR2) A753G, FASLG C844T, FASP G670A, TP53 Arg72Pro, and CD14 C260T) were investigated by PCR-RLFP and compared to healthy controls (n = 200, 24% females, age 63.4 ± 5.4 years).

Gender-specific analysis was carried out.

Results: Heterozygous, homozygous and wild type expression of the named polymorphisms did show almost identical distribution between patients with CAD and healthy controls. Among CABG patients, looking at clinical endpoints, there was statistical significance for FASP to suffer from recurrent angina, when patients had AA expression (n = 28, p = 0.004). We found that patients with homozygous expression of TP53 (CC, n = 3) were prone to get a reoperation after prior CABG (p = 0.03), but not reoperation. All CC carriers were men. Patients with GG genotype of TLR2 and GG genotype of FASP showed almost statistical significance (p = 0.09) for recurrent angina and reintervention. Over a period up to 15 years, reintervention rate was significantly different in homozygous genotypes of FASLG, FASP and TP53.

Conclusion: Polymorphisms of FASLG, FASP and TP53 may contribute to CAD progression after prior CABG.

Corresponding Author: Sandra Eifert, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Cardiac Surgery, Marchioninistra. 15, 81377 Munich, Germany, Sandra.Eifert@med.uni-muenchen.de

A 12 Kidney I/R-induced lung apoptosis

Heitham Hassoun, Frederick Moore

Despite advances in renal replacement therapy (i.e. dialysis), the mortality of acute kidney injury (AKI) has remained high, especially when associated with distant organ dysfunction such as acute lung injury (ALI). Mortality for combined AKI/ALI is up to 80% in critically ill patients. While the clinical presentation of AKI-associated ALI is characterized by increased pulmonary edema, a defining feature of the syndrome, the AKI-induced lung effects extend beyond simple volume overload. New experimental data have emerged in recent years focusing on the interactive effects of kidney and lung dysfunction, and these studies have highlighted the pathophysiological importance of both soluble and cellular factors as well as the complex nature of interorgan crosstalk. We have identified that ischemic AKI in mice leads to a specific pro-inflammatory and pro-apoptotic lung transcriptome that is independent of effects of uremia alone (AJP: Renal 2007), and using an initial genomic approach to discovery followed by a mechanistic approach to disease targeting, we demonstrated that TNFR1-dependent pulmonary endothelial apoptosis is a direct mediator of the distant organ dysfunction during experimental AKI (AJP: Renal 2009). Furthermore, recent studies from our lab suggest that pulmonary T cell trafficking and activation are sentinel events during kidney IRI, and while T cells appear necessary for kidney IRI-induced lung apoptosis, its effects may be independent from local T cell TNF-α production. The experimental data presented will provide new evidence for the deleterious cross-talk between the kidney and lung that arises, at least in part, due to an imbalance of the immune, inflammatory, and soluble mediator metabolism that attends AKI.

Corresponding Author: Heitham Hassoun, MD, The Methodist Hospital, Department of Surgery, Division of Surgical Critical Care and Acute Care Surgery, 6550 Fannin, Suite 1401, Houston, TX 77030, USA, hhassoun@tmhs.org

A 13 Cross-talk between adaptive immune cell death and innate immunity

Kevin Kasten1, Richard Hotchkiss2, David Hildebrand3, Charles Caldwell4

1Division of Research, Department of Surgery, University of Cincinnati, Cincinnati, OH, USA, 2Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO, USA, 3Division of Immunobiology, Cincinnati Children’s Hospital; Cincinnati OH, USA

Objective: Sepsis represents a clinical syndrome resulting from the body’s systemic inflammatory response to bacterial infection. It is difficult to treat and carries high rates of morbidity and mortality. The high rate of complications is often attributed to a failure of initial pathogenic clearance or susceptibility to a secondary infection, which results in end-organ tissue damage. The innate and adaptive immune systems are responsible for initial pathogenic clearance and maintenance of adequate immune response to subsequent pathogens, respectively. The innate arm involves an antigen non-specific response to control infection. There is a large body of literature demonstrating an aberrant innate immune response early in sepsis leads to end-organ damage. However, there is an emerging view that the adaptive immune response can be protective during the early host
response to sepsis. Here, we hypothesized that treatment with IL-7, a potent T cell anti-apoptotic cytokine would mediate the innate immune response to sepsis.

Material and methods: In this study, we subjected wild-type mice either treated with rhIL-7 or vehicle to cecal ligation and puncture (CLP).

Results: We investigated whether rhIL-7 treatment mediated the host innate immune response. First, we found that IL-7 treatment, given at the time of CLP, increased T cell Blc-2 expression as early as 3 h following the surgery as well as rescuing T cells from apoptosis. Next, we determined that serum IL-6 concentrations were significantly higher in the IL-7 treated mice at 6, but not 24 h after CLP. Importantly, bacteremia was decreased while general tissue damage was not significantly different between the IL-7 treated and untreated mice. We next observed increased in situ T cell production of IL-17, a cytokine involved in neutrophil recruitment, in mice treated with rhIL-7. Next, we observed systemic and local increases of IL-17 in the rhIL-7 treated mice. Consistent with increased IL-17, we determined that peritoneal concentrations of KC were increased in the IL-7 treated mice. Finally, we found increased neutrophil recruitment to the site of infection as well as increased neutrophil activation in the rhIL-7 treated mice.

Conclusion: These studies have advanced our knowledge of the crosstalk between the adaptive and innate immune arms during sepsis and may underlay novel potential therapeutic targets.

Corresponding Author: Charles Caldwell, PhD, University of Cincinnati, Department of Surgery, 231 Albert Sabin Way, Cincinnati, OH 45267, USA, charles.caldwell01@uc.edu

A 14
CCAAT/enhancer binding protein δ (C/EBPδ) impairs host defense during Streptococcus pneumoniae pneumonia

Jan Willem Duitman, Marcel Schouten, Sandrine Florquin, Angelique Groot, Tom van der Poll, Arnold Spek

Objectives: CCAAT-enhancer-binding protein delta (C/EBPδ) is transcription factor implicated in inflammation and apoptosis. However, the in vivo relevance of C/EBPδ for inflammatory disease has not been well elucidated yet. The current study aimed at elucidating the role of C/EBPδ during respiratory tract infection with Streptococcus pneumoniae (S. pneumoniae), the most common cause of community-acquired pneumonia. As secondary objective, we aimed to evaluate the underlying mechanism by which C/EBPδ affected the progression of S. pneumoniae infection.

Methods: Wild-type C57BL/6 mice and C/EBPδ−/− mice were infected intranasally (1 × 10^7 CFUs) or intravenously (i.v.; 0.5 × 10^5 CFUs) with S. pneumoniae. Mice were sacrificed at different time points after which bacterial outgrowth and inflammatory responses were determined both locally and systemically. The relevance of the observed differences was determined by a survival experiment, whereas the underlying mechanism was assessed by immunohistochemical stainings for epithelial barrier markers.

Results: S. pneumoniae pneumonia resulted in a up-regulation of C/EBPδ expression in lung tissue. C/EBPδ−/− mice showed an improved survival, which was accompanied by a lower bacterial load in the lungs at 48 h and a decreased dissemination of the bacteria to the systemic compartment at 24 and 48 h after inoculation. Lung inflammation was attenuated in C/EBPδ−/− mice at 48 h after inoculation, as indicated by cytokine/chemokine levels. Additional studies examining the effect of C/EBPδ on the early (6 h) inflammatory response to S. pneumoniae did not reveal an early accelerated or enhanced immune response. Intravenous injection of S. pneumoniae resulted in similar bacterial outgrowth and inflammatory responses in wildtype and C/EBPδ−/− mice suggesting that C/EBPδ does not affect bacterial clearance from the bloodstream. Interestingly, preliminary data show that C/EBPδ deficiency protects epithelial barrier integrity during S. pneumoniae pneumonia suggesting that bacterial dissemination into the bloodstream might be responsible for the aggravating effect of C/EBPδ.

Conclusion: These data suggest that C/EBPδ plays a detrimental role in host response to S. pneumoniae pneumonia by facilitating the bacterial growth and dissemination and concurrently enhancing the inflammatory response. Most likely, C/EBPδ-dependent epithelial barrier disruption plays a key role in both bacterial dissemination and concomitant inflammatory response.

Corresponding Author: Jan Willem Duitman, MSc, Academic Medical Center, Center for Experimental and Molecular Medicine, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, j.w.duitman@amc.uva.nl

A 15
The effect of anti-PAI-1 siRNA treatment upon PAI-1 protein and gene expression in NIH3T3 fibroblasts and mouse model of polymicrobial sepsis

Pierre Raenen, Georg Feichtinger, Katrin Weizelbäumer, Martijn van Griensven, Soheyl Bahrami, Marcin Osuchowski

Objective: Plasminogen activator inhibitor type 1 (PAI-1) is the main inhibitor of fibrinolysis and plays an important role in coagulopathy in critically ill patients. In sepsis, elevation of circulating PAI-1 is associated with higher mortality. The in vitro study aimed to select an anti-PAI-1 siRNA construct producing the most effective suppression of PAI-1 gene expression and protein synthesis in fibroblasts. The in vivo study aimed to define a viable therapeutic window for transient down-regulation of PAI-1 gene and protein expression in the acute phase of polymicrobial sepsis.

Patients and methods: NIH3T3 fibroblasts were transfected (lipofectamine) with 50 nM of either anti-PAI-1 or scrambled siRNA. PAI-1 protein/gene expression was measured at 24, 48 and 72 h post-transfection. 3 week old female CD-1 mice were used for in vivo experiments. First, the cecal ligation and puncture (CLP)-induced release of circulating PAI-1 was determined over 96 h. Next, mice were injected IV with 10 µg of siRNA complexed with polyethylenimine and were subjected to sub-lethal CLP 0, 24, 48 or 72 h later. Blood was sampled at CLP, 24 and 48 h post-CLP. Organs were harvested at 48 h post-CLP.

Results: In vitro, the most effective siRNA construct down-regulated PAI-1 gene expression by >40% resulting in a subsequent >80% (p < 0.001) repression of protein release at all time points. Circulating PAI-1 peaked at 24 h (475 vs. 8 ng/ml at baseline) and gradually decreased to 79 ng/ml at 96 h post-CLP. CLP triggered high PAI-1 gene expression in the heart and liver, but not in the lung or kidney. Of all treatment protocols tested, only siRNA administered 24 h prior to CLP markedly decreased circulating PAI-1. At 48 h post-CLP (but not earlier), the PAI-1 level was reduced to 54% (p = 0.06) compared to scrambled siRNA. This was accompanied by a sevenfold decrease of PAI-1 mRNA expression in the heart and liver. No effect of anti-PAI-1 siRNA was seen in the lungs.

Conclusion: The instant siRNA-dependent decrease of PAI-1 protein found in vitro was contrasted by its delayed repression in CLP sepsis. Only the pre-treatment 24 h prior to CLP led to siRNA-dependent down-regulation of circulating PAI-1 (at 48 h post-CLP) compared to earlier pre- or co-treatment. Additionally, the knockdown treatment was highly organ-selective i.e. the siRNA-dependent reduction of PAI-1 gene was apparent only in organs, in which its high expression was triggered by CLP. Supported by Marie-Curie IGRant.

Corresponding Author: Pierre Raenen, MD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA
Objective: The multiple organ dysfunction syndrome (MODS) is a frequent complication in septic patients. Yet the contribution of MODS to early septic mortality (ESM) is unclear. In order to outline the relationship of MODS and ESM, we assessed dynamics of organ function and metabolic parameters in dying (DYING) and surviving (SUR) mice during the acute phase (days 1–5) of polymicrobial sepsis.

A16
Comparison of organ function between dying and surviving mice during the acute phase of polymicrobial sepsis
Katrin Maria Weiselbaum, Dan Renick, Andrey Kozlov, Heinz Redl, Soheyl Bahrami, Marcin Osuchowski

Objective: The multiple organ dysfunction syndrome (MODS) is a frequent complication in septic patients. Yet the contribution of MODS to early septic mortality (ESM) is unclear. In order to outline the relationship of MODS and ESM, we assessed dynamics of organ function and metabolic parameters in dying (DYING) and surviving (SUR) mice during the acute phase (days 1–5) of polymicrobial sepsis.

A17
Long-term effects of sepsis: the influence of bacteremia and bacterial translocation on systemic adaptive immune responses
Timo Schwandt, Frank Juengerkes, Beatrix Schumak, Bernhard Holzmann, Laura Layland, Andreas Limmer

Objective: Bacterial translocation is a possible risk of abdominal surgery and may cause life-threatening consequences such as organ failure and septic shock. Patients surviving septic shock often suffer from opportunistic infections as well as defects in adaptive immunity.

Methods: Here we investigated the influence of bacteremia and bacterial translocation on systemic adaptive immune responses using murine models. To mimic abdominal surgery, mice were subjected to intestinal manipulation (IM). To study septic conditions, mice underwent colon ascendens stent peritonitis (CASP) or received E. coli intravenously or intraportally. We monitored the distribution of gut-derived bacteria by in vivo imaging (Xenogen) and additional microbiological assays and determined antigen-specific cytotoxic T lymphocyte (CTL) responses towards subsequent infection with recombinant adenviruses (rAV) or Listeria monocytogenes.

Results: We identified a strong correlation between the presence of bacteria in the spleen and a suppression of the CTL response, which was observed in mice that underwent CASP or were injected i.v. with E. coli. In contrast, the CTL response was not impaired in mice that were subjected to IM, or received E. coli by injection into the hepatic portal vein. Here, bacteria were detected in lung and liver but not in the spleens of mice.

Depletion experiments implied that Kupffer cells as well as soluble mediators such as tumor necrosis factor alpha played an important role in trapping and clearance of translocated bacteria in liver and lung. Importantly, we figured out, that mice deficient in TLR-4 or in both MyD88 and TRIF showed no impaired CTL response after systemic E. coli infection. Astonishingly, IL-10—known as an immunosuppressive cytokine—did not play any role in E. coli-induced suppression of CTL response but rather type I IFN were shown to represent essential effector molecules as in mice deficient in IFNAR or IRF3/7 generated normal CTL responses after E. coli infection.

Conclusion: We suggest that liver and lung fulfill a filter function to prevent systemic distribution of gut-derived bacteria. Failure of or bypassing these barriers might enable bacteria to access the spleen and thus cause systemic suppression of adaptive immunity, whereas the induction of local immunity was not affected. Suppression of CTL responses was strongly dependent on TLR-4 and mediated by downstream signaling of TRIF and MyD88 such as type I interferons.

Corresponding Author: Timo Schwandt, University of Bonn, Institute for molecular medicine, Sigmund-Freud-Str. 25, 53105 Bonn, Germany, t.schwandt@web.de

A18
The role of endogenous protein C during infection with Burkholderia pseudomallei
 Liesbeth Kager, W Joost Wiersinga, Marcel Schouten, Charles Esmon, Cornelis Van t Veer, Tom Van der Poll

Background: Melioidosis, an endemic disease in Southeast Asia, is caused by the gram-negative bacterium Burkholderia (B.) pseudomallei. Melioidosis is associated with pneumonia and bacterial dissemination to distant sites, often leading to severe sepsis. B. pseudomallei is also a potential bioterroristic agent. During pneumonia and sepsis a procoagulant state is elicited in the host, with activation of coagulation and downregulation of anticoagulant pathways. One of the major anticoagulants is activated protein C (APC), which also has cytoprotective and anti-inflammatory properties. Recombinant human APC has been shown to reduce mortality of patients with severe sepsis with a high likelihood of dying.

Objective: To investigate the role of endogenous protein C (PC) during murine infection with B. pseudomallei.

Methods: Three groups of mice were inoculated intranasally with B. pseudomallei to induce melioidosis and sepsis. They were treated with a monoclonal antibody (Mab) inhibiting both the anticoagulant and cytoprotective functions of PC (Mab 1609, a Mab inhibiting only the
A 19
**LQGV, an anti-inflammatory tetrapeptide derived from β-hCG, reduces CLP induced mortality and inflammation**

*JW van den Berg, J.W. van den Berg, W.A. Dik, R. Benner, J.N.M. Ijzermans, R.W.F. de Bruin*

Mortality in sepsis remains high and efforts to modulate inflammation so far failed to improve survival. The tetrapeptide LQGV, derived from the primary sequence of human Chorionic Gonadotropin, was recently shown to exert anti-inflammatory activity. Here the effect of LQGV on cecal ligation and puncture (CLP)-induced mortality and inflammation was examined in C57BL/6 mice. Low-grade CLP (40% ligation and double puncture) or high-grade CLP (80% ligation and double puncture) was used to induce sepsis. LQGV (5 mg/kg BW), PBS or dexamethasone (2.5 mg/kg/BW) were perioperatively administered. Survival as well as inflammatory markers were determined in plasma, peritoneum and lungs. Following high-grade CLP-induced sepsis mice also received antibiotics and fluid resuscitation. LQGV improved survival during the first 5 days following low-grade CLP. This was associated with reduced cytokine and E-selectin levels in peritoneal fluid, lungs and to a lesser extend in plasma. Furthermore, LQGV reduced pulmonary NF-kB activation and resulted in less pulmonary damage. LQGV combined with antibiotics and fluid resuscitation significantly improved survival following high-grade CLP as compared to antibiotics and fluid resuscitation alone. In conclusion, LQGV improves survival following CLP likely due to moderate down-regulation of inflammation and may be a valuable additive next to standard sepsis care.

*Corresponding Author: JW van den Berg, MD, Erasmus MC, Department of Immunology, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands, j.w.vandenber@erasumsmc.nl*

A 20
**Effects of modern crystalloid and colloid solutions on liver microcirculation and function in rodent sepsis**

*Tanja Stueber, Martin Schick, Jobst Isbary, Markus Pfeiffer, Norbert Roever, Christian Wunder*

Objective: Liver dysfunction plays a central role in remote organ injury during sepsis and has been identified as one of the major contributing factors for mortality. Volume resuscitation is one of the cornerstones of sepsis therapy to maintain hemodynamic stability and to improve microcirculation. It still remains unclear which solutions should be preferred in case of sepsis.

Methods: 36 anesthetized male Sprague-Dawley rats [285 ± 17 g bodyweight (BW)], underwent the CLP (cecal ligation and puncture)-procedure whereas control animals were sham operated. Animals of all groups (n = 5) received 0.5 ml/100 g BW/h NaCl 0.9%. Additionally, all CLP groups received 1 ml/100 g BW/h NaCl 0.9% (NaCl), Sterofundin® Iso (Steroiso), gelatin 4% (Gel) or 6% HES 130/0.4 (HES). After 24 h animals were re-anesthetized and intravitral microcirculation of the left liver lob was observed. Hemodynamic variables, cardiac output and blood gases were measured. Liver enzymes and coagulation parameters were determined to evaluate liver function. Statistical significance was determined by analysis of variance (ANOVA followed by post-hoc Duncan test, p < 0.05; mean ± SD).

Results: Mortality rate of all sepsis groups was 20% whereas NaCl showed the highest rate (50%) and SteroIso treated rodents survived completely. All sepsis groups had significant reduced sinusoidal diameters versus sham. HES infused rats had a significant increased sinusoidal velocity versus sham (0.29 ± 0.1 vs. 0.34 ± 0.1), whereas all other solutions exhibited significant decreased velocity [NaCl 0.22 ± 0.1; SteroIso 0.22 ± 0.1; Gel 0.23 ± 0.1 (μm/ms)]. Cardiac Index was significantly increased in colloid infused rats versus sham [Gel 468 ± 124; Hes 541 ± 60; sham 353 ± 67 (ml/min/kg)]. HES group had significantly increased DO2-I compared to all other sepsis groups. Albumin levels were significantly reduced in CLP treated rats. Gel had significantly elevated partial thromboplastin time [103 ± 39 vs. sham 49 ± 9 (s)] and INR (1.47 ± 0.5 vs. sham 0.85). Gel showed significant reduced level of glucose [36 ± 45 vs. sham 155 ± 40 (mg/dl)] and haemoglobin [8.6 ± 1.5 vs. sham 13.6 ± 0.7 (g/dl)]. Alanin-amoino-transferase and aspartate-amino-transferase showed a trend to increase in Gel treated rats.

Conclusion: The usage of different modern colloids and crystalloids in rodent sepsis leads to different effects in the liver. Gelatin 4% seems to derogate liver function, whereas 6% HES (130/0.4) optimized liver microcirculation when compared with crystalloid solutions.

*Corresponding Author: Martin Schick, MD, Department of Anaesthesiology, Department of Anaesthesiology, Oberderrabachersr.6, 97072 Waerzburg, Germany, schick_m@klinik.uni-waerzburg.de*

A 21
**Citrulline supplementation prevents organ failure in sepsis by improving the microcirculation**

*Karolina Wijnands, Hans Vink, Marcel Ho Kang You, Wim Buurman, Wout Lamers, Martijn Poewe*

Objective: Sepsis is characterized by microcirculation disturbances and endothelial glyocalyx degradation. Previous studies implicate an important role of the glyocalyx in nitric oxide (NO) production during sepsis. NO, a potent vasodilator, and citrulline (CIT) are produced from arginine (ARG) through NO-synthases (NOs). CIT can be recycled back to ARG, but CIT production is severely reduced during sepsis, resulting in diminished ARG de novo production and NO availability. The inflammatory NOS (iNOS) is upregulated during sepsis, using all available ARG, resulting in decreased NO for endothelial NOS (eNOS) and the microcirculation. Furthermore, sepsis is characterized by increased ARG breakdown, by arginase, leading to an extra decrease in ARG availability for eNOS. ARG supplementation did not improve the microcirculation in previous studies. However, CIT may be a potent therapeutic intervention in...
sepsis, as CIT increases the intracellular ARG pool, which is only available for eNOS and not iNOS.

Aim: To investigate the influence of CIT supplementation on the microcirculation in sepsis

Methods: Mice received either a continuous intravenous endotoxin (LPS, 50 μg total) infusion for 18 h alone or an 18 h LPS infusion combined with CIT during the last 6 h of endotoxin infusion. Side stream dark field imaging was used to evaluate the jejunal villi microcirculation. Images were obtained, and analysed for glycocalyx and microcirculation. Septic damage in tissue was quantified by immunohistochemistry of myeloperoxidase (MPO).

Results: The microcirculation was significantly improved in the LPS–CIT group compared to the LPS group ($p < 0.05$). CIT infusion resulted in an increased number of microvessels (diameter range 10–20 μm) compared to the LPS group. The red blood cell diameter in the LPS group was significantly larger (average difference of 0.4 μm, $p < 0.01$) compared to the LPS–CIT group, indicating less glycocalyx degradation in the LPS–CIT group during endotoxemia. Plasma ARG and CIT concentrations were significantly increased in LPS-CIT compared to LPS mice. CIT supplementation resulted in decreased MPO expression in the intestine compared to LPS treated mice.

Conclusion: Citrulline supplementation improves the microcirculation and prevents glycocalyx degradation during sepsis and may be a novel therapy to prevent organ failure during sepsis.

Corresponding Author: Karolina Wijnands, MD, Maastricht University Medical Center, Department of General Surgery, Universiteitssingel 50, 6229 ER Maastricht, The Netherlands, n.wijnands@ah.unimaas.nl

### A 22

**Systemic delivery of recombinant angiopoietin-1 ameliorates multiple-organ dysfunction syndrome in experimental abdominal sepsis**

*Philipp Kuempers, Alexander-Henrik Lukasz, Joon-Keun Park, Marijs van Meurs, Christian Koennecke, Sascha David*

Objective: Sepsis and endotoxic shock result in the development of SIRS causing MOD and often death. MOD is due to cellular dysfunction often not visible in histological examination. Characterisation of protein patterns of the affected organ is a crucial step in understanding intracellular changes causing cellular dysfunction. It has been shown by different authors that either mitochondrial or ER dysfunction can be the reason for MOD. The objective of this study was to investigate changes in the mitochondrial, ER, and cytosolic proteome of liver cells in response to LPS.

Materials and methods: Livers from Sprague-Dawley rats were obtained after 16 h of LPS-challenge (8 mg/kg, i.p.). Subcellular fractions were prepared by differential centrifugation of liver homogenates. Proteins were labelled with fluorophores and subsequently separated by two-dimensional electrophoresis (2D-DIGE). Spots differentially regulated between treated and control animals were identified by mass spectrometry methods.

Results: Upon LPS-challenge, in the mitochondrial fraction we observed upregulation of mitochondrial SOD, catalase and the s-chain of ATP synthase. In cytosol we observed upregulation of intact carbamoylphosphate synthase (CPS), 60 kDa heat shock protein, and one peroxiredoxin-1 spot. The most dramatic changes were observed in ER: many functional proteins were down-regulated (e.g. GRP78, protein disulfide isomerase A3, argininosuccinate synthase, transitional ER ATPase). At the same time, the expression levels of proteins responsible for antioxidant capacity were increased in mitochondria, slightly increased in cytosol, and definitely decreased in ER. We observed increased fragmentation of CPS, an important member of the urea cycle, in mitochondria and higher levels of intact CPS in cytoplasm of treated animals. This is in line with literature data describing that CPS is translocated from mitochondria and specific fragments are even detected in blood.

Conclusions: Our data suggest that protein patterns of ER are more sensitive to endotoxic shock than protein patterns of cytoplasm and mitochondria.

Corresponding Author: Alexander Lukasz, Medizinische Hochschule Hannover, Department of Nephrology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany, ALukasz@gmx.net

### A 23

**Aging is associated with protein C pathway suppression and increased coagulation during sepsis**

*Hiroshi Saito1, Hitoshi Takahashi1, Marlene Starr1, Bernard Evers1, Hartmut Weiler2, Charles Esmon2,3,4,5*

1*University of Kentucky College of Medicine, Lexington, KY, 2Blood Research Institute, Blood Center of Southeastern Wisconsin, Milwaukee, WI, 3Oklahoma Medical Research Foundation, 4Howard Hughes Medical Institute, 5University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*

Objective: Elderly patients with sepsis exhibit high mortality rates and increased thrombosis; however, mechanisms for the age-associated vulnerability to sepsis are not well understood. The protein C (PC) pathway is a negative feedback mechanism that regulates coagulation. This pathway is initiated when thrombin binds to thrombomodulin (TM) on the vascular endothelial cell surface where the complex converts the zymogen PC to activated protein C (APC). APC proteolytically inactivates FV and FVIIIa, thus inhibiting thrombin generation and propagation of the coagulation cascade. Clinically, septic patients with low APC levels are associated with a poor outcome. The purpose of this study is to determine whether sepsis-induced activation of the PC pathway is altered by aging.

Methods: Sepsis was induced in young (4-month) and aged (24-month) C57BL/6 mice by cecal ligation and puncture (CLP); mice were then monitored for survival for 10 days or sacrificed 24 h later for tissue harvesting. Levels of TM and fibrin (a marker of coagulation) were assessed by Western blot analyses. Plasma APC levels were determined with an immuno-capture assay using an APC-specific mAb and chromogenic substrate S2366. Mutant mice with reduced TM expression were also included in this study to assess the effects of TM deficiency during sepsis.

Results: Aged mice showed a higher mortality rate (100 vs. 23.1%, $p < 0.001$) with significantly elevated coagulation in the lung and kidney ($p < 0.05$) as compared to young mice. A 3.4-fold increase in plasma APC levels was observed in the young after CLP; however, APC levels in aged mice were not increased at all. TM expression in the lung was reduced in both young and aged mice after CLP with significantly more profound reduction in the aged ($p < 0.05$). TM-deficient mice showed significantly lower plasma APC levels ($p < 0.001$), increased fibrin formation ($p < 0.01$), and a higher mortality rate (100 vs. 34.6%, $p < 0.001$) as compared to wild-type control mice after CLP.

Conclusion: Aging is associated with profound down-regulation of TM expression during sepsis; this results in insufficient production of APC, increased coagulation, and high mortality in the aged.

Corresponding Author: Hiroshi Saito, PhD, University of Kentucky, Department of Surgery, 800 Rose Street, MS476, Lexington, KY 40536-0297, USA, hiroshi.saito@uky.edu
A 24
A cell culture model to analyze the influence of endotoxins on changes in gene expression of endothelial cells
 Dagmar Schwanzer-Pfeiffer, Eva Rossmanith, Dieter Falkenhagen

Objectives: This work is aimed to identify so far unknown factors in a cell culture model of endotoxemia. Effects of endotoxemia are widespread and several pathways are included, such as the activation of the endothelium and the recruitment of blood cells. The purpose of this study is to assess the influence of factors which are found by microarray analysis in an endothelial model of endotoxemia.

Materials and methods: THP-1 monocytes were stimulated with lipopolysaccharide (LPS) (4 h) for the production of a conditioned medium. This conditioned medium was used for the stimulation of human umbilical vein endothelial cells (HUVECs) (16 h) and changes in gene expression of stimulated HUVECs were analyzed by microarray analysis. Factors which indicated differences in the expression pattern, such as SVEP-1, SRPUL and KIAA, were selected for further analysis. To study their possible function, pro- and anti-inflammatory cytokines and complement factors were investigated after transfection of HUVECs with complementary siRNAs.

Results: Transient gene silencing revealed a significant increase in soluble E-selectin in case of SVEP-1 and SRPUL transfection of 19 and 14%, respectively. In addition, KIAA and SRPUL transfected cells showed a slightly increased expression of soluble ICAM-1 of about 10 and 11%. Concentrations of IL-6, IL-8, sVCAM-1, C3a and C5a showed no difference to the untransfected control. Quantitative RT-PCR and FACS analysis were performed to confirm aforementioned results.

Conclusions: In conclusion, we characterized some factors which seemed to have influence in cell adhesion and expression. SVEP-1 and SRPUL seemed to act as inhibitors of ICAM and E-selectin shedding, since the concentration of soluble factors of ICAM and E-selectin increased in the absence of either SVEP-1 or SRPUL.

Corresponding Author: Dagmar Schwanzer-Pfeiffer, PhD, Danube University Krems, Department Clinical Medicine and Biotechnology, Dr. Karl Dorrekstrasse 30, 3500 Krems, Austria, schwanzer-pfeiffer@donau-uni.ac.at

A 25
Murine susceptibility to bacterial infection following polymicrobial sepsis depends on innate immune cell production, recruitment and oxidative function and is independent of adaptive immunity
 Matthew Delano, Kindra Kelly-Scumpia, Robert Winfield, Philip Scumpia, Philip Efon, Lyle Moldawer

Objective: Current theory suggests that sepsis mortality is secondary to adaptive immune system dysfunction. However bacterial eradication is predicated on adequate innate immune system function. Neutrophils (PMN) and monocytes/macrophages (Mo/Mc) are the most abundant innate immune cells which eradicate pathogens by reactive oxygen species (ROS) production. We hypothesize that sepsis induces impairments in PMN/Mo/Mc generation and ROS production enabling subsequent infection and mortality independent of adaptive immunity.

Methods: C57BL/6 (B6), Rag2−/− (adaptive immune system deficient), CCR2−/− (monocyte migration deficient) and NCF-1 (NADPH oxidase deficient) mice were subjected to cecal ligation and puncture (CLP) (LD100) or sham procedure. At 3 and 7 days post-procedure, mice underwent either intranasal injection of P. aeruginosa (PA) (LD100) or intravenous L. monocytogenes (LM) (LD50). PA mouse (n = 5/group) bronchial alveolar lavage fluid (BALF) PMNs were collected at 24 h and LM mouse tissue Mo/Mc (n = 5/group) were collected at 5 days. Immune cells were tested for ROS production by dihydrorhodamine 123 incorporation and PMA stimulation via flow cytometry analysis.

Results: Day 3 post-CLP mice had a 60% greater mortality (p < 0.01 vs. sham) after PA. CLP PMN counts were 30–80% lower on days 3–7 (p < 0.01 vs. sham). BALF PMN counts 3 days after CLP and PA were less than sham (p < 0.001). Mice 3 days post-CLP receiving PA had reduced PMN ROS activity (p < 0.001) and increased BALF PA colonies (p < 0.001). NCF-1 mice failed to eliminate PA without prior adoptive transfer of wild type PMNs. After CLP and LM mice were devoid of LM colonies while sham mice demonstrated dramatic colonization (p < 0.001). Mice receiving ovalbumin-labeled LM 3 days after CLP had a greater ovalbumin-specific IgM response (p < 0.01 vs. sham). 3 days after CLP, splenic Mo/Mc had increased ROS (p < 0.001) and a 70% greater survival benefit over sham (p < 0.01). B6 and Rag2−/− mice after CLP were immune to LM mortality while CCR2−/− and sham B6 mice were not (p < 0.001). Conclusion: Using a CLP model of murine polymicrobial sepsis followed by PA pneumonia or LM bacteremia we demonstrated that PMN/Mo/Mc generation and ROS production are required for protection from subsequent bacterial infections. Using adaptive immune deficient Rag2−/− mice and Mo/Mc defective CCR2−/− mice we demonstrated the fundamental importance of intact innate immune function during subsequent bacterial eradication following sepsis.

Corresponding Author: Matthew Delano, MD, PhD, University of Florida, Department of Surgery, 1600 SW Archer Road, Gainesville, FL 32606, USA, delanmj@surgery.ufl.edu

A 26
Endotoxemia versus severe polymicrobial sepsis in rats: a retrospective comparison
 Patrick Scheiermann, Sandra Hoegl, Kim Boost, Bernhard Zwissler, Christian Hofstetter, Bertram Scheller

Objective: Endotoxemia and sepsis are different entities. Lipopolysaccharide (LPS) injection is still extensively used in short-term approaches to monitor hyperinflammatory immunological alterations. In contrast, clinically relevant sepsis models (i.e. CLP) induce systemic inflammation over a longer period of time. Cecal ligation and incision (CLI) is a new short-term sepsis model inducing polymicrobial sepsis within a few hours. In order to characterize similarities and differences between both experimental approaches, we aimed to compare retrospective data from endotoxemic and septic rats in regards to mortality, hemodynamics, blood gas analyses and proinflammatory cytokine patterns.

Material and methods: Endotoxemic (5 mg/kg LPS i.v., n = 8) and septic (CLI, n = 8) anesthetized/ventilated rats were matched. I.V. injection of 0.9%-NaCl (CTRL, n = 8) and laparotomy (SHAM, n = 8) represented the respective control groups. Mean arterial blood pressure (MAP), arterial pH and base excess (BE) were recorded continuously for 300 min. At the end of observation time, plasma samples were analysed for IL-1β, IL-6 and TNFα. Data are expressed as medians. Differences were considered statistically significant if *p < 0.05 using Kaplan–Meier Log-Rank test or Kruskal–Wallis analysis of variance of ranks with Dunn’s post-hoc test of all pairwise multiple comparisons.

Results: There were no significant differences at baseline between the groups. The chosen LPS-dose was not lethal. Mortality within the
A 27 Enhanced susceptibility for infection with S. pneumoniae during asplenia is related to the bacterial capsule

Jolanda Lammers, Alexander de Porto, Peter Hermans, Sandrine Florquin, Tom van der Poll

Objective: Patients without a spleen are believed to be susceptible for overwhelming infection with encapsulated bacteria. All guidelines to prevent post-splenectomy sepsis in these patients are focused on encapsulated bacteria, for example S. pneumoniae. The aim of this study was to determine whether the bacterial capsule is indeed responsible for poorer outcome after infection with S. pneumoniae in the asplenic host.

Methods: C57/BL6 mice were either Sham-operated or splenectomized (Splx). After 2 weeks, mice were inoculated intranasally (to induce pneumonia) with either wild-type serotype 2 S. pneumoniae (Splx) or the asplenic host.

Conclusion: Although proinflammatory cytokine levels are significantly higher after LPS-injection as compared to CLI-sepsis, this retrospective analysis shows that the CLI-procedure also induces systemic inflammation, arterial hypotension and metabolic acidosis within a comparable time. In contrast to LPS, CLI provides a polymicrobial insult. Hence, CLI features typical characteristics of severe sepsis and may represent a highly interesting experimental model to investigate short-term pathophysiological alterations in severe rodent sepsis.

Corresponding Author: Patrick Scheiermann, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Anaesthesiology, Marchioninistr. 15, 81377 Munich, Germany, pscheiermann@gmail.com

A 28 Rosmarinic acid attenuates inflammatory-induced organ injury in thermal injury and liver ischemia–reperfusion-injury

Joao Rocha, Maria Eduardo Figueira, Patricia Marques, Beatriz Silva Lima, Bruno Sepedes, Helder Mota Filipe

Rosmarinic acid (RA) is a naturally occurring polyphenolic compound that has been shown to inhibit activation of NF-kB and the complement system.

Here we evaluated the anti-inflammatory properties of RA on local and systemic models of inflammation. In order to study the effects of RA in local inflammation, a carrageenan-induced paw edema model in rats was performed. RA was administered at 10, 25 or 50 mg/kg (p.o.) and an extract of Rosmarinus officinalis (with RA as main constituent) was administered at 10 and 25 mg/kg (dosed as RA), 30 min before carrageenan injection. Inflammation was assessed by measurement of paw volume increase after 6 h. Male Wistar rats (100–150 g) were randomly allocated into 8 groups: Control, Carrageenan, RA10, RA25, RA50, E10, E25, Indomethacin).

Results demonstrate that administration of RA and extract at the dose of 25 mg/kg can reduce paw edema by over 60%, exhibiting a dose-response effect. Administration of the extract exhibited the same magnitude of effect, demonstrating that only RA contributed to the anti-inflammatory effect. RA effect on systemic inflammation was assessed using a rat model of thermal injury. Briefly, a 30% third degree skin burn was induced by immersing the dorsal shaved skin of the anaesthetized rat in 99°C water for 10 s. Organ injury was assessed by measurement of the serum levels of biochemical markers of liver and kidney injury. Our results show that pre-treatment (t = −15 min) with RA (25 mg/kg i.v.) reduced the increase on the level of biochemical markers (AST, ALT, LDH, and creatinine) for liver and kidney injury when compared to the Burn group. Measurement of serum cytokine levels after 6 h showed that RA pre-treatment reduced cytokine production increase induced by burn. RA effect on liver inflammation was evaluated in a rat liver ischemia–reperfusion (I/R) model. Rats were subjected to 45 min of ischemia to 3/4 of the liver followed by 2 h of reperfusion. In this model, RA was administered at 25 mg/kg (i.v.), 15 min prior to ischemia. Liver injury was accessed by measurement of the serum levels of liver injury markers. Results show that RA reduced the increase on the level of biochemical markers for liver injury (ALT, AST and LDH) when compared to the I/R group.

We demonstrate here that rosmarinic acid causes a substantial reduction of inflammation in both local and systemic inflammation models and propose that it may be useful in the therapy of inflammation-related injury.

Corresponding Author: Joao Rocha, MSc, University of Lisbon, Faculty of Pharmacy, Laboratory of Pharmacology, Avenida das Forcas Armadas, 1600-083 Lisbon, Portugal, jrocha@ff.ul.pt
A 29
Osteopontin impairs host defense during pneumococcal pneumonia
Gerritje van der Windt, Arie Hoogendijk, Marcel Schouten, Alex de Vos, Sandrine Florquin, Tom van der Poll

Objective: Streptococcus (S.) pneumoniae is the most frequently isolated pathogen responsible for community-acquired pneumonia. Osteopontin (OPN) is a phosphorylated glycoprotein that is involved in inflammatory processes during both innate and adaptive immunity. The aim of this study was to determine the role of OPN in the host response during pneumococcal pneumonia.

Methods: Wild-type (WT) and osteopontin knock-out (KO) mice were intranasally infected with a lethal dose (10⁴ colony forming units) of S. pneumoniae.

Results: OPN was constitutively present in lungs and plasma of naïve WT mice. Upon infection with S. pneumoniae pulmonary OPN concentrations were significantly elevated at 6 h and further increased at 48 h after infection, whereas plasma OPN levels increased more slowly and were elevated only at 48 h. OPN KO mice showed a prolonged survival relative to WT mice, which was accompanied by a diminished pulmonary bacterial growth and a reduced dissemination to distant body sites. In addition, at 48 h post infection pulmonary inflammation was reduced in OPN KO mice as reflected by lower inflammation scores and reduced chemokine concentrations. In contrast to pneumococcal pneumonia, OPN deficiency did not influence bacterial growth in primary pneumococcal sepsis induced by direct intravenous infection, suggesting that the detrimental effect of OPN on antibacterial defense during pneumonia primarily is exerted in the pulmonary compartment. Moreover, recombinant OPN stabilized S. pneumoniae viability in vitro.

Conclusion: These results suggest that the pneumococcus misuses OPN in the airways for optimal growth and to cause invasive disease after entering the lower airways.

Corresponding Author: Gerritje van der Windt, MSc, Academic Medical Center, Center for Experimental and Molecular Medicine, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, g.j.vanderwindt@amc.uva.nl

A 30
Protective role of PPAR-β/δ in cardiac dysfunction and organ injury/inflammation caused by endotoxemia in mice
Amar Kapoor, Yasunori Shintani, Massimo Collino, Lucy Bailey, Nimesh Patel, Bruno Sepodes

Objective: PPAR-β/δ is a transcription factor that belongs to the PPAR nuclear hormone receptor family. There is little information about the ligands of PPAR-β/δ and the effect of specific activation (e.g. GW0742) of PPAR-β/δ in animal models of shock.

Patients and methods: Here we used PPAR-β/δ knockout (KO) mice to investigate the role of this transcription factor in a lipopolysaccharide (LPS)-induced model of cardiac dysfunction and organ injury/inflammation.

Results: When challenged with LPS (9 mg/kg i.p.) for 16 h, KO mice exhibited a significant reduction in cardiac function when compared to (wild type) WT mice, as revealed by echocardiography. When compared to WT mice, KO mice exhibited significantly increased serum levels of creatinine (renal dysfunction) and alanine aminotransferase (ALT) (hepatocellular injury). In C57/BL6 mice, administration of the highly selective PPAR-β/δ agonist GW0742 (0.03 mg/kg i.v.) 1 h after LPS challenge significantly attenuated the cardiac dysfunction caused by LPS-induced endotoxemia, as revealed by echocardiography and the isolated-perfused Langendorff heart. Pre-treatment with a highly selective PPAR-β/δ agonist GSK0660 (0.1 mg/kg i.v.) 30 min before LPS reduced the cardioprotective effect of GW0742. When compared to mice challenged with LPS alone, GW0742 significantly attenuated creatinine serum levels and lung myeloperoxidase activity (lung inflammation).

Conclusion: These results suggest production of endogenous ligands of PPAR-β/δ which can afford a protective role in endotoxemia-induced cardiac dysfunction and organ injury as indicated by the loss of the receptor. Subsequently, activation of the receptor with a specific agonist, GW0742, inhibits the cardiac dysfunction and organ injury/inflammation caused by LPS-induced endotoxemia.

Corresponding Author: Amar Kapoor, William Harvey Research Institute, Translational Medicine and Therapeutics, Charterhouse Square, London EC1 M 6BQ, UK, a.kapoor@qmul.ac.uk

A 31
Systemic infection alters differentiation of dendritic cells in the bone marrow and mediates chronic dendritic cell dysfunction
Eva Pastille, Yang Zhang, Nadine Kroell, Michaela Bak, Fritz Ulrich Schade, Stefanie Barbara Flohe

Objectives: Sepsis is associated with the development of immunosuppression that leads to an enhanced susceptibility to secondary infections. During sepsis in mice, dendritic cells (DC) display a dysfunction that is characterized by an impaired capacity to secrete the Th1-polarizing cytokine IL-12 and to induce a protective Th1 cell response, while the production of IL-10 is enhanced. We investigated whether a defective differentiation of DC from bone marrow mediates DC dysfunction and the development of immunosuppression during sepsis.

Material and methods: Sepsis was induced in female BALB/c mice by cecal ligation and puncture (CLP). In Sham mice a laparotomy was performed alone. Four day after CLP bone marrow cells were isolated and cultured in the presence of GM-CSF to generate bone marrow derived dendritic cells (BMDC). Bone marrow derived dendritic cells were stimulated with bacterial oligonucleotides (CpG) and CD40 ligand. To test the capacity of BMDC to activate Th cells in vivo, CD4⁺ cells from D011.10 mice that are transgenic for an ovalbumin (OVA)-specific T cell receptor were injected i.v. in naïve mice and 24 h later OVA-loaded BMDC from septic or control mice were applied s.c.. After 7 days, lymph node cells were restimulated and cytokine levels were measured. The involvement of BMDC in the clearance of bacteria was tested by simultaneous intranasal injection of Pseudomonas aeruginosa and BMDC into naïve mice.

Results and conclusions: Upon stimulation with CpG and CD40 ligand BMDC from septic mice released extended levels of IL-10 but similar levels of IL-6 compared to BMDC from control mice. When injected subcutaneously, BMDC from septic mice showed a reduced capacity to promote Th1-cell polarization, while the proliferation of OVA-specific T cells in the draining lymph nodes was not affected. Intranasal application of BMDC from septic mice along with Pseudomonas aeruginosa into naïve mice disturbed the efficient clearance of the bacteria from the lung without having an impact on the recruitment of neutrophils. Lungs of mice treated with BMDC from septic mice contained less IL-12 and IFN-gamma in comparison to naive mice. The recruitment of neutrophils in the lungs of mice injected with sham BMDC. Thus, sepsis induces a reprogramming of DC precursors in the bone marrow towards dysfunctional DC that mediate immunosuppression through the inhibition of adaptive and innate immune responses.
A 32
A biofilm-penetrating, antimicrobial protein enables animal survival and biologic mesh protection in an infected model
Paul Montero, Terri Martin, Yuliya Yurko, Amy Lincourt, Alexey Vertegel, B Todd Heniford

Objective: Herniorrhaphy in an infected field is problematic, but biologic grafts have shown promise. Unfortunately, allografts tend to dissolve and xenografts lack ingrowth in an infected field. Herein we describe in vivo antibacterial properties of Lysostaphin, a naturally occurring antibacterial protein, when bound to biologic meshes in infected wounds versus uninfected controls.

Materials and methods: A rat model study design consisted of three variables: mesh type (Alloderm or Strattice), dosage of lysostaphin (none, low, or high dose), and infection (with or without 106 S. aureus). Each subgroup consisted of seven rats. Lysostaphin was adsorbed to mesh as previously described. A 3 x 3 cm² mesh was implanted on the fascia of a Lewis rat via midline incision. Mesh was explanted on day 28. An additional group of Alloderm was procured on day 60. Bacterial counts, histologic analysis, and tensiometry were performed. Standard statistical methods were used.

Results: For infected rats with Strattice, all animals died except those treated with Lysostaphin (100% survival). There was no difference in granulation between the Lysostaphin-treated infected grafts and uninfected controls. In infected Alloderm procured at 28 days, there were significantly less bacterial colonies (p = 0.003), neutrophils (p = 0.036), plasma cells (p = 0.0004), and lymphocytes (p = 0.003) with Lysostaphin treatment. There was an early reduction in overall inflammation and granulation (p = 0.001). Lysostaphin-treated, infected mesh did not affect fibroblast content (p = ns) or shear strength of the mesh (p = ns) compared to non-infected controls. By 60 days, all of the non-Lysostaphin, infected grafts had dissolved and essentially disappeared.

Conclusions: Lysostaphin, an organic, protein antimicrobial, can be applied to mesh and improves survival of infected rats with a xenograft while maintaining ingrowth. Histologic analysis demonstrates decreased numbers of bacteria and a dramatic reduction in wound inflammation yielding mesh salvage for the allograft in all animals. Treatment does not ultimately alter the shear strength, fibroblast content, or ingrowth of the mesh.

Corresponding Author: Paul Montero, MD, Carolinas Laparoscopic and Advanced Surgery Program, Department of General Surgery, 1000 Blythe Blvd, MEB 601, Charlotte, NC 28203, USA, paul.montero@carolinashcare.org

A 33
Impact of TRAIL in polymicrobial sepsis
Laura Klene, Katharina Czupka, Stephan Diedrich, Stefan Maier, Claus-Dieter Heidecke

Objective: In medical and surgical intensive care units severe sepsis and septic shock remain a major problem. Despite improvement of treatment mortality rate continues to be high because pathogenesis has not been fully understood yet. One phenomenon among others is elimination of lymphocytes by apoptosis during sepsis. Apoptosis can be induced via an intrinsic and an extrinsic pathway. The extrinsic way is initiated by activation of members of the TNF receptor family which posses an intracellular death domain. Among these are Fas, TNFR1 and the different receptors for TNF-related apoptosis inducing ligand (TRAIL). There is rising argument for Trail to influence several cells of the immune system. This is why we suppose a vital importance in polymicrobial sepsis.

Patients and methods: For all experiments Trail−− mice and C57B6 mice, as control group were used. CASP (16G) was performed as described before to induce sepsis in both groups. For survival analysis we used an 18G CASP. For further experiments organs (lung, liver, spleen, blood and peritoneal lavage) were harvested 20 h after CASP.

Results: First we checked for survival advantages. Comparable to previous findings 30% of the control mice survived. Numbers of survivors rose in the knock-out group up to 60%. (n = 11/group). Second we counted bacterial loads. Trail−− mice showed reduced bacterial loads in all investigated organs (n = 8/group). Third infiltration of granulocytes into the different tissues was measured. No significant differences were detectable between the observed groups (n = 5/group). Next we checked expression of DR5 in both Trail−− and control mouse. FACS analyses revealed enhanced expression of DR5 in both groups after sepsis induction. In the control group 1% of all cells expressed DR5 on their surface whereas in Trail−− percentages rose up to 3.5% of all cells (n = 6/group, p < 0.005).

Conclusion: In our recent studies we could show that Trail−− mice do have a survival advantage as survival analysis showed a protective effect of the knock-out raising survival from 30 to 60%. Furthermore bacterial assimilation was reduced. Even though there is better elimination of bacteria we were not able to observe a consequence on granulocyte invasion. Even though Expression of Trail was knocked out in both groups DR5 receptor was upregulated. We now suppose an alternative way of activation for this receptor. But further evidence had to be supplied.

Corresponding Author: Laura Klene, Ernst Moritz Arndt University Greifswald, Department of Surgery, Friedrich-Loeffler-Str. 23b, 17475 Greifswald, Germany, laura_klene@web.de

A 34
The arginine-NO metabolism in a prolonged murine sepsis model
Karolina Wijnands, Ernst van Faassen, Wim Buurman, Jacco Briele, Wout Lamers, Martijn Poeze

Objective: A possible explanation for sepsis-induced multiple organ failure (MOF) is a persistent abnormal organ microcirculation. Previously, in mice mainly intraperitoneally bolus injection of endotoxin was used as a model to study MOF. However, in the usual clinical setting, patients are exposed to a more gradual, but prolonged septic insult. Therefore, there is need to study the response of small animals to endotoxin during prolonged periods. A key factor in organ perfusion is nitric oxide (NO) production. NO and citrulline are produced to endotoxin during prolonged periods. A key factor in organ perfusion is nitric oxide (NO) production. NO and citrulline are produced from arginine breakdown, and citrulline can be recycled back to arginine. Metabolic pathways of arginine-NO metabolism in human sepsis are characterized by a decreased arginine de novo synthesis and increased arginine breakdown by arginase, leading to decreased arginine availability for NO synthesis.

Aim: to study the effect of prolonged endotoxic insult on the arginine-NO metabolism in the blood and tissue compartments of mice.

Methods: Mice received a continuous intravenous endotoxin (LPS, 50 μg total) infusion for 18 h. Endogenous NO levels were determined by in vivo spin trapping (30 min) of NO with Fe-DETC complexes. After trapping, the animals were sacrificed, arterial blood sampled and tissues snap frozen in liquid nitrogen. The trapping yield of the nitrosyl-Fe2+-DETC complex (MNIC) in tissues was quantified...
at 77 K. Amino acid concentrations in plasma were measured with HPLC. Septic damage in tissue was quantified by immunohistochemistry of myeloperoxidase (MPO).

Results: Tissues of LPS-treated mice showed two to threefold higher MNIC compared to controls (gut, liver, kidney, heart), correlated with increased MPO expression in the gut. In this prolonged model, the MNIC yields are significantly lower than in murine acute bolus endotoxin-induced sepsis. Whole-blood contained significantly higher quantities of MNIC and nitrosyl-Hb complexes in LPS-treated mice. Plasma arginine and citrulline concentrations of LPS challenged mice were significantly lower than in controls. In contrast, plasma ornithine levels were significantly higher.

Conclusion: Prolonged infusion of LPS has reproducible effects on the NO levels and arginine–citrulline metabolism in both blood and tissue compartments of mice. Enhanced endogenous NO levels are correlated with decreased plasma levels of the precursor arginine. Our murine model with prolonged infusion appears applicable to the human clinical situation to study NO metabolism of sepsis.

Corresponding Author: Karolina Wijnands, MD, Maastricht University Medical Center, Department of Surgery, Universiteitszeggel 50, 6229 ER Maastricht, The Netherlands, n.wijnands@ah.unimaas.nl

A 35
Treatment with the histone deacetylase inhibitor, valproic acid, worsens outcome to sepsis
Alex Cuenca, Kindra Kelly-Scumpia, Philip Scumpia, Dina Nacionales, Philip Efron, Lyle Moldawer

Objectives: Acetylation of chromatin is controlled by both histone acetyl transferases and histone deacetylases (HDACs). It is a highly regulated process that enables transcription and/or repression of genes during homeostasis and stress. HDAC inhibitors (HDACi) have been shown to reduce pathology in inflammatory disease and are thought to function by inhibiting one or more classes of HDACs. For example, valproic acid (VPA) has been shown to inhibit class I/IIa HDACs, whereas trichostatin A (TSA) has been shown to inhibit all four classes of HDACs. As HDAC’s have been shown to improve outcome in other inflammatory diseases, the primary goal of the study was to determine the effect of VPA on survival in cecal ligation and puncture (CLP).

Methods: The mouse model of polymicrobial sepsis, cecal ligation and puncture (CLP), was used to induce sepsis in C57BL/6 mice. Organs, splenocytes, blood and peritoneal exudates were obtained at 24 h post-CLP to assess the impact of sepsis on the expression of BTLA in various tissues (western analysis) and lymphocytes [flow cytometry (FACS)] and how BTLA gene deficiency affected blood bacterial burden. Comparative survival was also determined for septic WT and BTLA knockout mice over 15 days.

Results: Using CLP in the mouse we observed that 24 h after septic challenge there is a marked increase in the expression of BTLA in the thymus, spleen, lung and peritoneum. Initial studies attempting to delineate the cell sub-populations expressing BTLA during sepsis by FACS point at a significant rise of BTLA on septic mouse B-cells with trends towards an increase of BTLA expression on CD4+ and CD11c+ cell populations. Importantly, we have found that mice genetically deficient in BTLA expression efficiently clear bacterial burden. Comparative survival was also determined for septic WT and BTLA knockout mice over 15 days. Results: Using CLP in the mouse we observed that 24 h after septic challenge there is a marked increase in the expression of BTLA in the thymus, spleen, lung and peritoneum. Initial studies attempting to delineate the cell sub-populations expressing BTLA during sepsis by FACS point at a significant rise of BTLA on septic mouse B-cells with trends towards an increase of BTLA expression on CD4+ and CD11c+ cell populations. Importantly, we have found that mice genetically deficient in BTLA expression efficiently clear bacterial burden. Comparative survival was also determined for septic WT and BTLA knockout mice over 15 days.

Conclusion: Together these data indicate that BTLA plays an important role in septic morbidity/mortality that directly and/or indirectly affect aspects of both adaptive, e.g., T and B-cell function and innate host pathogen responsiveness.

Corresponding Author: Nicholas Shubin, Nicholas Shubin, Chun-Shiang Chang, Alfred Ayala

A 36
B and T lymphocyte attenuator (BTLA) is a contributor to the pathological progression of sepsis
Nicholas Shubin, Nicholas Shubin, Chun-Shiang Chang, Alfred Ayala

Objective: Sepsis develops through two-phases upon which the body initially elicits a strong pro-inflammatory response to infection, but is then followed by an anti-inflammatory response. The subsequent anti-inflammatory response often results in inflammatory cell dysfunction and an inability to clear infection, which can lead to multiple organ failure and death. Among the inflammatory cells that have reduced function during the anti-inflammatory phase of sepsis are T and B-cells. Activation of T and B-lymphocytes is regulated by co-inhibitory signaling through receptors such as CTLA-4, PD-1 and the relatively recently discovered receptor BTLA. Much like PD-1, BTLA contains cytoplasmic ITIM and ITSM domains that upon stimulation by its ligand HVEM activates the phosphatases SHP-1 and SHP-2 to stop cell activation and proliferation. BTLA is constitutively expressed at high levels on B-cells and may be induced on T-cells. Our objective is to therefore determine if BTLA is involved in the T and B-lymphocyte dysfunction observed during sepsis.

Methods: The mouse model of polymicrobial sepsis, cecal ligation and puncture (CLP), was used to induce sepsis in WT and BTLA deficient C57BL/6 mice. Organs, splenocytes, blood and peritoneal exudates were obtained at 24 h post-CLP to assess the impact of sepsis on the expression of BTLA in various tissues (western analysis) and lymphocytes [flow cytometry (FACS)] and how BTLA gene deficiency affected blood bacterial burden. Comparative survival was also determined for septic WT and BTLA knockout mice over 15 days.

Results: Using CLP in the mouse we observed that 24 h after septic challenge there is a marked increase in the expression of BTLA in the thymus, spleen, lung and peritoneum. Initial studies attempting to delineate the cell sub-populations expressing BTLA during sepsis by FACS point at a significant rise of BTLA on septic mouse B-cells with trends towards an increase of BTLA expression on CD4+ and CD11c+ cell populations. Importantly, we have found that mice genetically deficient in BTLA expression efficiently clear bacterial burden. Comparative survival was also determined for septic WT and BTLA knockout mice over 15 days.

Conclusion: Together these data indicate that BTLA plays an important role in septic morbidity/mortality that directly and/or indirectly affect aspects of both adaptive, e.g., T and B-cell function and innate host pathogen responsiveness.

Corresponding Author: Nicholas Shubin, Nicholas Shubin, Chun-Shiang Chang, Alfred Ayala

A 37
Endothelin mediated gut microcirculatory dysfunction during porcine endotoxemia
Andreas Andersson, Johan Fenhammar, Eddie Weitzberg, Alf Sollevi, Hans Hjelmqvist, Robert Frithiof

Objective: Microcirculatory changes are a fundamental part of the pathophysiology of septic shock. Endothelin-1 is a potent...
A 39
H2S blocks activation of NF-kBp65 in the human chondrocyte cell line C28/I-2
Burkhard Kloesch, Melissa Liszt, Johann Broell

Objective: Osteoarthritis (OA) is a type of arthritis that is caused by the breakdown and loss of the cartilage of one or more joints. Loss of the cartilage cushion causes friction between the bones, leading to pain and limitation of joint mobility and is often accompanied by inflammation of the joint capsule. Beside the treatment of OA with anti-inflammatory drugs (NSAID, “Biologica”, etc.), H2S has long been used as an alternative therapy for patients suffering from different rheumatic disorders. Yet, little is known about the molecular mechanisms of H2S in chronic inflammatory diseases like OA.

Methods: A human chondrocyte cell line (C28/I-2) was pre-incubated with an exogenous H2S donor (NaHS) before being stimulated with the cytokine IL-1β. After stimulation mRNA levels of IL-6 and IL-8 were quantified by real-time RT-PCR. Secretion of IL-6 and IL-8 protein was quantified by Enzyme-linked immunosorbent assays (ELISAs). Phosphorylation of the transcription factor NF-kBp65 was confirmed by the “NF-kB Transcription Factor Assay Kit” (Cayman Chemicals).

Results: C28/I-2 cells constitutively express and secret large quantities of IL-6 and IL-8. Stimulation with IL-1β leads to an increase in IL-6 and IL-8 expression. Data provided proves that in these cells IL-1β stimulated IL-6 and IL-8 expression is transiently blocked by the pre-treatment of cells with low concentrations of NaHS. One key finding is that H2S does inhibit phosphorylation of NF-kBp65 at Ser536 as well as p38 and ERK1/2 MAPKs were checked by Western blot experiments. Transactivation of NF-kBp65 was confirmed by the “NF-kB Transcription Factor Assay Kit” (Cayman Chemicals).

Conclusion: Presented data seem of importance to clarify underlying molecular mechanism of H2S and suggests H2S as an anti-inflammatory molecule for the treatment of chronic inflammatory processes.

Corresponding Author: Burkhard Kloesch, PhD, Ludwig Boltzmann Cluster for Rheumatology, Balneoology and Rehabilitation, Department of Molecularbiology, Kurbadstrasse 10, 1100 Vienna, Austria, burkhard.kloesch@gnix.at

A 40
TRIF deficiency in hematopoietic cells impairs host defense in Klebsiella pneumoniae pneumonia
Miriam van Lieshout, Cornelis van ’t Veer, Alex de Vos, Tom van der Poll

Objective: Klebsiella pneumoniae is a frequent causative agent in pneumonia. Toll/interleukin-1 receptor domain-containing
A 41
Phagocytosis and inflammation: what’s the connection?
David Underhill

Macrophages and dendritic cells use a panoply of receptors to detect microbial pathogens, and these receptors generate diverse intracellular signals that combine to define the nature of the net inflammatory response. Some receptors such as Toll-like receptors trigger primarily inflammatory cytokine and chemokine production, while other receptors such as the β-glucan receptor Dectin-1 additionally trigger responses such as phagocytosis and respiratory burst activity. While the former responses are useful even if a cell is not in direct contact with a microbe, the latter responses require contact to be effective. How does an individual receptor know the difference? We observed that while soluble β-glucan efficiently binds to Dectin-1, it does not activate the receptor. In contrast particulate β-glucan strongly activates the receptor. We demonstrate that upon binding to a particle of β-glucan, Dectin-1 is effectively segregated from membrane tyrosine phosphatases that suppress its activation. Signaling is initiated in the center of the “phagocytic synapse” structure that forms upon binding, and this structure is not formed when the receptor binds to soluble glucans.

Corresponding Author: David Underhill, PhD, Cedars-Sinai Medical Center, Immunobiology Resch. Inst., 110 George Burns Rd, Los Angeles, CA 90048, USA, david.underhill@csbs.org

A 43
Aging and innate immunity: is inflamm-aging = macroph-aging?
Elizabeth Kovacs

Advanced age is associated with an elevated basal inflammatory state referred to as “inflamm-aging.” This hyper-inflammatory condition is thought to predispose aged subjects to increased morbidity and mortality after infection or injury, as a result of excessive tissue damage and suppression of immune responses. We observed that after a 15% total body surface area burn injury or intraperitoneal administration of lipopolysaccharide (LPS), aged (18–22 month old) BALB/C mice have significantly higher mortality than young (3–6 month old) mice subjected to burn injury or given LPS. Additionally, aged mice had markedly elevated systemic and local inflammatory responses relative to young given the same insult. The inflammatory responses included aberrant leukocyte distribution and overproduction of pro-inflammatory cytokines, such as interleukin-6.
(IL-6) and tumor necrosis factor z (TNFz). These changes were seen in the peripheral blood, the liver and the lung among other organ systems. To begin to investigate the mechanisms responsible for the exaggerated inflammatory response in aged mice with or without injury, we compared macrophage cytokine production after in vivo and in vitro stimulation in the hope of determining if "inflamm-aging" resulted from overproduction of macrophage-derived mediators, "macroph-aging." In contrast to our in vivo observations, we found that in vitro LPS stimulation of macrophages harvested from the spleens of unmanipulated aged mice produced lower levels of IL-6 and TNFz than comparably treated cells from young mice. This observation was consistent regardless of in vivo insult. Furthermore, we noted that the attenuated in vitro pro-inflammatory response was not specific for LPS, but rather was similar after culture of macrophages with other Toll-like receptor (TLR) ligands. Age dependent defects in signaling pathways were found to occur at the level of mitogen activated protein kinases (MAPK). Additional studies will be required to determine the mechanisms responsible for the in vivo/in vitro discrepancy in cytokine production. Preliminary evidence suggests that the effects of both intrinsic and extrinsic factors are involved. [Supported by NIH R01 AG18859 (EJK), Illinois Excellence in Academic Medicine Grant, and the Falk Medical Research Trust].

Corresponding Author: Elizabeth Kovacs, Prof. PhD, Loyola University Chicago, Department of Surgery, 2160 South First Avenue, Maywood, IL 60153, USA, ekovacs@lumc.edu

A 44
The non-toll innate immune receptor dectin contributes to macrophage activation in chronic inflammation
Martha K. Cathcart, Deena Elsori, Praveena Thiagarajan

Macrophages are major participants chronic inflammation. One macrophage activation pathway that is believed to contribute to the pathology of chronic inflammation is the production of superoxide anion via the NADPH oxidase enzyme complex. We hypothesized that in the oxidative environment of a chronic inflammatory site that repetitive ligands might be generated that could trigger further macrophage activation via pattern recognition receptors. Zymosan, a yeast cell wall preparation, is a potent activator of macrophage NADPH oxidase. We have shown that zymosan activates NADPH oxidase through the non-toll pattern recognition receptor Dectin. Dectin engagement by zymosan causes downstream activation of SRC, SYK and PKCδ. Notably PKC-δ is a new member of the downstream signaling pathway for Dectin. To identify endogenous human ligands that are present in chronic inflammatory sites that can engage Dectin to activate human macrophage NADPH oxidase, we used an antibody to zymosan to search for similar endogenous, immunoreactive ligands in extracts of human atherosclerotic plaques. Endogenous ligands have been reported for some of the toll receptors but here to for none have been detected that interact with Dectin. Several ligands were detected in 2D Western blots using the anti-zymosan antibody. They were then identified by Mass Spec. Their direct binding to Dectin was verified by Biacore and by immunoaffinity pull-down. Their ability to activate macrophage NADPH oxidase was further confirmed using recombinant proteins. This process of generating endogenous ligands at a site of inflammation that can chronically activate macrophage NADPH oxidase likely contributes to tissue injury in chronic inflammation and may be responsible for the failure to resolve the inflammatory condition. These results suggest that localized interference with innate immune receptors may prove helpful in the treatment of chronic inflammatory disease.

(Supported by NIH R01 HL61971 to MKC)
Corresponding Author: Martha K. Cathcart, Prof. PhD, Cleveland Clinic Lerner College of Medicine, Department of Cell Biology, 9500 Euclid Avenue, Cleveland, OH 44195, USA, cathcam@ccf.org

A 45
Leucocytes, inflammation and host defense
Peter A. Ward

Objectives: To evaluate the strong proinflammatory state in rodents following cecal ligation and puncture (CLP).

Materials and methods: CLP was induced in rats and mice and appropriate methods were used to measure markers for leukocyte and vascular adhesion molecules on blood PMNs and endothelial cells.

Results: Endothelial cells had upregulated expression of ICAM-1 protein and enhanced production of cytokines/chemokines. PMNs had a "gain of function" related to increased surface expression of β1 and β2 adhesion molecules, which infers enhanced responsiveness to ligands for these adhesion molecules. PMNs were resistant to apoptosis, suggesting a longer life span both in vascular and extravascular compartments. In vivo blockade of IL-17A or C5a resulted in greatly reduced levels of plasma cytokines/chemokines. Production of C5a during sepsis resulted in adverse effects on both myeloid and non-myeloid cells. Interaction of PMNs with C5a at levels found in sepsis (10–100 nM) led to paralysis of signaling pathways (MAPKs) in these cells, causing defective innate immune responses. PMN contact with C5a resulted in two additional outcomes: formation and release of microparticles that were enriched in C5aR and internalization of C5aR/C5aR complexes, resulting in loss of C5a binding sites. Interaction of C5a with cardiomyocytes caused development of contractile and electrophysiological defects.

Conclusions: CLP induced a strong proinflammatory state in rodents as revealed by: enhanced ICAM-1 on endothelial cells and upregulated β1 and β2 adhesion molecules on blood PMNs, resulting in a "gain of function" for these cells; resistance of blood PMNs to apoptosis; dependency of the "cytokine storm" on C5a and IL-17A; C5a-dependent loss of innate immune functions of blood PMNs, together with C5a-dependent release of microparticles enriched in C5aR and C5a-dependent loss of contractility in vivo and in vitro of cardiomyocytes accompanied by electrophysiological abnormalities.

The data suggest that CLP-induced sepsis unleashes a series of harmful proinflammatory events, suggesting strategies for possible interventions in the setting of sepsis.

Corresponding Author: Peter A. Ward, Prof. MD, The University of Michigan Medical School, Department of Pathology, 1301 Catherine Rd, Ann Arbor, MI 48109, USA, pward@umich.edu

A 46
Dynamics of fibrinogen metabolism for 5 days following hemorrhagic shock and lactated ringer’s (LR) resuscitation in pigs
Wenjun Martini, David Chinkes, Shavaughn Colvin, Armando Rodriguez, Michael Dubick

Objectives: Fibrinogen availability plays an important role in restoring coagulation function after hemorrhage. We previously reported acute changes in fibrinogen metabolism after hemorrhage. The purpose of this study was to investigate long-term (5 days) dynamics of fibrinogen metabolism after hemorrhagic shock in pigs.

Material and methods: Sixteen pigs were randomized into the control (C) and the hemorrhage (H) groups. On day 1, hemorrhage was
induced in H by bleeding 35% of the total blood volume, followed by resuscitation with LR at 3 times the bled volume. Pigs in C were not hemorrhaged or resuscitated. Then a primed constant infusion of stable isotope 1-13C-phenylalanine (phe, 6 h) and d5-phe was performed in C and H with hourly blood samplings for quantification of fibrinogen metabolism. Hemodynamics and coagulation function were measured at baseline, after hemorrhage and resuscitation, and end of the infusion. The stable isotope infusion and measurements were repeated on day 2, 3, 4 and 5.

Results: All measurements were similar between C and H at baseline of day 1. Hemorrhage caused a decrease in mean arterial pressure and an increase in heart rate. Fluid resuscitation corrected these changes. Compared to baseline day 1, fibrinogen levels in H were decreased to 76 ± 6% by hemorrhage with resuscitation in day 1, increased to 217 ± 16% in day 2 (both p < 0.05), and remained at elevated levels afterwards. Clot strength in H was decreased by hemorrhage from 70 ± 1 mm (baseline day 1) to 66 ± 1 mm (p < 0.05) in day 1, and returned to baseline values in day 2 and afterwards. Compared to control day 1 (1.3 ± 0.1 mg/kg/h), fibrinogen synthesis in H was increased to 3.6 ± 0.1, 5.1 ± 0.5, 2.6 ± 0.4, 2.7 ± 0.5, and 2.3 ± 0.3 mg/kg/h in day 1, 2, 3, 4, and 5 (all p < 0.05). Compared to control day 1 (10.6 ± 14 mg/kg/h), fibrinogen breakdown in H was increased to 15.9 ± 1.4 and 16.5 ± 3.2 mg/kg/h in day 1 and 2 (both p < 0.05) but returned to control values afterwards. Fibrinogen half-life in H was shortened from 49 ± 6 h (control day 1) to 32 ± 2 and 31 ± 2 h in day 1 and 2 (both p < 0.05) and returned control values afterwards.

Conclusions: Hemorrhage caused accelerated fibrinogen turnover and the increase of fibrinogen synthesis peaked at day 2. These data suggest that fibrinogen supplementation may be necessary early in acutely injured and bleeding trauma patients.

A 47 Tranexamic acid for preventing progressive intracranial hemorrhage in adults with traumatic brain injury: a preliminary report

Surakrant Yutthakasemsunt, Piseke Lumbiganon, Nakornchai Phuenpathom, Bandit Thinkamrop, Warawat Kittiwatanagul, Ian Roberts

Background: Traumatic brain injury (TBI) is a major health problem with poor outcome especially with progressive intracranial hemorrhage (PIH) in severe patients. There are links between coagulopathic change after TBI and delayed traumatic hemorrhage revealed by CT brain. Antifibrinolytic treatment can reduce blood loss after surgery and perhaps in moderate to severe TBI by similar haemostatic responses. It is also justified to determine benefit for reversing hyperfibrinolysis after TBI. Tranexamic acid (TXA) has been shown to have significant clinical benefit in effectively reducing surgical bleeding with no effect on coagulation parameters and no demonstrated harmful effect in systematic reviews.

Objective: We designed to determine the effectiveness of TXA in preventing PIH in adults’ patients with moderate to severe TBI. We expected to see 50% relative treatment effect in 30% PIH in controlled group.

Methods: The study was a single hospital-based, randomised, double-blind, placebo-controlled, parallel group trial. It was approved by the Khon Kaen Hospital Ethics Committee and Khon Kaen University Ethics Committee with online trial registration. We enrolled 240 patients aged over 16 with moderate-severe (GCS 4–12) non-penetrating TBI within 8 h onset and no indication for emergency neurosurgical or major operation. We excluded patients with coagulopathy and creatinine above 2 mg%. The intervention was TXA (1 g bolus and 1 g maintenance for 8 h) with controlled placebo. The primary outcome was PIH revealed by CT about 24 h and secondary outcomes were clinically relevant variables until discharge.

Results: PIH was found 13.3% in Tranexamic and controlled group in order. There were nine patients without second CT (5 TXA/4 Placebo). TXA reduced PIH relatively 44.82, 10.81% absolutely (95% CI 0.01–0.21, p = 0.047) by ITT analysis and 44.35% RRR, 11.10% ARR (95% CI 0.01–0.21, p = 0.049) by PP analysis. There were 10%/15% of death in TXA/placebo group (33.3% RRR, 5% ARR with 95% CI −0.14–0.04, p = 0.329) while 17.5%/23.3% outcome in placebo. Also there were 25.8%/28.3% transfusion need during admission in TXA/placebo, respectively (8.82% RRR, 2.5% ARR with 95% CI −0.14–0.09, p = 0.771).

Conclusion: TXA has been shown statistical significant reduction for PIH in moderate–severe adults TBI while it does not reach our clinical expectation. There is no significant different effect from placebo on mortality and transfusion need.

Corresponding Author: Surakrant Yutthakasemsunt, MD, Khon Kaen University and Khon Kaen Regional Hospital, Faculty of Medicine, Mittraphap Road, 40000 Khon Kaen, Thailand, surakrant@yahoo.com

A 48 Rodent coagulation responses to trauma and haemorrhagic shock

Daniel Frith, Mitchell Cohen, Rupert Pearse, Chris Thiemermann, Karim Brohi

Objectives: Acute traumatic coagulopathy (ATC) is associated with a fourfold higher mortality, increased transfusion requirements and worse organ failure. It can develop in the absence of exogenous dilution or hypothermia. Increased levels of anticoagulant activated protein c have been identified in trauma patients with ATC. We have developed two rodent models of ATC in order to comprehensively characterise the pathophysiology of this condition.

Materials and methods: Male Wistar rats were anaesthetised with intra-peritoneal Phenobarbitone, subjected to hind limb crush, closed tibia/fibula fracture and had 35% of their estimated circulating volume removed. Male C57BL/6 mice were anaesthetised with isoflurane, subjected to laparotomy and bled to a mean arterial pressure of 25–35 mmHg. No resuscitation fluid was administered. Sham control animals were anaesthetised and monitored only. After 60 min blood samples were aspirated for gas analysis and coagulation factor assays. Ex vivo spiking tests with hydrochloric acid were performed to determine the influence of acidaemia on coagulation per se.

Results: Endogenous ATC was consistently demonstrated in response to combined trauma and haemorrhagic shock in both species. Prothrombin times (PT) were prolonged 1.4 times normal (p < 0.001 compared to sham controls) and partial thromboplastin times (PTT) were prolonged greater than 1.6 times normal (p < 0.001). Animals subjected to trauma and haemorrhage developed mild metabolic acidemia and elevated lactate levels (pH 7.15, l 8.5 mmol/l; p < 0.001). However, spiking tests demonstrated that this concentration of acid does not affect the PT (pH 7.15:17.2 vs. pH 7.35:17.4 s; p = ns) and only minimally prolongs the PTT (pH 7.15:18.1 vs. pH 7.35:16.0 s; p = ns).

Coagulopathy in rats was associated with significant (p < 0.001) reductions in factors II (59%), V (57%), VII (47%), VIII (71%), and X (41%) as well as depletion of antithrombin (50%) and protein c...
Methods: Anesthetized Yorkshire swine (n = 4/group) were subjected to 55% estimated blood volume controlled hemorrhage via carotid artery over 15 min. At 15 min (T15), T30 and T45, animals were resuscitated with HBOC-201, HBOC-201 + NTP (0.8 mcg/kg/min first infusion, 1 mcg/kg/min subsequent infusions), Hextend (HEX) or HEX + NTP. Each infusion was given concomitantly through separate lines over 10 min. At 60 min, hospital arrival was simulated and animals were eligible to receive blood or saline. Pigs were euthanized at 120 min. Hemodynamic parameters were continuously measured. Vasoactivity was assessed via mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), systemic and pulmonary vascular resistance indices (SVRI and PVRI).

Results: Baseline values were similar among groups. Survival was 100%, except for HEX (75%). Resuscitation with HBOC-201 + NTP significantly attenuated MAP and MPAP increases during the first and second infusion compared to HBOC-201 (p < 0.05). Towards the end of the third infusion, this effect was lost in the HBOC-201 + NTP group and pressures were equal to HBOC-201 alone. SVRI and PVRI were similar, cardiac output (CO) was lower in HBOC-201 + NTP group. There was no significant difference between HEX and HEX + NTP.

Conclusions: Concomitant infusion of NTP reduced HBOC-201 associated vasoactivity in pre-hospital resuscitation in this swine model of controlled hemorrhage. Addition of NTP to HEX control had no effect. This suggests that the NO scavenged during HBOC-201 infusion can be replenished with an NO donor, and potentially adverse events, i.e. vasoconstriction can be attenuated. If confirmed in more clinically relevant models of uncontrolled hemorrhage and polytrauma, these findings may advance regulatory approval for hemoglobin based oxygen carriers for trauma resuscitation, and significantly improve pre-hospital hemorrhagic shock treatment.

A 50
Non-erythropoietic analogues for the treatment of trauma–hemorrhage
Nimesh Patel, Kiran Nandra, Michael Brines, Anthony Cerami, Christoph Thiernemann

Erythropoietin (EPO) is a hormone that regulates the proliferation of erythroid progenitor cells by an anti-apoptotic mechanism in order to regulate erythrocyte production. EPO has been demonstrated to be beneficial in various models of ischemia/reperfusion injury such as stroke, myocardial infarction, hind-limb ischaemia, acute kidney injury, hemorrhagic shock (HS) and liver ischemia. There are two biologically distinct functions of EPO through its interaction with two very different types of receptors: the EPO receptor homodimer (involved in erythropoiesis) and the beta common heterocomplex (postulated to be involved in tissue protection). The novel peptide ARA290 has been modelled upon the 3D structure of the region of EPO presumed to bind to and initiate signalling of the beta common heterocomplex. Here, we investigate the effects of ARA290 (1 µg/kg i.v.) in 47 male wistar rats (Charles River) on the multiple organ injury induced by a rat model of HS when administered as late as 1 h into resuscitation. Mean arterial pressure was reduced to 35 ± 5 mmHg for 90 min followed by resuscitation with 20 ml/kg Ringer’s lactate for 10 min and 50% of the shed blood for 50 min. Rats were sacrificed 4 h after the onset of resuscitation. Arterial blood gas analysis was performed at the end of the experiment in order to quantify the degree of metabolic acidosis induced by HS. There was no significant difference in ABG results between any of the treatment groups; this consistency verifies that all the rats were subjected to the same degree of shock. Post-treatment with ARA290 was found to significantly attenuate the renal dysfunction, and hepatic and neuro-muscular injury caused by HS when compared to PBS treated controls (P < 0.05). In addition, upon comparison with sham-operated rats histological analysis of both the lung and liver demonstrated that post-treatment with ARA290 attenuates the degree of respiratory and hepatic damage induced by HS. Thus, these data indicate that ARA290 is potentially a very valuable therapy for HS after the resuscitation has commenced. Further experiments are required in animals deficient of the beta common heterocomplex to determine the mechanism of action.

Corresponding Author: Nimesh Patel, PhD, The William Harvey Research Institute, Translational Medicine and Therapeutics, Charterhouse Sq, London EC1 M 6BQ, UK, n.s.patel@qmul.ac.uk

A 51
Systematic review and meta-analysis of hemostatic resuscitation policies in multiple trauma
Dirk Stengel, Uli Schmucker, Gerrit Matthes, Axel Ekkernkamp, Julia Seifert

Objectives: Coagulopathy is a major cause of death in patients with multiple trauma. In addition to damage-control surgery, a high ratio of fresh frozen plasma (FFP) to packed red blood cells (PRBC) (so called hemostatic resuscitation) is currently advocated as a new concept of initial trauma management. No systematic review is available to estimate the potential survival benefit with this...
transfusion strategy. We set out to identify and summarize the available evidence on high-ratio (HR, that is, ≥1:1) FFP:PRBC versus low-ratio (LR, that is, <1:1) resuscitation in patients with multiple trauma.

Methods: We searched MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials for any comparative studies comparing HR with LR resuscitation. We manually searched the conference proceedings of important trauma meetings held in 2008 and 2009, as well as key periodicals. Our search algorithm complied with Cochrane recommendations for highly sensitive retrieval strategies, and was documented according to PRISMA guidelines. Numbers of participants, patient characteristics, FFP:PRBC ratios, and reported outcomes were extracted by two reviewers. Data were compiled by random-effects modeling, computing odds ratios (OR) with 95% confidence intervals (CI).

Results: No randomized controlled trials or quasi-randomized trials with 95% confidence intervals (CI). compiled by random-effects modeling, computing odds ratios (OR) with 95% confidence intervals (CI).

Corresponding Author: Dirk Stengel, MD, PhD, Unfallkrankenhaus Berlin, Department of Trauma and Orthopaedic Surgery, Warneer Str. 7, 10317 Berlin, Germany, stengeldirk@aol.com

A 52
Damage control resuscitation with a 1:1 ratio of plasma and packed red blood cells decreases systemic inflammation following hemorrhagic shock in mice
Amy Makley, Michael Goodman, Lou Ann Friend, Jay Johannigman, Alex Lentsch, Timothy Pr tits

Objectives: Hemorrhagic shock causes a dysfunctional inflammatory response, contributing to organ failure and mortality. The chemokines MIP-1α and MIP-2 are potent mediators of systemic inflammation and are implicated in hemorrhagic shock-induced organ injury. Clinically, resuscitation with blood components transfused in a high ratio of plasma to packed red blood cells (pRBCs) appears to improve outcomes following hemorrhage, but the effect of damage control resuscitation on inflammation is unknown. We hypothesized that resuscitation with plasma and pRBCs in a 1:1 ratio would attenuate inflammation and organ injury compared to other ratios of blood products. Materials and methods: Mice underwent femoral arterial cannulation and hemorrhage to a systolic blood pressure (SBP) of 25 ± 5 mmHg for 60 min. Mice were resuscitated to a goal SBP of 80 ± 5 mmHg with plasma and pRBCs collected from donor mice, separated and stored in citrate phosphate double dextrose and additive solution-3 prior to transfusion. Plasma, pRBCs, and ratios of plasma:pRBCs including 2:1, 1:1, and 1:2 were used for resuscitation. Serum was collected for cytokine analysis by ELISA and intestinal samples were harvested to measure capillary permeability by Evans blue technique. Results: Mice resuscitated with 1:1 demonstrated decreased serum levels of MIP-2 compared to mice resuscitated with plasma, pRBCs, 2:1, or 1:2. Mice resuscitated with equal volumes of plasma and pRBCs exhibited decreased serum levels of MIP-1x compared to mice transfused with only plasma or pRBCs. Mice resuscitated with 1:1 had decreased intestinal capillary leak compared to mice resuscitated with plasma or pRBCs alone as determined by Evans blue (Table).

Table

<table>
<thead>
<tr>
<th>Resuscitation</th>
<th>MIP-2 (pg/mL)</th>
<th>MIP-1a (pg/mL)</th>
<th>Evans blue (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma alone</td>
<td>1,072.3 ± 51.3</td>
<td>452.1 ± 111.7</td>
<td>8.8 ± 0.7</td>
</tr>
<tr>
<td>2:1 Plasma:pRBCs</td>
<td>543.5 ± 94.8</td>
<td>348.0 ± 70.4</td>
<td></td>
</tr>
<tr>
<td>1:1 Plasma:pRBCs</td>
<td>104.7 ± 27.1*</td>
<td>177.5 ± 35.1**</td>
<td>6.1 ± 0.5**</td>
</tr>
<tr>
<td>1:2 Plasma:pRBCs</td>
<td>840.1 ± 128.8</td>
<td>413.3 ± 93.3</td>
<td></td>
</tr>
<tr>
<td>pRBCs alone</td>
<td>1,085.0 ± 75.8</td>
<td>548.1 ± 41.9</td>
<td>8.6 ± 0.9</td>
</tr>
</tbody>
</table>

* p < 0.05 versus other groups.
** p < 0.05 versus plasma or pRBCs alone

Conclusions: Mice resuscitated with a 1:1 ratio of plasma:pRBCs showed evidence of decreased systemic inflammation and intestinal capillary leak following hemorrhagic shock compared to mice treated with other resuscitation strategies. The transfusion of blood products in a ratio of 1:1 may optimally attenuate systemic inflammation and mitigate end organ injury following hemorrhagic shock.

Corresponding Author: Amy Makley, MD, University of Cincinnati, Department of Surgery, 231 Albert Sabin Way, ML 0558, Cincinnati, OH 45267, USA, amy.makley@hotmail.com

A 53
Recombinant factor VIIa (rFVIIa) is effective to reduce bleeding and enhances survival in a coagulopathic, hypofibrinogenemic pig model with blunt liver injury
Oliver Grottke, Till Braunschweig, Leonie Zimmerman, Brian Lauritzen, Rene Tolba, Rolf Rossaint

Introduction: The present study investigated the efficacy of rFVIIa treatment under hypofibrinogenemia in a pig model with blunt liver injury. Animals and methods: All experimental procedures were approved by the governmental animal care and use office, Germany and adhere to the German legislation governing animal studies. Haemodilution induced coagulopathy was performed in 3 groups of 7 anesthetized pigs (31.7 ± 1.9 kg BW) using Ringer’s lactated solution and HES 130/0.4. Before inflicting liver injury with 220 ± 5 N, 1 animals were randomly assigned to receive either 70 mg/kg fibrinogen (group 3; Haemocompletan, CSL Behring) or placebo (group 1 and 2). Thirty seconds after injury, rFVIIa (180 µg/kg, group 2 and 3) or vehicle (group 1) was administered. Coagulation parameters and blood loss (BL) were monitored for 2 h. Histology was examined to evaluate the presence of thrombi. One-way ANOVA followed by Scheffe’s post hoc tests were used for multiple comparisons. Thromboelastometry parameters were analyzed using Kruskal–Wallis test with Bonferroni–Dunn correction. Data are expressed as mean ± SEM.

Results: Coagulation parameters following haemodilution were severely impaired (PT 20 ± 0.5 s, CFT 216 ± 8 s, MCF 38 ± 1 mm, z-angle 51 ± 1°) (P < 0.05). Fibrinogen substitution (group 3) significantly restored coagulation parameters (PT 12 ± 2 s; CFT
135 ± 7 s, MCF 52 ± 1 mm, z-angle 64 ± 1°). Following liver injury rFVIIa (group 2) significantly (P < 0.05) reduced BL (1.018 ± 54 ml) and improved coagulation parameters (PT 14 ± 1 s; CFT 144 ± 14 s, MCF 46 ± 1 mm, z-angle 63 ± 2°) as compared to control animals (BL 1.834 ± 124 ml, PT 24 ± 1 s; CFT 315 ± 12 s, MCF 32 ± 1 mm, z-angle 42 ± 2°). However, rFVIIa treatment after the substitution with fibrinogen reduced BL even further (631 ± 58 ml) and showed additive effects on coagulation (PT 10 ± 1 s; CFT 111 ± 5 s, MCF 56 ± 2 mm, z-angle 67 ± 1°). All animals treated with rFVIIa survived, whereas animals of the control group died within the observation period (65 ± 7 min) (P < 0.05). No signs of thromboembolism were observed. Discussion: rFVIIa under hypofibrinogenemia exhibited a positive impact in blood loss and survival rate. These effects were significantly improved after the prior substitution with fibrinogen.


Corresponding Author: Oliver Grottke, MD, RWTH Aachen University Hospital, Department of Anaesthesiology, Pauwelsstr. 30, 52074 Aachen, Germany, ogrottke@ukaachen.de

A 54
Zinc improves cardiac contractility following trauma–hemorrhage

Objective: Following major blood loss, prolonged and severe cardiovascular depression produces a cascade of events that can eventually lead to morbidity and mortality in trauma patients. Zinc, a cytoprotective essential trace element, has been suggested to produce beneficial effects on cardiovascular function, especially its anti-inflammatory, anti-oxidative, and anti-apoptotic effects in protecting cardiomyocytes, vascular endothelial cells, and the integrity of endothelial barrier function. We therefore hypothesized that zinc administration following trauma–hemorrhage (T–H) will have salutary effects on cardiac function under those conditions.

Patients and methods: To evaluate the effect of zinc on cardiac functions following T–H, the extensively used rat model of soft trauma, hemorrhage and resuscitation was used in this study. A single in vivo administration of ZnCl2 or an equal volume of vehicle was administered intravenously at the beginning of resuscitation following T–H. Mean blood pressure (MBP), maximal rate of left ventricular pressure increase (+dP/dt max), and maximal rate of left ventricular pressure decrease (−dP/dt max) were measured at 2 h after sham-operation (sham) or T–H.

Results: A significant decrease in MBP and ±dP/dt max following T–H was observed in the vehicle-treated group compared to shams. Administration of ZnCl2 following T–H ameliorated MBP and markedly improved ±dP/dt max. Progressively higher dose of ZnCl2 increased progressively the cardiac contractility in both ±dP/dt max until 10 mg/kg body weight (BW) of zinc was reached. Interestingly, administration higher than 10 mg/kg BW of zinc only can further increase +dP/dt max but had no effect on −dP/dt max. This enhanced cardiac contractility was sustained even 24 h after the administration of zinc. In addition, zinc also enhanced the survival rate of rats following T–H. Administration of 5 or 10 mg/kg BW zinc prevented T–H-induced death that occurred at the second or third day after the procedure.

Conclusion: These results demonstrate the zinc-mediated improvement in cardiac function and survival following trauma–hemorrhage.

The outcome of the survival study also serves as an index for the tolerable dose of zinc following T–H in rats. Evaluating the cardiovascular functions as well as inflammatory status in the absence and presence of zinc and the cell-based studies should provide new knowledge identifying the mechanism(s) by which zinc regulate organ effector responses following T–H. These studies will not only enhance our understanding of the effect of zinc on cardiovascular and inflammatory responses but also providing innovative approaches for preventing cardiovascular dysfunction following T–H and also other clinical conditions with intense inflammation (supported by a grant from the AHA to N-L C and NIH grant RO1 GM 39519 to IHC).

Corresponding Author: Nai-Lin Cheng, PhD, University of Alabama at Birmingham, Center for Surgical Research, 1670 University Boulevard, Birmingham, AL 35294, USA, Nai-Lin.Cheng@ccc.ualab.edu

A 55
New links between the complement and the coagulation system
Umme Amara, Umme Amara, Mirriam Kalbitz, Sebastian Weckbach, John Lambris, Markus Huber-Lang

Objectives: The complement system and the coagulation system are early activated instruments of innate immunity that provide defence after severe trauma. So far, the direct link, by which posttraumatic activation of coagulation/fibrinolytic proteases may interact with the complement cascade, remains in the dark. Therefore, the central serine proteases of the coagulation system were assessed for their potency to activate the complement system and to generate anaphylatoxins.

Methods: The central complement component C3, C5 and human serum from healthy volunteers were incubated in absence and presence of various coagulation factors and analyzed by C3a, C5a and MAC ELISAs, HMC1 and Neutrophils Chemotaxis Assays, MALDI-TOF, and complement hemolytic activity (CH-50) assay in order to detect and characterize biological active cleavage products.

Results: The coagulation/fibrinolytic proteases FXa, FXIa, FIXa, plasmin and thrombin were found to concentration- and time-dependently cleave the central proteins of the complement system, C3 and C5 in vitro and ex vivo while FVIIa, FXII, plasminogen, protein C, activate protein C failed to generate anaphylatoxins. The cleavage products C3a and C5a were chemotactic active for human mast cells (HMC-1 cells) and human neutrophils, respectively. The proteolytic activity of the coagulation/fibrinolytic proteases revealed an activity order of Ctrl < FXIa < thrombin < plasmin < FXIIa < FXa. In addition the protein sequence analysis of generated C3a and C5a by mass spectrometry displayed identical molecular weights of the naturally existing anaphylatoxins, indicating that the clotting proteases imitate the natural cleavage characteristics of C3 and C5. Addition of factor Xa to human serum as a more complex system led to a significant increase of C3a, C5a and MAC generation and decrease in hemolytic activity (CH-50). Strikingly, in presence of the coagulation inhibitors enoxaparin or fondaparinux, C3a and C5a production was virtually abolished.

Conclusion: These data provide evidence of an innate “serine protease system” with its three major columns coagulation, fibrinolysis and complement which might include novel therapeutic targets in counteracting the deleterious consequences of trauma-induced systemic inflammatory response.

Corresponding Author: Umme Amara, PhD, University Hospital Ulm, Department of Traumatology, Steinboevelstr. 9, 89075 Ulm, Germany, umneammara908@yahoo.com
A 56
Fibrinolysis in the setting of hemorrhagic shock: the role of admission base deficit
Max Wohlauer, Ernest Moore, Eduardo Gonzalez, Jeffry Kashuk, Angela Sauaia

Objective: The role of fibrinolysis in post-injury coagulopathy remains controversial. Rapid thrombelastography (r-TEG) provides a comprehensive, point of care assessment of hemostasis from the clot’s initiation phase to clot breakdown (fibrinolysis). We hypothesized that r-TEG could identify patterns of fibrinolysis in different injury pattern groups that might provide insights into the etiology of fibrinolysis in post-injury coagulopathy.

Patients and methods: 86 patients were studied retrospectively over a 16 month period. r-TEG was performed on non-citrated whole blood using tissue factor as an activator. Patients were evaluated from ED arrival through 24 h post-injury, and three groups were stratified by the following injury patterns: (1) primary head injury group: head AIS ≥ 3 and AIS ≤ 3 in other body regions (n = 41), (2) combined head and torso injury group: head AIS ≥ 3 and AIS ≥ 3 for other body regions (n = 17), and (3) primary torso injury group: head AIS ≤ 3 and AIS ≥ 3 for other body regions (n = 28). Primary fibrinolysis was defined by an r-TEG estimated percent lysis (EPL) >15%.

Results: Primary fibrinolysis was not identified in primary head injury compared to a 14.2% incidence in primary torso injury (0 vs. 14.2%, p = 0.01) (Table). Base deficit (BD) levels measured upon ED arrival where significantly higher in primary torso injury compared to primary head injury (13.9 vs. 6.1, p = 0.04) (Table). PF was associated with increased BD and lactate levels, regardless of injury pattern.

Conclusion: In this study, BD levels upon ED arrival were predictive of PF. The relationship between shock and PF suggests a mechanistic link not related to injury pattern but to the depth of shock, as measured by BD levels. Further study is required to elucidate the mechanisms of hemorrhagic shock leading to fibrinolysis.

<table>
<thead>
<tr>
<th>Injury pattern</th>
<th>Incidence of PF</th>
<th>Mean BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary head (n = 41)</td>
<td>0%</td>
<td>6.1 (±0.9)</td>
</tr>
<tr>
<td>Combined head and torso (n = 17)</td>
<td>5.8%</td>
<td>8.1 (±0.8)</td>
</tr>
<tr>
<td>Primary torso (n = 28)</td>
<td>4.2%*</td>
<td>13.9** (±2.3)</td>
</tr>
</tbody>
</table>

*p = 0.01 versus primary head injury group
**p = 0.04 versus primary head injury group

Corresponding Author: Max Wohlauer, MD, University of Colorado, Department of Surgery, 2631 E. 17th Ave., C302, Aurora, CO 80045, USA, max.wohlauer@ucdenver.edu

A 57
Experimental hemorrhagic shock: relevance of three animal models
Francoise Arnaud, Anke Scultetus, Ashrafal Haque, Bobby Kim, Kohuske Teranishi, Richard McCarron

Objective: Animal models are critical for testing hypothesis regarding new treatment strategies in hemorrhagic shock. Minimum mean arterial pressure (MAP) before resuscitation, maximum shock time or volume and time for fluid administration are parameters that require further scrutiny. We analyzed data from three severe swine hemorrhage models with relevance to military combat.

Methods: Three models were used to hemorrhage anesthetized Yorkshire pigs (30 kg). Liver laceration injury (LIV, n = 10) with free bleed for 15 min, and groin femoral injury by full transection (FT, n = 20) or by arterial puncture (AP, n = 12) with effective bleeding control at 2 min. Fluid resuscitation occurred at 15 min by administration of 30 ml/kg of oxygen carrier solution in LIV or 15 ml/kg colloid (Hextend) in FT and AP. Vital signs, blood pressure, and blood loss were recorded for 60 min.

Results: For respectively LIV, FT and AP, initial bleeding rate was 31 ± 15, 488 ± 164 and 461 ± 171 ml/min leading to a blood loss at 2 min of 7 ± 6, 34 ± 10 and 19 ± 6%EBV and MAP of 36 ± 12, 20 ± 10 and 32 ± 10 mmHg. At the end of the hemorrhagic phase and before fluid resuscitation MAP was 27 ± 11, 38 ± 18 and 39 ± 12 mmHg. At 60 min following fluid resuscitation total fluid blood loss was 72 ± 22, 42 ± 13 and 38 ± 16%EBV and MAP was 59 ± 15, 61 ± 22 and 29 ± 9 mmHg. All animals survived to 60 min. These findings indicate that LIV animals could survive low initial but continuous blood loss without definite bleeding control. Coagulation and regular administration of fluid contributed to the restoration of MAP in this group. In comparison with a more severe initial vascular injury, immediate effective bleeding arrest was necessary to restore MAP and limit blood loss. However, unstable clot with early high MAP could lead to secondary bleeding causing a MAP to decrease as in AP group.

Conclusions: These three models reflect different level of severity that should be considered while designing an experimental hemorrhage control model.

Corresponding Author: Françoise Arnaud, PhD, Naval Medical Research Center, NeuroTrauma Department, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500, USA, francoise.arnaud@med.navy.mil

A 58
Specific low-affinity binding of fibrinogen with young and old erythrocytes: CD47, a possible molecular target
Sofia De Oliveira, Henrique Sobral do Rosário, Carlota Saldanha

Objective(s): The main goal of our work is to find a molecular target for fibrinogen in red blood cells (RBCs) membrane. In this specific study our aim is to investigate the interaction between the membrane of erythrocytes, with different ages, and plasma fibrinogen in presence and absence of anti-CD47.

Material and methods: Human erythrocytes of different biological age, from healthy donors were separated in a percoll discontinuous gradient. After that whole population, old, and young RBC were labeled with: calcein (5 lM), anti-GlycophorinA (PE), and anti-CD47 (PE). Flow cytometry analyses were made at a BD FACS Calibur analyser. Data were analyzed in computer program Flow Jo. Furthermore, RBC fluorescence images were taken in a confocal microscope Zeiss LSM 510Meta and were analyzed in computer program ImageJ.

Results: Our results show the existence of a specific low binding affinity between RBCs membrane and fibrinogen. We report that this low binding connection is higher in younger than in older RBCs. We also have observed that the presence of the CD47 antibody diminish the interaction of fibrinogen with the membranes of whole population, young and old RBCs.

Conclusions: Fibrinogen is one of many inflammation mediators, and it is known as an acute phase protein. In this study, the interaction of fibrinogen with RBCs membrane was confirmed and visualized by confocal microscopy. Importantly, and for the first time, we have identified different levels of RBCs affinities for fibrinogen under different ages. Younger RBCs establishes a higher interaction with
fibrinogen than the older ones. Furthermore, we have observed a decrease in the fibrinogen interaction with all the three studied populations in the presence of anti-CD47. More studies will have to be made, however this last data suggests that CD47 could be a possible target for fibrinogen, or at least it may have a role in the interaction of these acute phase protein with red cell membrane. (Supported by Fundação para a Ciência e Tecnologia: PTDC/SAU-OSM/73449/2006)

Corresponding Author: Sofia De Oliveira, Institute of Molecular Medicine, Unit of Microvascular Biology and Inflammation, Av. Professor Egas Moniz, 1649-028 Lisbon, Portugal, sloliveira@fm.ul.pt

A 59

Effect of crystalloid hemodilution on coagulation using thrombelastography

Max Wohlauer, Ernest Moore, Eduardo Gonzalez, Christopher Silliman

Objective: It has been suggested that resuscitation strategies using crystalloids exacerbate coagulopathy secondary to plasma protein hemodilution. Thrombelastography (TEG) allows for an assessment of the entire clot lifespan, from activation of coagulation factors and platelets to fibrinolysis. The purpose of this study was to determine the independent contribution of in vitro hemodilution to coagulation integrity using TEG.

Methods: Clot formation was measured by thrombelastography (TEG) using blood from healthy volunteers. A total of ten samples were collected. Whole blood citrated samples were activated with kaolin and then re-calculated before initiating TEG. Native whole blood (NWB) was used as a control, and then diluted in a serial fashion with 25, 50 and 75% NaCl (0.9%), respectively.

Results: When compared to NWB, a 25 and 50% hemodilution caused a <30% decrease in G and MA, while R, delta and angle varied to an even lesser extent (Table). Although total clot strength (G) progressively decreased, critical hypocoagulable levels (G < 4.6) were not observed until whole blood was diluted by 75%. The platelet contribution (MA) to clot strength rather than involvement of plasma proteins (Delta) to initial clot formation was ultimately responsible for the observed coagulopathy.

Conclusion: Clot formation remains uncompromised until hemodilution levels reach 75%. These data suggest that a hemodilution of up to 50% (corresponding to a hematocrit of 20%) is not associated with clinically relevant coagulopathy. As hemodilution progresses, platelet dysfunction contributes more to coagulopathy than clotting factor abnormalities.

Table Mean (±SEM) coagulation parameters

<table>
<thead>
<tr>
<th>Variable (normal range)</th>
<th>Native whole blood (NWB)</th>
<th>Hemodilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25%</td>
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<tr>
<td>R (3–8 min)</td>
<td>7.1 (±1.1)</td>
<td>7.1 (±0.9)</td>
</tr>
<tr>
<td>Delta 0.6–1.2 min</td>
<td>0.9 (±0.3)</td>
<td>1.0 (±0.5)</td>
</tr>
<tr>
<td>Angle (55–78°)</td>
<td>69.0 (±8.8)</td>
<td>67.6 (±5.6)</td>
</tr>
<tr>
<td>MA (51–69 mm)</td>
<td>68.1 (±7.0)</td>
<td>56.5 (±7.8)</td>
</tr>
<tr>
<td>G (4.6–10.9 day/sc)</td>
<td>9.5 (±1.3)</td>
<td>7.0 (±0.8)</td>
</tr>
</tbody>
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Corresponding Author: Max Wohlauer, MD, University of Colorado, Department of Surgery, 2631 E. 17th Ave., C302, Aurora, CO 80045, USA, max.wohlauer@ucdenver.edu

A 60

Repression of equilibrative nucleoside transporter 1 and 2 dampens inflammatory acute lung injury

Julio C. Morote Garcia, Katharina Sprondel, David Koehler, Valbona Mirakaj, Holger Eitzeich, Peter Rosenberger

Objectives: Acute lung injury (ALI) is a devastating disorder characterized by hypoxemia and severe pulmonary inflammation. Extracellular adenosine has been implicated in attenuating tissue damage, acute inflammation and the preservation of the pulmonary barrier function. The equilibrative nucleoside transporters (ENTs) have significant impact on regulation of the levels of extracellular adenosine. Therefore, we investigated the role of ENT1 and ENT2 during ALI.

Materials and methods: Adenosine uptake was evaluated in endothelial (HMEC-1) and pulmonary epithelial (A549) cells after stimulation with pro-inflammatory cytokines (IL-6, IL-1β) and TNFα. Specific contribution of ENTs on adenosine uptake was gained by silencing with ENT1- or ENT2-siRNA. RT-PCR and Western blots defined the regulation of expression levels of ENTs during inflammation in vitro. Chromatin immunoprecipitation and luciferase-reporter assays identified the role of nuclear factor-kappa B (NFkB) on ENT expression. A murine model for ALI (NaCl/LPS-inhalation) was employed to reveal regulation of ENTs during acute inflammation in vivo. Inhalation of the ENT1 inhibitor S-(4-Nitrobenzyl)-6-thioinosine (NBTI) or the nonspecific ENT-inhibitor dipyridamole was carried out immediately following NaCl- or LPS-inhalation. A bronchoalveolar lavage (BAL) was performed and lung injury was evaluated by assessment of cell count, protein, myeloperoxidase activity, extracellular levels of adenosine and pro-inflammatory cytokines (IL-6, IL-1β, TNFα and MIP-1α).

Results: Our studies with HMEC-1 and A549 demonstrate attenuation of adenosine uptake as result of diminished expression of ENTs during acute inflammation in vitro. Studies with siRNA confirmed the major contribution of ENT2 as main adenosine transporter in lung. Furthermore, examination of the ENT2 promoters suggests a role of NFκB in ENT2 repression. Additional in vivo studies using a murine model for ALI showed that pharmacological inhibition of ENTs is associated with improved pulmonary barrier function, attenuated neutrophil infiltration and reduced release of pro-inflammatory cytokines during ALI.

Conclusion: Taken together, these findings reveal transcriptional repression of ENTs as an innate protective response during acute pulmonary inflammation and suggest pharmacological inhibition of ENTs by inhalation of dipyridamole as therapeutic approach against inflammatory disorders during acute lung injury.

Corresponding Author: Julio C. Morote Garcia, PhD, University Hospital Tuebingen, Anesthesiology and Intensive Care Medicine, Waldhoernlestr. 22, 72072 Tuebingen, Germany, julio.morote@medizin.uni-tuebingen.de

A 61

The selective 7nAChR agonist GTS-21 attenuates ventilator-induced inflammation and lung injury

Mathijs Kox, Esther Peters, Michiel Vaneker, Gerr-Jan Scheffer, Johannes van der Hoeven, Peter Pickkers

Objectives: Mechanical ventilation (MV) induces an inflammatory response that contributes to lung injury such as in ALI or ARDS. The efferent vagus nerve can limit the inflammatory response via the 7n nicotinic acetylcholine receptor (7nAChR), the so-called cholinergic anti-inflammatory pathway. We evaluated the effect of the selective 7nAChR agonist GTS-21 on pulmonary and systemic inflammation.
and lung injury induced by MV using clinically relevant ventilator settings (one-hit) and LPS administration followed by MV (second-hit). Material and methods: In the one-hit protocol C57BL/6 mice were i.p. injected with 8 mg/kg GTS-21 or placebo, 30 min later they were mechanically ventilated for 4 h (tidal volume 8 mL/kg; PEEP 1.5 cm H2O; FiO2 0.45). The second hit protocol was identical with the addition of an i.p. LPS injection (10 μg) 2 h prior to the start of MV. Untreated, not mechanically ventilated mice were used as controls. Results: In GTS-21-treated mice, the alveolar–arterial (A–A) gradient after MV was significantly reduced compared to ventilated placebo-treated animals (18.7 ± 0.8 vs. 20.8 ± 0.6, p = 0.04). MV resulted in an increase of all cytokines in plasma and lung compared to control mice. Plasma levels and lung mRNA expression of TNF-α were significantly lower in GTS-21-treated animals compared to placebo (plasma 196 ± 32 vs. 332 ± 51 pg/mL, p = 0.04; lung mRNA 0.76 ± 0.18 vs. 1.37 ± 0.15 compared to beta-actin, p = 0.02). Similarly, in lung homogenates a distinct trend was observed towards lower TNF-α levels in GTS-21-treated animals (54 ± 13 vs. 79 ± 6 pg/mg protein, p = 0.06). IL-10 levels in plasma and lung and lung IL-10 mRNA expression were unaffected by GTS-21. Pretreatment with LPS resulted in increased cytokine levels and mRNA expression. However, GTS-21 was not effective in attenuating the inflammatory response nor did it have an effect on the A–A gradient. Conclusion: MV with clinically relevant ventilator settings results in activation of the immune system. GTS-21 attenuates pro-inflammatory cytokine levels while the anti-inflammatory cytokine IL-10 is not inhibited. The reduced A–A gradient in GTS-21-treated animals indicates attenuation of lung injury. LPS pretreatment results in an amplified inflammatory response which is not affected by GTS-21. In conclusion, the cholinergic anti-inflammatory pathway may represent new treatment options for MV-induced lung injury.

Corresponding Author: Matthijs Kox, MSc, Radboud University Nijmegen Medical Center, Department of Intensive Care Medicine, Geert Grooteplein 10, 6500 HB Nijmegen, The Netherlands, m.kox@ic.umcn.nl

A 62 CD44 is protective during hyperoxia induced lung injury

Gerritje van der Windt, Marcel Schouten, Sacha Zeerleder, Sandrine Florquin, Tom van der Poll

Objective: Patients with acute lung injury or respiratory distress syndrome often require supplemental oxygen to maintain tissue oxygenation. Although this supportive treatment is necessary, it can also cause or worsen lung inflammation. CD44 is a transmembrane adhesion molecule that is present on a wide variety of cell types, including leukocytes and parenchymal cells, and an important player in leukocyte trafficking. The aim of this study was to determine the role of CD44 during hyperoxia induced acute lung injury. Methods: CD44 knockout (KO), wild-type (WT) and osteopontin KO mice were exposed to either >95% oxygen or room air for 24–72 h. Results: Whereas all WT mice survived the 72-h observation period, 37.5% of CD44 KO mice died. CD44 deficiency was associated with a profound influx of neutrophils into the bronchoalveolar space, in the presence of similar or even lower neutrophil numbers in lung parenchyma, suggesting that CD44 is important for containing neutrophils in the pulmonary interstitium during hyperoxia. In addition, CD44 deficiency resulted in enhanced interleukin-6 and keratinocyte-derived chemokine release into bronchoalveolar lavage fluid (BALF). CD44 KO mice further displayed evidence for increased vascular leak (reflected by higher protein levels in BALF) and injury of type II respiratory epithelial cells (higher BALF alkaline phosphatase levels).

Strikingly, CD44 protected against bronchial epithelial cell death, as shown by enhanced epithelial cell necrosis and increased BALF nucleosome levels in CD44 KO mice. Osteopontin, an important ligand for CD44, was constitutively expressed in BALF of naïve mice, increasing after 72 h of hyperoxia. However, osteopontin KO mice were indistinguishable from WT mice during exposure to hyperoxia for up to 72 h. Conclusion: These data suggest that CD44 protects against hyperoxia induced mortality and lung injury by a mechanism that does not rely on its interaction with osteopontin.

Corresponding Author: Gerritje van der Windt, MSc, Academic Medical Center, Center for Experimental and Molecular Medicine, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, g.j.vanderwindt@amc.uva.nl

A 63 Lung dimethylarginine dimethylaminohydrolase activity is decreased in ovine model of acute lung injury and contributes to long term pulmonary dysfunction

Linda Sousse, Yusuke Yamamoto, Pereneli Enkhaaatar, Young-Ming Yu, Lillian Traber, Daniel Traber

Objective: Thermally injured children who have sustained inhalation injury show evidence of restrictive lung disease during their recovery period. We hypothesize that lung arginase activity is increased by burn and inhalation injury, leading to increased collagen deposition in the lung and contributing to long-term pulmonary dysfunction. Methods: Ewes were randomly divided into the following groups: (1) Uninjured (no injury, no early excision/skin autografting), n = 6, and (2) injured (injury, early excision/skin autografting). The injured animals were subjected to 20% total body surface area burn and 36 breaths of cotton smoke under deep anesthesia. Following the injury, the animals were monitored for 2 weeks (n = 5) and 3 weeks (n = 7). Results: Dimethylarginine dimethylaminohydrolase-II (DDAH-II), an enzyme that degrades asymmetrical dimethyl-arginine (ADMA), which is an endogenous inhibitor of NOS, significantly decreases after 2 and 3 weeks in the lung compared with uninjured animals (0.08 ± 0.03 and 0.08 ± 0.04 vs. 0.33 ± 0.05, mean ± SEM, p < 0.05). DDAH-I decreases after 2 weeks in the lung compared with uninjured animals (0.45 ± 0.08 vs. 0.23 ± 0.17). Lung ADMA increases from 2 to 3 weeks (32.66 ± 9.12 vs. 48.01 ± 6.45). Expression of inducible nitric oxide synthase (iNOS) significantly decreases in the lung 2 weeks after injury compared with uninjured animals (4,505 ± 1,218 vs. 14,040 ± 3,520, p < 0.05). Lung arginase activity (0.44 ± 0.13 vs. 0.03 ± 0.001) and hydroxyproline (a marker of collagen deposition, 3.63 ± 0.571 vs. 10 ± 0.01, p < 0.05) increase with 3 weeks post-injury compared to the uninjured animals. The increases in arginase and collagen are associated with reduced gas exchange (PaO2/FiO2 509.7 ± 23.48 vs. 582.3 ± 17.91) and lung diffusion capacity (DLCO 15.12 ± 2.24 vs. 17.40 ± 1.52) after 3 weeks compared to uninjured animals.

Conclusions: The increase in DDAH-I and II results in increased ADMA. The elevated ADMA causes a decrease in NOS expression. The decrease in NOS expression causes an increase in arginase activity because NOS forms (omega)-hydroxy-nor-L-arginine (NOHA), which is an intermediate in the formation of NO and inhibits arginase. The increase in arginase activity contributes to the increase in collagen deposition, which leads to long-term pulmonary dysfunction. Interruption of this pathological chain reaction may be useful in preventing the long-term sequelae of the smoke inhalation and burn injury.

Corresponding Author: Linda Sousse, MSc, University of Texas Medical Branch, Anesthesiology, Pathology, 601 Harborbide Drive, Room 3.202, Galveston, TX 77555, USA, lesoussae@utmb.edu
A 64
Fibrin(ogen) degradation products are mediators of inflammation in human lung endothelial cells
Patrick Paulus, Peter Ellinghaus, Volker Laux, Nguyen Tran, Kai Zacharowski

Objectives: Systemic inflammatory conditions such as SEPSIS are associated with massive endothelial dysfunctions leading to organ failure. One major complication during SEPSIS is the failure of the lung leading to decreased gas exchange and thus increased peripheral organ failure due to oxygen malnutrition. Together with this, SEPSIS patients have coagulation disorders with high fibrin(ogen) (FGN) turnover leading to the production of small fibrin fragments (FFs). The latter are supposed to have a biological activity. Increasing evidence suggests that these FFs also play a role as mediators in pro-inflammatory signaling pathways. We hypothesize that FFs increase the vascular inflammatory response in the lung.

Material and methods: To test this hypothesis, human lung microvascular endothelial cells (HLMECs) were treated with FFs. FGN cleavage was performed by incubation with plasmin for 2.5 h prior to mRNA isolation. The inflammatory response was quantified by analysis of gene expression using real-time PCR. Genes mediating cell–cell interactions with ECs and leukocytes were investigated (e.g. intercellular adhesion molecule-1 (ICAM-1), endothelial cell leukocyte adhesion molecule-1 (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1), pro-inflammatory cytokines (Interleukin-6, Interleukin-8) and chemokines (monocyte chemotactic protein (MCP)-1, GRO-α and -β).

Results: Compared to untreated controls or HLMECs treated with FGN alone, HLMECs treated with FF generated by plasmin-cleavage of FGN show a significant upregulation of VCAM-1 (732-fold, p < 0.05/2.6-fold, p < 0.05), ELAM-1 (18.7-fold, p < 0.001/4.1-fold, p < 0.001), IL-6 (10.9-fold, p < 0.05/1.9-fold, p < 0.05), IL-8 (9.1-fold, p < 0.001/2.8-fold, p < 0.001), MCP-1 (3.7-fold, p < 0.01/2.2-fold, p < 0.01), GRO-α (7.9-fold, p < 0.001/2.6-fold, p < 0.001), GRO-β (12.5-fold, p < 0.01/4.3-fold, p < 0.01). HLMECs treated with plasmin alone show no significant changes in the latter genes’ mRNA expression.

Conclusions: Our data clearly indicate that cleavage of FGN results in the production of biological active FFs. These FFs have an overall pro-inflammatory effect on HLMECs. However, anti-inflammatory FFs have been described. The knowledge of the biological activity of FFs might be of value in SEPSIS patients, where coagulation is impaired. In these patients, the FGN turnover is higher and may thus lead to an increased production of FFs resulting in the damage of the lung microvasculature.

Corresponding Author: Patrick Paulus, MD, Johann Wolfgang Goethe University of Frankfurt/M, Clinic for Anaesthesiology, Intensive Care Medicine and Pain Therapy, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, Paulus@med.uni-frankfurt.de

A 65
Anti-inflammatory effect of liposomal paclitaxel (EndoTAG®-1) and other cationic liposomes in an animal model of LPS-induced ARDS
Susanne Herber-Jonat, Rashmi Mittal, Sara Kinert, Andreas W. Flemmer, Andreas Schulze

Objective: Paclitaxel has been shown to reduce lipopolysaccharide (LPS)-induced lung injury in mice. In addition, some studies have demonstrated the ability of cationic liposomes to bind to the acutely inflamed endothelium in the lung and other tissues, and to lead to a reduction in inflammation. Paclitaxel can be stably encapsulated in cationic liposomes and is available commercially as EndoTAG-1.

Hypothesis: EndoTAG-1 reduces the inflammatory reaction in the lungs subjected to LPS.

Material and methods: Prospective randomized study in ventilated Sprague-Dawley rats. After induction of lung injury (instillation of LPS/E. coli), the animals were given EndoTAG-1 (paclitaxel dose of 0.77 mg/kg), free paclitaxel (5 mg/kg), cationic liposomes with different charge densities (Lipo1: 100% DOTAP; Lipo2: DOTAP/DOPC 1:1) or 5% glucose (control). During the observation period (5 h), serial measurements of lung mechanics (Cst; TLC) were carried out.

Results: Treatment with EndoTAG-1 as well as with Lipo1 and Lipo2 led to a significant reduction in the inflammatory response as compared to controls. This was evident in (1) the total WBC count of lung lavage (1,700 ± 300; 2,400 ± 600 and 4,800 ± 1,100 vs. 9,000 ± 1,900, respectively, p < 0.05), and (2) wet/dry ratio (5.5 ± 0.1; 5.2 ± 0.1, and 5.5 ± 0.1 vs. 6.4 ± 0.4, p < 0.05). The administration of Lipo1 also had a significant effect on relative Cst after 5 h as compared to Cst in the control group (82.4 ± 6.0% vs. 62.0 ± 3.4% of initial Cst, p < 0.05).

Conclusion: Our results demonstrate an inhibition of the LPS-induced inflammation of the lung by EndoTAG-1 as well as empty liposomes (Lipo1 > Lipo2) and, thus, support the rationale of the possible use of cationic liposomes in the treatment of ARDS.

Corresponding Author: Susanne Herber-Jonat, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Neonatology, Marchioninistr. 15, 81377 Munich, Germany, susanne.jonat@med.uni-muenchen.de

A 66
Differential effects of intravenous antithrombin III as a single treatment or combined with nebulized tissue plasminogen activator and heparin following combined burn and smoke inhalation injury
Sebastian Rehberg, Yusuke Yamamoto, Linda Sousse, David Herndon, Daniel Traber, Perenlei Enkhbaatar

Objective: In the present randomized experiment we hypothesized that a combined therapy with intravenous (iv) recombinant human antithrombin III (rhAT III) and nebulized heparin and tissue plasminogen activator (TPA) is superior to sole iv rhAT III infusion for the treatment of combined cutaneous burn and smoke inhalation injury. This hypothesis was tested in an established ovine model.

Material and methods: After instrumentation for chronic monitoring, a tracheostomy as well as a 40% total body surface area third cutaneous flame burn and smoke inhalation injury (48 breaths of cold cotton smoke) were performed in 16 sheep under deep anesthesia. The sheep were then randomly assigned to receive an iv infusion of 6 U kg⁻¹ h⁻¹ rhAT III (started 1 h post injury; n = 6), an iv infusion of 6 U kg⁻¹ h⁻¹ rhAT III (started 1 h post injury) combined with nebulized heparin (10,000 IU every 4 h, started 2 h post injury) and TPA (2 mg every 4 h, started 2 h post injury; n = 4) or 0.9% NaCl iv (n = 6). All sheep were awake, mechanically ventilated and fluid resuscitated according to standard formulas during the 48 h study period. Data are expressed as mean ± SE at 48 h.

Results: Both strategies attenuated lung injury, as suggested by higher PaO₂/FiO₂ ratios (rhATIII: 199 ± 48 mmHg, combination: 360 ± 29 mmHg) compared with control animals (83 ± 6 mmHg, p < 0.05 each). This effect was more pronounced with the combined treatment.

The combination therapy, however, was associated with significantly lower pulmonary vascular resistance (115 ± 5 vs. 166 ± 17 dyn s cm⁻⁵ m⁻²) and pulmonary shunt fraction (22 ± 2 vs. 30 ± 4%) as...
compared to sole rhAT III infusion. Single rhAT III therapy, in turn, more profoundly reduced pulmonary as well as systemic vascular leakage, as represented by pulmonary lymph flow (16 ± 7 vs. 38 ± 5 mL h\(^{-1}\)) and net fluid balance (1,086 ± 572 vs. 3,274 ± 877 mL), respectively. The migration of neutrophils in the lung was only reduced by single rhAT III infusion (control: 330 ± 64% of baseline; rhAT III: 50 ± 22% of baseline; combination: 330 ± 94% of baseline).

Conclusion: The combination of rhAT III with nebulized heparin and TPA more effectively improved pulmonary function as compared to sole iv rhAT III. However, rhAT III induced anti-inflammatory effects and the reduction of vascular leakage were considerably attenuated by the combination. The possible systemic contamination with heparin and its subsequent interaction with rhAT III may represent a potential explanation for these findings.

Corresponding Author: Sebastian Rehberg, MD, The University of Texas Medical Branch, Department of Anaesthesiology, 301 University Blvd, Galveston, TX 77551, USA, Sebastian_Rehberg@web.de

A 67
R-roscovitine reduces lipoteichoic acid lung inflammation and enhances resolution of Streptococcus pneumoniae pneumonia in combination with antibiotic treatment
Arie Johan Hoogendijk, Joris J.Th. Roeelofs, Miriam H.P. van Lieshout, Dana C. Blok, Tom van der Poll, Catharina W. Wieland

Objectives: Streptococcus pneumoniae (S. pneumoniae) pneumonia remains associated with high morbidity and mortality. Additional strategies are needed, as antibiotic treatment is frequently insufficient in limiting inflammation induced lung damage. The cyclin-dependent kinase (CDK) inhibitor r-roscovitine was demonstrated to reduce inflammation in several models of inflammation. We studied the potential of r-roscovitine to modulate host defense in sterile inflammation and bacterial infection of the lung.

Methods: Isolated neutrophils were treated with 20 µM r-roscovitine and CDK and caspase 3 activity were determined by western blot analysis. Sterile lung inflammation was induced by administration of 100 µg lipoteichoic acid (LTA) intranasal (i.n.). Simultaneously 70 mg/kg r-roscovitine or vehicle was injected intraperitoneally. 24 h later bronchoalveolar lavage (BAL) was performed and differential cell counts were determined. Bacterial pneumonia was induced by inoculating 5 × 104 CFU S. pneumoniae i.n.. After 24 h 70 mg/kg R-roscovitine or vehicle was administered in combination with 20 mg/kg ceftriaxon. Mice were sacrificed after 48 h. In a second experiment mice were infected and treated at 24 and 72 h and sacrificed 96 h post-infection.

Results: Treatment with r-roscovitine reduced phosphorylated CDK substrates and increased cleaved caspase 3 levels in isolated neutrophils. During LTA induced lung inflammation r-roscovitine treatment significantly reduced the amount of PMNs in the BAL-fluid and cytokines levels in lung homogenates. After 48 h of bacterial pneumonia, r-roscovitine treated animals displayed enhanced pulmonary bacterial outgrowth. Both cytokine levels and lung damage scores were higher in the r-roscovitine treated group. Interestingly, 96 h post-infection, treatment with r-roscovitine resulted in lower bacterial outgrowth and chemokine levels in the lung.

Conclusions: We reproduced earlier findings that r-roscovitine reduces CDK activity and induces apoptosis in neutrophils. We demonstrated that r-roscovitine diminishes inflammatory responses in sterile inflammation; and found that r-roscovitine treatment in antibiotic treated bacterial pneumonia is detrimental early in infection but beneficial at later time points. We believe that the negative effect of r-roscovitine reflects the importance of neutrophil antibacterial defense early in infection. During resolution of infection, neutrophil apoptosis induced by r-roscovitine could present a way of damage control.

Corresponding Author: Arie Johan Hoogendijk, MSc, Academic Medical Center, Center for Experimental and Molecular Medicine, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, a.j.hoogendijk@amc.uva.nl

A 68
Directing responses after death: the unexpected link to TH17 cell development
Julie Magarian Blander

Adaptive immune responses rely on differentiation of CD4 T helper cells into subsets with distinct effector functions best suited for host defence against the invading pathogen. Interleukin (IL)-17 producing T helper cells (TH17) are a recently identified subset, separate from the T helper type 1 (TH1) and T helper type 2 (TH2) subsets. TH17 cells are induced in vitro by the cytokines transforming growth factor-β (TGF-β) and IL-6. However, it is not known which conditions in vivo would induce this combination of cytokines. Furthermore, it is enigmatic that a combination of pro-inflammatory and anti-inflammatory cytokines would be required to generate an effector TH17 response. Here I discuss our recent findings showing that the relevant physiological stimulus triggering this combination of cytokines is the recognition and phagocytosis of infected apoptotic cells by dendritic cells (Torchinsky M. et al. Nature 2009 5:458 (7234):78–82). Phagocytosis of infected apoptotic cells uniquely triggers the combination of IL-6 and TGF-β through recognition of pathogen associated molecular patterns and phosphatidylserine exposed on apoptotic cells, respectively. Conversely, phagocytosis of apoptotic cells in the absence of microbial signals induces differentiation of the closely related regulatory T-cells (Treg), which are important for controlling autoimmunity. Blocking apoptosis during infection of the intestinal epithelium with the rodent pathogen Citrobacter rodentium impairs the characteristic TH17 response in the lamina propria. Our results demonstrate that infected apoptotic cells are a critical component of the innate immune signals instructing TH17 differentiation, and point to pathogens particularly adept at triggering apoptosis that might preferentially induce TH17-mediated immunity.

Corresponding Author: Julie Magarian Blander, Prof. PhD, Mount Sinai School of Medicine, Immunology Institute, One Gustave L. Levy Place, New York, NY 10029, USA, julie.blander@mssm.edu

A 69
Hsp70 is released into circulation associated with export cellular vesicles and induces a robust activation of the immune system after injury
Antonio De Maio1, Daniel Vazquez1, Jonathan Okerblom2, Virginia Vega1, Whisler Charles2, Nelson Arispe2
1Department of Surgery, University of California San Diego, La Jolla, CA, USA, 2UCSD-IMSD Program, 3Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Objective: The cellular response to stress is mediated by the expression of heat shock proteins (hsp), which are directed at maintaining homeostasis. Hsp70, which is the major inducible form of all hsp, is involved in protein folding and assembly. In addition, Hsp70 stabilizes several cellular processes that are affected by the stress. Recent observations have shown that Hsp70 is detected outside cells, which appears to be part of an active
A 70
 Oxidative stress induces caspase activation and up-regulation of autophagy in mouse hepatocytes
 Qian Sun, Timothy Billiar, Melanie Scott

Reactive oxygen species (ROS) produced during ischemia/reperfusion and hemorrhagic shock activate cells to produce pro-inflammatory cytokines TNFα, IL-6 and IL-1β. It has been shown recently that IL-1β and IL-18 are cleaved and released through the activation of caspase-1 via the inflammasome. Regulation of inflammasome activation in hepatocytes is unknown but in other cell types the inflammasome can be negatively regulated through activation of autophagy pathways, which is protective in inflammatory bowel disease. In this study we investigated inflammasome activation and autophagy in mouse hepatocytes in response to oxidative stress.

Methods: Mouse hepatocytes were treated for 0.5, 1, 2, 6 or 18 h with hydrogen peroxide (H_2O_2) at concentrations of 125, 250, 500, 1, 2 mM. Similar sets of hepatocytes were pre-treated with pan-caspase inhibitor (z-VAD (OMe)-FMK) (50 mM) or DMSO control for 2 h before treatment with H_2O_2. Cell viability was determined by crystal violet assay. Whole cell lysates were collected and immunoblotted for caspase-1, autophagic markers LC3 and beclin-1 as well as apoptotic marker caspase-3.

Results: Caspase-1 was activated (cleaved) in hepatocytes after 1 h treatment with H_2O_2 and in a concentration dependent manner indicating inflammasome activation. LC3 and beclin-1 levels were also significantly increased over time after H_2O_2 treatment and caspase-3 activation increased with increasing H_2O_2 concentrations. Pre-treatment of hepatocytes with pan-caspase inhibitor inhibited caspase-1 and caspase-3 activation by H_2O_2 as expected, and also inhibited the up-regulation of beclin-1. Cell viability was not significantly different between comparable experimental groups.

Conclusions: Our data show that oxidative stress induces activation of the inflammasome and caspase-1, as well as activating both apoptosis and autophagy pathways in hepatocytes. Upregulation of autophagy marker beclin-1 is also dependent on caspase activation. Elucidating mechanisms of autophagy activation and inflammation in hepatocytes will be important for determining future treatments for organ failure and dysfunction after trauma.

A 71
Rapid expulsion of neutrophil extracellular traps (NETs) in vivo is a selective process requiring both toll-receptors and complement
Bryan Yipp, Lori Zbytniuk, Britney Scott, Paul Kubes

Objective: Both TLR2/4 and complement 3 (C3) mice have increased susceptibility to S. aureus infection, but the mechanisms underlying this vulnerability are not clear. S aureus causes severe infections in humans and neutrophils (PMN) are essential for defense against this organism. Extracellular release of nucleic acids (NETs) may represent a novel mechanism of PMN mediated host defense independent of conventional microbial killing. We investigated the mechanisms of NET formation and examined the requirement of both a functional TLR and complement cascade in mediating NET release in vivo in response to a S. aureus skin infection.

Methods: Spinning disk confocal intravital microscopy was used to directly visualize NET formation in a mouse model of a S. aureus skin infection. Skin microvasculature was visualized after surgical exposure of anaesthetized mice. Live S. aureus (1 × 10^7) was locally administered following baseline imaging. Experiments were digitally recorded and analyzed off-line. NETs were quantified using Volocity imaging software.

Results: Chemokine stimulation resulted in PMN emigration and tissue crawling without significant detection of the extracellular DNA dye (sytox). NETs were detectable within 20 min of local S. aureus injection. PMN were viable and displayed rapid crawling throughout the infected tissue. To confirm that NETs originated from the nucleus, a histone specific fluorescent antibody (H2AX) was used. A mitochondrial specific dye (MitoTracker) verified that these organelles are intact and intracellular, suggesting a minimal role in NET composition. In TLR2/4-/- and C3-/- mice, PMN emigrated to the site of infection, comparable to WT mice, however visual detection and NET area quantification was significantly reduced in both.

Conclusions: PMN can rapidly release DNA in response to a S. aureus infection in vivo. Histones are released with DNA suggesting that the nucleus is the primary source of NET-DNA. In addition, PMN mitochondria remain intact and intracellular during NET formation. Expulsion of nuclear contents does not affect viability as PMN continue to rapidly seek bacteria in the infected tissue. Both TLR2 and C3 are essential for NET production, however individually neither is sufficient to induce PMN to rapidly release NETs. These results suggest that neutrophils employ a selective and discriminate system to decide when to commit to expulsion of their nuclear contents.

Corresponding Author: Bryan Yipp, MD, University of Calgary, Department of Critical Care Medicine, 206, 429 14th ST, Calgary, AB T2 N 2A3, Canada, bryan.yipp@albertahealthservices.ca

A 72
Intracellular Hsp70 mediated inhibition of TLR4 signaling requires C-terminal interactions with the co-chaperone molecule chip
Amin Afrazi, Steven Gribar, Anna Evans, Chhinder Sodhi, Eugene Chang, David Hackam

Objective: Heat shock protein 70 (Hsp70) is a molecular chaperone that protects against cellular injury and has been shown by our lab to
serve as an intracellular inhibitor of TLR4 signaling in vitro and in vivo decreasing TLR4-mediated enterocyte apoptosis and inflammation in disease models of Necrotizing Enterocolitis (NEC) and endotoxemia through pathways that remain incompletely understood. C-terminal Hsp70 interacting Protein (CHIP) is an E3 ligase critical to Hsp70’s ability to target proteins for degradation via the proteasomal pathway. We now hypothesize that Hsp70 limits TLR4 signaling via direct interaction with TLR4 and CHIP leading to TLR4 ubiquitination and degradation.

Methods: Ileal mucosal scrapings from MyD88KO mice injected ip with LPS (1 mg/kg) were assessed for Hsp70 expression. LPS treated IEC6 enterocytes (50 μg/mL, 8–20 h) were assessed for Hsp70 and TLR4 expression +/- heat shock (42°C, 30’) and +/- proteasome inhibitor MG132 (20 μM) via SDS-PAGE and immunofluorescence (IF). Cell lysates were immunoprecipitated with either Hsp70 or Ubiquitin and assessed for TLR4 via SDS-PAGE. Cells were treated with MG132 or transfected with mutant CHIP or Hsp70 and treated with LPS +/- heat shock and assessed for TLR4 and cleaved caspase 3 expression to assess for apoptosis by SDS-PAGE and IF respectively.

Results: LPS injection caused a decrease in the expression of Hsp70 in the intestine in MyD88KO mice compared with WT animals. Treatment of IEC6 enterocytes with LPS significantly increased expression of Hsp70 as well as the colocalization of TLR4 and Hsp70 at 16 h. Immunoprecipitation of TLR4 with Hsp70 yielded a complex between these two proteins after LPS treatment, suggesting that a complex forms between Hsp70 and TLR4. LPS treatment led to an increase in the expression of TLR4 in IEC6. This increase is blocked by induction of Hsp70 via increased TLR4 ubiquitination and degradation. Strikingly, cells treated with the proteasome inhibitor MG132 or transfected with mutant Hsp70 or CHIP demonstrated increased LPS-mediated apoptosis and a loss of Hsp70 protection, confirming the importance of CHIP in the inhibitory effects of intracellular Hsp70 on TLR4 signaling in enterocytes.

Conclusion: These data identify a novel mechanism of TLR4 regulation in enterocytes through the induction of Hsp70 and the role of the co-chaperone CHIP, providing insights into the pathogenesis of impaired TLR4 signaling that characterizes diseases of intestinal inflammation such as NEC.

Corresponding Author: Amin Afrazi, University of Pittsburgh, Department of Surgery, 547 S Graham st apt 2, Pittsburgh, PA 15232, USA, afrazi.amir@medstudent.pitt.edu

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In vitro neutrophil chemotactic responses to sterile heat injury
Keir Pittman, Paul Kubes

Objective: To elucidate the nature and origin of chemotactic cues involved in targeting neutrophils to a sterile necrotic focus, and to elucidate the mechanism underlying the response.

Patients and methods: Human Embryonic Kidney (HEK) cells were isolated and then subjected to heat-injury for 15 s using a surgical cautery pen attached to a micromanipulator. Membrane disruption of the injured cells was ascertained by Trypan Blue inclusion. The injured cells were loaded into an underagarose gel chemotaxis assay. The assay was then used to test the directed chemotactic response of human neutrophils isolated from whole blood, both in the presence and absence of inhibitors to intracellular signalling pathways known to be involved in neutrophil chemotaxis.

Results: Interestingly, there was an in vitro neutrophil chemotactic response towards the foci of injured and dead HEK cells, suggesting that neutrophils are recruited by dead cells even in the absence of immune sentinel cells such as macrophages. Trypan blue analysis of the heated aliquots of HEK cells revealed that though the majority of cells were found to be necrotic, there was a percentage of viable cells remaining in each preparation. Time-course burn-injury experiments revealed that the further the extent of heat the HEK cells were subjected to, the greater the ensuing neutrophil chemotactic response, indicating that there is no role for the remaining viable cells. Furthermore, the pronounced neutrophil chemotactic activity was demonstrated to be dependent on G-protein coupled signalling, as the presence of pertussis toxin reduced the observed neutrophil migratory response.

Conclusion: We demonstrate that in vitro, neutrophil chemotactic responses to sterile, necrotic foci require G-protein signalling. This suggests that similar intracellular pathways underlying neutrophil targeting to pathogenic stimuli may also be involved in the response to sterile injury. We are currently undertaking experiments to establish the exact nature of this signal, and to determine if it originates from the necrotic cells themselves, or from viable cells in the periphery of the injury.

Corresponding Author: Keir Pittman, University of Calgary, Snyder Institute of Infection, Immunity, and Inflammation, Department of Immunology, 3330 Hospital Drive N.W., Calgary, AB T2 N 4N1, Canada, kapittma@ucalgary.ca

A 74
Hsp70 a potential prognostic marker for sarcomas
Gabrielle Multhoff, Sophie Lehtierer, Tobias Kraus, Indra Hesse, Peter Prodinger, Ingo Banke

Objective(s): Sarcomas consist of a great heterogeneity of different musculoskeletal malignancies. Most entities exhibit a high propensity for late primary or recurrent disease diagnosis, resulting in extensive primary tumor growth or metastasized stage with a poor prognosis. Despite the significant clinical improvements made over the past decades through novel imaging techniques and combined treatment concepts, patients with delayed detection and treatment of sarcoma continue to have a poor prognosis. In the case of osteosarcoma (OS), 5-year survival rate is only 10–20% (Gorlick et al. 2003). However, current diagnostic and treatment strategies lack of novel avenues such as molecular markers and therapy targets. Tumors differ from normal tissues in their capacity to express heat shock protein 70 (Hsp70) on the cell membrane. Hsp70 is frequently associated with a poor clinical outcome. In sarcomas, Hsp70 has not been evaluated systematically.

Material and methods: 40 human sarcomas (e.g. osteo-, chondro-, lipo-, Ewing’s sarcoma, malignant fibrous histiocytoma, synovial sarcoma) and their corresponding normal tissues were collected intraoperatively. Approval of the local Ethics Committee has been obtained. Immediately after explantation, the membrane Hsp70 levels were quantified using flow cytometric analysis with the Hsp70-specific antibody cmHsp70.1-FITC. Within each entity, mean and standard error of Hsp70 levels were compared to the respective healthy tissue. Statistical significance was determined (P < 0.05).

Results: All sarcomas showed elevated Hsp70 membrane levels compared to their corresponding normal tissues. The increased Hsp70 levels were significant in the case of chondrosarcoma (n = 15) and liposarcoma (n = 6). Interestingly, the membrane density of Hsp70 in liposarcoma and OS was higher than that of the tested carcinomas.

Conclusion(s): With this study, we show for the first time a membrane-positivity of Hsp70 in sarcomas. Since the rare incidence of some sarcoma entities, the Hsp70 membrane levels were found to be significantly increased in chondro- and liposarcoma as compared to normal tissues. This finding might have future clinical relevance with
A 75

Hsp70 is released in circulation in the form of stress export cellular vesicles after heat shock in rats

Daniel Vazquez, David Cauvi, Antonio De Maio

Objective: Expression of heat shock proteins (hsp) is induced in response to an array of physiologic, environmental, and disease conditions. Hsp have been shown to perform multiple roles including cytoprotection, immunomodulation, and assisting in the folding and translocation of polypeptides across membranes. Extracellular hsp, specifically Hsp70, have been reported to be key regulators of the host’s immune system in vitro, but their presence in vivo has been elusive. We hypothesize that Hsp70 is released into circulation within membranous structures after stress and can activate the immune system.

Methods: Adult male C56BL/6J mice or Sprague-Dawley rats were thermally stressed by increasing their core body temperature to 42°C. Membrane-bound Hsp70 was isolated by differential ultracentrifugation of plasma samples. The presence of Hsp70 in samples obtained from blood and broncho-alveolar lavage fluid (BAL) were measured by an ultra sensitive ELISA kit (Stressgen). Statistical analysis was performed by t-test.

Results: An increase in Hsp70 was detected in plasma samples obtained after 6 or 24 h of the thermal stress (p = 0.0002, 6 vs. 24 h). In contrast, Hsp70 was not detected in plasma samples of non-stressed rodents. Hsp70 was also detected in BAL samples of thermally-stressed mice (p = 0.0004 vs. control). In addition, Hsp70 was detected in the high-speed centrifugation pellet fraction from heat shocked animals by ELISA. Hsp70 was not observed in similar high-speed centrifugation fractions from plasma of control animals.

Conclusion: Our data suggest that Hsp70 is released into circulation after stress. Moreover, Hsp70 in plasma was found to be associated with membranes, which we have termed stress export cellular vesicles (SECV). Further work will be carried out to determine if these SECV modulate the immune system.

Corresponding Author: Daniel Vazquez, MD, University of California San Diego, Department of Surgery, 9500 Gilman Drive # 0739, La Jolla, CA 92093-0739, USA, devazquez@ucsd.edu

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Tumor-specific Hsp70 plasma membrane localization is enabled by the glycosphingolipid Gb3

Mathias Gehrmann, Daniela Schilling, Michael Molls, Gabriele Multhoff

Objective: Human tumors differ from normal tissues in their capacity to present Hsp70, the major stress-inducible member of the HSP70 family, on their plasma membrane. Membrane Hsp70 has been found to serve as a prognostic indicator of overall patient survival in leukemia, lower rectal and non small cell lung carcinomas. Why tumors, but not normal cells, present Hsp70 on their cell surface and the impact of membrane Hsp70 on cancer progression remains to be elucidated.

Methods: Tumor cells (CX+/−, Colo+/−, Hela+/−, Daudi) and primary fibroblasts from a Fabry patient were cultured at standard conditions and seeded regularly. Lipid compositions of whole cells or plasma membranes were analyzed by electrospray ionization tandem mass spectrometry (ESI-MS/MS). Colocalization of Hsp70 with lipids was tested by flow cytometry with a four color FACSCalibur or by a fluorescence capable microscope. Lipid vesicles with well-defined compositions were prepared with an extruder and incubated with proteins as indicated.

Results: Although Hsp70 has been reported to be associated with cholesterol rich microdomains (CRMs), the partner in the plasma membrane with which Hsp70 interacts has yet to be identified. Herein, global lipid profiling demonstrates that Hsp70 membrane-positive tumors differ from their membrane-negative counterparts by containing significantly higher amounts of globotriaosylceramide (Gb3), but not of other lipids such as lactosylceramide (LacCer), dodecasaccharidicceramide (DoCer), galactosylceramide (GalCer), ceramide (Cer), or the ganglioside GM1. Apart from germinal center B cells, normal tissues are Gb3 membrane-negative. Co-localization of Hsp70 and Gb3 was selectively determined in Gb3 membrane-positive tumor cells, and these cells were also shown to bind soluble Hsp70-FITC protein from outside in a concentration-dependent manner. Given that the latter interaction can be blocked by a Gb3-specific antibody, and that the depletion of globotriaosides from tumors reduces the amount of membrane-bound Hsp70, we propose that Gb3 is a binding partner for Hsp70. The in vitro finding that Hsp70 predominantly binds to artificial liposomess containing Gb3 (PC/SM/Cho/Gb3, 17/45/33/5) confirms that Gb3 is an interaction partner for Hsp70.

Conclusion: These data indicate that the presence of Gb3 enables anchorage of Hsp70 in the plasma membrane of tumors and thus they might explain tumor-specific membrane localization of Hsp70.

Corresponding Author: Mathias Gehrmann, PhD, Technical University of Munich, Klinikum rechts der Isar, Clinica for Radiotherapy, Ismaninger Str. 22, 81925 Munich, Germany, mathias.gehrmann@lrz.tu-muenchen.de

A 77

Heat shock proteins as a prognostic factor of acute kidney injury in children

Karl Reiter, Dennis Ballwieser, Philipp Pagel, Jan Tausendfreund, Judith Gloeckner-Pagel

Objective: Up to 50% of critically ill pediatric patients develop acute kidney injury (AKI). Despite constantly improving treatment options, AKI is still associated with high mortality in adult and pediatric patients. For multiple organ dysfunction syndrome in the course of sepsis or shock, developing AKI has been found to be associated with unfavorable outcome but little data is available on factors indicating and/or determining the prognosis of AKI. Heat shock proteins (HSP) play an important and extensively studied role during diverse cellular stress. Interestingly, certain point mutations (c.1267A>G) in the HSP72 gene have been found to result in a low-producer phenotype with an increased risk of AKI in premature infants. Furthermore, studies in animal models suggest that increased HSP72 levels may have a protective effect in renal ischemia. Based on these findings, we investigated if elevated HSP72 expression can be observed in pediatric patients and if it is associated with positive renal outcome during/after shock or sepsis.

Material and methods: Inclusion criteria: pediatric patients (<18 years) with shock, asphyxia or sepsis without preexisting renal disease. Mutation status was determined by restriction digest polymorphism assays of the HSP72 gene. HSP72 levels were measured in both urine and blood. Outcome was classified based on the development of AKI (criteria: doubling of serum creatinine).
Results: In the pilot phase of the study, we were able to recruit 16 patients. One of the heterozygous patients died during the study period. In the group of patients with HSP72 detected in urine, we found an initial peak followed by a rapid decrease over the following days. This observation is compatible with our hypothesis of an HSP72 on/off mechanism in renal cells triggered by ischemia/reperfusion or inflammatory stress. Our preliminary data seems to be consistent with the assumption that HSP72 mutations alter the expression level of the heat shock protein and its abundance in urine in pediatric patients. Our data also is in agreement with the hypothesis that elevated HSP72 levels in urine may be an indication of positive outcome.

Conclusions: The results from the pilot study are promising but a larger number of patients is required for a valid statistical analysis. We are currently in the process of recruiting more patients.

Corresponding Author: Karl Reiter, MD, Ludwig-Maximilians-University of Munich, Childrens Hospital, PICU, Lindwurmstr. 4, 80337 Munich, Germany, karl.reiter@med.uni-muenchen.de

A 78
Impact of graft-versus-host disease and influence of donor CD4+CD25+ regulatory T cells on the immune defence against fungal infections after allogeneic bone marrow transplantation
Bernd Echtenacher, Kristina Doser, Matthias Edinger, Petra Hoffmann

Objective: Graft-versus-host disease (GvHD) is a frequent and life-threatening complication after allogeneic bone marrow transplantation (alloBMT). It is initiated by the interaction of host antigen-presenting cells with mature alloreactive T cells in the graft leading to dysregulated pro-inflammatory cytokine secretion and finally to tissue damage and target organ destruction. Patients after alloBMT are severely immunocompromised and therefore also particularly prone to opportunistic bacterial and fungal infections. We have shown before that the co-transplantation of donor CD4+CD25+ Treg cells protects mice from lethal GvHD. We here tested the impact of GVHD on course and severity of an opportunistic fungal infection after alloBMT and its modulation by co-transplanted donor CD4+CD25+ Treg cells.

Animals and methods: We employed a completely MHC-mismatched murine C57BL/6 into BALB/c alloBMT model and infected recipients with or without GVHD with the clinically relevant pathogen Aspergillus fumigatus. Part of the animals received donor CD4+CD25+ Treg cells for GVHD prophylaxis. Results: After infection with Aspergillus fumigatus all animals with GVHD died within 10 days after infection, whereas 60% of the animals without GVHD survived for more than 35 days. Survival of recipients protected from GVHD after co-transplantation of donor CD4+CD25+ Treg cells was significantly better than that of unprotected recipients with GVHD. Interestingly, clearance of the fungus from the lung after i.v. infection, or from spleen and liver after i.v. infection, was rapid and comparable in mice with and without GVHD and no live fungus was detectable in moribund animals. However, when lymphocytes isolated from spleen and liver of infected animals were restimulated in vitro with germinating conidia, cells from animals with GVHD secreted significantly more pro-inflammatory TNF-α and IL-6 than those from control mice. Conclusions: Our data show that co-transplantation of donor CD4+CD25+ Treg cells protects mice not only from lethal GVHD but also from infection-related co-mortality. Furthermore, they support the hypothesis that an uncontrolled inflammatory immune response contributes to the high morbidity and mortality of opportunistic infections in GVHD.

A 79
Decreased lymphocyte proliferation is correlated with increased PD-1 and PD-1 ligand expression in septic shock patients
Fabienne Venet, Caroline Guignant, Fabienne Venet, Alain Lepape, Alfred Ayala, Guillaume Monneret

Objectives: Septic syndrome represents the leading cause of mortality in the ICU and remains major health care problem worldwide. The development of a state of immunosuppression in both patients as well as murine models of sepsis has been correlated with increased mortality and the development of secondary nosocomial infections. Programmed Death (PD)-1 and its ligands (PD-L1 and PD-L2) are ‘inhibitory co-receptors’. In septic mice, PD-1 deficiency is associated with decreased mortality [1]. The objective of this study was to investigate the relationship between PD-1 and its ligands expression and immune dysfunctions observed in septic shock patients.

Patients and methods: PD-1, PD-L1 and PD-L2 expressions were measured by flow cytometry on whole blood lymphocytes (CD4+ or CD8+) and monocytes from septic shock patients (n = 55) and healthy controls (n = 46). Lymphocyte proliferation (3H incorporation) was measured after stimulation with phytohemagglutinin (PHA) or tetanus toxin (TT). These parameters were also assessed in whole blood and peripheral blood mononuclear cells from healthy controls incubated 48 h with endotoxin (20 or 1,000 ng LPS/ml).

Results: In patients, a significant increase in the expression of PD-1 and its ligands along with a decrease in lymphocyte proliferation was observed relative to controls. Interestingly, the proliferative response to PHA was tightly correlated with PD-1 expression. Ex vivo incubation of healthy controls cells with endotoxin dose-dependently increased PD-L1 expression on monocytes whereas no effect was observed on lymphocytes or on PD-1 expression. In this model, this increased expression was also associated with a significant decrease in lymphocyte proliferation in response to TT stimulation.

Conclusion: Mechanisms responsible for immunodeficiency in septic shock are not completely understood. Our results suggest that increased PD-1, PD-L1 and PD-L2 expressions may be one regulatory pathway involved in immunological impairments after septic shock. Functional experiments with blocking antibodies are now required to investigate this point.


Corresponding Author: Fabienne Venet, PhD, Hospices Civils de Lyon, Immunology Laboratory, S, Place d’Arsonval, 69437 Lyon, France, fabienne.venet@chu-lyon.fr

A 80
B-lymphocytes contribute to early innate immune responses in bacterial sepsis
Kindra Kelly-Scumpia, Philip Scumpia, Alex Cuenca, Matthew Delano, Philip Efron, Lyle Moldawer

Objective: The interplay between the innate and adaptive immune systems is an integral component of pathogen host defense. Recently, T lymphocytes were found to hamper the early innate inflammatory response to viral infection and inflammasome activation whereas B lymphocytes have been shown to respond directly to bacterial toll like...
receptor ligands with cytokine and T cell independent antibody production. How the adaptive immune system modulates innate immune response to bacterial infection has been incompletely characterized. Here, we investigate which adaptive immune cell type is important for this response.

Materials and methods: The cecal ligation and puncture (CLP) model was used to induce polymicrobial sepsis. To determine which cell type is important in modulating the innate immune response to bacterial infection, survival studies were performed on C57BL/6, Rag1−/− (B and T cell deficient), μMT (B cell deficient) and TCR−/− (T cell deficient). B cells from the spleen and bone marrow were analyzed by flow cytometry. Serum cytokine levels were determined using multiplex analysis and peritoneal bacterial load was determined on peritoneal lavage fluid.

Results: Rag−/− mice have a 40% increased mortality following CLP compared to wild type mice (p < 0.001). This decreased survival is not associated with an exaggerated early inflammatory response. Six hours following CLP, Rag−/− mice produce similar concentrations of TNFa and IFNg and reduced levels of IL-6 and IL-1b compared to wild-type animals. Furthermore, by using μMT and TCR−/− mice, we identify B cells as the adaptive immune cell contributing to early cytokine production, bacterial clearance, and survival during bacterial sepsis. μMT mice have significantly lower serum cytokine levels including IFNg than wild-type mice. In addition, μMT mice have increased mortality (42%; p = 0.03) and peritoneal bacterial load following CLP compared to wild-type animals. Interestingly, TCR−/− mice display no significant difference in survival compared with wild-type animals and produce similar cytokine levels.

Conclusion: This study highlights the critical role of B cells in not only survival to experimental sepsis but also optimum clearance of bacteria and the generation of productive inflammatory responses.

Corresponding Author: Kindra Kelly-Scumpia, PhD, University of Florida, Department of Surgery, 1600 SW Archer Road PO Box 100019, Gainesville, FL 32609, USA, kindra@ufl.edu

A 81
Regulatory T cells demonstrate an injury-specific recall response

Goro Tajima, Marc Hanschen, Fionnuala O’Leary, Kimiko Ikeda, Adam Delisle, James Lederer

Objective: In a previous study, we demonstrated that burn injury caused rapid activation of FoxP3+ regulatory T cell (Treg) in injury-site lymph nodes in mice. Given this observation, we wished to test whether Tregs or conventional CD4 T cells might develop an injury-specific recall response. To accomplish this, we transferred CD4 T cells from sham or burn FoxP3-GFP knock-in mice into sham and burn congenic mice and tracked the expansion and activation of injury-experienced and inexperienced Tregs.

Methods: FoxP3-GFP mice underwent sham or burn injury. One or 4 weeks later, CD4 T cells were purified from the lymph nodes and spleens and then transferred into CD45.1 congenic mice. Recipient mice underwent sham or burn injury to represent a second injury response. After 7 days, transferred CD45.2 positive cells and FoxP3-GFP positive cells were detected by FACS to measure expansion and stained for cell-surface T cell activation and memory markers (CD62L, CD44, ICOS, and CTLA-4).

Results: When CD4 T cells from sham or burn FoxP3-GFP mice were transferred into naive recipient sham or burn mice, we found that the combination of burn CD4 T cells into burn recipient mice caused significantly greater FoxP3+ Treg expansion and activation than other combinations. This recall response by Tregs was much higher in Tregs transferred 1 week after injury than Tregs from 4 weeks.

Moreover, Tregs transferred at 1 week after the first injury showed a greater memory-like CD4 T cell phenotype (CD44high CD62Llow) than non-Tregs.

Conclusion: The observation that injury-experienced Tregs respond more vigorously to the second injury suggests that Tregs develop an injury-specific recall response. Conventional CD4+ T cells also showed a recall response to injury, but there were no significant difference between 1 and 4 weeks after injury. These findings are novel and support the concept that Tregs are injury-responsive and can develop an early, injury-specific memory-like response.

Corresponding Author: Goro Tajima, MD, PhD, Brigham and Women’s Hospital / Harvard Medical School, Department of Surgery (Immunology), 75 Francis Street, Boston, MA 02115, USA, gtajima@rics.bwh.harvard.edu

A 82
Injury induces differential signaling by CD4+ T-regulatory versus non-regulatory T cells

Marc Hanschen, Goro Tajima, Fionnuala O’Leary, Kimiko Ikeda, James Lederer

Objective: Although it is known that severe injury enhances the regulatory activity of CD4+ T regulatory cells (Tregs), the mechanisms responsible for Treg activation following injury remain unclear. Therefore we investigated whether injury differentially activates T cell receptor (TCR) signaling in Treg and non-Treg CD4+ T cells.

Methods: Instead of using Western immunoblots to measure TCR signaling, we optimized a flow cytometry method (phospho-flow cytometry) that allowed us to measure the phosphorylation of the TCR signaling molecules, ZAP-70, PKC-theta, NFATc1, and GSK-3beta in FoxP3+ Tregs and non-Treg CD4+ T cells. In a first set of experiments, we validated our approach in vitro by measuring the expression and phosphorylation of ZAP-70 and PKC-theta in anti-CD3 antibody stimulated splenic FoxP3+ Tregs versus non-Treg CD4+ T cells. Next, we addressed whether injury might induce intracellular signaling differences between Tregs and non-Tregs in vivo. In these experiments, C57BL/6 mice were subjected to 25% total body surface burn injury or sham treated and lymph nodes (LN) and spleens (SPL) were harvested to measure ZAP-70, PKC-theta, NFATc1, and GSK-3beta activation at early time points—15, 30, 60, 120, and 240 min—after sham or burn injury.

Results: We found that anti-CD3 antibody stimulation signaled a strong dose and time dependent up-regulation and phosphorylation of ZAP-70 and PKC-theta by Tregs and non-Tregs. Interestingly, we observed that Tregs showed higher levels of ZAP-70 and PKC-theta and that their activation was more pronounced compared to non-Tregs. Burn injury induced a significant differential signaling response in Tregs versus non-Tregs. As early as 15 min after injury, we observed a significant upregulation and phosphorylation of the TCR signaling molecules ZAP-70, PKC-theta, NFATc1, and GSK-3beta in Tregs prepared from draining LN. This effect could not be observed in Tregs derived from SPL. Interestingly, non-Treg CD4 T cells did not show an early signaling response to burn injury.

Conclusion: We demonstrate that flow cytometry can be used to accurately assess signaling differences in Tregs and non-Treg CD4+ T cells. We show that injury induces differential signaling by Tregs and non-Tregs and provide evidence to indicate that burn injury preferentially activates TCR signaling by Tregs in the LNs draining the injury site. These findings supply new insights into how injury influences the adaptive immune system.
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CD4 and gamma delta T cells are protective during the host response to sepsis

Andre Martignoni1,2, Johannes Tschoep1,2, Kevin R. Kasten1,2, Fabienne Venet1,2, Charles C. Caldwell1
1Division of Research, Department of Surgery, University of Cincinnati, Cincinnati, OH, USA, 2Department of Anesthesiology, Klinikum Groshadern, Munich, Germany

Objective: Sepsis leads to a number of systemic physiological changes associated with alterations in the immune system. During sepsis, systemic inflammation is observed with resulting tissue damage, inflammation, lymphocyte apoptosis, and subsequently, immunosuppression. It is well established that the immune response during sepsis is mediated by leukocytes associated with the innate immune system. However, although there is an emerging view that T lymphocytes can also mediate this response, the type of T cells involved has not been fully elucidated. We hypothesized that different subsets of T cells would either be protective or pathogenic during the host response to sepsis. Thus, we assessed the function of T cell subsets cells using a well-established murine model of sepsis.

Material and methods: In this study, we subjected various T cell deficient and wild-type (WT) mice to cecal ligation and puncture (CLP).

Results: We initially assessed whether CD4, CD8, regulatory or gamma delta T cell deficiency altered mortality as compared to wild type mice following CLP. We found that CD4 and gamma delta T cells were protective during sepsis and the absence of regulatory and CD8 T cell deficiency did not significantly change survival. We next focused upon the roles of CD4 and gamma delta T cells in septic mice and found that inflammation as determined by IL-6 and bacterial load was increased in septic mice deficient in CD4 or gamma delta T cells. Additionally, we observed decreased oxidative burst of peritoneal neutrophils isolated from CD4 T cell deficient mice but no differences in recruitment. In contrast, we found decreased peritoneal neutrophil recruitment from gamma delta T cell deficient mice but no differences in oxidative burst.

Conclusions: Thus, although septic CD4 T cell and gamma delta T cell deficient mice exhibited increased mortality, inflammation, and bacterial load, we propose that the underlying mechanisms for these observations are different. We speculate that a CD4 deficiency may mainly alter the Th1 response while the gamma delta T cell deficiency may alter the Th17 response. Further investigations are underway to elucidate these observations.

Corresponding Author: Charles Caldwell, PhD, University of Cincinnati, Department of Surgery, 231 Albert Sabin Way, Cincinnati, OH 45267, USA, charles.caldwell0@uc.edu

A 84

Regulatory lymphoid populations in trauma and sepsis: from T-reg to innate T-regs

Fabienne Venet, Guillaume Monneret, Alfred Ayala

Sepsis syndrome remains the leading cause of mortality in intensive care units. It is now believed that along with the body’s hyperinflammatory response designated to eliminate the underlying pathogen, mechanisms are initiated to control this initial response, which can become deleterious and result in immune dysfunctions and death. A similar state of immune suppression has also been described after numerous forms of severe trauma/injury. In this context, although the majority of clinical and basic science conducted so far has focused on the roles of myeloid cell populations, the contribution of T lymphocytes, in particular regulatory T cells, has been somewhat ignored. Here we will discuss the concept that both innate regulatory lymphocytes (γδ and NKT cells) and CD4+CD25+CD127low regulatory T cells are not only affected by injury and sepsis but also play a role in the control of immune responses both locally and systematically. This may be related to their capacity to interact with components of the innate and adaptive immune responses, via direct cell-cell and/or soluble mediators, as well as their ability to be activated non-specifically by bacterial products and/or cytokines in a TCR independent fashion.

It is our hope that a better understanding of the mechanism(s) through which these rare lymphocyte subsets exert their profound effect(s) on the immune response that we may help in improving our ability not only to diagnose but also to treat the critically ill individual.

Corresponding Author: Fabienne Venet, PhD, Hospices Civils de Lyon, Immunology Laboratory, 5, Place d’Arsonval, 69437 Lyon, France, fabienne.venet@chu-lyon.fr

A 85

Something innately different about PD-1 in sepsis

Alfred Ayala, Alfred Ayala, Guillaume Monneret, Fabienne Venet, Sean Monaghan, Xin Huang

Sepsis, a leading cause of death worldwide, is thought to involve expression of an overzealous inflammatory response (−− systemic/local pro-inflammatory cytokine/chemokine/mediator levels, etc.) and the concomitant development of an ineffective functional (↓ cytokine release capacity, ↓ reduce microbial phagocytosis, ↓ antigen presentation, etc.) innate immune response. Macrophage function is pivotal to the development of these two co-morbid aspects during sepsis; however, the mechanisms underlying these changes remain unclear. Here we will overview our recent observations made (Huang X, et al [2009] P.N.A.S. 106:6303-09; Elphick GF, et al [2008] J. Leuko. Biol. 84:58[abst.] suppl.; Wang YL, et al [2009] P.N.A.S. 106:6303-09; Elphick GF, et al [2008] J. Leuko. Biol. 84:58[abst.] suppl.; Gallant C, et al [2009] Inflam. Res. [TSIS abst.-in press]; Guignant C, et al [2010] Inflam. Res. [TSIS abst.-in press]) suggesting that a cell surface receptor known as Programmed Cell Death Receptor (PD)-1 (a member of the immunoreceptor tyrosine-based inhibitory motif [ITIM] family) and its ligands (PD-L1/PD-L2), that have been ascribed as primarily playing a role in T-cell mediated adaptive immune responses, also appears to be play a central role in not only modifying the outcome in response to septic challenge, but does so by restoring the innate antimicrobial immune response. Here we will discuss not only the data documenting the impact of both experimental sepsis in mice as well as septic shock and/or severe injury in humans on the expression of PD-1 on immune cells, but also the protective effect that gene deficiency or inhibition of signaling through the PD-1/PD-L1 provides in a murine model of septic shock, and also overview findings supporting the assertion that macrophage induced expression of PD-1 is central pathologic event contributing to septic morbidity. Together, these data suggest that PD-1 may not only be a effector/inducer of macrophage/ monocyte dysfunction, but may also be a potential diagnostic/ therapeutic target for consideration in designing measures to modulate the innate immune response, thereby preventing the detrimental effects of sepsis. Supported by NIH GM-46354 and GM-53209.

Corresponding Author: Alfred Ayala, Prof, PhD, Rhode Island Hospital/Brown University, Department of Surgery/Div. Surgical Research, 593 Eddy St, Aldrich 227, Providence, RI 02903, USA, AAYala@lifespan.org
Male TLR9 knockout (this model we tested the hypothesis that TLR9 contributes not only to organ responses observed following bilateral femur fracture. Using novel pseudofracture model which recapitulates the systemic and end inflammatory response in trauma models. However, the role of TLR9 severe and often fatal complications in multiple trauma patients. Toll-Imbalance in the post-traumatic inflammatory response leads to dysfunction A8 7 LAP to mature immunosuppressive TGF (PD-L1, CD47), and producing additional TSP-1 to activate surface target cell inhibitory receptors and by targeted TGF b inhibit host defense both through direct bidirectional triggering of target cell inhibitory receptors and by targeted TGF b delivery. Alteration of MO differentiation capacity can be triggered by excessive levels of injury released endogenous protein like TSP-1. Thus, elevated levels of TSP-1 can trigger more MO TSP-1 production altering their DC differentiation from stimulatory to inhibitory DC expressing surface latent TGF b, increased inhibitory receptors (PD-L1, CD47), and producing additional TSP-1 to activate surface LAP to mature immunosuppressive TGF b. This aberrant DC differentiation can mediate patients subsequent host defense defects. Corresponding Author: Carol L. Miller-Graziano, Prof. PhD, University of Rochester, School of Medicine and Dentistry, 601 Elmwood Ave, Box SURG, Rochester, NY 14642, USA, carol_miller-graziano@urmc.rochester.edu

A 86 Negative signalling in immune dysfunction seen in the septic patient Carol L. Miller-Graziano

The type and function of dendritic cells interacting with lymphocytes determines the degree and type of subsequent immune activation. During massive inflammatory challenges like trauma and sepsis, human peripheral blood monocytes serve as major precursors of myeloid dendritic cells seeding to the sites of inflammation. Thus, alterations in these circulating monocytes differentiation to dendritic cells (DC) will profoundly affect the type and degree of adaptive and innate immune responses in these patients. Our data shows that the subset of trauma patients developing T cell unresponsiveness and infection has dysfunctional monocyte (MO) to DC differentiation. These patients not only develop fewer DC but the DC differentiated are aberrant not only failing to enhance in vitro allogenic T cell proliferation but actually inhibiting T cell receptor induced proliferation (anti CD3) even in the face of appropriate costimulation signals (anti CD28). The T cell immunoinhibitory activity of these DC is mediated by their upregulation of inhibitory receptors like PD-L1 and CD47 concomitant to their production and surface expression of TGF b latency activated peptide (LAP) and production of the TGF b latency activator Thrombosponden-1 (TSP-1). These aberrant DC inhibit host defense both through direct bidirectional triggering of target cell inhibitory receptors and by targeted TGF b delivery. Alteration of MO differentiation capacity can be triggered by excessive levels of injury released endogenous protein like TSP-1. Thus, elevated levels of TSP-1 can trigger more MO TSP-1 production altering their DC differentiation from stimulatory to inhibitory DC expressing surface latent TGF b, increased inhibitory receptors (PD-L1, CD47), and producing additional TSP-1 to activate surface LAP to mature immunosuppressive TGF b. This aberrant DC differentiation can mediate patients subsequent host defense defects. Corresponding Author: Carol L. Miller-Graziano, Prof. PhD, University of Rochester, School of Medicine and Dentistry, 601 Elmwood Ave, Box SURG, Rochester, NY 14642, USA, carol_miller-graziano@urmc.rochester.edu

A 87 A role of TLR 9 signaling: late post-traumatic immune dysfunction Sophie Darwiche, Xiangcai Ruan, Melanie Scott, Rosemary Hoffman, Hans-Christoph Pape, Timothy Billiar

Imbalance in the post-traumatic inflammatory response leads to severe and often fatal complications in multiple trauma patients. Toll-like receptor (TLR) 9 has been implicated to contribute to the inflammatory response in trauma models. However, the role of TLR9 in delayed immune dysfunction following tissue trauma as seen with severe skeletal injury is unknown. To study this we have developed a novel pseudofracture model which recapitulates the systemic and end organ responses observed following bilateral femur fracture. Using this model we tested the hypothesis that TLR9 contributes not only to the initial inflammatory response following severe tissue trauma, but also to delayed immune dysfunction. Male TLR9 knockout (–/–) and wild type (WT) C57BL/6 mice (n = 9–14), were subjected to pseudofracture (crushed bone solution injection and soft tissue injury to the thigh musculature bilaterally). Control mice received no experimental manipulation. At 48 h, spleens were harvested for assessment of splenocyte proliferation and Th1 cytokine (Interferon-gamma (IFNγ)) release in response to the mitogen antiCD3e (1 µg/ml). Splenocyte proliferation was assessed through tritiated thymidine uptake as counts per minute (c.p.m.). Statistical significance was assessed by Student’s t test using p < 0.05.

Wild type mice showed a significant decrease in splenocyte proliferation at 48 h after pseudofracture (31.673 ± 4.494 c.p.m.) in comparison to controls (51.660 ± 4.310 c.p.m.). Splenocyte proliferation in TLR9–/– mice was similar (p < 0.05) to WT mice at baseline (45.623 ± 5.094 c.p.m.). However, in contrast to wild type mice, splenocytes from TLR9 knockout mice proliferated at the same rate as uninjured mice even after injury (57.034 ± 7.255 c.p.m.).

Splenocyte release of IFNγ was also significantly decreased (185.5 ± 24.7 pg/ml) in injured wild type mice in comparison with controls as expected (557.2 ± 82.7 pg/ml). However, this significant suppression was not seen in splenocytes from injured TLR9–/– mice (IFNγ: 533.1 ± 78.8 pg/ml) compared with TLR9–/– controls (IFNγ: 583.5 ± 135.5 pg/ml). Our novel pseudofracture model leads to delayed immune dysfunction typical of severe injury models. Our observation that delayed immune dysfunction fails to develop in TLR9 deficient animals indicates that TLR9 signaling contributes to the immune dysfunction seen in severe trauma and represents potential as a therapeutic target to limit injury-induced immune suppression.

Corresponding Author: Sophie Darwiche, MD, University of Pittsburgh Medical Center, Department of Surgery, 200 Lothrop Street, Pittsburgh, PA 15213, USA, darwiches@upmc.edu

A 88 Complement factor 3 deficiency attenuates hemorrhagic shock related systemic inflammatory response syndrome and remote hepatic injury Changchun cai, Gill Roop, Hyeon-Ae Eum, Zongxian Cao, Sophie Darwiche, Timothy Billiar

Objective: Complement (C) activation plays a key role in the adverse immune responses and remote organ injury of hemorrhagic shock (HS) with subsequent resuscitation. Blockade of C activation by antagonists or absence of effective complement factor may be protective. Complement factor 3 (C3) is required by all three known pathways for C activation. To establish the extent of the role of C activation and specifically C3 in HS-induced inflammation, we utilized C3 knockout mice.

Materials and methods: Our study consisted of treatment, sham, and control groups. Wild type C57BL/6 mice and C3KO mice underwent 1.5 h of HS, bilateral bone fracture (BFF), and soft tissue injury (STI), followed by 4.5 h of resuscitation respectively. To study the effects of C3 depletion, cobra venom factor (CVF) or vehicle (phosphate buffer saline) was given to C57BL/6 mice intraperitoneally 24 h before receiving 2 h of HS and 4 h of resuscitation. Serum alanine transferase (ALT), aspartate aminotransferase (AST) were measured by HESKA Dri-Chem 4000. Serum interleukin-6 (IL-6) and interleukin-10 (IL-10) levels were analyzed by enzyme-linked immunoabsorbent assay (ELISA), while serum high mobility group box-1 (HMGB-1) and hepatic heme oxygenase-1 (HO-1) were detected by western blot. Liver histological findings were assessed by H&E staining.

Results: The results showed C3–/– mice exhibited much less liver damage as reflected by ALT (207.60 ± 38.46 U/L versus 2,053.43 ± 284.79 U/L, P < 0.01) and AST (320.40 ± 50.72 U/L versus 2,041.20 ± 489.26 U/L, P < 0.01) levels. Liver histology showed well-preserved structure in C3–/– mice, while focal necrosis was seen in the wild type mice. C3–/– mice displayed much lower
A 89
Dendritic cells that infiltrate damaged skeletal muscle tissue reverse impaired Th-cell responses after trauma
Florian Wiradoerfer, Joerg Martin Bungen, Daniel Schmitz, Fritz Ulrich Schade, Stefanie Barbara Flohe

Objective: Upon injury granulocytes, macrophages, and dendritic cells (DC) sequentially infiltrate the damaged tissue. While granulocytes and macrophages are known to play a key role in repair and regeneration of damaged tissue, the function of DC is unclear. In case of an infection, DC take up antigens in the periphery and migrate into draining lymphoid organs. We investigated whether DC in the damaged tissue might modulate an immune response to a foreign antigen (ovalbumin (OVA)/LPS) in the draining popliteal lymph node (LN).

Materials and methods: A blunt soft tissue trauma of the gastrocnemius muscle of BALB/c mice was induced through a single drop-mass impact. Sham mice were anaesthetized only. At stated time points after treatment leukocytes were stained and analyzed by means of flow cytometry. For induction of antigen-specific Th cell responses, CFSE-labeled T cells from DO11.10 mice were i.v. injected into treated mice. One day later, OVA or OVA-loaded BMDC were applied into the m. gastrocnemius or into the hind footpad. T cell proliferation was determined according to the 50% dilution of CFSE upon each cell division. LN cells were restimulated in vitro with OVA peptide and cytokine secretion was assessed by means of ELISA. Results were compared using parametric two-tailed, unpaired Student’s t test.

Results and conclusions: DC in the damaged tissue increased in number from day 4 after trauma and continuously increased the expression of CD40 and CD86. After intramuscular application of FITC-labelled OVA, the absolute number of OVA-FITC+ DC was higher in draining LN from trauma than from sham mice indicating migration into the LN. Consequently from day 4 up to day 7 after trauma OVA-specific Th-cells in the LN of trauma mice secreted higher levels of IFN-gamma than cells from sham mice correlating with the infiltration of DC into the muscle at these time points. The proliferation of antigen-specific T cells remained unaffected. These findings were also observed when OVA-loaded BMDC were injected into the traumatized muscle instead of the soluble antigen. In contrast, upon application of OVA apart from the injured tissue (footpad), the OVA-specific Th1-cell response in the LN was suppressed. DC mature due to the local milieu in the injured tissue. After taking up an antigen they migrate into the draining LN where they mediate strong antigen-specific Th-cell responses in the draining LN and thereby might counteract T-cell suppression after severe injury.

Corresponding Author: Florian Wiradoerfer, University Hospital Essen, Department of Trauma Surgery, Virchowstr. 171, 45147 Essen, Germany, florian.wiradoerfer@uk-essen.de

A 90
Immediate local transplantation of mesenchymal stem cells into a severely injured skeletal muscle in rats improves the functional outcome comparable to delayed transplantation
Philipp von Roth, Tobias Winkler, Piotr Radojewski, Georg Matziolis, Georg Duda, Carsten Perka

Objective: Skeletal muscle trauma leads to severe functional deficits. Present therapeutic treatments are unsatisfying and insufficient post-traumatic regeneration is a problem in trauma and orthopaedic surgery. Stem cell therapy is a promising tool in the regeneration of muscle function after severe trauma. Our group showed increased contraction forces compared to a non-treated control group 3 weeks after mesenchymal stem cell (MSC) transplantation (TX) into a skeletal muscle trauma. Furthermore we demonstrated a dose-response relationship of the number of MSC and functional muscle regeneration. In addition we investigated the fate of the transplanted MSC labelled with very small iron oxide particles using 7 Tesla-MRI. Before further steps are taken into clinics ideal time of TX has to be identified. Due to the inflammatory environment in the initial phase of the trauma we hypothesized that a local injection of the cells immediately after injury results in a lower functional outcome compared to a delayed TX.

Methods: 30 female SD-rats received open crush trauma of the left soleus muscle. Group 1 received local TX of 2 × 10⁶ MSC immediately after trauma (group 2 and 3 received saline). Group 2 was transplanted with 2 × 10⁶ MSC seven day after the trauma (group 1 and 3 received saline). Functional muscle force testing was performed in vivo 3 weeks after TX.

Results: TX of 2 × 10⁶ MSC seven days after trauma improved the functional regeneration of the injured muscles as displayed in higher contraction forces (group 2: twitch p = 0.014, tetany p = 0.018). MSC-TX immediately after trauma enhanced the regeneration process to a similar extent with an increase of maximum twitch contraction forces by 73.3% (p = 0.006) and of tetanic contraction forces by 49.6% (p = 0.037). Comparison of contraction forces of muscles treated by immediate or delayed TX showed no significance (twitch: p = 0.93, tetany p = 0.73).

Conclusion: We could show the efficacy of MSC-TX for the treatment of severe skeletal muscle injuries. The most surprising finding was the similarity in functional muscle regeneration of early and delayed treated groups despite the fundamental differences of the local environment. Although a certain extent of inflammation is necessary to activate transplanted MSC in a host environment, we hypothesized that the initial processes of necrotic tissue removal would reduce their functional effect. We conclude that the effect of the MSC after immediate injection can partly be explained by their known immunomodulatory competences. Our results provide evidence for a large time window of MSC-TX after muscle trauma.

Corresponding Author: Philipp von Roth, MD, Charité University Medical Center, Center for Musculoskeletal Surgery, Chariteplatz 1, 10117 Berlin, Germany, philipp.roth@charite.de

A 91
Transcriptomic response of murine liver to severe injury and hemorrhagic shock: a dual platform microarray analysis
Rebecca Edmonds, Rebecca Edmonds, Yoram Vodovotz, George Tseng, Timothy Billiar

Objectives: The host response to trauma–hemorrhagic shock (T–HS) is a complex process characterized by the differential expression of
thousands of genes. In turn, numerous molecular pathways are engaged. The complexity of this process lends itself to investigation using microarray technology. We hypothesized that the liver, an organ well known to integrate immunologic and metabolic responses, would reveal the key pathways that are engaged following injury. We report the use of dual platform microarray analysis to optimize the characterization of the hepatic transcriptomic response in an injury severity model.

Methods: To do so, we used our previously described model of T–HS. C57BL/6 mice were divided into three groups to simulate a model of injury severity: (1) anesthesia; (2) 1.5 h femoral artery cannulation, 4.5 h resuscitation (R); (3) 1.5 h HS, bilateral femur fracture (BFF), 4.5 h R. Liver RNA was hybridized to Codelink and Affymetrix mouse whole genome microarray chips. Meta-analysis was employed to identify differentially expressed genes common to both platforms. Common genes with a cross-platform correlation greater than 0.6 (2,353 genes in total) were clustered into six clusters using k-means clustering. Clustered genes were analyzed by pathway analysis using ingenuity pathways analysis.

Results: Each cluster of genes and associated molecular pathways demonstrate a unique pattern of expression as severity of injury increases. Consistent with previous observations, minor surgical trauma results in upregulation of genes in the endoplasmic reticulum stress pathway, whereas increasing severity of injury is associated with up-regulation of genes involved in IL-6 and IL-10 signaling pathways. Interestingly, the most severe injury is characterized by marked upregulation of genes involved in apoptosis and cell death pathways. Significant genes identified include fas, p53, and caspases 2/3/7. Consistent with biological intuition, down-regulation of genes involved in metabolic and synthetic pathways occurs with severe injury.

Conclusions: This is the first study to examine the transcriptomic response to injury in a severity model using dual platform microarray analysis. Use of the dual platform approach increased the number of validated genes. This led to identification of the significant pathways, most notably those involved in cell death signaling, that are engaged as injury severity increases.

Corresponding Author: Rebecca Edmonds, MD, University of Pittsburgh, Department of Surgery, 200 Lothrop Street, Pittsburgh, PA 15212, USA, edmondsrd@upmc.edu

A 92
Damage of endothelial progenitor cells (EPC) by polymorphonucleated leukocytes (PMNL)
In vitro is mediated by direct interaction via CD54 and CD11b/CD18 and the release of reactive oxygen species
Sebastian Zimmer, Dirk Henrich, Ingo Marzi

Objective: EPC support the neovascularization of injured tissues. The differentiation of EPC is impaired under inflammatory conditions and EPC underwent apoptosis in the presence of proinflammatory mediators. PMNL invade injured tissues and are a source of inflammatory mediators and reactive oxygen species (ROS) upon stimulation. The interaction between both cell types was not addressed yet. The analysis of surface receptor expression revealed that interaction between both cell types is possible via CD54 on PMNL and its counterpart CD11b/CD18 on EPC. This study was conducted to elucidate the interaction between PMNL and EPC with respect to EPC damage and the identification of the involved surface receptors and the role of the NADPH oxidase.

Patients and methods: PMNL from healthy volunteers (n = 5) were obtained by density gradient centrifugation with Polymorphprep. Early EPC were differentiated from buffy coat. Killing of EPC was measured by flow cytometry, control and prestimulated (with FMLP or PMA) PMNL and DL-ac-LDL prestained EPC were incubated in a ratio of 20:1 for 3 h. Defect EPC were identified by DiL-fluorescence and uptake of 7-AAD. Role of reactive oxygen species was assessed by blockade of PMNL’s NADPH-oxidase with DPI and the role of CD54 on PMNL and CD11b/CD18 was assessed by blockade with specific antibodies prior incubation with EPC. Wilcoxon matched pair analysis, a Bonferroni corrected p < 0.05 is considered significant. Data were presented as mean ± SEM.

Results: A significant increase of defective EPC were observed after incubation with stimulated PMNL (control 5.9% ± 1.0, FMLP 13.7% ± 2.8; PMA 16.6% ± 1.7, all p < 0.05 vs. control). Blockade of CD11b or CD18 on EPC and CD54 on PMNL lead to a significant decline of defect EPC (CD11b −68% ± 14, CD18 −61% ± 19, CD54 −76% ± 26, all p < 0.05 vs. control). Blockade of NADPH oxidase in stimulated PMNL result in a significant inhibition of EPC death (PMA −75% ± 20, p < 0.05; FMLP −66% ± 33, both p < 0.05 vs. control).

Conclusion: Here we demonstrated that EPC can be harmed by activated PMNL through interaction between CD54 (PMNL) and CD11b/CD18 (EPC) and the release of reactive oxygen species. The results of this work should be taken under consideration for a future application of EPC in healing of injured tissues.

Corresponding Author: Sebastian Zimmer, Johann Wolfgang Goethe University of Frankfurt/M, Department of Trauma-, Hand- and Reconstructive Surgery, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, sebastianm@stud.uni-frankfurt.de

A 93
Isolated penetrating thorax injury leads to mild systemic activation of neutrophils without inflammatory complications
Kathelijne Groeneveld, Falco Hietbrink, Tim Hardcastle, Leo Koenderman, Luke Leenen

Objective: Acute respiratory distress syndrome (ARDS) is associated with systemic inflammation and is mediated by neutrophils. About five percent of patients with a thoracic injury develop ARDS. The goal of this project was to gain more insight into the pathophysiologic processes that link systemic neutrophil activation with inflammatory complications in the lung after isolated penetrating thoracic injuries. Our hypothesis is that the degree of innate immune activation, measured as neutrophil phenotype, is directly related to the occurrence of inflammatory complications of the lung.

Materials and methods: Fifty-seven patients with isolated stab and/or gunshot wounds of the thorax were included at the Tygerberg Hospital of the University of Stellenbosch. Blood samples were taken within 3 h of trauma and repeated six and 24 h after injury and analyzed for neutrophil phenotype with the use of flow cytometry. The presence of inflammatory complications (e.g. ARDS or sepsis/septic shock) was assessed during admission, and this was studied in context of neutrophil phenotypes. Statistical analysis was performed with Mann Whitney U.

Results: Two patients developed an inflammatory complication. The clinical follow-up of other patients was uneventful. In these last patients MAC-1 (CD11b) did not differ significantly from healthy individuals. Within the first 24 h after injury, the expression of IL-8 receptors CXCR-1 (CD181, p = 0.002) and CXCR-2 (CD182, 0.05 vs. control). Blockade of NADPH oxidase in stimulated PMNL result in a significant inhibition of EPC death (PMA −75% ± 20, p < 0.05; FMLP −66% ± 33, both p < 0.05 vs. control).

Conclusion: Here we demonstrated that EPC can be harmed by activated PMNL through interaction between CD54 (PMNL) and CD11b/CD18 (EPC) and the release of reactive oxygen species. The results of this work should be taken under consideration for a future application of EPC in healing of injured tissues.

Corresponding Author: Sebastian Zimmer, Johann Wolfgang Goethe University of Frankfurt/M, Department of Trauma-, Hand- and Reconstructive Surgery, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, sebastianm@stud.uni-frankfurt.de

A 93
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Altered expression of Fas-receptor on alveolar macrophages following blunt chest trauma: inflammatory effects of soluble Fas ligand

Daniel H. Seitz, Annette Palmer, Ulrike Niesler, Sonja T. Brauwer, Florian Gebhard, Markus W. Knoeferl

Objective: Lung contusion induces local and systemic inflammatory alterations. Fas dependent apoptosis of alveolar epithelial cells occurs after experimental blunt chest trauma. The aim of this study was to elucidate the involvement of the Fas/Fas ligand system in the inflammatory response after lung contusion.

Methods: Male CD rats were subjected to blunt chest trauma induced by a pressure wave or to the corresponding sham procedure. Alveolar macrophages (AM) and alveolar epithelial type 2 (AT2) cells were isolated at different time points. AM and AT2 cells were stained with antibodies against Fas or FasL. The expression of Fas and FasL on these cells was determined by FACS analysis. The mRNA expression for Fas and FasL in AM or AT2 cells was determined by RT-PCR. Furthermore, AM and AT2 cells isolated at 24 h after lung contusion or sham procedure were cultured in absence or presence of recombinant FasL (500 ng/ml). The release of IL-6 or IL-10 was determined by Elisa.

Results: In FACS analysis, Fas and FasL protein was significantly down-regulated on AM at 4 and 16 h after lung contusion, but not altered on AT2 cells. In contrast, the mRNA expression of Fas was significantly up-regulated in AM at 24 h after chest trauma, but did not change in AT2 cells. Fas ligand gene expression was markedly increased in AM as early as 4 h after lung contusion and significantly down-regulated in AT2 cells at 24 h after chest trauma. In cell culture experiments AM and AT2 cells isolated after blunt chest trauma released significantly higher levels of IL-6 and IL-10 than sham. FasL stimulation of AM cultures significantly enhanced IL-6 and reduced IL-10 release after blunt chest trauma. AT2 cell mediator release was not altered by stimulation with FasL.

Conclusion: Regarding the expression of Fas and FasL on AM, these cells seem to be protected from apoptosis early after blunt chest trauma. Furthermore, the inflammatory response of AM to chest trauma is altered by Fas Ligand stimulation. In contrast to AM, no stimulatory effects of FasL were observed in AT2 cells. Nevertheless, our data clearly indicate that AT2 cells contribute to the immunological response after chest trauma by the release of inflammatory mediators. (Supported by DFG KN 475/4-1).

Corresponding Author: Daniel H. Seitz, MD, University of Ulm, Department of Trauma Surgery, Hand, Plastic and Reconstructive Surgery, Steinhevelstr. 9, 89075 Ulm, Germany, daniel.seitz@uniklinik-ulm.de

Subsequent gene expression pattern in dendritic cells following multiple trauma is independent from injury severity and organ failure

Emanuel Geiger, Marcus Maier, Ann-Kathrin Nielsen, Thorsten Ottlinger, Dirk Henrich, Ingo Marzi

Objective: Severe trauma induces a systemic inflammatory response syndrome leading frequently to secondary organ failure. Besides various cytokines, dendritic cells (DC) play a key role within the systemic inflammatory response. DC are professional antigen presenting cells, reflecting an important linkage between the innate and...
adaptive immune system. Activation of DC leads to distinct transcriptional changes in genes relevant for signal transduction, apoptosis and mediator synthesis following trauma. Hence, the present study was performed to evaluate gene expression pattern in DC of multiple trauma patients and to analyze if there are any temporal changes early after trauma.

Patients and methods: The study was approved by the local ethics committee. Repeated blood samples were obtained from 10 multiple trauma patients (ISS 36 ± 10.4) starting on admission (day 0) until day 5. Messenger RNA was isolated from highly purified peripheral DC. Target cDNA and reference samples (monocytic cell line SIGM5) were cohybridized on a thematic medium-density microarray assessing 5331 inflammation-related transcripts. Results were confirmed by qRT-PCR in randomly selected genes. Data were normalized and transformed employing arcus sinushyperbolikus. Statistical analysis was performed using the principle component analysis and the significance was tested using ANOVA.

Conclusions: Transfusion of 1DO PRBCs induced significant decreases in Th (ALL 9.6 ± 1.0, SYN 13 ± 0.5, PBS 11.1 ± 1.1; p = 0.038) and Tef (ALL 6.8 ± 0.3, SYN 12.1 ± 0.8, PBS 9.1 ± 0.1; p = 0.005), increases in B cells (ALL 72.1 ± 1.3, SYN 48.6 ± 4.7, PBS 56.7 ± 3.2; p = 0.026) as well as a trend toward Treg cell expansion. 2IDO PRBC induced the opposite effect of 1DO PRBC, regardless of genotype. There were significant increases in Tef (ALL 15 ± 1.0, SYN 11.9 ± 0.7, PBS 7.6 ± 1.1; p = 0.002), decreases in Treg (ALL 5.2 ± 0.2, SYN 5.2 ± 0.4, PBS 9.5 ± 0.7; p = 0.001), as well as a trends toward Th cell expansion and B cell contraction. Transfusion of most any PRBC induced a relative loss of DC. There were no significant changes in total splenic WBC counts, implying percentage differences reflected absolute changes.

Conclusions: Transfusion of PRBC induces alterations in the recipients’ splenic leukocyte populations. Allogenicity appears to play a role in younger stored blood while storage age appears to be important independent of allo- or syngenicity. Interestingly, early DC alterations may be the result of the transfusion of any PRBC. In conclusion, PRBC transfusion generates WBC changes consistent with simultaneous immune suppression and activation that may be similar to other inflammatory insults such as sepsis.

A 98
Systemic inflammation following long bone fractures: what is the role of the fracture-associated soft tissue injury?
Philipp Kobbe, Philipp Lichte, Hans Christoph Pape

Objective: Patients with bilateral femur fractures are known to be at risk to develop the Systemic Inflammatory Response Syndrome with consequent multiple organ failure. The impact of the fracture-associated soft tissue injury in the induction of this systemic inflammatory response is poorly understood. To address this, the systemic inflammatory response and remote organ dysfunction following bilateral femur fracture with and without associated soft tissue injury were investigated.

Material and methods: Male C57BL/6 mice were subjected to a severe soft tissue injury of both thighs (STI-group), a bilateral femur fracture with minimal soft tissue injury (Fx-group), and the combination of both injuries (Fx + STI-group). Six hours after trauma animals were sacrificed and serum cytokine and ALT levels were measured. Hepatic inflammation was assessed by local IL-6 concentrations and hepatic myeloperoxidase activity (MPO). The wet-to-dry-weight ratio was used to measure hepatic permeability changes. Results: STI and Fx both induced a significant increase in serum IL-6 and IL-10 levels as compared to Sham animals; serum IL-6 and IL-10 levels were even significantly higher in the STI-group as compared to the Fx-group. Serum MCP-1 levels were significantly increased in the STI-group; however not in the Fx-group. Serum ALT levels as well as hepatic myeloperoxidase activity were significantly increased in the STI- and Fx-group; however both isolated injuries did not induce an increased hepatic IL-6 concentration or hepatic permeability changes measured with the hepatic wet-to-dry-weight-ratio. The combination of both injuries (Fx + STI) caused a significant increase in serum cytokines and hepatic injury as compared to isolated injuries.

Conclusion: Our date indicate that the soft tissue injury as well as the isolated bilateral femur fracture induce a systemic inflammatory response, which however is much more pronounced following soft
Objective: Multiple trauma is associated with a high mortality due to subsequent organ failure or sepsis. Fatty acid binding proteins (FABPs) comprise a group of nine organ-specific isoforms and in a recent pilot study we showed that L- and I-FABP can be used as an early marker for the detection of abdominal injuries. Aim of the present study was to evaluate the significance of FABPs as an indicator for the failure of a specific organ in the posttraumatic course and to evaluate an association between FABP levels with sepsis or mortality.

Patients and methods: Prospective study, 134 multiply trauma patients were included. 59 patients with abdominal injury (AI, AIS_Abb ≥2, ISS 34 ± 1) and 75 without abdominal injury (noAI, ISS 24 ± 1) and 75 without abdominal injury (noAI, ISS 24 ± 1). 60 healthy volunteers served as control group. Plasma I- and L-FABP levels were measured in the emergency room and the following 10 days using ELISA. The diagnosis of sepsis was determined according to the S2-guidelines of DSG. Organ failure and mortality were assessed by SOFA- and SAPS-2-score, respectively. The study was approved by the institutional ethics board. Statistics: Kruskal–Wallis, Spearman-Rang-Correlation, significance was considered at \( p < 0.05 \);

Results: On admission (d0) the median of L-FABP and I-FABP levels from patients with AI (258, 328 pg/ml, respectively) are significant higher compared to patients with noAI (30, 60 pg/ml, respectively). The cutoff level for L-FABP was 51 ng/ml (sensitivity 80%, specificity 75%) and for I-FABP 243 pg/ml (sensitivity 78%, specificity 61%) to detect AI. A rapid decline for both FABP’s to control levels was observed. In IL-6 knockout mice a significantly attenuated increase of cytokine levels was observed. In IL-6 knockout mice a significantly attenuated increase of IL-6 was demonstrated compared to wild type animals (Fig. 1, not shown). Furthermore, interstitial edema and PMN infiltration were determined after immuno-histochemical staining (LY-6G-specific monoclonal antibody rat-anti-mouse, BD Company, NJ USA). Study groups were compared by using one-way analysis of variances (ANOVA) followed by the Student’s t test. Statistical significance was considered at \( p < 0.05 \).

Conclusion: In wild type mice, already an isolated femoral fracture caused a significant increase of pro-inflammatory mediator release, pulmonary interstitial edema and PMN infiltration. Furthermore, the results of the present study support the pivotal role of IL-6 in the inflammatory response after trauma as TNF-z expression, interstitial edema and pulmonary PMN infiltration were significantly decreased in IL-6 knockout mice.

A 100

Effects of femoral fracture and trauma–haemorrhage on alveolar macrophages in IL-6-knockout mice

Philipp Mommsen, Philipp Mommsen, Tanja Barkhausen, Michael Frink, Christian Krettek, Frank Hildebrand

Objective: In multiple trauma patients, there is an ongoing discussion on the effect of femoral shaft fractures on the inflammatory response and the associated changes of pulmonary function. Alveolar macrophages are known to play a pivotal role in the pulmonary synthesis of cytokines. The present study investigates the influence of femoral shaft fracture isolated or in combination with trauma–haemorrhage on the activation of alveolar macrophages and the subsequent pulmonary infiltration of neutrophils (PMN).

Materials and methods: 36 male wild type (C57BL/6) and IL-6 knockout (B6; 12952-IL6klKO/f) mice (aged 10–12 weeks, weighing 20–22 g) underwent femoral fracture isolated or in combination with induced trauma haemorrhage followed by fluid resuscitation and splint fixation of the fracture. Animals were sacrificed 4 h after induction of fracture and trauma/haemorrhage. Sham group [S] (only anaesthesia), femoral fracture [FF] and trauma haemorrhage/femoral fracture group [TH/FF] were created consisting of six wild type and IL-6 knockout mice, respectively. Alveolar macrophages were isolated from BAL. After stimulation of isolated macrophages with 10 µg/ml LPS for 24 h, TNF-z and IL-6 concentrations were measured in cell supernatant. Furthermore, interleukin edema and pulmonary PMN infiltration were determined after immuno-histochemical staining (LY-6G-specific monoclonal antibody rat-anti-mouse, BD Company, NJ USA). Study groups were compared by using one-way analysis of variances (ANOVA) followed by the Student’s t test. Statistical significance was considered at \( p < 0.05 \).

Results: In wild type mice TNF-z and IL-6 release of stimulated alveolar macrophages were significantly increased after femoral shaft fracture compared to sham animals. After combined femoral fracture and trauma–haemorrhage a further significant increase of cytokine levels was observed. In IL-6 knockout mice a significantly attenuated increase of TNF-z was demonstrated compared to wild type animals (Fig. 1, not shown). Furthermore, interstitial edema and PMN infiltration was significantly decreased in IL-6 knockout mice.

Conclusion: In wild type mice, already an isolated femoral fracture caused a significant increase of pro-inflammatory mediator release, pulmonary interstitial edema and PMN infiltration. Furthermore, the results of the present study support the pivotal role of IL-6 in the inflammatory response after trauma as TNF-z expression, interstitial edema and pulmonary PMN infiltration were significantly decreased in IL-6 knockout mice.

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Effect of androstenediol on systemic chemokine levels in a two-hit model (trauma–haemorrhage/CLP)

Frank Hildebrand, Frank Hildebrand, Michael Frink, Philipp Mommsen, Christian Zeckey, Tanja Barkhausen

Introduction: The posttraumatic pro- and anti-inflammatory immune response and the associated release of inflammatory mediators (e.g. chemokines) are known to play an important role in the pathogenesis of posttraumatic multiple-organ-dysfunction-syndrome (MODS) and sepsis. Sex steroids have been shown to beneficially modulate the posttraumatic immune response. The precursor Androstenediol...
A 102

The infusion of norepinephrine impairs right ventricular ejection fraction in early endotoxemia

Hille Kisch-Wedel, Michael Flondor, Anja Hanser, Sabine Pillivathukal, Gregor Kemning, Bernhard Zwissler

Objective: The early diagnosis and immediate treatment of endotoxia might reduce mortality. Norepinephrine (NE) is frequently used in sepsis, but the effects of NE on right and left heart function have not been described in detail in the very early phase of endotoxia. Thus, we studied the effects of NE infusion on right (RV) and left ventricular (LV) heart function, ventilation, oxygen transport and blood counts in piglets 1 h after infusion of LPS.

Material and methods: RV function was assessed by measuring right ventricular ejection fraction (fast response pulmonary artery catheter), LV function was assessed by the conductance technique (endystolic elastance, LV ejection fraction, LV oxygen delivery, LV oxygen consumption). Additionally, the difference of pulmonary to arterial elastance, LV ejection fraction, LV oxygen delivery, LV oxygen consumption (14 ± 5–41 ± 6 ml min⁻¹ m⁻², p < 0.05) show that norepinephrine increased myocardial oxygen consumption.

Conclusion: The infusion of norepinephrine in early endotoxia further aggravated pulmonary hypertension and severely compromises RV function as estimated by the RV ejection fraction. The improvement of LV contractility by the infusion of norepinephrine might lead to a myocardial oxygen imbalance.

Corresponding Author: Hille Kisch-Wedel, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Clinic for Anaesthesiology, Marchioninistr. 15, 81377 Munich, Germany, Hille.Kisch-Wedel@med.uni-muenchen.de
A 105

Beneficial effects of macrophage activating lipopeptide (MALP)-2 in a murine sepsis model

Christian Zeckey, Frank Hildebrand, Thomas Tschernig, Michael Frink, Christian Krettek, Tanja Barkhausen

Objective: Effective and successful therapy in polymicrobial sepsis is still a major problem. Stimulation of the immune system via Toll-like receptors (TLR) 2 and 6 had beneficial effects on chronic inflammatory disorders and a severe peritonitis model when administered 4 days prior to induction. In the present study the hypothesis was tested whether the TLR2 and 6 pathway can also be used as a therapeutic agent applied either parallel to sepsis induction or several hours thereafter.

Material and methods: The TLR2 and 6 agonist macrophage activating lipopeptide (MALP)-2 was applied simultaneously to cecal ligation and puncture (CLP)-sepsis induction and 6 h thereafter in mice. Vehicle treated animals served as controls. Survival, activity, cytokine levels at different time points and pulmonary neutrophil infiltration were determined.

Results: Improved survival was found after both time points of MALP-2 application in comparison to untreated controls. The treatment resulted in reduced monocyte chemotactic protein (MCP)-1 levels in plasma, furthermore pulmonary infiltration by neutrophils was decreased. Activity levels were significantly increased.

Conclusion: Our results demonstrate a beneficial effect of MALP-2 as a therapeutic agent in polymicrobial sepsis in the CLP mouse model not only at sepsis induction but also 6 h thereafter. Accordingly, activity rates of mice were increased, indicating an overall improvement after CLP.

Corresponding Author: Christian Zeckey, MD, Hannover Medical School, Trauma Department, Carl-Neuberg-Str. 1, 30625 Hannover, Germany, zeckey.christian@mh-hannover.de

A 106

Lipid-rich nutrition modulates the inflammatory response in murine gram-negative sepsis

Tim Lubbers, Jacco-Juri de Haan, M’Hamed Hadfoune, Yiren Zhang, Wim Buurman, Jan Willem Greve

Objective: Regulation of the inflammatory response during septic conditions remains a major clinical challenge. Lipid-rich enteral nutrition has been demonstrated to modulate inflammation via activation of the autonomic nervous system, the so-called nutritional anti-inflammatory pathway. The current study investigates the anti-inflammatory potential and mode of action of lipid-rich enteral nutrition in a murine model of gram-negative sepsis.

Materials and methods: Male C57Bl6 mice were subjected to an intra-peritoneal bolus of lipopolysaccharide (LPS) from E. coli (2 mg/kg). Prior to LPS administration, mice were fasted or fed lipid-rich nutrition enriched with 30% phospholipids or control low-lipid nutrition. Antagonists to the cholecystokinin receptor and nicotinic receptor were administered to investigate the pathway of immune modulation. In addition, activation of the autonomic nervous system was determined by measuring mesenteric afferent discharge to both nutritional compositions ex vivo. Blood and tissue samples were collected at 90 min to investigate inflammation and intestinal epithelial cell damage, determined as plasma levels of ileum–lipid binding protein (I–LBP). A Mann–Whitney U test was used for between group comparisons, n = 8 for all groups.

Results: Lipid-rich nutrition attenuated systemic levels of TNF-\(\alpha\) (1.5 ± 0.2 ng/ml) compared with fasted (3.7 ± 0.5 ng/ml; \(p < 0.01\)) and low-lipid treated mice (2.5 ± 0.3 ng/ml; \(p < 0.05\)). In line, lipid-rich nutrition activated the autonomic nervous system more efficiently than low-lipid nutrition. Administration of cholecystokinin receptor antagonists (TNF-\(\alpha\); 3.9 ± 0.8 ng/ml) and nicotinic receptor antagonists (TNF-\(\alpha\); 3.0 ± 0.3 ng/ml) abrogated the anti-inflammatory effect of lipid-rich nutrition compared with vehicle (both \(p < 0.01\)). Furthermore, administration of lipid-rich nutrition prevented damage to small intestinal epithelium [I–LBP; 8.3 ± 2.3 mg/ml vs. (fasted) 27.1 ± 3.0 mg/ml and (low-lipid) 18.5 ± 3.2 mg/ml; both \(p < 0.05\)] and reduced levels of TNF-\(\alpha\) protein in liver and spleen compared with fasted and low-lipid nutrition (all \(p < 0.05\)).

Conclusion: The current study demonstrates that enteral nutrition enriched with fats attenuates organ-specific and systemic inflammation in murine gram-negative sepsis via the nutritional anti-inflammatory pathway. These findings implicate lipid-rich nutrition as an interesting intervention to modulate the inflammatory response in septic conditions.
A 107
Protective effect of a novel peptide targeting NF-kappaB p65 on collagen-induced arthritis in mice
Huaping Liang, Xia Fan, Qiang Wei, Xue Yang, Xi Wang, Yufu Wang

Objective: We have successfully design and synthesize a membrane-permeable hexapeptide, which can effectively interfere with DNA binding of NF-kappaB p65 subunit and alleviate phorbol myristate acetate-induced ear edema and zymosan A-induced peritonitis in mice. The therapeutic effect of this peptide on collagen-induced arthritis (CIA) in C57BL/6 mice was investigated in this study.

Methods: 2 days after the second immunization in mice, two hind ankle joints were given intra-articular injection of 5 μg peptide daily for consecutive three times. Two weeks later, the incidence of CIA and histopathological changes were determined. Cytokine messenger RNA (mRNA) expression was detected in synovial tissues by real-time polymerase chain reaction.

Results: As compared with vehicle or scrambled control group, the incidence of CIA, clinical scores and magnitude of joints erosion were significantly reduced in peptide treatment group. Synovial iNOS, COX-2, IL-1β, IL-6, and TNF-α mRNA was also lower than that in control group. In contrast, no difference was observed in IL-10 mRNA between these groups.

Conclusions: The novel hexapeptide we have developed possess effective anti-inflammatory activity for treating rheumatoid arthritis. This research is supported by the National High Technology Research and Development Program (“863” Program) of China (No. 2008AA02Z440).

Corresponding Author: Huaping Liang, Prof, MD, PhD, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Department 1, Changjiang Zhi Road 10&,#65292;Daping, 400042 Chongqing, China, huaping_liang@yahoo.com.cn

A 108
Glomerular glycocalyx disruption is associated with proteinuria and changes in ceftriaxone pharmacokinetics
Chiara Adembri, Selmi Valentina, Vitali Luca, Arrigucci Silvia, Tani Alessia, De Gaudio Angelo Raffaele

Objective: We have recently demonstrated that experimental sepsis is associated with impairment of glomerular endothelial glycocalyx [1]. In the present study, we aimed at evaluating whether an increased urinary protein loss provides a route for changes in distribution/elimination of ceftriaxone (CTZ), a highly protein-bound (>80%) third generation cephalosporin frequently used in abdominal infections [2].

Material and methods: At T0, polymicrobial sepsis was induced by cecal ligation and puncture (CLP) in rats (n = 6); control animals (CTRL, n = 2) received laparotomy only. At T1 (2 h from T0), all rats received CTZ (100 mg/kg, ip). At T2 (5 h from T0), rats were sacrificed and CTZ levels measured (by microbiological assay) in plasma, urine, peritoneal fluid and several tissue samples (kidney, lung, mesentery, brain etc.). Urinary protein levels were measured by using an immuno-fixation technique. Kidney specimens were processed for confocal microscopy examination and glomerular glycocalyx integrity was evaluated by using a mouse monoclonal antibody against Syndecan-1 (an integral membrane proteoglycan).

Results: Loss of glomerular capillary glycocalyx and proteinuria occurred early (from T2) in septic rats and were associated with changes in CTZ levels in several tissues and in CTZ renal elimination. Conclusion: Sepsis-related alteration in glomerular glycocalyx and increase in urine protein loss have an impact on CTZ pharmacokinetic parameters. It is likely that sepsis-related changes in distribution and elimination of CTZ determine a sub-optimal exposure of susceptible bacteria to this antibiotic, with consequent need of modifying dosing and/or frequency of administration.


Corresponding Author: Chiara Adembri, MD, PhD, University, Department of Critical Care Medicine, Viale Morgagni 85, 50134 Florence, Italy, chiara.adembri@unifi.it

A 109
Evolution of lethal and surviving responses in the two-hit mouse model of post-traumatic sepsis
Susanne Drechsler, Katrin Maria Weixelbauer, Martijn van Griensven, Heinz Redl, Soheyl Bahrami, Marcia Osuchowski

Objective: Sepsis is a frequent complication in trauma patients of all ages. To study the evolution of post-traumatic sepsis we set to develop a 2-hit model consisting of minimally lethal femur fracture and hemorrhage (TH, first hit) followed by polymicrobial sepsis (second hit) with approx. 50% mortality.

Animals and methods: Three month old CD-1 mice were subjected to cecal ligation and puncture (CLP, double puncture) and mortality was followed for 5 days. Monitoring was facilitated by daily, non-lethal blood sampling (20 μl by facial vein). Starting at TH, blood was collected every 24 h until day 7 (or death) for complete blood count (CBC) with differential and analysis of circulating organ function (OF) parameters.

Results: The initially selected 50% haemorrhage volume (HV, of total blood) produced an immediate (within 1 h of TH) 31% mortality (10/32), while reduction to the HV of 40% produced only 5% mortality (3/57). CLP mortality was identical regardless of HV. Using the CBC and OF values measured prior to CLP, we aimed to predict the post-CLP outcome (days 2–7 post-TH). All post-CLP animals were retrospectively identified as either dying (DIE) or surviving (SUR) by day 7 post-TH and their pre-CLP values were compared. All CBC and OF parameters were virtually identical between the DIE and SUR groups at the TH-, 24 h prior or immediately pre-CLP time points. Next, we monitored the daily, outcome-dependent (DIE vs. SUR) fluctuations of blood cells and OF in the acute sepsis (days 2–7 post-TH). DIE values represent these recorded within 24 h prior to death (on any post-CLP day), while daily SUR values include those repeatedly recorded in all mice alive by day 5 post-CLP. At 24 h post-CLP, DIE mice showed markedly higher RBC count and Hb concentration compared to SUR. Starting with day 2 post-CLP, the platelet count was lower in DIE animals (vs. SUR). Lymphocyte count was significantly lower in DIE vs. SUR mice only on day 4 post-CLP. Urea, ALT and LDH significantly increased in DIE vs. SUR between days 2–5 post-CLP.

Inflamm. Res.
Conclusion: We developed a reproducible, 2-hit mouse model of TH and CLP sepsis featuring daily, non-lethal monitoring of circulating parameters. The model is well suited for discerning disparities in responses between dying and surviving subjects and for testing of therapies against sepsis in the pre-clinical setting.

**Corresponding Author:** Susanne Drechsler, MSc, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingen Str. 13, 1200 Vienna, Austria, susanne.drechsler@trauma.blg.ac.at

**A 110**

**Role of oxidized phosphatidyl choline in oxidized LDL-induced activation of proinflammatory secretory phospholipase A2 group IIA**

*Elena Samoylova, Aleksandra Korotaeva, Galina Piksina, Nina Prokazova*

Objective: We have shown recently that oxidized but not native lipoproteins stimulate activity of proinflammatory secretory phospholipase A2 group IIA (sPLA₂(IIA)). Since oxidized lipoproteins potentially contain considerable amounts of oxidized phosphatidylcholine, we examined the effect of oxidized phosphatidylcholine (oxPC) and the competitive effects of oxPC and sphingomyelin (SM) on sPLA₂(IIA) activity.

Methods: Liposomes with radiolabeled phosphatidylethanolamine were used as a substrate for determination of purified human sPLA₂(IIA) activity. OxPC, phosphatidylcholine (PC) and SM were incorporated into liposomes or in LDL.

Results: OxPC considerably stimulated sPLA₂(IIA) activity which was monitored by measurement of fatty acid released from the substrate. SM inhibited sPLA₂(IIA) activity. OxPC in a dose-dependent manner abolished the inhibitory effect of SM. On the other hand SM suppressed the activating effect of oxPC in a dose-dependent manner.

Unoxidized PC caused no changes in the enzyme activity.

Conclusion: Thus, changes in the oxPC/SM concentration ratio in LDL may affect the regulatory mechanisms of sPLA₂(IIA) activity in human blood, inducing stimulation or inhibition of the enzyme. Influence on regulation of sPLA₂(IIA) activity can be useful in the development of new therapeutic approaches to the treatment of cardiovascular and inflammatory diseases.

**Corresponding Author:** Elena Samoylova, PhD, Russian Cardiology Research and Production Center of Rosmedtechnologiy, Experimental Cardiology, 3rd Cherepovskaya, 121552 Moscow, Russia, erihter@mail.ru

**A 111**

**Crucial role of vasodilator-stimulated phosphoprotein (VASP) during hepatic ischemia–reperfusion injury**

*David Koehler, Philipp Birk, Stefanie Laucher, Valbona Mirakaj, Peter Rosenberger*

Objective: Vasodilator-stimulated phosphoprotein (VASP) is a central cytoskeleton protein affecting actin dynamics and has key importance for physiological barrier function. VASP also controls the activation of platelets through phosphorylation. The activation status of platelets is of key importance during reperfusion injury, however the role of VASP during hepatic ischemia–reperfusion (IR) injury is poorly understood. Therefore, we pursued the functional role of VASP during hepatic IR using a murine model of hepatic IR injury.

Methods: Approval from the Regierungspräsidium Tübingen was obtained. WT, VASP−/− and bone marrow chimeric mice were used in a model of hepatic IR injury. After open laparotomy the hepato-duodenal ligament was identified and the portal triad was occluded using a hanging weight system. Occlusion resulted in ischemia in the median and left lobes for 30 min followed by 3 h of reperfusion. VASP-targeted siRNA repression was used in vivo during IR and 24 h prior to IR injury. VASP phosphorylation was performed with atrial natriuretic peptide (ANP) and prostaglandin E1 (PGE1) at the beginning of reperfusion. To determine the extent of hepatic damage, serum lactate dehydrogenase (LDH), aspartate (AST) and alanine (ALT) aminotransferase were measured. Triphenyl-tetrazolium chloride (TTC) staining and histological analysis were performed to confirm the results.

Results: Hepatic IR injury was significantly reduced in VASP−/− mice (LDH: WT = 409.2 ± 27.2 vs. VASP−/− = 246.9 ± 15.81 p < 0.001; AST: WT = 845.6 ± 93.6 vs. VASP−/− = 518.9 ± 47.21 p < 0.05 and ALT: WT = 158.3 ± 11.24 vs. VASP−/− = 85.00 ± 3.88; p < 0.001). VASP targeted siRNA repression confirmed these results. The chimeric animal studies identified myeloid-derived VASP deficiency as protective against IR-induced damage compared to tissue deficient VASP (LDH: WT → VASP−/− = 433.3 ± 31.6 vs. VASP−/− → WT = 199.7 ± 23.6, p < 0.001; AST: WT → VASP−/− = 1,052.0 ± 186.6 vs. VASP−/− → WT = 342.8 ± 65.7, p < 0.01 and ALT: WT → VASP−/− = 179.5 ± 16.8 vs. VASP−/− → WT = 310 ± 5.6, p < 0.001). VASP phosphorylation with ANP as well as PGE1 was associated with significantly reduced hepatic IR injury. TTC staining and histological analysis confirmed the serum marker measurements.

Conclusion: Taken together, these studies identified hematopoietic VASP as crucial in hepatoprotection during liver IR injury.

**Corresponding Author:** David Koehler, PhD, University Hospital Tuebingen, Department of Anesthesiology and Intensive Care Medicine, Wilhelmstr, 56, 72074 Tuebingen, Germany, david.koehler@medizin.uni-tuebingen.de

**A 112**

**Effect of flow in a dynamic model of endotoxemia based on a hollow fiber bioreactor**

*Giulia Mazzu, Dagmar Schwanzer-Pfeiffer, Anna Ciechanowska, Jan Wojcicki, Dieter Falkenhagen*

Objectives: The aim of this work is to study the effect of mechanical stress on endothelial cells when stimulated with lipopolysaccharide (LPS) to model endotoxemia. Knowing the importance of physical stimuli on the endothelium, the model was build to allow the co-culture and co-stimulation of HUVEC (human umbilical vein endothelial cells) and THP-1 monocytes, recreating the physiological in vivo mechanical stimulation and 3D structure of small diameter blood vessels.

Materials and methods: Various experiments were performed to investigate the effect of flow on, firstly, THP-1 and HUVEC separately and, secondly, in co-culture. THP-1 monocytes (at a density of 1 × 10⁶ cells/ml) were suspended in M-199 including 10% human plasma and 10 ng/ml LPS or control medium. HUVEC and THP-1 were then stimulated together with LPS to model endotoxemia. Knowing the importance of physical stress on endothelial cells when stimulated with lipopolysaccharide (LPS) to model endotoxemia. Knowing the importance of physical stimuli on the endothelium, the model was build to allow the co-culture and co-stimulation of HUVEC (human umbilical vein endothelial cells) and THP-1 monocytes, recreating the physiological in vivo mechanical stimulation and 3D structure of small diameter blood vessels.

**Corresponding Author:** David Koehler, PhD, University Hospital Tuebingen, Department of Anesthesiology and Intensive Care Medicine, Wilhelmstr, 56, 72074 Tuebingen, Germany, david.koehler@medizin.uni-tuebingen.de
medium for 20 h. Surface protein expression was analyzed by flow cytometry (FACscan, BD), whereas concentrations of TNF-α, IL-1β, IL-6, IL-8 and IL-10 in the supernatant were analyzed with Lumexin System (BioRad).

**Results:** The flow cytometric analysis showed up-regulation of ICAM-1 and CD142 due to flow conditions and presence of LPS in both cell populations. Stimulation with LPS caused also up-regulation of E-selectin and down-regulation of CD141 on HUVEC. Analysis of supernatant showed increased levels of TNF-α in a small amount by flow and in a larger amount by presence of LPS and a different pattern of interleukin concentration levels in comparison to the control.

**Conclusions:** Because of the more physiological culture conditions and the proved importance of the presence of flow on the cell response, our method is a promising tool to model inflammation processes caused by Gram negative bacteria, like sepsis.

**Corresponding Author:** Giulia Mazza, MSc, Danube University

**Krems, Center for Biomedical Technology, Dr. Karl Dorrekstrasse 30, 3500 Krems, Austria, giulia.mazza@donau-uni.ac.at**

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**A 113**

**Targeting of lung endothelium by cationic liposomes in an animal model of LPS-induced ARDS**

Susanne Herber-Ionat, Stefan Gsinn, Rashmi Mittal, Andreas Schulze

**Objective:** Cationic liposomes have been shown to target angiogenic endothelial cells in tumors, lungs and joints. This study sought to determine whether acutely inflamed lung endothelium preferentially binds cationic liposomes.

**Material and methods:** Experiments were performed in 14 male SD rats. The acute lung injury was induced by intratracheal instillation of lipopolysaccharides (LPS). Controls received 0.5 ml saline. Following instillation the rats were ventilated for 5 h. Four hours after instillation each rat received fluorescent labeled (Rhodamine 6-G) cationic liposomes as a bolus i.v. injection. The liposomes were allowed to recirculate for 1 h. Thereafter, a bronchoalveolar lavage (BAL) was done and the experiment was terminated (systemic and lung perfusion with NaCl and formalin). Accumulation of liposomes was assessed by quantitative confocal microscopy (left lung) and determination of the Rhodamine 6-G content of homogenized right lung tissue.

**Results:** Determination of inflammatory parameters in the BAL showed a significant induction of the inflammatory process in the LPS-treated rats versus controls (mean (±SD): WBC: 3,444 per μl (±1,420) vs. 1,314 per μl (±906), \( p = 0.007; \) IL-1β: 145.57 vs. 51.94 pg/ml, \( p = 0.026 \) and TNF-α: 3,468 vs. 42 pg/ml, \( p = 0.001 \), respectively). Cationic liposomes exhibited a significantly, up to twofold higher accumulation in the inflamed lung tissue as compared to healthy lungs as determined by confocal microscopy (\( p < 0.001 \)). However, this was inhomogeneously distributed among the left lung segments: A significant accumulation was seen in the apical 1.73 vs. 1.90, \( p = 0.004 \), and medial section 4.01 vs. 1.86, \( p < 0.001 \) (mean Fluorescent pixels/section in %), whereas no difference between groups was seen in the caudal lung parts. Determination of Rhodamine 6-G content in the lower lobes of the right lung did not show a significant difference between the groups (Rhodamine 6-G content in g/g lung tissue: 1.94 [1.09; 2.79] vs. 1.55 [0.59; 2.51]; mean [95% CI]).

**Conclusion:** Our findings indicate that acutely inflamed lung tissue in this model of lung injury binds cationic liposomes. This preferential uptake raises the possibility of using cationic liposomes to direct diagnostic or therapeutic agents selectively to the sites of acute inflammation in the lung.

**Corresponding Author:** Susanne Herber-Ionat, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Neonatology, Marchioninistr. 15, 81377 Munich, Germany, susanne.jonat@med.uni-muenchen.de

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**A 114**

**Array analysis of macrophage tolerance in peritonitis**

William Cheadle, Andreas Lenz, Carruba Chris, Jameson Mattingly

**Introduction:** In response to severe infections such as secondary peritonitis and sepsis, there may be alterations in the immune response of circulating leukocytes. This includes macrophage tolerance which can lead to reduced inflammatory responses and levels of proinflammatory cytokines upon subsequent stimulation. In this study we used a murine model of Klebsiella pneumoniae to examine changes in gene expression and protein level of key cytokines involved in the bacterial mediated inflammatory response.

**Methods:** Infection was induced in 12 male C57BL/6 mice through intraperitoneal inoculation of 10^5 colony forming units of K. pneumoniae. Mice were given antibiotic therapy throughout the duration of the study. Peritoneal exudate cells were obtained through peritoneal lavage and RNA was isolated. Samples were obtained at 4, 24, and 48 h following infection. The control group consisted of four mice not inoculated with K. pneumoniae. Following RNA isolation, we used reverse transcription and real time PCR to examine the relative expression levels of 84 proteins indicated by the ‘mouse TLR’ superarray from SA Biosciences. Data were further analyzed using Ingenuity Pathway Analysis. The data collected from the “Mouse TLR” superarray was analyzed using the 2^-ΔΔCT (fold change) and 2^-ΔCT (fold regulation) methods.

**Results:** Of the 84 genes examined, 28 were excluded from analysis based on the presence of too little mRNA or the absence of significant fold regulation. 38 genes had significant change. These genes displayed two patterns: up regulation at 4 and 24 h and down regulation at 48 h (\( n = 35 \)), or down regulation at 4, 24, and 48 h. Such ‘tolerized’ genes included IL-1α, IL-1β, IL-6, IL-10, NFκB, and TNF. Discussion: We have shown that in response to bacterial peritonitis, several inflammatory cytokines undergo changes in gene expression and protein levels consistent with microbial tolerance.

**Corresponding Author:** William Cheadle, MD, University of Louisville, Department of Surgery, 350 S Jackson, Louisville, KY 40207, USA, wg.cheadle@louisville.edu

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**A 115**

**The role of vasodilator stimulated phosphoprotein (VASP) phosphorylation in intestinal barrier dysfunction after hemorrhagic shock in rats**

Liuyang Zhang, Ye Zhang, Jing Xu, Huaping Liang

**Objective:** To investigate the role of vasodilator stimulated phosphoprotein (VASP) phosphorylation in intestinal barrier dysfunction after hemorrhagic shock in rats and its relationship with zonula occludens-1 (ZO-1) protein expression, which is associated with intercellular tight junction of epithelium.

**Methods:** With the hemorrhagic shock rats (after bleeding, mean arterial pressure of rats was maintained at 40 mmHg for a period of time according to the experimental group), the changes of intestinal barrier function, protein expression of VASP, phospho-VASP (Ser-157) and ZO-1 in small intestine mucous membrane were determined. the effects of PKA agonist cAMP, which phosphorylates
Objective: Ischemia–reperfusion (I/R) injury is the paradoxical exacerbation of myocardial tissue damage upon reestablishment of circulation after a period of ischemia. I/R injury is an evitable consequence of heart valve surgery via sternotomy and held responsible for an often occurring Systemic Inflammatory Response Syndrome (SIRS) postoperatively. This syndrome occurs, for unknown reasons, more frequent in patients with pre-existing heart failure. Our hypothesis for this discrepancy is a different myocardial inflammatory response to I/R injury in patients with and without preexisting heart failure. In this pilot study we examined the myocardial inflammatory response in those two patient groups. The feasibility and optimal times for blood sampling were also determined.

Material and methods: Paired blood samples, of patients with preexisting heart failure (n = 5) and patients without it (n = 5) undergoing mitral valve surgery, were obtained at fixed time-points until 24 h after reperfusion. Consecutive arteriovenous concentration differences of various factors were assessed directly over the reperfused heart to reveal specificity of the locally active inflammatory processes in human I/R injury. Inflammatory pathways were investigated by making use of the Bio-Plex human cytokine 27-plex panel. Endothelial activation was established by measuring ICAM-1 and vWF.

Results: Feasibility of sampling from a coronary sinus catheter (CSC) was confirmed by measuring oxygen saturation, which was in blood from the CSC almost 10% lower than in central venous blood. There was a significant release of IL-1β (p = 0.036), IL-6 (p = 0.001) and IL-9 (p = 0.005) 24 h after reperfusion. Our study did not reveal significant differences for other measured cytokines, neither arteriovenous nor between patients with and without pre-existing heart failure.

Conclusions: Arteriovenous differences for IL-1β, IL-6 and IL-9 were found, indicating a cardiac origin of these cytokines. Indications for plasma concentration differences of cytokines between patients with and without preexisting heart failure were found, but the differences were not significant. Feasibility of sampling from a CSC and optimal times for blood sampling were confirmed. The hypothesis that a different inflammatory response to I/R injury underlies the more frequent development of SIRS in patients with pre-existing heart failure needs further investigation.

Supported by NHF grant 2007B150
Corresponding Author: K.A. Kortekaas, MSc, Leiden University Medical Center, Department of Cardiothoracic Surgery, Albinusdreef 2, 2300 RC Leiden, The Netherlands, k.a.kortekaas@lumc.nl

A 117

Biglycan as a pro-inflammatory agent in myocardial ischemia/reperfusion injury?
Jan Mersmann, Jan Larmann, Vera Sprunck, Jens W Fischer, Gregor Theilmeier, Kai Zacharowski

Objective: Reperfusion injury after myocardial ischemia evokes an inflammatory reaction which can be modified by toll-like receptor (TLR) 2 deficiency or preconditioning (Favre, ATVB 2007; Mersmann, Crit Care Med 2009, in press) resulting in acute cardioprotection. The endogenous ligand responsible for TLR2 activation during reperfusion is however still unknown. The proteoglycan biglycan (Bgn) functions as a TLR2 agonist in macrophages (Schaefer, JCI 2005). We tested whether Bgn deficiency protects from reperfusion injury in a murine model of myocardial ischemia/reperfusion (MI/R).

Material and methods: With the permission of the local authorities on animal care, male wild-type (wt) or biglycan deficient (Bgn−/−) mice (10–14 weeks, C57BL/6J, n = 7) were subjected to surgical ligation of the left anterior descending coronary artery followed by 2 h of reperfusion. Infarct size, plasma troponin T, blood chemistry, plasma cytokine levels, and connexin 43 (Cx43) expression were determined.

The ECG was recorded during the observation period by an implanted telemetry transmitter. Comparisons were made using t tests after datasets passed normality testing. p < 0.05 was considered significant.

Results: No significant difference could be detected in infarct size or plasma troponin T, nor did cytokine levels suggest that the acute inflammatory reaction was altered by the absence of Bgn. With the exception of GPT standard blood chemistry values were also not affected by Bgn deficiency. Immunofluorescence revealed a significantly lower expression of gap junction protein Cx43 in Bgn−/− mice after MI/R (2,831 ± 187 vs. 1,404 ± 226 Cx43 pos. area/HPF (μm²), p = 0.0004). This was accompanied by a significantly lower heart rate during ischemia (407 ± 11 vs. 346 ± 13 bpm, p = 0.0033), which normalized during reperfusion. Heart rate variability or turbulence was unaltered.

Conclusions: The deficiency of the putative TLR2 ligand Bgn does not result in the same cardioprotective phenotype, as was observed in TLR2−/− animals. We therefore conclude that Bgn is at least not the most prominent endogenous ligand of TLR2 during MI/R. The intervention does however affect Cx43 expression in Bgn−/− mice. The observed lower heart rates during ischemia may be the result of the latter.

Corresponding Author: Jan Mersmann, MD, Johann Wolfgang Goethe University of Frankfurt/M, Clinic for Anaesthesiology, Intensive Care Medicine and Pain Therapy, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, mersmann@med.uni-frankfurt.de

A 118

Erythrocyte-derived superoxide formation after hemorrhage/truma and reperfusion
Clara Zižko, Astrid Postl, Heinz Redl, Andrey Kozlov, Soheyl Bahrami

Objective: Hypoperfusion and reperfusion are often associated with the occurrence of oxidative stress. Considering oxygen-dependent
hemoglobin autooxidation as a mechanism of superoxide generation, the aim of the present study was to investigate superoxide formation in erythrocytes as a possible source of oxidative stress after hemorrhage/trauma and reperfusion (HR).

Material and methods: Anesthetized rats were subjected to HR followed by restrictive reperfusion or a sham operation (n = 8 in each group). For superoxide quantification, infusion of the radical scavenger 1-hydroxy-3-carboxy-2,2,5,5-tetramethyl-pyrrolidine hydrochloride (CPh) during reperfusion was combined with electron paramagnetic resonance spectroscopy analysis. Erythrocytes were isolated at the end of reperfusion. Organ function parameters alaninaminotransferase (ALT), creatinine, lactate-dehydrogenase and creatinkinase were analyzed, as were blood gas parameters pH, pCO₂, pO₂ and negative base excess.

Results: We found that when compared to sham operated animals, HR significantly increased superoxide concentration in erythrocytes (p < 0.05) but not in plasma. In animals subjected to HR, superoxide formation in plasma correlated positively with ALT (p = 0.011, rᵢ = 0.857) and negatively with base excess (p = 0.015, rᵢ = -0.833). However, scavenging of superoxide did not ameliorate any pathophysiological changes.

Conclusion: We conclude that HR causes increased formation of superoxide in erythrocytes. Despite the correlation between plasma concentration and shock severity, erythrocyte-derived superoxide may not be the cause of HR-induced organ dysfunction.

Corresponding Author: Clara Zifko, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschinger Str. 13, 1200 Vienna, Austria, clara.zifko@trauma.lbg.ac.at

A 119

Hepatoprotective effects of a liver-specific growth factor compared to erythropoietin (EPO) during inflammation and ischemia

Maren Ilowski, Barbara Donabauer, Thomas Weiss, Karl-Walter Jauch, Wolfgang Thasler

Objectives: The liver is a highly metabolic organ with a great potential of regeneration. Beside mitogenic signals via hepatotropic growth factors like augmenter of liver regeneration (ALR), liver regeneration is also triggered through metabolites and anti-apoptotic mechanisms. During inflammation and ischemic states of the liver especially growth factors but also EPO has been reported to reduce apoptosis and support regeneration. Aim of this study was to compare the hepatoprotective effect of ALR and EPO in vitro regarding metabolic models.

Material and methods: Primary human hepatocytes (phH) and hepatic cell lines were exposed to different concentrations of ethanol and (pre) co-incubated with or without rhALR. The influence on cell proliferation was analyzed by MTT assay. As an indicator of irreversible cell death, the leakage of intracellular LDH into the culture medium was measured as well as the intracellular GSH/GSSG ratio (indicator of cellular oxidative stress). The amount of induced apoptosis was evaluated by FACS analysis with propidium iodide (PI) staining. In Western Blot studies the corresponding signal pathways were analyzed.

Results: Pre-incubation of culture media with rhALR showed an increase in proliferation of HepG2 cells and primary human hepatocytes. The collected data revealed a concentration dependent increase of cell damage by ethanol as well as a protective effect of rhALR (FACS analysis, enzyme leakage). This was also confirmed by measuring cytochrome c release into the cytosol. The results of the GSH/GSSH ratio showed an improvement of the redox status of the cells. Anti-apoptotic effects of rhALR were shown by increased phosphorylation of Akt, part of an important survival pathway. EPO has been shown to attenuate post ischemic hepatocyte liver damage and have a tissue-protective effect in animal models of a wide variety of tissues. In contrast, rhALR acts liver specific allowing both its mitogenic and protective effect.

Conclusions: Unlike EPO rhALR acts liver specific and exerts not only mitogenic but also protective effects through the PI3K/AKT signaling pathway. This effect implies a possible application of ALR in reducing damage during ethanol intoxication. Further tests will be needed to verify its protective effect with other toxicants as well as during ischemia/reperfusion.

Corresponding Author: Maren Ilowski, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Surgery, Marchioninistr. 15, 81377 Munich, Germany, maren.ilowski@med.uni-muenchen.de

A 120

Nebulization of gamma-tocopherol ameliorates lung injury in an ovine model of acute lung injury

Yusuke Yamamoto, Perenlei Ekhkbaatar, Szabo Csaba, Maret Traber, David Herndon, Daniel Traber

We have previously reported that nebulization of gamma tocopherol (gT) carried by flaxseed oil into the airway of an ovine model of acute lung injury was beneficial. In the present study, we hypothesized that nebulization of gT dissolved in ethanol would be more clinically relevant and will effectively improve pulmonary function following burn/smoke injury.

Method: Adult ewes (n = 12) were subjected to 40% TBSA, third-degree flame burn and insufflated with smoke (48 breaths of cotton smoke, <40°) under deep anesthesia. One g of gT dissolved in 2.2 mL of ethanol was continuously delivered by customized aerosolization device for 48 h, starting 3 h post-injury (gT group, n = 6). Untreated animals (n = 6) were nebulized with same amount of ethanol. Following the injury, all animals were placed on ventilator and monitored for 96 h. Weaning from the ventilator was initiated if PaO₂/FiO₂ was above 250 mmHg at 48 h post-injury. This experiment was carried out as a double-blind comparative study.

Results: PaO₂/FiO₂ (mean ± SD) was 310 ± 152 in the gT and 214 ± 158 in the untreated groups at 48 h. At 96 h post-injury, all gT animals were weaned from ventilator, while one untreated animal could completely weaned. Lung bloodless wet-to-dry weight ratio (mean ± SD) was 2.87 ± 0.87 in the gT and 4.22 ± 1.48 in the untreated groups. The lung tissue gT levels (mean ± SD) were 40.5 ± 15 and 0.13 ± 0.58 mmol/g in gT and untreated groups, respectively at 96 h post-injury.

Conclusion: The nebulization of gT carried by ethanol improved pulmonary oxygenation and markedly reduced the ventilator time in burn/smoke injured sheep. Delivery of gT into the lungs via ethanol may be a safe, novel, and efficient approach for management of ALI patients who have sustained oxidative damage to the airway.

Corresponding Author: Yusuke Yamamoto, MD, PhD, The University of Texas Medical Branch, Department of Anaesthesiology, Harbor Dr., Galveston, TX 77555, USA, yuyamamo@utmb.edu

A 121

Aerosolized surfactant generated by a novel noninvasive apparatus reduced acute lung injury in rats

Yu Sun, Zhaofan Xia, Yu Wang, Hengyu Li, Jiuhua Li, Shu Han

Objectives: Exogenous surfactant has been explored as a potential therapy for acute lung injury (ALI) and acute respiratory distress
syndrome. In the present study, a nebulizer driven by oxygen lines found in hospital wards was developed to aerosolize porcine pulmonary surfactant (PPS). We hypothesized that aerosolized surfactant inhaled through spontaneous breathing may effectively reduce severe lung injury.

Material and methods: Rats were injected with oleic acid (OA) intravenously to induce ALI, and, 30 min later, divided into five groups: Model (injury only), PPS aerosol (PPS-Aer), saline aerosol (Saline-Aer), PPS instillation (PPS-Inst), and saline instillation (Saline-Inst). Blood gases, lung histology, protein and TNF-α concentration in the bronchoalveolar lavage fluid (BALF) were examined.

Results: The particle sizes of PPS aerosol were smaller than 2.0 mm as determined by a laser aerosol particle counter. Treatment of animals with aerosolized PPS significantly increased the phospholipid content in BALF, improved lung function, reduced pulmonary edema, decreased total protein and TNF-α concentration in BALF, ameliorated lung injury, and improved animal survival. These therapeutic effects are compatible with those seen in the PPS-Inst group.

Conclusions: This new method of PPS aerosolization combines the therapeutic effects of surfactant with partial oxygen inhalation under spontaneous breathing. It is effective, simple, and safe for administration of exogenous surfactant.

Corresponding Author: Yu Sun, PhD, Chinese PLA Institute of Burn Surgery, Burn Surgery, Changhai Road, 21 Shanghai, China, littlefish0916@126.com

A 122
Exogenous porcine surfactants increase the infiltration of leukocytes in lung of rats
Yu San, Zhaofan Xia, Shihui Zhu, Bing Ma, Kaiyang Lv, Hengyu Li

Objectives: Several studies have investigated the influence of exogenous surfactants on inflammatory response in the lung, however results reported about effects of surfactants on the lung infiltration of leukocytes are controversial. Our previous study noticed that treatment of porcine surfactant (PS) significantly increased the lung infiltration of leukocytes in rats with acute lung injury (ALI). The objective of this study was to verify the effect of exogenous PS on the lung infiltration of leukocytes in vivo and investigate the possible mechanisms involved in vitro.

Material and methods: The number of leukocytes in bronchoalveolar lavage fluid (BALF) of rats with or without lipopolysaccharide (LPS)-induced ALI was determined after treatment with different concentrations of PS, dexamethasone (Dex) or PS + Dex. The effect of PS and Curosurf, a commercially available porcine surfactant, on human peripheral neutrophil migration was determined by the Boyden Chamber Assay.

Results: Instillation of PS significantly increased the number of leukocytes in BALF of normal rats and rats with LPS-induced ALI. Most of the increased leukocytes were neutrophils. Dex significantly decreased the number of leukocytes and TNF-α concentration in BALF caused by LPS, but did not significantly reduce the number of leukocytes induced by PS. In vitro experiments further demonstrated that both PS and Curosurf had direct chemotactic effects on neutrophils.

Conclusions: These results suggest PS contain chemocattractant(s) which induce the infiltration of leukocytes, especially neutrophils, into lung.

Corresponding Author: Yu Sun, PhD, Chinese PLA Institute of Burn Surgery, Burn Surgery, Changhai Road, 21 Shanghai, China, littlefish0916@126.com

A 123
The base deficit during experimental hemorrhagic shock is a predictor of acute lung injury severity
Max Wohlauer, Ernest Moore, John Eun, Miguel Fragoso, Frank Wright, Anirban Banerjee

Objective: Fifteen years ago, our lab published the observation that base deficit (BD) is an early predictor of acute lung injury (ALI) in the Surgical Intensive Care Unit. The currently accepted experimental hemorrhagic shock model (controlled exsanguination to an established mean arterial pressure), is problematic however, as the depth of shock does not reliably correspond to the magnitude of acute lung injury. We hypothesized that measurement of BD at the end of hemorrhagic shock will correlate with acute lung injury severity, leading to a more consistent and reproducible depth of shock in an animal model.

Methods: Blood is removed from anesthetized rats to induce hemorrhagic shock (MAP 30 mmHg × 45 min). An arterial blood gas is taken at the end of shock to measure the base deficit. The rats are resuscitated with NS and one half of the shed blood over 2 h, observed for 1 h, and then sacrificed. Evans blue dye is injected and used as an index of lung permeability.

Results: Lung injury increases in proportion to the base deficit during hemorrhagic shock (R² = 0.8846; N = 5).

Table

<table>
<thead>
<tr>
<th>Rodent</th>
<th>Base deficit during shock</th>
<th>Evans blue dye in BAL fluid (% of plasma)</th>
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<tbody>
<tr>
<td>Rodent 1</td>
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<td>2.6</td>
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<tr>
<td>Rodent 2</td>
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<td>Rodent 6</td>
<td>29</td>
<td>6.24</td>
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Conclusion: The base deficit at the end of shock is a predictor of acute lung injury, and can be a useful adjunct to standardize the depth of hemorrhagic shock in an animal model.

Corresponding Author: Max Wohlauer, MD, University of Colorado, Department of Surgery, 2631 E. 17th Ave., C302, Aurora, CO 80045, USA, max.wohlauer@ucdenver.edu

A 124
The effect of aerosolized hypertonic saline on lung injury following hemorrhagic shock
Max Wohlauer, Ernest Moore, John Eun, Miguel Fragoso, Erik Peltz, Anirban Banerjee

Objective: Intestinal ischemia and reperfusion play a central role in acute lung injury (ALI) and subsequent multiple organ failure (MOF) following hemorrhagic shock. Intravenous hypertonic saline (HTS) modulates inflammation and can attenuate ALI/MOF, however, lung-directed HTS therapy may be less prone to systemic complications and has not yet been investigated. We hypothesized that inhaled, aerosolized hypertonic saline therapy given at the onset
of resuscitation will decrease acute lung injury following hemorrhagic shock. Methods: Rats are placed on a T-piece (FI02 0.4, 2 LPM) at the start of hemorrhagic shock (MAP 30 mmHg × 45 min to achieve a base deficit greater than 20). Hypertonic saline (7.5% NS) is delivered via jet nebulizer (MiniHeart Hi-Flo Nebulizer. Westmed, Tucson, AZ) at a flow rate of 1–2 LPM for 15 min at the start of resuscitation, and at 1 and 2 h of resuscitation, respectively. Upon infusing a lethal dose of pentobarbital, a bronchoalveolar lavage is performed to measure lung vascular permeability using Evans blue dye (3 h postshock).

Results: Hemorrhagic shock caused marked lung permeability (5.41 percent Evans blue dye in BALF ± 0.72; N = 5), which was attenuated with administration of aerosolized 7.5% hypertonic saline (2.93% Evans blue dye in BALF ± 0.37; N = 5). The benefit of aerosolized hypertonic saline, using bronchoalveolar lavage fluid (BALF) protein as an additional marker of lung injury (23.80 mg/ml ± 3.90; N = 5), attenuated ALI considerably when compared to the untreated group (37.31 mg/ml ± 5.89; N = 5). Conclusion: Hypertonic saline nebulizer administration during resuscitation attenuates acute lung injury following experimental trauma/hemorrhagic shock. A clinical investigation to validate organ-directed hypertonic therapy for acute lung injury in the trauma population is needed.

Corresponding Author: Max Wohlauer, MD, University of Colorado, Department of Surgery, 2631 E. 17th Ave., C302, Aurora, CO 80045, USA, max.wohlauer@ucdenver.edu

A 125
Heat shock protein 70 is an endogenous modulator of the innate immune response
Philipp M Lepper, Daniel T Sawyer, Frederick GJ Gamper, Martha Triantafilou, Kathy Triantafilou

Mammalian responses to bacterial products can lead to an uncontrolled inflammatory response that can be deadly for the host. It has been shown that the innate immune system employs at least three cell surface receptors, TLR4, CD14 and MD2, in order to recognise bacterial products. We have previously shown that heat shock proteins (Hsps) are also involved in the innate immune recognition. Hsps are a family of highly conserved proteins that act as molecular chaperones and assist in proper folding, assembly and intracellular trafficking of proteins. How hsps reach the cell surface and how they are involved in the innate immune response still remain unclear.

In the present study we investigated their association with the TLR4/CD14/MD2 complex in response to bacterial products and provide evidence that the hsp70 and hsp90 associate with TLR4 in response to stimulation by bacterial products. These associations seem to take place within lipid rafts. The addition of exogenous recombinant hsp70 to cells in vitro results in a dose-responsive inhibition of the inflammatory signal cascade, including NF-kB activation, phosphorylation of mitogen-activated protein kinase (MAPK) proteins, and cytokine production in response to different microbial stimuli. We are currently beginning to explore their administration in vivo in order to limit the clinical signs and symptoms of inflammatory conditions. Our studies reveal that hsps may play an important role as endogenous regulators of the innate immune response.

Corresponding Author: Philipp M Lepper, MD, University Hospital of Bern, Department of Pneumology, Freiburgstrasse BHH, 3010 Bern, Switzerland, philipp.lepper@gmx.de

A 126
The acute phase response is mediated by the magnitude and mechanism of injury
Graciela Bauza\textsuperscript{1}, Nathan A. Wigner\textsuperscript{2}, Zhongyan Wang\textsuperscript{1}, Louis C. Gerstenfeld\textsuperscript{2}, Peter A. Burke\textsuperscript{1}

\textsuperscript{1}Departments of Surgery, Division of Trauma and \textsuperscript{2}Orthopaedics, Boston University School of Medicine, Boston, MA 02118, USA

Objective: The acute phase response (APR) is a complex physiologic response to injury and involves significant changes in the transcription of APR genes. However, the effects of mechanism and magnitude on the kinetics of the APR remain to be defined.

Material and methods: A standardized unilateral (uFF) and bilateral (bFF) femur fracture as well as a 20% total body surface area full thickness burn in C57-B6 male mice were utilized to induce the APR to injury. After injury, liver tissue and serum were harvested at 0, 6, 12, 24, and 48 h. Real-time PCR and ELISA were used to quantify mRNA expression of hepatic APR genes and serum IL-6 concentrations, respectively.

Results: The expressions of fibrinogen-gamma (FGG) and serum amyloid A (SAA), two major positive acute phase proteins, showed classic APR kinetics. In the femur fracture models, FGG expression increased at 6 h and peaked at 12 h, then decreased to control levels by 48 h. bFF (more severe injury) caused a delayed and prolonged FGG expression compared to uFF. The burn injury model showed a similar FGG expression pattern but the induction was also delayed and the response prolonged before returning to base line compared to both uFF and bFF. The expression of SAA had a similar expression pattern and kinetics as that of FGG in all injury models. Serum IL-6 levels reflected the magnitude of injury. In the femur fracture models, the peak level of IL-6 at 6 h post-injury in bFF was much higher than in uFF; burn injury, however, led to a smaller increase in IL-6 level compared to both uFF and bFF injury.

Conclusions: Greater magnitude of injury results in a delayed and higher expression of APR genes in the liver and a greater induction of IL-6 in the serum. Different mechanisms of injury exhibit similar but distinct hepatic APR and serum IL-6 responses. The differences and similarities in response to injury magnitude and mechanism reflect common regulatory pathways which require to further investigation.

Corresponding Author: Peter Burke, MD, Boston University School of Medicine, Department of Surgery, One Boston Medical Center Place, Boston, MA 02118, USA, peter.burke@bmc.org

A 127
The role of IL-10 in the development of acute organ dysfunction following hemorrhagic shock
Philipp Kobbe, Philipp Lichte, Hans Christoph Pape

Objective: In hemorrhagic shock and trauma, patients are at risk to develop systemic inflammation (SIRS) and multiple organ failure (MOF), which are thought to be related to the excessive release of inflammatory mediators. This study investigates the role of the immuno-modulatory cytokine IL-10 in the process of SIRS and MOF following hemorrhagic shock.

Methods: Male C57/BL6 and IL-10 KO mice were subjected to volume controlled hemorrhagic shock for 3 h followed by resuscitation. Animals were either sacrificed 3 or 24 h after resuscitation. To assess systemic inflammation, serum IL-6, IL-10, KC, and MCP-1 concentrations were measured with the Luminex\textsuperscript{\textregistered} multiplexing platform; acute lung injury (ALI) was assessed by pulmonary myeloperoxidase (MPO) activity and lung histology and acute liver injury was assessed by hepatic MPO activity, hepatic IL-6 levels, and serum ALT levels.
A 128

Contribution of alveolar macrophages to the regulation of local and systemic mediator release after blunt chest trauma

Ulrike Niesler, Annette Palmer, Janine Froeba, Daniel H. Seitz, Florian Gebhard, Markus W. Knoeferl

Objective: Blunt chest trauma plays an important role in multiple injured patients. Previous studies in a mouse model of lung contusion showed increased mediator concentrations in bronchoalveolar lavage fluids after trauma. These mediators contribute to the invasion of inflammatory cells into the lung. However, it is not known if these mediators are released by activated alveolar macrophages or by other pulmonary cells. This study was designed to determine the effect of alveolar macrophages on the inflammatory alterations after trauma.

Methods: Alveolar macrophages of male C57/HeN mice were depleted by intratracheal application of clodronate-loaded liposomes (CL). Control animals were instilled with PBS or PBS-loaded liposomes. 72 h after instillation, blunt chest trauma (induced by a single blast wave) or corresponding sham procedure were conducted. 2 or 24 h after injury, concentrations of IL-6, KC and MCP-1 were determined in bronchoalveolar lavage fluids (BAL), lung homogenates and plasma, using a multiplex cytokine/chemokine assay.

Results: At 2 h after blunt chest trauma, IL-6 concentrations in BAL, lung homogenates and plasma of CL-treated mice were significantly increased versus sham but decreased versus traumatized, non-depleted animals. KC concentration in BAL of CL-treated mice was significantly up-regulated and did not further change after trauma. At 24 h after trauma, MCP-1 concentrations in BAL and lung homogenates of CL-treated mice were significantly higher as in corresponding sham and traumatized animals without macrophage depletion.

Conclusion: These results indicate that alveolar macrophages are a major source of IL-6 early after blunt chest trauma. Since macrophage depletion did not alter KC release after lung contusion, other lung cells appear to be the source for this chemokine and its release seems to be negatively regulated by alveolar macrophages. The increase of MCP-1 concentrations observed in macrophage-depleted animals at 24 h after lung contusion appears to be caused by local or immigrated cells in the absence of negative regulation by resident macrophages. In summary, alveolar macrophages play a key role in regulating the inflammatory response after blunt chest trauma. (Supported by DFG KFO 200, KN 475/5-1).

Corresponding Author: Ulrike Niesler, University of Ulm, Department of Trauma Surgery, Hand, Plastic and Reconstructive Surgery, Steinhoevelstr. 9, 89075 Ulm, Germany, ulrike.niesler@uniklinik-ulm.de

A 129

A new mouse model to analyse the postoperative immune dysfunction after major surgery: ready to use?

Christian Kloecker, Pia Koerner, Stephan Diedrich, Stefan Maier, Claus-Dieter Heidecke

Objective: Abdominal surgery is regularly followed by immune dysfunction. The new mouse-model of surgically induced immune dysfunction (SID) was developed to characterize the effects of major surgery on the immune system. This study was designed to characterize its effects on edema, HMGB1-concentrations in serum and lavage and corticosterone levels.

Material and methods: Female C57Bl/6 mice were related to the following groups: laparotomy, SID or untreated (control group). SID was performed by manipulating the small intestine three times by two cotton swabs. To quantify the edema of the small intestine, the wet-dry-ratio of the whole small intestine (n = 5 mice per group) was determined 6 and 24 h after surgical procedure. Levels of HMGB1 in serum (n = 7 per group) and peritoneal lavage (n = 5 per group) and of corticosterone in serum were analysed by enzyme-linked immunosorbent assays 6 h and 24 h post operation.

Results: Within 24 h after SID operation mice developed a massive edema in the small intestine which was quantified by significantly increased wet-dry-ratios in the SID (24 h p.o.) group compared to the laparotomy group (p < 0.01). The degree of edema of the untreated, laparotomy and SID (6 h p.o.) operated mice were nearly identical. HMGB1 levels were significantly increased in the serum 24 h following SID compared to the laparotomy group (p < 0.05). The difference between the laparotomy group and the SID (6 h p.o.) group was even more clearly (p < 0.01). Concentrations of HMGB1 in peritoneal lavage were also increased. Elevated corticosterone levels were only significant for the SID (6 h p.o.) group in comparison to the untreated mice (p < 0.05).

Conclusion: We found increased edema of the small intestine following SID and quantified this macroscopic observation. This correlates with augmented levels of HMGB1 and elevated amounts of corticosterone 24 h following SID. Accordingly, SID induces not only a severe trauma reaction as described by elevated HMGB1-levels but also a postoperative stress situation. Therefore, SID fulfills several qualifications to study the effects of visceral surgery on the immune function.

Corresponding Author: Christian Kloecker, Cand. med., Ernst Moritz Arndt University Greifswald, Department of Surgery, Friedrich-Loeffler-Str. 23b, 17489 Greifswald, Germany, christian.kloecker@uni-greifswald.de

A 130

Propranolol suppresses apoptosis and production of inflammatory cytokines in LPS-stressed macrophage-like THP-1 cells

Marc Jeschke, Felicia Williams, Katsuhito Kita, Rong Chu, Gabriella Kulp, David Herndon

Objectives: Propranolol (β-blocker) decreases the incidence of sepsis and improves mortality of critically ill patients. In endotoxemic, as well as in thermally injured rats, propranolol attenuates the systemic
inflammatory response by decreasing the pro-inflammatory and increasing the anti-inflammatory cascade. The aim of the present study is to determine the effects of propranolol on cell survival, cell activity, apoptosis and pro-inflammatory response in a human macrophage like cell line (THP-1 cells) stressed with lipopolysaccharide (LPS).

Material and methods: Differentiated THP-1 cells were stressed with LPS and received either saline or propranolol. Cell viability was analyzed by MTT assay, apoptosis was detected using JC-1 assay and terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining. Caspase-3 activity was also measured by the fluorescence of Z-DEVD-R110. Tumor necrosis factor (TNF) and interleukin-1β (IL1-β) were measured to determine the effect of propranolol on pro-inflammatory cytokine expression.

Results and Conclusions: Propranolol increased cell viability and significantly reduced apoptosis in LPS-stimulated THP-1 cells. Trypan blue and TUNEL staining also supported the observation. Because caspase-3 plays a major role in apoptotic signaling, we measured the enzyme activity of caspase-3 by fluorescent substrate. We confirmed that the addition of propranolol reduced caspase-3 activity induced by high dose of LPS up to approximately 30–40% in a dose dependent manner (range 10–100 µM). Propranolol also significantly decreased TNF and IL1-β in differentiated THP-1 cells. Our results indicate that propranolol exerts anti-apoptotic effects in differentiated THP-1 cells, and it may reduce the expression of pro-inflammatory cytokines and apoptosis in endotoxemic human macrophages.

Corresponding Author: Marc Jeschke, MD, PhD, University of Texas Medical Branch, Department of Biochemistry and Molecular Biology, 815 Market Street, Galveston, TX 77550, USA, majeschk@utmb.edu

A 131

Role of caspase-1 in major trauma in mice: protects from organ damage and regulates systemic inflammatory responses

Christoph Menzel, Qian Sun, Patricia Loughran, Hans-Christoph Pape, Timothy Billiar, Melanie Scott

Objective: Severe injuries are associated with high morbidity and mortality due to posttraumatic, generalized inflammatory responses and development of multiple organ dysfunction. Activation of the inflammasome during trauma activates caspase-1, which cleaves and releases proinflammatory cytokines IL-1β and IL-18, which have been implicated in detrimental posttraumatic inflammatory responses. We investigated the role of caspase-1 in a model of major trauma in mice.

Methods: Wild type (WT) and caspase-1−/− mice were subjected to HS/BFF (1.5 h hemorrhagic shock + 4.5 h fluid resuscitation, plus bilateral femur fracture) or sham procedure (femoral artery cannulation only) or control (no manipulation) (n = 3–6). Plasma cytokine and AST/ALT level, as well as pulmonary myeloperoxidase (MPO) were measured. LY6G positive neutrophils and TUNEL (apoptosis) staining was assessed by immunohistochemistry in 4 µm liver sections. Whole cell liver lysates were immunoblotted for autophagy markers LC3 and Beclin-1. Statistical analysis by Student t test: p < 0.05 significant. Results presented as mean ± SEM.

Results: In HS/BFF, caspase-1−/− mice had significantly increased IL-6 (522 ± 136 vs. 161 ± 19 pg/mL) and IL-12 levels (407 ± 60 vs. 231 ± 47 pg/mL) when compared to WT. IL-18 was detectable at low levels in control WT mice (13.4 ± 10.9 pg/mL) and levels significantly increased after HS/BFF (39.7 ± 5.1 pg/mL). As expected, IL-18 was nondetectable in caspase-1−/− mice even at baseline (control). Caspase-1−/− mice had significantly increased ALT (4,705 ± 1,105 vs. 887 ± 197 IU/L) and AST levels (2,356 ± 472 vs. 867 ± 352 IU/L) compared with WT after HS/BFF, and also significantly more apoptotic hepatocytes (2.02 ± 0.37 vs. 1.08 ± 0.13%) and more accumulated neutrophils (1.05 ± 0.12 vs. 1.90 ± 0.43%) in liver compared with WT. Autophagy markers LC3 and Beclin-1 were also significantly upregulated in caspase-1−/− mice vs. WT after HS/BFF. Lung MPO was not different.

Conclusions: Mice without caspase-1 showed evidence of increased liver damage and organ dysfunction as well as increases systemic inflammatory responses in our severe trauma model compared with WT controls. Our data suggest that upregulation of both apoptosis and autophagy may contribute to organ dysfunction and that activation of caspase-1 is protective. These findings may have implications for future treatment of trauma patients.

Corresponding Author: Christoph Menzel, University of Pittsburgh Medical Center / Charitee Berlin, Department of Surgery/Trauma Surgery, Woerther Str. 4, 10435 Berlin, Germany, menzlec@upmc.edu

A 132

Changes of apoptotic signaling pathways in pancreatic cancer patients after enteral immunonutrition

Robert Slotwinski, Waldemar Olszewski, Sylwia Kedziora, Maciej Slodkowski, Anna Wluka, Marzanna Zaleska

Objective: Extensive tissue trauma and malnutrition results in disorders of programmed cell death influencing the patients susceptibility to infections. The purpose of our study was to assess the effect of pancreatic cancer surgery and immunonutrition on the apoptotic signaling pathways.

Material and methods: The randomized studies were performed in 88 patients after pancreatic cancer resection with preoperative standard or enteral immunonutrition. Lymphocytes expressions of Bcl-2, Bax, caspase-3, 6, 9, NFκB, PARP1/89 kDa, TNFR1/CD120a and CD95/Fas were assessed by Western-blot and flow cytometry.

Results: Before and after surgery the expression of Bcl-2, Bax, NFKB, PARP1 was significantly lower and expression of caspases, TNFR1 as well as percentage of CD95 cells significantly higher as compared with control group. Caspase 3 expression was significantly higher as compared with NFκB, PARP1 and TNFR1. In comparison to the standard nutrition preoperative immunonutrition increased Bcl-2 and NFκB expressions and decreased caspases and PARP1 expressions. In addition, we found a significant down-regulation of Bcl-2 expression after surgery, but insignificant in patients with preoperative immunonutrition.

Conclusion: Preoperative enteral immunonutrition has an modulative effect on apoptotic signaling pathways after pancreas resection and possesses antiapoptotic properties. This modulatory effect of glutamine and omega-3 fatty acids has no influence on patients outcome.

Corresponding Author: Robert Slotwinski, Prof. MD, PhD, Medical Research Center, Polish Academy of Sciences, Department of Surgical Research and Transplantology, 5 Pawinskiego Str., 02-106 Warsaw, Poland, robert_slotwinski@yahoo.com
A 133
Preserved detection of IL-17 and reduced detection of IFN-gamma in lymphocytes tCD4 in septic patients compared to healthy volunteers
Milena Brunialti1, Santos M1, Machado F2, Otelo Rigato1,3, Reinaldo Salomao1
1Infectious Diseases Discipline, Universidade Federal de Sao Paulo, 2Discipline of Anesthesiology, Sao Paulo Hospital, Universidade Federal de Sao Paulo, 3Intensive Care Unit, Sírio Libanes Hospital

Objective: Inflammatory response is modulated during sepsis and up and down regulation of cellular activity is observed, depending on the cells and functions evaluated. IFN-g and IL-17, cytokines characteristics of Th1 and Th17 lymphocyte subpopulations, play an important role in immune response, linking adaptive and innate immunity. The aims of this study were to evaluate the presence of these cells in septic patients compared to healthy volunteers and their association with prognosis.

Patients and methods: Thirty three patients with sepsis and eighteen healthy volunteers were included. The mean ages were 66.5 ± 15.4 and 60.3 ± 19.0 years, respectively. Twenty eight days mortality was 33%. The peripheral blood mononuclear cells were frozen and stored in liquid nitrogen. After thawed cell suspension was adjusted to 1 x 10⁶ cells/mL and stimulated with PMA/ionomycin at 37°C and 5% CO2 for 30 min. Brefeldin A was added to inhibit protein secretion and samples were incubated for further 15 h. An aliquot without stimulus was kept as negative control. Cells surface were stained to identify the population of lymphocytes CD3+CD8+ (TCD8) and CD3+CD4– (TCD4). The samples were permeabilized, intracellular stained to detected IL-17A and IFN-g and acquired in the FACSCanto flow cytometry. Data analysis was performed in the FlowJo software.

Results: Basal production of IFN-g and IL-17A was increased in TCD4 cells of septic patients when compared with healthy volunteers (P = 0.05 and P = 0.001, respectively). The percentage of TCD4 cells producing IFN-g after PMA/ionomycin stimulus was increased in the group of healthy volunteers when compared with patients (P = 0.038). IL-17A production did not differ between groups (P = 0.078). The proportion of TCD4+ producing IL-17 and IFN-g after PMA/ ionomycin did not differ between survivors and non-survivors (P = 0.661 and P = 0.117, respectively). Conclusion: The basal results with greater cytokines production in patients and HV, mean of percentage 28.14 ± 21.11 and 0.65 ± 1.21, respectively. Patients and methods: Thirty three patients with sepsis and twenty healthy volunteers (HV) were included. The means ages were 67.5 ± 17.3 and 59.20 ± 18.44 in patients group and healthy volunteers, respectively. Twenty eight days mortality was 36.4%. The peripheral blood mononuclear cells were frozen and stored in liquid nitrogen. After thawed cell viability was checked with trypan blue solution in Neubauer chamber. An aliquot of 4 x 10⁵ cells was stained on their surface with CD14, CD163 and CD206 and acquired in FACSCanto flow cytometry and FlowJo software, respectively. Twenty thousand events were acquired in CD14+ gate. Results: Septic patients presented increased percentage of monocytes expressing CD206 and CD163 compared to HV (P < 0.001 in both cases). The mean of percentage of CD206 positive monocytes in patients and HV were 3.82 ± 3.75 and 0.56 ± 0.72, respectively. In the same way, percentage of CD163 positive monocytes from septic group showed a significant increase compared with HV, mean of percentage 28.14 ± 21.11 and 0.65 ± 1.21, respectively.

A 134
Increased proportion of alternatively activated macrophages in septic patients compared to healthy volunteers
Milena Brunialti1, Maria Fernandes1, Eliezer Silva1, Otelo Rigato1, Reinaldo Salomao1
1Infectious Disease Discipline, Federal University of Sao Paulo, 2Discipline of Anesthesiology, Sao Paulo Hospital, 3Intensive Care Unit, Sírio Libanes Hospital

Objective: Macrophages, monocytes and polymorphonuclear cells from innate immune system are the first defense against infection. These cells have a central role in the development of inflammatory response in sepsis syndrome. Nowadays it is clear that monocytes/macrophages response is modulated during sepsis. As example, monocytes from septic patients can produce small amount of pro-inflammatory cytokines after LPS challenged in vitro, while in the same conditions the production of anti-inflammatory cytokines are preserved. A phenotype of alternatively activated macrophages, expressing CD206 or CD163, has been characterized as macrophages producing anti-inflammatory cytokines. The aim of this study was investigated the percentage of alternatively activated macrophages in the sepsis syndrome compared to healthy volunteers.

Patients and methods: Thirty three patients with sepsis and twenty healthy volunteers were included. The mean ages were 66.5 ± 15.4 and 60.3 ± 19.0 years, respectively. Twenty eight days mortality was 33%. The peripheral blood mononuclear cells were frozen and stored in liquid nitrogen. After thawed cell viability was checked with trypan blue solution in Neubauer chamber. An aliquot of 4 x 10⁵ cells was stained on their surface with CD14, CD163 and CD206 and resuspended in fixation buffer (PBS, 0.1% parformaldehyde). The data were acquired and analyzed in FACSCanto flow cytometry and FlowJo software, respectively. Twenty thousand events were acquired in CD14+ gate. Results: Septic patients presented increased percentage of monocytes expressing CD206 and CD163 compared to HV (P < 0.001 in both cases). The mean of percentage of CD206 positive monocytes in patients and HV were 3.82 ± 3.75 and 0.56 ± 0.72, respectively. In the same way, percentage of CD163 positive monocytes from septic group showed a significant increase compared with HV, mean of percentage 28.14 ± 21.11 and 0.65 ± 1.21, respectively.

Conclusion: There was a significant increase of alternatively activated macrophages in septic patients. These results showed that modulation of monocytes/macrophages function can be investigated by CD206 and CD163 surface expression during the sepsis syndrome, and not only by anti-inflammatory and pro-inflammatory cytokine detection. Financial support: Fapesp grant 2008/07511-2 and 2006/58744-1.

Corresponding Author: Milena Brunialti, PhD, Universidade Federal de Sao Paulo, Department of Medicine/Infectious Disease, Rua Pedro de Toledo, 781 15th floor, 4039032 Sao Paulo, Brasil, milena.brunialti@unifesp.br

A 135
In vitro effect of evodiamine on dendritic cells in inducing allogeneic T cell responses
Huaping Liang, Xia Fan, Qiang Wei, Xue Yang, Xi Wang

Objective: Dendritic cells (DCs) are professional antigen-presenting cells in the initiation and regulation of immune responses. Evodiamine, the major alkaloidal component isolated from Chinese herbal drug named Wu–Chu–Yu, exhibits various biological effects including anti-inflammatory activity. In this study, we detected the in vitro effect of evodiamine on murine bone marrow-derived dendritic cells in inducing allogeneic T cell responses.

Methods: Immature dendritic cells (iDC) derived from bone marrow of BALB/c mice were induced by GM-CSF for ten days in culture and received evodiamine. LPS, evodiamine + LPS treatment for another 24 h. then their capacity in inducing allogeneic T cell responses were determined by mixed lymphocyte reaction (MLR), expression of MHC class II, CD40, CD80, and CD86 on DC surface were measured using flow cytometry, and the cytokines in DC supernatants were
detected by antibody array (RayBio® Mouse Cytokine Antibody Array C Series 1000) and ELISA kits. Results: Compared with LPS induced mature DC (mDC), evodiamine can significantly enhance the ability of mDC to stimulate allogeneic T cells proliferation, while the expression of MHC class II, CD40, CD80, and CD86 on DC surface showed no statistical alterations between these two groups. Evodiamine can increase the secretion of Eotaxin-2, VEGF, DPPIV/CD26, IGF-I, IL-17B, R, MDC and Pro-MMP-9 in the absence of LPS, and elevate the levels of Eotaxin-2 and IL-13 in the presence of LPS stimulation. Conclusions: In vitro exposure to evodiamine, mDC may display enhanced capability in inducing allogeneic T cell responses. Its relationship with heightened levels of Eotaxin-2 and IL-13 need further investigation. This study was supported by the project of state key laboratory of trauma, burns, and combined injury (No. SKLZZ200802). Corresponding Author: Huaping Liang, Prof. MD, PhD, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Department 1, Changjiang Zhi Road 10&&65292; Daping, 400042 Chongqing, China, huaping_liang@yahoo.com.cn

A 136

Epstein barr virus (EBV) reactivation detected in blood and cultured dendritic cells of critically ill patients with septic/hemorrhagic shock
Maximilian Nass, Maximilian L. Nass, Moritz L. Huber, Xuefang Ren, Manfred E. Weiss, E. Marion Schneider

Objective: Herpes virus reactivation may be associated with trauma induced inflammation or severe sepsis in critically ill patients. The study addressed patients with hemorrhagic or septic shock. Using a sensitive multiplex PCR, viral replication and/or the determination of EBV latency states type I, II, or III were studied in defined leukocyte subpopulations. Patients and methods: DNA derived from plasma samples, cultured dendritic cells and early stages of spontaneously transformed B cells were studied for the presence of EBV-specific genes. The material was prepared from randomly selected samples of postoperative/posttraumatic patients with septic or hemorrhagic shock. EBV-specific genes EBNA1, 2, 3, BZLF1, BMRF1, LMP1 and the housekeeping gene ALAS1 were tested by a multiplex low-volume PCR followed by poly-acrylamide gel electrophoresis. Results: Out of 44 patients recruited over a 6-month period, 18 were in the septic shock, and 26 in the hemorrhagic shock group. Dendritic cell cultures were successfully established from 10 patients’ blood cell isolates (4 patients with septic and 6 with hemorrhagic shock). Upon prolonged cell culture, 11 of the septic and 26 of the hemorrhagic shock group patients showed spontaneous B-cell transformation. These B-cell isolates displayed a complete set of EBV-specific genes, whereas cultured dendritic cells either lacked any of the EBV-specific genes or were positive for EBNA2, or LMP1. In one case, we detected the EBV-replication gene BZLF1. Results obtained with plasma samples by multiplex PCR identified latency type II frequently, in a single isolate we found BMLF1. Conclusion: Using a newly established multiplex PCR adapted to low amount of DNA, we detected EBV specific genes in cultured dendritic cells of patients with septic as well as hemorrhagic shock. The dendritic cells contained either latency type I or latency type II genes, and in a single isolate we detected BMLF1. The spontaneously transformed B-cells were positive for the complete spectrum of genes indicating productive EBV infection. The results indicate that both, septic shock as well as hemorrhagic shock are conditions of severely impaired T- or NK cell immunity.

### Table 1  Patients’ samples studied for EBV specific genes by multiplex PCR

<table>
<thead>
<tr>
<th>DNA source</th>
<th>EBV latency I</th>
<th>EBV latency II</th>
<th>EBV latency III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cultured dendritic cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Corresponding Author: Maximilian Nass, University Hospital Ulm, Department of Anaesthesiology, Steinhoefelstr. 9, 89075 Ulm, Germany, maximilian.nass@uni-ulm.de

A 137

Effect of rapamycin on capacity of splenic dendritic cells from traumatized mice in inducing T cell responses
Huaping Liang

Objective: To study the ex vivo effect of rapamycin on capacity of splenic dendritic cells (DC) from traumatized mice in inducing T cell responses. Methods: DC were isolated from spleen 24 h after hemorrhage combined with closed femur fractures, then their autophagic activity, expression of MHC class II, CD40, CD80, and CD86 on DC surface, IL-12 and IL-10 levels in lipopolysaccharide (LPS)-stimulated DC supernatants and DC-induced mixed lymphocyte reaction (MLR) were measured, ex vivo effect of rapamycin on these parameters were observed. Results: Ex vivo administration of rapamycin could significantly reverse the suppression of autophagic activity of DC and DC-induced MLR after trauma, elevate the expression of MHC class II and IL-12 secretion, decrease IL-10 secretion, while unaltering the inhibited expression of CD40. Conclusions: Rapamycin can partially ameliorate ex vivo DC functions in traumatized mice, and further enhance the capacity of DC in inducing T cell responses. This study was supported by the national natural science foundation of China (No. 30772253) and project of state key laboratory of trauma, burns, and combined injury (No. SKLZZ200802). Corresponding Author: Huaping Liang, Prof. MD, PhD, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Department 1, Changjiang Zhi Road 10&&65292; Daping, 400042 Chongqing, China, huaping_liang@yahoo.com.cn

A 138

Circulating dendritic cells differ significantly in apoptosis compared to in vitro generated dendritic cells
Ramin Ebrahimi, Dirk Henrich, Kerstin Wilhelm, Marcus Maier, Ingo Marzi

Objectives: Prior studies showed functional and cellular differences between in vitro generated dendritic cells (DC) and ex vivo in vitro cultivated DC. In the present study the regulation of apoptosis after
LPS stimulation of ex vivo in vitro cultivated DC obtained from severely injured patients and healthy volunteers was compared with the apoptosis-regular of in vitro generated DC.

Patients and methods: On day 1 (d1) and day 4 (d4) after admission 25 ml blood was withdrawn from 10 severely traumatized patients (ISS 27 ± 7.8) and compared with blood samples obtained from 10 healthy volunteers. Ex vivo in vitro DC: PBMC were incubated for 6 h in presence/absence of LPS prior to the assessment of apoptosis in MDC and PDC by means of flowcytometry and annexin-V-PE staining.

Conclusion: Apoptosis of in vitro generated DC were not necessarily portable to in vivo DC. This study suggests that results obtained with in vitro generated DC significantly from the apoptotic cell decline observed in in vitro generated PDC.

The BCL2/BAX ratio was significantly reduced compared to circulating PDC but increased for PDC of healthy volunteers. Ex vivo in vitro DC: PBMC were incubated for 7 days and 4 days of incubation in a maturation preparation (Healthy serum, polytrauma serum, LPS, TNF-α). Apoptosis was determined as described above. Gene expression of BCL2 and BAX was assessed by realtime RT-PCR. For statistical evaluation the Kruskal–Wallis test was used (p < 0.05). The study was confirmed by the local ethics committee.

Results: Apoptosis of MDC from severe trauma patients (86% ± 28; d4) was significantly increased in comparison to MDC from healthy volunteers (32% ± 6). Incubation with LPS resulted in a significant decline of apoptotic MDC in patients (29% ± 5; d4). The BCL2/BAX ratio was significantly increased after severe trauma on day 1 (0.92 ± 0.26) and day 4 (0.88 ± 0.25) compared to healthy volunteers (0.27 ± 0.07).

In vitro generated DC showed a contrariwise increase of apoptosis in presence of LPS (68% ± 9) compared to control MDC (34% ± 2) independently from the maturation stimulus. The BCL2/BAX ratio was significantly reduced compared to circulating DC of healthy volunteers. For PDC a comparable tendency was shown. LPS reduced the apoptosis of circulating PDC but increased the apoptosis of in vitro generated PDC.

Conclusion: Apoptosis of ex vivo in vitro cultivated DC differed significantly from the apoptotic cell decline observed in in vitro generated DC. This study suggests that results obtained with in vitro generated DC were not necessarily portable to in vivo DC.

Corresponding Author: Ramin Ebrahim, Johann Wolfgang Goethe University of Frankfurt/M, Department of Trauma-, Hand- and Reconstructive Surgery, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, rebrahim@stud.uni-frankfurt.de

A 139

Differentiation of enzymatic from platelet hypercoagulability using the derived thrombelastography parameter delta
Max Wohlauer, Ernest Moore, Eduardo Gonzalez, Jeffry Kashuk

Objective: Thrombelastography (TEG) allows for rapid global assessment of coagulation function. Our previous work demonstrated that a hypercoagulable state identified by TEG’s $G$ value was associated with thromboembolic events in a cohort of critically ill surgical patients despite routine chemoprophylaxis. We hypothesized that a hypercoagulability state could be differentiated into enzymatic or platelet etiology through the use of thrombin velocity generation curves, specifically through time to maximum rate of thrombus generation (TMRTG) and the derived TEG parameter, Delta ($\Delta$).

Patients and methods: We retrospectively studied ten critically ill surgical patients receiving thromboprophylaxis for at least 72 h, by TEG using kaolin activated citrated samples. Heparinase and unmodified samples were run simultaneously. Thrombin generation velocity curves were plotted for each patient, and $\Delta$ was calculated as the difference between the TEG $R$ value (reaction time) and SP (split point). $\Delta$ represents the time to maximum rate of thrombus generation (TMRTG), reflecting the enzymatic contribution to clot formation.

The TEG parameter $G$ represents both enzymatic and platelet contribution to total clot strength. A hypercoagulable state was defined as $\Delta \leq 0.6$ min and/or $G \leq 11$ dyn/cm².

Results: Six patients received standard thromboprophylaxis doses with low molecular weight heparin (LMWH) and four with unfractionated heparin (UH). A hypercoagulable state was identified in unmodified samples via delta in 5 patients (60%); all of whom remained hypercoagulable following heparinase addition, suggesting chemoprophylaxis was ineffective. A mean TMRTG (+SEM) of 6.9 (+1.2) min was noted in all patients hypercoagulable via delta, versus 9.3 (+1.3) min in patients with a normal delta (Table). Of six patients with an elevated $G$, half had a normal delta suggesting the presence of platelet hypercoagulability. Hypercoagulable patients by delta had an increased mean (SEM) ICU stay and ventilator days when compared to patients with a normal delta, 32.8 (+7.7) and 25 (+6.5) versus 11 (+3.7) and 6.5 (+3.4), respectively (Table).

Table

<table>
<thead>
<tr>
<th>LMWH</th>
<th>Low molecular weight heparin</th>
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<tr>
<td>UH</td>
<td>Unfractionated heparin</td>
</tr>
<tr>
<td>R</td>
<td>Reaction time (3–8 min.)</td>
</tr>
<tr>
<td>MA</td>
<td>Maximum amplitude (51–69 mm)</td>
</tr>
<tr>
<td>G</td>
<td>Net clot strength (4.6–10.9 D/cm²)</td>
</tr>
<tr>
<td>TMRTG</td>
<td>Time to maximum rate of thrombus generation (6–12 min)</td>
</tr>
<tr>
<td>n</td>
<td>Number</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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</table>

Conclusion: Delta reflects the changes in thrombin generation as measured by TMRTG. Differentiation of enzymatic from platelet hypercoagulability can be accomplished by correlation of $\Delta$ to clot strength $= G$. Future studies will be required to validate these findings.

Corresponding Author: Max Wohlauer, MD. University of Colorado, Department of Surgery, 2631 E. 17th Ave., C502, Aurora, CO 80045, USA, max.wohlauer@ucdenver.edu

A 140

Effect of TachoSil® in a coagulopathic pig model with blunt liver injuries
Oliver Grotte, Till Braunschweig, Nora Daheim, Rolf Roxsaint, Rene Tolba

Introduction: The management of severely injured patients with life threatening bleeding requires a timely and targeted management to protect the trauma victim from exsanguination and secondary systematic complications. Thus we investigated the efficacy of a fibrinogen/thrombin-coated collagen patch (TachoSil®; Nycomed, Roskilde, Denmark) to terminate severe bleeding in a coagulopathic pig model with blunt liver injury.

Animals and methods: All experimental procedures were approved by the governmental animal care and use office, Germany and adhere to the German legislation governing animal studies. Severe coagulopathy was induced by exchanging 80% of the animals blood volume with HE 130/0.4 and lactated Ringer’s solution in 14 anaesthetized pigs (32.9 ± 2.1 kg BW). A grade III liver injury was induced with a force of 238 ± 19 Newton with a custom made instrument and free bleeding was allowed for 30 s. Animals were randomly assigned to receive either no patch (placebo, $n = 7$) or a TachoSil® patch ($n = 7$), which was positioned 30 s after injury on the area inflicted.
Coagulation parameters including thromboelastometry (TEG), hemodynamic variables and post treatment blood loss were monitored for 2 h post injury. The independent sample t test was used for intra-group comparison. TEG parameters were analyzed using the Wilcoxon test for unpaired data and for matched pairs. Data are expressed as mean ± SD.

Results: Coagulation after haemodilution was severely impaired as shown by significant increases of prothrombin time (20 ± 2 s), and alteration of TEG variables (CFT: 211 ± 23 s, MCF: 39 ± 1 mm; angle: 54 ± 4°) (P < 0.05). Without affecting the underlying coagulopathy total blood loss was significantly diminished in the TachoSil® group (419 ± 90 ml) as compared to the placebo group (1,775 ± 358 ml) (P < 0.005). All animals treated with TachoSil® survived, whereas 100% of the control group died before reaching the end of the observation period (P < 0.001). Histology showed an equal degree of injury with a tight adherence of the patch.

Conclusion: Despite severe coagulopathy the application of TachoSil® as first line treatment might be an effective therapeutic approach to terminate serious bleeding as damage control.

A 141
Higher concentrations of fibrinogen are not associated with a further reduction in blood loss in coagulopathic pig model with blunt liver injury
Oliver Grottke1, Till Braunschweig2, Rene Tolba*, Rolf Rossaint3
1Department of Anaesthesiology, 2Institute for Laboratory Animal Science, 3Department of Pathology

Introduction: Studies have shown that the early application of fibrinogen reverses haemodilution (HD)-induced coagulopathy. As no studies have investigated the impact on increasing concentrations of fibrinogen, we elaborated the impact of two concentrations of fibrinogen in a porcine model with HD-induced coagulopathy.

Animals and methods: Experiments were approved by the local governmental animal care and use office and adhere to the German legislation governing animal studies. HD was induced in 18 anaesthetized pigs (32 ± 1.6 kg) by exchanging 80% of their blood volume with HES 130/0.4 and lactated Ringer’s solution (1:1.2–1.5). After HD anesthesia (TEG) parameters were analyzed using Kruskal–Wallis tests post hoc tests were used for multiple comparisons. Thromboelastography (TEG) parameters were analyzed using Kruskal–Wallis tests (mean ± SD).

Results: TEG values after HD were severely impaired (Table 1) and dose-dependently restored after fibrinogen substitution. BL between the F70 (1,317 ± 113 ml) and F200 (1,155 ± 232 ml) group were comparable, but significantly diminished as compared to the control group (1,803 ± 248 ml) (P < 0.05). Animals from the control group died before the end of the observation period (59 ± 12 min), which was significantly (P < 0.05) shorter compared to the fibrinogen groups (F70: 67% survival; F200: 100% survival). Conclusion: In this specific animal model both fibrinogen dosages restore coagulation in vivo with an overall reduction in BL and no signs of adverse events, but the higher dosage of fibrinogen was not associated with a further reduction in BL.

<table>
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<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>FI (mg/dl)</td>
</tr>
<tr>
<td>Placebo 313 ± 29 59 ± 4</td>
</tr>
<tr>
<td>F70 291 ± 53 56 ± 6</td>
</tr>
<tr>
<td>F200 301 ± 20 50 ± 7</td>
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<tr>
<td>CFT (s)</td>
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<tr>
<td>Placebo 53 ± 6 220 ± 28</td>
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<td>F70 56 ± 5 235 ± 29</td>
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<td>F200 55 ± 3 212 ± 16</td>
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<td>MCF (mm)</td>
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<tr>
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</tr>
<tr>
<td>F70 67 ± 5 36 ± 4</td>
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<td>F200 70 ± 1 39 ± 2</td>
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* P < 0.05 versus placebo
† P < 0.05 versus F70

A 142
Medical need and timing of extracorporeal immune therapy in critically ill patients
Sascha Flohé, T. Loegter, J. Windolf

Severe deregulation of immune responses is discussed to be largely responsible for organ failure and insufficient infectious control. The immune system in critically ill patients may be disarranged in different ways depending on the causative event and the time course of the disease. A therapy or correction of the immune response in critically ill patients therefore cannot be standardized but has to be individualized taking the patient’s immune status into account. Therefore, the prerequisite for any proposed extracorporeal immune therapy is the monitoring of the patient’s immune response. This presentation will give an overview of applied tools to monitor the immune response in critically ill patients. This part will include markers that have already been introduced in routine patient care as well as novel markers currently under evaluation in preclinical studies. On the bases of this immune monitoring the potential approaches of an extracorporeal immune therapy will be discussed in an immune status dependent manner. Existing devices and theoretical therapeutic approaches will be discussed on the bases of the described disorders of the immune system under special consideration of the clinical applicability. Potential harmful side effects such as foreign surface activation as well as primarily non immunological disorders such as hypothermia that can be corrected by extracorporeal circuits will be discussed on the bases of the described disorders of the immune system.
A 143
Neutrophil apoptosis resistance and SIRS after major trauma: targeting the apoptosis signalling cascade by extracorporeal immune therapy
Adnana Paunel-Goerguelue, J. Windolf

Objective: SIRS and associated organ failure in critically ill patients are largely driven by hyperactivated apoptosis resistant neutrophils. We therefore examined whether agonistic targeting of neutrophil Fas by a biologic-device combination product may sufficiently induce apoptosis and limit neutrophil effector functions.

Patients and Methods: Blood samples from severely injured patients (ISS > 16) were collected from the day of admission until day 10 after trauma. The neutrophil apoptosis status, the inflammatory status of the patients and the ex vivo neutrophil effector functions were determined. Neutrophils were challenged ex vivo with immobilized agonistic anti-Fas IgM antibodies on open porous polyurethane foams to determine the immunomodulating potential of a biofunctionalized medical device.

Results: Hyperactivated neutrophils after trauma express high levels of the anti-apoptotic factors Mcl-1 and A-1 associated with apoptosis resistance. In conjunction, inflammatory markers such as MPO and PMNE as well as IL-8-mediated chemotaxis were significantly increased. Ex vivo cross-linking of neutrophil Fas by immobilized anti-Fas resulted in caspase-dependent apoptosis induction, immediate attenuation of chemotaxis, oxidative burst, and phagocytosis.

Conclusion: Targeting of neutrophil Fas by immobilized agonistic effector molecules efficiently overcomes apoptosis resistance and may limit neutrophil hyperactivation in critically ill trauma patients. Biologic-device combination products targeting neutrophil Fas might be an effective option in extracorporeal immune therapy.

Corresponding Author: Adnana Paunel-Goerguelue, PhD, Heinrich Heine University, Department of Traumatology and Hand Surgery, Universitaetsstr. 2, 40225 Dusseldorf, Germany, Adnana.Paunel-Goerguelue@med.uni-duesseldorf.de

A 144
Biofunctional surface engineering: enabling end sterilizable biologic-device combination products by a stabilizing postcoating technology
Jens Altrichter, Martin Scholz

Drug eluting stents have shown the enormous economic and medical potential of combination products by creating a five billion dollar market within 5 years. Even broader indications are anticipated from combination products consisting of a device and a biopharmaceutical protein like antibodies or growth factors.

One major drawback for the production of these biologic-device combinations products is the lack of safe terminal sterilization technologies, because proteins denature during irradiation or gas sterilization with ethylene oxide. Therefore, these products have to be produced under aseptic conditions, resulting in a sterility assurance level (SAL) of only 10E-3.

In order to overcome this obstacle, we developed a Stabilizing Postcoating Technology for the three-dimensional stabilization of proteins. This protective coating preserves the biological function of biologic-device combination products during sterilization and subsequent storage, resulting in reduced production costs and higher product safety (SAL 10E-6).

The Stabilizing Postcoating has been shown to protect even very large proteins of up to one million Dalton during beta or gamma irradiation with >25 kGy and even ethylene oxide.

One example that recently was evaluated in a prospective randomized controlled clinical trial is the Leukocyte Inhibition Module (LIM).

Objective: The leukocyte inhibition module (LIM) is a biofunctional medical device based on immobilized agonistic anti-Fas antibodies. LIM readily induces inactivation and apoptosis (programmed cell death) of activated neutrophils and can be easily integrated into the circuits of the heart–lung machine. The LIMFRA trial was done to confirm the findings from earlier studies showing the safety and efficacy of LIM in reducing inflammation in cardiac surgery.

Methods: LIMFRA was designed as a prospective randomized controlled single center trial with 100 elective on-pump cardiac surgery patients who received at least three bypass grafts. LIM (Leukocare AG, Munich) was introduced into the venous line of the heart–lung machine. In the control group (n = 50), the sham modules did not contain antibodies. Patients were followed up until 1 month after surgery. Main end points were safety and attenuation of inflammation, measured as leukocyte counts and plasma concentrations of neutrophil elastase.

Results: Each 50 CABG patients were operated with CPB containing either the LIM or the sham device. Mean CPB time was 95 ± 4 min, median was 85. No LIM-related SAEs were observed in this study. No antibody specific immune reactions against the murine anti-Fas antibody were found 1 month after surgery. As shown in earlier studies, intra- and postoperative inflammatory parameters were significantly reduced in the LIM group versus control group like the increases in neutrophil counts, neutrophil elastase, myeloperoxidase and lipid peroxidation (p < 0.05). When stratified for CPB time (90 min) the reduction in inflammation was even more pronounced in the stratification group with longer CPB time.

Conclusion: LIM proved to be a safe and effective biofunctional medical device to limit perioperative neutrophil-mediated inflammation. In contrast to previous approaches with leukocyte filters, LIM biologically prevents overshooting neutrophil effector functions by targeting the neutrophil Fas receptor without systemic application of drugs.

Corresponding Author: Ulf Abdel-Rahman, MD, Johann Wolfgang Goethe University of Frankfurt/M, Department of Thoracic and Cardiovascular Surgery, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, abdel-rahman@em.uni-frankfurt.de
A 146
The extracorporeal immune support system (EISS) for the treatment of sepsis
Martin Sauer

Objective: Neutrophil granulocytes are the first defense line in bacterial infections. However, granulocytes are also responsible for severe local tissue impairment. Recently, we published both in-vitro as well as animal data using an extracorporeal plasma treatment with a granulocyte bioreactor (1). Here we present a first in man study investigating whether this technology is safe in patients with septic shock. A further intention was to find suitable efficacy end points for subsequent controlled trials.

Design: Prospective uncontrolled clinical phase I/II study with 28-day follow up at three University hospital intensive care units.

Patients: Ten consecutive patients with septic shock. Mean ICU entrance scores were an APACHE II of 30 and a SAPS II of 66.

Interventions: All patients were treated twice within 72 h for 6 h with an extracorporeal bioreactor containing about 1.5 x 10E10 granulocytes from healthy donors. On average, 10 l separated plasma were treated by the therapeutic donor cells. Patients were followed up for 28 days.

Measurements and main results: Tolerance and technical safety during treatment, single organ functions pre/post treatment, and hospital survival were monitored. The extracorporeal treatments were well tolerated. During the treatments, the bacterial endotoxin concentration showed significant reduction. Furthermore, noradrenaline dosage could be significantly reduced while mean arterial pressure was stable. Also, c-reactive protein, procalcitonin, and HLA-DR showed significant improvement.

Conclusion: The extracorporeal treatment with donor granulocytes appeared to be safe and showed promising efficacy results, encouraging further studies.


Corresponding Author: Martin Sauer, MD, University of Rostock, Medical Faculty, Schillingallee 70, 18055 Rostock, Germany, martin.sauer@uni-rostock.de

A 147
Adjuvant sepsis therapy in renal failure
Kai Harenksi

Objective: Treatment of sepsis in patients with acute kidney injury remains crucial and mortality rates are reported to go up to 60% and higher. It has been hypothesized that the removal of inflammatory mediators from the blood might contribute to decrease mortality rates. Traditional therapies besides the chronic renal replacement therapy (CRRT) include drugs and surgical options. New, adjuvant treatments have emerged using either cytokine elimination or endotoxin adsorption to improve clinical outcome and chances of critical ill patients.

Patients and methods: For cytokine elimination a high cut-off membrane (SepteX) with an increased pore size was designed that allows hemofiltration of proteins up to 45 kDa and therefore is capable to reduce plasma cytokine levels. In a prospectively randomized trial 30 patients were included and the impact of SepteX on cytokine elimination, catecholamine consumption and clinical outcome (SAPS score) was investigated. Oxiris, a heparin coated membrane, adsorbs endotoxins and in a clinical trial including 25 patients the need for systemic anticoagulation was examined.

Results: The use of a high cut-off membrane (SepteX) contributes to significantly decrease plasma cytokine levels, norepinephrine dosage and to improve clinical outcome by reducing the SAPS II score compared to the use of a conventional highflux filter. The single use of Oxiris showed no differences regarding filter life time and no adverse events compared to the combination of Oxiris and systemic anticoagulation.

Conclusion: New filters contribute by cytokine elimination or endotoxin adsorption to improve clinical outcome of septic patients in acute kidney failure. Further collection of clinical data is mandatory to clearly identify those patients that would benefit the most of this adjuvant therapy.

Corresponding Author: Kai Harenksi, MD, Gambro Hospal GmbH, Produktmanager Intensive Care & MARS, Danziger Str. 23, 82194 Munich/Groebenzell, Germany, Kai.Harenksi@gambro.com

A 148
Extracorporeal immunotherapeutic procedures in critically ill patients
Hiroyuki Hirasawa, H. Hirasawa, S. Oda, E. Watanabe, M. Nakamura

It has been widely accepted that both pro- and anti-inflammatory hypercytokinemia plays a pivotal role in the pathophysiology of severe sepsis and septic shock. However, there is no description on the importance of hypercytokinemia in the pathophysiology of severe sepsis and septic shock, and on the efficacy of some countermeasures against hypercytokinemia in the treatment of severe sepsis and septic shock in the world-widely accepted “Surviving Sepsis Campaign Guidelines (SSCG)”.

We routinely check interleukin-6 (IL-6) blood level with CLEIA method as a biomarker of hypercytokinemia on every ICU patient every morning and found that IL-6 blood level significantly correlated with the severity of sepsis-related pathophysiology and mortality. Furthermore we also found that the successful application of some therapeutic approaches to severe sepsis and septic shock recommended in SSCG depended on the severity of hypercytokinemia. Among the patients with severe sepsis and septic shock whose initial IL-6 blood level on ICU admission was higher than 10,000 pg/mL, it was very difficult to accomplish some therapies recommended in SSCG, such as early-goal directed therapy and tight glycemic control, compared among the patients with severe sepsis and septic shock whose initial IL-6 blood level was lower than 10,000 pg/mL.

On the other hand, we have reported that continuous hemodiﬀusion with cytokine-adsorbing polymethyl-methacrylate membrane hemofilter (PMMA-CHDF) could effectively and continuously remove both pro- and anti-inflammatory cytokines from blood stream of critically ill patients. Therefore, we applied PMMA-CHDF on the patients with severe sepsis and septic shock regardless of the renal function. Following the application of PMMA-CHDF, the blood level of pro- and anti-inflammatory cytokines decreased significantly. And among the patients with septic shock, blood pressure recovered and blood lactate level normalized with PMMA-CHDF, indicating the improvement in tissue oxygen metabolism. Furthermore, immunop- analysis among those patients diagnosed with the ratio of HLA-DR expressing monocyte number to the total monocyte number, was significantly improved with the removal of anti-inflammatory cytokine, interleukin-10 (IL-10), with PMMA-CHDF.

Survival of the
patients with severe sepsis and septic shock treated with PMMA-CHDF addition to the recommendations in SSGC is significantly better than reported survival of the patients with severe sepsis and septic shock treated according to SSGC. Those results warrant the randomized clinical trial on the efficacy of PMMA-CHDF as cytokine and immune modulator in the treatment of the patients with severe sepsis and septic shock addition to SSGC.

Corresponding Author: Hiroyuki Hirasawa, Prof. Emeritus, MD, PhD, Chiba University Graduate School of Medicine, Department of Emergency and Critical Care Medicine, 1-8-1 Inohana, Chuo, 260-8677 Chiba City, Japan, hhirasawa@faculty.chiba-u.jp

A 149
Niche space regulates bacterial infection-induced hematopoietic stem and progenitor cell expansion in the absence of toll like receptor signaling
Kindra Kelly-Scumpia, Philip Scumpia, Matthew Delano, Alex Cuenca, Jason Weinstein, Lyle Moldawer

Objective: Injury, inflammation, and infection activate hematopoietic stem and progenitor cells (HSPCs) to increase the production of functional myeloid cells. The toll-like receptor (TLR) adaptor protein MyD88 is required for LPS-induced HSPC activation in vitro and vaccinia virus-induced HSPC activation in vivo. Here, we studied the mechanism of HSPC activation during in vivo bacterial infection.

Materials and methods: Eight to 12 week old female mice either received a single intraperitoneal injection of LPS, Staphylococcus aureus via retro-orbital injection, or underwent cecal ligation and puncture (CLP). The expansion of HSPC in the bone marrow was examined by flow cytometry using several cell surface molecules. C-kit interactions in the BM were disrupted by anti-c-kit antibody, ACK2. C-kit signaling was blocked by treating mice with imatinib mesylate. Ly6G high neutrophils were depleted using anti-Gr1 antibody. The role of TLR and type I interferon in the different infection models were investigated using MyD88−/− and MyD88−/−TRIF−/− double knockout mice. BM, blood and spleens were harvested at various time points for analysis.

Results: Our results demonstrate that despite endotoxin utilizing TLR4 signaling through either MyD88 or TRIF for in vivo HSPC expansion, MyD88 and TRIF signaling are dispensable for both CLP and S. aureus infection-induced HSPC expansion. Importantly, bacterial infection of wild type or MyD88−/−TRIF−/− mice is associated with increased BM niche space resulting from neutrophil release into the periphery. Additionally, depletion of BM Ly6G high neutrophils with anti-Gr1 treatment alone creates sufficient BM space to induce HSPC expansion. Moreover, detachment of primary HSCs from the BM niche, using ACK2 antibody, prevents infection-induced HSPC expansion leading to the failure of emergency myelopoiesis, persistently neutropenia, and the inability to clear secondary infections.

Conclusions: We find that creation of niche space by depletion of BM neutrophil stores is sufficient to induce HSPC expansion. These data show that HSC-niche interactions, and not MyD88 or TRIF pathways, regulate emergency hematopoietic responses to bacterial infection. This study also suggests that targeting hematopoietic failure, in particular LT-HSC failure, during severe infections may be a novel adjunct therapy to prevent nosocomial infections.

Corresponding Author: Kindra Kelly-Scumpia, PhD, University of Florida, Department of Surgery, 1600 SW Archer Road PO Box 100019, Gainesville, FL 32609, USA, kindra@ufl.edu

A 150
Bacterial superantigens enhance the pro-inflamatory cytokine release of the TLR2 agonist BLP in vitro and potentiate BLP associated lethality in vivo
David Kearney, Jinchuang Wang, Henry Paul Redmond

Objectives: Bacterial superantigens are gram-positive exotoxins that induce pro-inflammatory cytokine release in vitro, cause lethal shock in vivo, and can be detected in critically ill patients. They are one of the most powerful T-cell mitogens known, activating up to 20% of the T-cell repertoire. They are also known to act independently of T-cells, potentiating the effects of the TLR4 agonist, endotoxin. The objectives of this research were: (a) to investigate if superantigens could enhance the pro-inflammatory cytokine release of the TLR2 agonist bacterial lipoprotein (BLP) in human monocytes and peripheral blood mononuclear cells (PBMCs); (b) to examine the mechanisms behind this synergy, and (c) to examine whether superantigens enhance BLP lethality in vivo.

Materials and methods: Human monocytes and PBMCs were isolated from healthy volunteers. Cells were sequentially stimulated Staphylococcal enterotoxin A or B as well as BLP or endotoxin. Cytokine measurements were performed using ELISA. Cell surface expression of TLR2 and TLR4 on monocytes was assessed by FACSscan analysis. To determine the pathways involved in the superantigen priming process, inhibitors to NF-kB, P38 and ERK were used. Phosphorylated IkB-α and p38 were determined by Western blotting. Male C57BL/6 mice were used for the lethality studies.

Results: Priming of human monocytes or PBMCs with superantigens significantly enhanced the release of pro-inflammatory cytokines in response to either BLP or endotoxin (p < 0.001). The optimal duration for superantigen priming was 6 h. Superantigens significantly up-regulated the expression of TLR2 and TLR4 on monocytes. This priming effect could be completely blocked using inhibitors to p38 during the priming phase as opposed to NF-kB or ERK inhibition. This was confirmed with higher expression of phosphorylated p38 after superantigen priming and BLP or endotoxin stimulation. Superantigens (10 µg/mouse) enhanced the lethality of a sub-lethal dose of BLP (600 µg/mouse) in vivo (10/10 mortality) compared to mice without superantigen priming (0/10). Mice given superantigen alone did not demonstrate any signs of illness.

Conclusions: We have shown a novel method of bacterial superantigens’ action, through potentiating the effects of BLP stimulation on monocytes and PBMCs in vitro and enhancing the lethality of BLP in vivo. This synergy may help to explain the massive pro-inflammatory cytokine release seen in superantigen mediated septic shock.

Corresponding Author: David Kearney, MD, Cork University Hospital, Academic Surgery, Wilton, Cork, Ireland, dkearnage@gmail.com

A 151
Low dose LPS in vivo induces CD11b expression but not TLR4 expression on monocytes
Philipp Lichte, Reiner Oberbeck, Jan Grigolet, Manfred Schedlowski, Hans-Christoph Pape, Philipp Kobbe

Introduction: Recognition and induced cellular activation are mandatory for the control of bacterial infection. Details of this inflammatory response are poorly understood in septic patients. Lipopolysaccharide (LPS) induced activation of the immune system is a recognized model for septic inflammation.

Methods: We established a double blinded, randomized, placebo controlled cross over study. Eighteen healthy male test persons got an
intravenous injection of 0.4 ng/kg LPS (E. coli-Lipopolysaccharide/LPS/United States Pharmacoepia). In control conditions they got an equal dose of water. Between these two conditions had been at least 1 week and at maximum 3 weeks. Blood samples and vital parameters were taken before and 1, 1.75, 3, 4 and 6 h after injection. The blood samples had been analyzed by extensive FACS phenotyping and FACS detection of TLR4 and CD11b. Concentrations of IL-1, IL-1ra, IL-6, IL-10, TNF-α and IFN-γ were measured by an ELISA related beads technology.

Results: After injection of 0.4 ng/kg LPS vital parameters showed typical changes: heart rate and body temperature significantly increased between hour 2 and 4, blood leukocyte concentration significantly decreased whereas neutrophils significantly increased. Levels of IL-6 (p < 0.001), IL 10 (p < 0.001) and TNF-α (p < 0.01) increased within the first hours. The level of IL-1 showed no changes in opposite to IL-1ra which increased (p < 0.001). There was no difference in the expression of TLR-4 on monocytes between the placebo and the LPS group; however LPS injection induced a significant increase of CD11b expression (p < 0.01).

Conclusion: The results show that injection of low dose LPS (0.4 ng/kg) cause a typical and reproducible inflammatory response. Human studies showed different changes in TLR-4 expression. In patients with major trauma a downregulation of TLR-4 has been described. On the contrary even enhanced or preserved TLR-4 expression on monocytes has been shown in septic patients. In our study there were no changes in TLR-4 expression after low dose LPS injection despite of a typical inflammatory reaction. Obvious an increased expression of TLR-4 is not necessarily a part of the inflammatory response. The measured increase of CD11b expression is well matched with results in septic patients. The known enhanced LPS response and in acute septic patients might be independent from an upregulated TLR-4 expression but associated with an increased CD11b expression.

Corresponding Author: Philipp Lichte, MD, University of Aachen, Department for Orthopedic Surgery, Pauwelsstr. 30, 52074 Aachen, Germany, philipp.lichte@googlemail.com

A 152
Adenoviral gene transfer of the pattern recognition receptor nucleotide oligomerization domain 2 (NOD2) protects against toll like receptor-4 (TLR4) mediated signaling in a model of necrotizing enterocolitis (NEC)
Ward Richardson, Matthew Neal, Amin Afrazi, Richard Sigger, Chhinder Sodhi, David Hackam

Objective: Necrotizing enterocolitis (NEC) is the leading cause of death and disability from gastrointestinal disease in preterm infants, yet whose mechanism is incompletely understood. We have previously demonstrated that the receptor Toll like receptor 4 (TLR4), the receptor for lipopolysaccharide, plays a critical role in the development of NEC (J. Immunology 2008). Recent studies have identified a novel arm of the innate immune system consisting of the protein nucleotide oligomerization domain 2 (NOD2). Mutations in enterocyte NOD2 lead to exaggerated intestinal inflammation in vivo, suggesting a protective role for enterocyte NOD2 activation. We now hypothesize that NOD2 activation will attenuate TLR4 signaling in enterocytes leading to a reduction in the extent of intestinal inflammation during NEC.

Methods: Activation of TLR4 and NOD2 was accomplished in IEC-6 enterocytes using LPS (10–50 μg/ml) and muramyl di-peptide (MDP, 1–20 μg/ml) respectively. Experimental NEC was induced using 4 days of enteric feeds and intermittent hypoxia in mice. In addition mice were administered a dominant negative TLR4, wild-type TLR4, dominant negative NOD2, wild type NOD2, or gfp adenovirus. TLR4 and NOD2 signaling in vivo and in vitro were determined by qRT-PCR of iNOS expression in the gut mucosa. Apoptosis was assessed in tissue as well as cultured enterocytes via immunochemistry staining for the apoptotic marker cleaved caspase-3. Results: NOD2 activation with MDP reduced TLR4 signaling in IEC-6 enterocytes, as determined by reduced translocation of NFκB and reduced release iNOS. Strikingly the adenoviral gene transfer of the dominant negative NOD2 protein (containing a frame-shift mutation causing inactivation of the protein) caused a failure to induce LPS gene expression of key apoptotic suppressors showing a critical link between these two receptors in vivo. Furthermore, the induction of NEC required TLR4 activation, as NEC severity was reduced in mice administered the dominant negative TLR4 virus compared with littermates administered gfp and wild type TLR4 viruses. The physiological relevance of this was shown as the administration of the NOD2 adenovirus exacerbated NEC and caused a marked increase in iNOS.

Conclusion: NOD2 activation limits TLR4-mediated signaling leading to a reduction in LPS-induced intestinal inflammation. These findings suggest a potential therapeutic role for the NOD2 activation in the management of intestinal inflammation.

Corresponding Author: Ward Richardson, MD, Children’s Hospital of the University of Pittsburgh, Department of Pediatric Surgery, 4401 Penn Ave, Pittsburgh, PA 15224, USA, richardsonwm@upmc.edu

A 153
Cytoplasmic RNA sensors mediate synthesis of complement factor B after stimulation of macrophages with polynosine-polyctydlyic acid
David Kazcowski, Melanie Scott, So Kim, Joon Kwak, Rebecca Edmonds, Timothy Billiar

Objective: Local production of complement is critical to the host response in settings such as infection and ischemia/reperfusion. We have shown that pattern recognition receptor ligands, including the dsRNA analog polynosine-polycytidylic acid (poly I:C), stimulate synthesis of complement factor B, a key component of the alternative pathway. dsRNA is detected at the cellular level by either Toll-like receptor 3 (TLR3) or cytoplasmic RNA sensors, such as RIG-like helicases. The purpose of this study is to determine whether TLR3 or cytoplasmic RNA sensors mediate synthesis of factor B after stimulation of macrophages with poly I:C.

Material and methods: RAW264.7 macrophages were cultured at 2 × 10⁶ cells/well. Peritoneal macrophages were elicited from wild type (C57BL/6) and TLR3 knock-out (KO) mice using thioglycollate, and plated at 1 × 10⁶ cells/well. Cells were then stimulated with poly I:C. RNA was harvested 6 h after stimulation and used for RT-PCR specific for factor B. Protein from cell lysates or cell supernatant was analyzed for factor B via western blot. β-actin was used as a normalizing control.

Results: Stimulation of wild-type peritoneal macrophages with poly I:C (10 μg/ml) resulted in robust upregulation of factor B mRNA (12.2-fold, p < 0.05). Stimulation of TLR3KO macrophages with poly I:C (10 μg/ml) also resulted in upregulation of factor B mRNA (14.0-fold, p < 0.05), suggesting that TLR3 is not required for synthesis of factor B after stimulation with poly I:C. To test whether cytoplasmic RNA sensors mediate synthesis of factor B after stimulation with poly I:C, lipofectamine was used to facilitate entry of poly I:C into cells. Stimulation of RAW264.7 cells with low concentrations (0.1 μg/ml) of poly I:C alone led to mild upregulation of factor B (2.7-fold). Significantly greater (75.2-fold) upregulation was observed when poly I:C (0.1 μg/ml) was complexed with lipofectamine (p < 0.05). At higher concentrations of poly I:C (10 μg/ml) significantly more upregulation of factor B was also observed when

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poly I:C was complexed with lipofectamine (117-fold) compared to poly I:C alone (22.8-fold, \( p < 0.05 \)).

Conclusions: These results suggest that cytoplasmic dsRNA sensors, rather than TLR3, mediate synthesis of complement factor B after stimulation of macrophages with poly I:C. Understanding the mechanisms of local complement synthesis may lead to strategies that prevent tissue damage in diverse settings including sepsis, trauma, and ischemia/reperfusion.

Corresponding Author: David Kaczorowski, MD, University of Pittsburgh, Department of Surgery, 200 Lothrop Street, Pittsburgh, PA 15213, USA, kaczorowski@upmc.edu

A 154

Host lipoproteins and bacterial products synergistically enhance human vascular endothelial cells: possible mechanism leading to atherosclerotic plaque formation

Philipp M. Lepper, Frederick G.J. Gamper, Martha Triantafilou, Evtlambia Harakopakis, George Hajishengallis, Kathy Triantafilou

Inflammation and innate immune reactions are implicated in atherogenesis and plaque disruption. Toll-like receptors have been implicated as the initiators of the inflammatory response, but the precise triggers are not fully understood. Triggers, which have been suggested, include hypercholesterolemia, modified lipoproteins as well as infections with bacterial pathogens such as Chlamydia pneumonia or Porphyromonas gingivalis.

In this study we set out to fully characterise the ligand that triggers TLRs and the subsequent inflammatory response leading to atherogenesis. Using human vascular endothelial cells, our results suggest that host lipoproteins alone are not able to trigger a significant inflammatory response, whereas combinations of LDL or mmLDL and bacterial products seem to act synergistically in order to stimulate a substantial inflammatory response. Activation seems to require lipid raft function and formation of heterotypic receptor complexes comprising of TLR2, TLR6, and CD36 in the case of LDL, mmLDL, oxLDL or combinations of these with LTA, whereas in the case of P. gingivalis or Chlamydia LPS or combinations of these with lipoproteins require the formation of receptor complexes comprising of TLR1, TLR2, TLR6 and CD36. Surprisingly, oxLDL or combinations of oxLDL with bacterial products did not trigger a significant inflammatory response. Existence of LDL or mmLDL prior to the addition of bacterial products seems to augment the inflammatory response, suggesting that existence of hyperlipidemia followed by a subsequent bacterial infection could trigger an inflammatory response that leads to atherosclerotic plaque formation.

This is the first report to implicate a synergy of host and bacterial products in the inflammatory mechanisms of atherosclerosis.

Corresponding Author: Philipp M Lepper, MD, University Hospital of Bern, Department of Pneumology, Freiburgstrasse BHH, 3010 Bern, Switzerland, philipp.lepper@gmx.de

A 156

TLR4 inhibition improves intestinal microcirculation in experimental endotoxemia

Katrin Zimmermann, Dragan Pavlovic, Juan Zhou, Brent Johnston, Christian Lehmann

Objectives: Toll-like receptor 4 (TLR4) represents an important mediator of endotoxin-related signal transduction. Aim of our study was to evaluate whether TLR4 inhibition after onset of experimental endotoxemia is able to improve the intestinal microcirculation, which is crucial in the pathogenesis of septic multiple organ failure.

Materials and methods: We studied four groups of animals (Lewis rats, \( n = 10 \) per group): healthy controls (CON group), endotoxemic animals (15 mg/kg lipopolysaccharide, LPS group), endotoxemic animals treated with TLR4 antagonist (1 mg/kg CRX, LPS + CRX group), and CRX treated controls (CRX group). Intravital microscopy of the intestinal microcirculation was performed following 2 h of observation in all animals. Blood samples were taken for cytokine measurements at the end of the experiments.

Results: Following 2 h of endotoxemia we observed a significant increase of leukocyte adhesion in the intestinal submucosal venules and a reduced capillary perfusion of the muscular and mucosal layers

Objective: Pattern-recognition receptors (PRRs) collectively recognize molecular structures of invading microorganisms, followed by initiation of immune responses. PRRs comprise the toll-like receptor (TLR) family, including TLR4, which is essential for responses to bacterial lipopolysaccharide (LPS). As part of the adaptive immune system, Fc receptors (FcR) recognize antigen–antibody complexes and link antibody-mediated immune responses to cellular effector functions. In the present study, a linkage between TLR4 and FcγRIII (CD16) was evaluated in vitro and in vivo.

Methods: Neutrophils and macrophages from Wt and TLR4 mutant (mut) mice were incubated in vitro with LPS and IgG immune complexes (IgGC). Supernatants were analyzed for cytokine release by ELISA. Receptor interaction and activation of intracellular signaling pathways were evaluated by co-immunoprecipitation analyses.

In addition, two models of acute lung injury (ALI) were employed in Wt and TLR4mut or TLR4+/- mice, respectively. ALI was induced by intratracheal administration of either LPS or IgGC. To rule out a putative contamination with endotoxin, the LPS concentration of reagents was determined using Limulus lysate assay.

Results: In vitro activation of phagocytes by IgGC resulted in an association of TLR4 with FcγRII. Inversely, LPS incubation did not result in an association of both receptors. Neutrophils and macrophages from TLR4mut mice were unresponsive to either LPS or IgGC in vitro whereas the ability to produce cytokines in response to non-TLR4 agonists (zymosan, pam3cys) was intact. This phenomenon was accompanied by the inability of TLR4mut cells to phosphorylate tyrosine residues of the FcγRIII subunit. To transfer these findings to vivo, two different models of ALI were employed. As expected, LPS-induced ALI was abolished in TLR4mut and TLR4+/- mice. Unexpectedly, TLR4mut and TLR4+/- mice were also resistant to ALI following IgGC deposition. Finally, LPS was not detectable in any of the reagents. Furthermore, all experiments could be reproduced by using polymyxin-treated reagents.

Conclusion: In summary, TLR4 is involved in FcγRII signaling, and heterodimerization of TLR4 and FcγRII occurs in the presence of IgGC. Consequently, dysfunctional TLR4 signaling results in unresponsiveness of immune cells to both LPS and IgGC. These findings suggest that TLR4 and FcγRII pathways are structurally and functionally connected and that TLR4 is indispensable for FcγRII signaling.

Corresponding Author: Daniel Rittirsch, MD, University Hospital Zurich, Department of Trauma Surgery, Raemistr. 100, 8091 Zurich, Switzerland, drittirsch@googlemail.com

A 155

Cross-talk between TLR4 and Fcγ-receptor III (CD16)

Daniel Rittirsch, Michael Flierl, Markus Haber-Lang, Hans-Peter Simmen, Guido Wanner, Peter Ward

Objective: Pattern-recognition receptors (PRRs) collectively recognize molecular structures of invading microorganisms, followed by
of the intestinal wall. TLR4 inhibition reduced leukocyte activation and improved capillary perfusion significantly. Cytokine release was not affected.

Conclusions: Administration of the TLR4 antagonist improved intestinal microcirculation in a post-treatment model of experimental endotoxemia. The TLR4 pathway may be a target in clinical Gram-negative sepsis.

Corresponding Author: Christian Lehmann, Prof. MD, PhD, Ernst Moritz Arndt University Greifswald, Department of Anesthesia, Friedrich-Loeffer-Str. 23b, 17475 Greifswald, Germany, christian.lehmann@uni-greifswald.de

A 157
Toll-like receptor 7 (TLR-7) is a candidate receptor for the anti-inflammatory properties of Hsp72
Elyse Ireland, John Williams

Heat shock protein 72 (Hsp72) found within the extra-cellular milieu is termed a “danger signal” to the immune system as it is able to bind cell surface receptors leading to the up-regulation of pro- and anti-inflammatory cytokines. Several studies have shown that Hsp72 is able to bind many cell surface receptors, such as CD14, CD36, TLR-2, TLR-4 and TLR-7. TLR-7 is known to be stimulated by single-stranded RNA (ssRNA) from viruses and the synthetic agonist Imiquimod. TLR-7 is found in membranes of endosomes but recent studies have demonstrated its presence on the cell surface. The objective of this study was to determine which cell surface receptors could be bound by extra-cellular Hsp72, and determine the immune responses of macrophages in relation to the secretion of the cytokines TNF-a and IL-10. Hsp72 was pre-incubated with various concentrations of peptides derived from known receptor protein sequences for 1 h before being applied to U937 derived macrophages for 4 h, and supernatants assayed for the presence of TNF-a and IL-10. Pre-incubation of Hsp72 with various receptor peptides resulted in differential patterns of secretion of both TNF-a and IL-10. For example, a peptide derived from CD36 led to a dose-dependent abrogation of both cytokines, and a significant reduction in migration of macrophages. More interestingly, the use of two different TLR-7 peptides led to a significant dose dependent reduction in IL-10 secretion only. Migration of macrophages was not significantly reduced when compared to treatment with Hsp72 only.

In conclusion, the data presented here demonstrates that the anti-inflammatory properties of Hsp72 is potentially achieved through TLR-7 and that migration of macrophages is likely dependent upon TNF-a, which is indicative of tissue damage. Further work is required to elucidate whether Hsp72 has the potential to be used therapeutically in the treatment of inflammatory disorders.

Corresponding Author: Elyse Ireland, PhD, University of Chester, Biological Sciences, Parkgate Road, Chester CH1 4BJ, UK, eireland@chester.ac.uk

A 158
The toll-like receptor 4 mediates acute kidney injury in endotoxemic sheep
Johan Fenhammar, Mats Rundgren, Sigridur Kalman, Jakob Forestier, Stefan Eriksson, Robert Frithiof

Objective: This study was conducted to investigate the role of the toll-like receptor 4 (TLR4) in mediating acute kidney injury (AKI) in endotoxemic sheep. Sepsis is a common cause of AKI, and mortality among these patients is high. TLR4 signaling has been suggested to play a key role in the development of septic AKI and recently available, selective TLR4 antagonists have made it possible to investigate the importance of this receptor in a large animal model.

Material and methods: A prospective, randomized, placebo-controlled experimental study with fourteen conscious sheep was performed. Sheep were chronically prepared with exteriorized carotid arteries prior to the experiments. Urine was collected via a bladder catheter and aliquots taken for analysis every 2 h. Blood was sampled every 6 h. A pulmonary artery catheter and a catheter in the carotid artery were used for acquiring hemodynamic data. Sheep were randomized to receive a bolus dose (2 mg/kg) followed by a continuous infusion (4 mg/kg/24 h) of either the selective TLR4 antagonist, TAK-242 (n = 7) or vehicle (n = 7). E. coli lipopolysaccharide (LPS) infusion (50 ng/kg/min) together with 0.5 ml/kg/h Ringer’s acetate were started after the initial bolus dose was given and continued for 24 h. All sheep had free access to food and water during the entire experiment.

Results: LPS infusion resulted in a state of hyperdynamic circulation with increasing cardiac output, hypotension and tachycardia. Pulmonary hypertension developed rapidly and became progressively more prominent. Urine output decreased throughout the experiment and increasing arterial lactate levels were seen. TLR4 blockade with TAK-242 significantly attenuated the decrease in urine output compared to animals receiving vehicle. Furthermore, systemic circulation in the TAK-242 group was less affected by the LPS, with significantly higher arterial blood pressure, less tachycardia and lower pulmonary artery pressure. Arterial lactate was significantly lower in the TAK-242 group compared to placebo-treated animals.

Conclusion: These results indicate a critical role for the TLR4 pathway in impairing renal function during ovine endotoxemia. Further studies are needed to clarify the mechanism of TLR4 mediated septic AKI.

Corresponding Author: Johan Fenhammar, MD, Karolinska Institute, Karolinska University Hospital, CLINTEC, Department of Anesthesia and Intensive Care, Årstavägen 131, 12058 Ärsta, Sweden, john.fenhammar@karolinska.se

A 159
TLR9-mediated immunosuppression of B lymphocytes in late posttraumatic mice
Xiangcai Ruan, Sophie Darwiche, Melanie Scott, Changchun Cai, Timothy Billiar

Objective: Although T cell, Dendritic cell, and macrophage are dramatically altered following severe injury, it remains unclear whether trauma also effect B lymphocytes. Because Toll-like receptor (TLR) 9 has been implicated to contribute to the inflammatory response in trauma, we want to determine the role of TLR9-mediated the possible impairment of B lymphocytes following trauma in our novel pseudofracture (PF) model, which recapitulates the systemic and end organ responses observed following bilateral femur fracture, and allows for a late posttraumatic survive.

Methods: Male TLR9 knock-out (KO) and C57BL/6 wild-type (WT) mice (8- to 12-week) were randomly assigned to sham operation or PF. PF was induced by crushed bone solution injection and soft tissue injury to the thigh musculature bilaterally. Forty-eight hours later, blood were collected for IL-6 and IL-10 assay, and splenocytes were isolated and stimulated with LPS for B lymphocytes proliferation and IL-6 and IL-10 secretion. ANOVA analysis was performed.

Results: Stimulated B lymphocytes proliferation was decreased with 45.0% by the PF in WT mice (p < 0.05). However, PF in TLR9 KO mice did not affect the results. Levels of IL-6 and IL-10 significantly suppressed by PF in supernatant of WT B lymphocytes proliferation (99.4 and 88.6% respectively, p < 0.001), but not in that of TLR9 KO...
B lymphocytes, although they showed no significant fluctuations in serum of both WT and TLR9 KO mice at 48 h after PF.

Conclusions: Our results provide conclusive evidence that trauma contribute to a delay immunosuppression of B lymphocytes. TLR9 KO mice does not show the suppression indicating TLR9 signaling plays a key role in immnosuppression of B lymphocytes in late posttraumatic model. These data support further studies using inhibitory oligonucleotides.

Corresponding Author: Xiangcai Ruan, PhD, University of Pittsburgh, Department of Surgery, 3459 Fifth Avenue, Pittsburgh, PA 15213, USA, ruanx@upmc.edu

A 160
ST2 negatively regulates TLR2 signalling, but is not involved in BLP-induced tolerance
Liu Jinghua, Jing Hua Liu, Juliette M Buckley, H. Paul Redmond, Jiang Huai Wang

Objective: Activation of TLR signalling is critical for host innate immunity against bacterial infection. The orphan receptor ST2 functions as a negative regulator of TLR4 signalling and maintains LPS tolerance. We demonstrated that tolerance induced by BLP (bacterial lipoprotein), a TLR2 agonist, protects against microbial sepsis-related lethality. However, it is unclear whether ST2 negatively regulates TLR2 signalling, and furthermore whether BLP-induced tolerance is dependent on ST2.

Methods: Bone marrow-derived macrophages (BMM) isolated from wild-type and ST2-deficient mice were pretreated with culture medium (naive) or 100 ng/ml BLP (BLP-tolerised) for 24 h and stimulated with 1,000 ng/ml BLP for various time periods. HEK293-hTLR2 cells, stably expressing TLR2, were co-transfected with pNF-kB-Luc reporter vector and plasmid encoding ST2, and stimulated with BLP. Proinflammatory cytokines and NF-kB activation were assessed by ELISA and luciferase activity assay. TLR2, MyD88, IRAK1 expression and MyD88-IRAK complex formation were detected by Western blot analysis and immunoprecipitation.

Results: Upon BLP stimulation, significantly increased TNF-a release (p < 0.01) and enhanced MyD88-IRAK complex formation were observed in ST2-deficient BMM compared to wild-type controls. Overexpression of ST2 attenuated BLP-induced NF-kB activation by 40% (p < 0.05 vs. empty vector transfected cells), indicating a negative role of ST2 in TLR2 signalling. In response to a second BLP stimulation, a moderate but significantly attenuated TNF-a production was observed in BLP-pretreated ST2-deficient BMM (p < 0.01 vs. naive ST2-deficient BMM). Furthermore, down-regulated MyD88-IRAK complex formation, a molecular characteristic in BLP-tolerised cells, was evident in BLP-pretreated ST2-deficient BMM, suggesting BLP tolerance develops independent of ST2.

Conclusion: ST2 acts as a negative regulator in TLR2 signalling, but is not responsible for BLP-induced tolerance.

Corresponding Author: Liu Jinghua, PhD, Cork University Hospital, Department of Surgery, Wilton, Cork, Ireland, j.liu@ucc.ie

A 161
IFNs exaggerate LPS signaling by enhancing IRAK1 and TRAF6 interaction
Perenlei Enkhaaatar, Vivian Wolfe, Erika Stalets, Wong Hector, Zingarelli Basilia

Objective: The mechanism leading to synergy between antecedent viral infection and severe bacterial infection is not well understood but contributes to severe morbidity and mortality. Many viral infections create a permissive state for severe bacterial infection by facilitating bacterial penetration through disruptive protective barriers. Simultaneously, antiviral cytokines such as IFNa/b or IFNg can enhance inflammatory responses to challenge with live bacteria or TLR ligands leading to significant toxicity and mortality. Experimentally, we have found that pretreatment with rIFNa or rIFNg (without viral infection) followed by stimulation with TLR4 or TLR2 ligands prolongs and exaggerates activation of the TLR signalling molecules IKK, IkB, NFkB, and p38 leading to exaggerated TNF-a production. This occurred without a change in TLR2 or TLR4 cell surface expression suggesting that rIFNa/b and rIFNg affect signaling distal to the TLR but proximal to IKK. In this work our objective was to identify the mechanism for rIFNa/b or rIFNg modulation of responses to TLR ligands.

Methods: J774 cells (macrophage cell line) were treated with 2,000 U/ml of rIFNa/b or rIFNg for 16 h and the expression of several key TLR signalling proteins was assessed by Western blot. Next, IRAK1 and TRAF6 were assessed by WB at 5 min intervals after LPS (1 µg/ml), rIFNa + LPS or rIFNg + LPS. By co-immunoprecipitation the association between IRAK1 and TRAF6 was assessed over this same time course.

Results: Neither rIFNa/b or rIFNg altered total expression of TLR2, TLR4, MyD88, MAL, IRAK1, IRAK4, TRAF6, IKKa, IkB, p38, or IRF3. Cell lysates collected at 5 min intervals (0–35 min) after LPS showed that IRAK1 typically was degraded after 10–15 min. After rIFNa + LPS or rIFNg + LPS IRAK1 degradation significantly delayed out to 30 min. TRAF6 was not degraded after LPS or rIFNa + LPS or rIFNg + LPS. By co-immunoprecipitation, the interaction between IRAK1 and TRAF6 was enhanced and prolonged in rIFNa + LPS or rIFNg + LPS compared with LPS only.

Conclusions: Our data indicate that IFNs may enhance LPS-induced TLR signalling by altering the kinetics of protein:protein interactions in the TLR signalling cascade. Our previous data combined with this work suggest that the initial site at which IFNs change TLR signaling may be intimately involved in IRAK1 processing. Further characterization of this interaction will help uncouple the inflammatory synergy of viral and bacterial co-infection.

Corresponding Author: Lesley Doughty, MD, Cincinnati Children’s Hospital Medical Center, Department of Critical Care Medicine, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA, lesley.doughty@cchmc.org

A 162
LPS is not enough: an in vitro study of platelet activation via TLR4
Manja Appelt, Julia van der Linde, Stephan Diedrich, Katharina Czupka, Claus-Dieter Heidecke, Stefan Maier

Objective: In recent years platelets have become an important topic in inflammation and sepsis. To examine their function as immune cells we focused on their ability to react on host pathogens. Therefore activation marker as P-selectin (CD62P) as well as the aggregation with leukocytes was measured after exposure to LPS.

Material and methods: First platelet rich plasma (PRP) from healthy donors was stimulated with LPS (E. coli O127:B8, final concentration 10 µg/ml), TRAP-6 (Thrombin Receptor Activator for Peptide 6) (50 µmol/l) or both LPS and TRAP-6. Unstimulated PRP served as control group. Second we used whole blood from healthy donors and stimulated it on the one hand with LPS or TRAP-6 alone and on the other hand with both LPS and TRAP-6. Unstimulated blood served as control group. After 4 h incubation samples were stained with
antibodies to see aggregates of platelets and leukocytes in whole blood and activation by expression of CD62P in PRP and whole blood in flow cytometry.

Results: In stimulated PRP platelets showed a degranulation of their z-granules after stimulation with TRAP-6 but not after exposure to LPS alone. Simultaneous addition of LPS and TRAP-6 led to an increase of P-selectin expression in comparison to stimulation with TRAP-6 alone. This was clarified by a higher geometric mean of CD62P + cells \( p < 0.05 \). In whole blood a significant rise of monocyte-platelet- and granulocyte-platelet-aggregates was seen after exposure to TRAP-6, TRAP-6 + LPS and even after stimulation with LPS alone \( p < 0.05 \) in comparison to untreated control group. CD62P expression of platelets joint to the leukocytes was significantly higher after stimulation with TRAP-6 or LPS \( p < 0.05 \).

Conclusion: Platelets are involved in inflammation by activation through LPS alone in whole blood and LPS triggered activation through TRAP-6 in PRP. The activation marker and cell adhesion molecule P-selectin is upregulated and binds to PSGL-1 (P-selectin glycoprotein ligand-1) which is expressed on neutrophils and monocytes. This binding leads to platelet-leukocyte interaction and aggregate formation. CD62 expression did not occur in PRP after LPS stimulation while platelets in whole blood showed an increase. This can be due to the induction of tissue factor production in monocytes after stimulation with LPS (Meszaros et al., Blood 1994). TF leads to activation of factor VII and the initiation of the clotting cascade which finally results in thrombin synthesis and platelet activation again.

Corresponding Author: Manja Appelt, Ernst Moritz Arndt University Greifswald, Department of Surgery, Friedrich-Loeffler-Str. 23b, 17487 Greifswald, Germany, manjaappelt@web.de

A 163

TLR4 serves as a danger sensor for proliferating stem cells in the intestinal epithelium

Matthew Neal, Ward Richardson, Chinder Sodhi, Richard Siggers, Amin Afrazi, David Hackam

Objective: Necrotizing enterocolitis (NEC) is the leading cause of gastrointestinal morbidity and mortality in preterm infants, yet the underlying mechanisms remain largely unexplained. We have recently demonstrated a critical role for Toll-like receptor 4 (TLR4) in the pathogenesis of NEC, as TLR4 activation leads to reduced intestinal proliferation. We now seek to assess for a link between TLR4 and the intestinal stem cells (ISC), which are responsible for intestinal epithelial barrier renewal. Given that ISCs control intestinal epithelial renewal, we now hypothesize NEC results from intestinal stem cell failure and that TLR4 signaling mediates ISC proliferation. Material and methods: Experimental NEC was induced in newborn mice using combined enteric feeding and intermittent hypoxia and the terminal ileum was harvested for characterization of the stem cell markers LGR5 and OLFM4. In parallel, ISCs were harvested from newborn intestine using mechanical separation and differential centrifugation as well as by laser capture microdissection. To assess the role of TLR4 in ISC marker expression, a TLR4 knock-out (shTLR4) was created in an intestinal crypt cell line (IEC-6). To further characterize the intestinal stem cell response following injury, endotoxemia was induced via intraperitoneal injection of LPS (5 mg/kg) and mice were sacrificed after 3 h for harvest of the terminal ileum and mucosal scrapings.

Results: NEC was associated with a profound reduction in proliferation of the intestinal epithelium compared with breast fed control mice. NEC was also associated with a significant decrease of the intestinal stem cell markers: LGR5 (1.0 vs. 0.57, \( p < 0.05 \)) and OLFM4 (1.0 vs. 0.09, \( p < 0.01 \)). Analysis of the stem cell compartment obtained by laser capture microdissection showed that TLR4 expression was increased in the crypt compared to villi (1.67 vs. 1.0, \( p < 0.05 \)). In vitro, there was a significant decrease in the stem cell regulator LGR5 with TLR4 activation via LPS in a model of endotoxemia (1.0 vs. 0.47, \( p < 0.05 \)). Strikingly, however, in proliferating enterocytes with an induced deletion of TLR4 (knock-out (shTLR4) cells), LPS did not alter stem cell marker expression, confirming the role of TLR4 as a danger sensor for proliferating stem cells (1.11 vs. 1.0, \( p = 0.9 \)).

Conclusions: These data demonstrate that TLR4 acts as a danger sensor and inhibits intestinal stem cell proliferation, which may contribute to the failure of epithelial regeneration seen in NEC.

Corresponding Author: Matthew Neal, MD, University of Pittsburgh Medical Center, Department of Surgery, 1412 Senior Drive, Pittsburgh, PA 15227, USA, nealrn2@upmc.edu

A 164

Stimulation of the innate immunity via toll like receptor (TLR)-4 increases vascular inflammatory response to fibrinogen degradation products

Patrick Paulus, Peter Ellinghaus, Volker Laux, Nguyen Tran, Tiago Granja, Kai Zacharowski

Objectives: Systemic inflammatory conditions such as SEPSIS are associated with coagulation disorders and endothelial dysfunction leading to organ failure. In these patients, fibrinogen turnover is higher than in healthy individuals. Fibrin degradation results in the production of peptide fragments with biological activity. Increasing evidence suggests that fibrinogen fragments (FFs) play a role as mediators in pro-inflammatory signaling pathways. We hypothesize that the co-occurrence of an infection, stimulating the innate immunity and increased fibrinogen (FGN) turnover increases the vascular inflammatory response.

Material and methods: To test this hypothesis, we pre-incubated human umbilical vein endothelial cells (HUVEC) with LPS, a ligand for TLR-4, 0.5 h before incubation with FF. FGN cleavage is performed by incubation with plasmin for 2.5 h. The inflammatory response is quantified by mRNA expression, using real-time RT-PCR. Three groups of genes were investigated, i.e. genes mediating cell-cell interactions with ECs and leukocytes, pro-inflammatory cytokines and chemokines: intercellular adhesion molecule-1 (ICAM-1), endothelial cell leukocyte adhesion molecule-1 (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1), Interleukin-6, -8, macrophage inflammatory protein (MIP)-1, GRO-α, -β.

Results: Compared to untreated controls, HUVECs treated with FF generated by plasmin-cleavage of FGN show a significant ICAM-1 mRNA upregulation (2-fold, \( p < 0.05 \)), ELAM-1 (144-fold, \( p < 0.01 \)), VCAM-1 (72-fold, \( p < 0.001 \)), IL-6 (5-fold, \( p < 0.001 \)), -8 (21-fold, \( p < 0.01 \)), MCP-1 (7.7-fold, \( p < 0.001 \)), GRO-α (40-fold, \( p < 0.001 \)), -β (37-fold, \( p < 0.001 \)). When compared to HUVECs treated with FF alone, HUVECs preincubated with LPS 30 min before casting with FF and plasmin, show a significant VCAM-1 (1.8-fold, \( p < 0.001 \)) IL-6 (1.2-fold, \( p < 0.01 \)), MCP-1 (1.5-fold, \( p < 0.001 \)) mRNA upregulation. A corresponding negative control (LDL-R), which is known not to be regulated, shows no significant changes in the above groups.

Conclusions: Our data show for the first time that stimulation of TLR-4 is increasing the vascular inflammatory response in the presence of FF. Genes mediating the EC-leukocyte contact are significantly upregulated. This knowledge might be important in the treatment of patients with SEPSIS, where infections and coagulation disorders
A 165
Differential stimulation of trauma related toll-like receptors from peripheral blood mononuclear cells in children compared to adults
David Partrick, Ernest Moore, Ronald Harbeck, Richard Johnston

Objective: Toll-like receptors (TLRs) play a key role in innate immunity, and specific members of the TLR family (TLR 1/2, 2/6, 4, and 9) contribute to trauma-induced inflammation, which can result in multiple organ failure (MOF). Post-injury MOF occurs less frequently in children than in adults, and we therefore hypothesize that peripheral blood mononuclear cells (PBMCs) from children will exhibit a blunted pro-inflammatory cytokine response to in-vitro stimulation with trauma-TLR specific ligands.

Methods: PBMCs isolated from blood samples of 20 healthy children (age range 2–12 years) and 17 healthy adults (age range 24–66 years) were suspended in RPMI and plated in 96-well culture dishes at a concentration of 2 × 10^5 cells/well. Cultured cells were stimulated with TLR-specific ligands (Pam3CSK4: TLR 1/2; Zymosan: TLR 2/6; LPS: TLR 4; and ODN 2216: TLR 9) for 24 h. TNF-α levels in supernatants were measured by ELISA. Data are mean ± SEM.

Results: Stimulation of TLR 1/2 resulted in higher levels of TNF-α production in adults compared to children (498 ± 106 vs. 280 ± 48 pg/ml), although the difference did not reach statistical significance (P = 0.06) due to wide individual variation. TNF-α production in response to other ligands was similar between children and adults.

Conclusions: Age-related differences in TNF-α production resulting from TLR 1/2 stimulation in PBMCs could contribute to the lower incidence of MOF in injured children. However, further work is needed to more clearly define the role of TLR 1/2 in injury related inflammation and post-injury MOF. TNF-α production in response to stimulation of other trauma-associated TLRs is likely not implicated in age-related discrepancies in the incidence of post-injury MOF.

Table

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Adults</th>
<th>P value</th>
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<tbody>
<tr>
<td>TLR 1/2</td>
<td>280 ± 48</td>
<td>498 ± 106</td>
<td>0.06</td>
</tr>
<tr>
<td>TLR 2/6</td>
<td>1,330 ± 169</td>
<td>1,305 ± 134</td>
<td>0.91</td>
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<tr>
<td>TLR 4</td>
<td>1,114 ± 165</td>
<td>1,336 ± 172</td>
<td>0.35</td>
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<tr>
<td>TLR 9</td>
<td>984 ± 148</td>
<td>1,146 ± 235</td>
<td>0.62</td>
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PBMC TNF-α production in response to TLR-specific ligands. Mean ± SEM (pg/ml)

A 166
Synthetic ligands against Toll like receptors (TLR)-2-9 in TruCulture®—whole blood stimulation assays distinguish clinical stages of SIRS (trauma) and sepsis
Jelena Bindja, Manfred E. Weiss, Gerburg Stein, Nicole Schneiderhan-Marra, E. Marion Schneider

Background: In order to understand the nature of immune dysfunction in patients with sepsis manifesting after severe trauma, a robust whole blood ex vivo test system (TruCulture®) has been developed to determine secreted cytokines and soluble receptors following 24 h incubation.

Patients: Patients with major trauma, sepsis and severe sepsis, admitted to the ICU have been enrolled; healthy volunteer donors served as controls.

Methods: One milliliter of blood is drawn into separate syringes containing ligands to stimulate the following TLRs: TLR1/2(MALP); -2/6(Pam3Cys), -3(dsRNA), -4 (LPS), -5(flagellin), 7(ssRNA), and TLR9(CpGs typeA and typeB). The biomarkers TNF-α, IFN-γ, Interleukins-1α, -β, -4, -5, -6, -8, -10, -12, -13, -15, -16, -17, -18, G-CSF, GM-CSF, Eotaxin, TGF-β, MCP-1, MIP-1α, ferritin, sICAM, as well as metalloproteinases and soluble receptors, and the IL11RA are quantified by multiplexed sandwich immunoassays. The parameters: IL-1ratio and TNF ratio following stimulation with LPS, are here defined as the ratios of IL-1β [pg/ml]/IL-1α [ng/ml] and TNF-α [pg/ml]/sTNF-RII [ng/ml], respectively.

Results: When compared with healthy donors, most TLR induced cytokines were lower in trauma and even lower in sepsis patients. An exception was the TLR2 stimulation, which induced more inflammatory and anti-inflammatory cytokines as well as soluble receptors in trauma and sepsis than in healthy donors. Among the other TLR responses, TLR3 was most dramatically downregulated in patients with trauma and even more in sepsis patients. Calculating the ratios between active cytokines such as IL-1β and TNF-α and the respective soluble IL1RA (here named: IL-1ratio) and the soluble TNF-receptor, sTNF-RII (here named: TNF ratio), we found that a TLR4 activation by LPS results in a patient-type specific ratio of ligand to antagonists. Healthy donors had a median IL1 ratio of 1.48 and a median TNF ratio of 2.73, trauma patients had ten times and sepsis patients had a 100 times lower IL-1- and TNF ratios (Table 1). All differences are significant (p ≈ 0.01).

Conclusion: The here developed TruCulture® ex-vivo whole blood TLR stimulation test is valid to correlate a defined response pattern to the clinically established stages trauma and sepsis. Results may substantially contribute to signalling pathways leading to immune dysfunction.

Table 1

<table>
<thead>
<tr>
<th>Cytokine/antagonist ratio</th>
<th>Healthy</th>
<th>Trauma</th>
<th>Sepsis</th>
</tr>
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<tbody>
<tr>
<td>IL-1β/IL-1RA (=IL-1ratio)*</td>
<td>1.48 (1.27–6.1)*</td>
<td>0.18 (0.002–0.54)</td>
<td>0.09 (0.07–0.19)</td>
</tr>
<tr>
<td>TNF-α/sTNF-RII (=TNF ratio)*</td>
<td>2.37 (1.15–3.54)</td>
<td>0.43 (0.03–0.86)</td>
<td>0.06 (0.01–0.28)</td>
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* Median (range)

Corresponding Author: David Partrick, MD, The Children’s Hospital, University of Colorado, Department of Pediatric Surgery, 13123 East 16th Avenue, B-323, Aurora, CO 80045, USA, partrick.david@tchden.org

Corresponding Author: E. Marion Schneider, Prof. PhD, University of Ulm, Div. of Experimental Anaesthesiology, Department of Anaesthesiology, Steinheuelstr. 9, 89075 Ulm, Germany, marion.schneider@uni-ulm.de
Mitochondria exist in two interconverting forms, i.e. as small isolated particles, and as extended filaments, networks or clusters connected with intermitochondrial junctions. Here we provide evidence that in differentiating myoblasts endogenous nitric oxide (NO) generation controls mitochondrial shape; in the absence of NO mitochondrial fission occurs rapidly. The action of NO appears to be specifically addressed to mitochondrial fission since in PEG fusion assay organelle fusion was not modified by the treatment with the competitive NO synthase-inhibitor L-NAME.

A key protein involved in mitochondrial fission is the large GTPase DRP-1. DRP-1 translocation to the mitochondria and interaction with the specific docking protein hFis-1 promotes mitochondrial fission. DRP-1 translocation and mitochondrial fission were stimulated by L-NAME and inhibited by exogenous NO. In addition, NO inhibited DRP-1 GTPase activity. The effects of NO depended on generation of cyclic GMP a physiological mediator of NO actions. We also found that in differentiating myoblasts NO is required for the expression of myogenin and muscle specific myosin since L-NAME inhibited myogenic differentiation, and the prevention of cytochrome c release. This mechanism, which leads to the prevention of oxidative damage and apoptosis, is a major pathway for nitrite-mediated cytoprotection after IR. The implication of these data for endogenous nitrite signaling at the mitochondrial level, as well as the potential for nitrite-based therapeutics will be discussed.

Corresponding Author: Sruti Shiva, PhD, University of Pittsburgh, Department of Pharmacology, 3501 Fifth Avenue, Pittsburgh, PA 15261, USA, sss43@pitt.edu

Objectives: Autophagy is the regulated process cells use to recycle non-essential, redundant, or inefficient components and is an adaptive response during times of stress. In addition to its role in enabling the cell to gain vital nutrients in times of stress, autophagy can also be involved in elimination of intracellular microorganisms, tumor suppression, antigen presentation, and inflammatory response. In this study, we examined whether autophagy is associated with sepsis regarding inflammatory cytokine response.

Materials and methods: (1) Electron microscopy (EM) was performed on liver samples obtained from both an observational clinical cohort of six septic patients and four control patients. (2) ATG16L1 genotyping with TaqMan® assay was performed to examine whether the autophagy-related gene polymorphism was associated with susceptibility to severe sepsis as well as outcome of the patients with severe sepsis (genotyped severely septic patients (SS), control non-septic patients (NS), and healthy volunteers (HV) in our Japanese cohorts: n = 79, 89, and 276, respectively). Peak interleukin-6 (IL-6) blood levels during the ICU stay were daily measured and also compared by each ATG16L1 genotype in the septic patients.

Results: (1) EM demonstrated increased autophagic vacuoles in septic vs. non-septic patients. Randomly selected fields (3,000 μm²) from control and septic patients contained 1.2 ± 1.5 vs. 5.3 ± 3.3 (mean ± SD) complex lysosomal/autophagolysosomal structures per image, respectively (P < 0.001). Membrane alterations occur in a subpopulation of mitochondria in sepsis, but other hepatocyte organelles showed no consistent ultrastructural injury. (2) ATG16L1 genotypic distributions were not different among the three groups (AA:AG:GG (%), 71.1:25.0:4.0 in SS, 65.5:32.1:2.4 in NS, and 60.9:32.2:4.7 in HV). In the SS patient cohort, GG homozygotes had trend of increase of mortality compared with AA plus AG genotypes (66.7 vs. 27.8%, P = 0.161) and peak IL-6 blood levels (geometric mean; 24,070 vs. 6,820 pg/mL, P = 0.423).

Conclusions: Hepatocyte autophagic vacuolization increases during sepsis and is associated with mitochondrial injury. It is not possible to determine whether the increase in autophagic vacuolization is an adaptive response or a harbinger of cell death. It is, however, suggested that depletion of autophagy could cause excessive certain cytokine production leading to adverse outcome of severely septic patients.

Corresponding Author: Eizo Watanabe, MD, Chiba University Graduate School of Medicine, Department of Emergency and Critical Care Medicine, 1-8-1 Inohana, Chuo, 260-8670 Chiba City, Japan, watanabee@faculty.chiba-u.jp

The molecule nitrite (NO₂⁻), ubiquitous in the blood and tissues and present in the diet, was long considered to be merely a physiologically inert byproduct of nitric oxide (NO) oxidation. However, accumulating data suggests that this anion is an endocrine storage and the prevention of cytochrome c release. This mechanism, which leads to the prevention of oxidative damage and apoptosis, is a major pathway for nitrite-mediated cytoprotection after IR. The implication of these data for endogenous nitrite signaling at the mitochondrial level, as well as the potential for nitrite-based therapeutics will be discussed.

Corresponding Author: Sruti Shiva, PhD, University of Pittsburgh, Department of Pharmacology, 3501 Fifth Avenue, Pittsburgh, PA 15261, USA, sss43@pitt.edu
A 170
Nitrite protects sGC-dependently against morbidity and mortality associated with inflammatory shock in mice
Anje Cauwels, Anje Cauwels, Emmanuel Buys, Robrecht Thoone, Sruti Shiva, Peter Brouckaert

Objective: For a long time nitrite (NO\textsubscript{2}^-) was believed to be an inert oxidation metabolite and biomarker of the endogenous vasodilator nitric oxide (NO). Recently, however, nitrite was identified as an important biologic NO reservoir in both vasculature and tissues, contributing to hypoxic signaling, vasodilation and cytotoxicity after ischemia-reperfusion injury in heart, liver, kidney and brain. Reduction of nitrite to NO may occur enzymatically at low pH and oxygen tension by deoxymegoglobin, deoxymyoglobin, xanthine oxidase, mitochondria or NO synthase (NOS). Considering the fact that NO may exert protective effects in inflammatory and septic shock, and that circulating nitrite may function as a source of NO in hypoxic and/or acidic conditions that are present in ischemic microvascularity of vital organs during shock, we decided to test the protective capacity of nitrite on organ damage and toxicity associated with inflammatory shock.

Methods: We used sterile models of shock induced by TNF or LPS in female mice on a C57Bl/6 background. NaNO\textsubscript{2} pre- or post-treatments were done intravenously. To monitor morbidity, rectal body temperatures were measured frequently and mortality was recorded. In addition, separate groups of mice were sacrificed 2 or 6 h after challenge to analyze serum markers for organ damage, as well as mitochondrial parameters, ATP production and infiltration of myeloid cells.

Results: Low doses of intravenously injected nitrite significantly ameliorated hyperthermia, organ damage and mortality induced by a lethal TNF challenge. Mechanistically, nitrite-dependent protection was not associated with inhibition of mitochondrial complex I activity, as previously demonstrated for ischemia-reperfusion. On the contrary, nitrite rather protected mitochondrial complex I, complex IV and aconitase activity from TNF-induced damage. In addition, nitrite protection was largely abolished in mice deficient for the alpha1-subunit of soluble guanylate cyclase (sGC\textsubscript{1}), one of the principle intracellular NO receptors and signal transducers in the cardiovascular system. Interestingly, nitrite could also provide protection against toxicity induced by Gram-negative LPS, although higher doses of nitrite were required.

Conclusion: We show that nitrite can protect against toxicity in inflammatory shock via sGC-dependent signaling, which may include hypoxic vasodilation necessary to maintain microcirculation and organ function, and cardioprotection.

Corresponding Author: Anje Cauwels, PhD, Ghent University-VIB, Department for Molecular Biomedical Research, Technology park 927, 9052 Ghent, Belgium, anje@dnbr.UGent.be

A 171
Mitochondria seem not involved in the onset of myocardial depression in a rat acute sepsis model
Lonneke Smeding, Willem van der Laarse, Toke van Veelen, Martin Kneyber, Johan Groeneveld, Frans Ploetz

Objective: Sepsis is often associated with decreased cardiac function and mitochondrial damage and dysfunction seems to be involved. Mitochondrial damage can lead to opening of the mitochondrial permeability pore and subsequent leakage of cytochrome C into the cytoplasm. Opening of the pore is regulated amongst others by members of the B-cell lymphoma protein-2 (Bcl-2) family. It can be postulated that damaged mitochondria will be replaced. This study investigates the role of mitochondria in the onset of sepsis-induced myocardial depression in an acute model of sepsis.

Methods: Male Wistar rats were divided into a sepsis (n = 6) or control (n = 6) group. Animals in the septic group received lipopolysaccharide (LPS). Four hours after LPS injection, left ventricular developed pressure (P\textsubscript{dP/dt}) and contractility (+dP/dt) ex vivo were measured in a Langendorff set-up. Cytochrome C oxidase activity was measured immunohistochemically to determine mitochondrial function. Mitochondrial damage was examined by measurement of both cytochrome C leakage using immunohistochemistry and Bcl-2 protein expression. Mitochondrial transcriptional regulator peroxisome proliferator activated receptor gamma cofactor 1z (PGC-1z) and mitochondrial transcription factor A (TFAM) protein expression were examined to study mitochondrial biogenesis.

Results and conclusions: Rats who received LPS had a depressed cardiac function when compared to healthy animals, which was shown by a lower P\textsubscript{dP/dt} (p < 0.001) and +dP/dt (p < 0.01). However, no difference was found between septic and healthy animals in cytochrome C oxidase activity, cytochrome C leakage and Bcl-2, PGC-1z- and TFAM expression. Therefore, we concluded that mitochondria seem not involved in the onset of myocardial depression in this acute model of sepsis.

Corresponding Author: Lonneke Smeding, MSc, VU Medical Center, Department of Pediatric Intensive Care, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands, l.smeding@vumc.nl

A 172
Comparative proteome analysis of subcellular fractions of livers obtained from rats subjected to endotoxic shock
Ingrid Miller, Bernd Gesslbauer, Heinz Redl, Andrey V. Kozlov

Objective: Sepsis and endotoxic shock result in the development of SIRS causing MOD and often death. MOD is due to cellular dysfunction often not visible in histological examination. Characterisation of protein patterns of the affected organ is a crucial step in understanding intracellular changes causing cellular dysfunction. It has been shown by different authors that either mitochondrial or ER dysfunction can be the reason for MOD. The objective of this study was to investigate changes in the mitochondrial, ER, and cytosolic proteome of liver cells in response to LPS.

Materials and methods: Livers from Sprague–Dawley rats were obtained after 16 h of LPS-challenge (8 mg/kg, i.p.). Subcellular fractions were prepared by differential centrifugation of liver homogenates. Proteins were labelled with fluorophores and subsequently separated by two-dimensional electrophoresis (2D-DIGE). Spots differentially regulated between treated and control animals were identified by mass spectrometry methods.

Results: Upon LPS-challenge, in the mitochondrial fraction we observed upregulation of mitochondrial SOD, catalase and the alpha-chain of ATP synthase. In cytosol we observed upregulation of intact carbamoylphosphate synthase (CPS), 60 kDa heat shock protein, and one peroxiredoxin-1 spot. The most dramatic changes were observed in ER: many functional proteins were down-regulated (e.g. GRP78, protein disulfide isomerase A3, argininosuccinate synthase, transi-
notional ER ATPase).

At the same time, the expression levels of proteins responsible for antioxidant capacity were increased in mitochondria, slightly
increased in cytosol, and definitely decreased in ER. We observed increased fragmentation of CPS, an important member of the urea cycle, in mitochondria and higher levels of intact CPS in cytoplasm of treated animals. This is in line with literature data describing that CPS is translocated from mitochondria and specific fragments are even detected in blood.

Conclusions: Our data suggest that protein patterns of ER are more sensitive to endotoxin shock than protein patterns of cytoplasm and mitochondria.

Corresponding Author: Andrey Kozlov, Prof. PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUDA Research Center, Dornauerschingen Str. 13, 1200 Vienna, Austria, andrey.kozlov@libtrauma.org

A 173
Ultra structural changes in rat livers induced by endotoxic shock and underlying molecular mechanisms

Sylvia Nuernberger, Sanjeev Gupta, J. Catharina Davigneau, Oswan Hori, Heinz Redl, Andrey V. Kozlov

Objectives: The present study aimed at investigating the ultrastructural changes in livers of rat subjected to endotoxic shock.

Methods: Rats subjected to endotoxic shock were subdivided into four groups in accordance to their ALT blood levels, a marker of shock severity. Ultrathin sections of liver were stained with uranyl acetate and lead citrate and examined on a Zeiss EM 902 Electron Microscope. RT-PCR analysis was used for gene expression analysis.

Results: Electron microscopy examination did not reveal changes in mitochondrial structure; neither damaged nor swollen mitochondria were found. However, dramatic changes were observed in ER in close vicinity to mitochondria, appearing as dilated ER. There were no apoptotic changes in hepatocytes, but apoptotic bodies were found in white blood cells within the liver tissue. We also found no signs of autophagy, as previously reported in other sepsis models. Therefore the major observation was the dilated ER next to mitochondria, which could be the consequence of ER stress, or recently reported osmotic disequilibrium associated with Bak-BclXL interaction. While the mRNA levels of some ER stress markers were slightly upregulated (SCOTIN, GRP78), the protein levels of ER stress markers (GRP78, CHOP, Herp) remained unchanged. These findings suggest so-called unresolved ER stress, which it is induced, but not translated into a protein response. ER-stress signaling involves also members of the Bcl2 family, which contribute to the induction of apoptosis under conditions of unresolved ER stress. LPS induced upregulation of Bcl-2 family members at mRNA level suggesting activation of apoptotic pathways possibly mediated by ER dilation. Some markers of apoptosis were up-regulated at mRNA or protein level (BIM, NOXA), some down-regulated (BNIP3, BMF), and some unchanged (Puma, Bax), demonstrating that activation of cell death-signaling pathways remained incomplete. This is consistent with the fact that we did not observe morphological manifestation of apoptosis in hepatocytes.

Conclusion: These data show that the only ultrastructural changes in hepatocytes in response to severe endotox shock are associated with ER. We hypothesize that both ER stress and Bcl2 signaling pathway contribute to morphological changes in ER. The specific location of dilated ER in close vicinity to mitochondria suggests that pathways leading to ER dilation are activated by mitochondria.

Corresponding Author: Andrey Kozlov, Prof. PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUDA Research Center, Dornauerschingen Str. 13, 1200 Vienna, Austria, andrey.kozlov@libtrauma.org

A 174
Nitroglycerin inhibits mitochondrial respiration and induces ROS generation via a nitric oxide-independent mechanism

Peter Dangel, Susanne Haindl, Tricia Behling, Heinz Redl, Andrey Kozlov

Objectives: Nitroglycerin (NG) has been shown to protect lung, brain and other organs from ischemia/reperfusion injury, but until now the data on the mechanism of NG action remains controversial. Although biological effects of NG are thought to be based on the release of nitric oxide (NO), catalysed by aldehyde dehydrogenase 2 (ALDH2) and activation of cGMP-dependent pathways, not all existing data fit to this concept. We have previously shown that NG additionally modulates the respiratory function of mitochondria. The aim of this study was to clarify whether or not the effects of NG on mitochondria are NO-dependent.

Methods: Rat liver mitochondria were isolated by successive centrifugation steps. Respiratory parameters of isolated mitochondria were monitored using an Oxygraph-2k (OROBOROS Instruments, Austria) and reactive oxygen species (ROS) were detected by EPR. Glutamate plus malate and succinate were used as substrates for complex I and II, respectively. Choloralhydrate was used to specifically inhibit ALDH2, hemoglobin was used as an NO-trap, and blue light was used to dissociate protein-complexes of NO.

Results: Both NO-gas and NG inhibited mitochondrial respiration in a dose dependent manner. In doses causing approx. 50% inhibition of mitochondrial respiration (500 nM NO, 1 mM NG) NO, but not NO activated mitochondrial ROS production. Neither impaired mitochondrial respiration nor enhanced ROS generation were influenced by chloralhydrate. Nitrite, a possible intermediate of NG bioactivation, had no effect on either respiration or ROS production. Mitochondrial respiration impaired by NG was not ameliorated by blue light, while NO-inhibited mitochondria were completely reactivated by blue light. The presence of hemoglobin completely prevented mitochondrial dysfunction mediated by NO, but had no influence on either mitochondrial dysfunction or enhanced ROS generation mediated by NG.

Conclusion: Our data suggest that NG bioactivation undergoes at least two independent pathways; the NO-dependent leading to NO production and activation of cGMP pathways, and the NO-independent pathway leading to impaired mitochondrial respiration and enhanced ROS production.

Corresponding Author: Peter Dangel, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUDA Research Center, Dornauerschingen Str. 13, 1200 Vienna, Austria, peter.dangel@trauma.lbg.ac.at

A 176
Endoplasmic reticulum stress induction following trauma-hemorrhage

Irshad Chaudry, Irshad Chaudry, Bixi Jian, Raghavan Raju

Objectives: Trauma-hemorrhage induces hypoxia, cellular apoptosis and organ dysfunction. The proinflammatory cytokines, hypoxia and glucose deprivation following trauma-hemorrhage can activate endoplasmic reticum (ER) stress. As the physiological conditions consequent to trauma-hemorrhage are consistent with factors necessary to initiate endoplasmic reticum stress and unfolded protein response, our objective was to determine the ER stress response proteins in the liver following trauma-hemorrhage.

Corresponding Author: Andrey Kozlov, Prof. PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUDA Research Center, Dornauerschingen Str. 13, 1200 Vienna, Austria, andrey.kozlov@libtrauma.org
Materials and methods: Trauma-hemorrhage procedure was carried out as described previously in our laboratory (Hildebrand et al., Am J Path 2006:784). Briefly, male C3H/HeN mice 8–12 weeks old and weighing 19–25 g were bled rapidly to mean arterial blood pressure of 35 ± 5 mmHg and resuscitated with Ringer’s lactate. Sham operated animals served as controls. Liver was removed 24 h later. Bip, ATF6, PERK, phosphorylated PERK (PERK-p), PDI, IRE-1α and CHOP (C/EBP homologous protein, also known as GADD153) were analyzed in total liver proteins by Western blot. Apoptosis was measured by DNA fragmentation assay (Roche Diagnostics, Indianapolis, IN, USA) and TUNEL staining carried out using cryosections of the liver tissue (R&D Systems, Minneapolis, MN, USA).

Results and conclusions: We found that the expression of ER stress proteins, IRE1α, Bip, activated ATF6, PERK, phospho-PERK and PDI were significantly elevated in the liver after trauma-hemorrhage compared to shams. Furthermore, the protein expression of the pro-apoptotic transcription factor CHOP was also significantly elevated following trauma-hemorrhage. Apoptosis was confirmed by the observation of increased DNA fragmentation and TUNEL-stained nuclei. Thus, ER stress is activated following trauma-hemorrhage. Trauma-hemorrhage is followed by hypoxia, insulin resistance and excessive secretion of inflammatory cytokines, mainly by the Kupffer cells in the liver. Any or all of these conditions can trigger the activation of ER stress and UPR. Based upon these results, we hypothesize a possible role for ER stress in apoptosis and organ damage following trauma-hemorrhage.

(supported by NIH grants ROI GM 37127 to IHC and R21 AG031440-01A1 to RR).

Corresponding Author: Irshad Chaudry, Prof. PhD, University of Alabama at Birmingham, Center for Surgical Research, 1670 University Boulevard, Birmingham, AL 35294, USA, irshad.chaudry@ccc.uab.edu

A 177 Mitochondrial reactive oxygen species (ROS) in systemic immune response: interaction between mitochondria, endoplasmic reticulum and acute phase reaction
Andrey Kozlov, Ingeborg Kehrer, J. Catharina Davigneau, Heinz Redl

Objectives: The objective of this study was to find whether or not there is a link between mitochondrial ROS (mROS) and hepatocyte dysfunction induced by systemic immune response (SIR).

Methods: In vivo SIR was induced by LPS in rats, resulting in approx. 50% mortality by 16 h. In vitro model included incubation of hepatocytes with medium obtained after ex vivo incubation of white blood cells or whole blood with LPS.

Results: In vivo challenge with LPS was accompanied by a reversible inhibition of mitochondrial function, irreversible increase of mROS production, and drastically elevated tissue nitric oxide (NO) levels. Increased ROS production was detected both in whole liver and in isolated liver mitochondria by means of EPR. Exposure of control mitochondria to NO concentrations observed in vivo resulted in reversible changes in mitochondrial function, but did not activate ROS production. Ex vivo experiments were performed to better understand the impact of mROS. Incubation of either WBC or whole rat blood with LPS for different periods of time resulted in the appearance of specific time dependent patterns of cytokines in the incubation media. Hepatocytes were incubated with those media; mitochondrial potential and mROS production were determined by means of fluorescent confocal microscopy; the expression of relevant genes was determined by RT-PCR. The mROS, ER-stress markers

A 178 The use of interleukin-1 antagonists in the treatment of diabetes mellitus
Marc Donath

Onset of type 2 diabetes occurs when the pancreatic beta cell fails to adapt to the increased insulin demand caused by insulin resistance. Morphological and therapeutic intervention studies have uncovered an inflammatory process in islets of patients with type 2 diabetes characterized by the presence of cytokines, immune cells, beta cell apoptosis, amyloid deposits and fibrosis. This insulinitis is governed by IL-1 signalling. We propose that this insulinitis contributes to the decrease in beta cell mass and the impaired insulin secretion observed in patients with type 2 diabetes.

In this presentation we will review the evidence for insulinitis in type 2 diabetes, mechanisms inducing this inflammatory process, its physiological and pathological role and the ongoing clinical translation. Corresponding Author: Marc Donath, Endo & Diabetes, Department of Medicine, Raemistr. 100, 8091 Zurich, Switzerland, marc.donath@usz.ch

A 179 DNA sensing by the inflammasome
Veit Hornung

Host cytokines, chemokines and type I IFNs are critical effectors of the innate immune response to viral and bacterial pathogens. Several classes of germ-line encoded pattern recognition receptors have been identified which sense non-self nucleic acids and trigger these responses. Recently NLRP3, a member of the NOD-like receptor (NLR) family, has been shown to sense endogenous danger signals, environmental insults and the DNA viruses adenovirus and HSV. Activation of NLRP3 induces the formation of a large multiprotein complex in cells termed ‘inflammasome’ which control the activity of pro-IL-1β and pro-IL18 into their active forms. NLRP3, however, does not regulate these responses to double stranded cytolsic DNA. We identified the cytosolic protein AIM2 as a receptor for cytolsic DNA. AIM2 contains a HIN200 domain, which binds to DNA and a pyrin (PYD) domain, which associates with the inflammasome adapter molecule ASC to activate both NF-κB and caspase-1. Knock down of AIM2 down-regulates...
caspase-1-mediated IL-1β responses following DNA stimulation or vaccinia virus infection. Collectively, these observations demonstrate that AIM2 forms an inflammasome with the DNA ligand and ASC to activate caspase-1. We hypothesize that AIM2 plays a central role in innate immunity and host-defence to microbial pathogens, which can access the cytosolic compartment.

Corresponding Author: Veit Hornung, Prof. MD, University Hospital of Bonn, Institute for Clinical Chemistry and Pharmacology, Sigmund-Freud-Str. 25, 53127 Bonn, Germany, veit.hornung@uni-bonn.de

A 180

TLR and non-TLR mechanisms of ileus: sepsis and tissue trauma

Anthony Bauer, Bettina Buchholz, Takeshi Tsukamoto, R. Savahn Chanthaphavong, Hans Christoph Pape, Alexander Stojadinovic

Objective: Investigate the mechanisms of lipopolysaccharide/TLR4-mediated ileus and tissue trauma-induced ileus.

Methods: Bone marrow transplanted (BmTx) TLR4 chimeras, knockouts and an innovative subcutaneous trans-implanted syngeneic tissue-bone matrix (TBX) trauma model were used to assess the molecular and functional aspects of lipopolysaccharide (LPS) and tissue trauma-induced ileus.

TLR4 results: TLR4 ligation by LPS triggered a non-hematopoietic cell mediated early ileus. MyD88 deficiency completely protected mice from early endotoxin induced ileus while TRIF deficiency partially ameliorated ileus severity. LPS-induction of the primary downstream signaling element MyD88 was TLR4 dependent and was derived in equal amounts from both hematopoietic and the non-hematopoietic cells. Conversely, no induction of TRIF mRNA was detectable. Significant gene inductions of most inflammatory mediators were dependent on MyD88, while the TRIF pathway predominantly regulated the molecular level of CXCL10.

Conclusions I: Early endotoxin-induced ileus is TLR4-MyD88 non-leukocyte dependent. Both hematopoietic and non-hematopoietic cells contribute to LPS TLR4 sensitive inflammatory signaling within the intestinal muscularis. MyD88 and TRIF are non-redundant signaling pathways in early endotoxin-induced rodent ileus, but MyD88 is the essential adaptor molecule for transduction of TLR4-induced ileus and inflammatory signaling.

Trauma results: Increasing amounts of subcutaneous TBX (0–17.5% of body weight) progressively delayed gastrointestinal transit with higher amounts being lethal. In contrast, heated or decellularized TBX-17.5% had no effect. TBX-17.5% significantly suppressed both early and late ileus. The duration of gastrointestinal hypomotility is shown to be proportional to the severity of inflammation. Key characteristics in the inflammatory response are mast cell degranulation, activation of resident macrophages and influx of neutrophils in the intestinal muscularis. Peri-operative administration of lipid-rich nutrition has been shown to attenuate mast cell degranulation via activation of cholecystokinin receptors in several inflammatory models, including a rat model of postoperative ileus. In vitro experiments revealed that degranulation of mast cells is abrogated by stimulation of nicotinic receptors with acetylcholine. Additionally, lipid-rich nutrition reduced the intestinal manipulation-induced activation of intestinal macrophages and influx of neutrophils. Gastrointestinal motility was significantly promoted in a cholecystokinin-receptor dependent manner, by the inhibitory effect of lipid-rich nutrition on local inflammation.

Our data reveal a novel role for enteral nutrition in the prevention of postoperative ileus and may provide future insight in the beneficial effects of early enteral nutrition. Clinically, nutritional stimulation of the vagovagal anti-inflammatory reflex may be a new therapeutic option to treat postoperative ileus.

Corresponding Author: Tim Lubbers, MD, Maastricht University Medical Center, Department of General Surgery, Universiteitsring 50, 6200 MD Maastricht, The Netherlands, t.lubbers@ah.unimaas.nl

A 181

Nutritional treatment of postoperative ileus

Tim Lubbers

Postoperative ileus is a frequently occurring surgical complication, leading to increased morbidity and prolonged hospital stay. Abdominal interventions in particular are known to result in a protracted cessation of bowel movement. The introduction of enhanced recovery after surgery (ERAS) protocols has improved the course of postoperative ileus. An important pillar in ERAS is early enteral nutrition. Timely administration of nutrition via the oral route is believed to promote motility via release of neuropeptides and activation of digestive reflexes.

Recently, our group identified a novel application for enteral nutrition. Administration of lipid-rich nutrition was shown to reduce inflammation via a previously unidentified vagovagal reflex. The luminal presence of lipid-rich nutrition releases cholecystokinin, which stimulates cholecystokinin-1 receptors on vagal afferents. In turn, release of inflammatory cytokines is inhibited via efferent vagus-mediated activation of nicotinic receptors on inflammatory cells. An important event in the pathophysiology of postoperative ileus is the formation of inflammatory infiltrates in the intestinal muscularis. The duration of gastrointestinal hypomotility is shown to be proportional to the severity of inflammation. Key characteristics in the inflammatory response are mast cell degranulation, activation of resident macrophages and influx of neutrophils in the intestinal muscularis. Peri-operative administration of lipid-rich nutrition has been shown to attenuate mast cell degranulation via activation of cholecystokinin receptors in several inflammatory models, including a rat model of postoperative ileus. In vitro experiments revealed that degranulation of mast cells is abrogated by stimulation of nicotinic receptors with acetylcholine. Additionally, lipid-rich nutrition reduced the intestinal manipulation-induced activation of intestinal macrophages and influx of neutrophils. Gastrointestinal motility was significantly promoted in a cholecystokinin-receptor dependent manner, by the inhibitory effect of lipid-rich nutrition on local inflammation.

A 182

Prognostic value of NT-proBNP in patients with suspected severe infection in the emergency department

Joachim Wilhelm, Stefan Hettwire, Dorteje Hammer, Markus Schuermann, Henning Ebel, Karl Werdan

Objective: NT-proBNP is known to have a prognostic value, not only in patients with heart failure, but also in critically ill patients e.g. with severe sepsis and septic shock. In contrast to these ICU patients, those in the emergency department (ED) with a suspected severe infection may be in an earlier state with a lower severity of the disease. We tested the hypothesis that NT-proBNP is also of prognostic relevance in patients with suspected severe infection at the time of admittance to the ED.

Methods: Patients with suspected severe infection in the ED were included in the study. NT-proBNP and procalcitonin (PCT) levels
were determined, and the APACHE II score was calculated at admission. Patients with a PCT value $\geq 2$ ng/ml were considered as septic. The observational endpoint was 28-day-mortality. Measurements of NT-proBNP were provided free of charge by Roche Diagnostics, Germany.

Results: We analyzed 142 patients. 42.3% ($n = 60$) had a PCT value $\geq 2$ ng/ml and were considered as septic. Mean APACHE II score was $16.9 \pm 8.3$. Overall 28-day-mortality was 7.7% ($n = 11$). Mean NT-proBNP level was elevated to $6,784.1 \pm 13,253.1$ pg/ml (normal value $< 400$ pg/ml). Septic patients had significantly higher levels of NT-proBNP than non septic patients: $10,534.1 \pm 17,000.1$ vs. $4,040.3 \pm 8,786.6$ pg/ml, $p < 0.001$. Non survivors had significantly higher levels of NT-proBNP than survivors: $24,488.3 \pm 22,296.6$ vs. $5,300.9 \pm 11,199.6$ pg/ml, $p < 0.001$. Even in the subgroup without history of chronic heart failure (81%, $n = 115$) the mean NT-proBNP level of all patients was elevated to $4,667.2 \pm 4,950.5$ pg/ml. Also in this subgroup non survivors (5.2%, $n = 6$) had significantly higher levels of NT-proBNP than survivors: $13,690.7 \pm 14,278.7$ vs. $4,170.5 \pm 8,947.8$, $p < 0.01$. The AUC value of ROC curve analysis for mortality of NT-proBNP was 0.883 in the whole population and was also significant in the subgroup without chronic heart failure: 0.869.

Conclusions: In the early state of a severe infection, patients show elevated levels of NT-proBNP indicating an early impairment of myocardial function. The significant higher values of septic compared to non septic patients indicate the relevant impact of sepsis on cardiac function. The significant higher levels of NT-proBNP within non-survivors and the high AUC values underline the prognostic relevance of NT-proBNP at the time of admittance of patients with suspected severe infection, no matter if chronic heart failure was reported previously or not.

Corresponding Author: Joachim Wilhelm, University of Halle/Saale, Department of Medicine III, Ernst-Grube-Str. 40, 06120 Halle/Saale, Germany, joachim.wilhelm@medizin.uni-halle.de

A 183

Differential expression of TIMP-1 and MMP-9 in multiple major trauma

Mareen Brumann, Viktoria Bogner, Wolf Mutschler, Peter Biberthaler

Background: Metalloproteinases are secreted in response to a variety of inflammatory mediators and inhibited by tissue inhibitors of matrix-metalloproteinases (TIMP). Previous genome wide microarray studies showed that tissue inhibitor of metalloproteinases-1 (TIMP-1) is upregulated and differentially expressed depending on clinical outcome. The aim of the study was to evaluate the time course of TIMP-1 and MMP-9 expression in the early posttraumatic period. Therefore protein levels and the balance between TIMP and MMP activities were investigated in a large patient collective.

Patients and methods: Sixty patients presenting with blunt multiple injuries (ISS $> 16$ points) were included. According to injury severity patients were separated in two groups (group one: ISS 16–40, group two ISS 40–75). Blood samples were drawn on admission and 6, 12, 24, 48 and 72 h after trauma. For analyses of TIMP-1 and MMP-9 serum concentrations, sample dilutions of approximately 1:1,000 to 1:5,000 (for TIMP-1) and 1:100 to 1:500 (for MMP-9) were required and quantified by ELISA technology. Resulting data was statistically analyzed using Mann–Whitney Rank Sum Test (SigmaStat).

Results: Five of 60 patients did not survive the traumatic event (90-day survival). TIMP-1 showed a significant overexpression in group one (42 patients) and in group two (18 patients) in the very early posttraumatic period (0, 6, 12 h, $p < 0.05$). MMP-9 expression is significantly downregulated in group one in this period. Regarding the time course of MMP-9 expression, no significant differences were found in group two. The initial overexpression (TIMP-1) and downregulation (MMP-9) was followed by a constant level of protein expression without significant differences in both groups. No significant differences could be observed while comparing patients of group one (ISS $< 40$) and two (ISS $> 40$) regarding protein expression.

Conclusions: The very early posttraumatic period is characterized by an overexpression of TIMP-1 and a downregulation of MMP-9. In regard to these results, the inversely proportional balance between TIMP-1 and MMP-9 activities could be used to evaluate the very early posttraumatic period in multiple major trauma. Only severely injured patients (ISS $> 40$) do not show changes of MMP-9 expression level in the early posttraumatic period. Considering the results of this study the expression of TIMP-1 and MMP-9 is not associated with the severity of trauma and not significantly correlated with clinical outcome.

Corresponding Author: Mareen Brumann, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Department of Trauma Surgery, Nassaustr. 20, 80336 Munich, Germany, Mareen.Brumann@med.uni.muenchen.de

A 184

Stratifying mortality risk of multiple injured patients after 6 h post trauma by new target parameters in neutrophils

Viktoria Bogner, Thomas Giese, Wolf Mutschler, Peter Biberthaler

Objective: Patients after multiple, severe trauma suffer from posttraumatic immune system destabilization and subsequent multiple organ dysfunction. This closely influences the patients’ further clinical development. Precedent genome wide microarray profiling in monocytes after multiple injury revealed several potential outcome markers like BCL2A, MMP-9 and ETS-2. The impact and predictive value of this profile in the most represented immune cells—neutrophils—and their diagnostic value for stratifying the mortality risk of multiple injured patients in the initial post trauma period was intended to be evaluated.

Patients and methods: 40 multiple injury patients (ISS $> 16$ points) were included. Blood sampling and neutrophil separation was performed on admission of the patient and at 6, 12, 24, 48 and 72 h after trauma. Neutrophil expression levels of the target genes BCL2A, MMP-9, and ETS-2 by RT-PCR. Patients were assorted into groups according to the distinct clinical criterion “90-day survival”. Statistics were calculated by $t$ tests, Mann–Whitney-rank-sum test and receiver operating curves.

Results: Patients who did not survive the traumatic event (90-day survival) exhibit significant higher BCL2A levels at all blood sampling time points. Significant differences are observed already as early as 6 h after trauma in comparison to patients with a favourable outcome $p < 0.005$. ROC curve (data given as p, AUC and 95% confidence interval): $p < 0.0001, 0.78, 0.63–0.93$. Neutrophil MMP-9 significant differs in patients who deceased as compared to those who survived at 6 h: $p < 0.011$, ROC curve $p < 0.001, 0.75, 0.58–0.93$ and ETS2 expression was also significantly increased at 6 h after trauma in patients with unfavourable outcome $p < 0.03$, ROC: $p < 0.01, 0.71, 0.51–0.90$.

Conclusion: The present study demonstrates for the first time a subsequent, serial mRNA expression study of a regulating gene network in circulating neutrophils in patients after multiple, severe trauma. Selected genes of this network show a highly significant association with clinical outcome and thereby may have potential to classify high-risk patients in the very early post-injury period.
A 185
New markers of inflammation-induced renal injury subside when endotoxin tolerance develops in humans as measured by urine proteomics

Suzanne Heemskerk1,2, Annelies Draisma1, Coby Laarakkers1, Johannes van der Hoeven1, Rosalinde Masereeuw2, Peter Pickkers1
1Department of Intensive Care Medicine, 2Department of Pharmacology and Toxicology, 3Department of Clinical Chemistry, Radboud University Nijmegen Medical Centre, The Netherlands

Background: Sepsis has been identified as the most common cause of renal injury in intensive care units although the pathophysiology is not well understood. No large clinical studies are available that show an improvement of renal function in patients with sepsis and this may be related to the lack of early diagnostic tests that indicate the onset of renal injury.

Objective: The aim of the current study was to search for potential new early markers of renal injury during acute endotoxaemia and to investigate whether renal injury can be ameliorated by the induction of lipopolysaccharide (LPS) tolerance.

Methods: Healthy males received iv bolus injections of 2 ng/kg/day E. coli LPS for five consecutive days. We used Surface enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF MS). This approach allows for rapid high throughput profiling of multiple urine samples and detects low molecular weight biomarkers.

Results: Repeated LPS administrations induced a diminished glomerular filtration rate of 33 ± 7% (p = 0.02) on day 2 and an increase in serum creatinine of 11 ± 3% (p = 0.002) on day 3, which was associated with the appearance of 15 peak intensities in the urinary protein profile including an increase in b2-microglobuline levels (p = 0.04) 6 h after the first LPS administration. Four of the 15 peak intensities on day 1 correlated with serum creatinine levels on day 3; 3,950, 4,445, 6,723 and 7,735 mV (p = 0.05; 0.01; 0.02 and 0.05 respectively). With the development of LPS tolerance, renal function restored, reflected by a decrease in serum creatinine and b2-microglobuline levels to baseline (p = 0.2 and 0.4 respectively, between day 1 and 5), and by attenuated peak intensities in the urinary protein profile (p < 0.0001 for all 15 peak intensities).

Conclusion: In conclusion, renal injury occurs during repeated endotoxemia and can be predicted by new urinary markers using proteome research. The four markers that correlated with the extent of renal injury may represent potential new biomarkers for renal injury and need further identification. The inflammation-induced renal injury subsided when LPS tolerance developed after five consecutive days of LPS administrations.

Corresponding Author: Lucas van Eijk, MD, Radboud University Nijmegen Medical Center, Department of Intensive Care Medicine, Geert Grootplein, 6500 HB Nijmegen, The Netherlands, l.vaneijk@aig.umcn.nl

A 187
LBP and sCD14 patterns in a human in-vivo trauma model

Daniel Bastian, Margaret V. Tambursten, Ståle Petter Lyngstadåas, Olav Reikerås

Objective: This study on an aseptic human musculoskeletal trauma wants to elucidate the physiological reactions of soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP) after an elective, aseptic trauma. Endotoxin binding proteins have been targeting molecules in the treatment of SIRS and sepsis, but there is still a lack of knowledge about the physiological behavior after trauma.

Membrane CD14 together with LBP present endotoxin to its receptor TLR4/MD-2. sCD14 is able to present the LBP-lipopolysaccharide-complex to CD14 negative cells, furthermore sCD14 modulates the biological activity of circulating endotoxin. LBP can inhibit responses to LPS in higher concentrations, whereas it has a stimulating performance in low concentrations.

Patients and methods: The applied in-vivo model is a cementless total hip arthroplasty, which is a defined trauma to bone and muscles in conjunction with a certain amount of blood loss. Seven patients (four females) with coxarthrosis but without co-morbidity (ASA1) were operated. There was given high doses of Cephalotin per-operatively (8 g within 12 h). Venous blood samples were taken before operation, and 1 h, 3 and 6 days after surgery. LBP and sCD14 were measured by conventional ELISA. To correct for the hemodilution effect, each thereof abrogating the iron supply to the bone marrow, and inducing ‘anemia of inflammation’. The role of hepcidin in the development of acute anemia of inflammation observed in the first 2 weeks of human sepsis has not yet been identified. Therefore, we explored the association between hepcidin and sepsis-associated anemia.

Material and methods: 92 consecutive patients were enrolled after presentation on the emergency ward of a university hospital with sepsis, indicated by the presence of ≥2 SIRS criteria, and a proven or suspected infection. Blood was drawn at day 0, 1 and 2 after admission for the measurement of IL-6 and hepcidin-25 (by SELDI-TOF MS). IL-6 levels were correlated with hepcidin concentrations. Hemoglobin levels and data of blood transfusions during 14 days after hospital admission were retrieved and decreases in hemoglobin compared to admission levels were correlated to hepcidin levels. Patients who received a blood transfusion were excluded for correlation analysis.

Results: 53 men and 39 women with a mean age of 53.3 ± 18 were included. Hepcidin levels were highest at admission (19.0 [1.2–72.7] nmol/l) and decreased to normal levels in most patients within 3 days (9.6 [0.8–40.5] nmol/l). Hepcidin levels increased with more SIRS criteria (p = 0.049). Highest IL-6 levels were measured at admission (288.3 ± 45.0 pg/ml) and significantly correlated with hepcidin levels at admission (r = 0.38, p = 0.007) and on day 1 (r = 0.32, p = 0.029). Correlation analyses showed significant associations between hepcidin levels on day 1 and 2 and decreases in hemoglobin from day 7–14, with strongest correlation between hepcidin on day 2 and decreases in hemoglobin from day 9–11 (r ranging from −0.70, p = 0.001 to −0.86, p < 0.001, respectively).

Conclusions: Our study demonstrates that increased IL-6 concentrations in septic patients are associated with hepcidin concentrations. Moreover, the increased hepcidin concentrations observed in early sepsis negatively correlated with hemoglobin levels during the hospital stay of these patients. This suggests that hepcidin release is an important modulator of anemia in septic patients with systemic inflammation.

Corresponding Author: Lucas van Eijk, MD, Radboud University Nijmegen Medical Center, Department of Intensive Care Medicine, Geert Grootplein, 6500 HB Nijmegen, The Netherlands, l.vaneijk@aig.umcn.nl
Platelet aggregation plays an important role in the initial series of events leading to the development of sepsis. TREM-like transcript 1 (TLT-1) is a membrane protein localized specifically in the alpha-granules of the normal platelets. Upon activation a soluble form sTLT-1 is released in the plasma and can be quantified by elisa. Studies have suggested that TLT-1 regulates coagulation and inflammation at the sites of injury. We want to evaluate the value of soluble TLT-1 as a marker of sepsis.

Patients and methods: Patient admitted to the burn acute care unit a Parkland hospital, Dallas, Texas with more than 15% TBSA (total body size area) burn injuries or inhalation were prospectively enrolled. Clinical data was collected daily. Blood taken at 1, 3, 5, 7, 14 days, and soluble TLT-1 levels were measured in plasma by ELISA. Levels were compared between sepsis and no sepsis patients. Twelve healthy volunteers were included.

Results: Seventy patients were enrolled. Of those 25 developed sepsis and four died. There were not significant clinical or demographic differences between sepsis and no sepsis patients. Soluble TLT-1 levels were significantly increased at all time points in patients with sepsis when compared to no sepsis patients. TLT-1 was not detected in healthy individuals. Table shows median (25–75) TLT-1 levels (µg/ml). In addition, we found that the four patients who died had the highest levels of TLT-1.

Conclusions: Our data suggests that measurements soluble TLT-1 levels could be useful as a diagnostic and prognostic marker for sepsis, and possible for mortality prognosis.

Table

<table>
<thead>
<tr>
<th>Day</th>
<th>No sepsis</th>
<th>Sepsis</th>
<th>p Value</th>
</tr>
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<tr>
<td>1</td>
<td>410 [234–5,820]</td>
<td>744 [440–2,600]</td>
<td>0.01*</td>
</tr>
<tr>
<td>3</td>
<td>113 [17–344]</td>
<td>665 [364–1,723]</td>
<td>0.01*</td>
</tr>
<tr>
<td>5</td>
<td>518 [435–619]</td>
<td>1,084 [357–1,998]</td>
<td>0.03*</td>
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<tr>
<td>7</td>
<td>670 [307–711]</td>
<td>1,863 [786–4,122]</td>
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<tr>
<td>14</td>
<td>323 [146–473]</td>
<td>1,082 [303–1,579]</td>
<td>0.04*</td>
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</table>


core data: For sepsis in burn patients

Corresponding Author: Daniel Bastian, MD, Oslo University Hospital, Department of Orthopaedic Surgery, Sognsvannsveien, 27 Oslo, Norway, bastian_no@yahoo.com

A 188

TLT-1 TREM-Like Transcript-1 as Diagnostic Marker for Sepsis in Burn Patients

Fernando Rivera, Ming-Mei Liu, Sarah Burris, Agnes Burris, Joseph Minei

Objective: Trauma is still one of the major causes for mortality of people younger than 50 years. Severely injured patients die either as a direct consequence of their injuries or from the development of life-threatening complications such as multiple organ failure. To estimate the seriousness of the condition, we hypothesized a correlation with circulating nucleosomes. These complexes of DNA and histone proteins are released from dying and stressed cells into the blood circulation and are frequently found to be elevated in stroke, trauma and sepsis as well as in various cancers. Patients and methods: To estimate the disease severity and the prognosis of trauma patients, we prospectively determined circulating nucleosomes in serum of 201 patients among them 77 with multiple trauma (44 with Injury Severity Score (ISS) ≥ 16, 33 with ISS < 16) and 124 patients with single fractures (46 femoral neck, 57 femoral shaft and 21 ankle fractures) as controls. Samples were obtained at admission to the hospital, and in polytrauma patients additionally during the first week at intensive care unit (ICU; N = 20). Disease severity was objectified by the Simplified Acute Physiology Score (SAPS II), the Glasgow Coma Scale (GCS) and the ISS. Complications and final clinical outcomes were monitored during the stay at ICU.

Results: Polytrauma patients (ISS ≥ 16) showed higher values of nucleosomes than patients with single smaller (ankle) fractures. However, patients with more extended (femur) fractures showed elevated values as well. In polytrauma patients (ISS ≥ 16), there were no significant correlations between nucleosome levels and severity of disease, measured by SAPS II and GCS. However, nucleosome levels were associated with severity of traumatic brain injury. Further, polytrauma patients (ISS ≥ 16) who died during stay at ICU had higher values of nucleosomes at admission than patients who survived. Resulting AUC for predicting hospital mortality was 69.6% with a 40% sensitivity at 100% specificity. During the follow up of the first week after trauma, nucleosomes showed a further increase after 24–48 h in many patients, which was paralleled by increases of creatine kinase and C-reactive protein, sometimes after a temporary decline which may be influenced by therapeutic procedures. Conclusion: Circulating nucleosomes in serum showed to be relevant for estimating prognosis in patients with multiple trauma.

Corresponding Author: Stefan Holdenrieder, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Institute of Clinical Chemistry, Marchioninistr. 15, 81377 Munich, Germany, Stefan.Holdenrieder@med.uni-muenchen.de

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Prognostic Impact of Circulating Nucleosomes in Patients after Multiple Trauma

Juliane Roessner1, Stefan Holdenrieder1, Juergen Durner1, Konrad Wolf2, Juergen Halbach2, W. Hoffmann2, Eduard F. Hoechert2

1Institute of Clinical Chemistry, University Hospital Munich-Grosshadern, 2Trauma Center Munich-Schwabing, Germany

Background: Immune dysfunction following trauma and critical illness is a known entity. There are both periods of immunosuppression
as well as immune hyperactivity. Program cell death receptor (PD-1) is an inhibitory receptor of the immune system that has recently been identified as being upregulated on circulating leukocytes of patients and experimental animals after trauma or sepsis resulting in T cell and monocyte/macrophage anergy. Interestingly, studies also indicate that this receptor and its cell associated ligands (PD-L1 and PD-L2) can also be shed. However, it is unknown whether PD-1 and/or PD-L1 or PD-L2 are released as well as what the role of these soluble isoforms may play in the development of immune dysfunction observed in response to a secondary infectious complication. To address this we designed the following clinical study.

Methods: ICU patients requiring bronchoscopy, because of either known aspiration or a clinical pulmonary infection score greater than or equal to 5 were included in this institutionally IRB approved study. The normally discarded initial aliquot of lavage was utilized for all assays. ELISA was used to assess the levels of PD-1 in the BAL fluid. Western Blot was used to identify soluble PD-L1 and PD-L2 relative to beta-Actin expression and inflammatory cytokines were measured using a cytometric bead array. Clinical data, including microbiology, were collected. Samples (n = 20) were assign to groups based on presence or absence of pneumonia defined as >10,000 CFU/ml on quantitative culture.

Results: In the samples that were positive for pneumonia (n = 6) there was a trend towards a decrease in the amount of PD-1 (0.059 vs. 0.326 ng/ml). Alternatively, PD-L1 levels were significantly increased in the pneumonia samples (0.683 vs. 0.47, p = 0.05). Concordantly, TNF alpha was increased in the pneumonia group (202.7 vs. 69.68 ng/ml, p = 0.04) while IL-10 tended to be lower (39.46 vs. 55.1 ng/ml), but not significantly. Other inflammatory markers, IFN gamma, IL-6, IL-4, and IL-2 were unchanged.

Conclusions: Here we report, for the first time, that soluble PD-1 and one of its ligands, PD-L1, appear to be altered in ICU patients who have developed bacterial pneumonia. Since this occurs as part of secondary/subsequent response to a infectious challenge in an already injured/critically ill patient, it is suggested that this may represent novel compensatory-tissue specific response. However, further study is need to elucidate the actual pathologic, diagnostic and/or therapeutic significance of these observations.

Corresponding Author: Tjaakje Visser, MD, University of Utrecht, Medical Center, Department of Surgery, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands, T.visser@umcutrecht.nl

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Diagnostic test properties of a model-based clinical biomarker for sepsis in critical care
Jacquelyn Dawn Parente, Dominic Lee, Jessica Lin, J. Geoffrey Chase, Geoffrey M. Shaw

Objectives: To quantify the accuracy of an hourly, “clinical real-time” model-based insulin sensitivity (SI) clinical biomarker as a diagnostic test for severe sepsis or septic shock in adults in the intensive care unit (ICU), and compare it to the diagnostic test properties of procalcitonin (PCT).

Material and methods: A clinical biomarker for sepsis was developed from retrospective model-based insulin sensitivity glycemic control data from 36 adult patients with sepsis in the Christchurch Hospital (NZ) ICU. Readily available bedside clinical data were included in the clinical biomarker, including: temperature, heart rate, respiratory rate, blood pressure, SIRS score, and their respective hourly rates of change. The clinical biomarker was calculated to maximize discrimination between sepsis and non-sepsis cohorts. Kernel density estimates were used for development of joint probability density profiles for sepsis and non-sepsis data hours (213 and 5,858 respectively of 6,071 total hours) and for classification. The stratified bootstrap method and in-sample estimates were used to estimate bounds for prediction error of the classification model. Area under the ROC curve (AUC) was calculated to evaluate the discriminative ability of the test across a full range of probability cutoff values. From the ROC, the optimal probability cutoff value for classification was determined.

Results: The clinical biomarker for sepsis diagnosis at an optimal probability cutoff value of 0.319 has 94.4% sensitivity, 94.4% specificity, ROC AUC of 0.989, positive predictive value (PPV) of 37.78%, and negative predictive value (NPV) of 99.78% for insample data. The optimal probability cutoff value of 0.269 has 69.5% sensitivity, 74.6% specificity, ROC AUC of 0.783, PPV of 9.11%, and NPV of 98.52% for stratified bootstrap data. The overall result lies between these estimate bounds. The high ratio of non-sepsis to sepsis hours is clinically realistic, but provides too limited data for high positive predictive performance.

Conclusions: The clinical biomarker provides an effective real-time negative predictive diagnostic for sepsis with high accuracy. Comparing sensitivity and specificity the clinical biomarker equals or exceeds the performance of PCT, which is only typically measured daily. The hourly diagnostic presented is unique and can provide real-time early detection of sepsis. A prospective validation trial is ongoing at Christchurch Hospital.

Corresponding Author: Jacquelyn Dawn Parente, MSc, University of Canterbury, Mechanical Engineering, Private Bag 4800, 8140 Christchurch, New Zealand, jacquelyn.parente@pg.canterbury.ac.nz
A 193
Trauma induced 70 kDa heat shock protein (Hsp70) release: associations with injury severity and outcome
Gabor Nardai, Balazs Nagy, Szabina Guszejnov, Zoltan Prohaszka

Objective: Trauma induced inflammation is a very complex immunologic response. Numerous system, as immune cells, endothel, protein cascades and small molecules are involved in the process. Recent in vitro and animal experiments proved the significant role of the heat shock protein family during the activation of the innate and adaptive immune systems, but we have much less information about the clinical appearance and relevance of this contribution. In this trauma cohort study we characterized injury parameters associated to increased serum Hsp70 level after trauma and analyzed its relationship with outcome parameters.

Patients and methods: A single-center prospective study on adult, multiple injured patients, transferred to hospital within 3 h after trauma (n = 95) was performed. Blood was taken at admission and 1, 3, 6, 12, 24 h later for Hsp70 measurements. Samples were stored in freezer (−70°C) and Hsp70 levels were measures by ELISA later. Demographic data, injury description parameters were registered, haemodynamic status and therapeutic interventions were collected during the first 48 h, outcome parameters were followed until discharge or day 30. Statistical analysis: unpaired t test, Mann–Whitney and Chi-square tests, level of significance: p < 0.05*.

Results: We found that higher serum Hsp70 levels (>10 ng/ml) were measured after high energy, more extended, more severe injuries (ISS: 24 vs. 30*), major pelvic and abdominal trauma was more common (20 vs. 39%) and patients with higher Hsp70 at admission required more fluid and transfusion during the first day (7,500 vs. 8,900 ml*, 3.8 vs. 6.8 unit RBC*). Brain injured and older patients with similar ISS showed lower Hsp70 levels than non-brain injured or younger victims. Associations with complications: not only the occurrence of systemic inflammatory response and infections in high Hsp70 group (51 vs. 77%*, 53 vs. 62%) but septic complication related mortality was also higher (0 vs. 17%*).

Conclusion: Our results support the hypothesis, that Hsp70 is mainly released from damaged tissues (necrosis) after trauma and elevated Hsp70 level might have relevant contributions to the over-activated inflammatory response leading to septic complications.

Corresponding Author: Gabor Nardai. MD, PhD, Peterfy Hospital, Trauma Center, Anesthesiology and Intensive Care, Fiumei 17, 1081 Budapest, Hungary, nardai@hotmail.com

A 194
Myeloid derived suppressor cells in severely injured patients
Janesh Pillay, Vera Kamp, Tjaakje Visser, Peter Pickkers, Luke Leenen, Leo Koenderman

Objective: Myeloid derived suppressor cells (MDSCs) have been implicated to suppress immune responses in various animal models of cancer and infections. Severely injured patients suffer profound immune defects, which makes them susceptible to infections during ICU stay, increasing mortality and morbidity. As MDSCs have not adequately been characterized in humans, their occurrence in severely injured patients is unknown.

Patients and methods: Blood was sampled from healthy volunteers receiving 2 ng/kg LPS intravenously. In addition blood was sampled from severely injured patients requiring ICU admission. A distinct neutrophil subset was identified and sorted using FACS sort. These neutrophils were characterized by determining phenotype and functionality. Suppression of T cell responses was studied in various co-culture experiments and was measured by T cell proliferation and Interferon-gamma production. Identification of the pivotal role of neutrophil MAC-1 in mediating T cell suppression allowed us to assess this mechanism in severely injured patients.

Results: In a human model of systemic inflammation a distinct subset of CD16bright/CD62Ldim neutrophils appeared in the circulation. These cells elicited a hypersegmented morphology and normal anti-microbial capacity. This neutrophil subset were found to suppress T cell proliferation in response to PHA, CD3/CD28 or tetanus toxoid up to 70% when added in a 2:1 ratio. Suppression required cell contact between neutrophils and T cells mediated by formation of an immunological synapse requiring neutrophil MAC-1. Importantly these cells were found in severely injured patients and blocking MAC-1 resulted in an increased T-cell proliferation when this neutrophil subset was present.

Conclusion: Release of a large amount of suppressive neutrophils (up to 15% in systemic inflammation) can induce profound T-cell defects in severely injured patients and might contribute to the severe immune dysfunction seen in acutely ill patients. Identification of MAC-1 requirement for this suppression provides tools to target immune dysfunction in critically ill patients.

Corresponding Author: Janesh Pillay, MD, University of Utrecht Medical Center, Department of Respiratory Medicine, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands, jpillay@umcutrecht.nl

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CRP-VLDL complexes in sepsis
Guozheng Wang

C-reactive protein (CRP) can increase up to 1,000-fold in blood and form complexes with very low density lipoproteins (VLDL). These complexes are associated with worse outcomes for septic patients, suggesting a pathological role. In this study, we demonstrate that complex formation is heightened when CRP is over 200 mg/l and that CRP-VLDL complex levels are associated with sepsis severity as well as blood bacterial culture positivity. Using a mouse bacteraemia model, blood bacterial clearance is delayed by intravenous injection of in vitro generated CRP-VLDL complexes. The complexes are more efficiently taken up by activated U937 cells in vitro and Kupffer cells in vivo than VLDL alone. Both in vitro generated and naturally occurring CRP-VLDL complexes reduce phagocytosis of bacteria by activated U937 cells. Fcγ and scavenger receptors are involved in the uptake of both CRP-VLDL complexes and bacteria thereby a competitive mechanism for clearance is demonstrated. Interaction of phosphocholine groups on VLDL with CRP is the major driver for complex formation. Phosphocholine disrupts the complexes with reversal of their inhibitory effects on both phagocytosis and bacterial clearance. Thus, we propose that the increased formation of CRP-VLDL complexes is harmful and could be a novel therapeutic target in sepsis.

Corresponding Author: Guozheng Wang, MD, PhD, The University of Liverpool, Royal Liverpool University Hospital, Liverpool L7-8XP, UK, G.Wang@liverpool.ac.uk

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Phosphorylative post-translation changes of peroxisome proliferator activated receptor-γ; in lipopolysaccharide-stimulated macrophages
Basilia Zingarelli, Giovanna Piraino, Michael O’Connor

Objective: Peroxisome proliferator activated receptor-γ (PPAR-γ) is a nuclear transcription factor, which has potent anti-inflammatory
properties in addition to its regulatory role in lipid and glucose metabolism. However, PPARγ is markedly downregulated in several tissues during sepsis. Here we investigated whether activation of the extracellular receptor activated kinases 1/2 (ERK1/2) and subsequent post-translational phosphorylative changes are responsible for the reduced availability of the receptor after endotoxin challenge. Material and methods: J774.1A macrophages were stimulated with *E. coli* lipopolysaccharide (LPS, 100 ng/ml) up to 24 h. To investigate the role of ERK1/2, cells were pretreated 30 min prior to LPS with vehicle or U0126, a specific inhibitor of MEK that is an upstream regulatory kinase of ERK1/2.

Results: Stimulation of cells with LPS increased nuclear content of the active phosphorylated form p-ERK1/2 within 15 min up to 6 h (3.41 ± 0.07-fold increase over basal levels). Expression of p-ERK1/2 declined thereafter and returned to basal levels at 24 h after LPS challenge. LPS stimulation also induced an increase of nuclear content of PPARγ within 15 min. However, PPARγ content was downregulated thereafter with lowest levels at 6 h (0.32 ± 0.12-fold increase). At 24 h after LPS challenge, PPARγ protein was replenished. Because phosphorylation of serine residues may trigger the receptor degradation, we determined whether PPARγ might be phosphorylated. By immunoblotting analysis, phosphorylation of PPARγ correlated with downregulation of the total nuclear content of the receptor. Specifically, a weak band of phosphorylated PPARγ appeared at 15 min, which steadily increased up to 6 h (4.62 ± 0.18-fold increase), while it declined at 24 h after LPS stimulation. Pretreatment of the cells with U0126 (5 μM) significantly (p < 0.05) inhibited LPS-induced ERK1/2 activation (1.02 ± 0.03-fold increase) and phosphorylation of PPARγ (1.13 ± 0.24-fold increase), whereas it preserved the total nuclear content of the receptor (1.10 ± 0.17-fold increase) when compared with vehicle-treated cells. Pretreatment with U0126 (5–20 μM) resulted in a dose-dependent increase in PPARγ DNA binding activity in unstimulated control cells.

Conclusion: Our data suggest that ERK1/2 may directly phosphorylate PPARγ, contributing to its degradation. This finding also suggests that therapeutic intervention to enhance PPARγ functions may also target ERK1/2 activation.

Corresponding Author: Basilia Zingarelli, MD, PhD, Cincinnati Children’s Hospital Medical Center, Department of Critical Care Medicine, 3333 Burnet Avenue, MLC2005, Cincinnati, OH 45229, USA, basilia.zingarelli@cchmc.org

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Activation of nuclear factor-kappa B is differentially regulated in diet-induced obesity after sepsis

Jennifer Kaplan, Marchele Nowell, Paul Hake, Michael O’Connor, Alvin Denenberg, Basilia Zingarelli

Objective: Obesity is associated with an enhanced inflammatory response. In addition to the local adipose tissue inflammatory response in obesity, there is a systemic pro-inflammatory response. The mechanisms underlying this chronic inflammatory response remain unknown but data suggests nuclear factor-κB (NF-κB) may play a role. Therefore we sought to determine the effects of a high fat diet on the inflammatory response in sepsis. We hypothesized that obesity augments the NF-κB pathway is sepsis.

Material and methods: Six-week old C57BL/6 mice were randomized to a high fat diet (HFD) (60% kcal fat) or a standard diet (control) (16% kcal fat). Following 3 weeks of feeding, polymicrobial sepsis was induced by cecal ligation and puncture (CLP). Plasma and liver tissue were obtained for subsequent analysis at various time points after CLP. A p value of ≤ 0.05 was considered significant.

Results: Plasma levels of IL-6 and TNFα were increased in both normal chow and HFD mice at 18 h after CLP compared to time 0. There was no difference in plasma endotoxin levels between animals on a HFD versus controls. Nor was there a difference in bacterial load in the lung, liver, blood, peritoneal fluid or spleen between HFD versus control mice. Analysis of liver myeloperoxidase (MPO) activity indicated significant neutrophil infiltration in the liver at baseline (prior to CLP) in animals fed a HFD vs. control [median 1.26 vs. 0.59 U100 mg tissue, p < 0.05 by Mann–Whitney Rank Sum Test (MWRST)]. Liver MPO activity was higher in mice fed a HFD at 18 h after CLP versus control. Mice on a HFD had a significant increase in liver NF-κB activation at 18 h after CLP versus control (0.15 vs. 0.07 relative NF-κB units, p < 0.05 by MWRST) as evaluated by transcription factor assay and by electrophoretic mobility shift assays. In control mice, liver cytosol protein expression of phosphorylated IkBα (p-IkBα) was increased and corresponded with degradation of IkBα at 18 h after CLP as evaluated by western blot. However mice fed a HFD had no significant change in cytosol p-IkBα or IkBα protein levels.

Conclusions: NF-κB activation is increased in both normal and high fat mice after polymicrobial sepsis. However in high fat fed mice NF-κB activation occurs even in the absence of IkBα degradation. These results suggest that the signaling pathways leading to transcriptional activation of NF-κB in obesity may differ from pathways in non-obese mice following sepsis.

Corresponding Author: Jennifer Kaplan, MD, Cincinnati Children’s Hospital Medical Center, Department of Critical Care Medicine, 3333 Burnet Avenue, MLC2005, Cincinnati, OH 45229, USA, Jennifer.Kaplan@cchmc.org

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The ASC-dependent inflammasome protects against tubular injury and induces inflammation following progressive renal injury

Wilco Pulsakens, Loes Batter, Gwen Teske, Feryaz Sutterwala, Sandrine Florquin, Jaklien Leemans

Objective: Tubulo-interstitial injury is a common finding in the chronically failing kidney and can be characterized by a cascade of events, including inflammation and fibrosis. The intracellular multiprotein complex called “inflammasome” contributes to the induction of inflammation through maturation and subsequent release of effector cytokines. Although the mechanism of activation is still unclear, several endogenous danger ligands are identified that can activate the inflammasome upon tissue injury. Furthermore, it is known that inflammasome components, NALP3 and ASC are expressed in both inflammatory and epithelial cells. However, the role of the inflammasome in progressive renal injury is yet unknown.

Methods: C57BL/6 wild type and ASC−/− mice (n = 7/group) were subjected to unilateral ureter obstruction (UUO) by a permanent double ligation of the right ureter. The contralateral kidney served as an internal control. Mice were subsequently sacrificed 1, 3, 7 and 14 days post-UUO to determine renal injury, inflammation and renal fibrosis. Renal mRNA levels were determined by quantitative RT-PCR and renal cytokines were measured by ELISA.

Results: Following UUO, ASC mRNA remained constitutively present, whereas a strong increase of NALP3 mRNA was observed when compared to contralateral kidneys. Interestingly, ASC−/− mice displayed significantly enhanced levels of renal injury 1, 3 and 7 days post-UUO when compared to wild type mice. Fourteen days post-UUO maximal levels of tubular injury were reached in both groups and no differences could be observed. ASC−/− mice demonstrated 1 day post-UUO a diminished number, but 3 days post-UUO an
enhanced number of proliferating tubular epithelial cells. Moreover, 14 days post-UUO the number of apoptotic cells was reduced in ASC−/− mice. Moreover, ASC−/− mice displayed a reduced influx of granulocytes (1 and 14 days post-UUO), accompanied by reduced levels of the renal proinflammatory cytokine IL1β (t = 1). Macrophage accumulation was comparable to wild type mice at all time points. ASC deficiency did not affect accumulation of myofibroblasts and total collagen deposition in the kidney or renal TGFβ concentration at all time points. Preliminary data demonstrated that NALP3−/− mice had also significant higher levels of tubular injury compared to wild type mice 1, 3 and 7 days post-UUO.

Conclusion: The ASC-dependent inflammatory processes protect against tubular injury following progressive renal injury, associated with differences in the number of tubular epithelial cells that were proliferating or turned apoptotic. Moreover, the ASC-dependent inflammatory processes contributes to the initiation of an exaggerated inflammatory response following UUO, whereas no role in renal fibrosis was observed for the ASC-dependent inflammatory processes.

Corresponding Author: Wilco Pulskens, MSc, Academic Medical Center, Department of Pathology, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, w.p.pulskens@amc.uva.nl

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mRNA coding the VWF cleaving protease is decreased under proinflammatory conditions—reversal by co-incubation with activated protein C and selenate
Ralf Claus, Ralf Claus, Florian Conradt, Maik Sossdorf, Wolfgang Loesche, Michael Bauer

In sepsis, the severity-dependent decrease of the VWF-cleaving protease ADAMTS13 is a common phenomenon, which may contribute to aggregation of platelets/platelet consumption and the development of sepsis-associated thrombotic microangiopathy (TMA) and organ failure. Up to now, hepatic stellate cells (HSC) are considered to function as the primary source of ADAMTS13 protein. The underlying mechanisms of the decrease in sepsis remain unclear. Here we present data obtained in in-vitro experiments using cultured human HSC (LX2-line) and microvascular endothelial cells (HMEC) stimulated under proinflammatory conditions. Monolayers were exposed to (a) cytokines known to be plasma abundant/relevant during systemic inflammation (TNF, IL1beta, interferon gamma), (b) to bacterial endotoxin (100 ng/mL), (c) to a mixture of cytokines/ endotoxin, or (d) freshly prepared serum obtained from patients (n = 12) with severe sepsis/septic shock. Both cell lines expressed ADAMTS13 mRNA as quantitated using qPCR normalized to a set of unvaried genes. Overall, incubation with cytokines resulted in a decrease of ADAMTS13 mRNA to different extents ranging between 40–80% of basal transcription rate in between 24 h. Furthermore, in endotoxin treated cells, ADAMTS13 declined to 60% (HSC) or 65% of basal levels. This effect was more pronounced by the mixture of cytokines/endotoxin to levels of 55% (HSC) or 40% (HMEC). In monolayers treated with serum from patients with sepsis, only 10% (HSC) or 49% (HMEC) of basal level was determined. Both, the trace element selenium and activated protein C, which are used in the supportive therapy of patients with sepsis, ameliorates the decrease in serum treated HSC cells and increased the level of ADAMTS13 transcript in endothelial cells. Continuous infusion adapted to body weight also abolished the decrease of ADAMTS13 expression in hepatic tissues during the course of polymicrobial sepsis in mice.

In conclusion, we found that mRNA coding ADAMTS13 protein is also present in endothelial cells. Also we observed a marked decrease in both cell lines undergoing proinflammatory stimulation. This mechanism may contribute to the decline of proteolytic activity of ADAMTS13 in patients with sepsis and sepsis-associated TMA. Furthermore, the amelioration of this effect by selenate and APC may function as mechanisms resulting in a more favorable outcome observed in a number of clinical studies.

Corresponding Author: Ralf Claus, University Hospital Jena, Department for Anesthesiology and Intensive Care Therapy, Erlanger Allee 101, 07743 Jena, Germany, ralf.claus@med.uni-jena.de

A 200
System biology of multiple organ dysfunction: formation of ceramide-enriched macro-domain in SIRS/sepsis: hyperresponsiveness of sphingomyelinase deficient mice
Ralf Claus, Iris Suckert, Gordon Otto, Benedikt Acht, Michael Bauer, Ralf Claus

Generation of bioactive lipids such as ceramide (Cer) and the formation of Cer-enriched macrodomains are regarded as mediators of SIRS and development of multiple organ failure. Therefore we addressed the question whether there is a difference in the plasma activity of the secreted isoform of the Cer-forming enzyme sphingomyelinase (SMPD1) in patients with various degrees of SIRS/sepsis of different origin as well as in a murine loss of function model. We found plasma activity in critically ill patients (median 262.3 pmol/ml h) were significantly higher than age matched controls (123.6). In patients with fatal outcome activity increased (+77.4) in comparison to survivors (−252.1). A severity dependent increase was also analyzed in patients with MODS following elective cardiac surgery. Beyond immunological detection of increased pSMPD1 in septic patients, we found an increase in Cer-enriched macrodomains in endothelial cells after stimulation with patients’ plasma, endotoxin or TNF. Also we found formation of ceramide enriched macrodomains by immuno-staining using specific antibodies directed against Cer, CD14 and Fas. In a loss of function model we identified 315 transcripts differentially regulated in circulating white blood cells, liver and lung by use of microarray technology as well as in the cytokine pattern/organ function parameters following poly-microbial cavity infection. Furthermore, host response in ko-mice were more pronounced with respect to bacterial load in lung, liver and blood, plasma cytokine levels, thrombocytopenia as well as delayed migration of neutrophils into hepatic tissue. In conclusion, the results provide demonstration of a bio-functional relevant activity of SMPD1 resulting in altered signal transduction in SIRS, which may contribute to the development of MODS.

Corresponding Author: Ralf Claus, University Hospital Jena, Department for Anesthesiology and Intensive Care Therapy, Erlanger Allee 101, 07743 Jena, Germany, ralf.claus@med.uni-jena.de

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Leukotriene synthesis during opsonin-dependent phagocytosis by neutrophils treated with Salmonella typhimurium lipopolysaccharide species of various chemotype
Galina Sud'ina, Anna Zagryazhskaya, Sabine Lindner, Svetlana Galkina, Zoryana Grishina, Dieter Steinhilber

Objective: We investigated the influence of the lipopolysaccharide (LPS) carbohydrate chain length on phagocytosis of serum-opsonized zymosan (OZ) by human neutrophils using deep-core mutant,
complete-core and smooth LPS chemotypes. This is the first study of LPS chain length effect on leukotriene (LT) synthesis regulation during phagocytosis in human neutrophils.

Methods: Phagocytosis of serum-opsonized zymosan by human polymorphonuclear leukocytes (neutrophils) was an experimental model. Incubations for study of arachidonic acid (AA) metabolism were performed using [14C]-AA labeled neutrophils. Immunoblotting was used for 5-lipoxygenase (5-LO) and nitric oxide synthase (NOS) detection. Fluorescent and light microscopy was used for NOS isoform visualization.

Results: Priming of neutrophils with different LPS forms selectively enhanced OZ uptake by human neutrophils. The specificity of action of various LPS forms on OZ uptake was noticeably abrogated by MK886, 5-LO activating protein (FLAP) inhibitor, suggesting modulation of phagocytosis by 5-LO-derived LTs. Selective influence of different LPS chemotypes on OZ uptake correlated with these LPS effects on LT synthesis. Priming by various LPS forms did not influence 5-LO translocation and affected a little the release of the 5-LO substrate arachidonic acid. Direct measurement of nitrite release from neutrophils revealed the effects of LPS on NO synthesis during OZ phagocytosis. NO-donor diethylamine NONOate inhibited 5-LO activity in cell-free assay. Further mechanistic studies revealed that NO modulates cellular 5-LO activity in a guanylyl cyclase and protein kinase G dependent manner. Staurosporine, a protein kinase C inhibitor, increased NO formation and partially reversed the specific influence of various LPS forms on NO production. The results provide the evidence that different rates of OZ uptake by neutrophils, primed by different LPS chemotypes were noticeably modulated by different NO synthesis decreasing LT production during phagocytosis.

Conclusions: Our data suggest that leukotriene synthesis is regulated by various LPS chemotypes via multiple mechanisms, which result in different levels of neutrophil phagocytic activity. To summarize we proposed a model for the observed LPS effects on LT synthesis during phagocytosis of OZ which considers LPS efficacy in dependence on its structure.

The work was supported by grants: Russian Foundation for Basic Research grant 07-04-00410 and DFG (GRK757).

Corresponding Author: Galina Sud‘ina, PhD, Moscow State University, Belozersky Institute of Physico-Chemical Biology, Leninskie Gori, 119991 Moscow, Russia, sudina@genebee.msu.ru

A 202
Tolerance induced by gram-positive BLP and gram-negative LPS in murine macrophages leads to two categories of gene expression with opposite functions during inflammation

David Power, Jihau Wang, HP Redmond

Background: Inflammation and sepsis are a leading cause of morbidity. Dysregulated inflammation can lead to various pathological outcomes including SIRS, septic shock syndrome or even death. Yet inflammation and its regulation remain incompletely understood. The inflammatory response is stimulated chiefly at the level Toll-like receptors (TLRs) and these in turn induce transcriptional activation of thousands of genes. However, these genes encompass a wide scope of activity. We hypothesise that, given these diverse actions, additional, more specific mechanisms exist to regulate these genes.

Methods: Mouse bone marrow macrophages were divided into two arms, a BLP arm and an LPS arm. Each arm contains a control and treatment group. The control groups were incubated with culture medium for 24 h, whereas the treatment groups were pre-treated with 100 ng/ml BLP or LPS for 24 h. Both groups were subsequently challenged with 1,000 ng/ml of BLP or LPS for 6 h as appropriate. RNA was extracted. DNA gels and spectrometry were performed to ensure quality. DNA microarray analysis (Affymetrix 430 2.0 mouse genome arrays) and sub-group analysis was performed using the NCBI database.

Results: 45,101 genes examined in both arms. A significantly up- or down-regulated gene deemed to be a 1.5-fold change, or greater, in gene expression. In the BLP arm, 9,401 genes were significantly up- or down-regulated (18,972 in the LPS arm). 4,237 BLP induced genes were down-regulated and coded for pro-inflammatory effects (9,803 LPS induced genes). 5,165 BLP induced genes were significantly up-regulated and coded for anti-microbial effects (9,169 LPS induced genes). The top 50 genes of each group were further sub-classified depending on their functions. One striking difference observed between the BLP and LPS arms was the number of significantly up-regulated genes involved in the process of pathogen recognition (BLP ~3-fold greater than LPS).

Conclusion: TLRs do not completely account for all inflammatory regulation and therefore additional component specific regulation must exist in the inflammatory process. BLP induces more potent anti-microbial activity than LPS.

Corresponding Author: David Power, Cork University Hospital, Department of Academic Surgery, Wilton, Cork, Ireland, drdavidpower@gmail.com

A 203
C-peptide: a potential modulator of NF-kB in the lung

Ranjit Chima, Timberley LaMontagne, Paul Hake, Giovanna Piraino, Basilia Zingarelli

Objective: The transcription factor nuclear factor-kB (NF-kB) plays a major role in modulating the inflammatory response. C-peptide is a 31-amino acid peptide cleaved form proinsulin during insulin synthesis. Initially thought to be inert, recent work suggests that it may modulate the inflammatory response in the setting of endotoxemia and ischemia reperfusion. However at this time the mechanism of action of this peptide remains unclear. Hence, we investigated the effect of C-peptide administration on the activation of the pro-inflammatory mediator NF-kB in the lung following hemorrhage and resuscitation.

Material and methods: Hemorrhage was induced in rats (age 3–4 months) by withdrawing blood from the femoral artery to a mean arterial pressure of 50 mmHg. Animals were kept in shock for 3 h at which time they were rapidly resuscitated by returning their shed blood. At the time of resuscitation and every hour thereafter, one group of animals received C-peptide (280 nm/kg IV) while another group received vehicle. Lung NF-kB (p65 subunit) activation, neutrophil infiltration and plasma interleukin 6 (IL-6) were measured at 1, 2 and 3 h after resuscitation by a transcription factor ELISA, myeloperoxidase assay and multiplex assay respectively.

Results: In vehicle-treated rats hemorrhage and resuscitation significantly induced p65 activation in the lung which changed 4.2-fold at 1 h and 1.4-fold over basal levels at 3 h after resuscitation. Following hemorrhage and resuscitation, C-peptide-treated rats had a significant reduction in p65 activation in the lung when compared to vehicle-treated rats (p < 0.05). Specifically, p65 activation in the lung changed 1.5-fold at 1 h and 0.9-fold over basal levels at 3 h after resuscitation. At 3 h following resuscitation this difference in p65 activation with C-peptide treatment was associated with a significant reduction in lung neutrophil infiltration (497 ± 51 vs. 794 ± 78 U/100 mg lung tissue, p < 0.05) and plasma IL-6 level (47 ± 25 pg/ml vs. 408 ± 172 pg/ml, p < 0.05) when compared to vehicle-treated.
Conclusions: Our data suggest that C-peptide reduces NF-kB activation in the lung following hemorrhage and resuscitation. This effect is associated with a reduction in neutrophil infiltration and systemic IL-6 levels.

Control of intestinal homeostasis, colitis and colitis-associated colorectal cancer by the inflammatory caspases

Maya Saleh, Jeremy Dupaul-Chicoine, Garabet Yeretssian, Philippe Gros, Nicole Beauchemin, Bruce Vallance

Inflammatory caspases are essential effectors of inflammation and cell death. Here, we investigated their roles in colitis and colorectal cancer and report a bimodal regulation of intestinal homeostasis, inflammation and tumorigenesis by caspases-1 and -12. Casp1-/- mice exhibited defects in mucosal tissue repair and succumbed rapidly following dextran sulfate sodium (DSS) administration. This phenotype was rescued by administration of exogenous IL-18 and was partially reproduced in mice deficient in the inflammasome adaptor ASC. Casp12-/- mice, in which the inflammasome is derepressed, were resistant to acute colitis and showed signs of enhanced repair. Together with their increased inflammatory response, the enhanced repair response of casp12-/- mice rendered them more susceptible to colorectal cancer induced by azoxymethane (AOM) + DSS. Taken together, our results indicate that the inflammatory caspases are critical in the induction of inflammation in the gut following injury, which is necessary for tissue repair and maintenance of immune tolerance to commensals.

Relevance of HMGB1, MIF and apoptotic markers in predicting response to neoadjuvant chemotherapy of breast cancer patients

Oliver J. Stootzer1, Debora I.M. Fersching2, Stefan Holdenrieder2

1Haematological Oncological Outpatient Center, Munich, 2Institute of Clinical Chemistry, University Hospital Munich-Grosshadern, Germany

Objective: Effective diagnostic tools indicating early the efficacy of therapy are needed to improve the individual management of breast cancer patients receiving neoadjuvant cytotoxic chemotherapy. As inflammatory and cell death processes are present during cancerogenesis as well as during antitumor therapy we investigated circulating cytokines and apoptotic markers on their relevance for therapy prediction and monitoring.

Patients and methods: The courses of HMGB1, MIF and apoptotic markers Fas, DNase, nucleosomes, M30 and surviving were determined in prospectively collected sera of 52 consecutive breast cancer patients receiving preoperative, neoadjuvant chemotherapy. Biomarker levels were correlated with therapy response objectified by pathological staging at surgery after eight treatment cycles according to RECIST-criteria. In addition, biomarkers were measured in sera of 29 healthy controls.

Results: Levels of HMGB1, MIF, Fas, M30, and surviving were determined in prospectively collected sera of 52 consecutive breast cancer patients receiving preoperative, neoadjuvant chemotherapy. Biomarker levels were correlated with therapy response objectified by pathological staging at surgery after eight treatment cycles according to RECIST-criteria. In addition, biomarkers were measured in sera of 29 healthy controls.

Conclusions: Our data suggest that C-peptide reduces NF-kB activation in the lung following hemorrhage and resuscitation. This effect is associated with a reduction in neutrophil infiltration and systemic IL-6 levels.
**A 207**
 Plasma concentrations of high mobility group box protein 1 (HMGB1), sRAGE and circulating DNA in patients with acute pancreatitis

Yvette Mandi, Yvette Mandi, Gyula Forkas, Tamás Takaıcs, Katalin Kocsis

Objective: High mobility group box protein 1 (HMGB1), a late-acting proinflammatory cytokine, is secreted actively by inflammatory cells, and released passively from necrotic cells. From the aspect that both inflammation and necrosis are involved in the pathogenesis in acute pancreatitis, the aim of the study was a joint investigation of the plasma concentrations of HMGB1, its soluble receptor, sRAGE, and the circulating DNA as a marker of cell death.

Methods: 62 patients with acute pancreatitis (30 mild, 32 severe), 20 patients with sepsis and 20 healthy controls were enrolled in the study. HMGB1 and sRAGE plasma levels were measured by means of ELISA. Plasma DNA concentrations were estimated by real time quantitative PCR for the β-globin gene.

Results: Circulating HMGB1 level was significantly higher in patients with severe acute pancreatitis (13.33 ± 2.11 ng/ml) than in healthy controls (0.161 ± 0.03 ng/ml) or than in patients with mild pancreatitis (2.64 ± 0.185 ng/ml). Plasma concentration of sRAGE was highest in patients with sepsis (2.210 ± 252 pg/ml), while the levels of sRAGE correlated inversely with that of HMGB1 in patients with acute pancreatitis. Plasma DNA level was significantly elevated in patients with severe acute pancreatitis (2.206 ± 452 ng/ml).

Conclusion: A complex study of the plasma levels of HMGB1, sRAGE and circulating DNA can be informative in evaluations of acute pancreatitis with different levels of severity.

**A 208**
 Human S100A8 and S100A9 activate phagocytes via toll-like receptor 4 independent of rage

Thomas Vogl, Marc Wolf, Kirsten Roebrook, Christina Ehrhardt, Marieke A.D. van Zoelen, Johannes Roth

Objective: Endogenous damage associated molecular pattern (DAMP) proteins are known as important pro-inflammatory factors of the immune system which are released during cellular stress. Recognition of DAMPs involves the multiligand receptor for advanced glycation end products (RAGE) and Toll-like receptors (TLRs) in sensing not only pathogen associated molecular patterns (PAMPs) but also endogenous proteins. Members of the fast growing family of DAMP proteins are besides heat shock proteins, HMGB1 or defensins also some members of the family of S100 proteins, which promote inflammatory processes. It was claimed that RAGE is involved in almost all S100 protein activities. We here investigated the capacity of human S100A8 (MRP8, myeloid related protein 8) and human S100A9 (MRP14) on activation of human phagocytes. S100A8 and S100A9 form homodimers as well as heterodimers and belong to the S100 family of EF-hand calcium-binding proteins. Both proteins are the major cytoplasmic proteins of phagocytes and are released at sites of inflammation by activated or necrotic phagocytes.

Methods and results: While human S100A8/S100A9-complexes did not show any phagocyte activation, S100A8 homodimers as well as S100A9 homodimers induce strong pro-inflammatory mechanisms in these cells. Human S100A9 induces intracellular translocation of MyD88 and activation of IRAK-1 as shown recently already for murine S100A8. Finally NF-κB activation results in elevated expression of TNF-a as well as other pro-inflammatory genes. In blocking experiments with TLR4-specific monoclonal antibodies we demonstrate that both S100 proteins specifically signals via TLR4 receptor complex on human phagocytes. Using TLR4-complex transfected HEK293 cells and RAGE transfected HEK293 cells we clearly can exclude the involvement of RAGE for at least these two S100 proteins. Our in vitro results could be further confirmed in vivo. Mice lacking S100A8/S100A9 are protected against abdominal sepsis induced by E. coli.

Conclusion: Our present data clearly demonstrate the importance of TLR4 by which phagocytes promote their own activation via expression and secretion of endogenous ligands of this receptor.

**A 209**
 Mammalian double stranded DNA induces complement Factor B expression in macrophages through a receptor for advanced glycation endproducts (RAGE)- and myeloid differentiation factor 88 (MyD88)-dependent mechanism

So Dam Kim, Joon H. Kwak, David J. Kaczorowski, Timothy R. Billiar

Objective(s): We have previously shown that macrophage expression of complement Factor B (Factor B) can be upregulated by Toll-like receptor 3 (TLR3) and 4 agonist. This observation suggests an important link between innate immune activators and complement activation in tissues following injury or infection. Here we extend our previous work by showing that double stranded mammalian DNA (dsDNA) is a potent inducer of macrophage Factor B expression. A number of DNA sensors in immune cells have been described. Here experiments were carried out to identify the receptors on macrophages required to sense DNA for increased Factor B expression. Material and methods: We used RAW 264.7 macrophages and peritoneal macrophages from wild-type (C57BL/6), TLR9−/−, and myeloid differentiation factor 88 (MyD88)−/− mice. In some experiments, RAW 264.7 cells were transfected with control and receptor for advanced glycation end products (RAGE) siRNA 24 h before treatment. In other experiments, cells were pretreated with a soluble MyD88 inhibitor 1 h before treatment. In all experiments, macrophages were treated with calf-thymus DNA alone or with complexed transfection reagent (lipofectamine) to stimulate Factor B production and then RT-PCR and Western blot were performed. For western blots, protein samples were prepared from conditioned media and cell lysate.

Results: dsDNA strongly induced Factor B mRNA and protein expression in macrophages. No upregulation was seen in MyD88−/− macrophages or RAW 264.7 cells treated with a MyD88 inhibitor. However macrophages from TLR9−/− mice expressed Factor B to a similar level to that seen in wild-type macrophages in response to dsDNA. The response to dsDNA was significantly reduced when...
RAW 264.7 cells were transfected with RAGE siRNA compared to cells treated with control siRNA and negative control. Conclusion: These results indicated that a DNA receptor other than TLR9 is involved in the upregulation of Factor B. Since others have shown that RAGE signaling can be activated by DNA, these data suggest that RAGE is involved in the sensing of DNA for the upregulation of Factor B. MyD88 is known to be involved as signaling adaptor for a number of receptors. Whether RAGE engages MyD88 for the regulation of Factor B expression is unknown. Taken together, these findings point to an important link between endogenous activators of innate immune receptors and the regulation of a key component of the complement system.

Corresponding Author: So Dan Kim, MD, University of Pittsburgh, Department of Surgery, NW 607 MUH, 3459 Fifth Ave, Pittsburgh, PA 15213, USA, kims9@upmc.edu

A 210
Intravascular danger sensing guides neutrophils to sites of sterile cell death by Mac1-dependent adhesion and intravascular crawling
Braedon McDonald, Gustavo Menezes, Paul Kubis

Objectives: The pro-inflammatory danger signals released from necrotic cells have recently begun to be defined. However, relatively little is known about how inflammatory cells such as neutrophils sense these danger signals and home to sites of injury in vivo. We aimed to elucidate the mechanisms of recruitment that guide neutrophils to sites of sterile cell death using in vivo imaging in a novel model of focal hepatic necrosis.

Methods: A 200–400 mm necrotic lesion was induced in the livers of mice using a heated 30-gauge cautery needle. Spinning disk confocal intravital microscopy was used to examine the recruitment of fluorescently-labeled neutrophils towards necrotic lesions (labeled with propidium iodide) in vivo in real-time. Various antibodies, inhibitors, and knockout mice were used to investigate the roles of danger signaling pathways and cell adhesion molecules.

Results: Neutrophils were observed to rapidly (within 60 min) adhere to endothelium in liver sinusoids around necrotic lesions via Mac1-ICAM1 interactions. Neutrophil recruitment was dependent on extracellular ATP danger signals released from dead cells as a pre-treatment with apyrase significantly reduced neutrophil infiltration. Furthermore, recruitment was significantly impaired in Nalp3 knockout mice and mice pre-treated with a recombinant IL-1 receptor antagonist. Adherent neutrophils were then observed to migrate rapidly through sinusoids towards the dead cells via Mac1-dependent intravascular crawling, ultimately infiltrating directly into the lesion.

Chemokines MIP-2 and KC were expressed on endothelial surfaces around the lesions, and antibody blockade of these chemokines or deficiency of CXCR2 abolished chemotaxis into the necrotic lesions. Conclusion: Using in vivo imaging of the acute inflammatory response to sterile hepatic necrosis, neutrophil infiltration was observed to occur via a two-step mechanism. First, ATP released from necrotic cells and activation of the Nalp3 inflammasome pathway initiated neutrophil recruitment into the vicinity of the lesion by Mac1-ICAM1 dependent adhesion to endothelium. Subsequently, Mac1-dependent intravascular crawling (guided by an endothelial chemokine gradient) resulted in neutrophil accumulation precisely within the lesion rather than the surrounding healthy tissue. This rapid and precise mechanism of intravascular danger sensing may have evolved to prevent collateral damage during the sterile inflammatory response to focal necrosis.

Corresponding Author: Braedon McDonald, University of Calgary, Faculty of Medicine, Snyder Institute of Infection, Immunity, and Inflammation, 3330 Hospital Dr NW, HRIC 4A24A, Calgary, AB T2N 4N1, Canada, bamedona@ucalgary.ca

A 211
Hsp72 activates macrophage differentiation and is a chemottractant for leukocytes
John H.H. Williams, Helen Williams, Elyse Ireland

Hsp72 is found in the circulation of healthy individuals and is elevated following infection. It has therefore been proposed to be a danger signal to the immune system. The aim of this work was to investigate whether secreted Hsp72 was capable of interacting with leukocytes and influencing immune responses.

All experiments using human blood were approved by the University of Chester Ethics Committee. Experiments utilised LPS-free recombinant Hsp72 or Hsp72 derived from human cells. The effect of Hsp72 on the transformation of U937 monocytes to macrophage was observed by microscopy and flow cytometry. The effect of Hsp72 on the migration of U937 derived macrophages, or leukocytes from healthy volunteers, was performed in Boyden chambers. Hsp72 stimulated the transformation of U937 monocytes to macrophage based on morphological changes, including the presence of pseudopodia. Exposure of monocytes to Hsp72 resulted in significant increases in CD91, SRA-1 and CD36 (p < 0.001) and increased secretion of TNF-α (p < 0.001). Hsp72 released from apoptotic or necrotic Jurkat cells also stimulated increases in CD91, SRA-1 and CD36 expression in U937 monocytes (p < 0.001). This stimulation was inhibited by antibodies against Hsp72. When leukocytes from healthy volunteers were exposed to Hsp72 in Boyden chambers there was significant migration towards the protein by monocytes and neutrophils.

In conclusion Hsp72 is a chemottractant and causes differentiation of monocytes to macrophages. The use of human cell derived Hsp72 and the inhibition of this activity observed confirm that these data are not the result of LPS contamination. Hsp72 is clearly capable of interacting at several different levels with cells of the innate and adaptive systems and therefore may provide a useful therapeutic target against inappropriate reactions to sepsis.

Corresponding Author: John H. H. Williams, Prof. PhD, University of Chester, Chester Centre for Stress Research, Parkgate Road, Chester CH1 4BJ, UK, john.williams@chester.ac.uk

A 212
Analysis and targeting of HMGB1 and RAGE with heparin and heparan sulphates
Ari Rouhiainen, Heikki Rauvala

Objective: HMGB1 and its cell surface receptor RAGE (receptor for advanced glycation end products) are heparin binding proteins that mediate inflammatory reactions. Further, HMGB1 is a ligand of the proteoglycans syndecan-1 and phosphacan. We have utilized the heparin/heparan sulphate binding capability of HMGB1 in analysis of plasma samples from patients. Furthermore, we shown that ligand binding to RAGE can be targeted by heparin/heparan sulphate.

Methods: An HMGB1 assay that can separate HMGB1 from other HMGB proteins was used to analyse human plasma or serum samples. This assay is based on heparin-Sepharose precipitation and anti-HMGB1 Western blotting of HMGB1. The HMGB1 levels in samples derived from patients of sepsis/septic shock, liver transplantation and acute pancreatitis were analysed and compared to other inflammation and organ injury markers, and to soluble RAGE (sRAGE). Elevated
leve of HMGB1 were found in all pathological situations tested, and HMGB1 levels correlated to organ injury in liver transplantation. In acute pancreatitis, HMGB1 levels did not correlate to sRAGE levels whereas sRAGE levels correlated to disease severity and organ failure. Results: The effect of heparin and heparan sulphates on ligand binding to RAGE was studied in microwell binding assays. Heparin bound to V1 domain of RAGE with high affinity whereas there was no binding to C1 and C2 domains. Heparin, chondroitin sulphate E and soluble ectodomains of syndeacan-3 inhibited RAGE binding to amyloid-beta 1–42 peptide. The polymer size required for the inhibition was found to be 10–12 monosaccharide units. Conclusion: In conclusion, both HMGB1 and RAGE bind to heparin/ heparan sulphate. The ligand binding to RAGE can be inhibited by naturally occurring heparin and heparan sulphate structures.

Corresponding Author: Ari Rouhiainen, PhD, University of Helsinki, Neuroscience Center, Vilkinkaari 4, 14 Yliopisto, Finland, ari.rouhiainen@helsinki.fi

A 213 Caspase-1 processes HMGB1 and modulates its alarmin function during infection and septic shock
Philippe M. LeBlanc, Sylvie Perret, Yves Durocher, Maya Saleh

Objectives: Caspase-1 is a key effector in the induction of proinflammatory responses. It is activated within “inflammasomes” following the stimulation of Nod-like receptors (NLRs) by pathogen- or danger-associated molecular patterns (PAMPs/DAMPs). While caspase-1 cleaves pro-IL-1β and pro-IL-18 into their mature biologically active cytokine forms, its functions in inflammation and innate immunity are not confined to processing of these cytokines. Indeed, while casp1<sup>-/-</sup> mice are resistant to septic shock, IL-1β/IL-18-deficient mice are not. Our objectives were to identify and characterize novel caspase-1 substrates that mediate its actions in inflammation, microbial clearance, tissue repair and septic shock. HMGB1, a non-histone chromatin-binding protein, has been recently shown to act as an alarmin and a late-mediator of severe sepsis. Its nuclear function as a transcriptional regulator is suspended in response to cellular stress or injury. HMGB1 is released passively by dying necrotic and pyroptotic cells or actively in response to pro-inflammatory stimuli. Extracellular HMGB1 interacts with RAGE and Toll-like receptors to amplify the inflammatory response.

Methods and results: We have identified HMGB1 as a specific caspase-1 substrate and show that its processing modulated its alarmin function. HMGB1 was cleaved in vitro in a dose-dependent manner by recombinant caspase-1 and inflammasome- but not apoptosome-activated macrophage cell lysates. Site-directed mutagenesis mapped caspase-1 cleavage sites in HMGB1 and allowed the generation of fully activated caspase-1, such as casp1<sup>-/-</sup> or casp1<sup>-/-</sup> processed HMGB1 fragments to mice resulted in distinctive inflammatory responses.

Results: The effect of heparin and heparan sulphates on ligand binding to RAGE was studied in microwell binding assays. Heparin bound to V1 domain of RAGE with high affinity whereas there was no binding to C1 and C2 domains. Heparin, chondroitin sulphate E and soluble ectodomains of syndecan-3 inhibited RAGE binding to amyloid-beta 1–42 peptide. The polymer size required for the inhibition was found to be 10–12 monosaccharide units. Conclusion: In conclusion, both HMGB1 and RAGE bind to heparin/ heparan sulphate. The ligand binding to RAGE can be inhibited by naturally occurring heparin and heparan sulphate structures.

Corresponding Author: Ari Rouhiainen, PhD, University of Helsinki, Neuroscience Center, Vilkinkaari 4, 14 Yliopisto, Finland, ari.rouhiainen@helsinki.fi

A 214 Administration of soluble RAGE attenuates the inflammatory response in burned rats
Robert Kraft, Felicia Williams, Robert Cox, Siegfried Zedler, David Herndon, Marc Jeschke

Introduction: The receptor for advanced glycation end products (RAGE) is an important factor for the transmission of extracellular proinflammatory signals inside the cell. There it leads to a prolonged NFκB activation. Clinical investigations have shown that a difference exists in the systemic levels of soluble forms of RAGE (sRAGE) between multiple injured and severely burned patients. Thermally injured patients have significantly lower plasma levels of sRAGE during the initial phase post burn.

Hypothesis: RAGE is suspected to have the ability of a decoy function for proinflammatory mediators. Therefore we hypothesize that the administration of sRAGE leads to an improved inflammatory response.

Methods: Twelve male Sprague-Dawley rats received a 60% TBSA full thickness scald burn. Six animals were used as sham animals (no burn). The burned animals were randomized to the treatment groups saline (1.0 ml q 12 h ip) (n = 6) and sRAGE (2 μg/ml q 12 h ip) (n = 6), euthanized at 12, 24, and 48 h post burn. Blood and organs were harvested. Blood samples were analyzed using a multiplex cytokine assay. liver samples were HE stained and analyzed by light microscopy.

Results: Measured cytokine levels of IL-6, IL-10, IL-12 (p70) were significantly lowered (p < 0.05) at the time points 12 and 24 h post burn. GM-CSF and Eotaxin showed significantly lower (p < 0.05) at 12 h post burn. Liver tissue of the sRAGE treatment group showed an improved structure compared to the control group at the 48 h time point.

Conclusion: Our results suggest that the administration of sRAGE improves the inflammatory response post burn and has positive effect on liver tissue.

Corresponding Author: Robert Kraft, MD, University of Texas Medical Branch, Department of Surgery, 815 Market Street, Galveston, TX 77550, USA, rokraft@utmb.edu

A 215 Why are international guidelines for the management of acute cholangitis and acute cholecystitis required to be validated?
Masahiro Yoshida, Tadahiro Takada, Toshihiko Mayumi, Koichi Hirata, Toshio Tsuyuguchi, Yoshinobu Sumiyama

Background: Before the 1970s, the mortality rate of acute cholangitis was as high as >50%, and was significantly improved to <7% in the 1990s. However, in the cases of “Severe” acute cholangitis, the mortality rate remains high (20–28%). In the cases of severe acute cholangitis, prompt and appropriate clinical management are required. However, no standard criteria of diagnosis, severity assessment and clinical management guidelines for acute cholangitis have been established yet. There were some evidences that Charcot’s triad (fever, jaundice, right upper quadrant pain) could diagnose only 50–70% cases of all acute cholangitis. International consensus meeting (Tokyo) was held on April 1–2, 2006, with 29 panelists from abroad, 30 panelists from Japan and 200 audiences, to develop the
standard criteria of diagnosis, severity assessment and clinical management guidelines for the patients of acute cholangitis and cholecystitis (Tokyo Guidelines).

Objective: There were first guidelines that defined diagnostic and severity criteria, and management for biliary tract infection. We will evaluate theses guidelines for the members of related academic societies for its revisions.

Methods:

1. Questionnaire survey: We asked the following questions to the clinical practitioners who treat patients of biliary tract infection. (1) What kind of diagnostic criteria and severity assessment do you use? (2) How will you use antimicrobial agents (name of agents, dosage)? (3) What kind of biliary drainage (percutaneous, endoscopic or surgical) and what timing? (4) Methods of surgical intervention (laparoscopic or open surgery). Next, we will make international assessments for Tokyo Guidelines.

2. Prospective observational study (http://class.umin.jp/): From April 2009, for the world clinical practitioners, we have started a prospective observational study to evaluate differences between the Tokyo Guidelines and clinical practices of diagnostic criteria and the severity assessment of acute cholangitis. And we also found an international tendency for the management of acute cholangitis and cholecystitis.

Result: A preliminary domestic questionnaire survey of Japanese shows that after the guidelines have been published, more endoscopic procedure and less percutaneous procedure were performed for biliary drainage for acute cholangitis. On the other hand, earlier laparoscopic cholecystectomy was done in acute cholecystitis. In this study, we will show the results of the questionnaire survey, prospective observational study and international tendencies for the management of acute cholangitis and cholecystitis.

Conclusion: To evaluate the Tokyo Guidelines, we will start the questionnaire survey and the prospective observational study to find some international tendencies for the management of acute cholangitis and cholecystitis. Furthermore, we will assess the validity of the Tokyo Guidelines for management of acute cholangitis and cholecystitis for its revision.

Research subsidy: This project is conducted with a research subsidy sponsored by the Japanese Ministry of Health, Labor and Welfare.

Corresponding Author: Masahiro Yoshida, Prof. MD, PhD, International University of Health and Welfare, Department of Hemodialysis and Surgery, 6-1-14 Kounodai, 272-0827 Ichikawa, Japan, yoshida@iuhw.ac.jp

A 217

The clinical evaluation of the Tokyo Guidelines 2007 based on actual clinical cases

Masahiro Yoshida, Toshihiko Mayumi, Katusmi Hayashi, Hiroshi Hasegawa

Objective: Tokyo Guidelines for the management of acute cholangitis and acute cholecystitis, the world’s first guidelines for these diseases, was published in 2007. Two years have passed after publication, we have to evaluate this guideline in order to ensure the accuracy of this guidelines on actual clinical cases. For the purpose of clearing up this guidelines benefits and deficits, we evaluate our admission case records using Tokyo Guidelines 2007. In addition, we evaluate Japanese Guidelines too.

Patients and methods: We evaluate 74 cases that were clinically diagnosed acute cholangitis as an initial diagnose on admission and 81 cases that were clinically diagnosed acute cholecystitis as an initial diagnose on admission during the period from November 2004 to November 2005 before publication. We applied Tokyo Guideline’s diagnostic criterion and Japanese Guideline’s diagnostic criterion to 74 cholangitis cases and 81 cholecystitis cases, in addition, we compared the final clinical diagnoses of each case, we found the accuracy of these diagnostic criterion.

Results: Acute cholangitis (M:F = 39:35, 69.2 ± 15.2 years). 52 cases were matched for diagnostic criteria of acute cholangitis of Tokyo Guidelines. 51 of 74 cases were diagnosed with acute cholangitis as final diagnosis. Acute cholecystitis (M:F = 49:32, 69.0 ± 15.0 years). 67 cases were matched for diagnostic criteria of acute cholecystitis of Tokyo Guidelines. As a final diagnosis, 69 of 74 cases were diagnosed with acute cholecystitis as final diagnosis. We would like to report the details of each case and the sensitivity and specificity of Tokyo Guideline’s diagnostic criterion.

Conclusion: We compared initial diagnosis by clinicians, the diagnosis by using Tokyo Guideline’s diagnostic criterion, and final diagnosis by clinicians. From these results, Tokyo Guideline’s diagnostic criterion are better than Charcot’s Triad for acute cholangitis

A 216

Difference between Tokyo Guidelines and Japanese Guidelines for the management of acute cholangitis and acute cholecystitis

Toshihiko Mayumi, Masamichi Yokoe, Masahiro Yoshida, Tadahiro Takada, Yoshinobu Sumiyama

Objective and methods: Japanese Guidelines (JGL) for the management of acute cholangitis and cholecystitis were published in September 2005, and International Guidelines (Tokyo Guidelines: TGL) for the management of the diseases were published in January 2007. Diagnosis and severity criteria of acute cholangitis and cholecystitis are defined for the first time in the world. Here, we elucidate the differences of the two guidelines.

Results: Many differences exist between TGL and JGL. Diagnosis criteria of both acute cholangitis and cholecystitis of the two guidelines are different. Moderate acute cholangitis and cholecystitis of TGL is defined as the disease that does not respond to the initial medical treatment, whereas, that of TGL is defined as abnormality of laboratory data and image findings. Therefore, treatment strategies of the diseases are also different, although both guidelines recommend that therapies of the diseases depend on the severity of the diseases.

Conclusion: The diagnosis and severity criteria are defined based on expert’s opinions. Therefore, we need verify these guidelines using retrospective and prospective clinical data.

TGL diagnostic criteria for acute cholangitis


B. Laboratory data: 5. Evidence of inflammatory response. 6. Abnormal liver function tests.

C. Image findings: 7. Biliary dilatation, or evidence of an etiology (stricture, stone, stent, etc.). Suspected diagnosis: Two or more items in A. Definite diagnosis: (1) Charcot’s triad (2 + 3 + 4), (2) Two or more items in A + both items in B and item C.

Corresponding Author: Toshihiko Mayumi, MD, PhD, Nagoya University Graduate School of Medicine, Emergency and Critical Care Medicine, 65 Tsurumai, Showa, 466-8550 Showa-Ku, Nagoya, Japan, mtoshi@med.nagoya-u.ac.jp
A prospective study to validate the international guidelines for the management of acute cholangitis and acute cholecystitis
Toshio Tsuyuguchi, Osamu Yokosuka, Masahiro Yoshida, Tadahiro Takada

Objective: “Clinical practice guidelines for acute biliary tract infections in Japanese” were made in 2005. Subsequently, an International Consensus Meeting took place in Tokyo, on 1–2 April, 2006, to obtain international agreement on diagnostic criteria, severity assessment, and management for acute biliary tract infections. Then, Tokyo Guidelines for the management of acute cholangitis and cholecystitis (Tokyo GL) were made as the international guidelines in 2007. Because the recommendations by Tokyo GL are based upon a consensus meeting with the experts of many foreign countries varying in the medical environment, the validity of them requires evidence by the large-scale prospective study.

Patients and methods: Acute cholangitis tended to be severe or life-threatening conditions when compared to cholecystitis. Then, it is necessary to validate Tokyo GL recommendations for acute cholangitis immediately than those of cholecystitis. We planned an exploratory observational, prospective study for the diagnostic criteria and severity assessment in Tokyo GL, which focused on acute cholangitis.

Results: Official scientific title of the study is “Diagnostic criteria and severity assessment of acute cholangitis: A prospective observational study” (UMIN-CTR No. UMIN000002552). The inclusion criteria were the cases with acute cholangitis on the basis of each institution’s criteria (it is not necessary to conform to the Tokyo GL diagnostic criteria). The exclusion criteria were the cases with other active inflammatory diseases. The main outcome measures were validity of the Tokyo GL diagnostic criteria and severity assessment (duration of treatment, mortality and organ dysfunction incidence according to the grading of severity). The secondary outcome measures were other severity assessment factors (laboratory data etc.), etiologies of the cholangitis, biliary tract drainage methods and used antimicrobial agents. Because it is thought that the difference between institutions in diagnosis and the therapy has an influence on the outcome, it is necessary for a large number of cases to be enrolled as much as possible from many institutions. Given that this is a multicenter study, the data input methods should be simple and easy. Therefore, web registration system using INDICE (Information Network Internet Data and Information Center for Medical Research) was built, and a brief title of this study was named “CLASS Tokyo”.

Conclusion: The sensitivity, specificity and accuracy of the diagnostic criteria are evaluated objectively by the data which this study provides, and the validity of disease severity criteria can be examined. We are just after registration system operation, but the progress of the study is going to be reported at the meeting holding.

Corresponding Author: Toshio Tsuyuguchi, MD, Chiba University Graduate School of Medicine, Medicine and Clinical Oncology Graduate School of Medicine, 1-8-1 Inohana, Chuo, 260-8670 Chiba City, Japan, tsuyuguchi@faculty.chiba-u.jp

A 219
Therapeutic antibody vaccines for inflammatory diseases
Zhikang Peng

Background: Diseases mediated by excessive IgE/cytokine responses, such as asthma and autoimmune disorders, have increased significantly. The technique of blocking these cytokines with humanized monoclonal antibodies (mAb) as a passive immunotherapy has been used successfully in the clinical treatment of these diseases. However, the short-half life of the mAb, extremely high cost, and the development of antibodies to the infused mAb limit the use of this reagent, as these diseases are chronic and require long-term treatment. To overcome the above disadvantages, therapeutic antibody vaccines are currently being developed. Most of these vaccines are made by modifying the intact cytokine. Thus, vaccine-induced antibodies react against multiple epitopes of the target cytokine. As such, their use may be hindered by undesirable cross-reactions with other self-proteins containing similar epitopes. This is of particular concern when this strategy is used in humans.

Objective: To develop IgE/cytokine-peptide based virus-like particle vaccines for the long-term down-regulation of IgE/cytokine mediated inflammatory diseases and evaluate the vaccine-induced effects in animal models.

Methods: The vaccine was constructed by inserting a small peptide (8–16 amino acids), derived from the receptor binding site of the mouse target molecule, into a carrier, hepatitis B core antigen, via gene recombination methods. Mice with ovalbumin-induced airway inflammation or TNBS-induced colitis were used to evaluate the effects of the vaccines.

Results: The vaccine that was presented as virus-like particles induced strong and long-lasting antibody responses against the target cytokine without the use of an adjuvant. Immunization with an IgE vaccine prevented subsequent increase of IgE and down-regulated elevated IgE in sensitized rodents. Administration of a vaccine against IL-13 or IL-4 or TNF significantly suppressed airway allergic inflammation in asthmatic mice. Administration of a vaccine against the p40 subunit (shared by IL-12 and IL-23) or TGFβ1 or TNF significantly down-regulated intestinal inflammation and fibrosis in mice with chronic colitis. Recently, we developed an IL-10 peptide-based vaccine that enhances the bioactivity of IL-10. Administration of the vaccine effectively decreased airway allergic inflammation.

Conclusion: IgE/cytokine peptide-based virus-like particle vaccines may provide a potential approach to the long-term treatment of allergic and autoimmune diseases.

Corresponding Author: Zhikang Peng, Prof. MD, PhD, University of Manitoba, Department of Immunology, 532-715 McDermont Ave., Winnipeg, MB R3E-3P4, Canada, zpeng@ms.umanitoba.ca

A 220
Development of a live vaccine against tuberculosis
Bernd Eisele, Leander Grode, Hans-Heinrich Henneicke-von Zepelin, Christiane Desel, Jeroen Maertzdorf, Stefan HE Kaufmann

Objective: VPM1002 is a live vaccine against tuberculosis (TB). It is based on the well known Mycobacterium bovis Bacille Calmette-Guérin (BCG) strain which has been applied appr. 4 billion times worldwide. As BCG is not sufficiently effective to stop the spread of TB, two modifications have been implemented in VPM1002 to improve its immunogenicity. Our Phase I study in humans used multiparameter flow cytometry to characterize the quality of the
T cell response following immunization with our VPM1002 tuberculosis vaccine candidate or BCG.

Patients and methods: In a Phase I open label, randomized, controlled, dose-escalation study to evaluate safety and immunogenicity of VPM1002 in comparison with BCG. We enrolled 80 healthy male subjects stratified for history of BCG vaccination.

Results: Safety and tolerability revealed no serious adverse reactions from VPM1002 vaccination and only mild to moderate adverse reactions were reported. VPM1002 was very well tolerated in both cohorts the naïve and BCG pre-immunized volunteers. Higher total IFN-gamma production was measured in the VPM1002 group versus the BCG group. VPM1002 induced a good multifunctional CD4+ and CD8+ T cell response in comparison with BCG.

Conclusion: VPM1002 induces multifunctional T cell subsets which are thought to play a crucial role in protection against tuberculosis. At the same time VPM1002 is very well tolerated and presents a safety profile that is similar or even better than BCG. VPM1002 shows all characteristics for a safe, well tolerated and efficacious tuberculosis vaccine, which could replace BCG immunization in the future.

Corresponding Author: Bernd Eisele, MD, CEO, Vakzine Projekt Management GmbH, R&D, Mellendorfer Str. 9, 30625 Hannover, Germany, eisele@vakzine-manager.de

A 221
Kill the bacteria… and also their messengers?
Robert S. Munford

Although many host factors can modulate the bioactivities of bacterial LPSs, inactivation of these potent molecules in vivo is carried out by an unusual lipase, found in phagocytes, that removes two of the six fatty acyl chains from lipid A. We produced mice that lack this enzyme, acyloxyacyl hydrolase (AOAH), and found that they recover very slowly from parenteral exposure to LPS or Gram-negative bacteria: among the prolonged responses is a state of immune tolerance that persists for at least 4 months. During this time, the mice are hyper-susceptible to E. coli challenge. In contrast, transgenic mice that over-express AOAH recover more rapidly from LPS exposure and E. coli infection than do wildtype animals. Studies of tolerant macrophages from Aoah−/− mice indicate that they are chronically activated yet unable to respond normally to stimulation with LPS, peptidoglycan, or polyIC. These observations suggest that complete recovery from many Gram-negative bacterial infections may require both killing the bacteria and inactivating their most important “signal” molecule, LPS. Our findings also raise the possibility that variability in AOAH expression influences the rate at which human patients recover from Gram-negative bacterial diseases, and thus their risk for developing secondary infections.

Funded by Public Health Service grant AI18188 and by the Division of Intramural Research, NIAID, NIH, USA
Corresponding Author: Robert S. Munford, Prof, MD, PhD, Laboratory of Clinical Infectious Diseases, NIAID, NIH, USA, munfordrs@niaid.nih.gov

A 222
Genetically modified mesenchymal stem cell versus stem cell therapy alone following trauma-hemorrhage
Irshad Chaudry, Irshad Chaudry, Fariba Moeinpour, Selvarangan Ponnanzhagan, Sanjay Kumar, Kirby Bland

Objectives: Although inflammation and organ dysfunction occur following trauma-hemorrhage (T-H), it remains unknown whether administration of mesenchymal stem cells (MSC) following T-H produces any salutary effects. Accordingly, we determined the effects of bone marrow-derived MSC which were unmodified or genetically modified (GMMSC) to over-express estrogen receptor (ER)-β in a rat model of T-H.

Materials and methods: T-H was induced in the rat (60% circulating blood volume loss to decrease blood pressure to 35 mmHg for 90 min followed by fluid resuscitation). For GMMSC studies, we engineered MSC by transfecting an expression plasmid for ER-β. Rats received 4 × 10⁶ MSC or GMMSC via femoral vein following T-H, and fluid resuscitation was then provided. Four hour after resuscitation, rats were sacrificed; heart and liver tissues were harvested for measurement of ER, stress protein expression and cytokine production. In addition, hepatocytes were used for measurement of mitochondrial membrane potential.

Results and conclusions: MSC therapy following T-H significantly upregulated ER-α in the liver tissue and normalized hepatic mitochondria membrane potential. The elevated stress proteins (ATF6, CHOP) after T-H were also significantly downregulated and this was associated with significant downregulation of IL-6 and upregulation of IL-10 following T-H. Furthermore, GMMSC significantly upregulated ER-β in heart tissue compared to other groups and it further downregulated IL-6 and upregulated IL-10 compared to MSC therapy alone. Thus, although MSC therapy following T-H prevents injury by preventing the increase in pro-inflammatory cytokine and stress proteins levels, GMMSC has more potential to invoke anti-inflammatory process following T-H. Thus, GMMSC may be used as a novel adjunct for preventing tissue damage following T-H (supported by USPHS grant RO1 GM 39519 to IHC).

Corresponding Author: Irshad Chaudry, PhD, University of Alabama at Birmingham, Center for Surgical Research, 1670 University Boulevard, G094 Volker Hall, Birmingham, AL 35294, USA, irshad.chaudry@ccc.uab.edu

A 223
Use of hair root stem cells for skin regeneration and treatment of chronic wounds
Jan C. Simon, Regina Renner

An increasing number of chronic wounds in our society require strategies to improve wound healing and wound closure. One of several options is skin transplantation. In this talk, we focus on the transplantation of tissue engineered autologous epidermal sheets derived from outer root sheath cells of the patients’ hair. Out of the stem cells of the outer root sheath (ORS) of anagen hair, autologous keratinoocytes are cultured ex vivo in organotypic cultures to form a multilayered epidermal equivalent (EpiDexTM). These sheets are placed on the wound bed. Patients were observed twice a week in the first 2 weeks, then once weekly for 4 weeks, then every 4 weeks for up to 12 weeks after transplantation. We will report in detail on our clinical experience treating patients with chronic venous insufficiently including a responder/non-responder subgroup-analyses.

In conclusion, autologous keratinocyte transplantation with EpiDexTM can be performed easily and safely in patients with chronic wounds with satisfying results. Our data suggest that patients with small ulcer area <25 cm² might profit the most from this method.

Corresponding Author: Jan C. Simon, Prof, MD, University Hospital Leipzig, Department of Dermatology, Philipp-Rosenthal-Str. 23, 04103 Leipzig, Germany, jan.simon@medizin.uni-leipzig.de
A 224

Intraabdominal abscesses: image guided percutaneous drainage
Tobias Jakobs

Percutaneous drainage has already gained a wide acceptance in patients with abdominal and pelvic abscesses as it is considered a safe and effective treatment option. Percutaneous drainage is performed under either US or CT-guidance. If the initial puncture is carried out under US guidance the next steps of the procedure are completed either under fluoroscopy or CT-fluoroscopy guidance. This approach gives a chance to complete the procedure safer as all the necessary manipulations for the final catheter placement are easily carried out under imaging control. Catheterization is performed by one of two basic techniques, either the Seldinger-technique or the Trocar-technique. As a general rule, the Trocar-technique is employed for the large and superficially located abscesses while the Seldinger-technique is preferred for small and deeply located abscesses. It is very easy to perform the procedure in patients with pelvic abscesses if they are located in the proximity of anterior abdominal wall. However, it is sometimes a difficult to perform catheterization in patients with deeply located pelvic abscesses because of the surrounding structures such as pelvic bones, urinary bladder, bowel loops, iliac vessels and gynaecologic organs, which preclude safe access. Especially in these challenging cases online CT-Fluoroscopy guidance provides on excellent overview and enables the treating physician to perform the procedure without harming vulnerable structures. The transluminal approach can provide an important alternative to drain these groups of abscesses.

Corresponding Author: Tobias Jakobs, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Radiology, Marchioninistr. 15, 81377 Munich, Germany, tobias.jakobs@med.uni-muenchen.de

A 225

The impact of TLR ligands on the function and phenotype of myeloid-derived suppressor cell subpopulations
Daniel Noerenberg, Helen Bauer, Georg Wedekind, Philipp Bittner, Christine Zoglmeier, Carole Bourquin

Objectives: Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of bone marrow-derived immunosuppressive cells that expand during inflammation, trauma and cancer. Due to their remarkable ability to suppress T-cell responses, the induction of MDSCs is an important immune-evading factor used by tumors. In mice, MDSCs are phenotypically defined as Gr1+CD11b+ and can be subdivided into two subfractions with clear morphologic, molecular and functional differences. Our aim was to investigate the function and phenotype of MDSCs in tumor-bearing mice following innate immune activation.

Materials and methods: MDSCs were derived from mice bearing subcutaneous CT26 tumors. To isolate the highly sensitive Gr1+CD11b+ myeloid-derived suppressor cells we compared three different purification methods: magnetic-associated cell-sorting (MACS) and positive or negative selection using magnetic nanoparticles in cell suspensions.

Results: Passage through the magnetic column during MACS sort negatively affected cell viability. In contrast, cells separated using magnetic nanoparticles were vital and purity was greatly improved. Purification using a custom-made negative selection cocktail resulted in enrichment of untouched Gr1+CD11b+ MDSCs, preventing activation as a consequence of antibody binding.

In vivo treatment of C26 tumor-bearing mice with TLR9-activating CpG resulted in a shift towards a Ly6C expressing MDSC subset. Furthermore, a range of surface markers associated with maturation and activation of MDSCs was upregulated. Functionally, CpG treatment blocked the suppressive function of MDSCs as determined in T-cell proliferation assays. In vitro assays showed that the reduction of MDSC suppressivity depended on the presence of accessory cells activated by CpG, whereas direct treatment of isolated MDSCs in vitro resulted in an increase in suppressivity.

Conclusions: We provide evidence that activation of specific TLRs may have a profound impact on the phenotype and function of MDSCs, and that the effect of an in vivo therapy may differ greatly from that of an in vitro stimulation of isolated cells.

Corresponding Author: Daniel Noerenberg, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Division of Clinical Pharmacology, Ziemssenstr. 1, 80336 Munich, Germany, Daniel.Noerenberg@campus.lmu.de

A 226

CEACAM1 inhibits Toll-like receptor 2-triggered anti-bacterial responses of human pulmonary epithelial cells
Hortense Slevogt, Solveig Zabel, Bastian Optiz, Andreas Hocke, Norbert Sattorp, Bernhard Singer

Although Moraxella catarrhalis and Neisseria meningitidis are important human pathogens, they often colonize the human respiratory tract without causing overt clinical symptoms. Both pathogens express structurally unrelated proteins that share the capacity to stimulate carinoembryonic antigen (CEA)-related cell adhesion molecule 1 (CEACAM1) expressed on human cells. Here, we demonstrated that interaction of CEACAM1 with UspA1 expressed on M. catarrhalis or with Opa proteins on N. meningitidis reduced the Toll-like receptor 2 (TLR2)-initiated NF-κB-dependent inflammatory responses of primary pulmonary epithelial cells. These inhibitory effects were mediated by tyrosine phosphorylation of the ITIM of CEACAM1, and by the recruitment of the phosphatase Shp1, which negatively regulated TLR2-dependent activation of the phosphatidylinositol 3-kinase (PI3K)-Akt pathway. These findings reveal a CEACAM1-dependent immune evasion strategy through which bacteria “utilize” CEACAM1 to colonize the pulmonary epithelium.

Corresponding Author: Hortense Slevogt, MD, PhD, Charité University Medical Center, Department of Infectious Diseases and Pulmonary Medicine, Augustenburger Platz 1, 13353 Berlin, Germany, hortense.slevogt@charite.de

A 227

Tachyphylaxis limits the efficacy of the TLR7/8 agonist R848 for the immunotherapy of tumours
Laurin Roetzer, Christian Hotz, Andreas Voelkl, Nadja Sandholzer, Stefan Endres, Carole Bourquin

Small-molecule TLR7/8 agonists stimulate strong innate immune responses, prompting clinical trials investigating their potential for the immunotherapy of cancer. However, the optimal treatment regimen remains to be determined. As the innate activation induced by these ligands is short-lived, frequent applications are required. We examine here the efficiency of multiple injections of resiquimod (R848), a strong TLR7/8 agonist, that induces high but short-term serum levels of the proinflammatory cytokines IL-12 and...
IL-6. Repeated injections of R848 do not however lead to efficient antitumor therapy. Indeed, a single injection of R848 induces a refractory state of up to 5 days during which further R848 injections cannot activate cytokine production. In vitro studies showed that R848 treatment leads to homo- and heterotolerance towards stimulation with agonists for different TLRs. Tolerance induction is characterized by degradation of IRAK-1 within dendritic cells and increased production of IL-10. To prevent tolerance and enhance the efficacy of antitumor immunotherapy, we sought to alternate MyD88-dependent and independent agonists. Using a sequential stimulation of TLR3 and TLR7, we both prevent tolerance and enhance proinflammatory cytokine secretion. We propose that the sequential combination of TLR3 and TLR7 activation is a more efficient protocol for the immunotherapy of tumors.

Corresponding Author: Laurin Roetzer, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Department for Clinical Pharmacology, Ziemssenstr. 1, 80336 Munich, Germany, laurin.roetzer@googlemail.com

A 228
The TLR4 antagonist Eritoran tetrasodium reduces cardiac hypertrophy in a murine model of transverse aortic constriction
Heidi Ehrentraut, Carolyn Weber, Stefan Ehrentraut, Markus Schwederski, Rainer Meyer, Georg Baumgarten

Objectives: Toll-like receptors (TLRs) recognize both pathogenic organisms as well as endogenous ligands which are released following organ injury. It has been shown that TLR4−/− mice exhibit smaller infarct areas following induced cardiac ischemia-reperfusion and develop diminished left ventricular hypertrophy after transverse aortic constriction (TAC). In the present study we analyzed whether pharmacologic TLR4 antagonism with the lipid-A-analog Eritoran tetrasodium equally contributes to a reduction of cardiac hypertrophy. Material and methods: Male C57BL/6 mice (10–12 weeks age) were anesthetized (Isofluran 2.0 vol%) and intubated. A catheter was implanted into the jugular vein. Afterwards mice were TAC or sham operated. In the TAC group, the aortic diameter was reduced to 30% by means of a 6-0 silk suture and a 27G needle. Via catheter, mice received Eritoran tetrasodium (Eisai Research Institute of Boston Inc., Andover, MA, USA; 2.5 mg/ml vehicle; 5 mg/kg body weight) or placebo i.v. 5 min prior TAC/sham-procedure as well as 6, 12, 24, 36, 48, and 60 h after surgery. Three days after surgery hearts were excised, heart and lung weights were determined and cardiac tissue was preserved for RNA and protein quantification via real-time RT PCR, ELISA and gelatin zymography.

Results: After TAC the placebo group exhibited a significant increase of leftventricular weight (LVW): 104.2 ± 5.5 mg (M ± SEM); p < 0.01) compared to sham (78.7 ± 1.9 (placebo) and 82.3 ± 3.5 mg (Eritoran)) and TAC Eritoran tetrasodium groups (81.3 ± 4.2 mg). Furthermore, total heart weight (HW) and the LVW/body weight ratio differed significantly from all other groups [HW: 126.3 ± 7.3 mg (TAC placebo) p < 0.01 vs. 103.0 ± 5.7 (TAC Eritoran), 98.2 ± 3.0 (sham placebo), and 97.8 ± 3.9 (sham Eritoran)]. The gene expression of brain natriuretic peptide (BNP), a marker for left ventricular hypertrophy, was elevated twofold in TAC placebo mice compared to Eritoran tetrasodium treated TAC mice (p < 0.05) and tenfold compared to sham groups (p < 0.01). TAC surgery led to a distinct increase of IL-1β and IL-6 mRNA and protein expression in the placebo compared to the TAC Eritoran tetrasodium group (not significant). Furthermore, MMP-9 zymographic activity was highest in the TAC-placebo animals.

Conclusions: The pharmacological antagonism of TLR4 with Eritoran tetrasodium in a murine 3d TAC model prevents the development of cardiac hypertrophy.

Corresponding Author: Heidi Ehrentraut, PhD, University Hospital of Bonn, Department of Anaesthesiology, Sigmund-Freud-Str. 25, 53105 Bonn, Germany, h.ehrentraut@uni-bonn.de

A 229
In vivo application of Eritoran tetrasodium restores cardiac function during endotoxemia
Stefan Ehrentraut, Ralph Lohner, Markus Schwederski, Olaf Boehm, Georg Baumgarten, Pascal Kneuefmann

Objectives: Elucidate, whether in vivo application of the Toll-like-receptor 4 (TLR4) antagonist Eritoran tetrasodium (EISAI, Boston) successfully prevents lipopolysaccharide (LPS) induced hypotension, cardiac failure, loss of vascular resistance and cytokine production in the murine heart and influences the expression of the TLR4/CD14 complex.

Material and methods: 12–14 week old C3H/HeN mice were treated with either 2 mg/kg bodyweight (BW) LPS or 4 mg Eritoran + 2 mg LPS/kg BW, with LPS injected i.p. and Eritoran tetrasodium injected i.v. 6 h later, blood pressure, cardiac output and vascular response were measured using a Millar® catheter and a Mulvany myograph. Additionally, Physiotet® telemetry catheters were implanted and real time blood pressure and heart frequency were recorded previous and following LPS and Eritoran tetrasodium injection. After 2 and 6 h of stimulation hearts were harvested and cytokine expressions measured using realtime PCR and ELISA.

Results: LPS treatment lead to cardiac depression after 6 h resulting in loss of about 66% of blood pressure and heart frequency in the LPS group. Eritoran tetrasodium treated animals showed only 22% loss of blood pressure and a stabilized heart frequency. Ejection fraction and Cardiac output were both significantly reduced following LPS injection and restored in reaction to Eritoran tetrasodium. Cardiac contractility was significantly reduced by LPS and restored through Eritoran® tetrasodium. LPS led to a significant decrease of vascular contractility after 6 h of stimulation, reducing final vascular force by ca. 25%. Eritoran tetrasodium treatment did not improve vascular tone. Two hours of LPS stimulation led to a significant increase of proinflammatory cytokine mRNA-levels and a significant elevation in mRNA levels of the antiinflammatory IL-10, returning to baseline levels after 6 h. The TLR4/CD14 complex was also affected, with a significant increase of CD14 expression and a lowered expression of TLR4-mRNA. Injection of Eritoran tetrasodium increased the mRNA-levels of TNF-a and IL-6. Two hours after LPS injection, a significant increase of protein levels of TNF-a, IL-1b, and IL-6 was observable compared to the untreated control group. Eritoran tetrasodium injection did not show any significant effects on TNF-a, IL-1b and IL-6 protein levels.

Conclusions: The TLR-4 antagonist Eritoran tetrasodium reduces LPS induced cardiac depression. This appears to be due to stabilisation of cardiac function, more specifically heart frequency and less on vascular contractility. The underlying molecular interactions on cytokine levels need further investigation.

Corresponding Author: Stefan Ehrentraut, MD, University Hospital of Bonn, Department of Anesthesiology, Sigmund-Freud-Str. 25, 53105 Bonn, Germany, stefan.ehrentraut@ukb.uni-bonn.de
A 230
Gene expression profile of Toll-like receptor signaling pathway in LPS-tolerant human peripheral blood mononuclear cells
Marialice Mendes, Marialice Mendes, Maria Fernandes, Giovana Baggio-Zappia, Milena Brunaliiti, Reinaldo Salomão

Objective: To evaluate the expression of a set of 84 genes related to the Toll-like receptor (TLR) signaling pathway, in a model of LPS tolerance in human peripheral blood mononuclear cells (PBMC).

Methods: PBMC were obtained from healthy volunteers. Tolerance was induced by 1 ng/mL of LPS for 48 h and cells were challenged with 100 ng/mL of LPS for 2, 6 and 24 h. Non-stimulated and non-conditioned cells were run as negative and positive control, respectively. Tolerance was confirmed by diminished TNF-alfa secretion, measured by ELISA, 6 h after LPS challenge. TLR4 and CD14 expression was evaluated on monocytes by flow cytometry, following 48 h incubation with 1 ng/mL of LPS, in order to evaluate their expression at the moment of LPS challenge. PCR array comprising 84 genes belonging to the TLR signaling pathway was performed in each condition. Significant difference in gene expression was considered when fold change between groups was equal or higher than 2.

Results: No difference in the expression of TLR4 and CD14 on monocytes was observed between conditioned and non-conditioned cells after 48 h of incubation. Sixty of the 84 genes were activated by LPS and among them, 27 were made tolerant to LPS (fold change > 2). Genes coding for the surface receptors TLR2 and TLR4 were not tolerated, while the CD14 was down-regulated. All studied genes belonging to the TRIF-dependent pathway were tolerated. The MyD88 dependent upstream pathway was not tolerated. However, NF-kB and genes regulating its activation were inhibited in tolerated cells. On the other hand, the MAPKs pathway included tolerant and non-tolerant genes, predominating inhibition of the p38 and JNK pathways, while preserving the expression of ERK pathway during LPS tolerance. No tolerance was found for some anti-inflammatory genes, such as IL-10, while pro-inflammatory genes were found to be tolerated (TNF-alfa) and non-tolerated (IL-6).

Conclusion: The presence of tolerant and non-tolerant genes in human mononuclear cells shows that tolerance is a mechanism that modulates cell response to LPS, which is not directly controlled by the LPS binding to its receptor complex. Down-regulation probably occurs in an attempt to stop tissue damage caused by excessive inflammation, as demonstrated by inhibition of TNF-alfa and IL-1, while mechanisms involved in cell growth and survival are maintained, as demonstrated by preserved ERK pathway.

Financial support: FAPESP grant 06/58744-1 and 07/08656-7
Corresponding Author: Marialice Mendes, MSc, Universidade Federal de São Paulo, Department of Medicine/Infectiology, Rua Pedro de Toledo, 781, 4059032 São Paulo, Brasil, marialice.em@gmail.com

A 232
NF-κB activation is associated with activation of leukocytes after hemorrhagic shock/resuscitation in ethanol gavaged transgenic NF-κB eGFP reporter gene mice and is modulated by JNK inhibition
Sebastian Korff, S. Korff, M. Lehnert, C. Jobin, T. Borsello, I. Marci

Objectives: Hemorrhage and resuscitation (H/R) frequently lead to multiple organ failure and trauma patients with an alcohol related comorbidity are more susceptible for a fatal outcome putatively due to an altered immune response. Organ damage after H/R at least partly depends on activation of circulating leukocytes and is associated with activation of NF-κB and AP-1. This study examines the effect of ethanol before H/R on the pattern of NF-κB activation in the liver and in circulating leukocytes using a transgenic cis-NF-κB-eGFP reporter mouse. Furthermore, the role of AP-1 for the immune response and NF-κB-activation was addressed by specific blockade of the AP-1 activating kinase c-jun-N terminal kinase (JNK).

Methods: Male cis-NF-κB-eGFP mice were gavaged with alcohol (4 mg/kg body weight) or vehicle 12 h before H/R (90 min, MAP 30–35 mmHg, then resuscitation 60% of max. shed blood volume and 50% of max. shed blood volume as Ringer lactate over 30 min). JNK was blocked using D-JNKI-1 peptide (i.p. 11 mg/kg body weight) after hemorrhage before resuscitation. The mice were sacrificed 2 and 24 h after resuscitation. EGF content of circulating leukocytes was assessed by means of flow cytometry. Additionally CAE staining revealed hepatic neutrophil infiltration. To further characterise the inflammatory response, serum levels of AST and IL-6 were determined.

Results: Both H/R with and without ethanol gavage induced eGFP expression indicative of NF-κB activation in PMNL and monocytes as compared to sham groups. Application of D-JNKI-1 largely reversed this effect. In addition, D-JNK1 treatment prevented hepatic damage (AST levels and necrotic areas, p < 0.05), leukocyte infiltration (p < 0.05) and inflammatory changes (IL-6 levels, p < 0.05) both after H/R with and without ethanol gavage when compared to vehicle treated groups.

Conclusion: H/R lead to a strong NF-κB activation in peripheral leukocytes, this activation is not affected by ethanol gavage before shock. Inhibition of JNK leads to a tremendous decrease of NF-κB activation in circulating leukocytes and is associated with reduced...
hepatic leukocyte infiltration, hepatic injury and blunted serum IL-6 levels. Our findings demonstrate that JNK is an important modulator of the systemic inflammatory response. The dependence of NF-κB activity upon JNK modulation suggests a specific link between the JNK pathway and the NF-κB pathway that needs further to be elucidated (Supported by DFG MA 1119/3-3).

Corresponding Author: Sebastian Korff, Johann Wolfgang Goethe University of Frankfurt/M, Department of Trauma Surgery, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, sebastian.korff@kgu.de

A 233
Overexpression of IRAK-1 prevents BLP-induced tolerance by promoting NF-kB p65 nuclear transactivation
Liu Jinghua, Jing Hua Liu, Chong Hui Li, H. Paul Redmond, Jiang Hua Liu

Objective: Tolerance to bacterial cell-wall components including gram-positive bacterial lipoprotein (BLP) represents an essential regulatory mechanism during bacterial infection. BLP activates monocytes/macrophages through TLR2-mediated signal pathway. We demonstrated that BLP-induced tolerance, characterised by down-regulated proinflammatory cytokine expression, is associated with reduced IRAK-1 (interleukin-1 receptor-associated kinase 1) protein expression. However, it remains unclear whether IRAK-1 is a key molecule responsible for BLP-induced tolerance.

Methods: Human monocytc THP-1 cells were preincubated with culture medium (naive) or 100 ng/ml BLP (BLP-tolerised) for 24 h, and further stimulated with 1,000 ng/ml BLP for various time points. For IRAK-1 overexpression, BLP-tolerised cells were transfected with plasmid encoding IRAK-1 or empty vector as the control. Chromatin immunoprecipitation (ChIP) was performed to assess nuclear transactivation of NF-kB p65 at the TNF-a and IL-6 promoters, and ELISA was used to measure proinflammatory cytokine production.

Results: BLP stimulation caused a rapid recruitment of NF-kB p65 to both TNF-α and IL-6 promoters in naive cells, whereas significantly reduced p65 binding at these promoters were observed in BLP-tolerised cells. Notably, overexpression of IRAK-1-promoted NF-kB p65 binding to both TNF-a and IL-6 promoters, thus abrogating BLP tolerisation-attenuated NF-B p65 nuclear transactivation and resulting in an increased proinflammatory cytokine production in BLP-tolerised cells (p < 0.01 vs. empty vector transfected, BLP-tolerised cells).

Conclusion: Our data highlight a crucial role of IRAK-1 in BLP-induced tolerance and suggest the therapeutic potential of targeting this molecule during bacterial infection.

Corresponding Author: Liu Jinghua, PhD, Cork University Hospital, Department of Surgery, Wilton, Cork, Ireland, j.liu@ucc.ie

A 234
The key role of Annexin 1 in macrophage migration inhibitory factor counter-regulation of anti-inflammatory effects of glucocorticoids
Yu San, Zhaofan Xia, Shihui Zhu, Hongtai Tang, Jiahui Li, Jiangrong Zhang

Objectives: Macrophage migration inhibitory factor (MIF) is an important pro-inflammatory cytokine in many immunological diseases (IDs). As a counter-regulator of glucocorticoids’ (GCs) anti-inflammatory effects, MIF may weaken the therapeutic effects of GCs on IDs. The aim of this study was to investigate the mechanisms whereby MIF exerts its counter-regulatory effect on GCs.

Material and methods: Prostaglandin E2 (PGE2) and Leukotriene B4 (LTB4) production were measured by ELISA. Protein expression of Annexin 1, cytosolic phospholipase A2 alpha (cPLA2α) and phospho-cPLA2α were evaluated by Western blotting with recombinant MIF or without endogenous MIF expression using ribonucleic acid interference (RNAi) method.

Results: Recombinant MIF counter-regulated the inhibition of dexamethasone (Dex) on PGE2 and LTB4 production in RAW 264.7 macrophages stimulated with lipopolysaccharides (LPS) in a dose-dependent manner. Stimulation of RAW 264.7 macrophages with LPS resulted in a down-regulation of Annexin 1, while Dex or Dex plus LPS led to a significant up-regulation of Annexin 1 expression. The effect of Dex on Annexin 1 was counter-regulated by the administration of recombinant MIF. RNAi-mediated knockdown of the intracellular MIF further increased Annexin 1 expression of RAW 264.7 macrophages after incubation with Dex, and accordingly resulted in a low production of PGE2 and LTB4 through inhibiting the activation of cPLA2α.

Conclusions: Annexin 1 plays a key role in MIF counter-regulation of Dex inhibition on eicosanoids production, which indicates that exogenous Annexin 1 may partially reduce the effects of MIF on GCs, thus becoming a potentially effective steroid sparing therapy on IDs.

Corresponding Author: Yu San, PhD, Chinese PLA Institute of Burn Surgery, Burn Surgery, Changhai Road, 21 Shanghai, China, littlefish0916@126.com

A 235
Crosstalk between microglial and mesenchymal stem cells in neurological diseases: focus on neuroinflammation
Sabrina Schaefer, Cédric Boucherie, Emmanuel Hermans

Objective: Neuropathic pain is a persistent pain syndrome which is often refractory to pharmacological treatments. Pathophysiological mechanisms involve molecular changes in nociceptive neurons leading to abnormally sensitive and pathological spontaneous activity as well as to an activation of microglia and astrocytes, mainly in the spinal cord and trigeminal nucleus. These cells release mediators (e.g. pro-inflammatory cytokines like TNF-α/IL1-β, NO, prostaglandins (PGs) and excitatory amino acids) that in turn directly or indirectly act upon neurons in the nociceptive circuitry and amplify pain. In this context, we want to examine the possibility to use mesenchymal stem cells (MSCs) isolated from the bone marrow in cellular therapies of neuropathic pain. As it is known that MSCs possess neuroprotective and immunomodulatory properties, we are focussing on the crosstalk between MSCs and microglial cells with the general aim to investigate the role of inflammation on migration and fate of stem cells and the influence of stem cells on inflammatory processes in a rat model of neuropathic pain.

Material and methods: Female Wistar rats underwent partial sciatic nerve ligation (PSNL) which leads within days to neuropathic pain and causes a robust, local inflammation within the spinal cord. It is associated with measurable, abnormal sensations like allodynia (pain evoked by normally innocuous stimuli) and hyperalgesia (exaggerated pain in response to painful stimuli). On day 2, 3 and 4 after PSNL, when the activity of microglial cells peaks, animals received intra-thecal BrU-labelled MSCs. In order to compare pain perception, treatment- as well as control-groups were tested with a paw thermal stimulator and von-Frey filaments.
Results: Preliminary results show that the intrathecal transplantation of MSCs attenuates the elevated pain perception in animals suffering from neuropathic pain.

Conclusion: An animal model of neuropathic pain combined with intrathecal stem cell grafting allows us to study the direct influence of inflammation on stem cell recruitment as well as the beneficial effects provided by grafted MSCs. So far, we could observe a benefit from MSC transplantation in behavioural tests. Shortly, we will apply immunohistochemistry on cryosections of the lumbar spinal cord to investigate the migration-capacity of MSCs and their adaptation- and differentiation-profile as well as their influence on the proliferation of astrocytes and microglia.

This work was supported by the National Fund for Scientific Research (FNRS, Belgium) and by the Queen Elisabeth Medical Foundation (Belgium). SS is recipient of Marie-Curie Fellowship (EURON MEST-CT-2005-020589) and EH is Research Director of the FNRS.

A 237
Inducible nitric oxide synthase-dependent regulation of superoxide production in lipopolysaccharide-stimulated RAW 264.7 cells
Michaela Pekarova, Ivana Papezikova, Katerina Pejchalova, Lukas Kabala, Antonin Lojek

Objective: After stimulation, macrophages are significant source of NADPH oxidase-derived superoxide anion (O$_2^-$) together with the nitric oxide (NO) produced mainly by inducible nitric oxide synthase (iNOS). It is known that in addition to producing NO, iNOS can catalyze O$_2^-$ formation in the presence of low L-arginine or co-factors levels. In this case, the oxidation of L-arginine is not complete and the activity of the enzyme can be 'uncoupled'. By this mechanism macrophages can contribute to tissue injury by producing peroxynitrite (ONOO$^-$), a potent oxidant thought to be a key mediator of NO-mediated tissue injury in atherosclerosis, congestive heart failure and other disease states involving inflammatory oxidative stress. In our study we used two different approaches to obtain the data which can lead to the explanation of the iNOS-dependent mechanisms contributing to O$_2^-$ production in LPS-stimulated macrophages under in vitro conditions. Firstly, we established RAW 264.7 cell clones transfected with shRNA against iNOS and secondly, we used different synthetic NOS inhibitors.

Methods: RAW 264.7 cells were transfected with plasmids containing the shRNA construct against iNOS and negative control plasmid (Origene, USA) using an electroporation system. Nitrite accumulation in cell culture media was determined by using Griess reagent. Expression of iNOS protein was determined by using Western blot. Extracellular generation of O$_2^-$ by macrophages was measured utilizing the SOD-inhibitable reduction of cytochrome c. And scavenging properties of drugs and chemicals against O$_2^-$, OH, and NO were measured by using TRAP, ORAC, cytochrome c assay and electrochemical measurement.

Results: We demonstrated that down regulation of iNOS protein expression leads to the marked reduction of O$_2^-$ production in LPS-stimulated macrophages. Application of different synthetic NOS inhibitors similarly leads to decrease of O$_2^-$ production by macrophages. Importantly, we found that under normal in vitro conditions the activity of iNOS enzyme significantly contributes to O$_2^-$ production after 15 h incubation of macrophages with LPS.

Conclusion: Our results indicate that simultaneous activation of NADPH oxidase together with induction of iNOS can result to uncoupled state of iNOS early after macrophage activation with LPS and thus can reduce the production of NO and cause increase in O$_2^-$ and ONOO$^-$ formation.

A 238
ACAMP recognition by formyl peptide receptors dampens pro-inflammatory monocyte activation through JAK/STAT/SOCS signaling
Danute Pupjalis, Danute Pupjalis, Julia Goetsch, Dianer Kottas, Volker Gerke, Ursula Rescher

The immunosuppressive effects of apoptotic cells involve inhibition of pro-inflammatory cytokine release and establishment of an anti-inflammatory cytokine profile, thus limiting the degree of inflammation and promoting resolution. We report here that this is mediated through the release of the anti-inflammatory mediator annexin A1
from apoptotic cells. Annexin A1 functions an apoptotic cell-associated molecular pattern (ACAMP) molecule by functionally activating formyl peptide receptors (FPR) an target cells. Supernatants from apoptotic neutrophils containing annexin A1 or the annexin A1 peptidomimetic Ac2-26 significantly reduced IL-6 signaling and the release of TNFalpha from endothoxin-challenged monocytes in an FPR-dependent manner. Ac2-26 JAK-dependently activated STAT3, resulting in upregulated SOCS3 levels, and depletion of SOCS3 reversed the Ac2-26 mediated inhibition of IL-6 signaling. This identifies annexin A1 as part of the ant-inflammatory pattern of apoptotic cells and links the activation of formyl peptide receptors to established signaling pathways triggering anti-inflammatory responses.

Corresponding Author: Danute Pupjalis, ZMBe, Center for Molecular Biology of Inflammation, Von-Esmarchstr.56, 48149 Muenster, Germany, pupjalis@uni-muenster.de

A 239
Ferritin ions content in acute and chronic inflammatory diseases: re-inventing ferritin role
Valerio Cozza, Pier Luigi Spada, Pasquale De Sole, Cristina Rossi, Myrti Elisa Carvelli, Gabriele Sganga

Objective: The objective of this study is to elucidate the meaning of hyperferritinemia (HF) in states of acute inflammation (i.e. sepsis) or in chronic diseases (i.e. hemodialysis or hemochromatosis) through the analysis of ferritin ions content.

Patients and methods: 14 septic (SE) patients, 13 hemodialysed (HD), 16 Hereditary Hemochromatosis (HH) and 10 plasma pools from 100 healthy subjects were examined. Iron, Al, Zn, Cd, Mn, and Pb of serum Ferritin were measured by mass spectrometry (atoms/ferritin molecule).

Results: The measurement of the absolute values of metals inside the core of serum ferritin (from 2,000 to 10,000 atoms per ferritin molecule) indicates that the metal ion mainly present is aluminum. A statistically significant low content of ferritin iron in HF-HD patients was found, with a concomitant increase in aluminium. Septic patients show a higher percentage of iron content, as well as hemochromatosis patients. Cd, Mn, and Pb represent a minimum part of Ferritin core content.

Conclusion: The high ferritin levels of HD patients cannot be motivated by an inflammatory status or a high level of iron storage. Their high levels and the low ferritin iron content values might be due to the presence inside the ferritin core of oligoelements other than iron. Because, from a clinical point of view, ferritin is mainly, although not exclusively, considered as a iron storage indicator, in view of these results we think that the name of ferritin is highly misleading; as a matter of fact, it is strongly related to the iron content while, according to our hypothesis, ferritin should be rather considered as a regulatory protein for the availability of redox active ions (mainly aluminum, iron and zinc). Therefore we suggest to give to such protein a more functional non-misleading name such as RAI-RP (redox active ions—regulatory protein). Of course it is not simply a question of name but, considering the possible relationships of Al to Alzheimer’s disease and of Zn to the treatment of severe infantile diarrhoaeas, we think it is important to stress the functional role of ferritin as a regulator of these redox metals.

Corresponding Author: Valerio Cozza, MD, University of Rome Sapienza, Department of Surgery F. Durante, Piazzale Aldo Moro 5, 185 Rome, Italy, vcozza@libero.it

A 240
Oxidized LDL-induced activation of proinflammatory secretory phospholipase A2 group IIA may lead to restenosis development of coronary arteries after coronary angioplasty
Aleksandra Korotaeva, Samoylova Elena, Pirkova Aleksandra, Prokazova Nina

Objective: Development of restenosis in coronary arteries correlates better with catalytic activity than with serum content of proinflammatory secretory phospholipase A2 group IIA \([sPLA_2(IIA)]\) in patients after coronary angioplasty. However the mechanisms of \(sPLA_2(IIA)\) activation in human blood serum so far remain obscure. Since inflammation leads to an increase in \(sPLA_2(IIA)\) secretion and LDL oxidation we examined the effects of oxidized and native LDL on \(sPLA_2(IIA)\) activity.

Methods: Activity of purified human \(sPLA2(IIA)\) was determined using radiolabeled substrate. OxLDL was prepared by an overnight incubation of freshly isolated native LDL at 370°C. The degree of LDL oxidation was evaluated from the amount of conjugated dienes and lysophosphatidylcholine.

Results: Native LDL inhibited the activity of \(sPLA_2(IIA)\). By contrast oxidized LDL significantly stimulated the \(sPLA_2(IIA)\) activity and enhanced the release of fatty acids from the substrate. The effects of LDL depended on the degree of their oxidation. Minimally and moderately LDL activated \(sPLA2(IIA)\), but strongly oxidized LDL inhibited the enzyme.

Conclusion: The data indicate that native and oxidized lipoproteins regulate catalytic activity of \(sPLA_2(IIA)\). Activation of \(sPLA_2(IIA)\) by oxidized lipoproteins may be regarded as one of the mechanisms of restenosis development after coronary angioplasty in human coronary arteries.

Corresponding Author: Aleksandra Korotaeva, Prof. PhD, Russian Cardiology Research and Production Center of Rosmedtechnology, Experimental Cardiology, 3rd Cherepovskaya, 15, 121352 Moscow, Russia, a.korot@cardio.ru

A 241
Endoplasmic reticulum (ER) stress of the liver follows traumatic hemorrhagic shock in rats
J. Catharina Davigneau, Andrey V. Kozlov, Clara Zifko, Astrid Postl, Heinz Redl, Soheyl Bahrami

Objectives: It is generally accepted that traumatic hemorrhagic shock (THS) is associated with tissue hypoxia, a known trigger of ER stress. Reperfusion after THS is often associated with onset of inflammatory reactions and increased oxidative stress, which have also been shown to induce ER stress. Persistent ER stress mediates apoptosis and is possibly involved in delayed organ dysfunction, a frequent complication following THS. Methods: It was the aim of this study to investigate key points of the different ER stress pathways (spliced XBP-1 and GRP78 mRNA, induction of GADD153 mRNA) and occurrence of markers for apoptosis (ratio of Bax/Bcl-2 mRNA) in the liver at different time points following THS (directly, 40 min, 3, and 18 h after THS).

Results: We found that THS alone induced splicing of XBP-1 mRNA. Reperfusion was associated with a further increase, and upregulation of GADD153. Full reperfusion after shock was associated with a sustained upregulation of GRP78. ER stress markers remained
A 242

Hemim mediated upregulation of heme oxygenase-1 is accompanied by endoplasmic reticulum stress and mitochondrial dysfunction in rats

J. Catharina Davigneau, Amnika Cronstedt-Fell, Clara Zifko, Susanne Haindl, Soheyl Bahrami, Astrid Postl

Objectives: Heme is often used as an experimental approach to upregulate cytoprotective heme oxygenase (HO)-1. However, heme and heme degradation products can compromise essential subcellular functions. The aim of this study was to understand the impact of heme and the subsequent upregulation of HO on mitochondrial and endoplasmic reticulum (ER) dysfunction.

Methods: Mitochondrial function (membrane potential, respiration) and different markers of ER stress (splicing of XBP1 mRNA, GADD153, and GRP78) were assessed in-vitro (isolated mitochondria, hepatocytes) and in-vivo (liver) after heme challenge at different time points (8, 24 h) with and without simultaneous inhibition of HO with ZnPP, a porphyrine analogue.

Results: Function of isolated mitochondria was severely compromised by hemin, but not by ZnPP. In hepatocytes neither hemin nor ZnPP alone, but the combination of both induced mitochondrial dysfunction and ER stress. Treatment of rats with hemin upregulated HO activity, induced a transient ER-stress, and resulted in compromised mitochondrial function after 24 h. ZnPP treatment did not affect mitochondrial function, but resulted in increased ER stress after 24 h.

Conclusion: Inhibition of heme degradation compromises the proper function of mitochondria and ER. ER stress followed by heme treatment is transient, because upregulation of HO is associated with delayed ER protection, but leading to sustained mitochondrial dysfunction.

Corresponding Author: Andrey Kozlov, Prof. PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingen Str. 13, 1200 Vienna, Austria, andrey.kozlov@libtrauma.org

A 243

Mitochondrial reactive oxygen species is an important target for inflammatory mediators in liver

Ingeborg Kehrer, Romana T. Hartl, J. Catharina Davigneau, Asmita Banerjee, Heinz Redl, Andrey V. Kozlov

Objectives: Recently we have shown that mitochondria isolated from rats subjected to endotoxic shock generate significantly more mitochondrial reactive oxygen species (mROS) than control mitochondria. This was accompanied by damage to endoplasmic reticulum (ER) in close vicinity to mitochondria and increased levels of ER-stress markers in liver. The objective of this study was to better understand pathways causing ER stress and elevated mROS induced by inflammatory mediators.

Methods: Liver cells (BRL-3A) were incubated for 6 h with media obtained from white blood cells (WBC-cond.) or plasma from whole blood (blood-cond.), which was treated ex-vivo with 6 µg/ml LPS for either 0, 6, 12 or 24 h. The cells were double stained with mitotracker (MT633) and a ROS-sensitive fluorescent dye (CM-H2XROS) and examined by confocal microscopy. Gene expression was analyzed by real time RT-PCR.

Results: We showed a drastic increase in mROS in WBC-cond, a lower but remarkable increase in blood-cond. PCR analysis showed increased levels of IL-6, a marker acute-phase reaction, in WBC-cond. cells and blood-cond. cells. Markers for ER-stress (GRP78 and spliced XBP1) were up-regulated in WBC-cond. cells. In blood-cond. cells ER-stress marker showed a trend to increase, without significance. Detail examination revealed two types of cell response inflammatory mediators. While some cells contained mitochondria which displayed increased mROS production without a detectable membrane potential, other cells contained mitochondria showing increased levels of mROS and transmembrane potential. The changes described above were modulated by mitochondria targeted radical scavengers.

Conclusion: Our data suggest that during the inflammatory reaction WBC release inflammatory mediator(s) causing enhanced mROS generation, which are able to modulate IL-6 expression and ER stress in liver cells. Whole blood contains factor(s) reverting these effects.

Corresponding Author: Andrey Kozlov, Prof. PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingen Str. 13, 1200 Vienna, Austria, andrey.kozlov@libtrauma.org

A 244

Cardiomyocytes reduce nitrite predominantly via mitochondria

Peter Dungel, Asmita Banerjee, Daniela Dopler, Oleh Andrukhov, Heinz Redl, Andrey Kozlov

Objectives: It has recently been shown that a low dose of nitrate can protect the heart from ischemia/hypoxia injury, probably, via release of nitric oxide (NO), which improves tissue perfusion. In the vascularity nitrite-mediated vasodilation is based on the reduction of nitrite in red blood cells (RBC) to a bioactive intermediate (NO or N2O3) which diffuses into smooth muscle cells and induces vasodilation. Apart from regulation of vascular tonus, nitric oxide (NO) plays an important role in maintaining cardiac function. However, the mechanism of nitrite reduction in cardiomyocytes is still unclear. The objective of this study was to clarify the predominant mechanism(s) of nitrite bioactivation in an in vitro model of hypoxia in cardiomyocytes (HL-1) co-cultured with RBC.

Methods: Isolated RBC and HL-1 were incubated in various proportions with or without nitrite. Targeted inhibitors were used to investigate the relevance of specific enzymes. Nitrosyl complexes of hemoglobin (NO-Hb), NO, and cGMP were determined by electron spin resonance spectroscopy, confocal microscopy (DAF), and ELISA.

Results: RBC or HL-1 cells alone as well as in combination, reduce nitrite to NO, yielding NO-Hb complexes in an oxygen-dependent manner. Free NO, not bound to Hb, was only detected in HL-1 cells but not in RBC. Addition of nitrite to RBC resulted in the formation of a certain amount of NO-Hb, which was remarkably elevated if RBC were coincubated with HL-1, suggesting that one portion of NO-Hb is formed from NO generated in HL-1 and diffused to RBC.

Corresponding Author: Andrey Kozlov, Prof. PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingen Str. 13, 1200 Vienna, Austria, andrey.kozlov@libtrauma.org

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Nitrite reduction was accompanied by increased cGMP levels in HL-1 cells. With increasing concentrations of RBC in co-culture the cGMP levels in HL-1 cells were gradually decreasing. Myxothiazol, a specific mitochondrial inhibitor, abolished the increase in cGMP levels induced by nitrite, and significantly but not completely reduced the levels of NO-Hb complexes originated from HL-1. Allopurinol, an inhibitor of xanthine oxidase, and L-NAME, a NOS inhibitor, had no effect on cGMP levels.

Corresponding Author: Peter Dangel, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingen Str. 13, 1200 Vienna, Austria, peter.dangel@trauma.lbg.ac.at

A 246
Sox9-associated overexpression of IFIT3 leads to pancreatic cancer progression by activation of “pseudoinflammatory” pathways

Peter Camaj, Ivan Ischenko, Hendrik Seeliger, Georg Arnold, Karl-Walter Jauch, Christiane J. Bruns

Objective: The understanding of invasion, angiogenesis and metastasis is essential for the development of new targeted molecular therapy against cancer. Inflammation plays important role in tumour initiation and progression. Here we report the role of the transcription factor Sox9 for regulation of IFIT3 (interferon-induced protein with tetratricopeptide repeats 3) in inflammation-related and tumour-promoting protein in pancreatic cancer.

Material and methods: For in vivo and in vitro experiments we utilized the following human pancreatic cancer cell lines: low metastatic FG, high metastatic L3.6pl, and the stable transfected cell line FG-IFT3. To demonstrate effects on primary tumor growth and metastases in vivo we orthotopically injected the different cell lines in the pancreas of nude mice. To evaluate the VEGF depending angiogenic capacity of the different cell lines ELISA technology was used. By One StrEPr technology we were able to identify IFIT3-binding partners. Chromosomal immunoprecipitation (ChIP) using anti-Sox9 antibody, followed by PCR amplifying the IFIT3-promoter was used to identify the interaction of the IFIT3 promoter with the transcription factor Sox9. To investigate Sox9-dependent expression of IFIT3 (protein and RNA) we used stable transfected L3.6pl-Sox9-shRNA cells under control of the Tet-CMV promoter in presence or absence of tetracycline, respectively.

Results: Analysis of differential gene expression by gene array technology demonstrated that the IFIT3 gene is up-regulated in L3.6pl cells as compared to FG cells. Results of animal experiment and in vitro experiments clearly demonstrated tumor-promoting, pro-metastatic and pro-angiogenic features of IFIT3. RT-PCR has revealed that both treatment with IFnα as well as NFκB led to up-regulation of IFIT3-RNA expression. One StrEPr experiments identified JNK and STAT1 as binding partners of IFIT3. ChIP has demonstrated binding of the transcription factor Sox9 to the IFIT3 promoter. RT-PCR and immunoblot data demonstrated constitutive up-regulation of Sox9 expression in L3.6pl cells. By Western blotting and RT-PCR we could show that diminishing of Sox9 expression in stable transfected L3.6pl Sox9-shRNA cells leads to a significant down-regulation of IFIT3-expression on the RNA and protein level.

Conclusion: The inflammation associated protein IFIT3 is up-regulated in metastatic L3.6pl human pancreatic cancer cells and is in part responsible for the aggressive primary pancreatic tumor growth in vivo. This gene is up-regulated by IFnα and NFκB. Interestingly Sox9 binds to the IFIT3 and activates its expression. Since in L3.6pl cells Sox9 is constitutively over-expressed, IFIT3 is up-regulated independent on the presence of the cytokine IFnα. Therefore, the pro-inflammatory IFnα-signaling pathway is activated even without actual inflammation in absence pro-inflammatory cytokine. The activation of such a “pseudo-inflammatory pathway” seems to be in part responsible for pancreatic cancer progression.

Corresponding Author: Peter Camaj, PhD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Exp. Research Surgery, Marchioninistr. 15, 81377 Munich, Germany, nikto6@gmail.com

A 245
Geldanamycin derivative 17-AAG mediates radiosensitization in hypoxic tumor cells

Daniela Schilling, Christine Bayer, Rudolf Huber, Albrecht Bergner, Michael Molls, Gabriele Multhoff

Objectives: Hypoxia in solid tumors is associated with resistance to radiotherapy and poor prognosis. Hypoxia leads to stabilization of the a subunits of the hypoxia inducible factors (HIF) which has been implicated in the upregulation of proteins involved in tumor angiogenesis, invasion and radiosensitivity (e.g. VEGF, PAI-1). Since HIF-1α and HIF-2α are heat shock protein 90 (Hsp90) clients, Hsp90 inhibition is expected to block the hypoxic signaling pathway and inhibit tumor progression. Therefore, in the present study, we systematically investigated the influence of the Hsp90 inhibitor 17-AAG on the expression and/or secretion of the hypoxia-inducible genes—HIF-1α, HIF-2α, VEGF, PAI-1—and Hsp90 in a small (SCLC; H1339) and non-small cell lung cancer (NSCLC; EPLC-272H) cell line. Furthermore, we investigated the combined effect of 17-AAG and radiation on normoxic and hypoxic tumor cells.

Material and methods: Thirty minutes after 17-AAG administration, cells were exposed for different times to hypoxia (<1% O2). HIF-2α, Hsp70 and PAI-1 expression were investigated by Western Blot. Cellular HIF-1α expression and secreted Hsp70, VEGF and PAI-1 levels were quantified by ELISA. Hsp70 membrane expression was determined by flow cytometry. Clonogenic assays were used to determine survival fractions after treatment with 17-AAG, hypoxia and irradiation.

Results: Hypoxic incubation for up to 24 h induced HIF-1α expression only in EPLC-272H and not in H1339 cells. In contrast, HIF-2α expression was upregulated in H1339 cells by hypoxia. Incubation with 17-AAG led to a significant down-regulation of hypoxia-induced HIF-1α in EPLC-272H, and a weak down-regulation of HIF-2α expression in H1339 cells. PAI-1 and VEGF expression and secretion levels were down-regulated by 17-AAG in both cell lines. As a result of the Hsp90 inhibition, cytoplasmic and secreted but not membrane Hsp70 levels were strongly up-regulated by 17-AAG in both tumor cell lines. Low concentrations of 17-AAG (10 and 20 nM) reduced the clonogenic cell survival in H1339 cells stronger than in EPLC-272H cells. Furthermore, normoxic and hypoxic H1339 tumor cells, but not EPLC-272H cells, could be radiosensitized by treatment with 17-AAG.

Conclusion: By targeting multiple oncogenic Hsp90 client proteins with 17-AAG, hypoxic tumors might become more sensitive towards radiotherapy. In our lung carcinoma cell lines radiosensitization appears to be independent of 17-AAG mediated downregulation of HIF-1α.
A 247

Standardized multiparametric analysis of the eicosanoid response in human whole blood using tandem mass spectrometry

Ute Ludwig, Uta Ceglarek, Linda Kortz, Georg Martin Fiedler, Mathias Bruegel, Joachim Thiery

Objectives: Eicosanoids are lipid mediators that are primarily oxidized from arachidonic acid by enzymatic or non-enzymatic peroxidation and are discussed as central mediators of inflammatory processes. A systemic multiparametric analysis of the whole blood eicosanoid response to defined proinflammatory stimuli such as lipopolysaccharide (LPS) would allow to investigate the individual inflammatory response in various diseases, possibly resulting in new diagnostic and therapeutic strategies. Therefore, the aim of our study was to develop a standardized analytical protocol for the determination of the total eicosanoid response in LPS-activated human whole blood using tandem mass spectrometry and to investigate the variability of the eicosanoid response in healthy donors.

Material and methods: For standardization experiments, fresh human whole blood (Li-heparin, EDTA-blood, citrated blood) was drawn from healthy individuals and was incubated with different concentrations of LPS (0.1, 1, 10 μg/ml) for different time periods (6, 12 and 24 h). Whole blood was mixed with or without RPMI 1640 medium in proportions 1:5 and 2:1. Applying the developed standardized protocol, we determined the eicosanoid response of human whole blood (Li-heparin) from 15 healthy subjects after activation with LPS.

Results: Activation with LPS for 24 h resulted in a marked induction of major representatives of the cyclooxygenase-pathway. Within- and between day reproducibility of eicosanoid induction after LPS whole blood activation ranged from 4 to 21%. The interindividual variability of the measured eicosanoid concentrations in LPS activated whole blood from 15 healthy subjects ranged between 23 and 60%.

Conclusions: We developed a standardized protocol for reproducible LPS-activation of human whole blood and subsequent eicosanoid analysis by a multiparametric LC-MS/MS approach. Our first results indicate a remarkable interindividual variation in the eicosanoid response of healthy individuals to LPS, possibly affecting the disposition for inflammatory diseases. Using our standardized whole blood model, we are currently investigating the whole blood eicosanoid response of patients with sepsis.

Corresponding Author: Ute Ludwig, University Hospital Leipzig, Institute for Laboratory Medicine, Clinical Chemistry & Molecular Diagnostics, Liebigstr. 27, 04109 Leipzig, Germany, u.ludwig@medizin.uni-leipzig.de

A 248

Use of flow cytometry multiplex and ELISA to assess and characterize selected cytokine and other biomarker levels in serological samples from patients with severe sepsis

Jim Growcott, Aaron Dane, Jon Armstrong, Paul Newell, Fiona King, Steven Simonson

Objectives: Severe sepsis and septic shock have posed significant treatment challenges for many years. Recently, a number of biomarkers have emerged that may be predictive of likely outcome. We conducted a noninterventional study to establish the feasibility of measuring selected circulating cytokine levels using flow cytometry multiplex (FMC) and ELISA in such patients (pts) to detect and mitigate any critical issues before commencing clinical evaluation of a novel agent.

Methods and materials: This study was conducted in pts aged ≥18 yrs admitted to the ICU with severe sepsis at five US centers. Blood samples were collected: within 2 h of consent (baseline); at 8, 16, and 24 h thereafter; and once daily on days 2–5. Serum levels of tumor necrosis factor-α (TNFα), interleukin (IL)-6, IL-8 (by ELISA and FCM), IL-10 (by FCM), and procalcitonin (by immunoluminescence) were determined. Further blood samples were collected within 2 h at baseline and on days 2, 4, and 8 for measurement of cleaved cyto- keratin 18 (M30) and nucleosomal DNA (nDNA) plasma levels (both by ELISA). Levels of each biomarker were presented descriptively.

Results: 22 pts (mean age 60 [range 24–83] yrs; 11 men) were included. Most pts (n = 21; 95%) were White and the mean APACHE II score was 24.4 (range 7–50). One-third of pts had three organ system failures and over one-half had septic shock; three pts died. Quantifiable levels of TNFα, IL-6, IL-8, M30, and nDNA were found in serum and plasma using ELISA (baseline gmean ± SD: 4.06 ± 4.85, 219 ± 3,267, 138 ± 181 [all pg/mL]; 304 ± 525, 0.52 ± 0.81 [both units/L], respectively); IL-6, IL-8, and IL-10 were also detected using FCM (baseline gmean ± SD: 315 ± 3,707, 271 ± 16,665, 17.5 ± 39 [all pg/mL], respectively). However, FCM did not appear to have sufficient sensitivity to reliably quantify TNFα levels. Quantifiable levels of procalcitonin were detected using immunoluminescence (baseline gmean ± SD: 7.5 ± 33.7 ng/mL) and declined over the course of the evaluation.

Conclusions: FCM and ELISA reflect the same trends in biomarker levels in serological samples from pts with severe sepsis, but actual values differ substantially between the assay systems. FCM is able to assess multiple biomarkers using a small sample volume, but cannot reliably detect and quantify low biomarker levels, whereas ELISA provides this precision. FCM may be of utility in cases where blood volume is at a premium and precision is not critical.

Corresponding Author: Jim Growcott, AstraZeneca R&D, Alderley Park, Macclesfield SK10 4TF, UK, jim.growcott@astrazeneca.com

A 249

Assessment of circulating levels of full-length and caspase-cleaved cyto-keratin 18 and fragments of nucleosomal DNA in the plasma of patients with severe sepsis: a pilot study

David Moore, Alastair Greystoke, Fouchia But, Jim Growcott, Andrew Hughes, Caroline Dive

Objectives: Severe sepsis and septic shock have posed significant treatment challenges for many years. Recently, a number of circulating apoptosis biomarkers have emerged, such as full-length and caspase-cleaved cytokeratin 18 (CK18) and nucleosomal DNA (nDNA), that may be predictive of likely outcome. This non-interventional study aimed to assess the ability of ELISA assays of such biomarkers to provide clinically useful information to guide the management of sepsis.

Methods and materials: This study was conducted in patients (pts) admitted to the ICU with severe sepsis at five US centers. Blood samples for assessment of plasma levels of full-length and caspase-cleaved CK18 and nDNA (both by ELISA) were collected from pts within 2 h of consent (baseline) and on days 2, 4, and 8. Blood samples from 17 healthy volunteers acted as controls. Levels of each biomarker were presented descriptively.

Results: 22 pts (mean age 60 [range 24–83] yrs; 11 men) were included. Most pts (n = 21; 95%) were White and the mean APACHE II score was 24.4 (range 7–50). One-third of pts had three organ system failures and over one-half had septic shock; three pts
died. Baseline levels of all three apoptosis biomarkers were significantly higher in pts with severe sepsis versus controls (Table 1). CK18 levels decreased within 48 h following initiation of treatment of sepsis in pts who survived, whereas increases were observed in the same timeframe in pts who died within 28 days of admission (Table 2). Baseline nDNA and total soluble CK18 levels (caspase-cleaved and total soluble) were significantly (p ≤ 0.05) higher in pts who required renal support than those who did not (data not shown).

Table 1  Median baseline apoptosis biomarker levels

<table>
<thead>
<tr>
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<th>Patients (n = 22)</th>
<th>Controls (n = 17)</th>
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<tbody>
<tr>
<td>Caspase-cleaved CX18 (U/L)</td>
<td>258*</td>
<td>153</td>
</tr>
<tr>
<td>Total soluble CK18 (U/L)</td>
<td>955**</td>
<td>241</td>
</tr>
<tr>
<td>nDNA (optical density ×1,000)</td>
<td>510**</td>
<td>89</td>
</tr>
</tbody>
</table>

* p < 0.05
** p < 0.001 versus controls

Table 2  Percent change from baseline in apoptosis biomarker levels at day 2

<table>
<thead>
<tr>
<th></th>
<th>Survivors (n = 18)</th>
<th>Nonsurvivors (n = 3)</th>
</tr>
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<tbody>
<tr>
<td>Caspase-cleaved CX18</td>
<td>−16*</td>
<td>+36</td>
</tr>
<tr>
<td>Total soluble CK18</td>
<td>−15*</td>
<td>+168</td>
</tr>
<tr>
<td>nDNA</td>
<td>+7</td>
<td>+38</td>
</tr>
</tbody>
</table>

* p < 0.05 versus nonsurvivors

Conclusions: These results confirm that measurement of apoptosis biomarkers in severely ill pts with sepsis may provide clinically useful information for management of sepsis.

Corresponding Author: David Moore, Prof. MD, Paterson Institute for Cancer Research, Clinical & Experimental Research Group, Wilmslow Road, Manchester M20 4BX, UK, dmoore@picr.man.ac.uk

A 250
Reduced autophagic activity of splenic dendritic cells following trauma in mice
Huaping Liang, Xia Fan, Qiang Wei, Xue Yang, Xi Wang

Objective: Autophagy in antigen-presenting cells (APC) such as macrophages and dendritic cells plays an important role in innate and adaptive immunity against intracellular pathogens. It remains unknown whether or not dendritic cells’ autophagic activity is altered after serious trauma. This issue was investigated in this study.

Methods: A murine model of trauma (two hind femurs fracture plus hemorrhage of 35% blood volume) in BALB/c mice was performed, splenic dendritic cells (CD11c+) were isolated and their autophagic activity was detected by calculating the percentage of punctated monodansylcadaverine (MDC) staining cells and lack of autophagosomes. Moreover, induced autophagy by lipopolysaccharides treatment in dendritic cells from control group was enhanced while that from 1-day post-trauma group had resistance to this autophagy induction factor. There were no statistical differences in rapamycin-induced autophagy between these two groups.

Conclusions: These findings suggest that autophagy in dendritic cells, a protective mechanism in response to stress and inflammation was suppressed following traumatic injury.

This study was supported by the national natural science foundation of China (No. 30772253)

Corresponding Author: Huaping Liang, Prof. MD, PhD, Research Institute of Surgery, Daping Hospital, Third Military Medical University, Department 1, Changhai Zhi Road 10&#65292; Daping, 400042 Chongqing, China, huaping_liang@yahoo.com.cn

A 251
Serum markers of abnormal blood brain barrier function after traumatic brain injury
Jeff Bazarian, Brian Blyth, Gerry Shaw

Objectives: To evaluate the accuracy of serum S-100B, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and phosphorylated neurofilament heavy chain (pNFH) concentrations as measures of blood brain barrier (BBB) integrity after traumatic brain injury (TBI) compared to the gold standard of serum albumin quotient (QA).

Methods: The current gold standard for assessing BBB status is measuring QA = [albuminCSF]/[albuminserum]. Normal BBB permeability is defined as a QA ≤ 0.007. Sixteen patients with moderate to severe TBI (GCS < 12) and 6 patients with non-traumatic headache who had CSF collected by ventriculostomy or lumbar puncture (LP) were analyzed. Serum and CSF were collected at the time of LP for headache patients and 12, 24, and 48 h after ventriculostomy for TBI patients. Serum was also collected at time of presentation to the emergency department for TBI patients. Albumin was measured by nephelometry; S100B, UCH-L1, and pNFH were measured by ELISA.

The QA was calculated for all time points at which paired CSF and serum samples were available. Pearson’s correlation coefficient and area under the receiver operator characteristic (ROC) curve were used to determine the relationship between serum markers and QA.

Results: QA values for all headache subjects were normal with a mean of 0.006 (SD 0.002). The mean QA for TBI subjects was 0.047 (SD 0.062) at 12 h, 0.046 (SD 0.111) at 24 h, and 0.012 (SD 0.012) at 48 h. Mean marker values in controls were lower than for TBI patients at any time point. Using ROC analyses, a significant relationship was found between QA and UCH-L1 12 h after TBI (AUC = 0.76; 95% CI 0.55–1.00), but not 24 or 48 h. A significant relationship was found between QA and S100B 12 h after TBI (AUC = 0.79; 95% CI 0.57–1.02), but not 24 or 48 h. pNFH did not correlate with QA at any time point after injury. Combining S100B and UCH-L1 produced an AUC that was greater than with either marker alone (0.80, 95% CI 0.59–1.02)

Conclusions: Peripheral elevations of the brain specific protein UCH-L1 are associated with abnormal BBB status 12 h after moderate to severe TBI. This relationship is similar to that observed between serum S100B concentrations and QA despite the fact that S100B is thought to be released from peripheral tissues after multi-trauma. A combination of the two tests is a better predictor of abnormal BBB status than UCH-L1 alone.

Corresponding Author: Jeff Bazarian, MD, University of Rochester Medical Center, Emergency Medicine, 601 Elmwood Ave, Rochester, NY 14642, USA, jeff_bazarian@urmc.rochester.edu
A 252
Differential expression of triggering receptor expressed on myeloid cells 1 (TREM-1) and GM1 on monocyte subpopulations in septic patients
Jacqueline Oliva, Isabel Wong-Baeza, Eduardo Ferat-Osorio, Eli’ Guido-Guerra, Lourdes Arriaga-Pizano, Constantino López-Macías

Objective: The expression levels of triggering receptor expressed on myeloid cells-1 (TREM-1) and HLA-DR on monocytes have been studied as possible biomarkers in septic patients. However, monocytes are highly heterogeneous, and subpopulations can be defined by the expression levels of CD14, CD16 and GM1. So, in this study we determined the expression levels of TREM-1, HLA-DR and GM1 on CD14highCD16low and CD14lowCD16high monocyte subpopulations in septic patients, and evaluated their association with the outcome of the disease (survival/death).

Patients and methods: Blood samples were taken from 19 adult septic patients; 9 of these patients survived and 10 died. The first sample was taken within the first 24 h after diagnosis, and the second and third samples were taken 3 and 7 days later. Samples from 15 blood bank donors were also analyzed. Peripheral blood mononuclear cells were isolated by gradient centrifugation, stained with fluorochrome-labeled anti-CD14, anti-CD16, anti-HLA-DR, anti-TREM-1 and cholera toxin B-subunit (which binds GM1), and analyzed by flow cytometry.

Results: TREM-1 expression levels were increased on both monocyte subpopulations in septic patients, compared to healthy donors. However, this increase was only statistically significant on the CD14highCD16low subpopulation. No differences were found between survivors and non-survivors. A positive correlation between the expression levels of TREM-1 and GM1 on both subpopulations was observed. GM1 expression on the CD14highCD16low subpopulation was higher in survivors than in non-survivors. HLA-DR is decreased on both monocyte subpopulations in septic patients, compared to healthy donors, but no differences were observed between these subpopulations.

Conclusions: TREM-1 and GM1 are differentially expressed on monocyte subpopulations in septic patients; their expression levels are higher on the CD14highCD16low subpopulation, and a high GM1 expression on this subpopulation correlates with survival. Since GM1 is a constituent of lipid rafts, our results suggest that TREM-1 may co-localize with lipid rafts in septic patients, and that a loss of this signaling platforms (indicated by decreased GM1 expression) is associated with a failure to control sepsis.

Corresponding Author: Armando Isibasi, MD, PhD, IMSS Hospital de Especialidades, Unidad de Investigación Médica en Inmunología, Cuauhtemoc 330, 6720 Mexico City, Mexico, isibasi@prodigy.net.mx

A 253
Dynamics of the interaction of particulate matter with the internal lung surface: a model
Peter Gehr, Barbara Rothen-Rutishauser, Martin Clift, Fabian Blank

Epidemiologic studies give evidence that inhalation of fine particles and nanoparticles cause increased cardio-pulmonary morbidity and mortality. A series of structural and functional barriers protect the respiratory system against harmful particles: Surfactant and aqueous liquid layer, epithelial cell layer with macrophages on top, dendritic cells at the base, basement lamina. It is still not clear how the highly immunocompetent dendritic cells located at the base of the airway and alveolar epithelium take up inhaled and deposited antigens and how airway and alveolar macrophages on top of the epithelium may interact with the dendritic cells and with the epithelium.

Particles deposited on the surface of the airway and alveolar wall, i.e. on the surfactant film at the internal pulmonary air-liquid interface, are wetted and displaced toward the epithelium by surface forces which are exerted on them by the surfactant. Fine particles may be phagocytized by macrophages and dendritic cells. Whereas macrophages would clear these particles through mucociliary action, leaving the lungs via the airway system, dendritic cells would rather carry them to the specific immunological defence system.

Nanoparticles, however, may penetrate into the pulmonary tissue and they may be taken up by or enter cells by alternative mechanisms, i.e. by endocytosis, through pores or passively by adhesive interaction with cell membranes. It is assumed that it is by these mechanisms that nanoparticles can penetrate through the air-blood tissue barrier into the capillaries leaving the lungs via the vascular system. They will be transported by the blood stream to other organs.

With a triple cell co-culture model of the human epithelial airway wall, we could demonstrate that dendritic cells collect particles deposited on the internal pulmonary surface and transport them across the epithelium through their cytoplasmic processes. Macrophages, similar to dendritic cells, may push slender cytoplasmic processes through the tight junctions and between the epithelial cells to the base of the epithelial layer. Dendritic cells may also interact with particle loaded airway macrophages on top of the epithelium.

Based on our findings, we postulate that these two cell types, acting as sentinels in the airways and alveoli, build a trans-epithelial interacting cellular network, which makes them an efficient intraepithelial complex cellular defence system against inhaled particulate antigens.

Corresponding Author: Peter Gehr, Prof. PhD, University of Bern, Institute for Anatomy, Baltzerstr. 2, 3000 Bern, Switzerland, gehr@ana.unibe.ch

A 254
The thrifty epigenotype: an acquired and heritable predisposition for metabolic inflexibility
Reinhard Stoeger

Genes and environmental factors can protect from—or promote—the onset of complex metabolic disorders. A heritable predisposition to diabetes or obesity has historically been thought to have a genetic basis. However, despite large-scale screening efforts, only a limited number of common genomic variants have been identified and their relative impact on disease risk is small. Here, it is proposed that genomic programs regulating energy balance evolved to be buffered or ‘canalised’ against genetic variation, thereby providing an explanation for the difficulty to find sequence polymorphisms associated with diabetes or obesity.

Under this model, the current worldwide obesity epidemic is largely attributable to rapid changes in human diets and activity levels. These environmental changes, occurring in the context of a canalized genomic background, are proposed to produce epigenetic changes that significantly increase the risk of metabolic disease. Indeed, a large body of evidence implies that events during early life can permanently influence physiological processes and modulate disease risk in later life.

It is proposed that these effects are mediated by large genetic regulatory networks that acquire novel epigenetic states in response to environmental challenges encountered during developmental periods of early life. Such altered epigenetic states may be transmitted through the germ line, thus affecting the health of subsequent generations.
Corresponding Author: Reinhard Stoeger, PhD, University of Nottingham, School of Biosciences, College Road, Nottingham LE12 5RD, UK, Reinhard.Stoeger@nottingham.ac.uk

A 255
LPS trafficking and inactivation in mice and men?
Mingfang Lu, Robert S. Munford

Despite intense interest in host reactions to Gram-negative bacterial LPSs, very little is known about how these stimulatory molecules traffic in vivo. We used both radiolabeled and FITC-labeled LPSs to study the movement of LPS from a subcutaneous injection site, the footpad, to the draining popliteal and inguinal lymph nodes (DLN) in mice. Although LPS appeared in DLN within 3 min of injection into the footpad, drainage from footpad to DLN continued for over 6 weeks; both loss of LPS from the footpad and its appearance in the DLN were concentration-dependent, suggesting passive (cell-free) diffusion. In wildtype mice, over 80% of the LPS underwent inactivating deacetylation before it left the footpad. In mice that lacked acyloxyacyl hydrolase (Aoah−/−), in contrast, fully acylated LPS continued to drain for many weeks, stimulating massive lymph node enlargement (B cell proliferation) and polyclonal antibody production. Depletion of neutrophils or macrophages prior to LPS injection significantly slowed the deacetylation rate in wildtype animals. These results suggest that much of the LPS that enters the circulation from a local site of Gram-negative bacterial infection does so via lymphatic channels and that it is not carried by cells. They also support a major role for AOAH in LPS deacylation/detoxification in vivo. Since human myeloid cells produce much more AOAH than do murine cells, a prominent role for the enzyme in LPS deacetylation/detoxification also seems likely in humans. Strategies for investigating its role in humans will be proposed.

Corresponding Author: Robert S. Munford, Prof. MD, PhD, Laboratory of Clinical Infectious Diseases, NIAID, NIH, USA. lum3@niaid.nih.gov or munfordrs@niaid.nih.gov

A 256
S100 proteins as an alarmin for states of trauma and disease
Johannes Roth

Within the S100-family of calcium-binding molecules, myeloid related protein 8 (MRP8, S100A8) and MRP14 (S100A9) are pro-inflammatory proteins expressed and secreted by phagocytes, which play a pivotal role within the processes of trauma and sepsis. They are novel members of the DAMP-family and promote inflammatory processes. S100A8 and S100A9 are released at high concentrations at local sites of inflammation by activated neutrophils and monocytes. They exhibit pro-inflammatory effects in vitro at concentrations found during inflammation in vivo. It was supposed that RAGE may function as a general receptor for all S100 proteins. The pro-inflammatory effects of S100A8 and S100A9, however, depend clearly upon interaction with TLR4. S100A8 and S100A9 are overexpressed at local sites of inflammation and high concentrations are found in inflammatory exudates and serum during inflammation. The S100A8/ S100A9 complex has been proven to be useful as diagnostic marker of inflammation especially in arthritis, chronic inflammatory bowel disease, sepsis and polytrauma. They reflect activation of phagocytes more sensitively than conventional parameters of inflammation. As a consequence, there is a close correlation to the disease activity of various inflammatory disorders, making these proteins sensitive parameters for the monitoring of response to treatment in individual patients. Recently, we have identified S100A9 as the first molecular target of the DAMP-family for anti-inflammatory therapies.

Corresponding Author: Johannes Roth, Prof. PhD, University of Muenster, Institute of Immunology, Roentgenstr. 21, 48149 Muenster, Germany, rothj@uni-muenster.de

A 257
Crystals and the NALP3 inflammasome
Eicke Latz

Innate immunity evolved to recognize microbial infection and to respond to danger signals that appear under disease conditions. The most recently described innate immune receptor family is the Nod-like receptor (NLR) family. The NLR member NLRP3 and the adapter protein ASC form a multi-molecular complex termed the NLRP3 inflammasome. Inflammasomes control the activity of caspase-1, which cleaves and activates the pro-form of the inflammatory cytokines IL-1β and IL-18. The NLRP3 inflammasome can be activated by various membrane active bacterial toxins (e.g. nigericin, maitotoxin or gramicidin) or after phagocytosis of crystalline materials (e.g. silica, asbestos, monosodium urate or alum). The mechanisms by which the NLRP3 inflammasome is activated by physico-chemical diverse activators are not well understood. We demonstrate that crystals activate the NLRP3 inflammasome in a process that requires phagocytosis and we found that crystal uptake leads to lysosomal damage and rupture. Furthermore, sterile lysosomal damage is also sufficient to induce NLRP3 activation and inhibition of phagosomal acidification or inhibition or lack of cathepsins impairs NLRP3 activation. These results indicate that the NLRP3 inflammasome can sense lysosomal damage as an endogenous danger signal. Our results demonstrate a novel strategy of immune cells to recognize different classes of stimuli by a common, indirect mechanism.

Corresponding Author: Eicke Latz, MD, PhD, University Hospital of Bonn, Institute of Innate Immunity, Sigmund-Freud-Str. 25, 53127 Bonn, Germany, eicke.latz@umassmed.edu

A 258
Neutrophil granule proteins alarm the immune system
Oliver Soehnlein

Neutrophil granulocytes are mostly recognized for functions such as crawling on the endothelium, escaping from the circulation, eating and digesting bacteria and producing oxygen radicals. Undoubtedly, these functions are vital in host defense but perhaps equally important are the neutrophils functions in alarming the immune response, e.g. by recruiting and activating antigen presenting cells, such as monocytes, macrophages, and dendritic cells. Therein, prepacked, ready-made granule proteins hold a key position. Granule proteins are stored in neutrophil granule subsets which undergo limited exocytosis upon neutrophil emigration. Thereby granule contents are either deposited on the endothelial cell surface or seeded out in close proximity to inflammatory cells. In this location, adhesion and recruitment of mononuclear cells is stimulated involving G protein coupled receptors such as formyl peptide receptors. LL-37 and azurocidin are prominently involved in this response. Alpha-defensins, on the other hand, were shown to attract immature dendritic cells while they fail to recruit mature dendritic cells. In addition, neutrophil granule proteins
such as alpha-defensins and azurocidin induce macrophage activation ultimately enhancing bacterial uptake and killing as well as release of pro-inflammatory cytokines. Induction of dendritic cell maturation is yet another mechanism by which LL-37 and alpha-defensins contribute to shaping the immune response. Taken together, the instant release of preformed granule proteins from emigrating neutrophils promotes pro-inflammatory responses in antigen-presenting cells.

Corresponding Author: Oliver Soehnlein, MD, PhD, RWTH Aachen University Hospital, IMCAR, Pauwelsstr. 30, 52074 Aachen, Germany, osoehnlein@ukaachen.de

A 259
Single nucleotide polymorphism (SNP) of Toll-like receptor (TLR)4 influences hypothalamic-pituitary-adrenal (HPA) axis regulation in patients with postoperative systemic inflammation
Alexander Koch, Lutz Hamann, Ralf Schumann, Carsten Schwenke, Stefan Bornstein, Kai Zacharowski

Objective: Compared to wild type, TLR4 deficient mice demonstrate significant different regulation of the HPA axis under both, basal and stimulated (systemic inflammation) conditions. This underlines the link between innate immunity and HPA axis control. 6–14% of the European population are carriers of a TLR4 SNP. We investigated the effect of TLR4 SNP on ACTH and cortisol regulation in patients with systemic inflammation following cardiac surgery.

Patients and methods: Following ethical approval and patients consent we enrolled patients undergoing cardiopulmonary bypass surgery. Blood samples were obtained pre-op between 07:00 and 09:00 a.m. (A), at ICU admission (B) and on the first post-operative day between 07:00 and 09:00 a.m. (C). Samples were screened for TLR4 SNP (Asp299Gly and Thr399Ile), ACTH and cortisol levels. Demographic and clinical data were obtained. Time courses were analyzed by means of absolute changes from baseline (A) for time points (B and C) in a linear mixed model. Multiple visits per patient were taken into account. Data are presented as mean value with 95% confidence intervals (CI).

Results: 273 patients were enrolled. 230 did not carry a TLR4 SNP (contr.), 43 (15.8%) patients were detected to be carriers for a TLR4 SNP (TLR4 SNP). In both groups changes in cortisol levels were found to be significantly increased at time point B [contr.: 19.6 (95% CI, 15.5–23.7); TLR4 SNP: 19.8 (95% CI, 11.7–27.8)]. At time point C both groups still demonstrated significantly elevated but lower levels of ACTH (contr.: 25.0 (95% CI, 11.1 to 48.9)]. Furthermore there was a significant difference in ACTH level changes between the two groups at this time point. At time point C ACTH level changes in both groups were no longer elevated or did differ between the groups in a significant manner. Conclusion: We conclude that in patients carrying the TLR4 SNP, HPA axis control in terms of cortisol release is at least less dependent if not independent of ACTH release during systemic inflammation. Our findings could explain the diverse results of clinical trials (e.g. Corticosteroids) investigating HPA axis regulation and modulation during SIRS/sepsis.

Corresponding Author: Alexander Koch, MD, Johann Wolfgang Goethe University of Frankfurt/M, Clinic of Anaesthesiology, Intensive Care Medicine and Pain Therapy, Theodor-Stern-Kai 7, 60509 Frankfurt/M, Germany, a.koch@med.uni-frankfurt.de

A 260
Evidence for a novel gut-brain-immune axis: nutritional activation of a cholecystokinin-mediated afferent vagal pathway attenuates inflammation
Tim Lubbers, Jacco-Juri de Haan, M’hamed Hadjoune, Isabelle Verbaeys, Wim Buurman, Jan Willem Greve

Objective: Previously, we demonstrated that lipid-rich enteral nutrition modulates inflammation and prevents organ damage in a rat model of hemorrhagic shock and postoperative ileus. Although it is known that enteral nutrition activates various humoral and neural pathways to regulate homeostatic processes, the pathway via which lipid-rich nutrition triggers immuno-modulating effects remains to be unraveled. The current study investigates activation of the nutritional anti-inflammatory pathway by lipid-rich nutrition.

Materials and methods: Male Sprague–Dawley rats were subjected to hemorrhagic shock. Prior to shock, rats were fasted or fed lipid-rich nutrition enriched with phospholipids. Disruption of afferent vagal fibers with capsaicin (deafferentation) was used to investigate involvement of afferent fibers in the nutritional anti-inflammatory pathway. Peripheral activation of afferent vagal fibers via cholecystokinin (CCK)-mediated activation of CCK-1 receptors was investigated using administration of the selectively peripheral acting CCK-1 receptor antagonist, A70104 and PEG-CCK9, an agonist for peripheral CCK-1 receptors. Tissue and blood were collected 90 min after shock to assess systemic inflammation and intestinal integrity. A Mann–Whitney U test was used for between group comparisons, n = 8 for all groups.

Results: Deafferentation reversed the protective effect of lipid-rich nutrition on shock-induced systemic levels of TNF-α (145 ± 10 pg/ml vs. 60 ± 11 pg/ml [sham]; p < 0.001) and bacterial translocation (BT: 113 ± 20 CFU/g tissue vs. 33 ± 4 CFU/g tissue [sham]; p < 0.01). Furthermore, the protective effects of lipid-rich nutrition were negated by A70104 (TNF-α: 111 ± 20 vs. 37 ± 7 pg/ml [vehicle] and BT: 98 ± 19 CFU/g tissue vs. 30 ± 3 CFU/g tissue [sham]; both p < 0.01), indicating that lipid-rich nutrition triggers peripheral cholecystokinin-1 receptors on vagal afferents to modulate inflammation. These findings were supported by the fact that pre-treatment of fasted rats with PEG-CCK9 attenuated systemic levels of TNF-α and BT compared with vehicle (both p < 0.01).

Conclusion: These data demonstrate that enteral lipid-rich nutrition modulates inflammation and preserves intestinal integrity via peripheral CCK-mediated activation of CCK-1 receptors located on afferent vagal fibers. The current study reveals a novel gut-brain-immune axis and underlines the applicability of lipid-rich nutrition to treat inflammatory conditions.

Corresponding Author: Tim Lubbers, MD, Maastricht University Medical Center, Department of General Surgery, Universiteitsringel 50, 6200 MD Maastricht, The Netherlands, t.lubbers@ah.unimaas.nl

A 261
Lipid-rich nutrition preserves renal and intestinal damage via nicotinic receptors in a rat hemolysis model
Jacco-Juri de Haan, Bas Hansen, Iris Vermeulen Windstott, Tim Lubbers, Jan-Willem Greve, Wim Buurman

Objective: Massive hemolysis and rhabdomyolysis are emergencies frequently seen in trauma and surgical settings. Free circulating hemoglobin and myoglobin impair microcirculation via scavenging of intravascular nitric oxide (NO), a phenomenon that is associated with the development of organ damage. Previously, we demonstrated in a hemorrhagic shock model that lipid-rich enteral feeding prevents free circulating hemoglobin and myoglobin from impairing microcirculation via scavenging of intravascular nitric oxide (NO), a phenomenon that is associated with the development of organ damage.
organ damage via stimulation of the vagus nerve. Since the vagal neurotransmitter acetylcholine is a known vasodilator lancing acting independently of NO, here we investigate the effects of lipid-rich nutrition on intestinal and renal organ damage in a rodent model of acute hemolysis.

Material and methods: Hemolysis in Sprague-Dawley rats was simulated by administration of free hemoglobin (fHb). Following a bolus given at T = 0, fHb was infused continuously for 60 min to reach clinically relevant concentrations (37 ± 2 μM). Prior to fHb administration, rats were fasted or fed nutrition enriched with phospholipids. Chlorisondamine, an antagonist to peripheral nicotinergic acetylcholine receptors, was administered at 30 min before fHb. Microcirculatory changes in jejunum and ileum were evaluated using fluorescent microspheres. Urine and tissue samples were harvested at 120 min to assess renal and intestinal organ damage. Comparisons between groups (all n = 6) were performed with a Mann–Whitney U test.

Results: Lipid-rich nutrition reduced bacterial translocation compared with fasted animals (108 ± 8 CFU/g tissue vs. 154 ± 8 CFU/g tissue; p < 0.05). Furthermore, intestinal permeability to horseradish peroxidase was decreased in lipid-rich treated rats (2.9 ± 0.2 vs. 4.0 ± 0.2 μg/mL [fasting]; p < 0.01), which was accompanied by improved splanchnic perfusion compared with fasted animals (p < 0.05). In addition, urine concentrations of tubular injury marker N-acetyl-D-glucosaminidase (NAG) were reduced in lipid-rich fed rats (22 ± 4 U/mmol creat vs. 35 ± 4 U/mmol creat [fasting]; p < 0.05). Administration of chlorisondamine abrogated the protective effects of lipid-rich nutrition compared with vehicle (all parameters p < 0.05).

Conclusion: This study demonstrates that enteral lipid-rich nutrition preserves intestinal and renal integrity following administration of fHb in a nicotinic receptor-dependent manner. These findings implicate nutritional intervention as a novel therapeutic approach in patients with severe hemolysis or rhabdomyolysis.

Corresponding Author: Jacco-Juri de Haan, MD, Maastricht University Medical Center, Department of General Surgery, Universiteitssingel 50, 6229 ER Maastricht, The Netherlands, jj.dehaan@ah.unimaas.nl

A 263
Nutritional vagus activation: effects on early intestinal injury
Jacco-Juri de Haan

Gut wall integrity loss and local intestinal inflammation are associated with poor clinical outcome in surgical and trauma patients. Evidence for a causal relationship between intestinal compromise and inflammatory complications is provided by a growing number of experimental studies. Clinical confirmation of the occurrence and significance of intestinal integrity loss however has proven to be difficult due to lack of diagnostic tools. The identification of fatty acid binding proteins (FABP) as specific and sensitive markers of enterocyte damage is a promising advance in the detection of intestinal compromise. During non-abdominal surgery and following trauma, plasma FABP levels were demonstrated to increase rapidly. Moreover, FABP were related to the subsequent inflammatory response and the development of complications. Taken together, prevention of intestinal compromise is implicated as a potential target for therapies aimed at controlling systemic inflammation and improving outcome. A novel and promising approach to regulate the inflammatory response is lipid-rich enteral nutrition. We showed previously that lipid-rich nutrition activates a potent vagovagal anti-inflammatory reflex. Luminal presence of lipids results in release of cholecystokinin (CCK) that stimulates the afferent vagus nerve via CCK-1 receptors. Subsequently, cytokine release is inhibited via efferent vagus-dependent activation of nicotinic acetylcholine receptors on inflammatory cells. Attenuation of the inflammatory response is accompanied by reduced damage to several organs including the intestine.

Since loss of gut wall integrity occurs rapidly in surgical settings, we recently studied the effects of lipid-rich nutrition on early intestinal compromise. Already within a half hour following hemorrhagic shock, loss of gut barrier function was strongly inhibited by lipid-rich nutrition. Also the development of early enterocyte damage was prevented, as assessed by circulatory FABP. In line with clinical observations, the degree of gut barrier dysfunction correlated with the
inflammatory response. Lipid-rich nutrition significantly reduced local intestinal inflammation in a CCK-receptor dependent manner before systemic inflammation became detectable. These findings expand our insight in the protective potential of nutritional vagus activation in settings of splanchnic hyperperfusion. Surgical and trauma patients prone to develop a compromised gut may benefit from early intervention with enriched enteral nutrition.

Corresponding Author: Jacco-Juri de Haan, MD, Maastricht University Medical Center, Department of General Surgery, Universiteitsringel 50, 6229 ER Maastricht, The Netherlands, ff.dehaan@ah.unimaas.nl

A 264
Microarray technology: powerful tool for the discovery of new biomarkers
Wenzhong Xiao

We have designed a comprehensive high-density oligonucleotide array of human transcriptome (GG-H array) for high-throughput, cost effective, multiplexed analysis of the biological mechanism and biomarkers of disease. The contents of the array are further verified by deep RNA sequencing data. This array enables comprehensive examination of multiple components of human genomic response to diseases, including improved estimation of gene expression, quantitation of gene isoforms and identification of alternative splicing, examination of non-coding transcription, and other information (detection of coding SNPs and allele specific expression, antisense expression, and analysis of small interference RNAs). Patients admitted to hospitals with severe trauma and burn are studied using the GG-H array, and to date more than one thousand cell-separated patient samples of T-cells, monocytes, and neutrophils are processed.

Corresponding Author: Wenzhong Xiao, PhD, Massachusetts General Hospital, Harvard Medical School, Department of Surgery, 55 Fruit Street, Boston, MA 02114, USA, wzxiao@stanford.edu

A 265
In-vivo molecular imaging of vasculitis and atherosclerosis
Tobias Saam, Maximilian Reiser, Konstantin Nikolaou

This lecture provides a description of the various molecular imaging techniques for imaging atherosclerosis and vasculitis with a focus on PET/CT and MRI. The imaging methodology, evolving imaging technology, and development of novel targeted molecular probes relevant to the developing field of cardiovascular molecular imaging will be reviewed. Traditionally, the diagnosis, monitoring and prognostication of cardiovascular disease were based on techniques that measured the degree of luminal stenosis. However, the vulnerability or destabilization of atherosclerotic plaques has been directly linked to plaque composition and plaque morphology. Imaging modalities, such as MRI and PET/CT, that allow for evaluation of plaque composition at a cellular and molecular level, could further improve the detection of vulnerable plaque and may allow for monitoring the efficacy of antiatherosclerotic therapies. Targeted imaging of vascular inflammation or thrombosis may allow improved risk assessment of atherosclerosis by detecting plaques at high risk of acute complications. Although clinical experience remains limited, careful evaluation of safety as well as validation of diagnostic and prognostic value of these techniques in clinical trials is still needed.

Corresponding Author: Tobias Saam, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Clinical Radiology, Marchioninistr. 15, 81377 Munich, Germany, Tobias-Saam@med.uni-muenchen.de

A 266
Molecular imaging of atherosclerosis with adiponectin-targeted nanoconstructs
Rudolf Stollberger, Gunter Almer, Peter Opriessnig, Ruth Prassl, Harald Mangge

Introduction: Atherosclerosis is the major cause of mortality in the western world today. The morphologic assessment of atherosclerotic plaques and resulting stenosis are important tasks in vascular imaging and is used to characterize the temporary endpoint of arthrosclerosis. For deeper understanding of disease status and monitoring purposes it would be of high importance to get information about underlying functional parameters and processes. Inflammation is such a driving process. To get specific information on inflammation biomarker (e.g. adiponektin, Interleukin-10) targeted nanoconstructs were explored for imaging. The used nanoconstructs are based on Stealth®-liposomes which can be labeled with different imaging indicators and have also the potential to carry therapeutic agents (theranostic agents). The targeted nanoparticles were investigated towards their potential to characterize critical scenarios associated with anti-inflammatory counterregulation after critical perpetuation within atherosclerotic (AS) plaques applying in ApoE-deficient mice. Application to a Watanabe rabbit model using MRI contrast agents loaded to the nanoparticles is work in progress.

Methods: Aortas of wt and ApoE-deficient mice, fed a high fat diet, were dissected and stained with fluorescence-labelled biomarkers (BMs) and with BM-nanoconstructs to enhance the imaging performance. Ex vivo imaging was performed using confocal laser-scanning microscopy (CLSM). For all BM-conjugates Western Blot (WB) analysis was used to assess structural changes. Modified native gel electrophoresis was used for the characterization of the BM-targeted, fluorescence-labelled Stealth®-liposomes.

Results: CLSM imaging showed that all chosen BMs bind to the AS-plaque but not to the not unchanged vessel wall. The investigation of the nanoconstructs with WB showed that no critical structural changes occurred during the whole procedure. Successful coupling of native BMs to reactive PEGylated lipids exposed on the liposomal surface of fluorescence-labelled NPs was shown by native gel electrophoresis and WB analysis.

Conclusion and outlook: Results suggest a promising role of the applied BM-targeted NPs for enhanced AS-imaging in vivo and their potential use for new targeted therapeutic strategies in cardiovascular medicine. The rabbit model is currently fed with western diet and will be imaged using both Gd-DTPA and SPIO labelled nanoconstructs in the near future.

Corresponding Author: Rudolf Stollberger, Prof. PhD, University of Technology Graz, Department of Medical Engineering, Kronengasse 5, 8010 Graz, Austria, rudolf.stollberger@tuwien.at

A 267
Carbon nanotube capsules for medical imaging and therapy
Lon Wilson

Discovered in 2005, the “Gadonanotubes” are a new nanotechnology-inspired paradigm in MRI contrast agent (CA) design [1–3]. At a clinical imaging field of 1.5 T, Gadonanotubes exhibit a T1-weighted
relaxity of ~180 mM-1s-1 per Gd3+ ion or approximately 40 times greater efficacy than current Gd3+-ion-based clinical agents. Composed of naked/aqueous Gd3+-ion clusters (<10 Gd3+-ions per nanoscale cluster) confined within 20–80 nm segments of ultra-short single-walled carbon nanotube capsules (US-tubes), the Gadonanotubes (Gd3+@US-tubes) in Fig. 1 are among the first Gd3+-ion-based CAs not to employ metal chelate chemistry to provide efficacy and sequester Gd3+-ion toxicity. The only other Gd3+-ion CAs not to employ chelate chemistry are the related Gadofullerenes [4], but the Gadonanotubes are more likely to be developed for advanced applications such as molecular and cellular imaging.

Fig. 1 Schematic representation of a Gadonanotube (green spheres represent encapsulated Gd3+ ions, not to scale and Cl− ions are not shown)

The presentation will describe:

a. The synthesis and characterization of Gadonanotube materials.

b. Our current understanding of how Gadonanotubes function as T1-weighted (and T2-weighted) MRI CAs.

c. Progress in developing near-term and long-term in vivo applications for new Gadonanotube MRI CA materials, including therapy by RF-induced hyperthermia.

References


Corresponding Author: Lon Wilson, PhD, Rice University, 6100 Main Street, Houston, TX 77005-1827, USA, darango@rice.edu

A 269

Caveolin-1 promotes survival in Candida albicans sepsis by modulating Dectin-1 expression and signaling

Tammy Ozment-Skelton, David Williams, Mike Kruppa, Kevin Breuel, John Kalfbleisch, John Schweitzer

Objective: Candida spp. account for 10–15% of hospital acquired bloodstream infections, with C. albicans being the most common species identified. Diagnosis of Candida infection is challenging, and therefore frequently delayed. Thus, the mortality rate from invasive Candida infection exceeds 35%. Dectin-1 is thought to play a pivotal role in the response to fungal infections and is therefore a sentinel receptor for fungal pathogens. Dectin-1 is the pattern recognition receptor for glucans, a component of the fungal cell wall. Mice deficient in Dectin-1 have been found to be more susceptible to C. albicans and Aspergillus fumigatus infection. Caveolin-1 is a lipid raft associated protein that regulates the immune response to infectious agents such as Salmonella enteritica. The goal of the present study was to determine the role of caveolin-1 in the immune response to systemic infection with C. albicans.

Methods: Wild type (WT) and caveolin-1 knock-out (Cav−/−) mice were injected with 100,000 cfu of C. albicans iv and were followed for survival. Dectin-1 expression was measured on macrophages elicited from the WT and Cav−/− mice by flow cytometry. Phosphorylation of AKT was measured on WT and Cav−/− macrophage cell lysates treated with particulate glucan (10 mcg/ml) by Luminex.
Results: After injection with *C. albicans*, Cav$^{-/-}$ mice had a shorter onset of mortality (11 vs. 28 days), lower median survival time (22 vs. 42 days, $p < 0.02$) and decreased long term survival (0 vs. 20%, $p < 0.02$) compared to WT mice. The Cav$^{-/-}$ mice died from renal failure as evidenced by abscessed, scarred, and shrunken kidneys and severe dehydration. The WT mice suffered from CNS signs prior to death including head tilt and seizures. WT mice brains had focal necrotic lesions with macrophages but no evidence of *C. albicans*. Analysis of Dectin-1 expression on elicited macrophages revealed that Cav$^{-/-}$ cells had a 31% decrease in cell surface Dectin-1 when compared to WT ($p < 0.05$). Stimulation of WT macrophages with particulate glucan resulted in a 22.5% increase in phosphorylated AKT. The resting Cav$^{-/-}$ macrophages had 65% less phosphorylated AKT than the resting WT cells, and the activation of AKT was not increased with glucan treatment.

Conclusions: We conclude that caveolin-1 plays a role in the normal innate immune response to fungal infection. One possible mechanism for the protection conferred by caveolin-1 is by modulating Dectin-1 expression and signaling.

**Corresponding Author:** Tammy Ozment-Skelton, PhD, ETSU Quillen College of Medicine, Department of Surgery, PO Box 70575, Johnson City, TN 37615, USA, ozmentsk@etsu.edu

### A 270

**Aging decreases human leukocyte Dectin-1 responsiveness and co-operativity with TLR2**

David Williams, Tammy Ozment-Skelton, Melinda Steagald, Chuanfu Li, Julie Dunn, William Browder

Objective: Opportunistic fungal infections are increasingly common in the surgical ICU, particularly in aged patients. Evidence indicates that there is an age related increase in susceptibility to fungal infection, although the mechanism(s) have not been elucidated. The innate immune system recognizes fungal pathogens via pattern recognition receptor (PRR) receptors. Dectin-1 is the primary PRR for glucans, a fungal cell wall PAMP. Dectin-1 co-operates with TLR2 to elicit anti-fungal responses. Little is known about the effect of aging on Dectin expression and responsiveness. The goal of this study was to determine: (a) whether there were age related changes in human leukocyte Dectin levels; (b) the effect of age on leukocyte Dectin response to a fungal cell wall PAMP; (c) what, if any, effect aging plays in Dectin/TLR2 co-operativity. Patients and methods: Following informed consent, PBMCs were obtained from 73 normal healthy subjects between the ages of 18 and 81. Leukocyte Dectin-1 was assessed by flow cytometry. PBMCs from young (18–22 years) and aged (>60 years) individuals were incubated (24 h) with glucan, a Dectin agonist/fungal PAMP; Pam3CSK4, a TLR2 specific agonist or glucan/Pam3CSK4. LPS was employed as an inflammatory control stimulus. PBMCs incubated in media served as the negative control. Supernatants were analyzed for cytokine expression using a human multi-plexed bead immunoassay. Results: Human PBMCs did not show any significant age related change in leukocyte Dectin levels, although there was a slight downward trend after 50 years of age. However, PBMCs from healthy aged (>60 years) individuals showed decreased responsiveness to the Dectin agonist, glucan, and/or the TLR2 agonist, Pam3CSK4, when compared to young (18–22 years) individuals. Specifically, PBMCs from aged (>60 years.) individuals showed decreased ex vivo expression of IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p40/70, IL-17, TNFα and MIP-1α when stimulated with glucan, Pam3CSK4 and/or glucan/Pam3CSK4.

Conclusions: The data indicate that human leukocyte Dectin-1 levels do not significantly change as a function of age. However, PBMCs from aged (>60 years) individuals showed decreased responsiveness to Dectin-1 and Dectin-1/TLR2 ligands. Thus, Dectin-1 responsiveness and co-operativity with TLR2 decreases with age. This may explain, in part, the increased susceptibility of aging, critically ill patients to opportunistic fungal infections in the surgical ICU.

**Corresponding Author:** David Williams, Prof. PhD, East Tennessee State University, Department of Surgery, PO Box 70575, Johnson City, TN 37614, USA, williamd@etsu.edu

### A 271

**Prevention of systemic enterococcal infections: experimental vaccine approaches**

**Johannes Huebner**

Enterococci are important nosocomial pathogens, especially because of frequent intrinsic and acquired antibiotic resistances. There are often only limited treatment options available, and no preventive measures other than infection control exist. Alternative approaches for treatment and prevention of enterococcal infections are urgently needed, especially since vancomycin-resistance is increasing among hospital-adapted *Enterococcus faecium* clones and because of the possibility of the transmission of the vanA resistance determinant to other more virulent species (such as *Staphylococcus aureus*). Several components of the enterococcal cell wall are involved in virulence, and some of these antigens may be promising vaccine targets for passive and active immunotherapy. Our group has evaluated the protective efficacy of lipoteichoic acid (LTA) from *Enterococcus faecalis* for the immunotherapy and prevention of infections due to *E. faecalis*, *E. faecium*, and several other gram-positive species. Our results suggest that alanine residues on LTA play a minor role as epitopes for protective antibodies, although we could demonstrate that alanination seems to be important for biofilm formation, persistence in the blood stream, and resistance against antimicrobial peptides. LTA is exposed in about one third of *E. faecalis* and *E. faecium* strains, which are readily killed by antibodies raised against purified LTA. A novel capsular polysaccharide has been identified that is present in strains that are not susceptible to anti-LTA antibodies. This di-heteroglycan elicits opsonic antibodies that are able to kill strains that are not killed by anti-LTA antibodies. An antiserum raised against this antigen is protective in a mouse sepsis model against the homologous and two heterologous strains. A structurally different carbohydrate antigen has been found in *E. faecium* strains that are not killed by anti-LTA antibodies. Several surface proteins in enterococci are involved in virulence. Recent results suggest that the enterococcal surface protein Esp is not target of protective antibodies using a mouse sepsis model. However, a different protein involved in peptidoglycan modification elicited antibodies that were opsonic and protective.

A better understanding of the specific virulence factors that enable enterococci to colonize the host and cause infections will contribute to the development of new therapeutic and prophylactic approaches, which may help us to prevent spread and infections of enterococci in hospitals.

**Corresponding Author:** Johannes Huebner, Prof. MD, University Hospital Freiburg, Division of Infectious Diseases, Hugstetter Str. 55, 79106 Freiburg, Germany, johueb
**A 272**

**Prevention of GBS disease by monoclonal antibodies directed against antigens identified by ANTIGENome technology**

Martin B. Oleksiewicz, Beatrice M. Senn, Andreas L. Meinke, Zehra Visram, Dieter Reinscheid, Eszter Nagy

Objective: Group B Streptococcus (GBS, *S. agalactiae*) is a leading cause of neonatal sepsis and meningitis, reaching an incidence of up to 2.4/1,000 deliveries in risk populations even with antenatal screening and antibiotic prophylaxis of colonized mothers. Fatality can reach 5%, and approximately 20% of affected babies suffer permanent neurological sequelae. Prophylaxis has reduced the incidence of early-onset, but not that of late-onset disease. Thus, there is a need for improved prophylaxis. Vaccination of pregnant women faces significant regulatory hurdles, and transfer of antibodies to the foetus is in any case not effective before the 34th pregnancy week, leaving high-risk preterm babies unprotected. Therefore, we set out to provide preclinical proof-of-concept for passive immunoprophylaxis using murine monoclonal antibodies (mAbs).

Patients and methods: From healthy pregnant women with known GBS colonization status, we selected serum and cervical secretions with high immunoglobulin titers against GBS bacterial extract or culture supernatant. Human IgG and IgA purified from these samples were used to select bacterial surface-display libraries representing the fragmented genome of GBS NEM316 (serotype III). From antigenic GBS proteins thus discovered, highly conserved and antigenic proteins were selected for expression in *E. coli*, and these were further screened for protective effect by active and passive immunization in mice followed by lethal challenge with different GBS strains. Protective GBS proteins were used to generate murine mAbs by standard hybridoma technology.

Results: Six protective GBS proteins were identified by screening in murine lethal sepsis models using challenge with the main serotypes of GBS, and active vaccination or passive transfer of hyperimmune sera. Murine monoclonal antibodies against the antigens also provided significant protection against certain GBS strains that was comparable to or higher than that achieved by hyperimmune serum. A cocktail of mAbs induced broad protection against all GBS serotypes tested (Ia, Ib III and V).

Conclusion: This study provided preclinical proof-of-concept for passive immunoprophylaxis against invasive GBS disease, that warrants further development based on fully human mAbs.

**Corresponding Author:** Martin B. Oleksiewicz, PhD, Intercell AG, Department of Molecular Microbiology, Campus Vienna Biocenter 3, 1030 Vienna, Austria, moleksiewicz@intercell.com

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**A 274**

**Targeting inflammasomes in inflammation**

Eicke Latz

The innate immune system can recognize many situations that are dangerous for the body. Innate immune cells, such as macrophages, dendritic cells or neutrophils for example, are equipped with a myriad of germ-line encoded pattern-recognition signaling receptors that can respond to microbial products. Signaling by these receptors leads to the production of a concerted anti-microbial response with the production of many inflammatory mediators. The innate immune response aims to protect the host from infections. In recent years it has become increasingly clear that the innate immune system also functions to recognize dangerous situations that are not primarily associated with infections. For example, innate immune cells can respond to sterile tissue damage, tumor development, or cell death, during which host molecules become chemically altered or appear in areas that normally do not contain these molecules. These changes can be recognized by TLRs and by members of the NLR family of signaling receptors. The NLR member NLRP3 can form a caspase-1-activating inflammasome in response to many different chemically and structurally diverse activators, such as pore-forming toxins from microbes, medically relevant crystals or aggregates, and other chemical entities. Activation of the NLRP3 inflammasome leads to the activation and release of the pro-inflammatory cytokines of the IL-1 cytokine family. These cytokines are known to be important mediators of inflammation in a variety of acute and chronic inflammatory diseases. Therefore, the NLRP3 inflammasome is an important drug target and developing
strategies to screen for inhibitors of this receptor complex is crucially important for the discovery of molecules that interfere with NLRP3 activation. The mechanisms involved in NLRP3 inflammasome activation are not well understood. Current concepts and available assays to test for NLRP3 inflammasome activation will be discussed. Corresponding Author: Eicke Latz, MD, PhD, University Hospital of Bonn, Institute of Innate Immunity, Sigmund-Freud-Str. 25, 53127 Bonn, Germany, eicke.latz@umassmed.edu

A 275
Exploring the role of a TLR4 antagonist in severe sepsis and other inflammatory diseases
Daniel Rossignol, Alec Wittek, Melvyn Lynn

Toll-like receptor-4 (TLR4) has been shown to be activated by a wide variety of endogenous ligands such as heat shock proteins, hyaluronic fragments, and oxidized fatty acids as well as infection-related ligands such as endotoxin or lipopolysaccharide (LPS), a major constituent of the outer membrane of gram-negative bacteria. It is proposed that overly-robust stimulation of TLR4 by overwhelming infection (or a high endotoxin level) generates a proinflammatory response that ultimately proves harmful to the host. In other conditions, it has been proposed that less-intense activation of TLR4 may generate a low-level proinflammatory state that has been ascribed to diseases such as atherosclerosis, airway hyper-reactivity and ischemia-reperfusion injury. Eritoran (E5564), is a novel lipid A analogue/TLR4 antagonist that generates an inactive heterotrimer with the MD-2/TLR4 complex thereby blocking its activation by agonists.

Results from preclinical development as well as early Phase clinical trials indicate that eritoran is capable of completely blocking response to endotoxin in vitro as well as in a human in vivo endotoxemia model. These results have enabled us to model doses and dosage regimens to study the clinical effects of TLR4 antagonism for the prevention or treatment of inflammatory response due to coronary artery bypass graft surgery, as well as severe sepsis (including an ongoing multi-national clinical study in severe sepsis). In addition, we will review preclinical studies implicating TLR4 (and antagonism of TLR4) in a number of diseases proposed to be driven by activation of innate immunity.

Corresponding Author: Daniel Rossignol, PhD, Eisai Inc., Product Creation, 155 Tice Blvd, Woodcliff, NJ 07677, USA, dan.rossignol@eisai.com

A 276
Antagonists of Toll-like receptor 7 and 9 for autoimmune and inflammatory diseases
Sudhir Agrawal

In autoimmune diseases, immune complexes containing self-nucleic acids have been shown to induce inflammatory responses, mediated through Toll-like receptors (TLR) 7 and 9 including induction of TNF-α, IFN-γ, IP-10, and IL-6. Antibodies targeted to these selected cytokines have shown encouraging results in suppression of autoimmune disease symptoms. Our hypothesis is to use antagonist of TLR7 and 9 to block induction of these cytokines. This approach will allow to suppress induced levels of cytokines and not affect the constitutive levels. We have designed TLR7 and 9 antagonist candidates based on structure-activity relationship studies. Antagonist candidates have been shown to inhibit TLR7- and 9-mediated immune responses in vitro in mouse and human cell-based assays. Gene expression microarray profiling of human PBMCs treated with a TLR7 or TLR9 agonist in the presence or absence of antagonist have identified a number of genes in the NF-kB signaling, chemokinesis and inflammatory pathways that are down-regulated by the antagonist. In addition, systemic administration of an antagonist candidate in non-human primates followed by ex-vivo stimulation of PBMCs with TLR7 or 9 agonist leads to lower induction of cytokines including IL-6, TNF-α, IFN-γ, IP-10 and MIP-1β. In preclinical mouse models of lupus, collagen-induced arthritis and psoriasis, encouraging results with amelioration of disease associated parameters following antagonist treatment. Our studies also indicate that there is a cross-talk between TLR7 and 9 and both are inhibited with antagonist. Based on these studies, we have identified IMO-3100 as a lead TLR7 and 9 antagonist candidate for clinical development.

Corresponding Author: Sudhir Agrawal, Dr. Phil., Idera Pharmaceuticals Inc., 167 Sidney Street, Cambridge, MA 02139, USA, sagrawal@iderapharma.com

A 277
IL-33 induces IL-13-dependent cutaneous fibrosis
Stefan Pflanz, Andrew Rankin, John Mumm, Erin Murphy, Scott Turner, Ni Yu

IL-33 is constitutively expressed in epithelial barrier tissues such as skin. Although increased expression of IL-33/IL-33R has been correlated with fibrotic disorders such as scleroderma and progressive systemic sclerosis, the direct consequences of IL-33 release in skin has not been reported. To determine the effects of dysregulated IL-33 signaling in skin, we administered IL-33 subcutaneously and monitored its effects at the injection site. Administration of IL-33 resulted in IL-33R-dependent accumulation of eosinophils, CD3+ lymphocytes, F4/80+ mononuclear cells, increased expression of IL-13 mRNA, and the development of cutaneous fibrosis. Consistent with extensive cutaneous tissue remodeling, IL-33 resulted in significant modulation of a number of extracellular matrix associated genes including Collagen VI, Collagen III and Tissue Inhibitor of Metalloproteases 1 (TIMP-1). We establish that IL-33-induced fibrosis requires IL-13 using IL-13 knockout mice and eosinophils using AdIlGATA mice. We show that bone marrow derived eosinophils secrete IL-13 in response to IL-33 stimulation, suggesting that eosinophil derived IL-13 may promote IL-33-induced cutaneous fibrosis. Collectively, our results identify IL-33 as a previously unrecognized pro-fibrotic mediator in skin and highlight the cellular and molecular pathways by which this pathology develops.

Corresponding Author: Stefan Pflanz, PhD, MRL Palo Alto, Department of Immunology, 901 California Ave, Palo Alto, CA 94304, USA, stefan.pflanz@spcorp.com

A 278
IL-17/IL-22 in infection
Jay Kolls

Emerging evidence supports the concept that Th17 cells in addition to mediating autoimmunity have critical roles in mucosal immunity against extracellular pathogens. Both IL-23p19 KO and IL-17RKO lack enhanced susceptibility to the intracellular pathogens Listeria monocytogenes or Mycobacterium tuberculosis. However both are susceptible to the extracellular pathogen K. pneumoniae. IL-22 and IL-17A are both effector cytokines produced by the Th17 lineage and both cytokines are critical for maintaining local control of the gram negative pulmonary pathogen, Klebsiella pneumoniae. IL-17F also has a protective role but less so than IL-17A or IL-22. Although both
cytokines regulate CXC chemokines and G-CSF production in the lung, only IL-22 increased lung epithelial cell proliferation and increased transepithelial resistance to mechanical injury. These data support the concept that the Th17 cell lineage and their effector molecules evolved to effect host defense against extracellular pathogens at mucosal sites. In contrast to their role in mucosal immunity against planktonic bacteria, IL-23 and IL-17 contribute to tissue inflammation in chronic biofilm infection. This has been demonstrated for both biofilms produced by Pseudomonas aeruginosa as well as infections due to Aspergillus fumigatus. Recent data from our laboratory demonstrates that Th17 cells mediate airway inflammation that is characterized by the production CXCL1, CXCL2, CXCL5, neutrophil inflammation, goblet cell hyperplasia as well as airways hyperresponsiveness to methacholine. Interestingly both the production of IL-17 and IL-22 by Th17 cells as well as the tissue inflammation provoked by the adoptive transfer of Th17 cells is resistant to dexamethasone implicating these cells as having a role in some forms of steroid resistant inflammation. These data as well as mechanisms by which Th17 cytokines regulate anti-microbial responses in the epithelium will be presented.

Support by RO1HL079142 and P50HL084932.

**Corresponding Author:** Jay Kolls, MD, LSUHSC, Department of Genetics, 533 Bolivar St, New Orleans, LA 70112, USA, jkolls@lsuhsc.edu

**A 279**

**Strategies to Inhibit the Toxicity of Systemic TNF Treatment with Retention of its Antitumor Effect**

*Claude Libert*

The application of the spectacular antitumor effect of TNF is still limited to local treatment schedules because of the unacceptable toxicity of systemic TNF administration. This toxicity is based on the strong pro-inflammatory nature of TNF. Indeed, in many cell types, TNF stimulates several signals towards transcription of numerous genes, encoding important molecules of inflammation, such as cytokines, adhesion molecules and enzymes. All these signals result in acute inflammation and SIRS. Several lines of evidence from our group suggests that, in mice, the antimicrobial activities and induction of SIRS can be uncoupled. Indeed, mice can be protected against TNF toxicity with full retention of antitumor effects, by several strategies. These include inhibition of matrix metalloproteinases (MMPs) and induction of HSP70. The mechanism of action of HSP70 appears to involve protection of the glucocorticoid receptor, which is clearly malfunctioning in TNF-treated mice. Moreover, the identification of mediators of the induction of SIRS by TNF is a priority, as it can lead to novel interventions. We have identified IL-17 as an essential mediator, which, surprisingly seems to be induced and expressed in a specific cell type of the gut, the Paneth cells. More new mediators of TNF toxicity have been described and will be presented on the conference.

**Corresponding Author:** Claude Libert, Prof. PhD, Ghent University, Biomedical Molecular Biology, Technology park 927, 9052 Ghent, Belgium, Claude.Libert@UGent.be

**A 280**

**Obese trauma patients exhibit an attenuated early cytokine response to severe blunt injury**

*Robert Winfield, Matthew Delano, Alex Cuenca, Ronald Maier, Joseph Cucchiari, Lyle Moldawer*

Objective: Obesity is associated with a pro-inflammatory cytokine profile at baseline and a propensity to develop multiple organ failure (MOF) following major trauma. Elevated cytokine levels have shown value as predictive markers for post-injury MOF. We hypothesized that injured obese patients would display increased concentrations of pro-inflammatory cytokines when compared to patients of normal BMI, and that this would correlate with MOF.

Methods: We retrospectively reviewed prospectively collected multicenter data in the “Inflammation and the Host Response to Injury” trauma-related database. Subjects were divided into two groups on the basis of BMI (normal 18.5–24.9 kg/m$^2$ and obese, $\geq 30$ kg/m$^2$). Groups were compared on demographic, injury, and outcome data, including Marshall MOF score. Cytokine concentrations on post-injury days 0, 1, and 4 were compared between study groups and to those of healthy volunteer subjects.

Results: 74 adult blunt trauma victims (34 normal, 40 obese) and 13 healthy control subjects were evaluated. Obese patients were older than those of normal BMI (37 vs. 31 years, $p = 0.016$), but injured groups were similar in gender distribution, injury severity, and APACHE II score. Obese patients demonstrated an overall diminished cytokine response when compared to normal BMI patients with significantly lower concentrations of IL-10, IFN-γ, MIP-1x, TNF-α, and IL-1β in the first four post-injury days (Table 1). Obese subjects showed greater mean MOF score (6.3 vs. 5.3, NS) than normal BMI patients.

**Table 1** Post-injury cytokine concentrations (pg/mL) in normal and obese subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Obese</th>
<th>$p$</th>
<th>Normal</th>
<th>Obese</th>
<th>$p$</th>
<th>Normal</th>
<th>Obese</th>
<th>$p$</th>
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<tr>
<td><strong>Day 0</strong></td>
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<td></td>
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<tr>
<td>IL-4</td>
<td>184</td>
<td>98</td>
<td>0.070</td>
<td>219</td>
<td>130</td>
<td>0.078</td>
<td>140</td>
<td>90</td>
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<td>IL-6</td>
<td>241</td>
<td>200</td>
<td>0.591</td>
<td>242</td>
<td>108</td>
<td>0.058</td>
<td>86</td>
<td>52</td>
<td>0.206</td>
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<td>IL-8</td>
<td>36</td>
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<td>0.265</td>
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<td>IL-10</td>
<td>222</td>
<td>98</td>
<td>0.030*</td>
<td>141</td>
<td>109</td>
<td>0.434</td>
<td>282</td>
<td>109</td>
<td>0.022*</td>
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<td>IFN-γ</td>
<td>361</td>
<td>211</td>
<td>0.295</td>
<td>540</td>
<td>290</td>
<td>0.273</td>
<td>433</td>
<td>190</td>
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<td>MCP-1</td>
<td>132</td>
<td>171</td>
<td>0.066</td>
<td>146</td>
<td>120</td>
<td>0.061</td>
<td>66</td>
<td>114</td>
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<tr>
<td>MIP-1x</td>
<td>40</td>
<td>25</td>
<td>0.093</td>
<td>48</td>
<td>24</td>
<td>0.027*</td>
<td>32</td>
<td>20</td>
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<td>TNF-α</td>
<td>18</td>
<td>16</td>
<td>0.723</td>
<td>31</td>
<td>11</td>
<td>0.035*</td>
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<td>IL-1β</td>
<td>23</td>
<td>11</td>
<td>0.089</td>
<td>39</td>
<td>14</td>
<td>0.002*</td>
<td>23</td>
<td>12</td>
<td>0.152</td>
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</table>
patients, with a significantly increased cardiac component (2.9 vs. 2.4, 
\( p = 0.031 \)).

Conclusions: Despite the well-documented pro-inflammatory cyto-
kine profile present in obese individuals at baseline, obese patients 
sustaining severe injury show an attenuated early cytokine response 
when compared to patients of normal BMI. This is paradoxically 
associated with higher MOF scores in obese patients, suggesting that 
alternate mechanisms are responsible for increased MOF develop-
ment in this high-risk population.

**Corresponding Author:** Robert Winfield, MD, University of Florida, 
Department of Surgery, 1600 SW Archer Road, Gainesville, FL 32610, USA, robert.winfield@surgery.ufl.edu

### A 281

**Similar toxins induce different cytokine responses in nonhuman primate models of enterohemorrhagic *E. coli* toxemias**

*Shinichiro Kurosawa, D.J. Stearns-Kurosawa, Valta Collins, Scott Freeman*

Objectives: Infection with Shiga toxin-producing enterohemorrhagic
*Escherichia coli* (STEC) results in intestinal cramps and bloody 
diarrhea, followed 5–12 days later in some patients with develop-
ment of hemolytic uremic syndrome. Bacteremia is rare and two 
very similar bacterial toxins (Stx1, Stx2) govern organ damage. 
They are AB5 ribosome inactivating toxins that recognize CD77, 
inhibit protein synthesis, and induce ER stress and apoptosis in 
monocytes and epithelial cells, while paradoxically inducing cyto-
kine production in vitro. Stx2 is particularly associated with renal 
damage although mechanisms for this specificity are not understood. 
Rodents do not replicate human responses, so we evaluated effects 
of Stx toxemia in nonhuman primates, including inflammatory 
responses.

Materials and methods: Anesthetized baboons (Papio; \( n = 20 \)) were 
challenged with i.v. bolus of Stx1 (10, 50, 100 ng/kg) or Stx2 (10, 
50 ng/kg) and pathophysiology was followed over 7 days. Blood 
samples taken at T0 and periodically throughout and plasma stored 
for assay of biomarkers. Cytokine protein levels in plasma were 
quantified by xMAP™ multiplex fluorescent bead-based assays using 
a Luminex® 200IS system (Millipore, Billerica, MA). Luminex 
xPONENT® software (Luminex, Austin, TX) and non-human primate 
cytokine panel kits (Millipore). For each sample, the median fluo-
rescent intensity was analyzed with a weighted 5 parameter logistic 
and quantified relative to the standard curve for that cytokine.

Results and conclusions: Disease severity and mortality was dose-
dependent and, like patients, baboons were more sensitive to Stx2. 
A Stx1 lethal dose was 100 ng/kg at 2–3 days, whereas Stx2 was 
lethal at 50 ng/kg at 4–5 days. IL-6 and MCP-1 are markers of sys-
temic septic responses and increases were observed in baboons after 
challenge with the toxin. Toxin-specific responses were observed with 
IL-1Ra, G-CSF and IL-6 with increases observed after Stx1, but either 
no elevation (IL-1Ra, G-CSF) or much reduced (IL-6) after Stx2. 
TNFα was not detected in plasma after challenge with either toxin, 
suggesting that gut-derived endotoxin did not contribute to the 
inflammatory responses. STEC strains typically secrete both toxins in 
differing ratios, so the baboon models permit differentiation of toxin-
specific activities and clinical manifestations, which are impossible to 
identify in patients.

**Corresponding Author:** Shinichiro Kurosawa, MD, PhD, Boston 
University School of Medicine, Department of Pathology and Labo-
ratory Medicine, 670 Albany Street, Boston, MA 02118, USA, 
kurosawa@bu.edu

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### A 282

**MIF is a late mediator and marker of poor prognosis of sepsis**

*Yousef Al-Abed*

Severe sepsis is a serious clinical syndrome of organ damage following 
infection. The pathogenic sequelae of sepsis is attributable to a dys-
regulation of endogenous cytokines, produced by the innate immune 
system, which are both necessary and sufficient to cause the charac-
teristic manifestations of disease. One of these cytokines, macrophage 
migration inhibitory factor (MIF), has been implicated to have a critical 
role in severe sepsis pathogenesis. In patients with sepsis, the highest 
plasma MIF levels are observed in non-survivors. Similarly, in pre-
clinical models of sepsis, plasma MIF concentrations are significantly 
increased during disease. Moreover, mice rendered deficient in MIF by 
geneic knock-out are significantly protected from the lethality of 
sepsis, while administration of MIF antagonists (inhibitory anti-MIF 
antibodies or synthetic small molecule inhibitors) confer significant 
protection against the lethality of sepsis in wild type animals. In con-
trast to classical cytokines such as TNF and IL-1beta, we have 
discovered that serum MIF levels increase with delayed kinetics 
(peak at 36 h) following cecal ligation and puncture, an experi-
mental model of sepsis. Together, these findings identified MIF as a late 
mediator in sepsis with potential clinical use as a prognostic marker.

**Corresponding Author:** Yousef Al-Abed, The Feinstein Institute for 
Medical Research, Manhasset, NY 11030, USA, alabe2001@yahoo.com

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### A 283

**Fetuin-A protects mice against lethal sepsis by modulating endotoxin-induced HMGB1 release and autophagy**

*Huichao Wang, Shu Zha, Wei Li, Jianhua Li, Kevin Tracey, 
Andrew Sama*

Objectives: The pathogenesis of sepsis is complex, but in part mediated 
by bacterial endotoxin, which stimulates macrophages to release early 
(e.g., TNF, IL-1) and late (e.g., HMGB1) pro-inflammatory mediators. 
Various inflammatory stimuli (e.g., endotoxin, cytokines, and oxidative 
stress) similarly induce autophagy, a catabolic degradation process 
responsible for eliminating damaged cytoplasmic components during 
fication. A negative acute phase protein, fetuin (fetus protein in Greek), 
was recently characterized as a negative regulator of inflammation by 
modulating endotoxin-induced HMGB1 release and autophagy in 
sepsis (induced by cecal ligation and puncture, CLP) in vivo.

Methods: We examined its effects on endotoxin-induced HMGB1 
release and autophagy in vitro, and determined whether administra-
tion of exogenous fetuin protects mice against lethal experimental 
sepsis (induced by cecal ligation and puncture, CLP) in vivo.

Results: In vitro, fetuin (25–100 µg/ml) effectively inhibited endo-
toxin-induced (100 ng/ml) HMGB1 release (by 60–90%), but 
enhanced endotoxin-induced autophagy in macrophage cultures. In 
vivo, intraperitoneal administration of fetuin (100 mg/kg, once daily, 
for 3 days) beginning at +24 h post CLP, significantly increased 
animal survival rates from 40% (in saline vehicle group, \( N = 22 \) mice/ group) to 90% (in fetuin group, \( N = 22 \) mice/group, \( P < 0.05 \)).

Conclusion: Fetuin occupies a protective role in experimental sepsis 
by attenuating a late mediator of lethal systemic

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This study was supported in part by the National Institutes of Health 
(NIH, NIGMS, GM063075 to H.W.)
A 284
Discovery of a natural antagonist of macrophage migration inhibitory factor (MIF)
Yousef Al-Abed

Background: MIF is a proinflammatory cytokine that plays a critical role in the pathogenesis of multiple inflammatory diseases. In sepsis, plasma MIF concentrations are significantly higher in non-survivors than survivors and administration of MIF inhibitors (e.g., antibody
anti-MIF antibodies or MIF small molecule inhibitors) improve survival in preclinical models of this disease. Three-dimensional X-ray crystallography has demonstrated that MIF forms a homotrimer with a hydrophobic cavity formed between each adjacent subunit. This hydrophobic cavity is a critical domain since small molecules that fit into this site inhibit MIF’s pro-inflammatory activity in a dose-dependent manner. We have systematically explored the chemistry of synthetic small molecule MIF inhibitors and have developed increasingly selective and potent pharmacophore scaffolds. To date, however, no naturally-occurring inhibitory MIF ligand (MIFnl) has been reported. Hypothesis: We hypothesize the existence of a MIFnl that normally binds to and inhibits the proinflammatory activity of MIF. During sepsis, when plasma MIF levels increase, a MIF:MIFnl imbalance occurs, resulting in free MIF and increased inflammation; restoring this balance, by administering exogenous MIFnl, should improve sepsis survival. Results: We have discovered a MIF natural ligand (MIFnl) that binds in the hydrophobic cavity of MIF with high affinity (IC50, 15.8 μM) and attenuates MIF’s proinflammatory activity in a dose-dependent manner. Strikingly, MIFnl is a more potent inhibitor of MIF than ISO-1 (IC50, 25 μM), the benchmark, commercially available inhibitor of MIF. In plasma from patients with sepsis, we discovered an inverse correlation between the levels of MIF and MIFnl; namely, when MIF increases, MIFnl decreases. Moreover, in a rodent model of sepsis, administration of exogenous MIFnl significantly improved the 7 day survival rate (60 vs. 20%) relative to vehicle-treated mice.

Conclusion: Our data identify for the first time, the presence of a natural ligand antagonist of MIF that may play a critical role in regulating MIF activity. During severe sepsis, increased production and release of MIF leads to an imbalance of the MIF:MIFnl regulatory mechanism, resulting in the development of an overwhelming systemic inflammatory response leading to cardiovascular collapse and death. A better understanding of the kinetics of MIF:MIFnl regulation in patients with sepsis may lead to improved outcome in this devastating disease.

Corresponding Author: Yousef Al-Abed, The Feinstein Institute for Medical Research, Manhasset, NY 11030, USA, alabed2001@yahoo.com

A 285
Modification of co-stimulatory/inhibitory molecules in sepsis
Alfred Ayala, Alfred Ayala, Guillaume Monneret, Fabienne Venet, Nicholas Shubin, Xin Huang

Although advances have been made in our knowledge of the pathology of sepsis and the co-morbid development of multiple organ dysfunctions, much remains to be understood about the septic process if we are to develop better therapies. In this respect, immune dysfunction resulting from traumatic injury, in the presence or absence of a variety of pre-dispositional effectors (e.g., gender, age, diet, etc.), is thought to be a significant contributor to not only the development of multiple organ failure but also mortality associated with subsequent septic challenge. Intriguingly, recent studies from a number of laboratories looking at both animal models and traumatically injured and/or septic shock patients have begun to point to a unique family of immuno-receptors, known as co-stimulatory/co-inhibitory receptors, as being not only potentially important indices of immune cell dysfunction but possible contributors to the developing morbidity of the critically ill animal/patient. Here we will briefly overview the evidence that co-stimulatory/co-inhibitory, such as CD40/CD40L, CTLA-4, CD47, PD-1/PD-L, BTLA:HVEM; CD200/CD200R, CD24 and Siglec-10, etc., are not only altered in response to sepsis and/or injury but play a role in contributing to pathology associated with this state/syndrome (Huang X, et al [2009] P.N.A.S. 106:6303-09; Schwulst SJ, et al [2006] J. Immunol. 177:557; Nolan A, et al [2007] Amer J Resp Crit Care Med. 177:301; Bandyopadhyay G, et al [2007] Crit Care Med 35:794; Liu Y, et al [2009] Trends Immunol. 30:557-61; Gorczynski R, et al [2008] J Surg Res 145:87-96; Shubin N, et al [2010] Inflamm. Res. [TSIS abst.-in press]). Finally, we will also consider their potential as diagnostic and/or therapeutic targets in the injured and/or septic animal/patient. Supported by NIH GM-46354.

Corresponding Author: Alfred Ayala, Prof. PhD, Rhode Island Hospital/Brown University, Department of Surgery/Division of Surgical Research, 593 Eddy St, Aldrich 227, Providence, RI 02903, USA, Aayala@lifespan.org

A 286
From guidelines to clinical practice: barriers for improvement
Herwig Gerlach, Susanne Toussaint

The management of sepsis in hospitals is significantly better today than it was 10 years ago. However, sepsis-associated mortality rates due to multiple organ dysfunctions still remain unacceptably high, and new strategies in order to improve patient outcomes will still further have to be embraced. The recent improvement in outcomes of patients with severe sepsis and septic shock has been characterized by the successive introduction of multiple interventions and therapies and is an ongoing process. Large clinical trials especially in the last 2–3 years now allow the clinician to perform a partially evidence-based therapeutic strategy to approach single and/or multiple organ dysfunctions. It is believed that the current wave of clinical trial data relating to a number of new interventions should be viewed in the context of this trend towards ever-improving management of the condition. In intensive care medicine, work flows in acute situations such as septic shock are rarely specific and protocol-based. Communication is often indirect and arbitrary, information systems are complex, time resources are limited, and we are not able to transfer scientific evidence into improved processes. This is resulting in a considerable number of “errors”, thus inducing unstructured, variable health care, which finally may lead to a deterioration of patients’ treatment. To improve our reliability, we have to change the structure of work flows especially in acute situations. This makes it necessary to say good-bye to traditional behaviour, without giving up analytical thinking and constructive criticisms. On the one hand, the live-threatening situation of severe sepsis and septic shock is a classical example for daily errors, hence—on the other hand—an ideal start point for improving
quality of treatment, as well as increasing efficiency in terms of medical and economical success.

A set of core changes extracted from the SSC guidelines have been incorporated into a package of key elements or goals that, when introduced into clinical practice, have a high likelihood of reducing mortality due to severe sepsis. The aim of the sepsis bundle is two-fold: First, to eliminate the piecemeal application of guidelines that characterizes the majority of clinical environments today, and second to make it easier for clinicians to bring the guidelines into practice.

Corresponding Author: Herwig Gerlach, Prof. MD, PhD, Vivantes-Klinikum Neukoelln, Anesthesia and Intensive Care Medicine, Rudower Str. 48, 12351 Berlin, Germany, herwig.gerlach@vivantes.de

dower Str. 48, 12351 Berlin, Germany, herwig.gerlach@vivantes.de

A 278

Pathological alterations of human adipose tissue
Karine Clement

Obesity is a disease of society and economic transition spreading at an epidemic pace throughout the world. According to the World Health Organization, obesity is defined as an increased or abnormal accumulation of body fat mass to the extent that individual’s health is negatively affected. Overweight is considered as top at risk condition in the world and it is mandatory to identify the physiopathological causes involved in adipose tissue enlargement and related metabolic and cardiovascular health disorders. In this context adipose tissue has been under focus in the last decades and pivotal concepts have emerged from the studies of their complex biology. The adipose organ is not simply a site for passive energy storage. White adipose tissue (WAT) is composed of mature adipocytes, precursors (preadipocytes), endothelial cells, macrophages, mast cells, blood vessels, nerves and lymphatic and connective tissue. The phenotype, amount and biology of each WAT component are altered profoundly in human obesity, a disease with low-grade inflammation state. In addition to adipocyte metabolic dysfunction (i.e. modified lipogenesis and lipolysis capacity), cellular stress including inflammation, oxidative, reticulum endothelial stress and hypoxia are part of the biological alterations which attract and retain inflammatory cells within the WAT and promote adipocyte insulin resistance. Chemokines as MCP1 and Rantes/CCL5 not only contribute to inflammatory cell adhesion and transmigration in adipose tissue but also may exert physiological action as shown recently for CCL5 being antiapoptotic for adipose tissue macrophages. Human adipocytes and preadipocytes demonstrate profound modifications of their biology when co-cultured with human macrophage media. A pro-inflammatory state, increased lipolysis and resistance to insulin are observed in adipocyte while the capacity of preadipocyte to differentiate is altered in presence of inflammatory stimuli. Inflammatory preadipocytes also acquire capacities to migrate and to synthesize profibrotic components. The evaluation of transcriptomic interactions characterizing the adipose tissue of weight-stable obese subjects demonstrated the strong relationship linking inflammatory processes to extra cellular matrix (ECM) remodelling components. Our group showed that interstitial fibrosis accumulates in obese WAT as in many organs affected by low-grade inflammation in chronic diseases (i.e. liver, lung, kidney pathologies).

In this conference, some illustrative examples will be taken regarding the pathological alterations of the adipose tissue in human obesity including the local altered biology of the adipose tissue and the discovery of new biomarker linking enlarged adipose tissue to obesity complications.

References


Corresponding Author: Karine Clement, Prof. MD, PhD, Pitie Salpetriere, NUTRITION, 83 bd de l’Hôpital, 75013 Paris, France, karine.clement@psl.aphp.fr

A 288

The endothelium and platelets: major contributors to host response
Paul Kubes

The majority of TLR4 work has focused on the macrophages despite the fact that absence of these cells does not eliminate TLR4-mediated responses. Systemic LPS administration to mice results in numerous important responses including a profound neutropenia, thrombocytopenia with immune cells localizing to lungs and liver. Development of a mouse lacking TLR4 in all cells except endothelium (endotheli-TLR4) still caused neutropenia and increased neutrophil recruitment into the lungs and liver despite a complete absence of the cytokine storm within the vasculature. However in this case the neutrophils returned into the circulation within 24 h but only in the endothelialTLR4 mice. E. coli were administered to wild-type mice at levels that took 7 days to clear and caused 50% death. Endotheli-ATLR4 mice took half the time to clear the E. coli and resulted in no mortality. The migration of platelets to the lungs in response to LPS was dependent upon neutrophils trapped in lungs and platelet TLR4. The platelets bound to neutrophils and induced a novel killing mechanism, namely the release of nuclear DNA in the form of neutrophil extracellular traps. These NETs help trap bacteria but also damage surrounding tissue.

Corresponding Author: Paul Kubes, Prof, PhD, University of Calgary, Department of Physiology and Pharmacology, HRIC 4A16 3280 Hospital Drive NW, Calgary, AB T2N 4Z6, Canada, pkubes@ucalgary.ca

A 289

TLR and RIG-I-like helicase activation: impact on regulatory T-Cells
David Anz

The role of immune suppression by regulatory T (Treg) cells in the maintenance of immune homeostasis is well established. However,
little is known about how Treg cell function is inhibited upon viral infection in order to allow the development of a protective immune response. As viral RNA is a crucial mediator for activation of antiviral immunity, we examined the effects of immunostimulatory RNA and infection with RNA viruses on Treg cell function. We show that synthetic RNA oligonucleotides potently inhibit Treg cell–induced suppression in a sequence–dependent manner. This effect is entirely dependent on TLR7 activation of antigen–presenting cells (APC) and subsequent IL–6 production. In addition, stimulation with the RNA viruses encephalomyocarditis virus (EMCV) and Sendai virus that specifically activate the RNA–sensing helicases MDA–5 and RIG I also blocks Treg cell function. Interestingly, this effect is seen even in the absence of APC. Consistent with this, both Treg and Teff cells express RIG-I and MDA 5. Using MDA–5–deficient mice, we demonstrate that the loss of Treg cell function upon infection with EMCV is strictly dependent on MDA–5 expression by Treg cells. Thus, we show here that activation of a RIG–I–like helicase on Treg cells blocks their suppressive function.

Corresponding Author: David Anz, MD, Ludwig–Maximilians–University of Munich, Campus Innenstadt, Division of Clinical Pharmacology, Department of Medicine, Ziemssenstr. 1, 80336 Munich, Germany, david.anz@med.uni-muenchen.de

A 290
Point care diagnostics for assessment of acute coagulopathy
Martin Schreiber

Trauma is the leading cause of death after trauma and hemorrhage contributes to approximately 40% of these deaths. Aggressive and appropriate treatment of coagulopathy could significantly reduce the incidence of death after trauma while still preserving the blood supply. Standard coagulation assays are performed on plasma at 37°C and they do not reflect whole blood clotting or the effects of temperature on coagulation. In addition, these tests are generally not available to the clinician for prolonged periods of time after they are drawn and do not reflect the current status of patients who are rapidly hemorrhaging and receiving large amounts of fluid and blood products. High ratio blood component transfusions have been shown to improve outcomes in patients undergoing massive transfusion but these protocols may result in unnecessary blood product transfusion. Thrombelastography (TEG) is a point of care test that permits rapid, comprehensive assessment of whole blood clotting at the patient’s temperature. TEG assesses the viscoelastic properties of the clot and analyzes the life of the clot including onset of formation, rapidity of fibrin crosslinking, the strength of the clot and the rapidity of fibrinolysis. Analysis of individual TEG parameters permits identification of clotting deficiencies that are corrected by transfusion of specific blood components. Standard TEGs are performed utilizing kaolin activation and results are available in approximately 34 min. Rapid TEGs are done using tissue factor as the activating agent and accurate results are available in approximately 19 min. TEG parameters rapidly identify coagulopathy and have been shown to correlate with the need for transfusion and mortality. Retrospective data suggest that transfusion triggers guided by TEG parameters could result in earlier correction of coagulopathy and decreased blood product utilization overall compared to standard coagulation parameter triggers. In conclusion, TEG is a point of care test that permits rapid assessment of coagulation and specific component therapy potentially resulting in more rapid correction of coagulopathy and less blood product utilization overall.

Corresponding Author: Martin Schreiber, MD, Oregon Health and Science University, Department of Surgery, 3181 SW Sam Jackson Park Road, Mail Code L611, Portland, OR 97239, USA, schreibm@ohsu.edu

A 291
CD8+ T cells instigate endothelial cell injury and liver organ damage during sepsis through the Fas-FasL system
Noelle Hutchins, Chun-Shiang Chung, Alfred Ayala

Objective: Much remains to be understood about the interactions between T cells and liver sinusoidal endothelial cells (LSECs) during sepsis. Here, we use the mouse liver as a model organ; as it is susceptible to sepsis induced injury. It has also previously been shown that the number of various T-cell subpopulations in liver increases during experimental sepsis. Since activated (FasL expressing) T cells must traverse LSECs (a population cells that can up–regulate Fas in other systems) to reach the liver parenchyma; we hypothesized that activated T cells could mediate endothelial injury/death, that culminates in septic liver damage.

Materials and methods: To test this hypothesis, experimental polymicrobial sepsis was induced via cecal ligation and puncture (CLP) in male C57BL/6 mice (6–8 weeks). Twenty-four hours following CLP or Sham protocols the mice were euthanized and the liver was harvested. Non–parenchymal cells (NPCs) were isolated and stained for endothelial cell markers, CD31 and CD144 or Fas, and Annexin V (apoptotic markers) by flow cytometry. Purified/isolated liver CD8+ T cells (Miltenyi Biotec) derived from CLP or Sham mice were then co–cultured ex vivo with a mouse endothelial cell line, CRL–2167, to assess their effect on barrier function, via the ECIS system. Liver CD8+ T cell co–cultures ex vivo with CRL–2167 were also assayed for pro–inflammatory cytokines (by BD–Cytometric Bead Assay) and morphological changes to the monolayer determined by electron microscopy.

Results: Our results indicate that cells expressing CD144+, from the liver, exhibit an increased level of Fas and Annexin V following CLP. Septic mouse CD8+ T cells decrease endothelial barrier function and caused an increase of pro–inflammatory cytokine release when directly co–cultured. Septic mouse CD8+ T cells also directly attach to endothelial cell junctional proteins in comparison to Sham T cells. Conclusion: We conclude that endothelial cells undergo Fas–mediated apoptosis and that septic CD8+ T cells decrease endothelial barrier function, and attach at endothelial cell junctions to directly transmigrate and reach the site of infection. These results suggest that T lymphocytes may directly interact with the liver endothelium through the Fas–FasL system, leading to decreased permeability, a release of pro–inflammatory mediators, and ultimately liver dysfunction.

Supported by NIH–F31 DK83873 and R01 GM53209.

Corresponding Author: Noelle Hutchins, Rhode Island Hospital/Brown University, Department of Surgical Research, 591 Eddy Street, Providence, RI 02903, USA, noelle_hutchins@brown.edu

A 292
TLR4 regulates lung injury and neutrophil activation via TRIF, and to a lesser extent, MyD88–dependent signaling pathways in a mouse trauma–hemorrhagic shock lymph infusion model
Diego Reino, Rena Feinman, Da-Zhong Xu, Eleonora Feketeova, David Palange, Edwin Deitch

Objectives: Our previous work has shown that Toll–like Receptor 4 (TLR4), but not TLR2, mediates lung injury when porcine trauma–hemorrhagic shock (T/HS) mesenteric lymph is injected into a naïve mouse as a lung injuring reagent that simulates the lung injury seen in acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS). To further elucidate the cellular
mechanisms involved in mediating this lung injury, we investigated the roles of two TLR4 downstream signaling pathways, TRIF and MyD88, in modulating T/HS mesenteric lymph induced lung injury. We hypothesized that TRIFmut, and to a lesser extent MyD88mut mice, would be protected from lung injury after exposure to porcine T/HS lymph. Furthermore, we hypothesized that TRIF and MyD88 signaling would also play a pivotal role in the activation of blood derived neutrophils that may contribute to the inflammatory process of the injured lung.

Materials and methods: C57BL/6J-Ticam1 (TRIFmut), MyD88mut, and C57BL/6J wild type mice were injected with porcine T/HS mesenteric lymph or sham shock (T/SS) lymph (internal jugular vein cannulation, 15 min laparotomy, and 3 h lymph injection period). Mice were sacrificed after 3 h of lymph infusion and lung bronchoalveolar lavage fluid (BALF) was used to measure lung permeability using the EBD permeability assay. To assess neutrophil activation, 400 µl of whole blood was collected prior to sacrifice and analyzed for neutrophil respiratory burst (RB) by measuring CD11b expression using flow cytometric methods.

Results: Our results suggest that TRIF deficiency confers a 3.6-fold protection from T/HS lymph-induced lung injury compared to WT counterparts as measured by EBD lung permeability (2.68 ± 1.3 vs. 9.74 ± 1.3). A modest degree of protection (1.4-fold decrease) from lung injury was also seen after exposure to T/HS lymph in MyD88mut mice (6.97 ± 1.4 vs. 9.74 ± 1.3). PMN respiratory burst was also attenuated in both the TRIFmut and MyD88mut mice as compared to WT (T/HS) as noted in Table 1:

Table 1: EBD permeability and neutrophil activation after exposure to T/HS lymph

<table>
<thead>
<tr>
<th></th>
<th>WT (T/SS)</th>
<th>WT (T/HS)</th>
<th>TRIFmut (T/SS)</th>
<th>TRIFmut (T/HS)</th>
<th>MyD88mut (T/SS)</th>
<th>MyD88mut (T/HS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% EBD (mean = SD)</td>
<td>2.94 ± 1.0</td>
<td>9.74 ± 1.3</td>
<td>3.37 ± 1.6</td>
<td>2.68 ± 1.3</td>
<td>4.95 ± 0.6</td>
<td>6.97 ± 1.4</td>
</tr>
<tr>
<td>PMNRB (mean fluorescent intensity = SD)</td>
<td>219.38 ± 22</td>
<td>343.44 ± 59*</td>
<td>219.73 ± 31</td>
<td>232.14 ± 27**</td>
<td>189.81 ± 15</td>
<td>271.50 ± 33***</td>
</tr>
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*n p < 0.01 versus WT (T/SS), ** p ≤ 0.01 versus WT (T/HS), *** p < 0.01 versus WT (T/HS) (unpaired T test)

Conclusions: Taken together, our data suggest that both TRIF and MyD88 pathways contribute to the injurious effects of TLR4 in the lung after porcine T/HS mesenteric lymph infusion. However, TRIF activation appears to play a more prominent role than MyD88 in mediating lung injury. Similarly, both TRIF and MyD88 signaling was associated with an increased activation of neutrophils that may contribute to lung injury as measured by respiratory burst.

Corresponding Author: Diego Reino, MD, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Department of Surgery, 185 South Orange Avenue, Newark, NJ 07103, USA, reinodi@umdnj.edu

A 294

Macrophage migration inhibitory factor (MIF) and manganese superoxide dismutase (MnSOD) are early predictors for survival in severe septic patients

Thorsten Brenner¹, Stefan Hofer¹, Claudia Rosenhagen¹, Eike Martin¹, Ursula Hoffmann², Markus A. Weigand³

¹Department of Anaesthesiology, University of Heidelberg, Germany, ²First Department of Medicine, Faculty of Medicine, University of Mannheim, Germany, ³Department of Anaesthesiology and Intensive Care Medicine, University of Giessen, Germany

Objective: Severe sepsis, septic shock, and resulting organ failure represent the most common cause of death in intensive care medicine, with mortality ranging from 40 to 70%. Inflammatory mediators (Interleukin-6/IL-6, Macrophage Migration Inhibitory Factor/MIF), cell adhesion molecules (Intercellular Adhesion Molecule-1/ICAM-1, Vascular Cell Adhesion Molecule-1/VCAM-1) and redox active substances (Manganese...
Introduction: Infection and sepsis result in significant morbidity and mortality worldwide. The host response to infection involves complex regulation of inflammatory and immune responses. This regulation is to not only control infection, but also to prevent tissue injury and restore homeostasis. Studies illustrate that the initial organ dysfunction in sepsis involves minimal loss of structural integrity or cell death, thus allowing for the eventual recovery of function. Many adaptive cellular responses and signaling pathways have been implicated in these responses to sepsis. We hypothesize that autophagic signaling, a process of cellular autodigestion and recycling, is an adaptive response to sepsis that protects against cell death. Furthermore, that autophagic signaling is regulated by heme oxygenase (HO), a known important anti-inflammatory/apoptotic enzyme.

Objective: Demonstrate that the induction of autophagic signaling from a septic insult results in decreased cell death and that this induction is at least partially regulated by heme oxygenase.

Methods: Cecal ligation and perforation (CLP) or laparotomy and bowel manipulation without cecal ligation or puncture was performed on C57Bl/6 mice. To inhibit HO again mice were treated with tin protoporphyrin (SnPP, 50 μM/kg) 1 h prior to CLP. Autophagy was determined by Western blotting for autophagic proteins and electron microscopy demonstrating autophagosome formation. Apoptosis was determined by staining of autophagic proteins and electron microscopy demonstrating autophagosome formation. Apoptosis was determined by staining for activated caspase-3. In vitro studies were performed utilizing primary mouse hepatocytes treated with lipopolysaccharide (LPS) (100 ng/mL). HO activity was inhibited with SnPP (50 μM/mL). Autophagy was inhibited pharmacologically using 3 methyl-adenine (3-MA; 2 mM) or siRNA for VPS34, an upstream activator of autophagic signaling.

Results: CLP increased autophagic signaling in both liver and kidney tissues as demonstrated by increased expression and punctate staining for autophagic proteins and electron microscopy demonstrating autophagosome formation. Additionally, autophagosomes were visualized by electron microscopy. Inhibition of HO activity using SnPP decreased autophagic signaling, and conversely increased apoptosis. Furthermore, inhibition of HO activity led to increased tissue injury and

A 296

The protective role of autophagy and heme-oxygenase 1 against sepsis-induced apoptosis

Evie Carchman, Brain Zuckerbraun

Introduction: Infection and sepsis result in significant morbidity and mortality worldwide. The host response to infection involves complex regulation of inflammatory and immune responses. This regulation is to not only control infection, but also to prevent tissue injury and restore homeostasis. Studies illustrate that the initial organ dysfunction in sepsis involves minimal loss of structural integrity or cell death, thus allowing for the eventual recovery of function. Many adaptive cellular responses and signaling pathways have been implicated in these responses to sepsis. We hypothesize that autophagic signaling, a process of cellular autodigestion and recycling, is an adaptive response to sepsis that protects against cell death. Furthermore, that autophagic signaling is regulated by heme oxygenase (HO), a known important anti-inflammatory/apoptotic enzyme.

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Results: CLP increased autophagic signaling in both liver and kidney tissues as demonstrated by increased expression and punctate staining for autophagic proteins, which suggest autophagosome formation. Additionally, autophagosomes were visualized by electron microscopy. Inhibition of HO activity using SnPP decreased autophagic signaling, and conversely increased apoptosis. Furthermore, inhibition of HO activity led to increased tissue injury and

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Unravelling a novel role of myeloid-related proteins 8/14 (Mrp 8/14) in self-tolerance and cross-tolerance to gram-negative and gram-positive bacteria

Andrew Coveney, Andrew Coveney, Jianghuai Wang, Paul Redmond

Objectives: Myeloid related protein (Mrp) 8 and 14 form heterodimers that elicit an inflammatory response. It has recently been shown that Mrp8/14 is a ligand for Toll-like receptor 4 (TLR4), with Mrp8 being the active component. Endotoxin self-tolerance, a phenomenon which describes an attenuated inflammatory response to LPS after previous exposure to LPS, has been extensively investigated and is triggered through TLR4. LPS has been shown to also induce cross-tolerance with bacterial lipoprotein (BLP), where the inflammatory response to BLP, a TLR2 ligand is attenuated after previous exposure to LPS. This study examines the ability of Mrp8 to induce self-tolerance and cross-tolerance to BLP, through TLR4 and TLR2 in a dose and time dependent manner.

Corresponding Author: Andrew Coveney, Cork University Hospital, Dept. of Academic Surgery, Wilton, Cork, Ireland, acoveney@gmail.com
decreased survival. In vitro data shows a similar response. Hepatocytes demonstrate increased autophagic signaling in response to LPS. This is inhibited by SnPP, as well as by 3-MA or knockdown of VPS34. Moreover, inhibition of autophagy was associated with increased apoptosis.

Conclusion: Experimental sepsis in vivo or LPS in vitro increases autophagic signaling, which is dependent, at least in part, on heme oxygenase signaling. This induction of autophagy is important in the prevention of cell death and permanent loss of organ function.

Corresponding Author: Evie Carchman, MD, University of Pittsburgh Medical Center, Department of Surgery, 200 Lothrop Street, Pittsburgh, PA 15213, USA, carchmaneh@upmc.edu

A 297
Tolerance to lipopolysaccharide (LPS) is not regulated by toll-like receptor 4 (TLR4) expression on circulating CD14+ monocytes in a porcine model of severe gunshot injury and peritraumatic stress

Bard Lundeland1,2, Yngvar Gundersen1, Per-Kristian Opstad1, Ingjerl Thirane1, Per Vaagenes1,2
1Norwegian Defence Research Establishment, N-2027 Kjeller, Norway, 2Department of Anaesthesiology, Akershus University Hospital, N-1478 Lorenskog, Norway

Objective: In a porcine model of severe gunshot injury and peritraumatic stress we have earlier mapped the temporal pattern of activation of circulating immune cells. The cytokine response to a subsequent challenge with LPS is immediately reduced (“endotoxin tolerance”). LPS signals through TLR4, a genetically conserved pattern recognition receptor. This receptor is expressed on the surface of cells belonging to the innate immune system, i.e. monocytes. In the present study we have investigated how gunshot injury and peritraumatic stress influence the expression of TLR4 on CD14+ monocytes, and looked for a possible correlation with the cytokine response.

Methods: Ten anaesthetised pigs sustained two standardized rounds, one gunshot through right femur and one pistol shot through left upper abdomen. First aid treatment and acute surgery was started immediately. Blood samples were drawn before shooting and after 90 min, and were investigated in an ex vivo whole blood model. The samples were stimulated for 4 h with LPS 10 ng/ml or an equivalent amount of normal saline. The leukocyte response was evaluated after measurement of TNF-a in the supernatant. The expression of TLR4 on CD14+ monocytes was measured by flow cytometry.

Results: TLR4 expression tended to decrease from median fluorescence intensity (MFI) 811 before to 730 after shooting (NS). The corresponding values for the proinflammatory cytokine TNF-a after incubation in the ex vivo whole blood model was 2,109 pg/ml before and 326 pg/ml after the injury (p < 0.05). No significant correlation between TLR4 expression and cytokine concentrations was found.

Conclusion: Gunshot injury and peritraumatic stress significantly decreased the ability of inflammatory cells to respond with TNF-a secretion upon challenge with LPS. There was a non-significant tendency towards reduced TLR4 expression on CD14+ monocytes, but no covariation with TNF-a concentrations. The results imply that the TLR4 expression on circulating CD14+ monocytes plays a negligible role for the observed tolerance to LPS.

Corresponding Author: Bard Lundeland, Norwegian Defence Research Establishment, Department of Protection, Instituttveien 20, 2027 Kjeller, Norway, bard.lundeland@medisin.uio.no

A 298
Peritoneal cytokines and survival after surgical treatment of secondary peritonitis in the rat

Omar Buyne, Thijs Hendriks, Robert Bleichrodt, Roger Lomme, Ben De Man, Harry Van Goor

Objective: Secondary peritonitis mostly occurs after disruption of the integrity of the gastrointestinal tract. Despite optimal treatment, this condition remains associated with a high morbidity and mortality. Early identification of patients at risk for an adverse outcome would facilitate therapeutic management of peritonitis. Local expression of, and balance between, pro- and anti-inflammatory cytokines is related to severity and outcome of peritonitis. In this study abdominal cytokine levels for their use as a diagnostic and prognostic tool in peritonitis were evaluated.

Materials and methods: Data from two consecutive preclinical experiments on abscess formation in peritonitis were combined. In short: peritonitis was induced in rats by intraperitoneal injection of a suspension of sterile rat feces and Escherichia coli and Bacteroides fragilis surgical debridement was performed 1 h afterwards. Samples of abdominal fluid were taken at 24 and 72 h to determine levels of IL-6, IL-10 and TNF-alpha. After 5 days the animals were killed, intraabdominal abscesses were counted and survival rates were calculated. Statistical analysis was performed with respect to survival rates, differences between survivors and non-survivors, differences between measurements at two time points and correlation of cytokine levels. Prognostic potential was reflected by high diagnostic accuracy, represented by the area under the curve (AUC).

Results: There were 115 survivors and 48 non-survivors. Overall survival was 66%. Levels of all 3 cytokines were significantly (p < 0.001) higher in non-survivors. At 24 h significant (p < 0.0001) correlations between all cytokines were observed in a separate analysis of survivors and non-survivors. Correlations between cytokine levels weakened between 24 and 72 h. Strongly (p < 0.0001) increased mortality was observed if IL-6, IL-10 or TNF-alpha levels exceeded 2, 1 or 0.2 ng/ml. This remained true after halving the cut-off values. AUC values were high for all 3 cytokines with IL-10 showing the best characteristics with an AUC of 0.94 and 67% sensitivity at 95% specificity, obtained at a cut-off value of 1.26 ng/ml. Conclusion: The current data generate renewed interest to peritoneal cytokines as early markers for adverse outcome in secondary peritonitis. It should also be investigated if combinations of peritoneal cytokines, peritoneal and circulating cytokines or combinations with other markers can improve prediction of disease severity.

Corresponding Author: Omar Buyne, MD, PhD, Radboud University Nijmegen Medical Center, Department of Surgery, PO Box 9101, 6500 HB Nijmegen, Netherlands, O.Buyne@chir.umcn.nl

A 299
Hypertonic saline inhibits TLR4-mediated inflammatory signaling in human alveolar macrophages

Winston Choi, Anirban Banerjee, Ernest Moore, Roopali Shah, Sanchayita Mitra

Objective: While an innate immune response is essential for clearance of pulmonary pathogens, over-exuberant tissue inflammation may lead to irreversible architectural damage and gas exchange deficits. Animal models have shown that hypertonic saline blunts LPS-induced hyper-inflammatory states in alveolar macrophages (AMs) after
hemorrhagic shock and resuscitation. This protects the lung from secondary injury. However, the precise immunomodulatory mechanisms are yet to be elucidated in humans. Our hypothesis is that hypertonic saline inhibits human AM responses to TLR4 signaling by blunting pro-inflammatory transcription factor activation. Methods: CD14+ mononuclear cells were differentiated into an AM-like phenotype by incubation with GM-CSF (20 ng/mL) for 10 days. AMs were stimulated by LPS (100 ng/mL) for 8 h in isotonic RPMI (285 mMOSm), or incubated for 30 min in NaCl supplemented RPMI (400 mMOSm) prior to LPS stimulation. Supernatants were analyzed via ELISA for IL-6, MIP-1a and TNF-alpha. Short time course experiments were performed identically for up to 1 h of LPS stimulation. Whole cell lysates were probed via Western blot with antibodies to phosphorylated p38, ERK-1/2, and SAPK/JNK. Nuclear lysates were probed with antibodies to phosphorylated p65, and C/EBP beta. Results: TNF-alpha, IL-6 and MIP-1a showed significant decreases at 8 hs. Maximal ERK-1/2 phosphorylation occurred at 30 min, with a 90% decrease in hypertonic medium. NF-kB p65 translocation and phosphorylation decreased by >50% at 30 min in hypertonic medium. The ratio of the C/EBP-beta liver-enriched transcriptional activating protein (LAP) isoform to inhibitory (LIP) isoform in isotonic media reached a maximum of ~0.7 at 15 min. The LAP:LIP ratio remained <0.3 throughout the time-course in hypertonic media.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Isotonic (pg/mL)</th>
<th>Hypertonic (pg/mL)</th>
<th>n</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>15,700 ± 160</td>
<td>2,210 ± 230</td>
<td>2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>MIP-1a</td>
<td>1,330,000 ± 32000</td>
<td>20,200 ± 520</td>
<td>0.05</td>
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</tr>
<tr>
<td>TNF-a</td>
<td>1,520 ± 190</td>
<td>717 ± 120</td>
<td>3</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

Conclusion: Hypertonic conditioning decreases AM responses to TLR4 activation. Stimulated AM release of several key cytokines and chemokines was decreased by ~50–98%. This effect was likely produced via inhibition of ERK-1/2 activation, p65 nuclear translocation, and C/EBP activity. Hypertonicity altered the LAP:LIP ratio of C/EBP-beta isoforms, resulting in an increased proportion of inhibitory LIP protein. Further work will be needed to determine how these pathways contribute to the inhibition of specific genes at either the transcriptional level or further downstream. However, it is clear that hypertonicity is a powerful modulator of several inflammatory pathways relevant to acute lung injury.

**Corresponding Author:** Winston Choi, MD, University of Colorado at Denver, Department of General Surgery, 12700 E 19th Ave, Rm 6420, Aurora, CO 80045, USA, winston.choi@ucdenver.edu

### A 301

**Bacterial resistance through antibiotic-loaded bone cement?**

**Julian Fuermetz, Florian Vollrath, Joery Boehme, Stefan Klima, Christoph Josten**

Objective: Local antibiotic treatment using a temporary antibiotic-impregnated PMMA spacer for severe bacterial joint-infections or infected orthopedic implants is currently well accepted. But the long-term exposure to low doses of antibiotics after an initial high release is also seen critical by many. Persistence of bacterial growth on the foreign body and development of bacterial resistance seem to be possible dangers.

Methods: To objectify these dangers we examined 69 patients treated with an antibiotic-loaded PMMA spacer in the years 2006–2008. Different types of bacteria, local and systemic antibiotics, times of revision, final outcome and patients condition before the treatment were registered.

Results: The most common detected bacteria were staphylococcal strains and mixed infections. In 20 patients no bacteria were detected despite of a clear clinical infection. Multidrug-resistant organisms (e.g. MRSA) complicated the treatment in 16 patients. Seven of these patients acquired the multidrug-resistant bacteria during the treatment with a gentamicin–vancomycin-loaded spacer. The risk for a bad outcome was significantly higher in these patients.

Conclusions: Our results indicate that in vivo growth of multidrug-resistant specimen on gentamicin–vancomycin-loaded bone cement is possible. Long-term low dose local antibiotic treatment seems to favor the development of bacterial resistance. Multidrug-resistant bacterial strains and a compromised immune system highly endanger the patient. It has to be evaluated in following studies if other treatment options or the modification of the existing protocol can be helpful particularly for high risk patients.
A 302
Insufficient decrease of C-reactive protein after elective colorectal surgery predicts major septic post-operative complications
Xavier Guirao, Montserrat Juvany, Guzman Franch, Sara Amador, Ruben Hernandez, Josep Mª Badia

Objective: To evaluate the value of C-reactive protein (CRP) changes during the post-operative period in predicting major septic complications after elective colorectal surgery.

Materials and methods: 151 patients undergoing elective colorectal surgery were prospectively assessed for the incidence of major septic postoperative (PO) complications (deep incisional and organ-space surgical site infection and pneumonia). Levels of CRP were measured at PO day 2 and 5 and SIRS parameters (white count cells, and central temperature) were daily assessed. We compared levels of CRP and the expression of SIRS parameters in those patients with late (after PO day 5) complications (Group C+) and patients with uneventful course (Group C−). Levels of CRP and SIRS parameters at PO day 2 were additionally compared between patients with early (before PO day 5) and late complications. Data are summarized as mean ± SD. To analyze the data, we performed t Student test. The value of change in percentage of CRP values between PO day 2 and 5 (% CRP 2–5) in predicting late major septic complications was evaluated using a receiver operator curve analysis.

Results: 36 patients presented major PO complications, 5 early and 31 late. CRP values were significantly higher in complicated patients (200 ± 67 in Group C+ vs. 163 ± 77 mg/L in Group C− at PO day 2 and 213 ± 100 in C+ vs. 65 ± 59 mg/L in C− at PO day 5, p < 0.05). No patients expressed SIRS criteria (two or more altered parameters). At PO day 2, patients with early detected complications, significantly showed much higher levels of CRP (348 ± 71 and 200 ± 67 mg/L in early and late complicated patients, respectively, p < 0.001) and significantly expressed SIRS criteria of altered heart and respiratory rate. The area under curve of Δ% CRP 2–5 for predicting late PO complications was 0.93 for a cut-off point of minus 36% (sensitivity 0.9, specificity 0.86, positive predictive value 0.64, negative predictive value 0.97).

Conclusions: 90% of late complicated patients showed a decline in CRP levels less than 36% between PO day 2 and 5. A CRP decrease of 36% or more between the PO day 2 and PO day 5 predicts uneventful postoperative course in 97% of patients. Extremely high levels of CRP at PO day 2 might indicate early severe complications. Only patients with early complications significantly express SIRS criteria, mainly tachypneia and tachycardia.

Corresponding Author: Xavier Guirao, MD, Hospital General de Granollers, Department of Surgery, Avda. Frances Ribas s/n, 8402 Granollers, Spain, xguirao@teleline.es

A 304
The impact of C-reactive protein (CRP), IL-6, leptin, cortisol and caspase-3 on the decision of terminating planned abdominal repair in moderate and severe secondary peritonitis
Faruk Pehlivanli, Fatih Agalar, Canan Agalar, Tayfun Sahiner, Kacey Aysinurcu, Unase Bayukkocak

Background: The aim of this clinical prospective study was to investigate the impact of serum C-reactive protein (CRP), IL-6, leptin, cortisol and peritoneal caspase-3 on the decision of terminating planned abdominal repair in moderate and severe secondary peritonitis.

Materials and methods: Fifteen consecutive patients with moderate and severe peritonitis, and APACHE II score ≥10 were enrolled into the study. Serum CRP, IL-6, leptin and cortisol levels and peritoneal caspase-3 activities were measured in the samples taken during the first four planned relaparatomies including index laparotomy.

Results: For the patients that died, APACHE II scores at 48 h and age were significantly higher (p = 0.026; p = 0.001). For all patients, the
corresponding author: Fatih Agalar, Prof. MD, Kirikkale University

Keywords: STAR, Caspase-3, Peritonitis, IL-6, Cortisol
erate peritonitis treated with STAR.

As far as we are concerned, this is the first study implementing peritoneal caspase-3 levels of longer duration with more patients are needed.

Decrease in peritoneal caspase-3 activity may be useful in determining the number of peritoneal lavages. Accordingly, in patients with secondary moderate and severe peritonitis, four peritoneal lavages including the index operation are found to be sufficient. If peritoneal caspase-3 activity is to be chosen as a major criteria for abdominal closure in STAR patients, studies concerning peritoneal caspase-3 levels of longer duration with more patients are needed.

As far as we are concerned, this is the first study implementing peritoneal caspase-3 as a surrogate parameter for peritoneal apoptosis in decision making analysis of abdominal closure. Decrease in peritoneal caspase-3 activity may be useful in determining the number of peritoneal lavages. Accordingly, in patients with secondary moderate and severe peritonitis, four peritoneal lavages including the index operation are found to be sufficient. If peritoneal caspase-3 activity is to be chosen as a major criteria for abdominal closure in STAR patients, studies concerning peritoneal caspase-3 levels of longer duration with more patients are needed.

Conclusion: It was seen than there was no impact of CRP, IL-6, leptin, cortisol levels in decision making analysis for timing of abdominal closure. Decrease in peritoneal caspase-3 activity may be useful in determining the number of peritoneal lavages. Accordingly, in patients with secondary moderate and severe peritonitis, four peritoneal lavages including the index operation are found to be sufficient. If peritoneal caspase-3 activity is to be chosen as a major criteria for abdominal closure in STAR patients, studies concerning peritoneal caspase-3 levels of longer duration with more patients are needed.

A 305

The release of intracellular IL-1 alpha mediates the recruitment of infiltrating myeloid cells to the site of necrosis

*The Shraga Segal Department of Microbiology and Immunology and Faculty of Health Sciences and The Cancer Research Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel, 1Department of Medicine, University of Colorado Denver, Aurora, CO 80045, §These authors contributed equally to this work.

Objective: Recombinant IL-1 alpha, like IL-1 beta, mediates multiple inflammatory and immune responses. Unlike IL-1 beta, IL-1 alpha is active in its precursor form; it is present constitutively as an intracellular cytokine in healthy tissues without being actively secreted. Here, we describe the role of IL-1 alpha in mediating the inflammatory response to cells dying by apoptosis or necrosis.

Material and methods: We used an in vivo model of inflammation in which lysates of either UV-irradiated or non-treated cells, embedded in Matrigel, were injected into mice subcutaneously, thus simulating the exposure of tissue to apoptotic or necrotizing cells. The apoptotic or necrotic cells, used as stimuli, were either IL-1 alpha-transfected B16 cells or primary fibroblasts obtained from control or IL-1 alpha KO mice. Counting and characterizing the cells that infiltrated into Matrigel plugs allowed us to evaluate the nature of the local inflammatory response. In addition, neutralizing anti-IL-1 alpha antibodies were used in order to determine the specificity of the IL-1 alpha dependent response.

Results and conclusions: We have shown that IL-1 alpha released from necrotizing cells triggers early recruitment of cells of the myeloid lineage, whereas lysates of apoptotic cells, lacking IL-1 alpha, failed to trigger this infiltrative response. In apoptotic cells, in contrast to necrotic cells, IL-1 alpha is tightly bound to chromatin and is not released. The ability to recruit a cell infiltrate was ascribed only to the precursor of IL-1 alpha, containing the C-terminal receptor (IL-1R1) interacting domain, while the N-terminal propiece, which also translocates to the nucleus, failed to do so. The release of IL-1 alpha from necrotic cells suggests that IL-1 alpha is an important alarm cytokine initiating inflammation when tissue damage occurs. Neutralization of IL-1 alpha in such cases may prevent the induction of inflammation and the propagation of accompanied tissue damage.

Corresponding Author: Peleg Rider, Ben-Gurion University, Department of Microbiology and Immunology, Ben Gurion St-PO 653, 84105 Beer-Sheva, Israel, riderp@gmail.com

A 306

Type I IFNs drive TNF-induced lethal shock

Claude Libert, Liesbeth Huys, Lien Dejager, Filip Van Hauwermeiren

Defense against invading pathogens is dependent on a rapid and effective response by the host. One of the first steps after viral or bacterial infections is an abundant production of interferons (IFNs). IFNs are classified into three distinct groups based on amino-acid sequences and recognition by specific receptors: namely type I IFNs (with as most important representatives IFN alpha and beta) and type II IFNs (IFN-gamma). Type I IFNs all bind to a heterodimeric receptor, IFNAR1 and IFNAR2, and initiate several signalling cascades leading to the expression of inflammatory cytokines, type I IFNs and IFN-inducible genes. For a long time, type I IFNs have been known as potent antiviral molecules. But in the last years, a critical role for these IFNs was suggested in LPS-induced endotoxemia. Beside the type I and II IFNs, there is also a recently defined third group, the type III IFNs. Of this last group only a few data has been published. The group consists of three members, IFN-lambda1 (IL-29), IFN- l2 (IL-28A) and IFN- l3 (IL-28B).

The cytokine TNF in combination with Interferon-gamma (IFN-gamma), has a very spectacular antitumor effect. But TNF is also a very strong inducer of the systemic inflammatory response syndrome (SIRS) characterized by hypotension, hepatitis and bowel necrosis (5). We have found that a deficiency of type I IFNs leads to a very significant protection against in vivo deleterious effects of TNF. IFNAR1-/- mice suffer from minor hypothermia and recover soon, whereas control mice have a severe hyperthermia and lethality after injection of 30 µg TNF. Our data illustrate that type I IFNs are essential mediators in TNF-induced lethal inflammatory shock, possibly through induction of chemokines and WBC infiltration in tissues and enhancement of apoptosis, induced by TNF, in enterocytes and hepatocytes.

Corresponding Author: Claude Libert, Prof. PhD, Ghent University, Biomedical Molecular Biology, Technologypark 927, 9052 Ghent, Belgium, Claude.Libert@UGent.be

A 307

First functional characterization of a new MIF family member as a mediator of sepsis

Melanie Merk, Swen Zierow, Lin Leng, Wibke Schulte, Olivier Lesur, Richard Bucala

Objective: Macrophage migration inhibitory factor (MIF) is the first cytokine activity to be described, and it has been established to be an important regulator of innate and adaptive immunity. MIF is produced...
in response to diverse pro-inflammatory stimuli and it plays a pivotal, upstream role in host anti-microbial responses. While anti-MIF therapies are beneficial in models of sepsis and other inflammatory diseases, they seldom completely inhibit cellular activation responses. Furthermore, with the discovery of the MIF cell surface receptor in 2003, it was noted that genetic receptor deficiency produces a phenotype that is more pronounced in some models than MIF deficiency. This observation led to the hypothesis that there might be a second ligand for the MIF receptor.

Methods and results: On the basis of a genomic search, we hypothesized that a likely additional ligand for the MIF receptor is the closely homologous, MIF-like protein called D-dopachrome tautomerase (D-DT). D-DT shows 38% sequence identity with MIF, and their three dimensional structures previously were shown to be highly conserved. We expressed and purified the murine and human D-DT proteins in native form, and both show high-affinity binding to the MIF receptor with ensuing activation of the ERK MAP kinase and downstream pro-inflammatory pathways. Lipopolysaccharide challenge of mice leads to an increase in D-DT levels in the serum with a kinetic similar to that observed for MIF, and the specific neutralization of D-DT protects mice from lethal endotoxic shock. This protective effect is most likely due to the fact that neutralization of D-DT results in a decrease in the same pro-inflammatory cytokine levels in the serum (TNFa, IL-1β, IL12, IFNγ) that are known to be downstream of MIF signaling. We also show by specific ELISA that circulating D-DT levels are elevated in patients with sepsis.

Conclusion: D-DT is a novel mediator of inflammation that binds to the MIF receptor, activates similar pro-inflammatory pathways, and mediates lethal shock. Targeting both MIF and its homologous ligand D-DT might significantly improve the therapeutic value of MIF-directed therapies.

Corresponding Author: Melanie Merk, PhD, Yale University, Department of Internal Medicine, 300 Cedar Street, New Haven, CT 06511, USA, Melanie.Merk@gmail.com

A 308
Tissue fluid cytokines: a neglected source of damaged tissue healing regulators
Marzanna Zaleska, Waldemar L. Olszewski, Marta Cakala, Govinda Ambujam, Pradeep Jain

Objectives: Lymphocytes (Lc) and progenitors of macrophages (M) and dendritic cells (DC) extravasate at site of injury. The process of extravasation and directional migration is mediated by cytokines present in tissue fluid/L (TF/L).

Aim: To measure concentration of immune cell attracting cytokines in lower limb TF/L of patients with tissue inflammation.

Material and methods: Sixty patients with inflammatory and lymphostatic changes in soft tissues of lower limbs were selected. TF/L was collected from site of incision.

Results: CCL27 responsible for chemoattraction of Lc reached 732 pg/ml in TF/L and 285 pg/ml in serum (S). CCL21 240 pg/ml in TF/L and 76 pg/ml in S, MCP1 537 pg/ml in TF/L and 312 pg/ml in S and MIP1 88 pg/ml in TF/L and 71 pg/ml in S. Group 3, CCL27 reached 505 pg/ml in L and 285 pg/ml in S. In normal TF/L CCL27 reached 181 pg/ml in L and 621 pg/ml in S, CCL21173 pg/ml in TF/L and 175 pg/ml in S, MCP1 270 pg/ml in TF/L and 274 pg/ml in S, MIP1 64 pg/ml in TF/L and 10 pg/ml in S.

Conclusions: High levels of immune cell-attracting cytokines were found in inflammatory TF/L. We speculate that lack or decreased production of these cytokines may be responsible for impaired Lc, M and DC traffic from blood to non-healing wounds.

A 309
Resistance of SPRET/Ei mice to TNF-induced lethal shock
Claude Libert, Leen Puimege, Filip Van Hauwermeiren, Jan Staelens

Tumor necrosis factor (TNF) is a cytokine with a potent antitumor activity, but has also been identified as a central mediator in lethal shock. Injection of TNF induces a systemic inflammatory response syndrome leading to hypotension, liver failure and finally death. Consequently, the use of TNF as antitumor drug is limited to local treatments. TNF has also been shown to be centrally involved in the development of arthritis, IBD and MS.

We found that SPRET/Ei, an inbred mouse strain derived from M. spretus, and (BxS)F1 mice are extremely resistant to TNF-induced lethal shock. All TNF-induced metabolic changes (IL6, NO, ALT) occur at much reduced levels in both SPRET/Ei and (BxS)F1 mice. (BxS)F1 mice are also protected against a TNF/IFNγ antitumor therapy, while tumor regression still occurs.

In order to identify loci conferring resistance to TNF-induced lethality, we performed a backcross between (BxS)F1 and C57BL/6. We found loci on proximal chr2 and distal chr6, and a sensitivity locus on chr11. Any discussion of candidate genes is still speculative. However, the traf2 gene, coding for TRAF2, involved in activation of NF-kB and the JNK pathway, is located on proximal chr2. And an even more prominent candidate gene could be tnfrsf1a, coding for TNFR1, located on distal chr6.

An in vitro study using MEFs and primary macrophages showed that TNFRI is still functional in SPRET/Ei. M. spretus-derived cells were even more sensitive than C57BL/6 cells to TNF/AktD induced cell death. Furthermore, SPRET/Ei and (BxS)F1 were not protected against TNF/GalN-induced lethal hepatitis, which strictly depends on TNFRI. Currently we are performing a genetic analysis of the tnfrsf1a gene. Twelve sequence variations were found in the cDNA between SPRET/Ei and C57BL/6. Six of these are unique for SPRET/Ei. In the 5’ promoter region, we found 22 variations in SPRET/Ei versus C57BL/6. These may be responsible for differential binding of transcription factors and hence different levels of receptor expression in SPRET/Ei.

Corresponding Author: Claude Libert, Prof. PhD, Ghent University, Biomedical Molecular Biology, Technologypark 927, 9052 Ghent, Belgium, Claude.Liber@UGent.be

A 310
Link between MMP-7 and IL-1β release in endotoxemia
Roosmarijn Vandenbroucke, Ineke Vanlaere, Eline Dejonckheere, Philippe Van Lint, Claude Libert

Objectives: The family of matrix metalloproteinases (MMPs) currently consists of 25 zinc-dependent enzymes that play a role in many physiological processes, such as embryo implantation, bone remodelling and organogenesis, but also in many pathological processes, such as wound healing and inflammation. Although originally thought to cleave exclusively extracellular matrix (ECM) components, these proteases also have a wide variety of non-ECM substrates, such as cytokines and chemokines. MMP-7 is the smallest member of the
family. Multiple studies with MMP-7 knockout mice reveal MMP-7 as a critical mediator in inflammatory processes, illustrated by their reduced neutrophil influx, impaired re epithelialization of injured lungs and the lack of active "z"-defensins, concomitant with an impaired ability to kill enteric pathogens.

Materials and methods: Endotoxemia was induced by intraperitoneal injection of wild type and MMP-7 deficient C57Bl/6 mice with 17.5 mg/kg body weight of Salmonella enterica LPS (Sigma), diluted in endotoxin-free PBS. Cytokine levels in the serum were determined via the LumineX system (Biorad) or ELISA (R&D systems). RNA was isolated from tissues with the RNeasy kit (Qiagen) and reverse transcribed by using iScript cDNA synthesis kit (Biorad). For quantitative PCR, the SYBR Green Master mix (Applied Biosystems) and the Lightcycler-480 real time PCR system (Roche) were used.

Results: Using a mouse model of lethal LPS/TLR4-induced endotoxemia, we observed that the broad spectrum MMP inhibitor BB-94 protects mice against LPS. Additionally, the use of MMP-7 deficient mice showed that MMP-7 is one of the detrimental MMPs that drives the exaggerated inflammatory reaction following systemic endotoxin injection. MMP-7 is upregulated in several organs after LPS challenge and lung damage is attenuated in MMP-7 deficient mice. The lower mortality and morbidity in the absence of MMP-7 is likely a consequence of a blunted cytokine response as after LPS, IL-1β, IL-6, and IFN-γ levels are markedly lower in MMP-7 deficient mice.

Analysis of peritoneal exudates cells points towards IL1 as a key mediator in this observed MMP 7 LPS resistance as MMP-7 seems to be involved in IL-1β release.

Conclusion: Increased IL-10 levels during sepsis interfere with the maturation process of circulating mDC and may promote their migration toward the inflammatory sites.

Corresponding Author: Tilmann Ditting, MD, University Hospital Essen, Department of Gastroenterology and Hepatology, Hufelandstr. 55, 45122 Essen, Germany, tilmann.ditting@uni-duesseldorf.de

A 312

Lipopolysaccharide (LPS) affects the electrophysiological response to acid stimulation in rat dorsal root ganglion (DRG) neurons with renal afferents and sensitizes TRPV1-mediated CGRP release

Tilmann Ditting, Wolfgang Freisinger, Kristina Rodionova, Karl F. Hilgers, Roland Veelken

Objective: Renal sympathetic nerve activity (RSNA) is increased in sepsis. This is not just due baroreceptor unloading, but rather induced by the inflammatory process itself. Neither the consequences—beneficial or deleterious—nor the mechanisms leading to increased RSNA in sepsis are as yet determined. We hypothesized that (1) LPS and/or other inflammatory mediators interfere with electrophysiological properties of peptidergic afferent renal neurons (PARN) that play a role in the control of RSNA and (2) sensitize TRPV1 mediated release of calcitonin gene related peptide (CGRP).

Methods: (1) PARN were cultivated with or without LPS (E. coli O127/B8, 20 mg/l medium) from DRGs (Th11-L2), harvested from male SD rats 7 days after fluorescent labelling with DiI to enable identification of those neurons with renal afferents. PARN underwent acute stimulation (pH5) while measuring whole-cell currents at −80 mV, blockers of ASIC (Amiloride) and TRPV1 (Capsazepine) were added. (2) Kidney slices—incubated with or without LPS—were stimulated by Capsaicin and CGRP content of the tissue bath supernatant was measured (ELISA).

Results: (1) Transient, ASIC-mediated currents were not significantly affected by LPS; Sustained currents where significantly increased (−1.72 ± 0.45 vs. −3.62 ± 0.41 pA/pF; p < 0.01) and became resistant to TRPV1-blockade by Capsazepine (−0.88 ± 0.48 vs. −3.39 ± 0.65 pA/pF; p < 0.01). IL1β, TNFα, or IL2 had no effect. (2) Stimulation of kidney slices in organ bath by capsaicin increased supernatant CGRP content (6.0 ± 1.0 vs. 14.4 ± 2.1 ng/ml; p < 0.05), this response was augmented by LPS pre-incubation (6.0 ± 0.7 vs. 58.9 ± 20.6 ng/ml; p < 0.05).

Conclusion: (1) It is shown for the first time that LPS affects some electrophysiological properties of PARN. This might serve as one possible explanation for RSNA increase in sepsis. (2) Furthermore, the finding that LPS incubation not only changed acid-induced currents but also sensitized TRPV1-mediated CGRP release from afferent nerve endings in renal tissue underlines the functional relevance of the findings. (3) Whether the observed LPS effects are non-specific or rather due to, e.g., toll-like receptor 4 (TLR4) mediated mechanism remains to be determined.

Corresponding Author: Tilmann Ditting, MD, Friedrich-Alexander-University Erlangen-Nuernberg, Med 4, Nephrology and Hypertension, Loschgestr. 8, 91054 Erlangen, Germany, Tilmann.Ditting@t-online.de

A 311

Increased levels of interleukin 10 in serum of patients with sepsis syndrome are associated with altered homing properties of blood myeloid dendritic cells

Vito Cicinnati, Min Gong, Fuat Saner, Speranta Iacob, Andreas Paul, Susanne Beckebaum

Objective: Elevated levels of the immunoregulatory cytokine interleukin (IL)-10 and a decreased frequency of dendritic cells (DC) in blood of patients with sepsis have been consistently demonstrated in the literature. The aim of this study was to investigate the role of IL-10 for DC homeostasis in patients with sepsis.

Patients and methods: Frequencies and phenotypes of circulating mDC were assessed by flow cytometry in 17 septic patients and 14 controls after major abdominal surgery. The T-helper cell (Th)1/Th2 cytokine serum profile was investigated by cytometric bead array and systemic inflammatory chemokine levels were measured with enzyme-linked immunosorbent assay. In addition, mDC from healthy volunteers were exposed to interleukin-10 in vitro and their phenotype and migratory properties were analyzed by flow cytometry and chemotaxis assay.

Results: Direct ex vivo flow cytometric analysis of peripheral blood from septic patients revealed a significant reduction of circulating mDC which exhibited an immature phenotype with intrinsic activation of receptors for inflammatory chemokines. Compared with controls, septic patients had increased serum levels of IL-10 and inflammatory chemokines. IL-10 exposure in vitro induced downregulation of costimulatory and adhesion molecules, and CC-chemokine receptor (CCR7) in mDC; whereas receptors for inflammatory CC-chemokines, CCR1, CCR2 and CCR5, were upregulated. IL-10-treated mDC showed an enhanced migration toward inflammatory chemokines, whereas their migratory response toward lymph node chemokines decreased.

Conclusion: Increased IL-10 levels during sepsis interfere with the maturation process of circulating mDC and may promote their migration toward the inflammatory sites.

Corresponding Author: Vito Cicinnati, MD, University Hospital Essen, Department of Gastroenterology and Hepatology, Hufelandstr. 55, 45122 Essen, Germany, vito.cicinnati@uni-due.de
Interactive superabsorbing bandage in complex treatment of purulent wounds in patients with disturbed blood flow due to artery deformity and collateral blood flow of neuroischemic form of diabetic food syndrome (DFS)

Valery Kutskir, Irina Podyablonskaya, Leonid Novikov

Objective: to study influence of superabsorbing bandage (TenderWet 24 Active, Paul Hartmann, Germany) on term of coming of phase II of wound process and on epithelization speed in patients with puronecrotic forms of DFS.

Patients and methods: There were 50 patients of main group and 55 patients of reference group. All patients had a neuroischemic form of DFS with disturbed blood flow due to artery deformity (stenosis is >60%) and collateral blood flow. Diabetes mellitus (DM), type II was found out in 84 (80%) and in 80 (80%) patients of corresponding groups. Age, gender, severity of DFS course, accompanying diseases were almost the same in both groups. Autoplasty was performed either by islet method according to Yanovitch–Chainsky–Davis or by loose-split skin graft using a dermatome. Granulating wounds were closed by a transplant, its thickness was 0.3–0.4 mm. Minimum size of skin defect was equal to 3 cm², maximum—about 300 cm². In main group “Hydrotul” bandage was applied to a transplant or to donor area, in reference group a sterile cotton mesh was applied to a transplant, and donor area was covered by an aseptic bandage. In postoperative period donor area was dried up by warm air stream at the distance of 30 cm within 15 min during the first day. The procedure was performed thrice, both in the morning and in the evening. The interval between manipulations was 45 min. Wound dressing and evaluation of transplant condition was done in 3 days. After that wound dressings were performed every 2 days. An aseptic bandage from donor area was not removed until epithelization was completed.

Results: In main group skin graft engrafting was 91.52 ± 17.64%, in reference group—81.36 ± 22.29% (p = 0.010). In main group complete epithelization of wound surface took place on average by 12 day, in reference group—by 16 day. In main group epithelization of donor area took place by 13.76 ± 1.76 day, in reference group—by 19.38 ± 2.9 day (p < 0.01).

Conclusion: It is possible to perform a successful autoplasty by a split skin graft in patients with puronecrotic forms of DFS. The best results are obtained when one uses a “Hydrotul” bandage as it has atraumatic properties, and due to it optimum humid environment is maintained, it promotes engraftment and donor area epithelization and does not interfere with the visual control of skin graft condition.

A 315

Bacterial colonization of tissues of chronically ischemic lower limb

Waldemar L. Olszewski, Piotr Andziak, Maria Moscicka-Wesolowska, Marek Durlik, Ewa Swoboda, Ewa Stelmach

Objectives: Reaction of arterial wall to bacterial infection as etiological factor in pathogenesis of atherosclerosis remains a contentious issue. The majority of available data on identification of bacterial antigen originates from studies of coronary arteries but not of lower limbs arteries.

Aim: To investigate the presence of bacterial cells and microbial DNA with use of broad-range PCR, targeting conserved region (16 sRNA), Chlamydia pneumoniae (CP) and Helicobacter pylori (HP) in fragments of femoral and popliteal arteries of patients undergoing reconstructive surgery or amputation.

Methods: Fragments of arteries were harvested and cultured, from the remaining fragment DNA was extracted. PCR amplification was performed with primers for gene fragment coding bacterial 16s RNA, major outer membrane protein (ompA) of CP and urease gene of HP DNA with positive and negative controls. Routine bacteriological cultures of specimens were carried out.

Results: Using routine microbiological methods popliteal and femoral arteries contained isolates in 51% of cases (Staph. epidermidis, Enterococcus, Pseudomonas), carotid arteries 4.1% and aorta 0.7%. Microbial DNA (16sRNA) was detected in 64% of examined femoral and popliteal specimens. CP could be demonstrated in 69% of positively-tested patients while HP was detected in 3.8%. In carotid arteries 29% of cases contained bacterial DNA, 29% CP and 0% HP specific genes. Thirty one aortic specimens contained bacterial DNA, 65% CP and 18% HP.

Conclusions: Bacterial isolates and DNA were found in lower limb arteries. Aorta and carotid arteries only sporadically contained
isolates. The microbes colonizing limb vascular bundles may be responsible for complications after arterial surgery as anastomosis dehiscence and wound suppuration.

Corresponding Author: Waldemar L. Olzewski, Prof. MD, PhD, Medical Research Center, Polish Academy of Sciences, Department of Surgical Research and Transplantology, 5 Pawinskiego Str., 02-106 Warsaw, Poland, wlo@cmdik.pan.pl

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Blue light therapy by LED improves wound healing by enhancing neoangiogenesis and tissue perfusion

Peter Dungel, Rainer Mittermayer, Martina Hofmann, Joachim Hartinger, Heinz Redl, Martijn van Griensven

Introduction: Increasing interest exists in low level light therapy to enhance wound healing. Most studies are performed with red or infrared irradiation. In our recent publications, however, we showed that blue light (430 nm) can have significant influence on biologic systems, like nitric oxide (NO) metabolism, and is able to release NO from complexes with hemoglobin and mitochondrial protein complexes. Here, we investigated the effects of blue light on in vivo wound healing in an epigastric flap model in the rat.

Methods: Skin flap elevation (1.5 × 3 cm) was performed cranially to blunt dissection technique. The entire flap was randomly attached only to the left or right inferior epigastric neurovascular bundle. The fasciomyocutaneous flap was then sutured back to its original anatomical orientation with non-resorbable sutures using interrupted technique and quilting sutures. The flap was illuminated post-OP and on five consecutive days for 10 min with light-emitting diodes (LED) at 430 nm and an intensity of 50 mW/cm². On day 7 we analysed size of necrotic area and flap perfusion as well as histologic and immunohistochemical parameters.

Results: Treatment with blue light led to significant improvement of wound healing. In the light treated group necrotic areas were smaller (35%), and flap shrinkage, a parameter for the quality of wound healing, was significantly less pronounced compared to controls. Immunohistochemical analyses revealed that light had a profound effect on neoangiogenesis, which was increased by 29% in the perimuscular and by 82% (p < 0.05) in the subepidermal layer of the skin. Consistently, tissue perfusion was two times higher (p < 0.05) in the light treated group as determined by Laser Doppler Imaging.

Conclusion: Our data suggest that illumination with blue light can enhance the wound healing process. By improving the growth of new blood vessels and tissue perfusion, treatment with light can help to diminish pathophysiological complications, like necrosis, in wound healing. Illumination would provide an easily applied and cost-effective treatment for surface wounds.

Corresponding Author: Peter Dungel, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingen Str. 15, 1200 Vienna, Austria, peter.dungel@trauma.lbg.ac.at

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Expression of basement membrane components laminin-5 and type IV collagen in epidermal wound in severe sepsis and septic shock in humans

Marjo Koskela, Fia Gaeddaaes, Vesa Koivukangas, Matti Kallioinen, Aarne Oikarinen, Tero I Ala-Kokko

Objectives: An intact basement membrane at the dermal-epidermal junction is essential to the viability of the skin. Laminin-5 links the epidermis to the dermis and takes part for the repair or regeneration of the basement membrane at the dermal-epidermal junction. Type IV collagen is a critical microenvironmental factor in the basement membrane that is needed to sustain keratinocyte growth and survival. The effect of sepsis on wound healing and basement membrane is partly unknown. The hypothesis of this study was that the expression of laminin-5 and type IV collagen are disturbed in epidermal wound in sepsis.

Material and methods: Suction blister method and skin biopsies were used to study basement membrane formation in sepsis. In suction blister method prolonged vacuum leads to disruption of dermo-epidermal junction and creates a standardized wound. It is an in vivo model to study re-epithelization in humans. 31 patients with severe sepsis included the final analysis. 15 healing suction blisters were collected from 3rd to 7th day of healing. 16 skin biopsies from healthy abdominal skin were taken on the first and 12 on the 8th day of study. 14 healthy age-matched volunteers were used as controls. Immunohistochemical stainings were made using antibodies to laminin-5 and type IV collagen.

Results: Average age in septic patients was 63 years and most of them were male (73%). Mean APACHEII score on admission was 25 and average SOFA score 7.8. Laminin-5 expression in the skin decreased compared to controls on from the first to the 8th days of study but at 3 months it turned to baseline. Same influence was seen in type IV collagen expression in intact skin. In healing wounds type IV collagen expression was more prevalent on the edge of the regenerating epidermis and in the intact skin near the wound in severe sepsis compared to controls. Laminin-5 expression was more intense with controls since the fourth day of healing. In severe sepsis, laminin-5 expression was delayed compared to controls.

Conclusions: In conclusion Laminin-5 and type IV collagen expression in intact skin in sepsis is delayed and returns to baseline by 3 months, whereas in epidermal wound laminin-5 expression is delayed compared to healthy controls.

Corresponding Author: Marjo Koskela, MD, Oulu University Hospital, Department of Surgery, Jousenkaari 20, 90420 Oulu, Finland, marjo.koskela@oulu.fi

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Evaluation of a next-generation sprayable adhesion barrier to prevent postoperative abdominal adhesions

Nicolo Tamini, Armando Nardone, Giorgio Bovo, Luca Fattori, Angelo Nespoli

Introduction: Postoperative adhesions are one of the commonest causes of small bowel obstruction, a frequent surgical emergency. Various agents have been tested in order to prevent abdominal adhesions, but the results point to suboptimal effectiveness. The aim of this study was to evaluate the efficacy of a new polyetilenglicole (PEG) derivate in preventing intra-abdominal adhesions in a murine model.

Materials and methods: Twenty Wistar albino rats were divided into control (Group A) and treated group (Group B). Free access to water and food was allowed. All rats underwent laparotomy: abrasion of the cecal wall and of the peritoneum of the abdominal wall were performed. No haemostasis was attempted. In Gr. B the PEG derivate bio-gel was spread on the injured areas, otherwise the control group did not receive any type of local treatment or therapy. The animals were sacrificed on the 21st postoperative day. A different observer graded the adhesion macroscopically and resected the cecum of each rat for histological examination. Specimens were processed for the evaluation of three different histological characteristics: fibrosis, inflammation and angiogenesis.
Results: In Gr.A the results of histological evaluation are comparable as we expected with the timing of follow up required for this study (21 days). The treated (Gr.B) group showed a significant reduction of peritoneal adhesion formation (P < 0.05) compared to control group. Histological analysis confirmed a reduction, in Gr.B, of fibrosis and inflammation but only the reduction of the growth of new blood vessels revealed a statistically relevance (P < 0.05).

Conclusions: The PEG derivate biogel is effective in the reduction of postoperative adhesions and in the new vessels formation. This may be a promising result for reducing the morbidity and costs related to post-operative adhesions.

Corresponding Author: Nicolo Tamini, MD, Osp San Gerardo, Department of Surgery, Via Pergolesi 33, 20052 Monza, Italy, n.tamini@libero.it

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Ceruloplasmin oxidase activity in plasma from burn and non-burn trauma patients
Michael Dubick, Myung Park, Johnny Barr, James Atkins

Objective: Ceruloplasmin oxidase (CPO) activity converts ferrous iron to its less reactive ferric form. This is important in iron metabolism and may be protective after injury. CP is an acute phase reactant protein whose concentration increases during inflammation but it is unknown if the stimulus for increased production is related to a decrease CPO activity. CPO activity can be decreased by protein nitration and we have shown that the electron paramagnetic resonance signal of plasma CP is decreased during hemorrhage in rats, indicating a decrease in CPO activity. It is unknown if such a decrease occurs in humans in trauma. The objective of this study was to characterize CPO activity in burn and non-burn trauma patients and to assess its relation to iron status in these patients.

Material and methods: Under a BAMC IRB-approved protocol, plasma was collected from 11 healthy control subjects and from 24 burn and 35 non-burn trauma patients on admission to the ICU and at day 1, 3, 5 and 7. CPO activity was measured spectrophotometrically by the oxidation of pararhyleminediame. Soluble transferrin receptor (sTfR) levels were determined in these samples as an index of iron status in these patients using a commercial ELISA kit.

Results: Mean age of controls was 37 years compared to 40 years in non-burn trauma patients and 49 years in burn patients. Mean TBSA burned was 37% in burn patients. There were no significant differences in ISS score, ICU stay or total hospital days between the 2 groups of trauma patients. Plasma CPO activity in burn and trauma patients were about 37% lower than controls on admission. By day 1 in burn patients, CPO activity increased to nearly threefold higher than controls and remained elevated for the 7-day study period. In trauma patients, CPO activity rose slowly where activity was nearly twofold higher than controls on day 5 and 7. No significant differences in sTfR were noted among groups on admission, but levels in burn patients were lower than controls for the first 5 day after injury.

Conclusion: To our knowledge this is the first study to show an early decrease in CPO activity in burn and non-burn trauma patients. sTfR levels did not indicate a limitation of iron uptake by cells but the consequences on iron-induced oxidative stress is unknown. Further efforts should focus on determining whether CPO activity influences the effects of exogenous iron that may be administered inadvertently with transfusions during trauma resuscitation.

Corresponding Author: Michael Dubick, PhD, US Army Institute of Surgical Research, DCR, 3400 Rawley E Chambers Ave, San Antonio, TX 78234, USA, michael.dubick@amedd.army.mil

A 320
Vaccination and antibiotic management of post-splenectomy patients in The Netherlands
A. J. Jolanda Lammers, Daphne Veninga, Kiki Lombarts, Joost Hoekstra, Peter Speelman

Objective: After splenectomy, patients are at increased risk of sepsis with considerable mortality. The risk of sepsis can be reduced by immunizing these patients with pneumococcal, H. Influenzae B and meningococcal vaccines, as well as by prescribing antibiotic prophylaxis. The purpose of our study was to determine compliance with the international standards for the management of splenectomised patients in the Netherlands, by investigating (i) vaccination rates, (ii) prescription of antibiotics and (iii) information in discharge letters.

Patients and Methods: A retrospective review of medical records and discharge correspondence of 609 splenectomy patients from 1997 to 2008 was performed. Data were collected from 28 hospitals (i.e. 30% of all Dutch hospitals). Adherence to guidelines regarding vaccination and prescription of antibiotics was assessed by collecting the following data: patient demographics, indication for splenectomy, documentation of vaccine administration, timing of vaccination in relation to splenectomy and documentation of post-splenectomy infections. Discharge correspondence was checked for mentioning of relevant information to the general practitioner.

Results: 85.4% of post-splenectomy patients received pneumococcal vaccination, 39.4% received H. Influenzae type B and 32.3% received meningococcal group C vaccination. 34.5% of all performed splenectomies were indicated by iatrogenic lesions to the spleen. 12.4% of patients were discharged on prophylactic antibiotics. In less than 25% of cases adequate recommendations regarding post-splenectomy management were given to the general practitioner.

Conclusion: In the Netherlands compliance with recommendations for management of patients after splenectomy is insufficient. Fifteen percent does not receive vaccination against pneumococci and the majority of patients does not receive antibiotic prophylaxis. The development and implementation of a national guideline for vaccination strategies as well as recommendations for prescribing antibiotics in splenectomised patients is urgently required.

Corresponding Author: A. J. Jolanda Lammers, MD, Academic Medical Center, CEMM, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, a.j.lammers@amc.uva.nl

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Status on drug use patterns of arthritis in Bangladesh: a survey on patients in Shaheed Ziaur Rahman Medical College and Hospital of Bogra District

Arthritis is the most common disease in Bangladesh. The present status of arthritis is a very important factor from the global and national perspective. The aim of this study was to determine the prevalence of arthritis in consideration of age, sex, socio-economical status, and also determine the present therapeutic management in several forms of arthritis and related complications having by the patients. We accordingly conducted a survey in Shaheed Ziaur Rahman Medical College and Hospital of Bogra district, which is one of the largest medical college and hospital in Bangladesh. Interviews were conducted of the patients with the help of a semi-structured questionnaire. It was observed that 49% male and 51% female were affected by the arthritis. So female were more affected than male. In
During ICU stay, 85.3% of the patients required invasive ventilation, admission, and became constant thereafter. Correspondingly, we evaluated the impact of prolonged invasive organ support therapy during the acute phase. Hazard function was explored for invasive ventilation or renal replacement therapy was associated with poorer acute outcome. Duration of invasive ventilation (with a lag of 7 days) was also an important determinant of acute survival (linear term, hazard ratio 1.015 (per additional day), 95% confidence interval 1.019–1.030, p < 0.001). In contrast, duration of renal replacement therapy was unimportant for acute prognosis.

Conclusion: Duration of invasive ventilation is inversely related to acute survival, and is the only type of invasive ICU therapy, of which the duration affects life expectancy of critically ill surgical patients during the acute phase.

Methods: Retrospective analysis of prospectively collected data of an ICU patient cohort linked to a local database. Adult patients (n = 1462) admitted to a 12-bed ICU at a university hospital in Munich, Germany, between 1993 and 2005 who had an ICU length of stay of more than four days and who were followed-up until the end of the acute phase after ICU admission. Hazard function was explored by Weibull modelling and likelihood ratio tests. Cox-type structured hazard regression models were used to analyze linear, non-linear or time-varying associations of therapeutic variables with survival time during the period of hazard. Duration of different invasive therapies was evaluated by constructing specific vectors, which tested potential effects on outcome after lag-time of 7 days.

Results: Hazard rate declined exponentially up to day 195 after ICU admission, and became constant thereafter. Correspondingly, we defined the acute phase to last from day 0 to 195 after ICU admission. During ICU stay, 85.3% of the patients required invasive ventilation, and 16.1% a continuous renal replacement therapy. Beside the underlying disease and disease severity at ICU admission, the need for invasive ventilation or renal replacement therapy was associated with poorer acute outcome.

Corresponding Author: Christian P. Schneider, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Surgery, Marchioninistr. 15, 81377 Munich, Germany, christian.schneider@med.uni-muenchen.de
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Immunological and clinical biochemical factors for survival prediction of patients with sepsis syndrome

Vito Cicinnati, Fuat Saner, Min Gong, Speranta Iacob, Andreas Pail, Susanne Beckebaum

Objective: The aim of this pilot study was to investigate various immunological and clinical biochemical parameters associated with prognosis of patients with sepsis syndrome.

Patients and methods: Demographic, clinical and laboratory data were collected from 17 septic patients and 14 controls after major abdominal surgery. Frequencies of circulating dendritic cells (DC) and monocytes, phenotypes of myeloid DC (mDC) and monocytes, and the T-helper cell (Th1)/Th2 cytokine serum profile were investigated by flow cytometric assays. Systemic inflammatory chemokine levels were measured with enzymelinked immunosorbent assay. Univariate and multivariate statistical analyses were performed to identify immunological and clinical biochemical factors associated with patient outcome and a model for survival prediction was created.

Results: Reduced expression of CD86 in mDC (P = 0.04) and high Sequential Organ Failure Assessment (SOFA) score (P = 0.03) were two independent factors associated with sepsis. Reduced HLA-DR expression in mDC and high International Normalized Ratio (INR) were two independent predictors of mortality due to sepsis. Interleukin (IL)-10 serum levels were significantly higher in septic patients as compared to controls and were associated with poor prognosis. We created a model for the risk assessment of mortality due to sepsis within 14 days based on mDC HLA-DR expression, IL-10 serum level and INR with an area under the receiver operating characteristic of 0.97, a sensitivity of 100% and a specificity of 91.3%.

Conclusion: Our model for survival prediction based on immunological and biochemical parameters may improve clinical management of patients with sepsis syndrome.

Corresponding Author: Vito Cicinnati, MD, University Hospital Essen, Department of Gastroenterology and Hepatology, Hufelandstr. 55, 45122 Essen, Germany, vito.cicinnati@uni-duesseldorf.de

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Evaluation of reactive oxygen species and nitric oxide in peripheral blood monocytes and neutrophils from septic patients admitted at admission and after 7 days of follow up

Reinaldo Salomao1, Sidniea sousa1, Milena Brunialt1, Flavía Machado1, Otelo Rigato1,3
1Infectious Diseases Discipline, Universidade Federal de São Paulo, 2Discipline of Anesthesiology, Sao Paulo Hospital, Universidade Federal de Sao Paulo, 3Intensive Care Unit, Sirio Libanes Hospital

Objective: To evaluate the production of reactive oxygen species (ROS) and nitric oxide (NO) in peripheral blood monocytes and neutrophils from septic patients admitted to intensive care units.

Patients and methods: Forty nine patients were included, and in 30 of them blood samples were collected at day one (D0) and after 7 days (D7) of therapy. Nineteen healthy volunteers were included. The ROS and NO production were measured in peripheral blood by flow cytometry with DCFH-DA and DAF-FM-DA reagents, respectively. The basal condition and after Staphylococcus aureus and Pseudomonas aeruginosa stimuli were observed.

Results: ROS production in monocytes and neutrophils were increased in septic patient compared to healthy volunteers in all conditions analyzed (P < 0.001). Similar results were obtained with NO production in monocytes (P < 0.001). However, NO detection in neutrophils were increased only in the basal condition in sepsis group (P < 0.001). Increased monocyte’s ROS production was observed in D0 compared to D7 in all conditions (basal P = 0.001, P. aeruginosa P = 0.001 and S. aureus P = 0.002). In neutrophils increased ROS production were observed only after stimulation with S. aureus (P = 0.045). NO production by monocytes was higher in the sample from D0 compared to D7 in basal condition (P = 0.005) and after P. aeruginosa stimulus (P = 0.029), while in neutrophils it was higher in all conditions in D0 (basal P = 0.006, P. aeruginosa P = 0.022 and S. aureus P = 0.052).

Conclusion: Septic patients presented an increased ROS and NO production by monocytes and neutrophils, supporting a strong state of innate immune cells activation during sepsis. The lower production observed after 1 week of treatment can reflect the cellular adaptation during sepsis to control the toxicity of these mediators. The analysis of this dynamic response between survivors and non-survivors can contribute to clarify this phenomenon.

Financial support: Fapesp grant 06/58744-1

Corresponding Author: Reinaldo Salomao, Prof. PhD, Universidade Federal de São Paulo, Department of Medicine/Infectious Disease, Rua Pedro de Toledo, 781 15th floor, 4039032 São Paulo, Brasil, rsaolomao@unifesp.br

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Serum protein expression patterns in multiple injured patients are associated with extent of multiple organ failure and outcome

Christian Suren, Viktoria Bogner, Daniel Teupser, Sven Baumann, Wolf Matschler, Peter Biberthaler

Objectives: The development of posttrauma-systemic inflammatory response syndrome (SIRS) and subsequent multiple organ failure (MOF) is influenced by various components of the cellular and humoral immune systems. Screening analyses of serum protein expression might provide new insights into potential targets and mechanisms for the development of posttraumatic SIRS and MOF. The aim of this study was to discover serum protein patterns in correlation to the patients’ clinical course and definite outcome.

Material and methods: 36 multiple injured patients exhibiting an injury severity score (ISS) of >16 points were included. Blood samples were taken on admittance and after 6, 12, 24, 48, and 72 h. Serum was isolated by centrifugation. The serum protein mass spectra were measured using the Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The respective mass spectra were correlated to the extent of multiple organ failure (MOF score by Goris et al., cut-off 4 points) and the patients’ definitive clinical outcome (90-day survival).

Results: Using pseudo-prospective classification algorithms, we were able to allocate the patients’ clinical outcome with 51% sensitivity and 95.7% specificity. Pseudo-prospective classification of the extent of multiple organ failure using the protein expression patterns revealed a 91.7% sensitivity and 47.3% specificity.

Conclusion: For the first time, we have used the approach of MALDI-TOF MS for the identification and clinical correlation of specific protein mass spectra in multiple, major injured patients in the initial posttraumatic period. Some of these patterns show a significant correlation to multiple organ failure and clinical outcome. Further studies to identify and select the underlying proteins of the respective mass spectra are currently underway.

Corresponding Author: Christian Suren, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Department of Surgery, Nussbaumstr. 20, 80336 Munich, Germany, christian.suren@med.uni-muenchen.de
Circulating angiopoietin-2 correlates with distant organ injury and outcome in patients with primary acute liver failure

Alexander Lukasz, Johannes Hadem, Jan T. Kielstein, Sven Pischke, Michael P. Manns, Philipp Kuempers

Introduction: Endothelial activation leading to systemic vascular barrier breakdown play a key role in the pathogenesis of distant organ injury after acute liver failure (ALF). Angiopoietin-2 (Ang-2), a circulating antagonistic ligand of the endothelial specific Tie2 receptor, is rapidly released from Weibel-Palade bodies and has been identified as a non-redundant gatekeeper of endothelial activation. Here, we examine whether the release of circulating Ang-2 correlates with the extent of distant organ injury and outcome in patients with ALF.

Patients and methods: Ang-2 levels on admission were measured by immunoradiometric assay (IRMA) in sera from 20 healthy controls and 30 patients with ALF (median age 35 years, 50% had encephalopathy = grade 3. ALF etiologies were cryptogenic (30%), acute hepatitis B (20%), acetaminophen-induced hepatotoxicity (20%), and other causes (30%), treated between 2001 and 2009, respectively. Transplant-free recovery (death or liver transplantation) after 28 days was the primary outcome studied.

Results: Thirty-three percent of patients survived without transplantation, 10% died without transplantation, and 57% received a transplant (overall 28-day survival 90%). Median [IQR] Ang-2 serum concentrations were increasingly higher across the following groups: healthy controls (1.5 [1.4–2.2] ng/mL), patients with spontaneous recovery (3.0 [3.0–21.0] ng/mL), and patients that reached the composite end-point of death or transplantation (17.7 [8.4–40.2] ng/mL). Ang-2 release correlated strongly with lactate levels as a surrogate marker of liver damage (r = 0.66, P < 0.001). Moreover, Ang-2 levels were closely associated with the extent of distant organ injury as evidenced by a close correlation with pulse rate/mean arterial pressure index (r = 0.57, P = 0.0012), fraction of inspired oxygen (r = 0.5, P = 0.006), acute kidney injury (r = 0.58, P < 0.001), and Sequential Organ Failure Assessment (SOFA) score (r = 0.49, P = 0.009). Kaplan–Meier analysis demonstrated that a ROC curve-generated Ang-2 cutoff value of 7.2 ng/mL (AUC of 0.76 [95% CI: 0.57–0.95]) predicted transplant-free recovery during 28-day follow-up (Log-rank test: p < 0.05).

Conclusions: A marked imbalance of the Ang-Tie system in favour of Ang-2 may be used as readily available powerful predictor of outcome and may open new perspectives to individualize treatment in the ICU.

Corresponding Author: Nikolaos Memos, MD, University of Athens, Department of Propaedeutic Surgery, 114 Vas Sofias Ave, Hippokratio Hospital, 11527 Athens, Greece, nicosdoc@yahoo.gr

Prognostic value analysis of time dependent variables when longitudinal sampling is not possible: example from an experimental model of sepsis

Nikolaos Memos, Urania Dafni, Apostolos Bournetas, George Antonoglouros, Evangelos Messaris, Manousos Konstadoulakis Laboratory of Surgical Research, A Department of Propaedeutic Surgery, University of Athens. Department of Biostatistics, Faculty of Nursing, University of AthensSection of Statistics & Operations Research, Department of Mathematics, University of Athens

Objective: The aim of the study is to find a statistical methodology for analyzing prognostic factors that are time-dependent in cases that longitudinal sampling cannot be conducted.

Materials and methods: The dataset used came from an experimental model of sepsis (N = 105). The exact time of death was recorded from 21 animals died from the disease while the rest were sacrificed at various time points close to recorded death times. We then matched cases with euthanized animals using time of death as the matching variable. Blood samples were sampled from each animal at the time of the event.

Statistical analysis: Data is given as mean ± SD or the median with interquartile range. Correlation of variables with time was tested using Spearman’s correlation coefficient. Univariate analysis was performed using non-parametric methods. Conditional logistic regression was used for prognostic significance of time dependent covariates. Sensitivity analysis on the conditional logistic regression has been performed with Cox PH and Cox time dependent modeling using multiple imputations.

Results: Twenty one animals died from the disease and 84 were euthanized at various time points. Lymphocyte apoptosis has been strongly linearly correlated to time (Spearman rho =–0.4315, p < 0.001 for survivors and Spearman’s rho =–0.4735, p = 0.03 for non survivors). Lymphocyte and neutrophil apoptosis has been increased in non survivors related to survivors (p = 0.01 and p = 0.002 respectively). Individual matching analysis showed that neutrophil apoptosis has been associated with increased odds of death (OR 2.086 95% CI: 1.24–3.5) while lymphocyte apoptosis has been associated with increased odds of death in a statistical indicative manner (OR 2.35 95% CI: 0.8–6.5). Sensitivity analysis revealed that the estimates of matched case control analysis and Cox proportional hazards analysis are close if the variables are time independent. This is not the case when the covariates are time dependent where the estimates deviate. Multiple imputation data analysis showed individually matched case-control analysis is more sensitive than Cox modelling when the proportional hazards assumption is violated.

Conclusion: We propose the matched case control analysis for analysing prognostic significance of time dependent variables when the longitudinal sampling is impossible.

Corresponding Author: Nikolaos Memos, MD, University of Athens, Department of Propaedeutic Surgery, 114 Vas Sofias Ave, Hippokratio Hospital, 11527 Athens, Greece, nicosdoc@yahoo.gr
inhibitors were already found up or down regulated at the onset of shock and remained stable during the first 48 h. Conclusion: Endotoxin tolerance process, leading to immunoparalysis, is already present when septic shock is diagnosed. This result could contribute to improve bedside care management. Thus investigation of genes involved in ET mechanism in patient very early at the onset of shock could allow to find markers required for individualized treatment establishment.

Corresponding Author: Marie-Angelique Cazalis, BioMerieux, Joint Unit Hospices Civils de Lyon, HEE, pl Arsonval, pav P 5eme, 69347 Lyon, France, marie-angelique.cazalis@eu.biomerieux.com

A 330
Relevance of transforming growth factor-β1, interleukin-8, and tumor necrosis factor-α polymorphisms in patients with chronic pancreatitis

Gyula Farkas, Gyula Farkas, Peter Hofner, Yvette Mandi, Gyula Farkas Jr

Objectives: Cytokine regulation may be important as concerns the susceptibility to the development of chronic pancreatitis; transforming growth factor-β1 (TGF-β1) plays a central role in the pathogenesis of pancreatic fibrogenesis. The aim of our study was to analyse the relevance of TGF-β1, interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) polymorphisms in patients with chronic pancreatitis.

Patients and methods: Of the 83 patients enrolled in the study, 43 were treated medically and 40 patients underwent surgical intervention. Healthy blood donors (n = 75) served as controls. The polymorphisms of TGF-β1 +869 T → C and IL-8 -251 T → A were determined by the ARMS method, while that of TNF-α -308 was investigated by NcoI RFLP. Results: There was a higher frequency (50%) of the TT genotype of TGF-β1 +869 with a concomitant higher TGF-β1 level in the plasma (5.2 ± 1.7 ng/ml) in patients with chronic pancreatitis than in healthy blood donors (28%, and 2.8 ± 0.9 ng/ml respectively). The number of TT homozygotes differed significantly between the patients who underwent surgical intervention and the controls, and even between the operated and the non operated patients. The frequency of the T/A genotype with higher IL-8 production was significantly higher in both groups of patients than in the controls (58 and 58% vs. 40%). No correlation was found between the TNF-α -308 polymorphism and chronic pancreatitis.

Conclusions: The correlations of the TGF-β1 and IL-8 single nucleotide polymorphisms (SNPs) with chronic pancreatitis underline the importance of these cytokines in the pathomechanism of the disease. Moreover, it seems that the TT genotype of +869 TGF-β1 might be a risk factor for the development of a severe form of chronic pancreatitis, as a prognostic sign for a future surgical intervention or even reoperation.

Corresponding Author: Gyula Farkas, Prof. MD, PhD, University of Szeged, Faculty of Medicine, Department of Surgery, PO Box 427, 6701 Szeged, Hungary, dr.fgy@freemail.hu

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Quercetin administration reduces the pro-inflammatory cytokines expression in cirrhotic rats

Jesus Manuel Culebras*, Maria Jose Cuevas, Juliana Tieppo, Javier Gonzalez-Gallego, Norma Marroni, Maria Jesus Tudón

*JM Culebras. Institute of Biomedicine and Surgical Unit. CIBERehd and Hospital of León. SACYL. Altos de Nava 24071 León, Spain

Abstract: Hepatic inflammation is an important pathophysiological process that precedes the development of liver cirrhosis. Flavonoids are low-molecular weight compounds found in citrus fruits, olive oil, tea, red wine, seeds, and several medicinal plants. Anti-inflammatory properties of these compounds have been broadly studied. Quercetin is the major flavonoid found in human diet. A number of beneficial effects of quercetin on human health have been also shown and some studies have indicated an important role for quercetin in several diseases, including liver cirrhosis. This study aims to elucidate the effects of quercetin on the gene expression of pro-inflammatory factors, including transforming growth factor-beta (TGF-β), tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), in an animal model of liver cirrhosis.

Patients and methods: Rats were divided into four groups: animals submitted to common bile duct ligation (CBDL), sham (animals submitted to simulate CBDL), quercetin-treated sham, and quercetin-treated CBDL. Quercetin (50 mg/kg) was administered for 2 weeks starting on day 14 after surgery. On the 28th day, blood was collected for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP). Analysis of TGF-β1, TNF-α and IL-6 mRNA expression was performed in liver tissue by quantitative real time RT-PCR.

Results: Blood AST, ALT and ALP activities and mRNA levels of pro-inflammatory cytokines were significantly higher in untreated CBDL rats compared to sham rats. The flavonoid quercetin significantly lowered serum ALT, AST and ALP activities (indicators of hepatocellular damage) and consistently caused a reduction of mRNA levels of TGF-β1, TNF-α and IL-6, in treated cirrhotic animals compared with untreated CBDL rats.

Conclusion: Quercetin treatment, initiated following the establishment of liver injury in cirrhotic animals, has a clear anti-inflammatory effect in the subsequent severity of the CBDL-related inflammation. This result supports that trials of quercetin may be reasonable in patients with established liver disease and inflammation.

Corresponding Author: Maria Jose Cuevas, PhD, University of Leon, Institute of Biomedicine, Campus de Vegazana, 24071 Leon, Spain, mjcuev@unileon.es

A 332
Intravenous administration of freeze-dried sulphonated immunoglobulin increases serum levels of IGF-I and improves organ failures in patients with severe sepsis

Yoshizumi Deguchi, Takao Nakagawa, Hiroyasu Suga, Takayuki Sato, Naoko Harada, Kenji Okajima

Introduction: Insulin-like growth factor-1 (IGF-1) has been known to possess anti-inflammatory activity in addition to enhance the host defense capacity. Since Freeze-Dried Sulfonated Human Normal Immunoglobulin (venilon-I ©) increases IGF-I production in animal sepsis model, it is possible that venilon-I reduces organ failures in patients with severe sepsis. We examined this possibility in the present study.

Object and method: To examine if venilon-I enhances the production of IGF-I and improves the prognosis of sepsis in the clinical setting, 33 cases with high level of soluble E-selectin were surveyed among septic patients transported to our hospital from April 2008 to November 2009. They were divided into three groups, Sulfonated Immunoglobulin group (Venilon-I ® 5 g/day, 3 days: S-group), Polyethylene Glycol Treated Immunoglobulin group (Veno-globulin-IH ® and globenin-I © 5 g/day, 3 days: P-group) and non-administered group (C-group). We measured the level of SES and IGF-I on arrival and every other day (0,1,3,5,7 day). Systemic organ dysfunction in patients was evaluated using the SOFA score except GCS on each day. Furthermore, we also examine the prognosis on 28th day. T-test was utilized statistically.

Corresponding Author: Takao Nakagawa, Takayuki Sato, Naoko Harada, Kenji Okajima

Corresponding Author: Takao Nakagawa, University of Tokushima, Tokushima, Japan, nakagawa@medicine.tokushima-u.ac.jp
A 333
Glutamine administration reduces fibrosis in an animal model of inflammatory Bowel disease

Jesús Manuel Culebras*, Beatriz San Miguel, Irene Crespo, Norma Marroni, Javier González-Gallego, María Jesús Túñon

*JM Culebras, Institute of Biomedicine and Surgical Unit. CIBERehd and Hospital of León, SACYL, Altos de Nava 24071 León, Spain

Objective: Ulcerative colitis (UC) and Crohn’s disease (CD) are the two major forms of inflammatory bowel disease (IBD) and are characterized by non-specific inflammation and intestinal tissue damage. The etiology of both diseases is still unclear. In inflamed intestine, healing of the damaged wall requires reconstruction of the tissue framework and remodeling of extracellular matrix (ECM) components. However, deposition of excessive collagen, which is one of the major components of the ECM, may result in fibrosis. Glutamine is the most abundant amino acid in the bloodstream; it helps to protect the lining of the gastrointestinal tract known as the mucosa. Glutamine deficiency may play a role in the development of IBD. Transforming growth factor-beta (TGF-beta) has a key role in the induction of fibrosis. Following activation of the TGF-beta receptors, intracellular signal transduction is mediated by a variety of Smad protein. Smad-3 acts as an activator of signal transduction, whereas Smad-7 has an inhibitor effect. Furthermore, TGF-beta provides a strong stimulus for the synthesis for connective tissue growth factor (CTGF), a potent stimulator of the production of collagen. The aim of this study was to assess the ability of glutamine to reduce the severity of fibrosis in animal model of colitis and to analyze its effects on different fibrogenic factors.

Material and methods: Colitis was induced in male Wistar rats by intracolonic administration of 30 mg of 2,4,6-trinitrobenzene sulfonic acid (TNBS). Glutamine (25 mg/dl) was given by rectal route daily for 7 days in a volume of 3 ml. On the eighth day the rats were killed and the distal 8 cm of the colon was collected. Analysis of TNF-alfa, TGF-beta, CTGF, Smad-3 and Smad-7 expression was performed in gut tissue by quantitative real time RT-PCR.

Results: Glutamine significantly reduced gross damage and histopathological scores of fibrosis in the colon of animals receiving TNBS. TNBS administration induced a marked increase of TNF-alfa, TGF-beta, Smad 3 and CTGF and an inhibition of Smad-7 mRNA. All these effects were significantly inhibited by administration of glutamine.

Conclusion: The results of our experiments indicate that glutamine, by down-regulating increased expression of several genes and modifying intracellular signaling pathways that contribute to the accumulation of matrix proteins, may be interesting candidate for the treatment of fibrosis in IBD.

Corresponding Author: María Jesús Túñon, PhD, University of León, Institute of Biomedicine, Campus de Vegazana s/n, 24071 León, Spain, mjtuang@unileon.es

A 334
Th1/Th2-Type shift after abdominal trauma in plasma of multiple injured patients

Oleg Heizmann, Manfred Köhler, Gert Muhr, Daniel Oertli, Christian Schinkel

Objective: Abdominal injuries have a severe impact on early morbidity and mortality after major trauma. We have previously shown that adverse outcome after major trauma was not associated with a shift towards Th2-type cytokines in plasma rather with a general suppression of all mediators.

Patients and methods: To investigate this within the most critically ill subgroup of patients with abdominal trauma we studied 73 patients (age 36 (14–80) years; ISS 34 (17–66) pts) with documented abdominal injuries and compared them with a group of polytraumatized patients without abdominal injuries (n = 153; age 41 (11–81); ISS 26 (16–66)) as well as with healthy controls (n = 112). Plasma levels were determined for Interleukin-4 (IL-4), IL-6, IL-10, IL-11, IL-12 (p4070), and IL-18 by ELISA technique.

Results: All patients showed significant higher plasma levels than controls except IL-4 that remained on control level. Trauma without abdominal injuries compared to the abdominal trauma group showed significant higher plasma values except IL-4 and IL-10 which might be explained by a higher ISS in the abdominal trauma group. When matching the results for ISS a shift towards a Th2-type cytokine response was shown in non-survivors during the first week after trauma (Table). Highest cytokine levels were seen in patients with severe liver injuries.

Table

<table>
<thead>
<tr>
<th></th>
<th>IL-4</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-11</th>
<th>IL-12(p70)</th>
<th>IL-18</th>
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<tbody>
<tr>
<td>Survivors</td>
<td>5 (0–1,125)</td>
<td>68 (3–665)</td>
<td>32 (0–3,000)</td>
<td>45 (0–5,000)</td>
<td>7 (0–1,000)</td>
<td>90 (0–3,310)</td>
</tr>
<tr>
<td>Non-survivors</td>
<td>8* (0–115)</td>
<td>32* (12–1,890)</td>
<td>46* (0–255)</td>
<td>66*** (0–1,510)</td>
<td>5* (0–415)</td>
<td>71** (0–955)</td>
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*p<0.05; ** p<0.01, *** p<0.005
Conclusion: A significant shift towards Th2-type response for plasma cytokines was shown for the subgroup of non-survivors after major and abdominal trauma. Further in vitro studies are mandatory to prove our results on a cellular level.

Corresponding Author: Oleg Heizmann, MD, University Hospital Basel, Department of Surgery, Spitalstr. 21, 4108 Basel, Switzerland. oheizmann@uhbs.ch

A 335
Interferon-regulatory-factor-4 (IRF4) prevents postischemic acute renal failure by suppressing cytokine and chemokine release in intrarenal immune cells
Saraswati Lassen, Maciej Lech, Onkar Kulkarni, Hans-Joachim Anders

Background: Acute renal failure (ARF) is still a serious medical problem that causes substantial morbidity and mortality. Ischemia/reperfusion (IR) activates innate immunity, leads to sterile inflammation and causes kidney injury. Negative regulatory mechanisms are necessary to suppress uncontrolled immune reactions. IRF4 suppresses the production of proinflammatory cytokines and chemokines by monocytes/macrophages after LPS stimulation. We therefore hypothesized that IRF4 protects from postischemic ARF and that lack of IRF4 predisposes to postischemic ARF.

Methods: Bilateral (30 min) or unilateral (45 min) renal pedical clamping was carried out for C57BL/6 (WT) and IRF4-knockout (IRF4−/−) mice to induce ARF. Inflammation parameters like cytokines/chemokines, neutrophil counts and tubular injury was measured 1, 5 and 10 days (d) after clamping. Macrophages were depleted in mice by injecting clodronate and etanercept was injected to block TNF effects. Macrophages were stimulated with reactive oxygen species (ROS) to check IRF4 induction in various time points.

Results: IRF4 expression was significantly increased in macrophages after ROS stimulation at 24 h. Similar to that, higher IRF4 mRNA levels were observed in the kidney and these levels were raised constantly until 10 days after clamping. TNFα levels were observed in the kidney and these levels were raised after ROS stimulation at 24 h. Similar to that, higher IRF4 mRNA levels were measured 1, 5 and 10 days (d) after clamping. Macrophages were depleted in mice by injecting clodronate and etanercept was injected to block TNF effects. Macrophages were stimulated with reactive oxygen species (ROS) to check IRF4 induction in various time points.

Conclusions: IRF4 expression was significantly increased in macrophages after ROS stimulation at 24 h. Similar to that, higher IRF4 mRNA levels were observed in the kidney and these levels were raised constantly until 10 days after clamping. TNFα levels were observed in the kidney and these levels were raised after ROS stimulation at 24 h. Similar to that, higher IRF4 mRNA levels were measured 1, 5 and 10 days (d) after clamping. Macrophages were depleted in mice by injecting clodronate and etanercept was injected to block TNF effects. Macrophages were stimulated with reactive oxygen species (ROS) to check IRF4 induction in various time points.

A 336
Immunodulatory effect of rFVIIa on the LPS-induced cytokine production of human monocytes
Lena Schreyer, Heiko Trentzsch, Siegfried Zedler, Peter Camai, Christian Sommerhoff, Eugen Faist

Objective: Exogenous coagulation is initiated by interaction between tissue factor (TF) and activated factor VII (FVIIa) generating major amounts of thrombin. Moreover, inflammation triggers up regulation of TF on cells such as monocytes and endothelial cells, thus linking inflammation and coagulation. Following treatment with recombinant FVIIa (rFVIIa) used as rescue therapy in patients with traumatic haemorrhage, reduced rates of organ failure and ARDS have been observed. We hypothesized that immunomodulatory effects of rFVIIa might be responsible for this effect. Therefore, we have investigated the effects of rFVIIa on the LPS-induced cytokine release in a cell-culture model.

Methods: Human monocytic MonoMac6 cells (MM6) or CD14+ PBMC were stimulated with 200–1,000 ng/ml LPS with or without pre-incubation with 1–20 μg/ml rFVIIa. The cytokines were specified intracellularly by flow cytometry and in the supernatant by a Luminox 100 System. We assessed levels of IL-1β, IL-8, IL-10 and TNFα in MM6 and IL-1β, IL-6, IL-8 and TNFα in human CD14+ cells. To scrutinize the involvement of TF, we also assessed MM6 with increased TF-expression following TNF-stimulation or transfection.

Results: rFVIIa had a significant decreasing effect on the LPS-induced TNFα and IL-8 production of naive MM6. But cytokine release of human CD14+ cells or TNFα-stimulated MM6 was not affected by rFVIIa. TF-transfected MM6, however, liberated more TNFα when stimulated with LPS + rFVIIa instead of LPS only. Regarding the impaired cytokine-response of the transfectants compared to the reaction of wild type cells, this result has to be considered with caution. When MM6 were incubated with thrombin before LPS-challenge, we observed increased IL-10 levels but unchanged TNFα.

Conclusion: Our results suggest a reduced proinflammatory response to LPS in MM6 treated with rFVIIa. This effect was not consistent with findings in human CD14+ cells. The observed effect could not be enhanced by increasing TF on the cell-surface. However, data indicate changes in the response of transfected MM6. Based on our findings, we assume a more complex role of rFVIIa in the crosstalk between inflammation and coagulation. The TF-transfection of another monocytic cell-line might lead to a better understanding of the thrombin-unrelated effects of rFVIIa on the inflammatory response and the influence of TF-expression on this effect.

Corresponding Author: Lena Schreyer, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Surgery, Marchioninistr. 15, 81377 Munich, Germany, lena.schreyer@med.uni-muenchen.de

A 337
Molecular pathogenesis of sepsis in newborns
Johannes Roth

Immaturity of innate and adaptive immune responses in the perinatal period has been suggested to predispose the neonate to increased infectious morbidity and mortality from a variety of organisms. However, recent data provide evidence that in contrast to adults, survival from polymicrobial sepsis in neonates does not depend on an intact adaptive immune system and is not improved by T cell-directed adaptive immunotherapy. Dysregulation of various immunoregulatory genes and cytokines have been demonstrated in neonatal mononuclear cells which may in part contribute to the immaturity of neonatal cell-
mediated immunity. Despite recent identification of specific pattern recognition receptors (PRR) for distinct microbial structures, data indicating their relevance in human infectious diseases are limited. We found that the basal expression of TLR2 was only slightly lower in phagocytes of healthy neonates compared to healthy adults. Analyzing neonates with sepsis, however, we found an impressive up-regulation of TLR2 on neonatal blood phagocytes already at initial presentation of symptoms. In addition, we show a strong up-regulation of S100A8 and S100A9 in neonatal sepsis. S100A8 and S100A9 are novel members of the DAMP-family (danger associated molecular patterns) and promote inflammatory processes which clearly depend upon interaction with TLR4. The S100A8/S100A9 complex has been proven to be useful as diagnostic marker of inflammation especially in chronic autoimmune diseases and sepsis. Recently, we have identified S100A9 as the first molecular target of the DAMP-family for immuno-modulatory therapies. Thus, the definition of TLR expression patterns and their internal ligands might open a new field of therapeutic targets for neonatal sepsis.

Corresponding Author: Christoph Buehrer, Prof. MD, Charité University Medical Center, Department of Neonatology, Augustenburger Platz 1, 13353 Berlin, Germany, christoph.buehrer@charite.de

A 339
Intravenous immunoglobulin (IVIG): between disappointment and hope
Khalid Haque

Objective: To review the role of IVIG in the treatment of neonatal sepsis.

Patients and method: Neonates and review of literature
Discussion: Following the establishment of ‘Germ’ based theory of infectious disease in the eighteenth and late nineteenth century, the main mode of therapy had been to provide antibodies and boost host defence until the development of antibiotics in 1930s. Neonatal sepsis remains the unconquered frontier in neonatal medicine today, with mortality in the preterm around 18–20% that has not changed for last three decades. With increasing survival of smaller and smaller babies and increasing antibiotic resistance adjuvant/alternative therapies have been sought. IVIG is one such immune-modulatory and host defence boosting therapy. This presentation will discuss the rationale behind the use of IVIG, critically review published data and the seven meta-analyses published so far. Meta-analysis of all the 12 published studies that show a clear benefit [odds ratio of 0.43 (95% CI 0.29–0.63)] will be presented.

Conclusion: Use of polyclonal IVIG, in particular IgM-enriched IVIG as an adjuvant to standard therapy significantly reduces mortality from neonatal sepsis with very little or no untoward effects. Hence, it is suggested that IVIG should be included as an adjuvant to standard therapy ‘package’ in the treatment of neonatal sepsis.

Corresponding Author: Khalid Haque, Prof. MD, University of London, Department of Neonatology, 92, Grange Road, London GU2 9QQ, UK. khalidnh99@yahoo.com

A 340
Interplay between innate immunity, inflammation and host lipid metabolism in the pathophysiology of Dengue shock syndrome
Patricia Couissinier-Paris, Stephanie Devignot, Cedric Sapet, Veasna Duong, Aurelie Bergon, Philippe Buchy

Objectives: We report the results of a genome-wide expression profiling study aimed at deciphering the molecular mechanisms supporting dengue shock syndrome (DSS), a life-threatening systemic inflammatory syndrome occurring in a fraction of patients infected by dengue viruses, thought to be due for a large part to detrimental host response to infection. This critical illness is indeed a world public health problem and a leading cause of children mortality and morbidity in tropical and sub-tropical countries, and clinical measures to predict progression to DSS or to improve its clinical support are limited due to poor knowledge of host mechanisms altered in DSS patients.

Methods and patients: Based on highly controlled study design and careful statistical analysis, we compared the whole blood genome-wide expression profiles of 48 prospectively recruited and matched Cambodian children of whom 19 progressed to DSS while other had clinically uncomplicated forms of dengue infection. Further search of molecular patterns associated to DSS was carried out to get an overview of molecular mechanisms altered in those patients at the time of cardiovascular decompensation.

Results: We report for the first time a large gene signature highly relevant of the DSS phenotype, of which integrated analysis allowed us deciphering a large part of the whole blood transcriptional events occurring in DSS children at the time of cardiovascular decompensation. We show first that, similarly to patients harbouring other critical illnesses such as severe sepsis, DSS patients have altered lymphocyte responses but over-expressed compensatory anti-inflammatory and repair responses. More importantly, we described previously unreported pro-inflammatory mechanisms transcriptionally active in DSS patients. We show that the pro-inflammatory transcriptional responses identified are all related to host innate immunity and involve a number of host innate defense genes, genes
from term (n = 17) and preterm (n = 17). pDCs were characterized by BDCA2 and CD123. Upon stimulation with type A CpG IFN-α production was quantified by intracellular IFN-α staining using FACS analysis.

Results: We found comparable amounts of absolute pDC and myeloid dendritic cell typ1 (MDCs)1 numbers and expression of the surface markers BDCA2 and BDCA1 between the three groups. Upon stimulation with type A CpG preterm CB pDCs, being BDCA2- and CD123-positive displayed significantly lower INF-α production. Moreover, the percentage of pDCs capable of producing IFN-α was reduced compared to pDCs from term neonates and adults.

Conclusion: While equal in absolute numbers, pDCs in preterm neonates produce significantly less IFNα after TLR9 stimulation compared to term neonates and adults. Moreover, the percentage of pDCs that can be stimulated with type A CpG is significantly reduced. These in vitro observations of reduced responsiveness and impaired functional capacity of pDCs might help to explain why preterm neonates are more susceptible to severe course of bacterial and viral diseases.

Conclusion: This study is to our knowledge the first one providing an overview of host responses altered at the time of cardiovascular decompensation in patients presenting this life-threatening form of severe dengue outcome in children. We suggest that the pro-inflammatory mechanisms identified, may open new perspectives for clinicians to improve both predictive prognosis of patients at risk to progress to DSS and therapeutics proposed to patients presenting with DSS.

A 341
Preterm neonates display altered plasmacytoid dendritic cell function: a cause of severe infections?
Simone Schueller, Kambis Sadeghi, Susanne Diesner, Marseille, France, parisp@imtssa.fr;parispatricia@yahoo.fr

Objective: Bacterial and viral infections are associated with high rates of morbidity and mortality in neonates, especially in preterm newborns. There are few data on the pathophysiology of neonatal infections and the detailed mechanisms of the altered immunological functions leading to severe courses of infections are yet a question of debate. Plasmacytoid dendritic cells (pDCs), although low in numbers encoding a diversity of endogenous danger signal molecules (DAMPs), as well as molecular patterns characteristic of lipid-laden monocytes at the interface between host lipid metabolism and innate immunity, which are all known to play key roles in sterile and other non sterile inflammatory diseases.

Conclusion: This study is to our knowledge the first one providing an overview of host responses altered at the time of cardiovascular decompensation in patients presenting this life-threatening form of severe dengue disease and providing evidences for a major contribution of innate immunity in severe dengue outcome in children. We suggest that the pro-inflammatory mechanisms identified, may open new perspectives for clinicians to improve both predictive prognosis of patients at risk to progress to DSS and therapeutics proposed to patients presenting with DSS.

Corresponding Author: Simone Schueller, MD, Medical University of Vienna, Department of Surgery, Waehringer Guertel 18-20, 1090 Vienna, Austria, simone.schuettler@meduniwien.ac.at

A 342
In-line filtration reduces sirs in critically ill children
Martin Boehne, Thomas Jack, Bernadette E. Brent, Armin Wessel, Michael Sasse

Objectives: Sepsis, systemic inflammatory response syndrome (SIRS) or organ failure often complicate the clinical course on an intensive care unit. Particulate contamination of infusion solution may contribute to the clinical deterioration of these patients. Particles have been shown to induce thrombogenesis, deterioration of microcirculation and modulation of immunoresponse. The use of in-line filtration with micro-filters almost completely prevents particulate infusion. We assessed the effect of in-line filtration on the reduction of major complications in critically ill children (Clinical Trials.gov ID NCT 00209768).

Patients and methods: In a randomised, prospective trial 807 paediatric patients admitted to the interdisciplinary PICU of a tertiary university hospital were assigned to either control or interventional group, the latter receiving in-line filtration (infusion filter Pall ELD96LLCE/NOE96€, Braun Intrapur Lipid/ Intrapur Neonat Lipid) throughout whole infusion therapy. Prior to this study, infusion regimen was optimised to prevent precipitation and incompatibilities of solutions and drugs. Primary objectives included a reduction in the incidence of sepsis, thrombosis, systemic inflammatory response syndrome (SIRS), organ failure (liver, lung, kidney, circulation) and mortality.

Results: 807 children (343 female, 464 male) with a heterogeneous background of underlying diagnoses and a Gaussian distribution to either control (406 patients) or in-line filtration group (401 patients) were included. According to the study criteria a significant reduction in the incidence of SIRS for the interventional group (95% CI, 145 vs. 200 patients, P < 0.001) was evident. No differences were demonstrated for the occurrence of sepsis, thrombosis, organ failure (liver, lung, kidney, circulation) or mortality between the control and interventional group.

Conclusion: The occurrence of SIRS often complicates the treatment in intensive care medicine. Inline-filtration is most effective reducing the incidence of SIRS and offers a novel therapeutic option.

Corresponding Author: Martin Boehne, MD, Medicinische Hochschule Hannover, Department of Paediatric Cardiology and Intensive Care Medicine, Carl-Neuberg-Str. 1, 30625 Hannover, Germany, boehne.martin@mh-hannover.de

A 343
Inhibition of systemic inflammation by plasma protein C1-esterase inhibitor in a human inflammation model
Tjaakje Visser, Mirrin Dorresteijn, Anky Koenderman, Luke Leenen, Peter Pickkers, Leo Koenderman

Introduction: An overwhelming inflammatory reaction underlies the pathogenesis of acute respiratory distress syndrome and multiple organ failure. These complications are frequently seen after trauma or during severe sepsis. Early inhibition of the acute inflammatory response may reduce the risk of organ failure.

Animal models have shown that the acute phase protein C1-esterase inhibitor (C1-INH) can act as a potent endogenous inhibitor of
systemic inflammation. Therefore, we investigated the anti-inflammatory effect of C1-INH in human model of acute systemic inflammation.

Methods: In a randomised double-blind placebo-controlled trial, E. coli polysaccharide (LPS 2 ng/kg) was injected intravenously in 20 healthy male volunteers, to induce an acute systemic immune response. Thirty minutes after injection of LPS C1-INH (Cetor®; 100 U/kg) or placebo was administered. Inflammation was quantified by induction of cytokines and CRP.

Results: Treatment with C1-INH induced a significant decrease in LPS-induced inflammation. The release of pro-inflammatory cytokines and CRP was reduced by C1-INH (IL-6 1521 ± 209 vs. 932 ± 174; TNF-α 1213 ± 187 vs. 827 ± 167; MCP-1 1616 ± 1302 vs. 3373 ± 228 pg/ml; CRP 29 ± 2 vs. 39 ± 4 mg/dl; p < 0.05), whereas the release of the anti-inflammatory cytokine IL-10 was significantly increased (73 ± 11 vs. 121 ± 18 pg/ml, p < 0.01).

Conclusion: C1-INH modulates the acute humoral inflammatory response induced by LPS challenge in humans. During experimental endotoxemia, the release of pro-inflammatory cytokines was attenuated by C1-INH, whereas the release of the anti-inflammatory cytokine IL-10 was potentiated. Treatment with C1-INH might provide an interesting tool to inhibit systemic inflammation, thereby, preventing life threatening inflammatory complications.

Corresponding Author: Tjaakje Visser, MD, University of Utrecht, pre@uni-leiden.nl

A 344
Role of the anaphylatoxin c5a in bone marrow dysfunction during experimental sepsis
Ralf Reutter, Fleig Vera, Kolbitz Miriam, Acker Barbara, Gebhard Florian, Huber-Lang Markus

Objective: The bone marrow (BM) plays a crucial but so far rather disregarded role in the development and progression of sepsis and sepsis-induced multi-organ-failure (MOF). As principal components of innate immunity, both, the BM and the complement system exhibit early alterations during the sepsis course. However, the role of the complement activation product C5a on the BM function is still unknown. Therefore, aim of this study was to specify the effects of C5a on BM function during experimental sepsis.

Methods: Experimental sepsis was performed by cecal ligation and puncture (CLP) in Wistar rats (250 g BW). After CLP, the animals were treated with 400 µg of either preimmune IgG or anti-C5a IgG and compared to both, controls and sham-operated littermates (n = 5–7 for each group). Femoral BM and peripheral blood were then analyzed for cellular recruitment and ex vivo growth capacity. Furthermore, BM cell recruitment was assessed by an ex vivo model of a continuously perfused femoral bone in presence or absence of C5a.

Results: All CLP animals exhibited a substantial peripheral leukopenia. In contrast, normalization of the sepsis-induced left-shift was observed by blockade of C5a. Furthermore, the total BM cell count was found to be significantly decreased in untreated CLP rats while anti-C5a treatment reversed this effect. Ex vivo BM cultures revealed a significant increase in granulocyte-macrophage colony forming units (GM-CFU) after CLP, which was significantly ameliorated by C5a blockade. Remarkably, isolated BM cells from normal rats exhibited a dose-dependent growth of GM-CFU upon exposure to increasing concentrations of C5a. Regarding cellular recruitment from BM, presence of 100 ng/ml C5a enhanced the total cellular count in the perfusion solution. In contrast, BM from CLP-animals exhibited a significantly reduced cellular recruitment rate unresponsive to any additional C5a stimulation.

Conclusion: These data suggest for the first time that the complement activation product C5a plays an essential role in the development of functional BM changes during sepsis.

Corresponding Author: Ralf Reutter, University of Ulm, Department of Traumatology, Steinhoevelstr. 9, 89075 Ulm, Germany, ralf.reutter@uni-ulm.de

A 345
Post-burn 17β-estradiol administration significantly decreases the early pulmonary inflammatory response, and improves lung function following experimental severe burn injury
Jane Wigginton, Joshua Gatson, James Simpkins, Paul Pepe, Ahamed Idris, Joseph Minei

Objective: In patients with severe burn injury, the most common cause of morbidity and mortality is pulmonary dysfunction followed by multi-organ failure. To date, therapies aimed at preserving lung function in this patient population are lacking. We therefore tested estrogen, a known anti-oxidant, anti-apoptotic, and anti-inflammatory drug as a potential therapy for mitigation of lung injury and pulmonary dysfunction following severe remote thermal injury in a rat model.

Patients and methods: 149 male Sprague-Dawley rats were randomized into 3 groups (5 sham, 72 burn/placebo, 72 burn/17β-estradiol) and sacrificed at one of 8 time points over the first 18 h (0.5, 1, 2, 4, 6, 8, 12, 18 h). The rats received a 40% total body surface area 3rd degree torso burn, then were administered a single dose of placebo versus 17β-estradiol (0.5 mg/kg) at 15 min post-burn. Lung tissue was collected and analyzed by ELISA for the pro-inflammatory cytokine IL-6. Another 24 animals (8 sham, 8 burn/placebo, 8 burn/17β-estradiol) handled in the same manner were subjected to whole body plethysmography, arterial blood gas sampling, and myeloperoxidase (MPO) activity measurement at 18 h post-injury.

Results: Administration of 17β-estradiol significantly decreased lung IL-6 at all time points, with levels nearly identical to sham animals (average 73.5 pg/mg). Those rats receiving placebo steadily increased lung IL-6 to 760.6 pg/mg at 18 h, while those receiving estradiol maintained levels of 75.9 pg/mg at the same time point. MPO activity levels were 0.337 units/mcg in the sham animals, 0.446 units/mcg in those receiving estradiol, and 0.8537 units/mcg in the placebo animals. While the minute ventilation (MV) of both the sham and estradiol treated animals remained unchanged from baseline to the end of the 18 h post-burn measurements, those rats receiving placebo more than doubled their MV (115.1 cc/min baseline to 270.3 cc/min at 18 h). Mean PaO2 levels at 18 h in those animals receiving estradiol were again similar to those receiving a sham burn (76 vs. 81.2 mmHg, respectively), while those receiving placebo had a PaO2 level of 64.05 mmHg. Two placebo animals died at 12–15 h, while all sham and estrogen animals survived.

Conclusion: Early estrogen administration following severe remote burn injury controlled the IL-6 response in the lung tissue, significantly preserved lung function, and decreased mortality at 18 h post-burn.

Corresponding Author: Jane Wigginton, MD, UT Southwestern Medical Center at Dallas, Department of Surgery, 5323 Harry Hines Blvd, Dallas, TX 75390, USA, jane.wigginton@UTSW.edu
A 346

Direct hemoperfusion with polymyxin B-immobilized fiber column (PMX-DHP) can improve the prognosis and medical expense

Yoshihiro Edamoto, Ryuichiro Suda, Keigo Kumazawa, Masanori Hashimoto, Yukio Saito, Toshio Shimizu

Direct hemoperfusion with polymyxin B-immobilized fiber column (PMX-DHP) has been applied to patients with endotoxemia or septic shock in Japan since 1989. The aims of this study were to assess the efficacy of PMX-DHP in relation to cost effectiveness.

Methods: 44 septic shock patients (PMX treatment) who underwent PMX-DHP between 2001 and 2006 were evaluated with the outcome and cost retrospectively comparing with 36 patients without PMX-DHP (Control). The patients were classified into four groups according to APACHE-II score and SOFA score: those whose APACHE-II score <15 (low risk group n = 15), APACHE-II score >30 or SOFA score >10 (poor prognosis group n = 4), APACHE-II score 15–24 and SOFA score <7 (intermediate group n = 40), and the other patients (high risk group n = 31).

Results: Mortality rate (30 vs. 50%), length of hospital stay (62.7 vs. 139 days) and ICU stay (7.7 vs. 13.5 days), medical expense (59,730 vs. 127,039 €) in intermediate risk group with PMX treatment were significantly improved from those of control (P < 0.05). Conversely low risk and poor prognosis group did not reveal any improvement of mortality rate (0 vs. 0%), length of ICU stay, and medical expense. Conclusions: Our data suggest that PMX treatments are associated with a reduction of the mortality rate and the medical expense in intermediate and high risk group. This may be one of indications of that the use of PMX-DHP for the cure of septic shock.

Corresponding Author: Yoshihiro Edamoto, MD, PhD, International Medical Center of Japan, Department of Surgery, 1-21-1 Toyama, Shinjuku, 162-8655 Tokyo, Japan, yoedamot@imcj.hosp.go.jp

A 347

Tail vein injection of the human interleukin-1 homologue F7b cDNA in mice reduces local and systemic inflammation in ConA-induced hepatitis

Philip Bufler, Michaela Fink, Kai Wagner, Christoph Mauksch

Objective: IL-1F7b is a novel homologue of the IL-1 cytokine family. We demonstrated that IL-1F7b shares critical amino acid residues with IL-18 and binds to the IL-18-binding protein enhancing its ability to inhibit IL-18-induced interferon g. IL-1F7b was also shown to bind to the IL-18Ra, however, no IL-18Ra-dependent agonistic or antagonistic function was discovered. We reported recently that after LPS-stimulation IL-1F7b translocates to the nucleus and reduces the expression of proinflammatory cytokines. The aim of this study was to investigate the role of IL-1F7b in ConA-induced hepatitis.

Methods: Human IL-1F7b cDNA was cloned into the expression plasmid pTarget which contains a constitutively active CMV-promotor. 5-week-old female C57BL/6j mice were rapidly injected with either 20 mg of empty pTarget or pTarget-IL-1F7b in 2 ml of Ringer’s solution into the tail vein (“hydrodynamic injection”). The plasmid pLac was co-injected at a ratio of 1:20 for in vivo transfection control. After 48 h the mice were injected with ConA 200 mg into the tail vein to induce hepatitis. 2 h after ConA-injection a blood sample was taken for cytokine measurement. 24 h after ConA-injection the mice were anesthetized and injected with luciferin to monitor in vivo luciferase-activity for transfection control. Another blood sample was obtained and the mice were sacrificed. The liver was stored for histology, immunohistochemistry and cytokine analysis.

Results: DNA-injected mice expressed high levels of luciferase-activity predominantly in the liver. Transgene IL-1F7b was detected in the liver lysate of mice injected with IL-1F7b plasmid by western blotting. 2 h after ConA-injection significantly reduced serum levels for IL-1α, IL-6, IL-5 and IL-9 were measured in IL-1F7b-expressing mice. IL-6 (p = 0.009) was reduced in the liver lysate 24 h after ConA-injection. All mice developed severe acute hepatitis as shown by histology. However, no difference in the histological score was observed between the two groups. In parallel, no difference in serum ALT was detected.

Conclusion: In vivo expression of human IL-1F7b in mice reduces local and systemic inflammation in ConA-induced hepatitis. This observation supports the in vitro-generated hypothesis of IL-1F7b acting as an anti-inflammatory cytokine. Patchy expression of IL-1F7b-protein after tail vein injection might explain the lack of reduced histological severity score despite reduced IL-6 in within the liver.

Corresponding Author: Philip Bufler, MD, PhD, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Children’s Hospital Hannes’sches Kinderspital, Lindwurmstr 4, 80337 Munich, Germany, Philip.Bufler@med.uni-muenchen.de

A 348

Effect of mediator modulation with specific or selective adsorbents on endothelial cell activation in an experimental cell culture model of sepsis

Viktoria Weber, Anita Schildberger, Tanja Buchacher, Eva Rossmanith, Dieter Falkenhagen

Objectives: Modulation of inflammatory mediators by therapeutic apheresis with specific or selective adsorbents may be a supportive therapy in septic patients. The aim of this study was to assess the influence of adsorptive mediator modulation on endothelial activation in a cell culture model for Gram-negative sepsis.

Materials and methods: Monocytic THP-1 cells were stimulated with 10 ng/ml lipopolysaccharide (LPS) from Pseudomonas aeruginosa in media containing 10% human plasma for 4 h. The medium containing LPS and factors secreted by THP-1 in response to stimulation (conditioned medium) was harvested and applied to human umbilical vein endothelial cells (HVECs). Two approaches were used for mediator modulation: (a) a specific adsorbent for tumor necrosis factor-α (TNF) based on Sepharose beads functionalized with anti-TNF antibodies and (b) a selective polystyrene divinylbenzene copolymer which was coated with human serum albumin to improve biocompatibility. The specific TNF adsorbent (or a control adsorbent without antibody) was applied to HVEC either 1 or 3 h after stimulation, or TNF was removed from the conditioned medium before HVEC stimulation. The polystyrene divinylbenzene copolymer was only used to remove mediators from the conditioned medium before HVEC stimulation. Endothelial activation was monitored for up to 16 h by measuring NF-κB activity, cytokine secretion, adhesion molecule expression, and release of plasminogen activator inhibitor (PAI-1).

Results and conclusions: Average TNF concentrations in the conditioned medium were 800 pg/ml. Specific TNF adsorption resulted in a significant decrease of NF-κB activity in HVEC within 2 h of adsorption. In addition, IL-6 and PAI-1 secretion, and ICAM-1 and E-selectin expression on HVECs were significantly decreased after TNF adsorption, and pre-adsorption had a stronger effect than adsorption after 1 or 3 h. Pre-treatment of conditioned medium with
the selective adsorbents (simultaneous adsorption of various cytokines) resulted in significantly reduced HUVEC secretion of IL-6 and IL-8, and reduced ICAM-1 and E-selectin expression indicating reduced endothelial activation. Studies to measure the effect of other specific adsorbents are underway. The cell culture model is suited to monitor the efficacy of adsorption of individual factors in blood purification and thus may support the development of new therapies for Gram-negative sepsis.

Corresponding Author: Viktoria Weber, PhD, Danube University Krems, Department Clinical Medicine and Biotechnology, Dr. Karl Dorrekstrasse 30, 3500 Krems, Austria, viktoria.weber@donau-unl.ac.at

A 349
Green tea polyphenols reduce hepatic apoptosis after hemorrhage/resuscitation (H/R) by suppression of NF-kappaB activation and JNK phosphorylation

Borna Relja, Mark Lehnter, Eva Toettel, Lara Breig, Dirk Henrich, Ingo Marzi

Objective: H/R induce an inflammatory response and free radical formation that are associated with hepatocellular damage. Previously, we demonstrated, that plant polyphenols (i.e. green tea extract, GTE) possess high antioxidative capacity in the liver after H/R in vivo. Nuclear Factor kappaB (NF-kappaB) is an ubiquitous rapid response transription factor involved in inflammatory ad apoptosis reactions. NF-kappaB exerts its action by expressing cytokines and cell adhesion molecules. Here, we investigated the effect of GTE and the role of NF-kappaB in the pathogenesis of liver injury induced by H/R.

Material and methods: Female Lewis rats received daily chow containing 0.1% GTE or regular chow 5 days before H/R. Then, rats were hemorrhaged to a blood pressure of 30 ± 2 mmHg for 60 min and resuscitated. 2 h later, serum alanine aminotransferase (ALT) and IL-6 levels were evaluated. Immunohistochemical analysis of apoptosis (M30-staining, caspase-3 cleaved CK18/necrosis (TUNEL), I-CAM and polymorphonuclear leucocyte (PMN) infiltration were performed. In addition, immunoblotting of liver tissue revealed the phosphorylation of IkapapaBalpha (inhibitor of IkB) and c-Jun N-terminal kinase (JNK). A p < 0.05 was considered significant (ANOVA).

Results: GTE pretreatment blinded serum ALT increase after H/R (1385 ± 191 IU/L) by 62% (p < 0.05). Liver apoptosis and necrosis were largely dimished after GTE pretreatment as compared to ctrl after H/R. Serum IL-6 level increased to 1272 ± 220 pg/ml in ctrl after H/R and was largely blocked by GTE (565 ± 193 pg/ml, p < 0.05). Hepatic ICAM-1 staining revealed strong expression after H/R as compared to sham and GTE pretreated rats after H/R. PMN infiltration in the liver strongly increased after H/R to 9 ± 1 (positive cells/high power field) in the ctrl group but was largely dimished after GTE pretreatment (3 ± 1, p < 0.05). H/R induced phosphorylation of JNK and increased phosphorylation and degradation of IkapapaBalpha. Treatment with GTE decreased the phospho-JNK and phospho-IkapapaBalpha after H/R to basal/sham levels.

Conclusion: Plant polyphenols significantly decrease the inflammatory response and hepatic damage induced by H/R. H/R induce JNK, NF-kappaB and caspase-3 activation. This study provides the first evidence showing that plant polyphenols exert their antiinflammatory and antianpoptotic effects by down-regulation of NF-kappaB and JNK.

(Supported by DFG MA 1119/3-3).

Corresponding Author: Borna Relja, MSc, Johann Wolfgang Goethe University of Frankfurt/M, Department of Trauma Surgery, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany, info@bornarelja.com

A 350
Open abdomen treatment with vacuum-therapy-randomized pilot-trial comparing fascial closure and survival with abdominal-dressing versus vacuum-pack-technique-rationale and design of the ABDOVAC-trial

Florian Herrle, Jens-Olaf Jonescheit, John Kirby, Peter Kienle, Stefan Post, Marco Niedergethmann

Background and objectives (Trial-Rationale): Open abdomen treatment in surgery has been established worldwide for severe intraabdominal sepsis and other conditions like abdominal-compartment-syndrome or polytrauma. A diversity of temporary abdominal closure techniques exist. Today even without sufficient high-level evidence there is a clear tendency to treat open abdomen with vacuum-devices. 1995 Brock and Barker described and applied the self-made “Vacuum-pack”-system with until now promising results at low costs. The commercial KCI “Abdominal-dressing” ready-to-use-kit is also used frequently but cost-intensive. The need for evidence is high: many individual case series about vacuum-therapy in open abdomen exist but no sound randomised trial. The clinical and economical impact of such a trial could be high. Individual benefit would be important if survival is improved and patients can be spared a large ventral hernia and their consequences. Objective of this pilot trial is to examine overall feasibility, mortality and fascial closure rate of these two vacuum-therapies.

Methods (Trial-Design): Prospective randomised pilot trial (n = 20, 12 months recruitment, 12-week follow-up). Stratification before intraoperative randomization will be performed between patients with and without secondary peritonitis. Interventions will be “Vacuum-Pack” as experimental group versus “Abdominal-Dressing”-kit as control group. The co-primary endpoint is in-hospital-mortality and/or delayed fascia-to-fascia abdominal closure rate. Important secondary endpoints are: Vac-related morbidity, costs for Vac-Therapy and total hospital stay, quality of life (SF-12, EQ-5-D), recurrent abdominal wall hernia-rate at 12 weeks.

Results: The trial has been approved by the local ethics committee Mannheim and is registered (NCT00834314, http://www.clinicaltrials.gov). Funding is provided by DFG-Grant HE 5918/1-1. To minimize conflict of interest vacuum-kit-materials will be provided by two competing companies (KCI and Medela). Recruitment will start Nov 2009. In March 2010 preliminary data and feasibility aspects can be reported at the TSIS.

Conclusions: The ABDOVAC-Pilot trial is currently the first randomised-controlled trial to compare two commonly used but empirically-based vacuum-techniques for open abdomen therapy. A multicentre trial is intended if study design proves feasible in this condition. TSIS 2010 would be the ideal platform for fruitful discussion and future multicentre enrolment for this trial.

Corresponding Author: Florian Herrle, MD, University of Mannheim Medical Center, Department of Surgery, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany, florian.herrle@umm.de

A 351
The use of the Wittmann patch facilitates a high rate of fascial closure in severely injured trauma patients and critically ill surgery patients

Martin Schreiber

Introduction: The open abdomen following severe intraabdominal trauma and emergency surgery is a major operative challenge. It is
A 352
A multicentre study of bacteraemia using a new commercial universal 16S rRNA PCR test
Samir Sakka¹, Nele Wellnhausen, Anna-Julia Kochen, Claudia Disque, Helge Muehl, Susanne Gebert
¹Klinik der Stadt Köln, Krankenhaus Merheim, Ostmerheimerstrasse 200, 51109 Cologne, Germany

Objective: Bloodstream infection is a life-threatening condition with a high mortality rate, especially in intensive care and neutropenic patients. Standard diagnostics is based on blood culturing (BC). However, limitations of BC include relatively low sensitivities and a long time-to-result for the identification of the pathogen, generally over 2 days and more. On the grounds of data from a multicentre study using a universal 16S rRNA gene PCR assay, SepsisTest™, molecular diagnosis is discussed as a rapid and sensitive tool for the detection and identification of pathogens supportive of BC.

A new commercial PCR test, SepsisTest™, for direct detection of bacteria in whole blood was compared to BC in terms of sensitivity, specificity, predictive values and time to positivity (TTP) of bacterial infections of the blood stream of critically ill patients.

Patients and methods: The test, SepsisTest™ (Molzym, Bremen), comprises the extraction and 16S rRNA gene PCR detection of bacterial DNA in whole blood samples. Bacteria in positive samples were identified by sequence analysis of the amplicon. In a prospective multicentre study 342 blood samples from 187 patients with systemic inflammatory response syndrome (SIRS), sepsis, or neutropenic fever were included.

Results: Compared to BC, the diagnostic sensitivity and specificity of PCR/sequencing was 87.0 and 85.8%, respectively. The positivity rate of PCR/sequencing (25.7%) was higher than BC (15.8%). Of 31 PCR/sequencing-positive, BC-negative patients, most of whom received antibiotics, the PCR results of 25 were judged as true or possible to bacteraemia. Using a routine testing workflow, time to positivity of the PCR-test was on average decreased by 40 h for anaerobe/fastidious infections and by 54 h for yeast infections.

Conclusions: The PCR approach enables the detection and identification of bacteraemia in blood samples within a few hours. Despite the indispenability of BC diagnostics, the rapid detection of bacteria by SepsisTest™ appears to be a valuable tool, allowing earlier pathogen-adapted antimicrobial therapy in critically ill patients.

Corresponding Author: Karen Hinsch, PhD, Molzym GmbH+ Co. KG, Product Management, Mary-Astell Str. 10, 28359 Bremen, Germany, hinsch@molzym.com

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A 353
Diagnostic and prognostic value of biochemical markers in critically ill patients on ICU
Katharina Biller, Peter Fae, A.K. Walli, Peter Fraunberger

Objective(s): The best precondition for successful therapy of a sepsis is to recognize the inflammation as early as possible and to detect and eliminate the possibly existing focus. The aim of the present study was to prospectively evaluate the relevance of the biochemical markers IL-6, PCT, CRP and cholesterol for early and specific detection of inflammatory complications in intensive care unit patients.

Patients and methods: Plasma levels of IL-6, PCT, CRP and cholesterol were measured in blood samples from 125 consecutive ICU patients who fulfilled criteria of SIRS or Sepsis according to the ACCP/SCCM criteria. Values were correlated with severity of disease, incidence of infection, length of hospital stay, mortality and clinical course. Clinical worsening was defined as increasing catecholamine requirement and/or oliguria and/or rise in temperature for more than one degree, confusion, decline of thrombocytes and decline of the liver function.

Result: All complications were associated with an increase of IL-6 levels. Increase of IL-6 levels could be observed 24–48 h before other biochemical markers and before clinical signs of infection. IL-6 and PCT levels were significantly higher in patients with infection. Furthermore PCT levels correlated with severity of sepsis according to ACCP/SCCM. Cholesterol levels were significantly lower in patients with infection and showed an inverse correlation with severity of sepsis. On the day of admission IL-6 levels were significantly higher and cholesterol levels were significantly lower in patients who did not survive.
Conclusion: As an early inflammatory alarm marker IL-6 is more adequate than CRP or PCT. IL-6, PCT and cholesterol are all useful to detect an infection. Only IL-6 and cholesterol levels are useful markers to predict survival. For assessing the severity of sepsis PCT appears to be a good marker. Further studies will show whether combination of these markers may better predict clinical outcome.

**Corresponding Author:** Katharina Biller, Medizinisches Zentrallaboratorium GesmbH, Medical Central Laboratory, Carinagasse 41, 6800 Feldkirch, Austria, katharina_bill@web.de

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**A 354**

**Sex-related differences do not improve survival of female patients following severe trauma: a matched pair analysis**

Heiko Trentzsch, Rolf Lefering, Robert Kraft, Siegried Zedler, Eugen Faist, Stefan Piltz; and the Traumaregister der Deutschen Gesellschaft für Unfallchirurgie (TR-DGU).

Animal models suggest that females have better outcome, lower risk of organ failure (OF) and sepsis after trauma. However, clinical data does not fully comprehend this paradigm. Objective of our study was to contribute sound data to this controversy.

Matched pair analysis was performed using Trauma Registry of the Deutsche Gesellschaft für Unfallchirurgie (TR-DGU) data. From 1993 to 2006, 29,353 cases from multiple centers were included in a prospective and standardized fashion. All primarily treated patients with injury severity score (ISS) >9 were selected for matching. It accounted for mechanism, injury severity of head, thorax, abdomen and extremities according to Abbreviated Injury Scale, shock in the field (blood pressure [RRsys] ≤90 mmHg) and age-group yielding into 3887 pairs of a male (M) with a corresponding female (F). We assessed survival, OF, sepsis, hospital days (LOS), ICU days, ventilator days, management in the field or in the trauma room, age, comorbidities, RRsys on admission, admission labs (hemoglobin [HB], base excess [BE], thromboplastin time [Q] and partial thromboplastin time [PTT]). Findings were tested for differences using t-test for quantitative data and Chi2-test for categorical data. Significance was excepted at \( p < 0.01 \) (*). Data is given as mean.

Pairs were comparable in age, ISS and comorbidities. RRsys (pre-clinical and on admission) and HB were lower in F*. There was no difference in BE, Q or PTT. F showed less sepsis (6.7 vs. 9.1%, odds ratio (OR) 1.45, (95%-confidence interval [CI] 1.21–1.74)\(^*\), singleOF (33.1 vs. 36.3%, OR 1.15 (CI 1.05–1.27)\(^*\)), pulmonary OF (15.6 vs. 19.8%, OR 1.33 (CI 1.17–1.51)\(^*\)) and multiOF (17.6 vs. 21.8%, OR 1.18 (CI 1.05–1.33)\(^*\)). Mortality was M 17.8%, F 16.1%, OR 1.14 (CI 1.01–1.28). Mortality in pts diagnosed with sepsis was M 21.8%, F 18.3%, OR 1.25 (CI 0.8–1.94). LOS (M 26 days; F 25 days) was even, but M stayed longer in ICU (11 vs 9 days*) with more ventilator days (7 vs. 5 days*). In the field, M received more often chesttubes (5.5 vs. 4.1%\(^*\)). Intubationrate, use of vasopressors and fluid resuscitation were even. On admission, M were more often operated on (79.7 vs. 76.4%\(^*\)), fluid resuscitation showed no difference.

Women have lower risk of sepsis and OF following severe trauma. Better tolerance of shock may explain this finding. Eventually, men may have had higher systemic burden from surgical procedures. Men were more prone to pulmonary complications. Women however, despite such advantages had no improved outcome.

**Corresponding Author:** Heiko Trentzsch, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Surgery, Marchioninistr. 15, 81377 Munich, Germany, heiko.trentzsch@ed.uni-muenchen.de

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**A 355**

**Hypertonic saline in the ICU reduces inflammation and increases shedding of receptors responsible for microcirculatory inflammation**

Jose L. Pascual, Weiwei Ding, Carrie Sims, Babak Sarani, Annamarie Horan, William Schwab

Systemic inflammation is an important cause of ICU-related mortality and may result from activation of endothelial cell (EC) and neutrophil (PMN) expression of surface adhesion receptors. Hypertonic saline (HTS), in severely ill ICU patients, may reduce PMN adhesion receptors but no human study has evaluated its effects on ECs. Furthermore, the impact of HTS on systemic inflammation is also unknown.

Objectives: To determine if ICU patients receiving HTS develop reduced indices of systemic inflammation associated with shedding of EC/PMN adhesion receptors.

Methods: This prospective, observational study was approved by the University IRB prior to initiation. Clinical variables were collected from 13 ICU subjects treated with a 5% HTS bolus, for resuscitation \((n = 9)\) or to treat traumatic brain injury \((n = 4)\). Blood samples were taken before HTS administration (baseline), immediately after \((\text{time} = 0)\), and at 6, 12, and 24 h post dosing. Soluble ICAM-1 (sICAM-1) [EC receptor responsible for EC adhesion to PMN] and L-selectin (sL-selectin) [PMN receptor responsible for PMN rolling on EC] were evaluated as markers of shed surface receptor. TNFAlpha, IL-1beta, and IL-6, [Systemic pro-inflammatory markers] were also evaluated. Target molecules were quantified by standard ELISA protocols. Pre- and post-dosing levels of each were compared using the Mann–Whitney test. Significance was defined as \(* p < 0.05.\)

Results: Mean clinical scores were: APACHE:22, ISS:36 and GCS:7.\(^*\), IL-1beta. \((n = 9)\) or to treat traumatic brain injury \((n = 4)\). Blood samples were taken before HTS administration (baseline), immediately after \((\text{time} = 0)\), and at 6, 12, and 24 h post dosing. Soluble ICAM-1 (sICAM-1) [EC receptor responsible for EC adhesion to PMN] and L-selectin (sL-selectin) [PMN receptor responsible for PMN rolling on EC] were evaluated as markers of shed surface receptor. TNFAlpha, IL-1beta, and IL-6, [Systemic pro-inflammatory markers] were also evaluated. Target molecules were quantified by standard ELISA protocols. Pre- and post-dosing levels of each were compared using the Mann–Whitney test. Significance was defined as \(* p < 0.05.\)

Conclusions: HTS administration in critically ill patients increases endothelial and neutrophil shedding of receptors responsible for EC/ PMN interactions and microcirculatory inflammation. HTS also reduces circulating indices of systemic inflammation. HTS may serve to reduce systemic inflammation observed in critically ill patients through a blockade of endothelial and neutrophil activation.

**Corresponding Author:** Jose L. Pascual, MD, PhD, University of Pennsylvania, Morpionalp, 3400 Spruce St, 2 Dalles, Philadelphia, PA 19104, USA, jose.pascual@uphs.upenn.edu

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**A 356**

**Increased aromatase expression may be responsible for increased estrogen levels in the brain following severe traumatic brain injury**

Jane Wigginton, Joshua Gatson, James Simpkins, Paul Pepe, Ahamed Idris, Joseph Minei

Objective: In traumatic brain injury (TBI), the persistent demise of neuronal and glial populations leads to decreased brain function. Endogenous and exogenous estrogens may protect these vulnerable cells. Following brain injury, inflammation and oxidative stress trigger an increase in endogenous estrogens levels, thought due to...
increased aromatase expression converting testosterone to estradiol. In a human observational study of cerebrospinal fluid (CSF) following severe TBI, we found that CSF estradiol levels rise rapidly, and are much higher than serum levels in those with a good outcome (up to 2,000 pg/ml in male patients’ CSF). Normal CSF estradiol levels are 1/10 that of their serum level (~2–5 pg/ml). In this study, we hypothesized a novel mechanism for the significant increase in estradiol in these TBI patients, namely an upregulation of aromatase expression due to increased intracranial pressure.

Patients and methods: We subjected primary astroglia to a clinically relevant increased pressure for TBI patients (25 mmHg) for 1, 3, 6, 12, 24, 48, and 72 h. Total aromatase protein levels were measured using Western analysis, and aromatase RNA levels were measured using RT-PCR. In the human observational study, we assessed the serial levels of CSF estradiol from patients with severe TBI (GCS 3–8) who required placement of a therapeutic ventriculostomy. CSF and serum estradiol levels were measured with the IMMULITE® immunoassay system.

Results: In male and female TBI patients, individuals with a good outcome showed a significant, rapid increase in the levels of estrogen in the CSF compared with the serum levels. As a possible explanation, increased pressure applied to the astroglias cultures resulted in a significant increase in aromatase RNA levels (twofold) at the 3 h time-point. At 6 and 12 h there was a significant increase in the protein levels of aromatase (~95 and 90%, respectively) following increased pressure.

Conclusion: In our TBI patients with a good outcome, there was a rapid increase in estradiol levels in the CSF at early time-points (~16 h). It is possible this elevation is a protective mechanism triggered by the higher intracranial pressure seen in these patients, prompting a significant escalation in aromatase production leading to conversion of testosterone to protective estradiol, as both RNA and protein levels were increased in astroglia in the presence of clinically relevant increased pressure (25 mmHg).

Authors: Jane Wigginton, MD, UT Southwestern Medical Center at Dallas, Department of Surgery, 5323 Harry Hines Blvd, Dallas, TX 75390, USA, jane.wigginton@UTSW.edu

A 357

Sympathetically mediated cardiac effects of hypertonic resuscitation
Robert Frithiof, Rohit Ramchandra, Sally Hood, Clive May

Objective: Resuscitation with small volume hypertonic NaCl is an established treatment of hemorrhagic shock. The volume expansion achieved with the resuscitation is important, but previous studies have indicated that the full hemodynamic effect depends on additional mechanisms. A prolonged hemorrhage effectively attenuates sympathetic nerve activity (SNA) causing hypotension and bradycardia. Sodium is a powerful modulator of SNA and therefore we hypothesized that resuscitation with hypertonic NaCl would reestablish sympathetic nerve activity and improve cardiovascular function.

Material and methods. Initially, nine ewes were pre-experimentally prepared with intrafascicular electrodes in renal and cardiac sympathetic nerves for measurement of SNA. Hemorrhage was performed in conscious sheep by withdrawal of blood from the jugular vein (20 ml/kg in 20 min). Thereafter they were resuscitated for 10 min with either 2 ml/kg 1.2 M NaCl or 12 ml/kg isotonic NaCl IV. Secondly, to isolate the cerebral effects of hypertonic NaCl resuscitation, five sheep received a reduced volume of the hypertonic resuscitation in the carotid arteries (IC) (1 ml/min bilaterally) after hemorrhage. Finally, six sheep, prepared with an ultrasonic flow probe around the pulmonary artery and a left ventricular catheter, were hemorrhaged and resuscitated for 2 min with 2 ml/kg 1.2 M NaCl with and without a preceding IV infusion of the beta-blocker propranolol.

Results: Hemorrhage caused a characteristic biphasic response in heart rate (HR) and SNA with an initial increase followed by an abrupt fall when the blood loss reached a critical volume. Cardiac and renal SNA were then completely abolished and mean arterial blood pressure (MAP) and cardiac output (CO) significantly reduced. Resuscitation with hypertonic NaCl IV improved cardiac SNA and MAP more rapidly and to a higher level than isotonic resuscitation, although the plasma volume expansion was the same. When hypertonic NaCl was given IC cardiac SNA, HR and MAP were significantly increased, indicating a cerebral mechanism for the effect. Finally, propranolol attenuated the increase in HR, CO and dP/dTmax caused by IV hypertonic NaCl resuscitation.

Conclusion: Our data demonstrates that hypertonic NaCl resuscitation after hypotensive hemorrhage improves cardiac function by stimulating cardiac SNA through direct effects on the brain.

Corresponding Author: Robert Frithiof, MD, PhD, Karolinska Institute, Department of Physiology, von Euler's väg 8, 11240 Stockholm, Sweden, robert.frithiof@ki.se

A 358

Resuscitation with hypertonic saline-dextran attenuates leukocyte and endothelial cell activation and adhesion molecule expression in traumatic brain injured patients
Shawn Rhind, Shawn Rhind, Pang Shek, Andrew Baker, Laurie Morrison, Sandro Rizoli

Objectives: Resuscitation with hypertonic saline has proven successful in optimizing cerebral perfusion and reducing intracranial hypertension in severe traumatic brain injury (TBI) patients. Beyond favorable hemodynamic and osmotic properties, hypertonic solutions exert immunomodulatory effects that may convey neuroprotection against secondary inflammatory injury by dampening excessive leukocyte activation, adhesion and infiltration into damaged brain.

Patients and methods: This prospective, randomized controlled trial investigated the impact of prehospital resuscitation of severe TBI (GCS ≤ 8) patients, with paramedic administration of a single 250-mL bolus infusion of 7.5% hypertonic saline combined with 6% dextran-70 (HSD) versus 0.9% normal saline (NS), on selected cellular and soluble leukocyte and endothelial-derived activation/adhesion molecules. Serial blood samples were drawn from 65 adult patients (30 HSD, 35 NS) at the time of hospital admission and at 12, 24, and 48-h post-resuscitation. Flow cytometry and immunoassay was used to analyze polymorphonuclear neutrophil (PMN) and monocyte cell-surface adhesion/degranulation molecules (CD11b, CD62L, CD66b, CD63), and serum concentrations of soluble (s)L-selectin, vascular and intercellular adhesion molecules (sVCAM-1, sICAM-1, sE-selectin). Twenty-five healthy control subjects were studied for comparison. Statistical significance (P ≤ 0.05) assigned by repeated measures ANOVA.

Results: Upon admission, PMN counts were significantly lower in patients receiving HSD versus NS. Relative to control, NS-treated patients showed up to twofold higher surface expression of CD62L, CD11b and CD66b on PMNs and monocytes that persisted for 48-h. HSD blunted expression of these cell-surface activation/adhesion molecules at all time-points to levels approaching control values. Admission concentrations of endothelial-derived sVCAM-1 and sE-selectin were reduced in HSD patients. Levels of sL-selectin were initially lower in all patients, but exhibited a delayed rise after HSD treatment. Conclusions: These findings support an important protective role of early HSD resuscitation in attenuating upregulation of
leukocyte/endothelial cell inflammatory molecules, which may help ameliorate secondary neuroinflammatory processes after TBI. Funded by Defence R&D Canada.

Corresponding Author: Shawn Rhind, PhD, DRDC Toronto, Department of Immunology, 1133 Sheppard Avenue West, Toronto, ON M3M3B9, Canada, shawn.rhind@drdc-rddc.gc.ca

A 359

Hypoalbuminaemia is an independent risk factor for the development of surgical site infection following gastrointestinal surgery: a multi-institutional study

Derek Hennessey, John Burke, Tara Ni-Donochu, Conor Shields, Des Winter, Kenneth Mealy

Background: Surgical site infection (SSI) is an infection occurring in an incisional wound within 30 days of surgery and significantly impacts patient recovery and hospital resources. Objective: This study sought to determine the relationship between preoperative serum albumin and SSI.

Patients and methods: A study of 524 patients who underwent gastrointestinal surgery in four institutions was performed. Patients were identified using a prospective SSI database and hospital records. Serum albumin was determined preoperatively in all patients. Hypoalbuminaemia was defined as albumin <30 mg/dL. Data is presented as median (interquartile range) and differences between groups was examined using Mann–Whitney U and Fishers exact tests and multiple logistic regression analysis.

Results: 105 patients developed a SSI (20%). The median time to the development of SSI was 7 (5–10) days. Having an emergency procedure (P = 0.003), having a procedure over 3 h in duration (P = 0.047), being ASA grade 3 (P = 0.03) and not receiving preoperative antibiotics (P = 0.007) were associated with SSI whilst having a laparoscopic procedure reduced the likelihood of SSI (P = 0.004). Patients who developed a SSI had a lower preoperative serum albumin (30 (25–34.5) vs. 36 (32–39), P < 0.001). On multivariate analysis, hypoalbuminaemia was an independent risk factor for SSI development (RR 5.68, 95% CI: 3.45–9.35, P < 0.001). Patients who developed a SSI had a lower preoperative serum albumin (30 (25–34.5) vs. 36 (32–39), P < 0.001). On multivariate analysis, hypoalbuminaemia was an independent risk factor for SSI development (RR 5.68, 95% CI: 3.45–9.35, P < 0.001). Albumin <30 mg/dL was associated with an increased rate of deep versus superficial SSI (P = 0.002). The duration of inpatient stay was negatively correlated with pre-operative albumin (R² = -0.319, P < 0.001).

Conclusions: Hypoalbuminaemia is an independent risk factor for the development of SSI following gastrointestinal surgery and is associated with deeper SSI and prolonged inpatient stay.

Corresponding Author: Derek Hennessey, MD, St James’s Hospital, Institute of Molecular Medicine, St James’s Street, 2 Dublin, Ireland, derek.hennessey@gmail.com

A 360

Long-term survival after surgical critical illness: the impact of prolonged preceding organ support therapy

Christian P. Schneider, Jan Fertmann, Simon Geiger, Helmut Kuechenhoff, Karl-Walter Jauch, Wolfgang H. Hartl

Objective: Nothing is known about the effect of preceding ICU-related therapies on long-term outcome. The objective, therefore, of the present study was to identify the prognostic importance of preceding invasive ventilation, renal replacement therapy and catecholamine therapy for long-term survivors after surgical critical illness. Methods: We performed a retrospective analysis of prospectively collected data of an ICU patient cohort linked to a local database. Adult patients (n = 1462) admitted to a 12-bed ICU between 1993 and 2005, who had an ICU length of stay of more than 4 days, were followed-up until the end of the second year after ICU admission. Hazard function was explored by Weibull modelling and likelihood ratio tests. Cox-type structured hazard regression models were used to analyze linear, non-linear or time-varying associations of therapeutic variables with 2-year survival time of a patient subgroup, which had survived the period of high hazard.

Results: Hazard rate declined exponentially up to day 195 after ICU admission, and became constant thereafter. 808 patients reached this stable stage of their disease forming the study population. 648 of these patients (80.2%) were still alive at the end of the second year after ICU stay. Underlying diseases were major determinants for long-term outcome. Long-term mortality was significantly associated with the acute extent of physiological derangement during ICU stay (maximum APACHE II score), but was independent from the duration of preceding invasive organ support.

Conclusion: In surgical patients with a prolonged ICU length of stay, an exorbitant mortality exists for about half a year after ICU admission. Later on, life expectancy of surviving patients is largely determined by the underlying disease and, to a minor degree, by the acute extent of homeostatic disturbance during ICU stay. The duration of preceding invasive therapies does not limit long-term survival.

Corresponding Author: Christian P. Schneider, MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Surgery, Marchioninistr. 15, 81377 Munich, Germany, christian.schneider@med.uni-muenchen.de

A 361

SOFA-score is predictive of outcome in severely injured patients irrespective of the leading injury

Adrian Billeter, Matthias Turina, Christoph Duebendorfer, Valentin Neuhaus, Marius Keel, Hans-Peter Simmen

Objective: Arterial lactate values, base excess (BE), lactate clearance, and sequential organ failure assessment score (SOFA) have been shown to correlate with outcome in severely injured patients. However, the patient populations studied are often heterogeneous to the type of injury, and the role of the above parameters in different subsets of trauma patients remains unclear. The goal of our present study is to assess the predictive value of the above four parameters in patients suffering from traumatic brain injury (TBI) as opposed to patients suffering from injuries not related to the brain.

Material and methods: 724 adult patients with an injury severity score (ISS) ≥16 were enrolled, excluding all burn patients, secondary referrals at any time point and patients receiving comfort therapy only. The patient collective was divided into trauma patients without TBI (group 1, NTBI), patients with isolated TBI (group 2), and patients with a combination of TBI and non-TBI injuries (group 3).

Results: The mean age of all patients (77% male) was 40 years at a mean ISS of 32 (16–75). Mortality ranged from 13% (group 1) to 24% (group 3). Admission and serial lactate/BE values were higher in non-survivors in groups 1 and 3 (all p < 0.01), but not in group 2. Subsequently septic patients had higher lactate and BE levels >4 h after admission except for patients with isolated TBI, in whom no distinction could be made. Admission SOFA-scores were higher in non-survivors in all groups (p ≤ 0.023); in subsequently septic patients, a distinction between septic and non-septic patients was only possible in groups 1 and 3 (p ≤ 0.005). Stepwise logistic regression and ROC-curve analysis revealed SOFA-score as best predictor for death irrespective of the leading injury. Lactate and BE showed good predictive
value in patients suffering from hemorrhagic shock (mainly group 1), whereas in patients with isolated TBI, initial GCS remained the most powerful predictor of mortality. 

Conclusion: Scores based on a range of physiologic data such as the SOFA-Score shows superior performance in predicting outcome in trauma patients suffering from injuries other than isolated TBI compared to single parameters such as lactate or BE. The observed differences are greatest in patients with profound physiologic derangements following excessive bleeding. Patients with isolated TBI, however, must be analyzed separately due to the overall poor performance of most prognostic parameters.

Corresponding Author: Adrian Billeter, MD, University of Zurich, Department of Trauma Surgery, Raemistr. 100, 8091 Zurich, Switzerland, adrianbilleter@bluewin.ch

A 362
A web based multi-center patient data registry for open abdominal management
John P. Kirby, Florian Herrle, Jens-Olaf Jonesheit, Douglas J.E. Scheurer

Objectives: (1) Formulate how private industry and academic medicine can properly work together to study the problem of the difficult to close abdomen. (2) Utilize clinical practice guidelines for a standardized set of data points for a patient de-identified, web-based data registry for open abdomen and closure therapeutic techniques. (3) Utilize data registry reviews for a future prospective, randomized trial of abdominal closure techniques.

Patients and methods: Review of Barnes-Jewish Hospital Acute and Critical Care Surgery database of patients admitted to the 24 bed Surgical Intensive Care Unit after exploratory laparotomy. Basic patient demographics, reasons for exploratory laparotomy, interventions at surgery, initial open abdominal management, SICU course, final closure technique and clinical outcomes will be tabulated.

Results: The Acute and Critical Care Service performed over 260 exploratory laparotomies. Of those available for review: more than a third had elevated BMI, more than half had 2/3 components of the lethal triad for staged laparotomy, and the remaining had peritoneal contamination for staged laparotomy open abdominal management. Final closure techniques varied over the life-span of the database: negative pressure dressings, primary fascial closures, absorbable mesh closures with delayed split thickness skin grafting, controlled tension fascial closures and fascial closure with biologic augmentations.

Conclusions: Although individual institutions and professional societies have published practice recommendations for open abdominal management and closure techniques; none, including this database review, have sufficient statistical power for authoritative recommendations. Although increasingly accepted, leaving a patient’s abdomen open at the end of the initial operation remains a clinical judgment. Once left open, optimal closure timing and technique is dependent upon a wide array of variables including injury response status, ventilator wean progress, either immunologic deficits, body habitus, resuscitation volumes, and local tissue limitations. Given current research funding availabilities and that many of these techniques now rely either completely or in part upon commercial surgical technologies, proper involvement of private industry will need to be formulated to execute needed research. We conclude that randomized, prospective multi-centered trials should be orchestrated for a Closure of the Abdomen Techniques Study: C.O.A.T.S.

Corresponding Author: John Kirby, Prof, MD, Washington University St. Louis, Department of Surgery, 660 South Euclid Avenue, Campus Box 8109, St. Louis, MO 63110-1093, USA, kirbyj@wudosis.wustl.edu

A 363
A population based study on the influence of viral infections among ICU patients in the United States
Makesha Miggins, Anjum Hasan, Sam Hohmann, Phil Efron, Huazhi Liu, Darwin Ang

Objectives: 1. To examine the effects of viral infections on the outcomes of immunocompetent intensive care unit (ICU) patients. 2. To identify potential variables amenable to intervention among those infected with viruses.

Materials and methods: A population based retrospective cohort study of academic medical centers in the United States using The University HealthSystem Consortium (UHC) database. The index population consisted of immunocompetent ICU patients age 18 yrs or older who were not admitted for primary diagnosis of infection but who likely developed bacterial and or viral infection in the hospital between 2006 and 2009. The patients were divided into four cohorts; individuals without a documented infection (negative), those with viral, those with bacterial, and those with both bacterial and viral infections (combination) according to culture results. Outcomes were compared among the cohorts using multiple variable logistic and linear regressions. Data were adjusted for age, gender, race, and hospital length of stay.

Results: Approximately 250,000 patients meeting study inclusion criteria were identified. Of those, 10,587 had a positive viral culture. Culture validated viral infections were associated with an increased likelihood of mortality over patients who had negative cultures, adjusted OR 1.89 (1.72, 2.07). Patients infected with viruses did not have an increased risk of adverse outcomes compared to those who were infected with bacterial infections. However, bacterial and viral co-infections had a combined effect on mortality OR 7.38 (6.81, 8), as well as ARDS 1.59 (1.29, 1.97), respiratory failure 1.19 (1.09, 1.30), and diarrhea 1.28 (1.06, 1.53).

Conclusions: Viral and bacterial co-infections among immunocompetent ICU patients appear to have an increased overall effect on several key adverse outcomes compared to viral or bacterial infections alone. Knowledge of the role viruses play in these poor outcomes may lead to alterations in medical practice patterns and improved patient outcomes.

Corresponding Author: Makesha Miggins, MD, University of Florida, Department of General Surgery, 1600 SW Archer Road, Gainesville, FL 32610, USA, makesha.miggins@surgery.ufl.edu

A 364
Clearing polytrauma patients for major surgery: which parameters are the best?
Philipp Lichte, Philipp Kobbe, Ivan Tarkin, Hans-Christoph Pape

Purpose: In polytrauma, the aim of fracture management is to perform initial definitive stabilization whenever the patient is in adequate condition. Several clinical parameters have been described to assess the patient’s clinical condition. We tested which parameters best describes the patient condition.

Methods: Prospective cohort study. Inclusion criteria: New Injury Severity Score (NISS) >16 points, or 3 fractures and Abbreviated Injury Scale (AIS) ≥2 points and another injury (AIS ≥2 points), and age 18–65 years. Exclusion: patients in unstable or critical condition. Parameters tested for grading of whether or not a patient is cleared: Systolic blood pressure, volume replacement, no. of blood units/6 h, AIS of all body regions, thoracic trauma score, lung contusion, platelet count, Prothrombin time (PTT). Endpoints consisted of...
clinical complications (Pneumonia, Sepsis, ARDS, ALI, Multiple organ failure).

Statistics: Parameters were tested for normal distribution. A sensitivity and specificity analysis was performed for all variables individually. In addition, variable combinations (double and triple factors) were tested to see if they led to an increase in sensitivity. Specificity values are in parentheses below:

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<td>Single factors:</td>
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<td>AIS Chest Score (&gt;2)</td>
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<td>PTT Elevated (&gt;45 s)</td>
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<td>AIS Chest Score OR Fluids Score (&gt;1.5 L received)</td>
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The systolic blood pressure and the numbers of blood units administered were not sensitive in predicting complications.

Conclusion: The most sensitive individual parameters were the AIS chest score, elevated PTT levels, fluid requirements and the presence of a lung contusion at time of admission. By using a combination of three factors, the predictive value for developing complications was improved.

Significance: A combination of conventional scores and physiologic parameters is useful to judge the clinical condition and clear a patient for surgery.

Corresponding Author: Philipp Lichte, MD, University of Aachen, Department for Orthopedic Surgery, Pauwelsstr. 30, 52074 Aachen, Germany, philipp.lichte@googlemail.com

AbstractID: 274

A 365

Differential expression of MAP kinases and C-Jun in patients with adverse outcome after multiple trauma

V. Stoecklein¹, V. Bogner², P. Richter³, C. Suren¹, D. Teupser², J. Thiery⁴, W. Mutschler¹, P. Biberthaler³

¹Department of Traumatology and Orthopedic Surgery, Ludwig-Maximilians-University Munich, ²Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig

Objective: Posttraumatic immune system dysfunction like SIRS and subsequent MODS is still a significant cause of morbidity and mortality. Monocytes are known to play a pivotal role for this pathologic state. It was the goal of our study to investigate the role of the MAP kinases p38 and JNK and their downstream substrate c-Jun in monocytes of patients with multiple injuries. Previous work by our group had implicated this pathway in adverse outcome using genome-wide mRNA microarray screening. Patients and methods: 37 patients with an ISS >16 points were enrolled in our study. Blood was collected at six time-points: at admission (45 s) 66%, 73%, after injury), 6, 12, 24, 48 and 72 h after trauma. Monocytes were isolated and nuclear protein was extracted. Protein expression was measured using the Bio-Plex Phosphoprotein Detection System (Bio-Rad). Resulting data was statistically analyzed with SigmaStat (Systat). A p value of p < 0.05 was considered significant.

Results: Seven patients died within the 90-day follow-up period. Patients who succumbed to their injuries exhibited increased p38 MAPK expression at all six time-points when compared to surviving patients. At 48 and 72 h the differences between the two groups were statistically significant (p = 0.017 and p = 0.024). JNK expression was higher in deceased patients at three of six time-points. No statistically significant differences between the two groups could be detected. C-Jun expression was higher in patients who died at all time-points. Statistically significant differences between the two groups were demonstrated at admission as well as 24, 48 and 72 h after trauma (p = 0.018, p = 0.014, p = 0.021, p = 0.014).

Conclusion: We were able to show that the MAP kinase pathway and one of its major downstream substrates seem to play an important role for an adverse outcome after multiple trauma. Especially the transcription factor c-Jun is differentially expressed in patients who died. This is supported by the findings of a previous mRNA expression study. P38 MAPK and JNK, which are upstream activators of c-Jun, were differentially expressed as well. C-Jun, acting as a component of the AP-1 transcription factor complex, governs many important functions in the immune system like transcriptional control of cytokine gene expression. Therefore, studies to clarify the role of c-Jun in the immunologic pathophysiology after trauma and the mechanisms and kinetics of its activation further upstream seem to be promising.

Corresponding Author: Veit Stoecklein, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Department of Traumatology and Orthopedic Surgery, Nussbaumstr. 20, 80336 Munich, Germany, veit.stoecklein@med.uni-muenchen.de

A 366

Early neutrophils dysfunction precedes late onset septic complications in multi trauma patients


Objective: Severely injured trauma patients are prone for the development of post-operative septic and non-septic inflammatory complications. Early identification of patients at risk will aid the prevention of these late life-threatening complications. The aim of this study was to test whether the extend of the initial (<12 h) immunological response after injury was related to the late (>6 days) development of immune dysfunction and septic complications such as sepsis and septic shock.

Materials and methods: We performed a prospective, observational, cohort study. We validated our results with an independent international replication cohort. In the Netherlands we included a consecutive series of severely injured trauma patients requiring ICU admission. In South Africa a replication cohort of severely injured trauma patients (ISS >16) was included. Blood samples of multi trauma patients were analyzed for neutrophil phenotype with the use of flowcytometry. We measured neutrophil activation by determining expression of sensitive activation markers and the responsiveness for the innate immune stimulus fMLP.

Results: Of the 109 included patients, 48 developed sepsis and thirteen died. In patients who developed sepsis, neutrophils showed a
lower expression of active FcγRII and a low responsiveness for the innate stimulus fMLP immediately (<12 h) after trauma. This low responsiveness was visualized by a low induction of active FcγRII by this stimulus. This data reflects systemic dysfunction of the innate immune system. In addition, neutrophils showed decreased FcγRIIIB (CD16) expression, indicative for recruitment of immature cells.

Conclusion: These results show that multiple injuries lead to a dysfunctional systemic innate immune system within 12 h. Also, this study shows that these results do not depend on the geography/racial background of the patients’ population, hospital protocols and health care facilities. Systemic neutrophils with a low responsiveness towards fMLP in the context of activation of FcγRII on neutrophils could be a predictor for the development of inflammatory complications such as septic shock as this low responsiveness preceded the onset of clinical symptoms by almost a week. This opens possibilities for preventive measures for limiting inflammation in those patients with most pronounced immune dysfunction immediately after trauma.

Corresponding Author: Kathelijne Groeneveld, MD, University of Utrecht, Medical Center, Department of Surgery, Heidelberglaan 100, 3508 GA Utrecht, Netherlands, k.m.groeneveld@umcutrecht.nl

A 367
‘Spine damage control’: a safe and effective treatment modality for unstable spine fractures in multiply injured patients
Philip Stahel, Michael Flierl, Ernest Moore, Kathryn Beauchamp, Anthony Dwyer

Objective: The “ideal” timing and modality of unstable spine fracture fixation in polytrauma patients remains controversial. While the concept of “damage control orthopedics” has been widely implemented in multisystem trauma, a “spine damage control” (SDC) approach for unstable spine injuries remains unexplored. The present study was designed to evaluate the safety and efficacy of an institutional SDC protocol for multiply injured patients with unstable spine fractures.

Patients and methods: Our institutional SDC protocol mandates immediate posterior spine fracture fixation within 24 h of admission, followed by a staged anterior 360° completion fusion, if indicated. From 10/2008 to 10/2009, 50 consecutive polytrauma patients with unstable spine fractures were prospectively entered into a database. Nineteen patients were treated by SDC, while 31 patients underwent unstable spine fractures were prospectively entered into a database. Nineteen patients were treated by SDC, while 31 patients underwent unstable spine fractures within 24 h, “delayed surgery”/DS group. The two cohorts were analyzed for demographics, length of operative time, intraoperative blood loss, total hospital/ICU length of stay, ventilator-dependent days, and postoperative complications.

Results: Both cohorts were comparable with regards to age, distribution of spine fracture level, and intraoperative blood loss. The DS group displayed a trend towards a higher injury severity compared to SDC patients (ISS 25.3 vs. 19.6 points, n.s.). The mean time to initial spine fixation was 14.4 h (SDC) vs. 95.9 h (DS). The SCD cohort had significantly reduced operative time (1.8 vs. 3.5 h, P < 0.05), significantly reduced length of hospital stay (18.4 vs. 30.3 days, P < 0.05), ventilator-dependent days (4.9 vs. 6.7 days, P < 0.05), and incidence of urinary tract infections (5 vs. 22%, P < 0.05). The SDC group displayed a non-significant trend towards reduced ICU length of stay (8.9 vs. 9.9 days), reduction of pulmonary complications (16 vs. 23%) and pressure sores (6 vs. 0%), but higher incidence of postoperative hardware problems (16 vs. 3%).

Conclusion: A standardized SDC approach represents a safe and efficacious treatment strategy in multiply injured patients with unstable spine fractures. Larger multicenter trials will have to be designed to formally validate the safety and efficiency profile of SDC.

Corresponding Author: Philip Stahel, MD, Denver Health Medical Center, Department of Orthopaedics, 777 Bannock Street, Denver, CO 80204, USA, philip.stahel@dhh.org

A 368
Acute hemolysis in surgical patients is independently associated with renal tubular damage and predicts acute kidney injury
Iris Vermeulen Windsant, Maarten Snoeijis, Sebastiaan Hanssen, Geert Willem Schurink, Wim Buurman, Michael Jacobs

Objective: Acute kidney injury (AKI) negatively affects patient outcome after trauma and extensive surgery. Hemolysis is a common phenomenon in these patients, resulting in increased plasma free hemoglobin (fHb) levels. Recent studies demonstrated fHb as a potent scavenger of intravascular nitric oxide, the most important endogenous vasodilator. Therefore, hemolysis is associated with microcirculatory impairment resulting in organ damage. Since renal tubular cells are particularly vulnerable to changes in tissue perfusion, we investigated the relation between acute hemolysis, renal tubular damage and AKI in a clinical setting of major surgery.

Material and methods: 35 patients undergoing surgical repair of thoracoabdominal aortic aneurysms with cardiopulmonary bypass were consecutively included. Plasma fHb and the tubular injury marker N-acetyl-β-D-glucosaminidase (NAG) in urine were measured at 8 perioperative time points. Postoperative AKI was defined according to the AKIN classification which is based on perioperative serum creatinine changes. Multivariable linear regression analysis enabled correction for confounding variables. Receiver operating characteristics (ROC) curves were used to analyze the predictive value of plasma fHb and urine NAG for AKI.

Results: During surgery, plasma fHb and urinary NAG increased in all patients. Peak levels of plasma fHb and urinary NAG were reached at 2 h and 15 min after cessation of cardiopulmonary bypass, respectively (P < 0.001 compared to baseline for both). AKI was diagnosed in 19 patients (54%). Peak fHb and NAG concentrations were significantly higher in patients with AKI (P < 0.05). Total plasma fHb release was independently related to urinary NAG levels (P = 0.001), which in turn was independently associated with postoperative serum creatinine increase (P = 0.002). Importantly, both peak plasma fHb and urine NAG levels predicted postoperative AKI (area under ROC-curve 0.73, P = 0.04 and 0.76, P = 0.01, respectively).

Conclusion: In this study, an independent association was demonstrated between acute hemolysis and renal tubular injury. In addition, peak fHb concentrations predicted postoperative AKI. These findings cast new light on the pathophysiology of AKI and identify hemolysis as a potential therapeutic target to preserve renal function and improve general outcome in patients after trauma and extensive surgery.

Corresponding Author: Iris Vermeulen Windsant, MD, Maastricht University Medical Center, Department of Surgery, Universiteitskliniek, 6200 MD Maastricht, The Netherlands, i.vermeulenwindsant@ah.unimaas.nl

A 369
Targeting cytokines in chronic inflammatory disease: treatment rather than cure?
Fionula Brennan

The involvement of cytokines in the molecular events associated with chronic inflammation in particular in rheumatoid arthritis (RA)
A 370

Toll-like receptor antagonists: therapeutic interventions/clinical trials in severe sepsis/septic shock
Daniel Rossignol, Alec Wittek, James McShane, Melvyn Lynn

A wide variety of inflammatory events of sepsis can be attributed to activation of the innate immune system, and specifically, TLR4. Activation of TLR4 may be by direct means—from infection-related ligands such as endotoxin or lipopolysaccharide (LPS) or indirectly from a variety of endogenous ligands such as heat shock proteins and hyaluronic fragments that signal cell distress and necrosis (due to inflammatory response or trauma). It is proposed that overly-robust stimulation of TLR4 by overwhelming infection (or a high endotoxin level) generates a proinflammatory response that ultimately proves harmful to the host. Eritoran (E5564), is a novel Lipid A analogue/TLR4 antagonist that forms an inactive heterotrimer with the MD-2/TLR4 complex. Preclinical development and early-Phase clinical studies including translational research studies uncovered a number of unique characteristics of eritoran that required expanded clinical research and development. Consequently, PK and PD models were established that enabled selection of two dose levels (Q12 h dosing) for Phase II clinical evaluation.

In a Phase II study, the safety and efficacy of eritoran was evaluated in 300 patients with severe sepsis and 20–80% predicted risk of mortality (PROM) by APACHE II score. Two treatment regimens, 45 or 105 mg of eritoran, or placebo, were given over 6 days. Results demonstrated an acceptable safety profile and a 6.7% reduction in 28-day all-cause mortality (study not powered to demonstrate statistical significance) in patients with severe sepsis. These results have supported the development and current implementation of a multinational Phase III study in patients with severe sepsis.

Corresponding Author: Daniel Rossignol, PhD, Eisai Inc., Product Creation, 155 Tice Blvd, Woodcliff, NJ 07677, USA, dan.rossignol@eisai.com
for their ability to affect MSC migration at normal (5%) and reduced oxygen levels (1%). The substances were also tested for their capacity to stimulate cell proliferation and to induce cell differentiation. Under normoxia, TNF-$\alpha$, bFGF, VEGF, PDGF, SDF-1, FGF, enhanced MSC migration, IGF-1, IL-4, INF-$\gamma$, had no effect. Under hypoxia, INF-$\gamma$ gained the ability to enhance MSC migration, whereas VEGF and SDF-1 lost it. Generally it was found that hypoxic conditions increase the migratory ability of MSC.

We also investigated so called nutraceuticals (resveratrol, epigallocatechin gallate (EGCG), reversine, valproic acid, 5-Aza-2'-deoxycytidine and sodium selenite) on the migratory ability of MSC but found no significant effect on migration or differentiation profiles, but all mildly enhanced cell proliferation.

Pre-incubation of MSC with INF-$\gamma$ as under inflammatory conditions, decreased the effects of bFGF-stimulated migration.

Analysis of the substances secreted by the MSC themselves showed that the secretory profile changes in aged MSC and can be partially restored under hypoxic conditions.

**Corresponding Author:** Elisabeth Meyer, MD, University of Berlin Charité, Campus Benjamin Franklin, Institute for Hygiene and Environmental Medicine, Hindenburgdamm 27, 12203 Berlin, Germany Elisabeth.Meyer@charite.de

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**A 374**

**Assessment of T-cell function**

Gaëlle Monneret

Septic syndromes still remain a major but largely under-recognized healthcare problem worldwide accounting for thousands of deaths every year. Despite numerous clinical trials, most of therapies have failed to mitigate the devastating effects of sepsis. It is now agreed that the initial hypotheses for sepsis pathophysiology have been too simplistic. Sepsis deeply perturbs immune homeostasis by concomitantly inducing a strong inflammatory response and a major anti-inflammatory process, acting as a negative feedback. Several lines of evidecnes indicate that this inhibitory response secondly becomes deleterious in patients who survived initial resuscitation, as it may be directly responsible for worsening outcome by decreasing resistance to secondary nosocomial infections or viral reactivation. In this context, while the majority of clinical and basic science conducted so far has focused on innate immune cell depressed functions (especially monocytes), the contribution of T lymphocyte anergy has been somewhat ignored. This presentation focuses on lymphocyte dysfunctions described so far in patients, on biomarkers usable to diagnose and monitor those alterations (CD3 expression, % of circulating regulatory T cells, T cell repertoire, functional testing...) and on potential new therapeutic strategies aimed at restoring a functional lymphocytic response after sepsis.

**Corresponding Author:** Gaëlle Monneret, PhD, Hospices Civils de Lyon, Department of Immunology, Hopital E. Herriot, 69003 Lyon, France, gaëlle.monneret@chu-lyon.fr

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**A 375**

**Cathepsin X and PMN elastase as potential inflammatory markers**

Dorit Naegler, Marianne Jochum

Understanding the pathophysiology of inflammatory processes and the complex mechanisms of host defense is essential for the development of effective therapies aimed at inhibiting components of the mediator cascade. In particular, early evaluation of prognosis in various inflammatory conditions is difficult and the predictive value of available inflammatory markers is quite uncertain. Here, we have investigated the proteolytic enzymes cathepsin X—a cysteine protease—and PMN elastase—a serine protease—for their potential use as inflammatory markers. With regard to its proteolytic specificity, cathepsin X is a carboxypeptidase and may be able to modulate inflammatory processes through C-terminal processing of peptide hormones such as kinins. In contrast, elastase is an endopeptidase with broad substrate specificity and considerable degradative potential which is rapidly inhibited by z1-PI and eliminated from the circulation. Cathepsin X is mainly released from monocytes/macrophages which are activated at a somewhat later stage of the inflammatory process, while elastase originates from the primarily activated polymorphonuclear leukocytes (PMNs).

In patients suffering from multiple trauma, both, cathepsin X and PMN elastase levels were increased in the circulation during the early postrau traumatic phase. Within the first 72 h after trauma, plasma levels of cathepsin X rose significantly, particularly in patients who did not survive the postrau traumatic period. Notably, cathepsin X levels in plasma remained elevated for up to seven days after multiple trauma or surgical procedures. Elastase levels peaked during the first 6 h after trauma, followed by a decrease and a second peak after 48 h in patients with severe complications. Similar to cathepsin X, increases in elastase levels were higher in nonsurvivors. These results confirm our previous data with regard to the prognostic value of PMN elastase in patients with multiple trauma and identify cathepsin X as a novel inflammatory marker.

In conclusion, the quantification of proteases released from both monocytes/macrophages and PMNs during inflammatory processes either alone or in combination with other inflammatory markers may allow the early identification of patients at high risk of developing complications.

**Corresponding Author:** Dorit Naegler, PhD, Ludwig-Maximilians-University, Campus Innenstadt, Division of Clinical Chemistry and Clinical Biochemistry, Department of Surgery, Nussbaumstr. 20, 80336 Munich, Germany, dorit.naegler@med.uni-muenchen.de

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**A 375**

**Neopterin to predict outcome in patients with trauma and sepsis**

Dietmar Fuchs, Martin Ploder, Katharina Kurz, Erich Roth

Objective: Immune system activation and inflammation accompany immune dysfunction in trauma and sepsis patients. In this group of patients increased neopterin concentrations in body fluids predict unfavourable outcome. Thereby the determination of neopterin by using immunoassays is stable and reliable and fulfils all the criteria of quality control for laboratory diagnostic applications.

Results: The increased neopterin derives from monocyte-derived macrophages and dendritic cells and relates to an enhanced pro-inflammatory Th1-type immune response. Thus, neopterin concentrations are closely associated with the immunopathogenetic pathways of sepsis, e.g., neopterin concentrations correlate strongly with circulating cytokines TNF-$\alpha$ and IL-6 [1] but also with features of the immunocompromised host such as the diminished capacity of monocytes to respond to ex vivo stimulation with LPS [2]. Increased neopterin concentrations also coincide with the activation of immunosuppressive biochemical pathways such as the induction of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) which is indicated by an enhanced kynurenine to tryptophan ratio (kyn/trp). Like the elevated neopterin concentration, also an enhanced kyn/trp is found to be associated with poor outcome of patients after trauma and with sepsis [3].
Conclusions: The findings could represent a key for the understanding of the negative contribution which an overwhelming innate and adaptive immune response and the production of pro-inflammatory cytokines might have in patients at risk for septic complications. Aside from the tolerogenic activity of activated IDO, also the enhanced neopterin production can contribute to adverse effects of activated macrophages, e.g., by enhancing oxidative stress [4].


Corresponding Author: Dietmar Fuchs, Prof. PhD, Medical University of Innsbruck, Division of Biological Chemistry, Biocenter, Fritz Pregl Str. 36, 6020 Innsbruck, Austria, dietmar.fuchs@i-med.ac.at

A 376

HMGB1 as a potential therapeutic target for sepsis

Haichao Wang, Ph.D. Shu Zhu, M.D., Ph.D., Andrew E. Sama, M.D.

Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection. The inflammatory response is partly mediated by innate immune cells (such as macrophages, monocytes and neutrophils), which not only ingest and eliminate invading pathogens, but also initiate an inflammatory response by producing various proinflammatory mediators. In response to infection, a ubiquitous nucleosomal protein, HMGB1, is actively secreted by innate immune cells, and functions as a proinflammatory alarmin(g) danger signal by recruiting, alerting, and activating various innate immune cells. Consequently, extracellular HMGB1 sustains a rigorous inflammatory response, and contributes to the pathogenesis of inflammatory diseases. A growing number of HMGB1 inhibitors ranging from neutralizing antibodies, endogenous hormones, to medicinal herb-derived small molecule HMGB1 inhibitors (such as nicotine, glycyrrhizin, tanshinones, and EGCG) are proven protective against lethal infection and ischemic injury. Here we review emerging evidence that support extracellular HMGB1 as a proinflammatory alarmin(g) danger signal, and discuss a wide array of HMGB1 inhibitors as potential therapeutic agents for sepsis.

Corresponding Author: Haichao Wang, PhD, North Shore University Hospital, New York University School of Medicine, Emergency Medicine, 350 Community Drive, Manhasset, NY 11030, USA, hwang@nshs.edu

A 377

Surgical treatment for diabetes mellitus type 2

Bruce M. Wolfe

The incidence of type 2 diabetes mellitus (T2DM) has increased in parallel to the increase in obesity among both children and adults indicating a relationship between obesity and T2DM. Review of the natural history of T2DM is critical to the potential for benefits with different surgical interventions. Insulin resistance is the initial metabolic alteration which is reflected a greater insulin secretion than is expected to maintain glucose homeostasis in comparison to normal subjects. There are 2 components to insulin resistance: diminished peripheral uptake particularly by muscle and failure of suppression of gluconeogenesis by the liver in the presence of glucose availability or hyperglycemia. The insulin resistance may be apparent in the fasting state and may be semi-quantitated by the ratio of fasting glucose and insulin (HOMA). A post-prandial or fed state however may be necessary to fully delineate the presence of insulin resistance such that HOMA is not an entirely reliable parameter of insulin resistance. Initially hyper-secretion of insulin is sufficient to maintain normal peripheral glucose concentration, such that clinical diabetes is not apparent. Over a several year time course however, the capacity of the pancreas to maintain the insulin hyper-secretion or increasing severity of insulin resistance leads to increasing degrees of hyperglycemia. Thus, intermediate fasting or post-prandial hyperglycemia may be present prior to establishment of a clinical diagnosis of diabetes. As insulin secretion decreases and/or insulin resistance increases, the condition progresses to clinical diabetes and its secondary manifestations. Over still further time, generally many years, ultimate islet failure may occur, such that insulin therapy is required to overcome substantial insulin resistance. In this situation a distinction between type 1 and type 2 diabetes is not clearly made.

Metabolic factors contributing to insulin resistance and obesity include chronic inflammation with secondary mediators effecting glucose metabolism, changes in adipokine secretion including increased leptin, and diminished adiponectin, hyper-secretion of glucagon and direct inhibition of insulin action in muscle by free fatty acids. Weight loss leads to improvement or clinical remission of T2DM by decreasing carbohydrate intake, decreasing inflammatory mediators associated with obesity, resolution of altered secretion of adipokines and glucagon and diminished FFA action. Thus, the surgical treatment of T2DM leads to remission by changes in dietary intake, weight loss, and changes in gut hormone secretion. These gut hormone changes involve the proximal gut (as discussed by Rubino) and the distal gut stimulation of GLP-1 and PYY.

Research is needed to establish clinical means for establishing the pathophysiology of T2DM in a specific patient so that a prediction can be made regarding the potential for islet stimulation and increased insulin production. This assessment will be particularly important in identifying those T2DM’s whose obesity is less severe than the typical bariatric surgical patient (BMI <30).

Corresponding Author: Bruce M. Wolfe, Prof. MD, Oregon Health and Science University, BTE 223, 3181 SW Sam Jackson Park Road, Portland, OR 97239-3098, USA, wolfeb@ohsu.edu

A 378

Balanced crystalloids and colloids: to be or not to be

David Story

What do we mean by balance? Balance has several meanings including equilibrium and harmonious adjustment of parts. If our target for fluid therapy is the intravascular extracellular fluid, none of our fluids are balanced, rather some cause less imbalance than others. Further, the degree of imbalance that a patient can tolerate will depend on the patient and their circumstances. If we are to individualize patient therapy we need to consider each component of the fluid and how we can harmoniously adjust the parts. For a fit patient having more minor surgery normal saline or saline with starch or gelatine could be given. However even in this scenario the
A 379

**Effect of co-morbidity and bleeding on outcome in patients with major trauma**

Sebastian Wutzler, Arash Wafaisade, Rolf Lefering, Edmund Neugebauer, Marc Maegele, Ingo Marzi

Objective: Mortality after trauma has been shown to be influenced by individual host factors. The effect of age and preexisting medical conditions (PMCs) as well as acute coagulopathy of any cause has not been fully elucidated. We therefore analysed the independent predictive value of specific PMCs for in-hospital mortality after adjustment for injury severity, injury pattern, age, and presence of other PMCs.

Patients and methods: Records of 11,142 trauma patients (18 years of age or older, injury severity score >16, years 2002 to 2007) documented in the Trauma Registry of the German Society for Trauma Surgery were analyzed to assess the association of PMCs with inhospital mortality. Multiple logistic regression models were used for this analysis. Additionally, earlier studies by our group further stratified the effect of early coagulopathy in patients sustaining multiple injuries.

Results: PMCs were affirmed for 3,836 of the 11,142 patients studied (34.4%). An independent statistical association with increased in-hospital mortality was found for 6 PMCs after adjustment for age and the Revised Injury Severity Classification score, respectively, ie, heart disease, obesity, hepatitis/liver cirrhosis, malignancies, coagulation disorder, and peripheral arterial occlusive disease stage IV. The association with mortality varied with different injury patterns. Coagulopathy upon admission was present in 34.2% of all patients. Patients with coagulation disorder had an odds ratio of 1.68 (CI95% 1.17–2.43) for hospital mortality.

Conclusion: Specific PMCs, among them coagulation disorder of any cause, were associated with increased mortality after trauma independent from injury severity and age. Knowledge of the identified relevant PMCs could help the medical team to be able to assess the mortality risk profile of trauma patients in a more detailed and quantifiable way. An early aggressive management of coagulopathy including a balanced administration of blood products is advocated.

**Corresponding Author: Sebastian Wutzler, MD, Johann Wolfgang Goethe University of Frankfurt/M, Department of Trauma-, Hand- and Reconstructive Surgery, Theodor-Stern-Kai 7, 60596 Frankfurt/M, Germany, sebastian.wutzler@kgu.de**

A 380

**Coagulation management during liver transplantation**

Klaus Goerlinger, Daniel Dirkmann, Hannes Muller-Beissenhirtz, Andreas Paul, Matthias Hartmann, Fuat Saner

Objective: Coagulation disorders and massive bleeding are frequent during liver transplantation (LTX). Moreover, blood transfusion is associated with increased morbidity, mortality, and costs. Therefore, in 2000 we implemented thromboelastometry for point-of-care coagulation management in visceral surgery and LTX. In 2004 we developed an algorithm based on thromboelastometry and targeted therapy with coagulation factor concentrates [1, 2, 3]. The goal of our study was to prove if this management is effective in reducing transfusion requirements and costs.

Patients and methods: In our retrospective study we analysed the intraoperative usage of blood products and coagulation factor concentrates and their respective costs from 1999 to 2009 in visceral surgery and LTX at our hospital. Cost calculation was based on prices in December 2009.

Results: From 1999 to 2009 transfusion requirements of red blood cells (RBC) decreased from 3,454 to 1,365 units per year by 60%, fresh frozen plasma (FFP) from 4465 to 499 by 89%, and platelet concentrates (PC) from 433 to 181 by 58%. At the same time usage of fibrinogen concentrate increased from 68 to 745 g per year, prothrombin complex concentrate from 66 to 239 kU, whereas antithrombin concentrate decreased from 151 to 33 kU. No off-label-use of rFVIIa occurred during this period. Reduction of costs for blood products in 2009 compared to 1999 amounted to 498,355€, whereas increase of costs for coagulation factor concentrates amounted to 228,188€. Overall, this resulted in cost-saving of 270,167€ in 2009 compared to 1999 (36%). During the whole study period (2000–2009) cost-saving amounted to 1,765,280€ in visceral surgery and LTX (overall 21,814 surgeries, including 1105 LTX).

Number of LTX per year increased from 97 in 1999 to 143 in 2009 whereas increase of costs for coagulation factor concentrates resulted in distinct reduction of intraoperative blood transfusion requirements and is cost-saving in visceral surgery and LTX.
Reference

Corresponding Author: Klaus Goerlinger, MD, University Hospital Essen, Dept. of Anaesthesiology and Intensive Care Medicine, Hufelandstr. 55, 45122 Essen, Germany, klaus@goerlinger.net

A 381
Indications for and risks of pre-thawed fresh frozen plasma
Martin Schreiber

Exsanguination is the second leading cause of death after trauma and the leading cause of preventable death in civilian and military casualties. Transfusion of high ratios of plasma to packed red blood cells (PRBCs) has been shown to improve survival in patients who require massive transfusion. This effect is greatest when the ratio is achieved early after trauma. In order to maintain its coagulation factor activity, plasma must be frozen soon after blood donation and thawed over approximately 25 min prior to its use. This logistical requirement makes it difficult to maintain high ratios of plasma to PRBCs. One solution to this problem is to maintain a supply of thawed universal donor AB plasma at all times. Units of thawed plasma can be kept for up to 5 days and studies reveal that coagulation factor activity is relatively well preserved during this time period. Potential risks of utilizing pre-thawed plasma include increased plasma waste and transfusion related acute lung injury (TRALI). Several series reveal that one of the beneficial results of a high ratio strategy is decreased overall blood product usage secondary to early correction of coagulopathy. In addition, waste of pre-thawed plasma rarely occurs in busy centers where utilization is high. TRALI has become the leading cause of transfusion related death. It primarily occurs after transfusion of plasma containing products from previously pregnant female donors. Most blood banks have stopped using fresh frozen plasma derived from female donors to reduce the risk of TRALI but use of platelets from female donors continues. A potential future solution to this problem is to maintain a supply of thawed universal donor plasma. The use of lypophilized plasma. Lypophilized plasma can be stored in a broad temperature range for years and reconstituted in less than a minute facilitating the maintenance of high ratio transfusions. In conclusion, the use of pre-thawed plasma is indicated to achieve high transfusion ratios in patients undergoing massive transfusion. The benefits of pre-thawed plasma outweigh the potential risks.

Corresponding Author: Martin Schreiber, MD, Oregon Health and Science University, Department of Surgery, 3181 SW Sam Jackson Park Road, Mail Code L611, Portland, OR 97239, USA, schreibm@ohsu.edu

A 382
Netrin-1 reduces LPS-induced acute lung injury through the adenosine 2B receptor
Valbona Mirakaj, Lausher Stefanie, Mielke Carina, Morote Julio Cesar, Koehler David, Rosenberger Peter

Objective: Acute pulmonary inflammation may result in acute lung injury (ALI). A hallmark of ALI is the infiltration of leukocytes into the alveolar space. Netrin-1 is an endogenous anti-inflammatory protein controlling the migration of leukocytes. The role of netrin-1 during acute inflammation is not thoroughly investigated. Therefore we studied whether netrin-1 influences ALI.

Methods: HMEC-1 and A549 were exposed to pro-inflammatory cytokines and netrin-1 mRNA and protein expression determined. The putative netrin-1 promoter and NFκB truncation were cloned into a PGL-4 luciferase vector. Approval was obtained by the IRB and the Regierungspraesidium Tuebingen. WT, Ntn−/− and A2BAR+/− animals were exposed to LPS or NaCl, and then subsequently to nebulised vehicle or exogenous netrin-1. Netrin-1 expression, bronchoalveolar lavage (BAL) for cell count and protein content, myeloperoxidase activity and acute lung injury score were determined. Expression levels of pro-inflammatory cytokines within the BAL were determined.

Results: Netrin-1 mRNA was significantly reduced in HMEC-1 and A549 through inflammatory cytokines. Protein analysis confirmed these results. The luciferase activity of the putative netrin-1 promoter was significantly reduced in a NFκB dependent fashion. Ntn−/− animals demonstrated significantly increased pulmonary inflammation compared to Ntn−/+ animals following LPS inhalation. In WT mice, inhalation of exogenous netrin-1 resulted in a significant reduction of pulmonary inflammation (BAL cell count × 106: NaCl 0.13 ± 0.02; LPS + NaCl 1.95 ± 0.13; LPS + netrin-1 0.7 ± 0.13). In the A2BAR−/− mice, netrin-1 did not reduce the BAL cell count (BAL cell count × 106: NaCl 0.10 ± 0.01; LPS + NaCl 2.16 ± 0.4, LPS + netrin-1 1.69 ± 0.18). These results were reflected in the histological sections of pulmonary tissue. Expression levels of TNF−, IL−1β and IL-6 within the BAL were also reduced through exogenous netrin-1 in an A2BAR dependent fashion.

Conclusion: Netrin-1 expression is significantly reduced during acute pulmonary inflammation. Administration of exogenous netrin-1 results in decreased pulmonary inflammation, an effect dependent on the A2BAR.

Corresponding Author: Valbona Mirakaj, MD, PhD, University Hospital Tuebingen, Department of Anaesthesiology, Waldhoernlestr. 22, 72072 Tuebingen, Germany, valbona.mirakaj@klinikum.uni-tuebingen.de

A 383
Monoaminoxidase A in sepsis and septic shock
Gordon Otto, Gordon Philipp Otto, Maik Sossdorf, Stephan Scholz, Ralf Alexander Claus, Michael Bauer

Objective: The use of hydrocortisone (HC) for the treatment of septic shock is controversial. Interestingly, micro-array analyses from the CORTICUS trial showed that monoaminoxidase-A (MAOA) is one of the most strongly upregulated transcripts in patients (pts) with septic shock receiving HC. MAOA is involved in generation of reactive oxygen species in mitochondria as well as in metabolism of catecholamines. Therefore, MAOA could play an important role in infection defence as well as catecholamine metabolism in pts with septic shock receiving HC. This study investigates MAOA in pts with septic shock as well as potential benefits of specific inhibition with Clorgylin (CL).

Material and Methods: For determination of MAOA expression citrate anticoagulated blood was fixed, permeabilized and stained with polyclonal MAOA antibody fluorescently labelled with Dy485-XL. Total RNA was isolated with PAXgene Blood RNA Kit. MAOA mRNA was quantified by quantitative PCR. Phagotest was...
performed according to the manufacturers instructions. Ex vivo stimulation was done with whole blood of healthy individuals using HC and LPS.

Results:

I. RT-PCR showed significant increase of mRNA encoding for MAOA derived from circulating white blood cells in pts with sepsis versus healthy controls ($p < 0.05$). The same hold true for protein expression of MAOA. In granulocytes MAOA expression was 75% ($p < 0.04$) and in monocytes 65% ($p < 0.01$) higher in pts with sepsis compared to healthy controls.

II. While in pts with septic shock without HC treatment MAOA was upregulated threefold, pts with septic shock receiving HC exhibited a fivefold increase ($p < 0.05$).

III. Inhibition of MAOA by CL enhanced phagocytosis by monocytes in an ex vivo stimulation assay of healthy controls up to 70%. In pts with septic shock receiving HC an increase of 16 % was observed.

Conclusions: In circulating white blood cells MAOA is strongly upregulated during sepsis which is more pronounced in pts with septic shock receiving HC. Moreover, MAOA seems to play an important role for phagocytic activity. Inhibition of MAOA enhances phagocytosis by monocytes in pts with septic shock and HC treatment up to 20%. The molecular mechanisms are still unknown. However, inhibition of MAOA may help to overcome adverse effects of HC treatment i.e. secondary infections and may provide a novel therapeutic intervention for pts with septic shock.

Corresponding Author: Gordon Otto, MD, Jena University Hospital, Department of Anesthesiology and Intensive Care Medicine, Erlanger Allee 101, 07747 Jena, Germany, gordon.otto@med.uni-jena.de

A 384
Application of infection control measures for multiresistant ESBL-producing bacteria among hospitalized general surgery patients: an institutional study
Ines Rubio-Perez, Myrian Pichiale, Elena Martin-Perez, Diego Domingo, Angels Figuerola, Eduardo Larrañaga

Objective: The increasing emergence of multiresistant microorganisms is becoming a major problem worldwide. Nosocomial infection by such pathogens is associated with prolonged hospital stay and costs, and even increased mortality. In the surgical setting, it can complicate the postoperative course and outcome of our patients. The aim of this study was to investigate the epidemiology of ESBL-producing bacteria in a General Surgery ward, and to evaluate the compliance of the infection-control protocol validated by the Department of Preventive Medicine in our institution.

Patients and methods: A descriptive, retrospective study of all the positive clinical isolates for ESBL-producing bacteria in hospitalized General Surgery patients was performed, with data from a 2-year period (January 2007–December 2008). Different clinical and microbiological variables were studied.

Results: 31 surgical patients, 14 (45%) female/17 (55%) male, with a mean age of 66 ± 17 years, presented nosocomial infection by ESBL-producing bacteria. Incidence rate of: 6.69 × 1,000 hospitalized General Surgery patients. Outstanding clinical data were: use of blood/urinary catheters (31; 100%), previous antibiotic treatment (26; 84%), endotracheal intubation (22; 71%), previous hospitalization (20; 64%), neoplasia (16; 52%). Clinical diagnosis of patients were: colorectal pathology (8; 26%), pancreatitis (6; 19%), appendicitis (3; 10%), proctology (4; 13%), peritonitis (2; 6%), others (8; 26%). The multiresistant bacteria identified were: E. coli (22; 71%), K. pneumoniae (8; 26%), E. cloacae (13%). Clinical isolates were: pus/abscess (13; 28%), surgical wound (11; 23%), urine (8; 17%), blood (5; 11%), sputum (4; 8%), pressure ulcers (2; 4%), others (4; 9%). Isolation measures were applied in 28 patients (90%). Antimicrobial susceptibility test results showed high resistance rates to Amoxicillin/clavulanic, Quinolones, Trimethoprim-sulfamethoxazole and Tobramicin, and 100% susceptibility to Carbapenems.

Conclusions: These results indicate that the early detection of multiresistant bacteria resulted in a high rate of application of isolation measures in surgical patients, with a low incidence of infection by ESBL bacteria maintained in the 2-year period. The implementation of institutional protocols and a multidisciplinary approach with close collaboration between the surgeon, the microbiologist and the Department of Preventive Medicine is of great importance in order to achieve a reduction in surgical infection rates, prevent the spread and avoid outbreaks in the hospital environment.

Corresponding Author: Ines Rubio-Perez, MD, Hospital de La Princesa, Department of General Surgery, c/Diego de Leon 62, 28006 Madrid, Spain, i.rubio@aecirujanos.es

A 385
CRISPLD2, a novel endotoxin-binding molecule, is a diagnostic as well as a therapeutic target in the treatment of endotoxic shock
Zhi-Qin Wang1, Hong-Quan Jiang1, Xin Zhang1, Michael K Hoffmann2

1Chinese National Human Genome Center at Shanghai, 250, Bi Bo Road, Shanghai 201203, China; 2Department of Microbiology and Immunology, New York Medical College, Valhalla, NY 10595, USA

Objective: We have recently described the endotoxin-binding features of a major serum protein called CRISPLD2/Crispld2 (human/mouse and rat cysteine-rich secretory protein LCCL domain containing 2, J. Immunol. 2009. 183: 6646–6656). CRISPLD2 shares a high endotoxin affinity with another serum protein, LPS-binding protein (LBP), but while LBP mediates endotoxin activity, CRISPLD2 blocks it. CRISP-LD2 prevents endotoxin from exercising immunological functions, such as the induction of endotoxic shock. Experiments were conducted in a mouse model to assess the predictive value of Crispld2 serum levels for the outcome of endotoxin induced sepsis, and to evaluate the therapeutic efficacy of recombinant human CRISPLD2 in the prevention of endotoxic shock. The assessment of human CRISPLD2 levels relative to human shock susceptibility is in progress (preliminary data will be presented).

Materials and methods: Endotoxin shock was induced in Balb/C mice by intraperitoneal injection of 400 μg LPS (company). Human recombinant CRISPLD2 was produced in this laboratory. Polyclonal CRISPLD2 antibody was produced in rabbits. CRISPLD2/Crispld2 serum concentrations were determined by ELISA.

Results:
1. Crispd2 serum concentrations correlate negatively with the susceptibility to endotoxic shock.
2. Passive recombinant CRISPLD2 administration suppresses the induction of lethal endotoxic shock in mice.
3. Measures taken to induce Crispld2 production and to enhance crispld2 serum concentrations protect against endotoxin shock lethality.

4. Prolonged depletion of gram negative commensal bacteria, the biological source of endotoxin, downregulates crispld2 levels and enhances the risk of endotoxic shock lethality.

Conclusions: CRISPLD2/Crispld2 is a physiological negative regulator of bacterial endotoxin. We propose that serum CRISPLD2 concentrations may serve as diagnostic indicators of endotoxin shock susceptibility. As a therapeutic measure, the elevation of CRISPLD2 concentrations can be achieved through the injection of an inducer of CRISPLD2 release, or through passive CRISPLD2 administration.

Corresponding Author: Zhi-Qin Wang, Chinese National Human Genome Center at Shanghai, Functional Genomics, 250 Bi Bo Road, 201203 Shanghai, China, wangzq@chgc.sh.cn

A 386

Interleukin 33 and soluble ST2 serum levels in liver failure
Georg Roth, Matthias Zimmermann, Peter Faybik, Hubert Hetz, Claus Krenn, Hendrik Ankersmit

Objective: IL-33, a member of the IL-1 family induces the production of pro-inflammatory and Th2-associated cytokines and may also serve as an ‘alarmin’ similar to HMGB1. Soluble ST2 has been implicated as a decoy receptor, to attenuate Th2 inflammatory responses. Hepatic over-expression of IL-33 has been recently linked to liver fibrosis. The relevance of both molecules in hepatic failure is unknown.

Material and Methods: The trial was a prospective preliminary study in a university hospital surgical ICU. Eleven patients with acute liver failure (ALF) and twelve patients with acute-on-chronic liver failure (ACLF), who were admitted to the ICU. 14 patients with chronic hepatic failure (CHF) awaiting liver transplantation and 13 healthy individuals served as controls. IL-33 and soluble ST2 concentrations were determined by ELISA.

Results: The concentration of IL-33 and soluble ST2 was significantly higher in ALF, ACLF and CHF patients as compared to the controls. Soluble ST2 serum concentration was significantly elevated in ALF and ACLF as compared CHF, moreover soluble ST2 was significantly higher in CHF as compared to healthy controls. IL-33 and soluble ST2 serum levels correlated significantly ($r = 0.6117$, $P < 0.0001$). Moreover there was a significant correlation between IL-33 serum levels and alanine aminotransferase (ALT) activity in CHF, ALF and ACLF patients ($r = 0.432$, $P = 0.0171$).

Conclusion: Our data provide evidence for elevated levels of IL-33 and soluble ST2 in the sera of CHF, ALF and ACLF patients. Elevated soluble ST2 levels found in patients suffering from acute or chronic hepatic could be an sign of immune hyperactivation, and / or a mechanism to down-regulate inflammation. Moreover, soluble ST2 may be useful to discern acute from chronic hepatic failure or to monitor the course and the severity of the disease.

Corresponding Author: Georg Roth, MD, Medical University of Vienna, Department of General Anesthesia and Critical Care, Waehringer Guertel 18-20, 1090 Vienna, Austria, georg.roth@meduniwien.ac.at

A 387

Mrp8—A novel DAMP (damage associated molecular pattern), through TLR2, is a critical mediator of sepsis related lethality
Justin Kelly, J. H. Wang, H. P. Redmond

Objectives: Investigate the role played by a novel DAMP, Mrp8 (myeloid related protein) in modulating the immune response.

Methods: Human monocyctic THP1 cells were treated with BLP, LPS, hMrp8 and hMrp8/14 complex. Cytokines in culture supernatants were measured (ELISA). Peritoneal macrophages, circulating blood PMNs and bone-marrow derived macrophages (BMM) taken from wild-type and TLR2-/- mice were plated and stimulated with BLP and mMrp8. Phagocytic receptors CR3 and Fcγ R were assessed. Cytokines in culture supernatants were measured. Human embryonic kidney cells stably expressing hTLR2 were co-transfected with pNF-kB-luciferase vector and control vector, and were stimulated with either hMrp8 or BLP to act as a positive control. NF-kB activation was assessed by measuring luciferase activity using a digital luminometer. We blocked specific elements of the TLR2 signal pathway in ERK, p38 and SN50 and measured the resultant cytokine release when stimulated with hMrp8 and BLP.

Results: BLP and LPS augmented a spontaneous release of Mrp8/14 across all time points. hMrp8 caused a significant increase in pro-inflammatory cytokine TNF-α release. BLP and mMrp8 cause massive over expression of both CR3 and Fcγ R on peritoneal macrophages and peripheral PMNs, and a significant release of proinflammatory cytokines from BMM in wild-type mice (not seen in TLR2-/- mice). Exogenous hMrp8 activated NF-κB ($>15$-fold increase vs. naïve cells, $p < 0.01$) via a TLR2 dependent signal transduction pathway. Blocking elements of TLR2 cascade attenuated proinflammatory cytokine release.

Conclusion: Mrp8 is an endogenous ligand of TLR2 signalling and critical mediator of sepsis related lethality.

Corresponding Author: Justin Kelly, MD, Cork University Hospital, Department of Surgery, 72 Friars Walk, Cork City, Ireland, justinjoshkelly@gmail.com

A 388

Modulation of the inflammatory response in an experimental model of abdominal sepsis
Luca Fattori, Pietro Padalino, Alessandro Germini, Angelo Nespoli

Background: Our previous studies showed that administration of recombinant interleukin-2 (IL-2) and granulocyte-macrophage-colony stimulating factor (GM-CSF) significantly improved survival in an animal model of abdominal sepsis. To evaluate possible mechanisms of action, we studied peritoneal levels of TNF-α, IL-6, IL-10 and nitrates.

Methods: Peritonitis was induced by cecal ligation and puncture (CLP) in Sprague-Dawley male rats. Animals were divided into 6 groups: A: naïf, B: sham, C: CLP, D: CLP plus GM-CSF, E: CLP plus IL-2, F: CLP plus GM-CSF and IL-2. 1 ng/gr of GM-CSF and 10 UI/gr of IL-2 were systemically injected 30 min before CLP.

Conclusion: Mrp8 is a novel DAMP that interacts with TLR2 and may play a critical role in sepsis-related lethality. Blocking elements of the TLR2 signal transduction pathway may be a potential therapeutic strategy.

Corresponding Author: Angelo Nespoli, MD, Department of Surgery, University of Rome, Rome, Italy, angelo.nespoli@uniroma1.it

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Each group consisted of 12 animals. TNF-α, IL-6, IL-10 and nitrates were detected in the peritoneal lavage after 4 and 24 h from induction of sepsis using an immuno-enzymatic technique. A purified cellular cultures constituted by 95% of peritoneal macrophages were stimulated by 10 μg/ml E. coli LPS. Two way analysis of variance was used.

<table>
<thead>
<tr>
<th>Mediators</th>
<th>Without LPS</th>
<th>After 4 h LPS</th>
<th>After 24 h LPS</th>
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<tr>
<td>Group A</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TNF-α pg/ml</td>
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<td>IL-10 pg/ml</td>
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<td>IL-6 pg/ml</td>
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<td>233</td>
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<tr>
<td>Nitrates microM</td>
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<tr>
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<tr>
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Conclusion: The simple laparotomy induces inflammation as elevation of TNF-α and IL-6 concentrations showed in Group B. Laparotomy and CLP produce pro-inflammatory mediators as high IL-6 levels indicates in Group C. Laparotomy, CLP, GM-CSF e/or IL-2 (Group D and E) determine an pro-inflammatory stimulus and a high anti-inflammatory activity as IL-10 concentration shows. LPS is more effective than CLP but it is incapable to stimulate IL-10 production. Immuno-modulation caused by GM-CSF plus IL-2 is able to reduce TNF production even if LPS is present. High levels of nitrates have been found in all groups only after a 24 h LPS exposition. The contemporary use of GM-CSF plus IL-2 (Group F) seems to reduce inflammation activity. At the same time peritoneal macrophages, 24 h after induction of peritonitis, maintained their capacity to release both pro- and anti-inflammatory mediators after LPS induced second hit. In this case we could foresee an “immuno-modulation” able to reduce sepsis negative effects.

Corresponding Author: Luca Fattori, MD, Osp San Gerardo, Department of Surgery III, Via Pergolesi 33, 20052 Monza, Italy, luca.fattori@fastwebnet.it

A 389

Mesenteric lymph ligation prevents the burn-induced functional down-regulation of cardiac L-type Ca2+ currents

Garima Dosi, Koich Takimoto, Mingshan Jiang, Justin Sambol, Edwin A Deitch, Atsuko Yatani

Objective: Depressed left ventricular (LV) contractile function develops 4–24 h following major burn injury. Studies from our laboratory have shown that, in a rat burn model (≥40% of total body surface area burn), mesenteric lymph duct ligation (LDL) prior to burn injury prevents the development of acute cardiac dysfunction. The cellular mechanisms of the cardio-protective effects of LDL seem to involve in altered cellular Ca2+ regulation.

Methods: Using electrophysiological methods, we assessed myocardial morphometric changes, i.e., the cardiomyocyte size, myocyte contractility and L-type Ca2+ channel current (ICa) properties in LV myocytes isolated from (1) sham burn (control), (2) sham burn with LDL (sham + LDL), (3) burn, and (4) burn with LDL (burn + LDL) rat hearts at 4 and 24 h following burn injury. In addition, we compared the expression level of alc mRNA that encodes the pore-forming subunit of the L-type Ca2+ channel, and its auxiliary subunits which are implicated in trafficking of the Ca2+ channel to the plasma membrane, i.e., β1, β2 and a2d2 mRNAs in LV tissues of these groups by autoradiographic densitometry and normalized to the GAPDH mRNA level in the same sample.

Results: Baseline myocyte contraction was significantly reduced during the 4–24 h post-burn period (≥20%) as compared with control, sham + LDL or burn + LDL hearts. This pattern of changes was similar to that observed at the whole heart level. Myocyte size from post-burn hearts was not significantly different from other groups, suggesting that cellular remodeling was not a major factor in burn-induced contractile dysfunction. Conversely, basal ICa density was significantly decreased in burn group (≥30%) compared with control, sham + LDL or burn + LDL hearts, without changes in voltage-dependent properties. The mRNA expression levels of β1, β2 or a2d2 were not significantly different among the groups whereas alc mRNA was increased (≥15–20%) in both burn and burn + LDL hearts at 4 and 24 h post-burn, indicating that the increase in alc mRNA may be a cellular adaptation to compensate for burn-induced decrease in ICa density.

Corresponding Author: Garima Dosi, MD, UMDNJ-NJMS, Department of General Surgery, 185 South Orange Avenue, Newark, NJ 07103, USA, dosi.garima@gmail.com

A 390

A potential new serum marker for concussion injury in professional ice hockey sport: Pilot Study on S-100

Peter Biberthaler, Kai Fehske, Gerrit Oedekoven, Viktoria Bogner, Wolf Mutschler

Objective: Minor traumatic brain injury is a relevant problem in contact sports, such as American football, ice hockey and boxing. In this context, concussion injury has been identified as one of the major reasons for posttraumatic sequelae. Hence, professional sport team physicians take out professional players after concussion injury, which implicates substantial sportive and financial problems for the team. So far, no quantitative and rapid available parameter is available to identify those individuals at risk. In this respect, the measurement of S-100 has been identified recently for identification of patients suffering from intracranial bleeding after TBI. So far, it remains unclear if measurement of S-100 might allow for...
identification of concussion injuries in contact sport. Hence, the aim of this study was to measure S-100 after concussion injuries in ice hockey players and compare the results to standard clinical tests.

Patients and methods: Into this study, we included all professional players (n = 23) of a national league team in Germany and draw samples during non-training summer period as negative intra-individual control and after a traumatic event. We calculated the ratio between the serum concentration of S-100 (Elecsys S100, Roche Diagnostics GmbH) after a substantial concussion injury and the individual negative control concentration during training pause and after sharp physical activity. Furthermore, concussion victims were analyzed using clinical SCAT neurological examination form. All data is given as mean ± SD. S-100 values are given in [µg/L].

Results: During the winter season 2008/2009 4 concussion events and complete blood samples as well as SCAT data were recorded. All players had positive result in SCAT testing and stopped playing. The S-100 control values without training were 0.1 ± 0.02, after training 0.1 ± 0.4 and after concussion 0.2 ± 0.08. The individual slope between negative control and training was 1.3 ± 0.52, between concussion and training 2.3 ± 0.83 and between concussion and untrained [SG1] control 2.7 ± 0.62, respectively.

Conclusions: Although still preliminary, our data suggest for the first time that measurement of S-100 might allow for valid quantitative results for identification of individuals suffering a substantial concussion injury while performing contact sport. Hence, we recommend that further studies on extended collectives are performed to secure the potential diagnostic value of S-100 measurement in contact sports.

Corresponding Author: Peter Biberthaler, MD, Ludwig-Maximilians-University of Munich, Campus Innenstadt, Department of Trauma and Ortho, Nussbaumstr. 20, 80336 Munich, Germany, peer.biberthaler@med.uni-muenchen.de

A 391
Improving the quality of perioperative antimicrobial prophylaxis through active surveillance
Kemal Rasa, Elif Hakko, Tayfun Enuenlue, Metin Akmakçı

Objectives: Surgical site infections (SSIs) not only increase the morbidity and mortality rates but also the health care costs of the surgical patients. Despite the solid evidence of effectiveness of prophylactic antimicrobials to prevent SSIs, previous studies have demonstrated inappropriate usage. CDC implemented the National Surgical Infection Prevention Project (SIP) in collaboration with the Centers for Medicare and Medicaid Services in 2002 which promotes prophylactic practices; and the results of this project clearly demonstrates that compliance to optimum antimicrobial prophylaxis guidelines significantly improve surgical outcomes. Inspired from this success story we launched our local SIP implement an active surveillance system on perioperative antimicrobial prophylaxis and aimed to increase the quality of prophylaxis practices.

Methods: An infection control nurse prospectively evaluated all surgical patients’ charts (1,782 patients) and determined the proportion of patients (1) who had parenteral antimicrobial prophylaxis initiated within 1 h before the surgical incision; (2) who were given a prophylactic antimicrobial agent that was consistent with current published guidelines; and (3) whose antimicrobial prophylaxis was discontinued within 24 h after surgery. The results were shared individually with the surgeons on a monthly basis.

Results: 2009 January results revealed that an antimicrobial dose was administered to 84.9% of patients within 1 h before incision; a correct antimicrobial was administered to 99.5% of the patients; and prophylaxis was discontinued within 24 h of surgery for 79.5% of patients, and all three parameters were correctly implemented at only 68.1% of the patients. At the end of 9 months the results were improved to 100, 98.1, 95.8, and 93.1%, respectively.

Conclusion: Active surveillance with a dedicated team, increased awareness and collaboration with surgeons and anesthesiologists provide a significant improvement at the use of prophylactic antimicrobials. This evidence encourage us to believe that substantial opportunities exist and without any doubt these improvements will have a positive impact on SSI rates and outcomes.

Corresponding Author: Kemal Rasa, MD, Anadolu Medical Centre, Department of General Surgery, Anadolu Caddesi No 1 Bayramoglu Cikisi Gebze, 41400 Kocaeli, Turkey, kemrasa@yahoo.com

A 392
Is perioperative povidone-iodine wound application effective in the reduction of surgical site infections? A meta-analysis
Isabelle Fournel, Michel Tiv, Montaine Soulis, Catherine Hua, Karine Astruc, Ludwig-Serge Aho-Gléle

Objective: The effectiveness of intra-operative povidone-iodine (PVI) application in the reduction of surgical site infections (SSI) remains controversial. Thus, we performed a meta-analysis to assess the effect of intra-operative PVI application compared to no antiseptic solution (saline or nothing) on the SSI rate.

Methods: Our meta-analysis was conducted according to the QUORUM statement. We included randomized controlled trials that compared intra-operative PVI application versus no PVI application in surgery patients with SSI as primary outcome.

Results: Twenty-four randomized controlled trials totalling 5004 patients (2,465 patients with PVI and 2,539 patients without PVI) were included: 15 in the main analysis and 9 in sensitivity analysis. SSI rate was 8.0% in the PVI group and 13.4% in the control group. Intra-operative PVI application significantly decreased the SSI rate (RR 0.603 [95%CI: 0.427–0.852], p = 0.004) and consistent results were observed in subgroup analyses according to the modality of PVI administration (spray or irrigation), the type of surgery and the moment of PVI application (before or after wound closure).

Conclusion: Our results suggested that the use of intra-operative PVI may reduce rates of SSI.

Corresponding Author: Isabelle Fournel, MD, CHU Dijon, Epidémiologie et Hygiène Hospitalières, 1 Boulevard Jeanne d’Arc, 21000 Dijon, France, isabellefournel@yahoo.fr

A 393
Prevalence of Candida non-albicans infections in surgical intensive care unit patients: a preliminary report
A. Marinis1, S. Antonopoulou2, G. Gkiokas1, E. Logothetis2, E. Kouskountzi2, D. Voros1

1Second Department of Surgery and 2Department of Bacteriology and Biochemistry, Aretaieion University Hospital, National and Kapodistrian University of Athens, Greece

Objectives: Due to a recent trend in the epidemiology of yeast infections towards mainly Candida non-albicans species, we analyzed the prevalence of these infections in surgical patients admitted to the intensive care unit.

Material and methods: The charts of patients admitted and hospitalized in our Surgical Intensive Care Unit (SICU) who had a positive culture of blood or other body fluids for yeasts during a 3, 5-year-period (1 January 2006 to 31 May 2009) were retrospectively reviewed. Yeast isolates were
identified by VITEK-2 (Biomerieux) and sensitivity was tested for five antimycotic agents (Ampho-B, 5 FC, Itraconazole, Fluconazole and Voriconazole).

Results: 51 surgical patients were found to have one or more yeast infections. These patients included 20 females and 31 males, with an age range from 19 to 85 years. All patients underwent a major surgical procedure (general surgery procedures in 45 and vascular procedures in 6) and were admitted to the SICU due to postoperative complications, such as hemorrhage and sepsis. Twenty-nine patients (57%) were infected by a non-albicans species, mainly by C. glabrata (23.5%), C. parapsilosis (11.7%) and C. tropicalis (9.8%). Initial treatment included mainly fluconazole (50%) and amphoterine-B (40%). Mortality was 33%.

Conclusions: In our study we observed a modification of the usual pattern of distribution between the several species of Candida with an increased prevalence of the non-albicans species with main predominance of candidemias (59%) and respiratory infections (23.5%). However, during the last 2 years a gradual decrease of yeast infections in SICU and especially the non-albicans species has been demonstrated and is believed to be associated with a better understanding and treatment of them. Optimization of patients’ status, close monitoring and support, consideration of possible risk factors for the development of yeast infections and administration of pre-emptive antifungal agents are key factors for lowering morbidity and mortality rates. Surgical patients, with concomitant risk factors (diabetes, prolonged total parenteral nutrition and mechanical ventilation, administration of broad spectrum antibiotics for long periods of time, etc) are considered to be very suitable candidates for developing yeast infections.

Corresponding Author: Athanasios Marinis, MD, PhD, Aretaieion University Hospital, Second Department of Surgery, 76 Vassilisis Sofias’s Ave, 11528 Athens, Greece, drmarinis@gmail.com

A 394

Gentamicin attached to microdispersed oxidized cellulose in the local treatment of acute wound infections - an experimental study

Petr Lochman, J. Páral

Objective: Surgical site infections (SSI) are the second most frequent healthcare-associated infections and the commonest postoperative complication in surgery. Micro-dispersed oxidized cellulose (MDOC) is a random copolymer of polyanhydroglucose and polyanhydroglucuronic acid. It is a trademark of HemCon Company (USA) and is already used for its proven hemostatic effect. We decided to use this effect and to combine it with antimicrobial one of attached gentamicin in the treatment of acute wound infections.

The aim of this study was to examine the effect of topically used gentamicin attached to a biodegradable carrier formed by nanofibrous micro-dispersed oxidized cellulose in acute wound infection treatment and to compare it with Garamycin Schwamm®.

Material and methods: Twelve female domestic swine (35–45 kg of weight) were used in our experimental study. Each experiment took 7 days. After intramuscular premedication with ketamine 30 mg/kg (Narkamov®, Zentiva, Czech Republic), azaperone 40 mg/kg (Stressnil®, Janssen Pharmaceutica, Belgium) and atropine 0.5 mg (Atropin Biotika A.U.V.®, Biotika, Slovakia), the animal was put under general intravenous anesthesia maintained with ketamine. After preparation of the operation field, eight full-thickness dermal defects, 5 cm long with side incisions and fascial injury, were created in the paravertebral area (four wounds on each side) of the animal. Contusion of skin margins using pean forceps was performed to imitate the most common wound type in routine practice. After that, 0.5 ml of a microbiological agent suspension in a density of 10⁶ CFU/ml was inoculated into seven wounds; the last one was left clean as a control. Each microbiological agent was tested separately in two animals for both the MDOC in nanofibre form with gentamicin and Garamycin Schwamm, also in 12 treated wounds (2 were left clean and 2 were infected controls). After a 45 min period, beds of MDOC with gentamicin or Garamycin Schwamm (5 x 1.6 cm containing 10.83 mg of gentamicin) were placed into six infected wounds. Sites of treatment were not rotated. The wounds were not closed and left for open healing like contaminated or dirty wounds. We preferred open healing due to the better possibility to evaluate wounds macroscopically by clinician and for repeated taking of microbiological swabs. The entire operation area was covered by gauze and a surgical towel. After 24, 48 and 168 h swabs were made and macroscopic assessment by clinician was performed. At the end of the experiment some tissue samples of wound margins for histopathological examination were taken and the animal was euthanised by intravenous application of T-61® (Intervet Canada Ltd., Canada).

Results: The results of cultivations (on blood agar) of microbiological swabs from the wounds taken after 24, 48 and 168 h were compared. No statistically significant differences were noted (Table 1).

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<thead>
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<th>E. coli</th>
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<th>Ps. aeruginosa</th>
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<tr>
<td>24 h</td>
<td>48 h</td>
<td>168 h</td>
</tr>
<tr>
<td>Garamycin Schwamm</td>
<td>12/12</td>
<td>12/12</td>
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<tr>
<td>Gentamicin + MDOC</td>
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Although the microbial results were similar, macroscopic assessment showed differences between both of the tested materials. Nanofibre MDOC with gentamicin was fully absorbed in 94.4% of treated wounds after 48 h and in 100% of those after 168 h. All the wounds were macroscopically clean, healed with crust and did not show signs of local infection already after 48 h. Garamycin Schwamm was fully absorbed in 5.6% of wounds after 48 h and in 16.7% after 168 h. Additionally, 83.3% wounds showed local signs of infection at the end of the experiment, especially if the collagen carrier was not absorbed (Table 2).

<table>
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<tr>
<th>E. coli</th>
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<td>Garamycin Schwamm</td>
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<td>Gentamicin + MDOC</td>
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Group I, clean wound, tested material fully absorbed; group II, clean wound, tested material not fully absorbed; group III, wound with signs of infection, tested material not absorbed.

Table 2 Macroscopic assessment at the end of experiment (after 168 h)—counts and percent of wounds in each group
Conclusion: Topically used gentamicin attached to micro-dispersed oxidized cellulose in nanofibre form seems to be effective in soft tissue infections treatment thanks to its antimicrobial effect, excellent resorption of the carrier compared to collagen, as well as due to influence on blood clot formation.

Corresponding Author: Petr Lochman, MD, University of Defence, Faculty of Military Health Sciences, Department of Field Surgery, Trebesska 1575, 50001 Hradec Kralove, Czech Republic, lochmer@seznam.cz

A 395
What is the value of microbial culture results in the management of surgical infections?
Ioannis Gerogiannis, Apostolos Kambouroudis, Michelle Christodoulidou, Eleni Paschalidou, Christos Karkos, Thomas Gerassimidis

Objective: To document the type of hospital microbial flora in patients who are referred to the Department of Surgery of a University Hospital and to evaluate the need for microbial culture collection and its role in the management of surgical infections.

Material and methods: We collected 156 microbial culture results from 89 patients undergoing gastrointestinal (n = 44 patients, group A) or vascular surgery (45 patients, group B). The majority of specimens had been collected from wounds (n = 90), whereas the remaining were from infected gangrenous tissue (n = 11), blood (n = 10), drains (n = 8), central venous catheters (n = 8), peritoneal fluid (n = 6), various fluid collections (n = 4), bile (n = 4), bronchial secretions (n = 4) and thrombus (n = 2).

Results: The most common microorganisms isolated were Acinetobacter baumannii, Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus and Pseudomonas aeruginosa. In 19 group A patients (43.2%), antibiotic therapy had to be changed due to culture results. This change was dictated by the sensitivity in 5 of these patients. In group B, antibiotic therapy had to be changed in 22 (48.9%) patients due to culture results. In 5 of these, the reason was a different sensitivity. In 23 specimens, multi-drug resistant strains were isolated, such as A. baumannii, K. pneumoniae and P. aeruginosa and involved patients who had been transferred from ICU or had multiple previous hospitalizations. In group A, the average antibiotic treatment period was 16.9 (range 2–70) days; the average hospital stay was 20.6 (2–70) days with a mortality rate of 6%. In group B, the average antibiotic treatment period was 20 (2–43) days, hospital stay was 16 (3–44) days and the mortality rate 4%. Deaths in both groups involved patients in septic shock who had been transferred to ICU and suffered from multi-organ failure.

Conclusion: Microbial culture results are essential to guide antibiotic therapy in surgical infections and to document each hospital’s microbial flora and multi-drug resistant strains.

Corresponding Author: Ioannis Gerogiannis, MD, Hippokrateion General Hospital, 5th Department of Surgery, 49, Konstantinoupolios Str., 54642 Thessaloniki, Greece, oldjohn@hotmail.com

A 396
The role of oxidized regenerated cellulose in preventing infections at surgical site: prospective, randomized study in 98 patients affected by a contaminated wounds
Sergio Alfieri, Dario Di Miceli, Roberta Menghi, Giuseppe Quero, Caterina Cina, Giovanni Battista Doglietto

Background: Surgical site infections (SSI) have significant clinical and economic repercussions since they are still the most common cause of nosocomial infections in surgical patients. It is important to bear in mind the growing number of SSI due to antibiotic-resistant microorganisms.

Methods: The study included 98 patients who underwent intestinal recanalization procedures between December 2003 and December 2008, with the stoma as the surgical site. Authors considered several risk factors for SSI. The patients were divided into two groups. In group A (50 pts), the surgical wound, previous site of the stoma, was packed with oxidized regenerated cellulose (ORC), whereas in group B (48 pts) gauze soaked in iodine was used. Microbial contamination was evaluated with 3 swabs (in subcutaneous tissue and the dermis), in the operating room before wound packing and on the second and third postoperative day (before suturing the skin).

Results: There were no cases of wound dehiscence and no clinically evident superficial or deep SSI in either group. Analysis of all data revealed that there was no or reduced bacterial contamination in the second and third swab in 33 patients (66%) of Group A versus 12 patients (25%) of Group B (p = 0.0059). Conclusions: Although it is necessary to consider all factors which can have an influence on SSI and use all the means shown to be effective to reduce the risk of SSI, there is a rationale for using ORC to reduce the risk of infection, especially in patients who undergo “dirty” surgery.

Corresponding Author: Sergio Alfieri, MD, Catholic University of Sacred Heath, Surgical Science, Largo A. Gemelli 8, 168 Rome, Italy, s.alfieri@rm.unicatt.it

A 397
Influence of treatment delays on the perforation rate in acute appendicitis
Wytze Lamers, Wytze Lamers, Adrienne van Randen, Patrick Bossuyt, Jaap Stoker, Marja Boermeester

Objective: To evaluate the association between treatment delays and perforated appendicitis and identify factors related to prolonged time to treatment.

Methods: Data were prospectively collected in a multi-centre diagnostic accuracy study investigating the added value of imaging after clinical evaluation in patients with acute abdominal pain. For the current analysis we selected patients with appendicitis who underwent appendectomy. Perforation was assessed macroscopically at surgery. We evaluated the pre-hospital delay; duration of diagnostic work-up; time from imaging to appendectomy as possible determinants of the risk of perforated appendicitis using logistic regression modelling. Total in-hospital delay was defined as the time from presentation at the ED to appendectomy. We examined the effects of the following patient features on pre-hospital delay using linear regression: age; gender; referral method (self-referred vs. referred by general practitioner); gastrointestinal complaints; and diffuse abdominal pain.

Results: Two hundred sixty-six patients underwent an appendectomy in the acute setting, of whom 57 patients (21%) had perforated appendicitis. The mean time between onset of complaints and appendectomy was 56 h for perforated cases and 38 h for non-perforated cases (p < 0.001). The mean in-hospital delay did not differ significantly between perforated and non-perforated cases: 9.6 versus 9.1 h (p = 0.66). The perforation rate was 12% in patients in whom the time from complaints to appendectomy was two days and 31% in patients with a total time to appendectomy of 4 days. Pre-hospital delay was significantly associated with the risk of perforated appendicitis (odds ratio 1.03 per hour), but in-hospital delay was not. Older patients had a longer pre-hospital delay. Self-referrals and patients experiencing nausea had a shorter pre-hospital delay.

Conclusion: Only the pre-hospital delay was significantly associated with the risk of perforated appendicitis, and was influenced by age,
referral method and gastro-intestinal complaints. In contrast, the in-hospital delay was less variable and not significantly associated with the risk of perforated appendicitis.

Corresponding Author: Wytze Lameris, MSc, Academic Medical Center, Department of Surgery, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, w.lameris@amc.uva.nl

A 398
Tapas study: timing of antibiotic prophylaxis and surgical site infection
Eefje de Vries, W. M. Ankum, C. N. van Dijk, M. W. Hollmann, M. A. Boermeester

Introduction: Surgical site infection (SSI) is a common complication of surgery: reported incidence rates range from 2 to 20%, the variation largely depending on case mix. SSIs are a major cause of morbidity, mortality and healthcare costs. Among many process measures that decrease SSI rates, the effect of preoperative administration of antibiotic prophylaxis (AP) has been demonstrated most extensively. However, the timing of AP remains controversial. Two recent large prospective trials presented conflicting data; one recommended AP within 30 min of incision, whereas the other recommended AP between 30 and 60 min from incision. In this prospective observational cohort study, we will aim to determine the optimal interval between AP and incision to decrease the risk of SSI.

Methods: In a large cohort of gynaecological, general surgical and orthopaedic procedures, data will be collected concerning timing of antibiotic prophylaxis, surgical site infections and possible confounding factors (among others: ASA score, body mass index, medication use (steroids), manner of shaving and disinfection of operative site, duration of surgery, wound class, number of door movements and persons present in the operating room, measures taken to protect temperature (bairhug, warm infusion fluids), patient temperature at end of surgery and perioperative oxygen pressure.) The interval between AP and incision will be divided into 15 and 30-min categories. The incidence of SSI will be compared between the different interval groups. All potential confounding factors will be described according to their distribution across the interval groups. Factors that show a significant maldistribution across interval groups, as well as factors that are known to be of influence in SSI, will be included in a multivariate logistic regression model.

Conclusion: The present study will be the first one to include procedures from general surgery, gynaecology and orthopaedic surgery. It will be interesting to see how SSIs are distributed over timing categories in a setting where the standard for timing of AP is ≥30 min, instead of ≤120 min, like in the other large European study researching this subject. In addition, no other study has included the number of preoperative door movements as a possible confounding factor.

Corresponding Author: Eefje de Vries, MD, Academic Medical Center, Department of Surgery, Meibergdreef 9, 1105 AZ Amsterdam, Netherlands, e.e.devries@amc.uva.nl

A 399
Environmental memory: culture results from a surgical ICU show pathogen preference for particular locations
Stefan Engstrom, Patrick Norris, Ken Debelak, Addison May, Erik Boczko

Objective: Anecdotal observations suggest that infections appear to “prefer” particular clinical spaces and recur there. We examine the relationship between spatial distribution and recurrence of infection through a statistical analysis of electronic medical record data.

Methods: All final microbiology culture results from surgical intensive care patients taken over a 53-month period were examined. Subsequent positive cultures of the same organism within the same patient were weighted so as not to constitute completely independent observations, and only one independent observation (of a duplicate) was allowed per week. The observations were stratified by bed. Differences in incidence by bed were assessed using a variety of hypothesis tests, including a contingency table analysis. Five isolation beds, designated for holding patients with known infections, were identified and analyzed separately. We also differentiated between beds in dual occupancy rooms (n = 12) and those in one room with six beds.

Results: Out of 4,837 unique patients, 925 had one or more positive cultures, 115 different pathogens were represented.

• A contingency table analysis over all rooms and organisms (that had at least 20 observations) shows that there is an overall highly significant difference in the composition of positive cultures across all beds (p < 10⁻⁴).

• When we restrict the analysis to isolation room beds (n = 5, accounting for 55% of positive cultures) the observed organisms are more uniformly distributed but a statistically significant difference remains (p < 0.01).

• An exact multinomial test was used to identify spatial deviations per pathogen. The results indicate that Acinetobacter baumannii (p = 0.0014), Enterococcus faecalis (p = 0.019), and Serratia marcescens (p = 0.048) exhibit significant spatial preferences.

• The isolation rooms presented the most positive cultures (11% of the total per bed). The dual occupancy rooms contributed 2.9% per bed while the beds in the 6-bed room produced 1.7% of the total number of positive cultures per bed.

Conclusion: A straightforward statistical analysis of microbiology data within the SICU indicates that microorganisms are asymmetrically distributed among all beds, and among isolation beds as a subset. More powerful techniques such as categorical time series analysis are warranted to understand this apparent “preference” of certain pathogens for particular locations.

Corresponding Author: Stefan Engstrom, PhD, Vanderbilt University, Biomedical Informatics, 2209 Garland Avenue, Nashville, TN 37232, USA, stefan.engstrom@vanderbilt.edu

A 400
The inflammatory radicular cysts have higher concentration of TNF-α in comparison to odontogenic tumour
Vladimir Jurisic, Terciz Tanja, Jurisic Milan

Objective: TNF-α is a pleiotropic cytokine that is considered as a primary modifier of inflammatory and immune reaction in response to various inflammatory diseases and tumour. It is known that radicular cysts are result of inflammatory process in the peri-apical tissues associated with necrotic and infected pulps, while the aggressive behaviour and high recurrence rate of odontogenic keratocysts (OKC) suggests neoplastic potential and promoted the WHO Working Group to classify its as benign tumor with odontogenic epithelium.

Patients and methods: We here compared TNF-α concentration in 43 radicular cysts and 15 odontogenic tumor, obtained from patients undergoing surgery, under local anesthesia, and after aspiration of cystic fluid from non-ruptured cysts. The concentrations of TNF-α in
the cystic fluids were measured by the enzyme-linked immunosorbent assay, ELISA, obtained from (Diacclone, Besancon, France). The degree of epithelial proliferation in cysts well was analyzed in respect to number of the epithelial layer cells and described as low, moderate and high. For immune-histochemistry of peri-cystic tissues following monoclonal antibodies where used: anti-CD3, anti-CD20 and anti-CD68 (all from Sigma, USA).

Results: TNF-α is elevated in both cysts fluid, but higher values were found in radicular cysts in comparison to odontogenic tumor. The significantly higher concentration of TNF-α were associated with smaller radicular cysts, higher protein concentration, higher presence of inflammatory cells in peri-cystic tissues, degree of vascularisation and cysts wall thickness (Mann–Whitney test, \( p < 0.05 \)). No correlation was found, based on these parameters in odontogenic keratocyst, but all cysts have detectable concentrations of TNF-α.

Conclusion: We here indicated that difference in the concentration of TNF-α exist between inflamed cysts and odontogenic tumors.

Corresponding Author: Vladimir Jurisic, MD, University of Kragujevac, School of Medicine, PO Box 124, 34000 Kragujevac, Serbia, vvd@mailcity.com

A 401
Prediction of the need for relaparotomy after the initial laparotomy in secondary peritonitis
Jordy Kiewiet, Oddeke van Ruler, Kimberly Boer, Hans Reitsma, Marja Boermeester

Objective: Laparotomy remains the cornerstone of treatment for patients with abdominal sepsis due to secondary peritonitis. After the initial laparotomy a common challenge arises: timely and adequate identification of patients in need of a relaparotomy. There are few evidence-based leads for decision making. The objective of this study is to provide evidence-based leads to aid the selection of patients with secondary peritonitis for a relaparotomy by means of a prediction model.

Patients and methods: Data from a randomized controlled trial comparing two surgical strategies for relaparotomy were used. Selection of variables appropriate to use in a prediction model was based on previous reports in literature, common clinical sense and univariable logistic regression analysis. Based on a multivariable logistic regression analysis a final model was constructed including only variables suitable for a clinically applicable model. The predictive capacity of the model was assessed with the area under the curve (AUC) of a receiver operating characteristic curve. A cut-off value corresponding with a sensitivity of 90% was calculated to enhance the interpretability in clinical practice.

Results: A total of 182 patients were included in the analysis, consisting of 46 patients with a positive relaparotomy and 136 with a negative relaparotomy or survivors without a relaparotomy. A prediction model was constructed with six predicting variables: heart rate, temperature, stool passing, extent of contamination, need for inotropic agents and hemoglobin level. The AUC of this model was 0.80 indicating a reasonable discriminatory capacity. With a cut-off score of 30 points the sensitivity was 89%, but the specificity was only 47%.

Conclusion: Despite reasonable discriminatory capacity the cut-off analysis illustrates that the prediction model cannot provide an absolute outcome whether or not to perform a relaparotomy. This would lead to intolerably high rates of unnecessary relaparotomies (66%). Therefore, the prediction model should be regarded as an evidence-based support in the decision making process, preferably as part of a decisional rule including or directing imaging use.

Corresponding Author: Jordy Kiewiet, PhD, Academic Medical Center, Department of Surgery, Meibergdreef 9, 1053 DV Amsterdam, The Netherlands, j.j.kiewiet@amc.uva.nl

A 402
New antibiotic targets: virulence and quorum sensing
Evangelos J. Giamarellos-Bourboulis

Sepsis is one of the leading causes of death worldwide. Data coming from the Hellenic Sepsis Study Group (http://www.sepsis.gr) clearly show that patients with sepsis are divided into two broad categories based on the rationale for empiric antimicrobial therapy: those with sepsis presenting after an intensive care unit (ICU) and those with sepsis presenting after ICU admission. Causative pathogens in the latter group are multidrug-resistant (MDR) or even pandrug-resistant (PDR) isolates. Ventilator-associated pneumonia (VAP) is the most common cause of sepsis inside the ICU. Macrolides possess several characteristics that allow to modulate the inflammatory response of the host to the offending pathogen. Part of their properties is exerted on the mononuclear cells of the host and another part is exerted on virulence quorum sensing of MDR Gram-negatives mainly Pseudomonas aeruginosa. The latter species colonize the airways and is a common cause of VAP. In the Greek setting where Gram-negatives predominate as pathogens for sepsis, we underwent a double-blind, randomized placebo-controlled trial in 200 patients with VAP; 100 patients were allocated to placebo treatment and another 100 patients to clarithromycin. All patients were treated with standard antimicrobial therapy and the clarithromycin regimen was 1 g infused within 1 h through a central catheter for three consecutive days. Bacterial causes of VAP were defined in 65% of enrolled patients after quantitative cultures of tracheobronchial secretions; MDR Acinetobacter baumannii was isolated in two thirds of cases and MDR P.aeruginosa in one-third of cases. Results revealed that patients allocated to clarithromycin had: (a) a significant fivefold decreased risk for death by septic shock and multiple organ dysfunction; and (b) a significant shorter time to resolution of VAP by a median of 5.5 days. These data advise about the need to introduce clarithromycin in the therapy of the septic host particularly in a setting of VAP.

Corresponding Author: Evangelos J. Giamarellos-Bourboulis, Prof. MD, PhD, Attikon University Hospital, 4th Department of Internal Medicine, 1 Rimini Str., 124-62 Athens, Greece, giamarel@ath.forthnet.gr

A 403
Hypoxia and inflammation as regulators of MSC migration
Alexandra Stolzing, Stephan Ishak, Jerome Lay

As mesenchymal stem cells (MSC) can home to and repair injured tissue, enhancement of MSC migration may hold promise as therapeutic strategy, especially in the elderly where such migration is impaired. Evaluating the migration capacities of primary MSCs isolated from rat bone marrow in two different in vitro models (scratch test and transwell assay) we tested several cytokines involved in inflammatory processes for their ability to affect MSC migration at normal (5%) and reduced oxygen levels (1%). The substances were also tested for their capacity to stimulate cell proliferation and to induce cell differentiation. Under normoxia, TNF-α, bFGF, VEGF, PDGF, SDF-1, FGF, enhanced MSC migration, IGF-1, IL-4, INF-γ, had no effect. Under hypoxia, INF-γ gained the ability to enhance MSC migration, whereas
VEGF and SDF-1 lost it. Generally it was found that hypoxic conditions increase the migratory ability of MSC. We also investigated so called nutraceuticals (resveratrol, epigallocatechin gallate (EGCG), reversine, valproic acid, 5-Aza-2’-deoxycytidine and sodium selenite) on the migratory ability of MSC but found no significant effect on migration or differentiation profiles, but all mildly enhanced cell proliferation. Pre-incubation of MSC with INF-γ as under inflammatory conditions, decreased the effects of bFGF-stimulated migration. Analysis of the substances secreted by the MSC themselves showed that the secretory profile changes in aged MSC and can be partially restored under hypoxic conditions.

A 404
ER stress markers in inflammation and ischemia
J. Catharina Davigneau, Ingrid Miller, Soheyl Bahrami, Heinz Redl, Andrey V. Kozlov

Objectives: Essential functions of the liver (protein synthesis, detoxification) are fulfilled by the endoplasmic reticulum (ER). Many critical care diseases are associated with hepatic dysfunction, suggesting involvement of the ER. The objective of this study was to investigate the impact of ER stress, a major cause of ER dysfunction, in in-vitro and in-vivo rat models of shock.

Methods: We have studied markers for ER stress (splice variant of XBP1 mRNA), the unfolded protein response (UPR) and its downstream effects, the induction of pro-survival factors (GRP78) or the shift to a pro-apoptotic phenotype (CHOP and Bax/Bcl-XL ratio) at RNA or protein levels in parallel to functional parameters (Cyp450 1A1 activity).

Results: Circulatory failure, a major feature of shock, which was induced by haemorrhage, resulted in significantly increased levels of spliced XBP1. The subsequent reperfusion further aggravated ER stress, induced GRP78 mRNA, and resulted in a pro-apoptotic shift. However, ER function was not compromised.

Endotoxic shock, a model for systemic immune response (SIR) syndrome includes not only circulatory dysfunction but also inflammatory reaction. Thus we expected a similar induction of ER stress. However, endotoxic shock only moderately increased splicing of XBP1 and GRP78 mRNA. These markers were also up-regulated in cultured hepatocytes treated with conditioned media, which was obtained by incubation of rat white blood cells with LPS, indicating an inflammatory origin of this response. However, GRP78 protein was not increased and although we detected a pro-apoptotic shift, this was not accompanied by a translocation of AIF to the nucleus. It appears that UPR was not completely executed in SIR and that liver cells remained between fully functional and execution of cell death, a state which we called: functional ER failure.

Conclusion: Our data show that the investigated markers are suitable for monitoring ER-stress and its downstream effects in shock models. We found that hemorrhagic and endotoxic shock induced ER stress to a different degree. Hypoxia was a strong inducer of ER stress and UPR, which restored proper ER function or induced apoptosis. However, SIR induced ER stress was relatively moderate, and possibly too weak to result in full recovery of ER function or induction of apoptosis. This yielded alive but not functional cells and may be a mechanism underlying SIR mediated liver dysfunction.

A 405
SVLP technology in synthetic vaccine design
Arin Ghasparian

Nanoscale biomolecular assemblies, such as viruses or Virus-like Particles (VLPs) are interesting targets for biomimetic chemistry. In the field of nanotechnology there is great interest in the synthesis and properties of nanoparticles in the 10–100 nm size range, not least for potential applications in medicine. A new approach to artificial nanoscale biological-like assemblies or “Synthetic Virus-like particles” (SVLPs) has been developed for use in vaccine design. The approach exploits totally synthetic lipopeptide building blocks that are capable of self-assembling into nanostructures resembling small viral capsids in their size (ca. 20 nm) and shape. Synthetic antigens including haptens, peptides and antigen mimetics can be coupled to the lipopeptide building blocks for multivalent display on the particle surface. SVLPs are highly immunogenic in small animals without co-administration of an adjuvant. Recognition of the surface-exposed antigens by multiple B-cell receptors may lead to cross-linking of the receptors on the cell surface, a process that provides a powerful B-cell activation signal. SVLPs can also be engineered to contain pathogen-specific T-helper epitopes and toll-like receptor (TLR) ligands. The combination of T-helper epitopes and B-cell epitopes derived from the same protein or at least pathogen offers the prospect of inducing anamnestic antibody responses mediated by memory CD4+ T cells while inclusion of a TLR ligand potentially could further enhance the immune response. Rational molecular design methods may also be employed for the design of synthetic antigen mimetics (SAMs). Here the 3D structures of key protective epitopes may serve as starting point for the design of B-cell epitope mimetics that reproduce the conformational properties of the epitopes. Stabilization of the conformation may be achieved, for example, by incorporation of side-chain cross-links or by grafting peptide loops onto appropriate scaffolds. Inclusion of B-cell epitope mimetics may be beneficial for the induction of antibodies with improved capability to cross-react with the parent antigen on the intact pathogen. The presentation will illustrate these points with recent examples from our research on SVLPs.

Corresponding Author: Arin Ghasparian, PhD, University of Zurich, Institute of Organic Chemistry, Winterthurerstr. 190, 8057 Zurich, Switzerland, arin_g@access.uzh.ch

A 406
Inflammation and mitochondrial fatty acid beta-oxidation link obesity to early tumor promotion
M. Canan Arkan

Obesity is associated with increased risk for developing pancreatic cancer however the exact molecular mechanisms that link these two disease entities remain nebulous. We demonstrate that high fat diet predisposes mice with oncogenic K-ras activation to accelerated pancreatic intraepithelial neoplasia (PanIN) development and early tumor promotion is closely associated to increased inflammation and dramatic changes in energy metabolism. Through enhanced metabolic rates and increased mitochondrial fatty acid β-oxidation, obesity regulates host and tumor energy metabolism in these mice thereby suggesting alterations in inflammatory and bioenergetic pathways may represent the underlying cause.

Corresponding Author: M. Canan Arkan, MD, Technical University of Munich, Klinikum rechts der Isar, 2nd Department of Medicine, Ismaninger Str. 22, 81675 Munich, Germany, Canan.Arkan@lrz.tumuenchen.de
A 407

TLR4 in acute lung injury
Rena Feinman

Acute lung injury (ALI) and the development of the multiple organ dysfunction syndrome (MODS) is a major cause of death in trauma patients. Using rodent, porcine, and non-human primate trauma-hemorrhagic shock (T/HS) as well as burn models, injurious non-microbial factors released from the stressed gut during shocked states has been implicated as the initial triggering events that contribute to the development of the systemic inflammatory response syndrome, ALI and MODS. Since Toll-like receptors (TLR) act as sensors of tissue injury as well as microbial invasion and TLR4 signaling occurs in both sepsis and noninfectious models of ischemia/reperfusion (I/R) injury, we hypothesized that factors in the intestinal mesenteric lymph after trauma hemorrhagic shock (T/HS) mediate gut-induced lung injury via TLR4 activation.

The concept that factors in T/HS lymph exiting the gut recreates ALI is evidenced by our findings that the infusion of porcine lymph, collected from animals subjected to global T/HS injury, into naïve wildtype (WT) mice induced lung injury. Using C3H/HeJ mice that harbor a TLR4 mutation, we found that TLR4 activation was necessary for the development of T/HS porcine lymph induced lung injury as determined by histology, Evan’s blue lung permeability, and myeloperoxidase levels. Furthermore, TLR4 deficiency significantly decreased the induction of the injurious pulmonary iNOS response in alveolar and interstitial macrophages after the infusion of T/HS lymph compared to their WT counterparts. Additional studies in TLR2 deficient mice showed that TLR2 activation was not involved in the pathology of T/HS lymph induced lung injury. As proof of principle, the effects of TLR4 deficiency on ALI were assessed in an actual T/HS model. TLR4 deficiency attenuated the influx of neutrophils and the iNOS response in alveolar and interstitial macrophages. To further elucidate the cellular mechanisms involved in mediating this lung injury, we investigated the roles of two TLR4 downstream signaling pathways, TRIF and Myd88, in modulating T/HS lymph-induced lung injury. Reduced lung permeability was evident in both TRIF and Myd88 deficient mice, albeit TRIF activation appears to have a more pronounced lung injurious effect than Myd88. However, TRIF and Myd88 deficiency reduced PMN respiratory burst to a similar extent. Taken together, our findings suggest that injurious, non-microbial factors in the intestinal mesenteric lymph after T/HS are capable of recreating gut-induced lung injury via TLR4 activation.

Corresponding Author: Rena Feinman, Prof. PhD, UMDNJ-New Jersey Medical School, 185 South Orange Ave MSB-G594, Newark, NJ 07103, USA, feinmarr@umdnj.edu

A 408

A new generation of β-lactamase inhibitors
Carole A. Sable

Gram negative organisms are important causative pathogens in serious infections. High level resistance, including multi-drug or pan-resistance, has been reported in multiple species. IDSA recently identified a group of Gram negative pathogens called “ESKAPE” bacteria that are of serious concern due to relatively limited treatment options. Unfortunately, inappropriate initial empiric antibiotic therapy is associated with increased mortality, increasing the need for new therapies with activity against resistant Gram negative pathogens. β-lactam antibiotics have traditionally been the mainstay of treatment for Gram negative infections, but the evolution and spread of β-lactamases has significantly compromised the utility of penicillins and cephalosporins. β-lactamases may be divided into 4 functional classes based on substrate specificity: Ambler classes A through D. Carbapenems are currently the treatment of choice for infections caused by Gram negative pathogens, particularly where class A extended-spectrum beta-lactamases (ESBLs) are suspected, but the appearance of serine carbapenemases (KPC and OXA enzymes) may threaten the long term utility of this class.

One successful strategy that has been employed to overcome resistance mediated by β-lactamase production has been the co-administration of a β-lactamase inhibitor (BLI) with a partner β-lactam antibiotic. Currently available BLIs include clavulinate, tazobactam, and sulbactam. All three are inhibitors of class A enzymes, the most abundant class of β-lactamases, but do not restore activity against important, recent β-lactamases such as Amp C or KPC. NXL104 is a non- β-lactam, β-lactamase inhibitor that inhibits enzymes by acylation of the catalytic serine and forms a stable carbamoyl linkage. It has a broad spectrum of activity against class A and C β-lactamases, including serine carbapenemases (KPC), ESBLs, and Amp C. In vitro and in animal models NXL104 restored the activity of ceftazidime (CAZ) against broad panels of resistant Gram negative pathogens, including those that produce multiple β-lactamases. In an in vitro PK/PD hollow fiber model, time above a critical concentration (<0.5 μM) was found to be the parameter predictive of efficacy. NXL104/CAZ has been evaluated in 4 week regulatory toxicology studies and a large safety margin was demonstrated with no target organ identified in either species. Non-clinical studies suggest that NXL104 has no potential for cardiac toxicity.

NXL104 is available as an intravenous formulation and is administered in a 4:1 ratio of CAZ/NXL104. NXL104 has been evaluated in ~100 healthy subjects as single and multiple doses alone and in combination with CAZ. The half life of NXL104 is ~2.5 h and it is ~90% renally excreted as unchanged drug. NXL104 is not a substrate for or an inhibitor of CYP450 enzymes so drug–drug interactions are not expected. NXL104 plus CAZ is currently being evaluated in two Phase II studies: in complicated urinary tract infections versus imipenem and in complicated intra-abdominal studies (with metronidazole) versus meropenem. Results from these studies are expected in 2010. If developed successfully, CAZ/NXL104 would be an alternative to carbapenem for empiric therapy of Gram negative infections, particularly where resistance due to β-lactamases is suspected.

Corresponding Author: Carol A. Sable, Novexel S.A., Chief Medical Officer, 2250 Hickory Rd., Plymouth Meeting, PA 19462, USA, Carole.Sable@novexel.com

A 409

Extracellular matrix scaffolds support airway progenitor cell growth and differentiation
Thomas W. Gilbert 1,2

1McGowan Institute for Regenerative Medicine, Department of Surgery, University of Pittsburgh, 2Department of Cardiothoracic Surgery, Children’s Hospital of Pittsburgh, University of Pittsburgh Medical Center

The interactions between airway stem cell populations and extracellular matrix is an important topic of ongoing research in which there is still a great deal to learn, particularly in regards to development of new therapies for airway injury and disease. Recently, we have begun to investigate the potential for extracellular matrix scaffolds to facilitate or enhance epithelial repair in the trachea and lung of mice. In a mouse model of tracheal reconstruction with a decellularized mouse trachea, ECM was shown to promote rapid epithelialization of the graft with differentiated epithelium and increased expression of keratin 14, a marker for activated basal cells.
A 410
Self and non-self recognition through C-type lectin Mincle
Sho Yamasaki

Macrophage-inducible C-type lectin (Mincle) is expressed mainly in macrophages and is induced after exposure to various stimuli and stresses. We found that Mincle selectively associated with the Fc receptor g-chain (FcgR) and activated macrophages to produce inflammatory cytokines and chemokines. Mincle-expressing cells were activated in the presence of dead cells, which was blocked by anti-Mincle mAb. SAP130, a component of small nuclear ribonucleoprotein (snRNP), was identified as a Mincle ligand that is released from dead cells. Intriguingly, Mincle also recognizes ‘non-self’ pathogenic fungus, Malassezia, and is required for inflammatory response to this fungus. In addition, we have recently found that Mincle recognizes pathogenic bacteria, Mycobacterium tuberculosis as well. An unique mycobacterial glycolipid, trehalose dimycolate (TDM), was identified as a Mincle ligand. Thus, Mincle may function as a multitask receptor that senses “danger” derived from both damaged self (damage associated molecular patterns: DAMPs) and invading non-self (pathogen associated molecular patterns: PAMPs). The molecular basis and physiological advantages of these recognitions will be discussed.

Corresponding Author: Sho Yamasaki, Prof. PhD, Kyushu University, Medical Institute of Bioregulation, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan, yamasaki@bioreg.kyushu-u.ac.jp

A 411
Autologous bone marrow cell therapy of stroke in an ovine model
J. Boltze1,2,3, A. Färschler6,5, B. Nitzsche1, A. Dreyer1, V. Zeisig1, T. von Geymüller1

1Department of Cell Therapy, Fraunhofer Institute for Cell Therapy and Immunology, Leipzig2Institute for Clinical Immunology and Transfusion Medicine, Leipzig3Institute for Regenerative Medicine, Leipzig4Department of Neuroradiology, University Hospital rechts der Isar, Technical University of Munich5Centre for Diagnostic Radiology

Objectives and background: The purpose of our study was to evaluate the therapeutic efficacy of autologous bone marrow cell (BMC) application in the sub-acute stage of stroke in large animals. The model itself was designed to mimic the situation of human stroke patients, potentially providing enhanced translational power for experimental protocols including cell therapies. The promising therapeutic potential of BMC therapies for stroke has recently been demonstrated in numerous rodent trials. However, many promising experimental protocols were successfully evaluated in rodent experiments but failed in subsequent clinical trials. Thus, transfer to clinical application requires close-to-practice large animal models of stroke. We evaluated benefit of autologous BMC transplantation 24 h upon stroke onset in a novel sheep model of focal cerebral ischemia which also allows for control of lesion size and subsequent functional deficits.

Methods: 30 adult rams weighting 51–104 kg were subjected to permanent middle cerebral artery occlusion (MCAO) for stroke induction. 100 mL of bone marrow were harvested from the iliac crest and BMCs were subsequently obtained by density centrifugation. A minimum of 4.0 x 10E6 cells gained per kilogram bodyweight (kgBW) was defined as inclusion criterion for cell treated subjects. Following baseline behavioral phenotyping, magnetic resonance imaging (MRI) and positron emission tomography (PET), 15 animals were randomly subjected to intravenous autologous nuclear BMC treatment 24 h after MCAO. 15 sheep served as controls. Functional outcome was continuously observed by behavioral phenotyping. Lesion size development and brain atrophy were monitored by MRI as well as 15O-water- and 18F-deoxyglucose PET performed at days 14 and 42 before brains were removed for further histological investigation.

Results: In 4 animals, less than 2.0 x 10E6 cells per kgBW were obtained. Those subjects were excluded from the treatment group, but also monitored by MRI for 42 days. In BMC treated animals (n = 11), an enhanced functional improvement was observed as compared to control animals (p < 0.01). Despite a spontaneous tendency of motorfunction improvement, non-treated animals suffered from moderate to severe motor and sensory dysfunctions like ataxia, absent startle reflexes and spatial hemineglect for the entire observation period. MRI investigations showed similar lesion size in both groups at day 1 (p = 0.59) and reduction of lesion size/hemispherical atrophy in cell treated rams 42 days upon MCAO (p < 0.01), but not at day 14. These findings could be confirmed by 15O-water- and 18F-deoxyglucose PET (p < 0.05) and macroscopic pathological lesion volumetry (p < 0.05). Interestingly, transplantation of less than 4.0 x 10E6 BMCs failed to induce lesion size reduction. Modulation of the glial scar as well as diminished signs of axonal degeneration and leukocyte infiltration were found in cell treated subjects (p < 0.05). No tumor formation was observed upon BMC administration.

Conclusions: Autologous BMC administration 24 h following stroke is safe and effective in sheep and might therefore be evaluated as a novel treatment option for stroke in upcoming clinical trials. The study revealed first indications that the therapeutic effect is related to a cell-dose-dependent neuroprotection, probably leading to reduction of lesion size at later stages. Results of histological analysis further indicate that functional recovery is related to modulation of post-stroke effects as gliosis rather than to neural cell replacement. The model itself is feasible for translational studies in stroke research. To confirm this statement, the talk will also give a further ovine model study example to evaluate an experimental therapeutic procedure for acute intervention.

Corresponding Author: Johannes Boltze, MD, Department of Cell Therapy, Fraunhofer Institute for Cell Therapy and Immunology, Penickstr., 04103 Leipzig, Johannes.Boltze@izi.fraunhofer.de

A 412
Pathologic impact of ER stress: regulation by Hsp72
Sanjeev Gupta

Endoplasmic reticulum (ER) stress is associated with cell death and pathology of cerebral ischemia and neuronal diseases, such as Parkinson’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis and prion-related disorders. Enhanced expression of HSP72 has been shown to reduce tissue injury and improve survival in experimental models of stroke, sepsis, renal failure, and myocardial ischemia. HSP72 can act at several different steps in the apoptosis cascade. For example, HSP72 can inhibit cytochrome c and Smad3/4ABLO release from mitochondria, inhibit apoptosis inducing factor (AIF) translocation to the nucleus and interfere with recruitment of pro-caspase-9...
into the apoptosome. However, the molecular mechanisms by which HSP72 expression inhibits ER stress-induced apoptosis are not clearly understood. Here we show that HSP72 inhibits ER stress-induced cytochrome c release and loss of mitochondrial membrane potential. Further, we show that HSP72 enhances the ER stress-mediated production of spliced X Box Binding Protein-1 (XBP1) at RNA and protein levels. We observed increased expression of several UPR target genes downstream of spliced XBP1, which was associated with the formation of a stable protein complex between HSP72 and IRE1. The interaction between HSP72 and IRE1 is mediated by the ATPase domain of HSP72 and cytosolic C-terminal region of IRE1. The ability of HSP72 to bind with IRE1 correlates with increased induction of XBP1-target genes and inhibition of ER stress-induced apoptosis. Attenuating the production of spliced XBP1 either by dominant negative IRE1 or by XBP1 targeting shRNAs specifically abrogated the inhibition of ER stress-induced apoptosis by HSP72. Our data shows that binding of HSP72 to IRE1 enhances IRE1/XBP1 signalling at the ER and inhibits ER stress-induced apoptosis. These results suggest a novel mechanism by which HSP72 reduces apoptosis in pathological settings involving ER stress.

Corresponding Author: Sanjeev Gupta, PhD, Apoptosis Research Centre, School of Natural Sciences National University of Ireland-Galway (NUIG) Galway, Ireland, Sanjeev.gupta@nuigalway.ie

A 413
Bacteriophages as an antibacterial strategy

David R. Harper

Bacteriophages have a long history of use as antibacterial agents, dating back to 1919. However, early uses were uninformed and unsuccessful, largely due to failure to comprehend the actual nature and exquisite specificity of these agents. It was only with the informed use of bacteriophages in the 1980s that their potential became clear. During the 1990s attempts were made to import Soviet-era technologies, but these were unable to meet the exacting standards required to obtain approval for use in the European Union and the United States. More recently, a number of safety trials for use on body surfaces and in the gut have been carried out. However, it was only in 2007 that the first fully regulated clinical trial of the efficacy of a bacteriophage therapeutic was completed. This trial, targeting ear infections caused by Pseudomonas aeruginosa, was carried out in the United Kingdom by Biocontrol Limited, with the results published in August 2009. The trial was successful in demonstrating both safety and efficacy, and is now supporting progression to larger trials for a range of therapeutic approaches.

While these initial trials are targeting topical uses, there is a considerable body of animal studies that indicate that bacteriophage therapeutics are able to be used systemically. Some of these have shown suitability for oral dosing, while others have indicated successful transit of the blood-brain barrier. It is planned to evaluate such approaches.

A 414
The role of HIV and viral hepatitis in polytraumatized patients

Frank Hildebrand, Michael Klein, Christian Krettek

Mortality after multiple trauma is not only related to the injury itself but also to individual factors of the patient. These individual factors can significantly impair the physiological response to trauma and include age, gender and injury-independent preexisting medical conditions. Among those, viral hepatitis (hepatitis B or C) or human immunodeficiency virus (HIV) infections are supposed to result in more complications such as infections due to profound immunosuppression and coagulation disturbances.

In North America and Europe, the prevalence of hepatitis B ranges between 0.3 and 1.6%, whereas a prevalence of hepatitis C between 1.8 and 13.8% has been described. In an European Level I center, no significant influence of viral hepatitis on mortality and the clinical course (duration of mechanical ventilation, stay on intensive care unit (ICU), total hospital length of stay) has been observed. In contrast, liver cirrhosis caused by viral hepatitis was identified to be associated with significantly impaired outcome in multiple trauma patients. The underlying mechanism seems not only to be the reduced production of coagulation factors in patients with liver disease but might also be influenced by immunologic alterations.

The prevalence of HIV infections among trauma patients in North America and Europe has been reported to range between 0.2 and 4.3%. Despite a higher incidence of respiratory, renal and infectious complications in HIV-positive patients, the majority of studies did not find a significant impact of HIV on posttraumatic mortality. The impact of HIV on duration of mechanical ventilation, stay on ICU or total hospital length of stay after trauma is discussed controversially. Some studies found no differences between HIV-positive and -negative patients, whereas others described a prolonged ventilation time and stay on ICU. Even a low CD4 count seems not to influence the posttraumatic course, as infectious complications in HIV-positive patients were described to be more related to injury severity than to CD4 count. Only in surgical patients with a CD4 cell count less than 50 µl and a high viral load (>30,000 copies/ml) an association with adverse outcome has been observed. In conclusion, contrary to earlier beliefs about poor surgical outcome in the HIV-positive group, the majority of the recent studies conclude that the HIV status should not influence management decisions regardless of the patient’s CD4 count.

Corresponding Author: Frank Hildebrand, MD, Trauma Department, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany, Hildebrand.Frank@mh-hannover.de

A 415
F-18-fluorodeoxyglucose positron emission tomography of colonic anastomosis: a possibility to detect anastomotic leakage? A Pilot study

P. H. E. Taeven1, L. F. de Geus-Oei2, T. Hendriks1, W. J. G. Oyen2, R. P. Bleichrodt1

1Department of Surgery, Division of Gastro-Intestinal Surgery, Radboud University Nijmegen Medical Centre, 2Department of Nuclear Medicine Radboud University Nijmegen Medical Centre

Background: F-18-fluorodeoxyglucose positron emission tomography (FDG-PET) may be a promising imaging technique to detect anastomotic bowel leak. FDG uptake is increased in inflammatory processes with granulocyte and macrophage activity, e.g. abdominal abscesses. The aim of this study was to assess postoperative FDG uptake in colonic and
coloacal Anastomosis in patients without suspicion of active infection or anastomotic leakage.

Methods: Design of a prospective observational pilot study in order to assess normal FDG uptake in the Anastomosis after colonic or colorectal resection. Patients older than 17 years of age undergoing elective colorectal surgery with primary anastomosis were included. All included patients underwent FDG-PET of the abdomen which was performed 2–6 days postoperatively.

Results: Fifteen patients met the criteria of inclusion. Postoperative FDG-PET showed no increased uptake in 8 patients and in 6 there was only physiological bowel uptake. None of the patients developed a clinical relevant anastomotic leakage within the first 30 days after surgery.

Conclusion: FDG uptake in colonic or colorectal anastomosis is low within the first 6 days after surgery. Therefore, this technique might be useful in further research to evaluate the value of FDG-PET in the detection of anastomotic leakage in an early stage postoperatively.

Corresponding Author: Robert P. Bleichrodt, MD, PhD, Department of Surgery, Division of Abdominal Surgery, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, R.Bleichrodt@chir.umcn.nl

A 416
Immunocastrceptive vaccination: a success story for therapeutic vaccines
Peter J. Delves

The idea of using vaccines to control fertility has generated interest for many decades. It has long been known that the generation of antibodies against sperm can be a cause of failure to conceive in some infertile couples. Indeed, Baskin demonstrated in 1932 that immunisation of fertile women with their partner’s sperm prevented conception for up to 1 year of subsequent observation. In addition to gamete antigens, the possibility of using hormone-based vaccines has also received considerable attention. One notable success in relatively recent times was the Phase II clinical trial conducted by Talwar and colleagues who demonstrated an extremely high rate of efficacy, with no significant side effects, in females that were medium to high responders to a human chorionic gonadotropin vaccine. Contraceptive vaccines have also been developed for use in animal populations. These include a number of gonadotropin-releasing hormone vaccines (e.g. GonaCon™) and vaccines based upon oocyte zona pellucida antigens (e.g. SpayVac™). Such vaccines have shown efficacy in a range of different animal species. A new generation of vaccines that are being developed combine a contraceptive effect with an anti-infection strategy, such as simultaneous raby and fertility control in wild dogs.

Corresponding Author: Peter J. Delves, Prof. MD, University College London, Windeyer Institute of Medical Sciences, Department of Immunology, 46 Cleveland St, Room 444, London WIT 4JF, UK, reg@ucl.ac.uk

A 417
The endotoxin activity assay EAA: measurement of endotoxin activity in whole blood by neutrophil chemiluminescence
Hans Guenther Wahl, D. Middendorf, B. Friederichs

Objective: Evaluation of a new whole blood endotoxin activity assay. Patient and methods: EAA measures the endotoxin activity in whole blood by priming host neutrophil respiratory burst activity via complement opsonon LPS-IgM immune complexes. The EA assay consists of three tubes containing luminol and zymosan. Assay reactions are initiated by the addition of 40 μL whole blood to each tube. One tube (blank) reflects baseline in the absence of exogenous immune complexes. A second tube (test) contains a specific anti-LPS IgM that stimulates neutrophil activity in proportion to the concentration of LPS in the blood. The third tube (max) contains specific anti-LPS IgM and an excess of LPS so that the chemiluminescence reflects the maximum response of the individual patient sample. The luminol reaction in the presence of immune complexes emits light energy, which is measured by a luminometer and converted into an endotoxin activity (EA) value reported as the ratio of the test (minus blank) to the max (minus blank). If the level is high (>0.6 EAA units) the patient is at high risk for severe sepsis with invasive Gram negative infection. If the level is low (<0.4) the patient is not endotoxic and Gram negative infection can be ruled-out.

Results: Within-run imprecision (n = 10, patient samples), determined for different EA ratios between 0.38 and 0.70 showed coefficients of variation (CVs) in the range from 11.9 to 18.6%. EA ratios, determined for ten patients in the range from 0.11 to 0.72 EAA units with two different test lots showed differences in the measurements between 0 and 16.8%. EAA cut off (mean + 2 SD) was determined for a healthy population of 43 men and 57 women (31.5 ± 14.0 years) as 0.42 EAA units.

Conclusion: EAA is a rapid and valid whole blood chemiluminescence method to detect circulating endotoxin in critically ill patients.

Corresponding: Hans Guenther Wahl, MD, University of Giessen and Marburg, Medical Laboratory Wahl, Paulmannshoeper Str. 14, 58515 Lucenscheid, Germany, hg.wahl@medlabwahl.de

A 418
An approach to new antibiotics for avoiding resistance: PMX-30063
R. Eric McAllister

Objective: PK and safety evaluations in man.

Background: PMX-30063 is a small non-peptide molecule unrelated to any existing antimicrobial drug. It was designed to mimic the membrane interactions of host defense proteins, killing bacteria by direct external physical action independent of metabolic processes. Therefore, resistance is less likely, and has not been seen in serial passage studies. All Staphylococci have been sensitive in vitro, including all multi-drug resistant strains.

Patients and methods: Two phase-1 studies have been analyzed: one ascending single-dose (N = 22), and one multi-dose comparing 3 regiments (Q4H, Q24H, and Q12H; total N = 50). Pharmacokinetics (PK) from the first study determined the design of the second. Normal healthy male volunteers aged 18-55, were confined to the clinic for the entire observation period. One clinic conducted both studies. PMX-30063 was infused into a peripheral vein at a constant rate over 1 h.

Results: The single-dose study advanced to a maximum of 2.5 mg/kg, limited only by a syndrome of peripheral sensory symptoms (‘’tingling‘’, ‘’numbness‘’) and no observable or measurable signs. This syndrome was never disabling in any way, and resolved without treatment in hours to days. Using a score based on intensity, distribution, and duration, these effects were linearly correlated with dose. PK (2 compartments) indicated dose-linearity for Cmax and a half-time for elimination from the plasma of 13 h, with simple exponentials fitting the data well.

The multi-dose study (5 doses) ascended to 0.6 mg/kg Q24H, when the sensory syndrome was again prominent. Minor asymptomatic changes in hepatic enzymes (ALT, AST) were seen, resolving...
Simvastatin in Experimental Bacterial Meningitis
Frank Winkler

Accumulating evidence suggests that statins have antiinflammatory and neuroprotective effects in a variety of human diseases and animal models. In the brain, these beneficial effects have been demonstrated for animal models of traumatic brain injury, multiple sclerosis, and stroke. Furthermore, statins are capable of changing the expression of various genes in the brain. These so-called pleiotropic effects are cholesterol-independent.

In recent years, a large number of clinical retrospective and prospective cohort studies have suggested that statins may have a positive role in the treatment of patients with sepsis, but also in infections like viral and bacterial pneumonia. Animal experiments have confirmed that statin therapy can markedly attenuate the clinical course of experimental sepsis, mainly by preservation of cardiac function, hemodynamic status, and microperfusion. This was accompanied by a reduced systemic inflammatory response as measured by a reduction in blood cytokines. On the other hand, inflammatory lung infiltrates were increased by statin treatment in a murine model of pulmonary Chlamydia pneumoniae infection. Clinical and preclinical data on the effects of statin treatment on the pathogenesis and course of severe infections in other organs, including the CNS, are still missing.

We therefore designed a study to assess the therapeutic potential of simvastatin in an established animal model of pneumococcal meningitis, an acute infection characterized by high morbidity and mortality despite effective antibiotic treatment. Treatment with simvastatin dose-dependently decreased CSF leukocyte counts, a marker for CNS inflammation. In addition, hypothermia was completely abolished in the 40 mg/kg simvastatin group. In contrast, a neurological clinical score, and intracranial complications like increase in intracranial pressure and blood–brain barrier breakdown were not altered by the treatment. In conclusion, simvastatin attenuated CNS leukocyte recruitment and systemic complications of experimental pneumococcal meningitis.

Corresponding Author: Frank Winkler, MD, University of Munich, Campus Grosshadern, Department of Neurology, Marchioninistr. 15, 81377 Munich, Germany, Frank.Winkler@med.uni-muenchen.de

Role of adipose tissue turnover in obesity
Kirsten Spalding

Owing to the increase in obesity, life expectancy may start to decrease in developed countries for the first time in recent history. In humans the generation of fat cells (adipocytes) is a major factor behind the growth of adipose tissue during childhood. The factors determining the fat mass in adults, however, are not fully understood. Increased fat storage in fully differentiated adipocytes, resulting in enlarged fat cells, is well documented and thought to be the most important mechanism whereby fat depots increase in adults. Very little is known about the maintenance adipocytes in humans, how different fat depots are maintained and how (or if) this is altered in obesity. Recently we developed a method that is based on the incorporation of 14C from nuclear bomb tests into genomic DNA, which allows for the analysis of cell and tissue turnover in humans. Using this novel methodology we now have a strategy for studying cell turnover in humans. One tissue of great interest and significant clinical relevance is adipose tissue. Excess adipose tissue, resulting in obesity, is currently one of the most serious threats to human health on a global level. Understanding fat cell turnover has important implications for understanding the development of obesity. Does the development of obesity mainly involve the growth of pre-existing adipocytes, or does it also include an increase in the number of adipocytes? These results will be discussed.

Corresponding Author: Kirsty Spalding, Prof. PhD, Karolinska Institute, Karolinska University Hospital Huddinge, Department of Cell- and Molecular Biology, Berzelius vag 35, SE 171 77 Stockholm, Sweden, Kirsty.spalding@ki.se

The multipotent advantages of adult progenitor cells following spinal cord injury
Jerry Silver

Macrophage-mediated axonal dieback presents an additional challenge to regenerating axons after spinal cord injury. Adult adherent stem cells are known to have immunomodulatory capabilities, but their potential to ameliorate this detrimental inflammation-related process has not been investigated. Using an in vitro model of axonal dieback as well as a dorsal column crush model of spinal cord injury we found that multipotent adult progenitor cells (MAPCs) can affect both macrophages and dystrophic neurons simultaneously. MAPCs alter the activation state of macrophages effectively preventing the induction of axonal dieback. In addition, MAPCs promote neurite outgrowth, induce sprouting, and further enable axons to overcome the negative effects of macrophages by increasing their intrinsic growth capacity. Our results demonstrate that MAPCs have therapeutic benefits after spinal cord injury and provide specific evidence that adult stem cells exert positive immunomodulatory and neurotrophic influences following acute injury to the nervous system.

Corresponding Author: Jerry Silver, Case Western Reserve University, School of Medicine, Department of Neurosciences, Cleveland, OH 44115-2634, USA, jss10@po.cwru.edu

Statins in sepsis: the first clinical results
Peter S. Kruger

Prior to incorporating statins into routine clinical practice for patients with sepsis more prospective clinical data is required to better understand the pharmacology and therapeutic targets of these agents. Evidence has emerged from basic science research that HMG Co A Reductase inhibitors (statins) have beneficial effects independent of their lipid lowering properties. These include anti-inflammatory and immunomodulatory roles that might be associated with a reduced mortality in sepsis. Observational studies have suggested that patients
on statins for heart disease may have fewer infections and that these infections may be less severe. However, not all studies support such an associated benefit and some studies also suggest that stopping statins in patients with infections (as suggested by current prescribing guidelines), may worsen outcomes. Continued prospective evaluation of more than 2,000 patients admitted to our hospital with presumed infection has not demonstrated any clinically significant difference in manifestations of inflammation (SIRS criteria) or hospital outcomes between patients taking prior statin therapy and those not usually on a statin. Interestingly, our preliminary pharmacokinetic studies of atorvastatin in patients with sepsis suggest significantly increased plasma levels. Further research is required to explore the cause and potential pharmacodynamic impact of these elevated levels. It is possible statins may have differing biological effects at higher levels to those seen at conventional plasma concentrations. Inhibition of hepatic cytochrome P4503A4 enzymes may explain in part the increased plasma levels. We have recently shown a modest increase in Rosuvastatin plasma levels in patients with sepsis. This would suggest factors other than cytochrome 3A4 inhibition must also be involved. Several randomised trials of statin use in sepsis are underway around the world. The Continued use of Atorvastatin in Sepsis trial (CAS Trial) randomised 150 hospitalised sepsis patients taking prior statin therapy to continue atorvastatin or placebo, and final data analysis is now complete. The prospective data from this and other randomised trials will provide a sound basis for further exploration of this fascinating field of experimental biology. This may provide new insights to the role of lipids and the endothelium in the response to infection. 

### A 423
**Alpha-1-antitrypsin for protecting insulin producing beta cells in islet allograft transplantation and in the treatment of diabetes**

**Eli C. Lewis**

During the progression of type 1 diabetes and also upon pancreatic islet transplantation, the cells that produce insulin in our body fall under the attack of an unopposed inflammation-driven immune response. Circulating alpha-1-antitrypsin (AAT), an endogenous anti-inflammatory mediator, exhibits reduced activity in diabetic individuals. Plasma-derived human AAT is administered to individuals with varying degrees of genetic AAT-deficiency over extended periods of time and is considered safe. We have found that when added to pancreatic islets, AAT protects from cytokine-induced injury, and also from fatty acid and HMGB1-induced injury, while blocking the release of nitric oxide, chemokines and proinflammatory cytokines. In vivo, AAT protects islet allografts from acute rejection in immune-intact animals and facilitates antigen-specific T regulatory cells. The elaboration of tolerance in animals has been further studied in multiple cell types. For example, in the presence of AAT dendritic cells migrate while maintaining a semi-mature phenotype. AAT reduces co-stimulatory molecule expression in macrophages and induces the release of IL-1 receptor antagonist. AAT inhibits B cell isotype-switching in vivo and diminishes antigen-induced IgG levels. Exogenous AAT therapy can be replaced by plasmid-derived hAAT-expression; while achieving 100-fold lower circulating levels of hAAT, animals accept allografts and their sera contain greater levels of IL-1 receptor antagonist. When added to culture, sera from hAAT-expressing animals inhibit macrophage responses. In the presence of AAT, cultured islet supernatants contain elevated VEGF levels and promote blood vessel sprouting, coinciding with the fact that VEGF is degraded by active elastase. This phenomenon was also observed in primary hAAT-expressing transgenic epithelial cells grafted in animals.

It is concluded that the beneficial effects of AAT during islet transplantation and during the progression of diabetes represent an elegant harnessing of native molecular activities that are exerted during acute phase responses, as tissues enter recovery mode and hold acquired immunity at bay; the safety record of AAT in humans is explained by its ability to preserve tissue damage and prevent pathogen invasion, demonstrated by reduced frequency of bacterial lung infections in hAAT-treated AAT-deficient patients.

*Corresponding Author: Eli C. Lewis, Ben-Gurion University of the Negev, Soroka Medical Center, Department of Clinical Biochemistry, Rm 4-73, Beer-Sheva 84101, Israel, Lewis@bgu.ac.il*

### A 424
**MMPs plead guilty to the lethality in endotoxemia**

**Eline Dejonckheere**

In mammals, the Toll-like receptor 4 is stimulated by lipopolysaccharides of Gram-negative bacteria as well as by endogenous alarm proteins. When the production of proinflammatory cytokines after TLR4 stimulation is not tightly controlled, an inflammatory cascade that may lead to shock, multiple organ failure and death is initiated. SIRS (systemic inflammatory response syndrome), the systemic inflammation caused by an infection or traumatic tissue injury, is a major cause of death in intensive care units, despite intensive research. Using a mouse model of lethal LPS/TLR4-induced endotoxemia, we found that a broad-spectrum inhibitor of matrix metalloproteases completely protects mice to hypothermia and death. This protection was accompanied by a strong reduction of circulating IFN-γ, MCP-1, IL-12, IL-1 and IL-18 levels. Matrix metalloproteases (MMPs) constitute a family of 25 members and are involved in different processes, such as carcinogenesis and angiogenesis. Some MMPs are also involved in inflammatory diseases, such as inflammatory hepatitis. Several matrix metalloproteases were found to be centrally involved in endotoxemia, as mice deficient of MMP-2, MMP-3, MMP-7, MMP-8, MMP-12 and MMP-13 showed a different response to LPS-induced death and hypothermia. We conclude that MMPs are a potential drug target in TLR4-dependent inflammatory pathologies.

*Corresponding Author: Eline Dejonckheere, PhD, Ghent University, VIB, DMBR, Technologypark 927, 9052 Ghent, Belgium, eline@dmbr.vib-ugent.be*

### A 425
**What can stem cell technology accomplish in stroke?**

**Frank Emmrich, Johannes Bolzle**

Stem cell transfer has been used for treatment of ischemic disorders such as myocard infarction or stroke. However, the use of embryonic or fetal grafts in humans is restricted by ethical considerations, severe logistical problems, and the fact that intracerebral administration of homologous embryonic stem cells can lead to teratocarcinomas as demonstrated in rodents (Erdo et al. 2003). Therefore, alternative stem cell sources for treatment of neurological disorders have been evaluated in animal models, among them human bone marrow (Chopp and Li 2002) and human umbilical cord blood (HUCB, Newman et al. 2003; Boltze and Emmrich 2005).
In our study we investigated the potential of stem cells from different sources to promote functional recovery following experimental stroke by middle cerebral artery occlusion (MCAO). The experiments were performed in spontaneously hypertensive (SH) rats, known for a risk profile comparable to stroke patients. For cell therapy cryopreserved cells were administered systemically within the first days after MCAO. Behavioural tests (Beamwalk, RotaRod, Stairway) were performed together with a neurosurgical severity score (mNSS) to assess neurofunctional disabilities. In addition, the experiments were carefully documented by magnetic resonance imaging (MRI).

We could demonstrate a nearly complete recovery from functional deficits with treatments performed within the first 3 days after artery occlusion. Additionally, in vitro experiments with a neuronal cell line demonstrated a direct protective effect conferred by our cell preparations. Less glial reactivity and smaller lesions were observed in vivo, while in the groups with little behavioural recovery (irrelevant control cells) reactive astrocytes were more numerous, and the lesions were larger.

To prepare for clinical studies we have recently established a large animal stroke model in sheep by surgical MCAO of various sizes. Autologous bone marrow cells have been used for systemic i.v. cell therapy 24 h after the stroke incident. Significant improvement could be observed in the following 6 weeks by functional behavioural tests as well as by sequential MRI imaging und subsequent positron emission tomography (PET).

Abbreviations: SH = spontaneously hypertensive; MCAO = middle cerebral occlusion; CSFE = Carboxy-fluorescein diacetate, succinimidyl ester; HUCB = human cord blood, GFAP = glial fibrillary acidic protein

Corresponding Author: Frank Emmrich, Professor MD, Fraunhofer Institute for Cell Therapy and Immunology (IZI), Translational Centre for Regenerative Medicine (TRM), University of Leipzig, Johannisallee 30, 04103 Leipzig, frank.emmrich@medizin.uni-leipzig.de

A 426

Differential patterns of endotoxemia and lipopolysaccharide-binding protein concentrations in patients suffering from blunt trauma versus severe burn injury

Caroline Moegele, Siegfried Zedler, Heiko Trentsch, Peter Zverenich, Michael Eder, Eugen Faist

Background: Lipopolysaccharide (LPS) from the cell wall of gram negative bacteria is highly toxic and the most important microbial trigger of septic shock. The acute phase protein lipopolysaccharide-binding protein (LBP) catalyzes in low concentrations the transfer of LPS to the cellular receptors increasing the sensitivity of cells toward endotoxin.

Objective: Utilizing the rapid whole blood Endotoxin Activity Assay™ (Spectral Diagnostics Inc., Toronto, Canada) and Immulite 1000 (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) this study compared time dependent changes in whole blood endotoxin levels with plasma LBP concentrations within the first 10 days after traumatic injury.

Methods: In a prospective study, 12 severely burned patients (mean TBSA of 34.4%; partial and/or full thickness injury) and 15 polytraumatized patients (mean ISS of 29.9 points) were included. EDTA blood was drawn immediately after injury (day 0), if possible, and on consecutive days 1, 3, 5, 7 and 10 post trauma. With EAA™, we measure endotoxin activity in whole blood through priming the host neutrophil respiratory burst activity via complement opsonized LPS-IgM immune complexes. The luminal reaction in the presence of immune complexes emits light energy measured by a luminometer. The cut-off value to rule-out gram negative infection is set at 0.40.

Results: In both patient populations endotoxin was detected, with EAA values by trend higher in burns. On day 3 nearly all burn patients showed endotoxemia. This finding persisted during the further observation period. In contrast, polytraumatized patients displayed most differential infection patterns. One third was endotoxin negative on day 3. In 11 polytraumatized patients out of 15 at least on one day during the observation period, high endotoxin levels were detected. In burns, LBP concentrations were steadily increasing from 4.90 ± 0.76 (day 0) to 32.50 ± 3.95 µg/mL on day 10. LBP values correlated significantly with endotoxin activity (r = 0.56; p < 0.001). In contrast, in patients with multiple injuries, LBP peaked on day 3 (27.0 ± 2.77 µg/mL) with no correlation to endotoxin levels.

Conclusion: In the burn population rather uniform endotoxin levels were observed. Unlike with burns, in blunt trauma, characterized through most heterogeneous injury patterns, it appears to be most difficult to predict septic complications, solely based on the assessment of EAA™ and LBP. EAA™ represents in our conviction a reliable method to measure endotoxin in peripheral blood. Synchronous increase of LBP and EAA™ values represents a unique diagnostic tool to differentiate between bacterial infection and sterile inflammation.

Corresponding Author: Eugen Faist, Prof. MD, Ludwig-Maximilians-University of Munich, Campus Grosshadern, Department of Surgery, Marchioninistr. 15, 81377 Munich, Germany, eugen.faist@med.uni-muenchen.de

A 422

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A 426

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A 427

Comparative analysis of cytokine production by synoviocytes in response to Toll-like receptor ligands and sterile inflammatory mediators

Ashwini Maratha, Sinead Miggin

During the inflammatory episodes associated with Rheumatoid arthritis (RA) and late-stage Osteoarthritis, the activated synovial fibroblast (SF)s secrete a wide variety of proinflammatory cytokines, chemokines, and matrix-degrading enzymes, which perpetuate the chronic inflammatory state and lead to progressive, irreversible damage of the affected joint. Among the proinflammatory cytokines that are present at high levels in the synovial fluid of inflamed joints are TNFα and IL-6.

Toll-like receptor (TLR)s are innate immune receptors that, when activated, secrete a plethora of cytokines including IL-6 and TNFα. Herein, we sought to establish the role of TLRs in the perpetuation of the RA and late-stage Osteoarthritis (OA). To this end, we assessed TLR functionality in RA and OA synovial fibroblast (SF)s in response to pathogen-associated molecular patterns (PAMPs) and sterile inflammatory mediators by cytokine profiling. We found that differences exist between RA/OA SFs when compared to normal SFs in terms of their cellular responses to various TLR ligands and to sterile inflammatory mediators. These differences give an insight into the disease mechanisms that perpetuate the pathological processes associated with OA and RA and offers a greater understanding of the molecular mechanisms involved in chronic inflammatory pathology.

Kindly supported by the Science Foundation Ireland (to SM O8/RFP/BIC1548) and the Health Research Board of Ireland.

Corresponding Author: Sinead Miggin, PhD, Immune Signaling Group, Institute of Immunology, Department of Biology, National University of Ireland Maynooth, Ireland, sinead.miggin@nuim.ie
DOI 10.1007/s00011-010-0172-x

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