12th INTERNATIONAL CONFERENCE

Sunday, October 3 – Thursday, October 7, 2004,
The Sagamore, Bolton Landing, NY, USA

MISSION STATEMENT

The Inflammation Research Association is a non-profit organization instituted to bring together scientists of all degree and experience levels with an interest in inflammation research, to encourage communication and discussion of scientific and technological advances that can be used to develop new therapeutic agents for the wide diversity of serious diseases with inflammatory processes.

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((Contents, 6 Seiten))

WELCOME

October 3, 2004

Dear Colleague,

On behalf of the IRA Officers and Directors, the Organizing Committee, and the many important IRA Liaisons from inflammation research laboratories around this country and several other countries, it is a great pleasure to welcome you to the 12th International Conference of the Inflammation Research Association.

For over thirty years, the IRA has worked to bring together scientists of all degree and experience levels with an interest in inflammation research for sharing of high-quality scientific and technological advances. Our work is particularly relevant to the discovery and validation of causal mechanisms of a wide diversity of serious diseases with inflammatory processes, and for translating that knowledge into new therapeutic agents. This meeting is organized around these principles and also emphasizes the strategic goals of the 2002-2004 term of the IRA. Namely, our goals are to have an excellent balance of timely and important biology, chemistry and other issues surrounding the challenging task of discovering novel anti-inflammatory agents; to feature themes evolving in part from our program of regional IRA meetings across the country; to be inclusive of and promote networking among pharmaceutical, biotech, clinical, academic and international colleagues; and to have a lot of fun.

Our 12th International Conference kicks off with a Plenary Lecture from Garret A. Fitzgerald, MD, entitled "Lessons and Opportunities from Drug Development in the Arachidonic Acid Cascade," which will present exciting and timely insights for the future. Our Main Symposia cover Nuclear Hormones/Receptors, Innate Immunity and Medicinal Chemistry, and include novel approaches to inflammatory disease as well as new information on known target systems. As has been our custom in past meetings, our exciting New Drugs Symposium features late-breaking data from clinical trials of new therapies. We are pleased to host a guest society evening session sponsored by the American Chemical Society entitled "Targeting Protein Kinases: Tools, Approaches and Case Studies in Inflammation." Our Minisymposia and Poster Sessions derive from the submitted abstracts from our international and multidisciplinary members and participants, and cover a wide range of diseases with inflammatory mechanisms, as well as in vivo and in vitro biology and chemistry approaches to discovery of novel medicines to treat those diseases.

We are extremely pleased to have your participation and fellowship at this meeting. We think you will find that, in addition to great science, you will have ample time for networking via our scientific program, social events and familial atmosphere. We know from our own experiences that many of the scientific contacts and colleagues you discover here will become and remain important life-long friends.

Stephen A. Stimpson, Ph.D.
President
Inflammation Research Association

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Symposia Abstracts

Hot Topics In Nuclear Receptor Biology

Co-Chairpersons: **Julie Stimmel, PhD** (GlaxoSmithKline)
David Ray, **MRCP PhD** (Manchester University)

As we learn more about the biology of nuclear receptors, new indications for potential therapeutic strategies become apparent. Historically, work on Vitamin D receptor has focused on mineral and bone metabolism. This symposium will cover recent work that implicates the Vitamin D receptor in regulation of the immune system and, in particular, T cell-mediated immunity, with the implication that this receptor may play an important role in the treatment of autoimmune disorders. The orphan nuclear receptor, liver X receptor (LXR), was initially validated as a target for atherosclerosis by evidence that this receptor regulated several genes involved in cholesterol transport. Another presentation in this symposium will cover data showing that in addition to a role for this receptor in atherosclerosis, LXR may also be a target for inflammation. The glucocorticoid receptor (GR) is a powerful repressor of proinflammatory processes. Studies will be presented analyzing how ligand recognition by the GR mediates cofactor recruitment, and both NFkB interaction and transrepression. The peroxisome proliferator activated receptor (PPAR) family comprises three members, alpha, gamma, and delta. Initial work defined important roles in fat metabolism, and insulin sensitivity, but more recently they have been implicated as targets in inflammation. A symposium presentation will cover cofactor peptide profiling assays as a new way of differentiating PPAR ligands. The majority of nuclear receptor research has focussed on transcriptional, or nuclear actions. However, a number of phenomena occur rapidly, and are not dependent on gene transcription. The symposium will include new data defining membrane and cytosolic signaling actions of estrogens, with implications for other nuclear receptor action as well.

SA01

THE ROLE OF VITAMIN D AND THE VITAMIN D RECEPTOR IN THE DEVELOPMENT OF T CELLS. Margherita T. Cantorna. The Pennsylvania State University, University Park, PA 16802.

Vitamin D is an important immune system regulator. The active form of vitamin D ($1,25(OH)_2D_3$) has been shown to inhibit the development of Th1 driven autoimmune diseases like inflammatory bowel disease (IBD). T cells are both direct and indirect $1,25(OH)_2D_3$ targets. Th1 cell responses are suppressed, both in vivo and in vitro, by $1,25(OH)_2D_3$ while Th2 cell responses and other Th1 inhibiting pathways (regulatory T cells) are upregulated by $1,25(OH)_2D_3$ treatment. IL2 and IL4 production are central to the effectiveness of $1,25(OH)_2D_3$ to inhibit autoimmunity, since in the IL2 KO and IL4 KO mouse $1,25(OH)_2D_3$ treatment was ineffective. $1,25(OH)_2D_3$ treatment is not broadly immunosuppressive since Th2 driven experimental asthma, immunity to *Candida albicans,* and immunity to *Herpes simplex* virus were unaffected by $1,25(OH)_2D_3$ treatment. The vitamin D deficient and vitamin D receptor (VDR) deficient host develops accelerated Th1 driven diseases like IBD. Interestingly, the Th2 driven disease experimental asthma fails to develop in VDR deficient mice. Vitamin D is a selective regulator of the immune system and the outcome of $1,25(OH)_2D_3$ treatment, vitamin D deficiency, or VDR deficiency depends on the nature (infectious disease, asthma, autoimmune disease etc.) of the immune response. Supported by NIH-NINDS 1R01 NS38888-01A4 and Crohn's and Colitis Foundation of America Senior Research Award.

SA02

Glucocorticoid receptor domain function and repression of NFkB.

David Ray, FRCP PhD, and Helen Garside PhD Endocrine Sciences Research Group and Centre for Molecular Medicine, University of Manchester, UK

Glucocorticoids powerfully inhibit NFkB function. We sought how ligand recognition by the glucocorticoid receptor (GR) regulates this effect. Using GST pull-down and BRET approaches we identify an unexpected effect of ligand binding on protein-protein interaction between the GR and the p65 component of NF-κB, with the antagonist RU486 inhibiting efficient recruitment. In addition we identified significant differences in transcriptional co-modulator recruitment to the C terminal of the GR when bound to either dexamethasone, or RU486. We used chromatin immunoprecipitation to measure recruitment of the GR to a well-characterised NFkB response element from the IL-8 gene. Dexamethasone promoted recruitment of the GR to DNA bound NFkB, but RU486 had only a minimal effect. Thus ligand recognition affects the GR DNA binding domain interaction with NFkB. Understanding this allosteric switch caused by ligand binding has implications for anti-inflammatory therapeutic developments.

SA03

LXRs: nuclear receptors at the crossroads of lipid metabolism and inflammation.

Antonio Castrillo* and Peter Tontonoz.
Howard Hughes Medical Institute at the University of California Los Angeles.

Macrophages are essential modulators of lipid metabolic pathways and innate immune signaling. Lipid and inflammatory pathways induced in activated macrophages are central to the pathogenesis of human diseases including atherosclerosis.
The Liver X Receptors (LXRs) are members of the nuclear hormone receptor superfamily and play a key role as intracellular cholesterol sensors. LXRs are crucial transcriptional regulators of cholesterol metabolism and determinants of atherosclerosis susceptibility. Recent studies have revealed the existence of crosstalk between macrophage inflammatory pathways and LXR signaling. LXR ligands inhibit the LPS- or cytokine-induced expression of inflammatory genes such as iNOS and IL-6 by interfering with NF-kB signaling. In addition, Activation of Toll-like receptors by microbial ligands inhibit the expression of LXR-dependent cholesterol efflux genes through a mechanism involving the transcription factor IRF3. These results have implications for the pharmaceutical control of inflammation and the pathogenesis of atherosclerosis. Collectively, these observations position LXRs at the crossroads of lipid metabolic and inflammatory pathways and suggest that these receptors may serve to integrate these pathways in the control of macrophage gene expression.

SA04

MNAR integrates ER action in Src and PI3K mediated cell signaling

Boris J. Cheskis
Wyeth Research, 500 Arcola Road, Collegeville, PA 19426.

Traditionally, action of steroid hormones is attributed to fine-tuning of transcription. However, in the past few years there has been a dramatic increase in evidence that supports rapid, "nongenomic" action of various steroid hormones. We have recently identified a novel nuclear receptor interacting protein designated as MNAR (**M**odulator of **N**ongenomic **A**ctivity of estrogen **R**eceptor). We show that endogenous MNAR, ERα, Src and p85 interact and that this interaction leads to activation of Src/MAP and PI3K/Akt pathways. Mutation analysis and functional evaluation of MNAR and the use of ERα and cSrc mutants reveals that the coordinate interactions between MNAR, ER and Src are responsible for Src activation. Depletion of MNAR from MCF-7 cells inhibits ER-mediated gene expression. Alternatively, enhanced MNAR expression leads to stimulation of ER transcriptional activity and proliferation of MCF7 cells. Since specific inhibitors of Src, MEK and PI3 kinases block the ability of MNAR to augment ER transcriptional activity, we speculate that this activation of ERα is due to stimulation of the phosphorylation cascades promoted by activation of Src and PI3 kinases. These data reveal that ER "genomic" and "nongenomic" activities are mutually dependent and that MNAR integrates ERα action into the regulation of the Src/MAP and PI3/Akt kinase cascades

SA05

Novel Assay Approaches to Define PPAR Modulators. Julie B. Stimmel, Nuclear Receptor Discovery Research, GlaxoSmithKline, Research Triangle Park, NC 27709

Peroxisome Proliferator-Activated Receptors (PPARs) are transcription factors of the nuclear receptor family that are involved in the regulation of glucose, lipid and cholesterol levels. The ligands that bind to these receptors are important regulators in multiple metabolic pathways that include fatty acid and carbohydrate metabolism. PPARα is the target for lipid lowering fibrate drugs, while PPARγ is the target for anti-diabetic agents of the thiazolidinedione (TZD) class that includes troglitazone, pioglitazone and rosiglitazone. Recent evidence suggests that PPARδ-selective ligands may promote reverse cholesterol transport and thereby decrease cardiovascular disease associated with metabolic syndrome X. The discovery of PPAR ligands has been achieved primarily through binding and cell-based reporter assays. Efficacy of putative ligands in an appropriate animal model was the final determining factor before a compound proceeded into human evaluation. It is not practical nor cost-effective to screen ligands in animal models, thus better predictive in vitro assays need to be established to focus on a minimum number of compounds that proceed for animal evaluation. Recently, it has been shown that biological effects of a ligand on a specific nuclear receptor are dependent not only on the affinity of the ligand for the receptor, but also on the coregulator (coactivator/corepressor) context of the target cell line or tissue. Using a set of PPAR ligands, we have determined the activity of these compounds in binding, cell-based reporter assays, cell-based efficiacy assays, in addition to cofactor profiling assays. A small subset of these PPAR ligands has efficacy data in animal models. We have used multivariate clustering techniques to characterize cross-assay profiles of these ligands and attempted to define the relationship of unique profiles to efficacy in animal models.

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The American Chemical Society (ACS) is a nonprofit organization with a membership of approximately 160,000 chemists and chemical engineers. The ACS publishes scientific journals and databases, convenes major research conferences, and provides educational, science policy and career programs in chemistry.(http://www.chemistry.org).

Targeting Protein Kinases: Tools, Approaches and Case Studies in Inflammation

Co-Chairpersons: **John Rediske, PhD (Novartis)**
Les McQuire, PhD (Novartis)

Protein kinases play a central role in many signalling pathways and therefore have the potential to contribute to a wide range of diseases and other biological processes. Recent investigations reveal that there are over 500 human kinases, making them one of the largest classes of druggable targets. This symposium will provide useful insights into the tools and approaches that are being employed in the identification and development of kinase inhibitors for the treatment of inflammatory diseases. The talks will highlight chemoinformatics-chemogenomics, assay design, virtual screening, target validation, and key case studies related to inflammation.

SA06
State of the art tools for drug discovery

Dr. Mark A. Murcko
Vice President/Chief Technology Officer
Vertex Pharmaceuticals, Inc.
130 Waverly Street
Cambridge, MA 02139

Emerging computational and target family focused technologies are having a significant impact on the predictability of the drug discovery process. For example, the application of pattern recognition methods to lead generation and optimization increases drug-likeness and thus the probability of success for candidate small molecule therapeutics. The integrated application of genomic data, HTS, structural, medicinal chemistry, and statistical methods to drug target families can also result in drug candidates for multiple indications. This presentation will focus on these and additional technologies emerging as key drivers of the „post-genomic" drug discovery process.

SA07
Chasing Chemical Space: In Pursuit of New Tools to Explore the Chemical Biology of Kinases in Inflammatory Disease.

William. A. Metz, Ph.D., Discovery Chemistry
Aventis Pharmaceuticals

Signal transduction, the biochemical cascade by which extracellular signals are transmitted to the cell nucleus, is a key process in many biological events. Recently most pharmaceutical companies, including Aventis, have moved to a target class approach to early drug discovery with kinases identified as one of the key areas. New tools to extend the knowledge of chemical space for intuitive design of novel small molecules have been pursued and developed to increase potency, build in selectivity (i.e.; discovery of allosteric sites, and substrate inhibitors) and improve the pharmacological profile of new kinase inhibitors. This talk will examine the many different methods recently developed for the design of kinase inhibitors as therapeutic agents for inflammatory disease targets.

SA08
6-Substituted PyridoPyrimidines : Second Generation Inhibitors of p38 MAP Kinase Advanced to Human Clinical Studies.

David M. Goldstein, Ph.D., Department of Medicinal Chemistry,
Roche Palo Alto, CA

P38 mitogen activated protein (MAP) kinase is an intracellular enzyme involved in the regulation of cytokine biosynthesis and signaling. Several p38 kinase inhibitors have been reported to be in early Phase II studies, the results of which may validate p38 as a therapeutic target in rheumatoid arthritis. This presentation will focus on lead generation approaches resulting in the discovery of another novel and proprietary scaffold distinct from RO3201195, our first generation P38 inhibitor advanced for clinical studies. The use of crystallographic information of inhibitors bound in the active site of the enzyme to optimize both the selectivity and potency will be highlighted. In addition, early clinical experience with an orally active and selective 6-substituted pyridopyrimidine inhibitor of P38 will be disclosed.

SA09
Discovery of BMS-509744, a Selective Itk Inhibitor that blocks T-cell Activation and Reduces Murine Lung Inflammation

Jagabandhu Das, Ph.D.
Bristol-Myers Squibb Pharmaceutical Research
Institute, Princeton, NJ

Itk (Emt, Tsk), a member of the Tec family of non-receptor tyrosine kinases is expressed mainly on CD4+ T-cells. Mice deficient in Itk exhibit defects in T-cell signaling and development leading to reduced IL-2 production. Selective Itk inhibitors may therefore have utilities as an immunosuppressive agent in the treatment of rheumatoid arthritis, graft rejection, and other T-cell mediated immunological disorders. Discovery tools and lead optimization strategies leading to a selective Itk inhibitor BMS-509744 will be presented.

New Insights into Innate Immunity

Co-Chairpersons: **Jim Mobley, PhD** (Pfizer Global R&D)
Gabriel Nunez, PhD (University of Michigan)

The innate immune response is considered to be the first line of immunological defense, functioning primarily during the initial stage of an immune response. As the response matures, the adaptive immune response takes over, and the innate response is no longer a factor. Recent studies into the biology of pattern recognition receptors and their role in chronic inflammation have begun to cast doubts on this paradigm. Receptors expressed on macrophages and neutrophils that are specific for bacterial and viral ligands have been found to be upregulated in chronic inflammatory sites. Stimulation of these receptors by exogenous or endogenous ligands could be mediating the prolongation of several chronic inflammatory conditions. This session will cover several pattern recognition receptors normally involved in innate immunity that have been implicated in specific chronic inflammatory diseases.

SA10

Toll-like Receptor 4-Deficiency Reduces Atherosclerosis and Alters Plaque Phenotype in Apo E Null Mice without Changing Circulating Cholesterol Levels

Kathrin S. Michelsen, Michelle H. Wong, Wenxuan Zhang, Juliana Yano, Terry Doherty, Tripathi B. Rajavashisth, Prediman K. Shah, and Moshe Arditi*

Division of Pediatric Infectious Diseases and Division of Cardiology, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA.

Background: Toll-like receptors (TLR) play an essential role in recognition of microorganisms and in the initiation of an innate immune response. Recently TLR expression has been reported in murine and human lipid-rich atherosclerotic lesions but the causal role of TLR or the common TLR-signaling molecule MyD88 signaling in atherogenesis remains unclear.

Objective and Methods: To determine whether TLR4 is causally related to atherogenesis we generated double knockout mice by crossing $ApoE^{-/-}$ with $TLR4^{-/-}$ mice. Both, double knockout and $ApoE^{-/-}$ mice were fed a high cholesterol diet and sacrificed at six month of age. Extent of aortic atherosclerosis was measured in en-face preparations of descending aorta after Oil-red O staining using computerized morphometry. Aortic sinus lipid, macrophage content and COX-2 expression was measured after oil-red O staining and immunohistochemistry. Systemic cytokine and chemokine concentrations were measured in the serum by ELISAs.

Results: TLR4-deficiency was associated with a nearly 25% reduction in aortic atherosclerosis (% aortic surface covered by plaque): $ApoE^{-/-};TLR4^{-/-}$: 12.6 ± 3 % vs. $ApoE^{-/-};TLR4^{+/+}$: 16.6 ± 2 %, $p < 0.01$). Furthermore, TLR4-deficiency was associated with a 55 % reduction in lipid content of aortic sinus plaques (% of plaque area) ($ApoE^{-/-};TLR4^{-/-}$: 2.9 ± 2.2 % vs. $ApoE^{-/-};TLR4^{+/+}$: 5.9 ± 2.7 %, $p < 0.05$). TLR4-deficiency had no significant effect on plasma cholesterol levels (mg/dl) ($ApoE^{-/-};TLR4^{-/-}$: 617 ± 403 vs. $ApoE^{-/-};TLR4^{+/+}$: 779 ± 274) or lipid subfractions by HPLC. Immunocytochemistry also showed reduced expression of COX-2, an inflammatory gene, in aortic sinus plaques of $ApoE^{-/-};TLR4^{-/-}$ mice as compared to profound COX-2 expression seen in $ApoE^{-/-};TLR4^{+/+}$ mice. Macrophage infiltration in $ApoE^{-/-};TLR4^{-/-}$ mice was also significantly reduced by 65 % compared to control mice ($ApoE^{-/-};TLR4^{-/-}$: 0.7 ± 0.5 vs. $ApoE^{-/-};TLR4^{+/+}$: 2.1 ± 1.0, $p < 0.01$). Serum concentration of the chemokine MCP-1 was significantly reduced in the double knockout mice compared to control mice ($p < 0.05$).

Conclusions: These data suggest an important role for TLR4 and the innate immune system in the development of atherosclerotic plaques in a murine model of hypercholesterolemia induced atherosclerosis. Supported by NIH grant HL 66436 to MA.

SA11

New Development in Formylpeptide Receptor Research

Ji Ming Wang*

Laboratory of Molecular Immunoregulation, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702

The N-formylpeptide receptor (FPR) is a classic chemotactic receptor that has served as a model for understanding the structure, function and signal transduction properties of other types of leukocyte chemotactic receptors, including chemokine receptors. FPR was first defined biochemically in 1976 as a high-affinity binding site on the surface of neutrophils for the prototypic N-formylpeptide fMLF, and the receptor was cloned in 1990. Neutrophils also possess low affinity fMLF binding sites and a low affinity fMLF receptor, named FPR-like 1 (FPRL1), was cloned in 1992. Both receptors are expressed at high levels on neutrophils and monocytes, and both mediate neutrophil chemotaxis. Since classical studies carried out in the 1970s and 80s had suggested that the N-formyl group was a critical determinant of ligand binding to FPR, and since bacterial and mitochondrial proteins are the only sources of N-formylpeptides in nature, it has been widely held that these receptors evolved to mediate trafficking of phagocytes to sites of bacterial invasion or tissue damage. This notion is supported by the ability of N-formylpeptides to induce release of antimicrobial mediators and oxidants from phagocytes, and by the reduced resistance of FPR knockout mice to infection with *Listeria monocytogenes*. Recently, additional more complex roles for these receptors have been proposed as FPR, and to an even greater extent FPRL1, have been shown to interact with a menagerie of structurally diverse pro- and anti-inflammatory ligands associated with diseases, including amyloidosis, Alzheimer's disease, prion disease and HIV. Why and how do these receptors recognize such diverse ligands and which ones are most important *in vivo*? How are these receptors regulated in cells of the host defense? These are the basic questions currently under investigation that may lead to new therapeutic targets in disease.

SA12

The NOD Protein Family: Role in Innate Immunity and Inflammatory Disease

Gabriel Nunez*, Yasunori Ogura, Mathias Chamaillard, Takeoshi Tanabe, Theresa Dowds, and Naohiro Inohara.

Department of Pathology and Comprehensive Cancer Center, University of Michigan, Ann Arbor, USA

NODs are members of a family of cytosolic proteins with structural homology to Apaf-1 and plant disease resistance (R) gene products. R proteins control the defense response of plant cells to invading pathogens. We have identified 24 NOD genes in the human genome. NODs including NOD1 and NOD2 contain variable N-terminal effector domains, a centrally located nucleotide-binding oligomerization domain (NOD) and C-terminal leucine-rich repeats (LRRs). Mutation in three NOD proteins (NOD2, Cryopyrin and CIITA) are associated with inflammatory disease or immunodeficiency. NOD1 and NOD2 recognize conserved but distinct structural motifs in bacterial peptidoglycan through their LRRs and induce the activation of NF-kappaB. Activation of NF-kappaB through NOD1 and NOD2 is mediated through RICK/RIP2, a serine/threonine kinase that interacts with the IkappaB kinase (IKK) complex. Mutation and genetic variation in the LRRs of NOD2 are associated with susceptibility to Crohn's disease, a common inflammatory disease of the bowel. NOD2 variants associated with disease are deficient in NF-kappaB activation induced by bacterial components. NOD2 expression is enhanced by proinflammatory cytokines and bacterial components via NF-kappaB, a mechanism which may contribute to the amplification of the innate immune response. Systematic mutational analyses revealed a general mechanism for recognition of pathogens by the LRRs of NOD2. Mutation in the NOD domain of Cryopyrin are associated with several autoinflammatory syndromes. Our results indicate that Cryopyrin is involved in the activation of caspase-1 through the adaptor molecule ASC. Thus, NOD proteins including NOD1, NOD2 and Cryopyrin function as intracellular receptors for microbial components leading to the activation of a cellular response against the pathogen.

SA13

The White Death Hypothesis of Rheumatoid Arthritis James L. Mobley, Inflammation Pharmacology, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105

The human genome carries within it the scars of our constant battle against infections microorganisms. Random mutations that once allowed our ancestors to survive epidemics and plagues have been passed down to successive generations. In most cases, there is a cost to be paid for survival through mutation. Although the bubonic plague or "Black Death" is notorious for the toll it took on the population of Europe in the middle ages, another epidemic, the "White Death" of tuberculosis (TB) is responsible for millions of deaths worldwide over the past 300 years. With one in four deaths due to TB in Western Europe and the United States in the 19^{th} century, this disease undoubtedly acted as a powerful genetic selective force. An examination of the available literature on the epidemiology of deaths due to tuberculosis (TB) from 1780 to 1900 reveals a striking straight-line correlation to the epidemiology of rheumatoid arthritis (RA) today. This suggests that rheumatoid arthritis (RA), and possibly other autoimmune diseases, are modern day manifestations of TB selective pressure.

Inflammation Targets: Medicinal Chemistry Strategies for Lead Identification and Optimization

Co-Chairpersons: **Jerry Skotnicki, PhD** (Wyeth)
Shripad Bhagwat, PhD (Celgene)

An essential component in a mechanism-based drug discovery project is to demonstrate action at the defined molecular target. Identification of ligand-protein interactions helps build correlation between specific biochemical processes and pharmacological events. This information can be exploited by medicinal chemists in the design of compounds to block or augment these biochemical processes. In addition, medicinal chemists tackle other drug discovery issues such as physicochemical properties, pharmacokinetics, and a tolerable margin between efficacy and safety of compounds.

The Medicinal Chemistry Symposium will focus on the identification and solution of drug discovery issues at various pre-clinical junctures (exploratory, discovery, development) across a broad spectrum of inflammation topics. The scope of the presentations will embody the utilization of all tools in the medicinal chemistry arsenal for compound optimization with respect to potency and selectivity, to provide information concerning the boundaries of compound manipulation, and to eliminate undesirable chemical and pharmacological properties.

SA14

Discovery of Orally Active CCR1 Antagonists

Kenneth G. Carson, Geraldine C.B. Harriman*, Bruce D. Jaffee
Millennium Pharmaceuticals, 35 Landsdowne St. Cambridge, MA 02139

CC Chemokine Receptor (CCR1) is a member of the G-protein-coupled receptor (GPCR) superfamily, and is involved in leukocyte recruitment, predominantly of monocytes, macrophages, and activated T-cells. CCR1-mediated cell recruitment to chemokines such as Mip-1α and RANTES has been implicated in several inflammatory diseases, including Rheumatoid Arthritis (RA) and Multiple Sclerosis (MS). The discovery of a series of small-molecule CCR1 antagonists will be discussed. The Structure-Activity Relationship (SAR) development from screening hit through pharmacologically active lead compounds will be described. Results from this effort led to the identification of an orally active, potent and selective inhibitor of CCR1.

SA15

Discovery of a New Class of Anti-Inflammatory: Indole cPLA$_2$α Inhibitors

John McKew, Katherine Lee, Mark Behnke, Lihren Chen, Megan Foley, Paresh Thakker, Lane Wooder, Kun Wu, Sam Sum, Steve Tam, Marina Shen, Wen Zhang, Elisabeth Murphy, Manju Ramarao, James D. Clark

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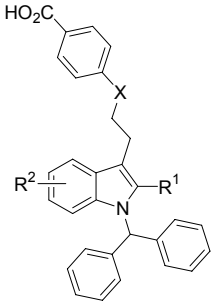
Abstract:
Cytosolic Phospholipase A$_2$α (cPLA$_2$α) selectively cleaves the *sn-2* position of arachidonyl-glycerophospholipids to generate free arachidonic acid. This arachidonic acid is in turn metabolized to a variety of inflammatory mediators including leukotrienes, prostaglandins and thromboxanes. The lysophospholipid remaining after arachidonic acid cleavage can be acetylated to form yet another inflammatory mediator, platelet activating factor, or PAF. Selective inhibition of cPLA$_2$α would provide a novel therapeutic with applications in many disease states including osteoarthritis, rheumatoid arthritis, and asthma. The development of a class of novel and selective indole based inhibitors of cPLA$_2$α is presented. This class of cPLA$_2$α inhibitors is unique in that SAR defined a specific pharmacophore that is completely unrelated to lipophilicity. These inhibitors represent a breakthrough in that they show excellent correlation between activity in isolated enzyme assays and that seen in whole blood assays. These are also the first class of inhibitors that show a predictable correlation between *in vitro* potency, exposure and *in vivo* efficacy. Examples that delineate the required pharmacophore, demonstrate good correlations in the assays and show *in vivo* efficacy will be presented.



SA16

Pyran-Containing Sulfonamide Hydroxamic Acids: Potent MMP Inhibitors That Spare MMP-1 – SAR and Early Clinical Results

Lawrence A. Reiter, Ralph P. Robinson, Kim F. McClure, Peter G. Mitchell, Ivan G. Otterness, Richard J. Griffiths, Jennifer Liras, Arturo Lopez-Anaya, Maria-Dolores Vazquez-Abad, Marie-Pierre Hellio Le Graverand-Gastineau, James Hilbert, Mary J. Saltarelli, Eve Pickering, and Thasia Woodworth*

Osteoarthritis (OA) is characterized by the degradation of articular cartilage. While the events leading to the development of OA remain to be defined, the collagenases (MMP-1 and MMP-13) have been implicated to play a role in the pathology of the disease. However, clinical investigations with non-selective MMP inhibitors revealed a side effect (MSS) which has been postulated to derive from inhibition of MMP-1. In an effort to identify therapeutically useful disease modifying agents for the treatment of OA, we sought compounds that potently inhibit MMP-13 while sparing MMP-1. This lead us to investigate a series of sterically hindered sulfonamide hydroxamic acids with relatively large P_1' groups. The metabolically more stable compounds in the series contain either a monocyclic or bicyclic pyran ring adjacent to the hydroxamate group. Two compounds were advanced into development with one of these continuing into clinical trials. While MSS was observed in the clinic, leading to the conclusion that MMP-1 alone is not responsible for the production of this side effect, biochemical evidence of efficacy was observed suggesting that more selective agents could be useful therapeutic agents.

SA17

The Discovery of Orally Active Aniline Amides as p38 MAP Kinase Inhibitors

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Herein, we describe our initial efforts in developing a potent, selective triaminotriazine aniline amide as an inhibitor of p38α MAP kinase. Our initial hit was identified through screening the Pharmacopeia ECLiPS compound collection. The lead compound from this series possesses oral activity in *in vivo* models of acute and chronic inflammatory disease and represents a unique structural class compared to known p38 inhibitors. X-ray crystallography demonstrates that this compound accesses the ATP binding pocket of p38α, forming a key hydrogen bond through a water molecule to the backbone NH of Met109. Further modification of the triazine core structure to optimize interaction with Met109 led to the identification of novel structures possessing superior physicochemical properties. A description of SAR development, *in vivo* activity, profiling and X-ray crystallography will be presented.

SA18

A NOVEL ANTI-INFLAMMATORY TREATMENT FOR TRAUMATIC BRAIN INJURY BASED ON APOLIPOPROTEIN-E

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Apolipoprotein E (ApoE) modifies clinical outcome in acute and chronic neurological diseases. We have characterized a peptide derived from amino acids 133–149 of the receptor binding region of ApoE that exhibits the full anti-inflammatory activity of the holoprotein. To evolve this peptide into a more suitable pharmaceutical form, we used truncation studies, alanine scanning, and non-conservative substitution studies to define the residues of apoE133-149 that are required for biological activity. Comparison of these QSAR data with holo-apoE structure suggested that the integrity of the alpha helix was critical for biological activity of this peptide. We created analogs containing helix breakers and helix stabilizers to substantiate the need for alpha helical character for functional activity. Peptides with helix-destabilizers resulted in complete loss of activity, whereas peptides with helix stabilizers resulted in 20 to 50 fold increases in activity in cell culture and in whole animal models of TBI. The added benefit of these stabilizers is a reduction from 17 to 12 and possibly 8 amino acids for a potent anti-inflammatory that significantly improves behavioral performance in traumatic brain injury.

New Drugs: Targeting Inflammation in Disease

Co-Chairpersons: **Rodger McMillan, PhD** (Astra-Zeneca)
Ron Magolda, PhD (Wyeth)

In this symposium we will focus on therapeutic agents targeting a wide array of diseases to emphasize the reality that inflammation spans all organs and disease states through common pathways and processes. The charter of this session is to expand our perspective of novel drug discovery to include the practical aspects of achieving “First time in Human” clinical studies (Phase I), and designing Phase II studies to provide “Proof of Concept in Human” as a potential therapeutic. Each talk will appeal to the broad audience of biochemists, pharmacologists, medicinal chemists and clinical colleagues.

SA19

Selective Estrogen Receptor-Beta Agonists Are Potent Antiinflammatory Agents

Michael S. Malamas (a), Richard Mewshaw (b), Heather A. Harris (b), James C. Keith, Jr. (c), Robert McDevitt (a), Iwan Gunawan (a), Christopher P. Miller (c), Leo M. Albert (c), Yelena Leathurby (b), Eric Manas (b) and Ron Magolda*(a), Chemical & Screening Sciences and Woman's Health & Bone Research, Wyeth Research, Princeton, NJ & Collegeville, PA, USA

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The discovery of a second subtype of the estrogen receptor (ERβ) in 1996 provided the impetus to identify its physiological role in mediating estrogen action. The lack of ERβ selective agonists tools have prevented characterization of this receptor. Employing a structure-based approach (X-ray crystallography data, molecular modeling) where we were able to exploit a single amino acid difference between the two ERs (ERβ Ile_{421} to ERαMet_{373}), we have designed a series of highly potent and selective agonists for ERβ. We have also characterized their activity in several clinically relevant rodent models. This presentation will describe the design and synthesis of a several highly selective ERβ agonists along with their novel pharmacological profile that offers new insights into the role of ERβ.

SA20

SA21

ICE inhibitors for the treatment of inflammatory and autoimmune diseases.

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Vertex Pharmaceuticals Incorporated, Cambridge, MA 02139 USA

Interleukin-1ß converting enzyme (ICE, caspase-1) processes the inactive precursor forms of both IL-1β and IL-18 to the biologically active, pro-inflammatory cytokines. Vertex and Aventis Pharmaceuticals have collaborated on development of ICE inhibitors with nanomolar potency against isolated ICE and >100-fold selectivity against other non-ICE subfamily caspases. These compounds inhibit the production of IL-1ß and IL-18 by whole blood or peripheral blood mononuclear cells stimulated by endotoxin or *Staphylococcus* extract with IC_{50} values in the range 200-5000 nM, but display little anti-apoptotic activity. Prodrugs of these ICE inhibitors allow for oral administration and rapid release of the active metabolite ICE inhibitors in animals and humans. In a wide variety of animal models of inflammatory, autoimmune and other disorders in which IL-1β and IL-18 are suspected mediators, such as murine collagen-induced arthritis, collagenase-induced osteoarthritis and oxazolone-induced dermatitis, oral administration of these drugs results in reduced tissue cytokine levels, inflammation and tissue damage.

Pralnacasan (HMR3480/VX-740), the first selective ICE inhibitor to enter clinical development, is currently in PhII trials in rheumatoid arthritis (RA) and osteoarthritis (OA). In PhI testing in healthy volunteers and PhII testing in RA patients, pralnacasan has exhibited excellent safety and tolerability and dose-dependent pharmacokinetics and inhibition of IL-1ß production in an *ex vivo* whole blood assay. In a PhIIa trial in RA, pralnacasan produced statistically significant dose-dependent reductions in serum markers of inflammation, including C-Reactive Protein, erythrocyte sedimentation rate, serum amyloid-A and matrix metalloprotease-1. Furthermore, pralnacasan treatment resulted in a dose-dependent increase in the 20% response rate on the American College of Rheumatology (ACR20) composite endpoint and allowed for dose-dependent reduction in the use of oral corticosteroids. Further PhII trials in RA and OA have been conducted and will be discussed. Vertex has completed initial PhI testing of a second ICE inhibitor, VX-765, and has used reduction of the levels of serum IL-18 levels in healthy volunteers as a biomarker for dose selection. The initiation of PhII testing in a primary indication will be discussed. ICE inhibition is a promising novel oral anti-cytokine strategy with potential for the treatment of a wide variety of inflammatory and autoimmune disorders.

SA22

Development of LEA29Y and the Clinical Potential of Co-stimulation Blockade in Solid Organ Transplantation

D. Scott Batty*, Jr., MD, FACS, Director, Global Medical Affairs for LEA29Y, Bristol-Myers Squibb, P.O. Box 4000, Princeton, NJ 08543

Solid organ transplant has made much progress over the last decade in treating and preventing acute rejection of the transplanted allograft. With this success has come the recognition that the very regimens responsible for this success have created issues of compromise of long term health for the recipients. Deteriorating renal function, hypertension, and new onset of diabetes mellitus are but a few of these consequences for the patients required to take permanent immunosuppressive agents.

During this time, progress in the understanding of the basic interactions of transplant immunology, and the sequence of events that leads to organ rejection have identified new targets for immunosuppressive strategies. Co-stimulation blockade is one of those strategies. Co-stimulation blockade prevents the interaction of CD28 on T cells, and CD80/86 on the antigen presenting cells (APC), thus preventing the initial step in the activation of T cells.

Targeting of this interaction produced initially the fusion protein CTLA4Ig. This novel fusion protein was optimized for use in solid organ transplant in the form of a related compound, LEA29Y. While maintaining efficacy in prevention of acute rejection of current regimens, clinical trials have shown a marked improvement in long term renal function, as well as other long term health-related side effects. Phase II data showed equivalent acute rejection rates of 18%, when comparing LEA29Y or cyclosporine as part of a four drug regimen. Further, LEA29Y demonstrated a significantly improved measured creatinine clearance at one year (66.9 ml/min vs. 49.3 ml min) between LEA29Y and cyclosporine, respectively. LEA29Y also showed improved profiles when comparing systolic blood pressure, non-HDL cholesterol profiles (36.1% LEA29Y patients receiving lipid-lowering medications vs. 53.1% for cyclosporine patients), and the onset of post-transplant diabetes mellitus (1.5% vs. 6.8% respectively). At one year, a marked reduction of chronic allograft nephropathy was seen, again favoring LEA29Y treated patients. 20.3% of LEA29Y patients showed these changes on protocol biopsy at one year, vs. 30.1% of cyclosporine treated patients. LEA29Y will soon enter Phase III trials to verify the benefits seen in the Phase II trial.

SA23

Biological Characteristics of Anti-α4 Integrin Monoclonal Antibody (Natalizumab) a Selective Adhesion Molecule (SAM) Inhibitor for the Treatment of Multiple Sclerosis and Crohn's Disease.

Tony Arulanandam*, Biogen Idec

The interaction of VLA-4 expressed on leukocytes with it's counter ligand VCAM-1 expressed on endothelial cells plays a critical role in the migration of leukocytes to inflammatory sites in the brain. Likewise the interaction of the α4ß7 integrin with it's counter ligand MadCAM expressed on mucosal endothelium regulates the infiltration of leukocytes into the gut during inflammatory bowel disease. Animal disease model studies conducted with an anti-α4 integrin monoclonal antibody (mAb) in guinea pig and rodent EAE models and cotton-topped tamarin model of colitis demonstrated the early promise of anti-□4 integrin mAb therapy for multiple scelrosis and inflammatory bowel disease. Randomized placebo controlled phase II clinical studies conducted with a humanized IgG4 anti-α4 integrin antibody (Natalizumab AN100226) showed a significant reduction in both the number of brain lesions and clinical relapses in multiple sclerosis patients and significant response rates were observed in crohn's disease patients. Based on these encouraging clinical data, large multicenter double blinded placebo controlled Phase III clinical trials in multiple sclerosis and crohn's disease are being conducted. A summary of the biological properties of Natalizumab, it's ability to function as a selective adhesion molecule (SAM) inhibitor of VCAM-1 and MadCAM and not affect complement mediated effector functions, as a desirable property for the treatment of autoimmune diseases will be discussed.

Mini-symposia and Poster Session Abstracts

A001

Exploration of the diaryl-pyrrolizine scaffold towards selective COX-2 inhibitors

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A discovery program towards inhibitors of COX-1, COX-2 and 5-LOX led to the identification of 6,7-diaryl-pyrrolizine-5-yl acetic acid derivatives. As a promising drug candidate, Licofelone ([2-(4-Chloro-phenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl]-acetic acid) was selected which inhibits all three enzymes in the submicromolar range. Currently, Licofelone is in phase III of clinical development for the treatment of osteoarthritis. The diaryl-pyrrolizine scaffold was further explored towards the generation of selective inhibitors of COX-2. Potency of COX-1, COX-2 and 5-LOX inhibition was assessed in cellular assays. A substantial loss of 5-LOX-inhibition was observed when the acetic acid moiety was substituted by either a methyl group or a halogen. COX-2 selectivity was achieved by introduction of a methylsulfone or sulfonamide group into the para-position of the 7-phenyl ring. IC_{50}-values towards COX-2 in the range of 10 nM were achieved with a COX-2/COX-1 selectivity between 10 and approx. 500. Compounds with promising in vitro activity were further characterized in in vivo models of acute and chronic inflammation. Despite similar in vitro data, the anti-inflammatory efficacy in the applied experimental models varied between modest and good activity.

A002

ML 3403 – Pharmacological characterization of a potent p38-MAP kinase inhibitor

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ML 3403 ({4-[5-(4-Fluoro-phenyl)-2-methylsulfanyl-1H-imidazol-4-yl]-pyridin-2-yl}-(1-phenyl-ethyl)-amine) was selected as a promising p38-inhibitor. Its pharmacological properties were investigated and compared to those of SB203580. The p38-mediated phosphorylation of immobilized ATF-2 was inhibited with an IC_{50} of 380 nM (SB203580: IC_{50}=760 nM). At a concentration of 10 µM, a relevant inhibition was observed for the protein kinases JNK2α2 (80%) and JNK3 (98%), and for the cytochrome P450 isoenzymes 3A4, 1A2, 2C9 and 2C19. In human mononuclear cells (MCs) and whole blood (WB), the LPS-induced synthesis of TNFα and IL-1β was inhibited with IC_{50}-values of 200 nM/2700 nM (TNFα) and 30 nM/1000 nM (IL-1β). The addition of serum albumin to MC culture medium had a slight influence on the potency. Following oral administration of ML 3403 to male BALB/c mice, the GalN/LPS-induced production of TNFα was suppressed dose-dependently with an ED_{50} of 1.33 mg/kg (SB203580: ED_{50} 2.7 mg/kg). The pharmacological profile of ML 3403 has been considered promising, and deserves further exploration of this structural motif.

A003

COX inhibitor prodrugs tropic for macrophages and neutrophils exert enhanced anti-inflammatory activity in the collagen induced arthritis model.

Michael Wolff, Hans-Jürgen Gutke, Thomas Meindl, Jan Guse, Simona Margutti, Christian Flohr, [1]Michael Seed, [2]Stefan Laufer, [2]Wolfgang Albrecht, Michael Burnet. Synovo GmbH, [1]William Harvey Institute, [2]Merckle GmbH,*

In vivo anti-inflammatory activity of carboxylate COX inhibitors is limited by rapid elimination. However, longer half lives risk COX-1 mediated gastric toxicity. We reason that activity could be increased if drug physical properties were altered to allow preferential partition into macrophages and neutrophils (WBC). We prepared prodrugs of diclofenac and screened them using whole human blood. Prodrugs with WBC:Plasma partition >10-fold were optimized for uptake and ester stability. Pharmacologically inert macrocycle (azilide) conjugates were assessed for enhanced efficacy in murine collagen induced arthritis either therapeutically (after onset of signs) or prophylactically (2 d post boost). In both modes, the prodrugs achieved complete suppression of arthritis at tolerated doses with optimal activity at 15µmolkg^{-1}d^{-1} (≡ 4.5mgkg^{-1} diclofenac). The prodrugs exert >2-fold (p.o.) or >4-fold (i.p.) potency against paw inflammation vs. diclofenac - >50% of prodrug is eliminated unhydrolysed and diclofenac elimination is retarded. We propose that the prodrugs increase efficacy via improved pharmacokinetics partly related to biased disposition of the prodrug toward immune cells.

A004

Inhibitors of the Interaction Between Leukocyte Function-Associated Antigen-1 (LFA-1) and Intracellular Adhesion Molecule-1 (ICAM-1). Kenneth J. Barr,* Minna Bui, Emily Hanan, Jose Rafael Martell, Johan Oslob, Kumar Paulvannan, Wang Shen, Chul Yu, Min Zhong, Jenny Zhu, Jennifer Arbitrario, Michelle Arkin, Erin K. Bradley, Teresa Chen, Brian C. Cunningham, Marc Evanchik, Mike Flanagan, Ute Hoch, Karen Huen, Jennifer Hyde, Jeff Kumer, Teresa Lac, Christopher Lawrence, Saileta Prabhu, Hans E. Purkey, Jeffrey A. Silverman, Dave Stockett, Jasmin Wright, Travis Bastow, Steve Chen.

The integrin known as leukocyte function-associated antigen (LFA-1) ($\alpha_L\beta_2$, CD11a/CD18), a heterodimeric transmembrane glycoprotein expressed on all leukocytes, serves as a cell-surface adhesion receptor for intracellular adhesion molecules (ICAMs) –1, -2, and –3. The intercellular interaction between LFA-1 and ICAM-1 (CD54) has been shown to support inflammatory and T-cell specific immune responses through mediating cell adhesion, leukocyte transmigration, and augmentation of T-cell receptor signaling. It has been proposed that disrupting LFA-1/ICAM-1 may have a mitigating effect on a variety of inflammatory and autoimmune disorders, including rheumatoid arthritis, multiple sclerosis, chronic obstructive pulmonary disease, inflammatory bowel disease, and others, as well as provide protection from transplant rejection. The recent FDA approval of the humanized anit-CD11a monoclonal antibody efalizumab, co-developed by Genentech and Xoma initially for the treatment of psoriasis, has served as validation for LFA-1/ICAM-1 as a target for this indication. Several companies are currently investigating whether similar results can be achieved using small molecule therapeutics. As such, we herein report the discovery of a novel, potent and selective chemotype that disrupts LFA-1/ICAM-1, presumably through binding to the I-like domain of the β2 (CD18) subunit of LFA-1.

A005

Complex Primary Human Cell Based Assays for the Discovery and Development of Novel Therapeutics for Vascular Inflammation

Ellen L. Berg*, Eric J. Kunkel, Ivan Plavec, Vangelis Hytopoulos, and Eugene C. Butcher. BioSeek, Inc., Burlingame, CA 94010

Unexpected drug activities discovered during clinical testing establish the need for better characterization of compounds with respect to human disease biology early in the discovery process. Biologically Multiplexed Activity Profiling (BioMAP) is an approach to characterize drug function based on statistical analysis of protein expression data sets from complex primary human cell-based models of inflammatory disease. BioMAP profiling, using four model systems containing primary human endothelial cells and peripheral blood mononuclear cells in different environments relevant to vascular inflammation and immune activation, can detect and discriminate multiple functional drug classes including glucocorticoids and other nuclear hormone receptor ligands, TNF-alpha antagonists, inhibitors of HMG-CoA reductase, calcineurin, IMPDH, PDE4, PI-3 kinase, hsp90, and p38 MAPK among others. Here we describe the application of BioMAP profiling for discrimination of p38 MAPK inhibitors according to secondary / off-target activities and the development of clinical hypotheses for kinase target family inhibitors.

A006

Novel, Orally Active Small Molecule Inhibitors of PDE4: A Functional and Biological Assessment.

Kate Blease, Miles Houslay, York-Fong Cheung, Maria Celeridad, Brydon Bennett, Paul Omholt and Jeff McKenna. Celgene, 4550 Towne Centre Court, San Diego, CA

The phosphodiesterase super-family consists of 11 known isoenzymes, each possessing different cyclic nucleotide specificities. The PDE4 isoenzyme, of which there are 4 isotypes, is the most widely expressed PDE in inflammatory cells, and catalyzes the breakdown of cAMP. This abstract describes a novel series of small molecule inhibitors of PDE4 activity that also show potent anti-inflammatory properties. The prototype compound, CC001, showed a relatively non-selective profile of PDE4 isotype inhibition, A, B, C and D (910nM, 820nM, 380nM and 120nM respectively) but no inhibition of PDE1-5 and 6. The IC_{50} of TNF-α inhibition from LPS-stimulated hPBMCs with CC001 was 200nM *in vitro*, and efficacy of this compound was demonstrated following oral administration in an *in vivo* model of LPS-induced TNF-α release in the rat (ED_{50}; 1mg/kg). To assess the emetic potential, CC001 was tested in a ferret emesis study, and was found to be highly emetic at 10mg/kg. Additionally a high Rolipram binding site affinity (HARBs/PDE4) ratio of 23.3 was observed, which correlated with the high emetic potential of this compound. Improvement of the HARBs/PDE4 ratio could be demonstrated with related compounds, indicating less emetic potential within the compound series. Additionally the PDE4 isotype profile was altered in these later compounds: CC002 showed a 10 fold selectivity for PDE4C. Enhanced *in vitro* (IC_{50}; 1nM) and oral *in vivo* activity (ED_{50}; 0.3mg/kg) with respect to TNF-α inhibition was also observed with this compound. In conclusion we show the development of a series of potent PDE4 inhibitors, with differing isoenzyme profiles that may result in enhanced biological activity and reduced emetic potential.

A007

The Role of the NF-κB Signaling Pathway in Activation of ENA-78 in Response to Inflammatory Cytokines

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Epithelial cell neutrophil-activating protein 78 (ENA-78) is a chemotactic cytokine that is up-regulated in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Our group and others have identified an essential NF-κB binding site in the promoter region of ENA-78. Using reporter gene constructs in both immortalized and primary cells, we have shown that deletion or mutation of this site results in the inability to induce ENA-78 expression in response to the inflammatory cytokines IL-1β and TNF-α. To further study the regulation of ENA-78, we established A549 epithelial cell lines stably expressing siRNA against different proteins in the NF-κB signaling pathway. These cell lines are currently being studied to determine which proteins play a pivotal role in ENA-78 regulation.

A008

Investigation of Cytokine Production and Collagen-induced Arthritis in SAPK4 (p38δ) Gene Knock-Out Mice

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p38α MAP kinase is a key enzyme involved in regulating the production and actions of proinflammatory cytokines. SAPK4 (p38δ) is a closely related p38 isoform that is expressed in macrophages and T cells present in rheumatoid arthritis synovial tissue. To investigate possible roles of SAPK4, knockout (KO) mice were generated and effects on LPS-induced cytokine production and joint inflammation in collagen-induced arthritis (CIA) were assessed. Following an LPS challenge, plasma cytokines (TNFα, IL-6 and IL-1β) were increased to a similar extent in WT and KO mice. Furthermore, thioglycollate elicited peritoneal macrophages from WT and KO mice produced similar amounts of LPS-induced cytokines. Finally, KO and WT mice developed CIA with similar incidence and severity. These results suggest that relative to p38α SAPK4 does not play an important role in regulating the production of cytokines in response to acute microbial stimuli and is not required for immunoregulatory processes contributing to more chronic inflammation.

A009

De Novo Design, Synthesis and Evaluation of 1,5-Disubstituted-1-*H*-Indazoles as Inhibitors of p38 MAP Kinase

Ganghyeok Kim, Mark Munson, Mareli Rodriguez, Jim Rizzi, Gary Hingorani, Suzy Brown, Jennifer Otten, Darin Smith, Guy Vigers, Barb Brandhuber, Jianhong Wang, Michelle Goyette, and Larry Burgess

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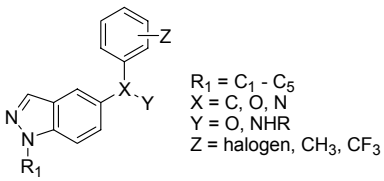
p38 MAP kinase is a key down stream signaling protein of the mitogen activated protein kinase family that triggers the biosynthesis of pro-inflammatory cytokines such as tumor necrosis factor and interleukin-1. Inhibition of p38 MAP kinase has been one of the key approaches toward the development of treatments for chronic inflammation such as rheumatoid arthritis, inflammatory bowel disease and psoriasis.
The *de novo* design, synthesis, x-ray co-crystal structure, and *in vitro* activities of 1,5-disubstituted-1-*H*-indazoles as inhibitors of novel p38 MAP kinase will be presented.



A010

PHARMACOLOGICAL ANALYSIS OF THE INFLAMMATORY RESPONSE INDUCED BY LATEX OF *CALOTROPIS PROCERA*

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Introduction: *Calotropis procera* (Ait). R.Br. (Asclepiadaceae) produces contact dermatitis and iridocyclitis on accidental exposure. But the mechanism underlying the pro-inflammatory response is unknown. In the present study, pharmacological characterization of the inflammatory response induced by the dry latex (DL) of *C. procera* has been carried out in acute and chronic models of inflammation and the effect of anti-inflammatory drugs was evaluated.
Results: Subcutaneous injection of 0.1ml of 1% DL into the subplantar surface of rat paw produced a significant inflammation with a peak response at 1h accompanied by an increase in vascular permeability. NSAIDs were more effective than steroidal drugs while anti-histaminics and anti-serotonergic drugs completely inhibited the DL induced oedema. Aminoguanidine and IL-1β were other inhibitors. In the other model, 2.5% of DL when injected into a 6 day old rat air pouch caused a time dependent increase in granulation tissue, exudate volume, cellular infiltration, protein concentration and PGE_2 levels. The prostaglandin mediated inflammatory response of DL was inhibited by selective COX-2 inhibitors and steroidal drugs. Further, DL was also found to contain histamine.
Conclusions: Our study indicates that latex of *C. procera* is a potent phlogistic agent mediating its inflammatory response by the release of histamine, prostaglandins, nitric oxide and the presence of histamine in the latex and can be used a model to evaluate anti-inflammatory compounds. (AS is a SRF of CSIR).

A011

Histamine modulates mast cell degranulation through indirect mechanism in *Toxocara canis*-infected rats. [1]Daniela Carlos*, [1]Anderson Sá-Nunes, [1]Camila M. Peres, [2]Maria C. Jamur, [2]Constance Oliver and [1]Lúcia H. Faccioli. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, [2]Faculdade de Medicina de Ribeirão Preto, São Paulo, Brasil. E-mails: danicar@fcfrp.usp.br; faccioli@fcfrp.usp.br
Histamine, a chemical mediator of allergy and inflammation, regulates functions of immune and inflammatory cells by modulating the activity of mast cells, T cells, monocytes, macrophages, neutrophils and eosinophils. The aim of this study was to investigate whether functional alterations on mast cells due to *T. canis* infection would be favouring degranulation of these cells. Peritoneal cells were recovered from non-infected and *T. canis*-infected rats intraperitoneally with 20 ml PBS. The mast cell population was immunomagnetically isolated from the peritoneal washing using monoclonal antibody (mAb) AA4 coupled to beads. Isolated mast cells were incubated for 30 minutes at 37º C with PBS, compound 48/80, histamine, serotonin or with lymphoid cell or macrophage culture supernatants stimulated with histamine. Incubation of isolated mast cells from non-infected and infected groups with histamine or serotonin directly did not induce degranulation of these cells. However, isolated mast cells from infected group incubated with lymphoid cells supernatants stimulated with histamine released high amounts of β-hexosaminidase and TNF-α. On the other hand, β-hexosaminidase and TNF-α release was reduced in the supernatant of isolated mast cells from infected group after incubation with macrophages supernatants stimulated with histamine. Therefore, histamine induces mast cell degranulation through of an indirect manner, which involves the participation of lymphocytes and macrophages. Financial support: CNPq, FAPESP.

A013

New, Optimized Cell-based Assays to Monitor Cellular p38/JNK Activities with the Two-color Fluorescent Imaging System

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P38s and JNKs are the two distinct MAP kinases that have been validated clinically and/or pre-clinically as viable therapeutic targets for multiple inflammatory disorders such as rheumatoid arthritis. In the course of developing JNK- or P38-specific inhibitors, the unmet challenge is the assessment of cellular potency and selectivity when potential chemical lead candidates possess dual JNK/P38 potency. Herein we report the successful development of new, optimized cell-based assays specific for the endogenous p38 and JNK activities in human transformed and primary cells. Cellular P38/JNK activities are monitored via phosphorylation levels of their downstream substrates (HSP27/ c-Jun respectively) by Western blotting and/or In-Cell Western assays with the two-color fluorescent Odyssey Imaging System. These assays allow a simultaneous determination of the EC50s of potential lead compounds against P38/JNK activities in cells with acceptable throughput. These quantitative assays have been further validated with reference compounds, demonstrating their utility in compound screening.

A014

Identification and characterization of potent and selective antagonists of the Th2 cell-associated chemokine receptor CCR4

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Th2 cells are thought to play a pivotal role in the pathophysiology of allergic inflammatory disease. The chemokine receptor CCR4 is expressed on Th2 cells, and its interaction with two chemokine ligands CCL17 and CCL22 is believed to regulate the migration of Th2 cells to inflammatory sites. Therefore CCR4 antagonists may have therapeutic potential in the treatment of Th2 cell-mediated diseases such as asthma and atopic dermatitis. We have developed a series of non-peptidic inhibitors that block the binding of both CCL17 and CCL22 to CCR4. *In vitro* characterization of these compounds in cell-based assays utilizing CCR4 transfectants and primary $CCR4^+$ T cells demonstrates that they are devoid of agonist activity, and block CCR4 mediated signaling such as calcium flux and chemotaxis. No interaction was seen with a panel of related chemokine receptors. Such compounds should be useful in further understanding the role of CCR4 in the allergic inflammatory response.

A015

Design And Syntheses Of Novel Small Molecule Peripherally Restricted Mu Agonists For The Treatment Of Inflammatory And Incisional Pain.

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Opioid receptors exist both in the CNS and the peripheral terminals of primary afferent neurons. Recently a substantial literature has demonstrated that opioids can produce potent and clininically measurable antihyperalgesia by activation of the opioid receptors on the peripheral terminals of primary sensory neurons. The discovery of opioid "peripheral analgesia" provides an opportunity to design new analgesics that produce no central side effects such as respiratory depression, dependence, dysphoria, nausea, and sedation, but retain potent analgesic actions. Such drugs should not cross the blood-brain barrier, and they would neither affect motor functions, nor have NSAID-like side effects such as gastrointestinal irritation. In this presentation, we will report our efforts in the discovery of novel small molecule peripherally restricted mu agonist targeting on inflammatory pain states. We will describe the design and synthesis of a structurally novel, highly potent and peripherally-restricted mu opioid agonist, DiPOA ([8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic acid) and its in vitro and in vivo pharmacological properties. DiPOA represents the first peripherally-restricted, small molecule mu opioid agonist that is non-sedating, anti-hyperalgesic and effective against both inflammatory and incisional pain when administered systemically. The presentation will also discuss SAR around DiPOA, the drug design strategies for inflammatory targets and the method of peripherallization by introducing a zwitterion moiety into a centrally active mu agonist.

A016

Activation of PPAR γ inhibits IL1β-induced mPGES-1 expression in human synovial fibroblasts by interfering with Egr-1

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Membrane-associated prostaglandin E synthase-1 (mPGES-1) catalyzes the conversion of PGH_2 to PGE_2, which plays a critical role in inflammation. Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated transcription factor and was shown to regulate a number of inflammatory genes in several cell types. In this study, we examined the effect of PPARγ ligands on IL-1β-induced mPGES-1 expression in human synovial fibroblasts (HSF). PPARγ ligands 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (15d-PGJ_2) and the thiazolidinedione troglitazone (TRO), dose-dependently suppressed IL-1β-induced PGE_2 production, as well as mPGES-1 protein and mRNA expression. 15d-PGJ_2 and TRO suppressed IL-1β-induced activation of the mPGES-1 promoter. Overexpression of wild-type PPARγ further enhanced, whereas overexpression of a dominant negative PPARγ alleviated, the suppressive effect of both PPARγ ligands. Furthermore, pretreatment with an antagonist of PPARγ, GW9662, relieved the suppressive effect of PPARγ ligands on mPGES-1 expression, suggesting that the inhibition of mPGES-1 expression is mediated by PPARγ. We also demonstrated that PPARγ ligands suppressed Egr-1-mediated induction of the activities of the mPGES-1 promoter and of a synthetic reporter construct containing three tandem repeats of an Egr-1 binding site. Electrophoretic mobility shift and supershift assays for Egr-1 binding sites in the mPGES-1 promoter showed that both 15d-PGJ2 and TRO suppressed IL-1β-induced DNA binding activity of Egr-1. These data define mPGES-1 and Egr-1 as novel targets of PPARγ and provide further support for the promising application of PPARγ ligands in the treatment of inflammatory disorders.

A17

Therapeutic Utility of Selective iNOS Inhibition

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Expression of inducible nitric oxide synthase (iNOS) results in the sustained production of NO and NO-derived metabolites eliciting cytotoxicity and tissue damage that contribute to the pathophysiology of diseases including arthritis and other inflammatory conditions. The pharmacological activity of a selective iNOS inhibitor, L-N^6-(1-iminoethyl)lysine 5-tetrazole amide, and its active metabolite, L-N^6-(1-iminoethyl)lysine, were characterized. L-N^6-(1-iminoethyl)lysine is a potent, selective, irreversible inhibitor of purified recombinant human iNOS and inhibits iNOS activity in human cartilage explants and primary lung epithelial cells. In vivo, L-N^6-(1-iminoethyl)lysine 5-tetrazole amide reduces the severity of adjuvant arthritis in the rat, cartilage destruction in the ACL transection model in the dog, pain in the meniscetomized rat model of osteoarthritis, and exhaled NO following LPS or antigen challenge in the rat. Following administration to normal volunteers and mild asthmatics, it produces a rapid, prolonged inhibition of exhaled NO. Taken together, these studies demonstrate that this selective inhibitor of iNOS has pharmacological activity and therapeutic potential in the treatment of inflammatory diseases.

A018

Requirement of GM-CSF for the effective development of an antigen-induced inflammatory reaction

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Data from several inflammation models indicate that granulocyte-macrophage-colony stimulating factor (GM-CSF) can be a key inflammatory mediator. Convenient models in readily accessible tissues are needed to enable the GM-CSF-dependent cellular responses to be elaborated. We show here that, in contrast to the response to thioglycolate, an antigen-specific methylated-BSA-induced peritonitis in GM-$CSF^{-/-}$ mice was severely compromised. The reduced response in the latter peritonitis model was characterized by fewer neutrophils and macrophages, as well as by deficiencies in the properties of the remaining macrophages, namely size and granularity, phagocytosis, allogeneic T cell triggering, and cytokine production. B1 cells were more evident in the GM-$CSF^{-/-}$ antigen specific exudates. We propose that these findings contribute to our understanding of how GM-CSF acts as a proinflammatory cytokine, and indicate that the nature of the stimulus is quite critical in determining whether a particular inflammatory mediator, such as GM-CSF, plays a role in an ensuing inflammatory reaction.

A019

Differing roles for tPA and uPA in thioglycolate-induced peritonitis

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The plasminogen activators (PAs), tissue-type (tPA) and urokinase (uPA), are involved in cleavage of plasminogen to plasmin. While the PA/plasmin system is involved in fibrinolysis, we have shown differing roles for tPA and uPA in collagen-induced arthritis. The aim of this study was to investigate their roles in thioglycolate (TM)-induced peritonitis. $tPA^{-/-}$ and $uPA^{-/-}$ mice were injected intraperitoneally with TM. 4 day TM-elicited exudates from $tPA^{-/-}$ mice contained significantly fewer Mac-1^{hi} macrophages compared with those from $tPA^{+/+}$ mice. In contrast, there was no difference in the TM-elicited exudates response from $uPA^{-/-}$ and $uPA^{+/+}$ mice. *In situ* LPS activation 3 hours prior to harvesting TM-elicited exudate cells led to a reduction in Mac-1^{hi} macrophages recovered from $tPA^{-/-}$ and $tPA^{+/+}$ mice, with increased cellular adhesion and fibrin staining on the peritoneal lining, particularly in $tPA^{-/-}$ mice. We propose that the impaired cellular infiltrate seen in $tPA^{-/-}$ mice in response to TM is due to the accumulation of fibrin on the mesothelial lining of the peritoneal cavity to which the Mac-1^{hi} macrophages adhere.

A020

Pharmacological characterization of arthritis pain in a rat model of menisectomy-induced osteoarthritis. Cortes-Burgos, L*, Settle, S, Sanders, J, Bennett, L, Manning, P, Connor, J and Muthian, S. Pfizer Inc, St. Louis, MO, USA

A model of meniscectomy-induced osteoarthritis (OA) was generated to study OA pain. Rats underwent right hind limb partial medial meniscectomy and pain assessed using 3 different measures: tactile allodynia, mechano-hyperalgesia and hind limb weight distribution. A selective COX-2 inhibitor, 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58236), a selective COX-1 inhibitor, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-58560), acetaminophen and the anti-convulsant gabapentin, was assessed. SC-58236 significantly increased allodynic paw withdrawal and mechano-hyperalgesic thresholds in the arthritic limb. Hind leg weight distribution differential was also significantly decreased. SC-58560 was ineffective in all pain assessments. Acetaminophen was efficacious in mechano-hyperalgesia and hind limb weight differential but not in tactile allodynia. Gabapentin was partially effective in all three pain assessments. Meniscectomy-induced osteoarthritis in the rat results in chronic pain that responds to standard therapies and may be useful for evaluating the mechanisms of pain and efficacy of pharmacological agents to treat signs and symptoms of OA.

A021

High fractional inhibition by an irreversible cathepsin S inhibitor is required to modulate mouse immune responses

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Cathepsin S (Cat S) is a cysteine protease expressed mainly in spleen and antigen presenting cells including B-cells, dendritic cells and macrophages. Cat S cleaves the MHC class II invariant chain (Ii) p10 fragment to allow peptide binding in the MHC class II peptide binding groove. In this study, we have tested the effects of an irreversible Cat S inhibitor, CRA-3316(4-methyl-*N*-[(1*S*,2*E*)-1-(2-phenylethyl)-3-(phenylsulfonyl)-2-propenyl]amino]-1-(phenylmethyl)ethyl]-1-piperazinecarboxamide), in a mouse model of immune response. CRA-3316 has an IC50 of 2 nM against mouse Cat S, 6.2 nM in a mouse splenocyte enzyme occupancy assay and 107 nM in an *in vitro* mouse whole blood enzyme occupancy assay. CRA-3316 was active in a functional mouse antigen presentation assay (A20 B-cells and DO.11.10 hybridoma, IC50=0.6 nM) and a mouse isolated splenocyte Ii p10 accumulation assay (MIC= 10 nM). *In vivo* in a single dose study, 24 hrs post delivery of 10, 30, 100 mpk ip of CRA-3316 to C57BL/6 mice, splenocyte Cat S enzyme occupancies of 35, 82, 100 % respectively were observed. As well in this study, accumulation of the Ii p10 fragment in the spleen occurred at 30 and 100 mpk. CRA-3316 was also tested at the same doses in a 10-day mouse model of immunosuppression in which mice were primed with NP-OVA on day 2. After 10 days, CRA-3316 at 30 mpk affected mouse immune responses as measured by a ~50% inhibition of lymph node cell proliferation in response to an OVA recall, and a ~50% reduction in the numbers of germinal centers. Spleen Ii p10 accumulation occurred at 100 mpk. CRA-3316 affected measures of mouse immunosuppression, thereby validating this functional model for future use with reversible inhibitors having activity toward Cat S. Based on these data we suggest that a high fractional inhibition of Cat S is required to cause immunosuppression in the mouse.

A022

Title: *Hepatic Acute Phase Reaction in Histamine Deficient Gene Targeted Mice*

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Histamine is a versatile mediator that — according to *in vitro* studies — affects the synthesis of inflammatory cytokines and acute phase proteins. Histidine-decarboxylase knockout mouse (HDC-/-) is a model of *in vivo* investigation of the physiologic and metabolic integration of the acute phase response. These mice do not synthesise histamine and feeding them with histamine-poor diet they are almost completely histamine-deficient.

We compared the serum concentrations of representatives of acute phase plasma protein as well as the levels IL-6 and IL-1α in wild type and HDC -/- mice during local (turpentine induced) or systemic (LPS induced) inflammation. The level of some acute phase proteins significantly differed in wild type and HDC -/- mice while others remained unaffected. The IL-6 levels are also differ in the wild type and histamine deficient animals, suggesting that the effect of histamine is attained through IL-6, although direct effect is not disclosed yet.

A023

Triterpenoid inhibition of matrix metalloproteinase expression

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Abstract: We have reported that CDDO, a synthetic derivative of the triterpenoid family, inhibits matrix metalloproteinase (MMP) -1 and -13, suggesting anti-arthritic potential. Recently, MMP-9 expression by macrophages was proposed to have a role in atherosclerosis. We report a comparison in joint cells of MMP inhibition by CDDO with a chemically similar triterpenoid, TP-222; and on TP-222's effects in macrophages.

Material and methods: Human chondrocytes, synovial fibroblasts and murine RAW 264.7 macrophages were cultured +/- TP-222 or CDDO for 24 hours, and then IL-1 or LPS added for 18 hours. RNA was harvested and subjected to quantitative real-time PCR for MMP expression.

Results: In all cell types, 300nM TP-222 or CDDO were sufficient to decrease MMP expression. In chondrocytes, TP-222 decreased IL-1-induced MMP-1 and MMP-13 expression by 36 and 74%, whereas CDDO resulted in inhibition of 15% and 47%. Similar results were found in synovial fibroblasts. In RAW cells, TP-222 decreased LPS-induced MMP-9 (76%) and MMP-13 (95%) expression.

Conclusion: TP-222 was the more effective inhibitor of MMP expression in chondrocytes and fibroblasts. TP-222 was effective at inhibiting macrophage expression of MMP-9 and -13. Triterpenoid inhibition of the expression of MMPs relevant to arthritis or atherosclerosis indicates their potential as therapeutics for these diseases.

A024

Role of microsomal prostaglandin E synthase-1 in prostaglandin production and cytokine release in alveolar and peritoneal macrophages.

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Cyclooxygenase-2 (COX-2) and prostaglandin E synthase-1 (mPGES-1) are inducible enzymes shown to be involved in the production of inflammatory PGE2. Macrophages synthesize high levels of PGE2 and may contribute to rheumatoid arthritis. We compared the prostaglandin-producing capacity of LPS-treated alveolar and resident peritoneal macrophages from mPGES-1 null and wild type mice. Striking differences in the LPS-stimulated PGE2 output were observed between the two cell types, with resident peritoneal macrophages producing 300-fold more PGE2 than alveolar macrophages. Although the presence of mPGES-1 was required for the synthesis of the majority of PGE2, resident mPGES-1 null peritoneal macrophages retained some PGE2 production capacity. The loss of mPGES-1 did not result in a significant increase of other prostaglandins (TXB2, PGD2, PGF2alpha and 6-keto PGF1alpha). Addition of exogenous arachidonic acid to resident macrophages from wild type and mPGES-1 null mice demonstrated a requirement for mPGES-1 and an ability of mPGES-1 to produce PGE2 in a COX-1-dependent manner. In an experiment where resident peritoneal macrophages from mPGES-1 null and COX-2 null were co-cultured, no increase in synthesis of PGE2 beyond basal levels was observed. Finally, examination of cytokines produced by alveolar and resident peritoneal macrophages shows that mPGES-1 deletion results in a small increase in TNF alpha release and minimal changes to levels of IL-12 p70, IL-10, IL-6 and MCP-1.

A025

15d-PGJ$_2$ inhibits IL-1β-induced cyclooxygenase-2 expression in human synovial fibroblasts through a histone deacetylase-independent mechanism

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Objective. 15d-PGJ$_2$ is a natural ligand for peroxisome proliferator-activated receptor γ (PPARγ) and has been reported to inhibit the expression of a number of inflammatory genes in several cell types. However, its effects on cyclooxygenase-2 (COX-2) expression remains controversial. In the present study, we investigated the effects of 15d-PGJ$_2$ on IL-1β-induced COX-2 expression in human synovial fibroblasts (HSFs).

Results. 15d-PGJ$_2$ inhibited IL-1β-induced COX-2 protein and mRNA expression, as well as COX-2 promoter activation. The suppression of COX-2 protein expression was abrogated by the PPARγ antagonist, GW9662, suggesting that this effect is mediated by PPARγ. The induction of COX-2 by IL-1β is associated with hyperacetylation of histone H3 and H4 at the COX-2 promoter. Interestingly, 15d-PGJ$_2$ selectively blocked IL-1β-induced histone H3 acetylation. This reduction was demonstrated to not correlate with the recruitment of histone deacetylase (HDAC) to the COX-2 promoter. Also, treatment with the specific HDAC inhibitor, trichostatin A, did not relieve the suppressive effect of 15d-PGJ$_2$, indicating that HDACs are not involved in the inhibitory effect of 15d-PGJ$_2$ on COX-2 expression. Furthermore, 15d-PGJ$_2$ blocked IL-1β-induced recruitment of the histone acetylase (HAT) p300 to the COX-2 promoter, which may be the mechanism for decreased histone H3 acetylation and COX-2 expression. In line with this, overexpression of p300, but not of a mutant p300 lacking HAT activity, relieved the inhibitory effect of 15d-PGJ$_2$ on COX-2 promoter activation.

Conclusion. Our data suggest that 15d-PGJ$_2$ can inhibit IL-1β-induced COX-2 expression in a PPARγ-dependent, HDAC-independent mechanism, likely by interfering with the HAT p300.

A026

WITHDRAWN

A027

Chronic administration of selective bradykinin B_1 receptor antagonists attenuate hyperalgesia in non-obese diabetic (NOD) mice

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The present study aimed at evaluating the implication of the bradykinin B_1 receptor (BKB_1-R), a receptor upregulated during the inflammatory progress of diabetes, in the development of diabetic hyperalgesia in a model of spontaneous autoimmune type 1 diabetes in non-obese diabetic (NOD) mice. Only female NOD mice with blood glucose level > 20 mmol l^{-1} were used. The nociception was assessed using the hot plate and tail immersion thermal tests and was followed from the 3rd to the 28th week of age. The results showed that NOD mice develop a significant time-dependent hyperalgesia starting from the 8th week of age with the maximum effect observed at the 16th week. The hyperalgesic activity reached a plateau from the 16th to the 24th week of age (n = 50; P < 0.001). Chronic i.p. administration of the selective peptidic BKB_1-R antagonists R-715 (Ac-Lys-[D-ß Nal^7, Ile^8] desArg^{9}bradykinin; 400 µg kg^{-1}) and R-954 (Ac-Orn-[Oic^2, α-Me Phe^5, D-ß Nal^7, Ile^8] desArg^{9}bradykinin; 200 µg kg^{-1}), twice daily for 7 days, significantly attenuated the diabetes-induced hyperalgesia in NOD mice. The percent of hot plate basal latency increased to 90.20 ± 0.92 and 92.04 ± 1.34 % for the R-715 and the R-954-treated NOD mice, respectively, compared to 72.23 ± 1.29 % for untreated NOD mice (n = 12-18; P < 0.001). Similarly, the percent of tail immersion basal latency was 89.86 ± 1.23 and 90.55 ± 1.18 % for the R-715 and the R-954-treated NOD mice, respectively, compared to 70.77 ± 1.46 % for untreated NOD mice (n = 9-19; P < 0.001). These results provide further evidence for the involvement of the B_1-R in the diabetic hyperalgesia observed in NOD mice and confirm our previous findings in the STZ-induced type 1 diabetes model in CD-1 mice.

A028

ROLE OF IKK BETA IN OSTEOCLASTOGENESIS AND RESORPTION OF BONES

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Osteoclasts are the principal, if not exclusive, bone resorbing cells, and their activity has a profound impact on skeletal health. These cells are members of the monocyte/macrophage family. RANKL, a member of the tumor necrosis factor superfamily, is most abundantly expressed by bone marrow stromal cells. Interaction of RANKL with its receptor RANK on osteoclast-precursor cells activates all three MAPK pathways as well a PI-3K and the NF-κB family of transcription factors. NF-κB plays a pivotal role in the regulation of many immune and inflammatory processes including those involved in rheumatoid arthritis (RA) pathology. IKKβ is essential for the inflammatory cytokine-induced activation of NF-κB. We used a potent and selective small molecule inhibitor of IKKβ ---MLN120B to further elucidate the biological function of IKKβ in human osteoclasts. MLN120B can block NF-κB signaling in many major cell types that are involved in the genesis of RA. In the osteoclast, IKKβ is critical for both differentiation as well as early ostoclastogenesis, which indicates a mechanism distinguished from p38 inhibition or inhibition by Alendranate. In two rat arthritis models, Adjuvant (AA) and Collagen (CIA) induced arthritis, bioavailable IKKβ inhibitor MLN120B can significantly protect the bone lost as seen by micro CT scanning. In summary, we used potent, selective IKKβ inhibitor MLN120B to demonstrate the critical role of IKKβ in human osteoclast differentiation, function and apoptosis. Data further support the rationale of developing selective IKKβ inhibitors as a therapeutic drug target for bone related diseases, such as RA, OA, psoriatic arthritis and osteoporosis.

A029

Induction of microsomal prostaglandin E_2 synthase-1 (mPGES-1) in carrageenan-induced paw edema: a contribution to both peripheral and CNS responses

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It has been shown that peripheral inflammation in the rat paw causes stimulation of cyclooxygenase-2 (COX-2) and of PGE_2 synthesis in the central nervous system (CNS). We have evaluated the changes in prostanoid levels and in the expression of the various prostanoid synthases in the inflamed paw and CNS tissues. In the paw, increases in TXB_2 and PGE_2 were associated with the induction of COX-2 and mPGES-1, while the expression of cytosolic PGE_2 synthase (cPGES) and mPGES-2 did not change. In the cerebrospinal fluid (CSF), we observed increases in PGE_2, 6-keto-$PGF_{1\alpha}$, PGD_2 and TXB_2 during the early phase. PGE_2 was also elevated in the CNS tissues with a strong induction of the expression of mPGES-1 both in spinal cord and brain. The other synthases (cPGES, mPGES-2, prostacyclin, PGD_2 and TXB_2 synthases) were not induced in the CNS. The results indicate that the levels of several prostanoids in addition to PGE_2 are elevated in the CSF during peripheral inflammation and that mPGES-1 induction contributes to the stimulation of PGE_2 production in both peripheral and CNS tissues.

A030

Molecular and Cellular Mechanisms underlying Airway Inflammation and Hyperreactivity in a Murine Model of Asthma

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Blockade of CD86 but not CD80 function via specific antibody treatment has been shown to suppress airway inflammation in allergen-challenged mice. Our study describes some of the molecular and cellular events that regulate CD86 expression and the induction of pulmonary inflammatory responses in ovalbumin (OVA)-sensitized mice. Maximum CD86 and MHC II antigen upregulation on resident antigen presenting cells (APCs) occurred 6 hours after the second of the three local challenges in OVA-sensitized mice. Activation of p38α MAPK in the lung preceeded CD86 upregulation whereas activation of AKT occurred later and in parallel with down regulation of CD86. Pre-treatment of OVA-sensitized mice with an antisense oligonucleotide specific for p38α MAPK down regulated CD86 gene expression, suppressed the co-stimulatory activity of APCs and development of airway eosinophilia following allergen challenge. These studies demonstrate the coordinated activation of signaling pathways in APCs, Th2 cells, and effector inflammatory cells in response to allergen challenge.

A031

P38 inhibitors as potential therapeutics to treat cartilage degeneration and pain associated with osteoarthritis

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Osteoarthritis (OA) is the most common rheumatic disease and is characterized by a progressive loss of joint integrity resulting in decreased joint function and pain. There is evidence that inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) play a significant role in the progression of OA. Inhibition of intracellular signaling pathways such as the mitogen activated protein kinases (MAPK) may be a potential approach to treat the symptoms and to preserve joint structure in OA. Several p38 MAPK inhibitors were evaluated for their inhibitory effects on joint destruction in the rat iodoacetate (RIA) model of OA and for analgesic benefit in the Hargraeve's model of hyperalgesia. SKB-203580 and VX-745 administered orally at a dose of 50 mg/kg resulted in the significant ($p < 0.05$) inhibition of joint degeneration in the RIA model of 45% and 31%, respectively. SKB-203580 demonstrated a dose related inhibition of joint degeneration of 30, 25 ,12 and 8% at 50, 25, 10 and 5 mg/kg, p.o. b.i.d. in the RIA model. Similarly, both p38 inhibitors significantly ($p < 0.05$) inhibited the pain response (paw withdrawal time) in the Hargraeve's assay when administered orally at 30, 10 and 3 mg/kg. These data suggest that p38 MAPK inhibitors may be useful for both structural benefits and pain relief in OA.

A032

Apoptosis Induced by Paclitaxel, Tobacco Smoke Condensate and Hydrogen Peroxide in Lung Epithelial Cells (A549).

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Cigarette smoke is known to be a complex mixture of >4000 constituents, dispersed between the volatile and non-volatile phase, and inhaled exposure causes lung inflammation and cell injury. Here, we investigate the effects of some components of cigarette smoke, as inducers of apoptosis in A549 cells. Apoptosis is characterised by initial changes in the mitochondria, leading to chromatin condensation and ultimately DNA fragmentation. Cells were treated with 1µM paclitaxel, 80µg/ml tobacco smoke condensate (TSC), 1µM nicotine and 10µM hydrogen peroxide, for 24 and 48 hours. Attached and detached cells were stained with 4',6-diamidino-2-phenylindole (DAPI), and visualized by fluorescent microscopy, % of normal and apoptotic cells were determined. Results indicated morphological changes, with time, but quantification proved difficult. DNA was isolated and electrophoresed on 1% agarose gels. A DNA ladder was present from cells treated with paclitaxel, TSC and hydrogen peroxide, but not nicotine. The presence of a DNA ladder indicates that late stage apoptotic events are occurring in these cells on exposure to certain components of tobacco smoke.

A033

MyD88 dependent and independent pathways in murine chronic destructive arthritis

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MyD88 is a Toll/IL-1 receptor (TIR) domain-containing adaptor molecule known to have a central role in both IL-1/IL-18 and TLR signaling. The present study investigated the role of MyD88 in acute and chronic destructive SCW arthritis using MyD88 deficient mice. Joint swelling, histopathology, T-cell and B-cell responses were examined. Joint swelling was strongly reduced in the MyD88 deficient mice in acute SCW arthritis. Histology confirmed the crucial role of MyD88 in acute joint inflammation. Chronic relapsing SCW arthritis is highly T-cell dependent, since IFN-γ-/-, RAG-2-/-, mice did not develop the chronic stage. Induction of chronic SCW arthritis in MyD88-/- mice revealed that joint swelling, shortly after each reactivation, was not reduced, implying MyD88 independency. MyD88ko mice did not develop severe chronic arthritis and joint destruction was absent. Both T- and B-cell responses to SCW fragments were not found in MyD88-/- mice. The present study showed that MyD88 is an essential adaptor molecule in acute and erosive chronic destructive SCW arthritis, although acute flares after each reactivation were partly MyD88 independent. Targeting of MyD88 may be a novel therapy in RA.

A034

HISTAMINE H_4 RECEPTOR ANTAGONISTS

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Antihistamines (histamine H_1 receptor antagonists) are a mainstay treatment for atopic allergy, yet they are only partially effective in relieving the symptoms of the disease. They also have very limited value for the treatment of asthma, despite the well-characterized bronchoconstrictory effects of histamine. The recent discovery of a fourth histamine receptor (H_4), expressed mainly on hematopoietic cells led us to develop selective H_4 antagonists to investigate a potential role in allergy and inflammation. The antagonists have high affinity of the human receptor with K_i values in the low nanomolar range. There is at least 1000-fold selectivity over H_1, H_2 or H_3 receptors and no cross-reactivity against 50 other targets. Compounds from this series have oral bioavailability in rats and dogs. We find that histamine induces chemotaxis but not degranulation of mouse bone marrow derived mast cells in vitro and in vivo, and that H_4 receptor antagonists can block the histamine-induced migration. In addition, the H_4 receptor also mediates chemotaxis and adhesion molecule upregulation in eosinophils. The expression and function of the H_4 receptor on several of the hematopoietic cell types that are implicated in the development and symptomatology of allergy and asthma, suggests that pharmacological targeting of the H_4 receptor, either alone or in combination with H_1 receptor antagonists, may prove useful for treating both conditions.

A035

Serum amyloid P-component induction kinetics in murine tuberculosis.

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Acute-phase response is known to occur during human tuberculosis (TB), and the concentration of C-reactive protein, a human acute-phase reactant (APR), increases upto 100-fold. However, the induction of acute-phase response and changes in the levels of serum amyloid P-component (SAP), a major APR in mice, during murine TB are not known. Herein, we report the SAP induction kinetics during murine TB, and the effect of isoniazid (INH) treatment on it. BALB/c mice were intravenously inoculated with live/heat-killed *Mycobacterium tuberculosis* H37Rv (1 x 10^6 CFUs), and their lung mycobacterial loads and SAP levels were determined by plating and rocket immunoelectrophoresis, respectively. Two days after *M. tuberculosis* H37Rv infection, mice showed elevated SAP levels (84.6±9.8 µg/ml) which normalized by day +4 (40.6±5.7 µg/ml), peaked (336.4±38.2 µg/ml) on day +21, and then plateaued thereafter till their death. Mice injected with heat-killed *M. tuberculosis* H37Rv, on day +2, alsoshowed SAP levels similar (76.8±8.4 µg/ml) to those injected with viable bacilli; however, on day +21, SAP levels in these mice returned to background levels. The enhanced-SAP levels correlated well with the increased lung mycobacterial load (6.2±0.9 x 10^7 CFUs on day +21). In INH-treated (25 mg/kg/day x 30; orally) *M. tuberculosis* H37Rv-infected mice, SAP levels increased upto day +14, and then gradually returned to background levels by day +30. These data show the induction of acute-phase response during murine TB resulting, in part, in increased SAP production.

A036

Human osteoarthritic synovial adipose tissue as a drug target: multiplex assay of corticosteroid-sensitive cytokine production from tissue explants.

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Inflamed synovium is considered a potential drug target in osteoarthritis. Adipose tissue is associated with synovial membrane in most osteoarthritic synovial tissue. Screening of drug candidates in arthritis involves the isolation of the synovial membrane from associated adipose tissue. To explore the importance of examination of adipose tissue in drug candidate screening in arthritis we have examined a spectrum of cytokine production in these joint tissue types. We measured cytokine production from both human osteoarthritic synovial membrane and associated adipose tissue separately. Tissues were identified by distinct densities and adipocyte morphology. Cytokines were measured by Luminex™ multiplex assay at 24 hour incubation of tissue explants. Cytokine production was examined under unstimulated, lipopolysaccharide (LPS,100ng/ml) and phorbol 12,13myristate acetate (PMA,10^{-7}M)-stimulated conditions, in the presence and absence of budesonide (10^{-6}M). Both synovium and associated adipose tissue secreted the following cytokines: TNFα, IL-1, -4, -6, -8, -10, IFNγ, and GM-CSF. Neither tissue secreted either IL-2 or IL-12 under unstimulated, LPS or PMA-stimulated conditions. Budesonide reduced (20- 70%) adipose tissue cytokine responses to LPS and PMA. We conclude that adipose tissue is a potentially important drug target in osteoarthritis and suitable for target validation.

A037

Oil of Mustard Induces Acute Colitis That is Prevented by Cannabinoid Receptor Agonists.

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Oil of mustard (OM) is a potent neuronal activator that when given intracololinically elicits visceral hyperalgesia. The extent to which OM is also pro-inflammatory in the GI tract is not known. We show that mice (CD-1) given a single administration of 0.5% OM in 50 uL of 30% ethanol develop a severe colitis that is maximum at day 3 and essentially absent by day 14. Colon shrinkage (2.8 ± 0.5 cm), colon thickening, weight increases (160 ± 40 mg), severe diarrhea with occult blood, and body weight losses (10.6 ± 2.8 %) peaked at day 3. The distal colon had evident inflammation and ulceration. Histology revealed epithelial loss, cell recruitment, edema and destruction of mucosal and smooth muscle architecture. OM-induced neuronal stimulation is reduced by cannabinoid agonists and CB1R-/- mice have exacerbated experimental colitis. Consistent with these reports, the mixed CB1/CB2 agonist, WIN 55212-2 and the CB1R-specific agonist ACEA reduced ($P < 0.05$) OM–induced colon weight gain (70-100%), shrinkage (61–70%), damage (55–80%), diarrhea (76–86%) and bleeding. These findings support the emerging idea that cannabinoid receptors mediate protective mechanisms in experimental colitis and reinforce the importance of neuronal activation in intestinal inflammation.

A038

T cell IL-17 bypasses IL-1-dependent Cartilage Damage in a Macrophage-Driven Arthritis Model

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Objective: IL-1 is a crucial cytokine in cartilage destruction. T cell IL-17 is a proinflammatory cytokine with IL-1-like activities. The purpose of this study was to examine the capacity of T cell IL-17 to circumvent IL-1-dependent cartilage damage in experimental arthritis.
Results: Local overexpression of IL-17 during murine streptococcal cell wall (SCW) arthritis turned this acute, macrophage-driven joint inflammation into a severe, chronic arthritis with aggravated cartilage damage. In IL-1α/β-deficient (IL-1-/-) mice, both joint inflammation and cartilage damage were significantly suppressed after SCW-induction. In contrast, in the presence of IL-17, IL-1-deficiency had no influence on influx of inflammatory cells after SCW arthritis induction and comparable degree of cartilage damage was found as in wild-type mice.
Conclusions: These data clearly show the capacity of T cell IL-17 to turn an acute inflammation into a severe, chronic synovitis. Moreover, T cell IL-17 can replace the catabolic function of IL-1 regarding cartilage damage, directly or via interplay with other macrophage-derived factors.

A039

Identification of a Novel Nonsteroidal Glucocorticoid Modulator.

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Glucocorticoids (GC) such as dexamethasone and prednisolone are among the most potent anti-inflammatory agents in clinical use. However, chronic use of GC's is limited due to potentially adverse side effects which can include osteoporosis, glucose intolerance, lipid redistribution and acute psychosis. Identification of novel glucocorticoid receptor (GR) ligands that maintain the anti-inflammatory efficacy of currently marketed steroids while demonstrating a potential for an improved side effect profile is highly desirable. This presentation describes the discovery of 3-Chloro-4-[4-(5-fluoro-2-methoxy-phenyl)-2-hydroxy-4-methyl-2-trifluoromethyl-pentyl]-benzonitrile **1,** a novel nonsteroidal GR ligand. Similar to dexamethasone, compound **1** demonstrated high affinity for GR and potent repression of interleukin (IL)-1 stimulated IL-6 production in human foreskin fibroblasts (HFF). In contrast to dexamethasone, compound **1** demonstrated a modest induction of aromatase production, a potential side effect marker in the same cellular system. Therefore, this class of ligands may offer a therapeutic advantage over the currently marketed glucocorticoids.

A040

SMALL MOLECULE INHIBITORS OF PROINFLAMMATORY CYTOKINE RELEASE
BIOISOSTERIC REPLACEMENT OF IMIDAZOLES BY ISOXAZOLES

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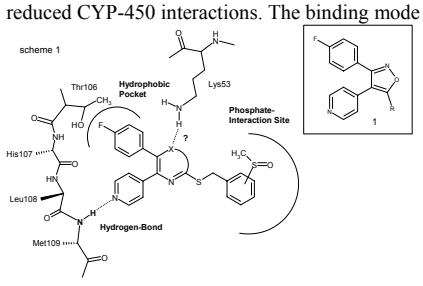
SB 203580 is a prototype inhibitor of p38 MAP kinase, the key enzyme for signal transduction and release of proinflammatory cytokines TNFα and IL-1β. A serious drawback of SB-derived inhibitors is the high interaction potential of the Imidazole-system with CYP-450 enzymes. Our aim was the bioisosteric exchange of the imidazole system by Isoxazoles (1) while retaining essential interaction sites with the p38 protein. This lead to more active compounds with reduced CYP-450 interactions. The binding mode is outlined in scheme 1.



A041

Tetrasubstituted Imidazole Inhibitors of cytokine release Optimizing the N-1 position for p38 MAP kinase inhibition and low CYP-450 interaction

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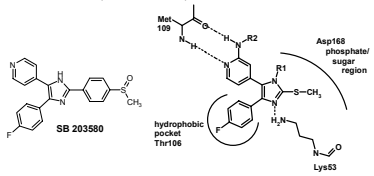
Pyridinylimidazoles are known as potent inhibitors of p38 MAP Kinase. The structures known so far have the disadvantage of inhibiting CYP450 enzymes. Responsible for this interaction are two structural properties of these compounds: the pyridinyl- and the imidazole –ring. Aim of this work was to modify SB-type structures to optimize p38 inhibition while lowering CYP450-interference at the same time. Our strategy was to substitute the pyridinyl-ring by aminoalkyl- and aminoacyl-substituents (R1) and to introduce alcohols, ethers, amines, acetales and sulfides at the N1 position (R2).
Most potent compounds showed 20-fold increase in potency and strongly reduced CYP-450 inhibition (1A2, 2D6).



A042

Design of Purine-Like ATP-Competitive Kinase-Inhibitors

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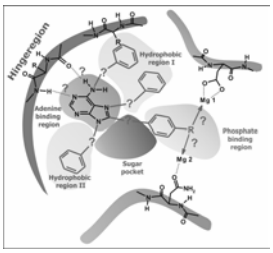
The protein kinase family represents both a huge opportunity and a challenge for drug development. Reversible protein phosphorylation is the main strategy for controlling activities of eukaryotic cells. Uncontrolled signalling has been implicated in inflammation, oncogenesis, arteriosclerosis, and psoriasis.
Structure elucidation of complexes of ATP or the ATP-analog AMP-PNP bound to protein kinases have revealed that there are regions within the binding cleft that ATP does not fully occupy. The regions not occupied by ATP show structural diversity between members of the kinase family.



This diversity provides opportunities for the discovery or design of selective small molecule ATP-competitive inhibitors. We synthesized about 50 derivatives by systematic variation of alkylaryl- and/or heteroalkylaryl-substituents at the purine-template.
These structures were tested on inhibitory potency on a panel of 15 most relevant kinases (upstate-kinase profiler)

A043

Inflammatory Cell Signaling

Structural Basis for the Selective Inhibition of JNK1 by the Scaffolding Protein JIP1 and SP600125(anthra[1,9-cd]pyrazol-6-(2H)-one).

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Objectives: To investigate the mechanism of selective regulation of JNK by JIP1 which is a scaffolding protein assembling the components of JNK cascade, we determined the crystal structure of human JNK1 complexed with pepJIP1 and SP600125. **Methods**: The protein expressed in bacteria was purified and crystallized for structure determination. The binding affinity was measured by Isothermal Titration Calorimetry. **Results**: From the crystal structure at a resolution of 2.35Å, the van der Waals contacts by the 3 residues (Pro157, Leu160, and Leu162) of pepJIP1 and the hydrogen bonding between Glu329 of JNK1 and Arg156 of pepJIP1 are critical for the selective binding. Binding of the peptide also induces a hinge motion between the N- and C- terminal domains of JNK1 or JNK2 and distorts the ATP-binding cleft, reducing the affinity of the kinase for ATP and inhibiting the catalytic activity of the kinase. In addition SP600125, an ATP-competitive inhibitor of JNK, effectively occupies the hydrophobic pocket of the ATP binding site. **Conclusions**: The three dimensional structure will provide basis for development of more potent and selective JNK inhibitors.

A044

Inoculation of a DNA expressing IL-12 in mice inhibits eosinophilic inflammation but not airway hyperresponsiveness

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The present study investigated the effect that intradermal injection with pcDNA-IL-12 or pcDNA3 has on eosinophilia, cytokine production and airway hyperresponsiveness in *Toxocara canis* infection mice, 24 and 48 days post-infection. DNA-IL-12 and pcDNA3 administrated via gene gun were done 3 times to Balb/c mice and were inoculated with 200 eggs of *T.* canis. The infected animals pre-treated with pcDNA-IL-12 presented inhibition of eosinophil migration into the BALF and peripheral blood and showed significant increase in IL-12 and IFN-γ levels in lung homogenates. At 24 days post-infection, the IgG was reduced, specific IgG2a was unaffected, the production levels of IL-4, IL-5 and IL-10 were unchanged and there was no inhibition of airway hyperresponsiveness. 48 days post-infection, the pcDNA-IL-12 reduced IL-10 levels. In contrast, the pcDNA3 did not induce IL-12 production by lung cells but did induce IFN-γ production, reduced airway eosinophil numbers and significantly inhibited airway hyperresponsiveness. These results indicate that pcDNA-IL-12 modulates responses between Th2 and Th1 helper T cells and may be therapeutically beneficial eosinophilic inflammation. In order hand, pcDNA3 administration may be therapeutically beneficial in eosinophilic inflammation and hiperresponsiveness.

A045

Glycosylation Influences Ligand Recognition by the Receptor for Advanced Glycation End Products (RAGE)

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RAGE is a membrane-bound receptor that recognizes a number of ligands. We have investigated the extent of RAGE glycosylation and the impact on recognition of a new ligand, the S100 protein, S100A9. RAGE transfected cells bind S100A9; soluble RAGE reacts with S100A9 in ELISA activity assays; and soluble RAGE is recovered in pull down assays using glutathione-S-transferase-S100A9 fusion protein. RAGE is glycosylated at a single site, utilising Asparagine 25. Interaction between RAGE and S100A9 is mediated in part through a further modification of the N-linked carbohydrate. Binding of RAGE to other RAGE ligands has individual requirements that include the presence or lack of RAGE glycosylation. We conclude that RAGE glycosylation influences the specificity of receptor-ligand recognition.

A046

DEXAMETHASONE INHIBITS CYTOKINES PRODUCTION AND EOSINOPHILIA IN MICE INFECTED WITH *Strongyloides venezuelensis*

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The dexamethasone (Dex) presents an anti-inflammatory action and is used worldwide to treat urticaria, allergy and asthma treatment. When human patients with chronic strongyloidiasis are submitted to treatment with this drug, they develop a disseminated form of this parasite. So we investigated the role of dexamethasone in the production of cytokines and eosinophilia in mice infected with *S. venezuelensis.* BALB/c mice were s.c infected with 1500 infective larvae of *S. venezuelensis* and daily treated or not with 2 mg/kg of dexamethasone s.c. Non-infected mice were used as control. On days 1st, 3rd, 5th, 7th, 14th and 21th after infection, the animals were killed and the number of eosinophils in the blood, peritoneal cavity fluid (PCF) and bronchoalveolar lavage fluid (BALF) and cytokines (pg/mg of lung) were quantified. The eosinophil numbers and IL-3, IL-4, IL-5, IL-12 and IFN-γ levels increased significantly in all mice infected when compared with control group in all days analyzed. The treatment of infected animals with dexamethasone significantly inhibited eosinophils number in blood and migration to PCF and BALF. On one hand, the IL-3, IL-5 cytokines were significantly inhibited by Dex treatment, while IL-4, IL-12 and IFN-γ presented an increase when compared with infected mice. Our data show that dexamethasone is a potent immunesupressor drug able to inhibit eosinophilia and cytokines Th_2 pattern; however cytokines Th_1 increased during the immune response in strongyloidiasis. **Financial support:** CNPq/FAPESP.

A047

Effects of an Anti-GM-CSF Monoclonal Antibody on Experimental Autoimmune Encephalomyelitis.

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Efforts were undertaken to investigate the effects of a rat anti-mouse GM-CSF monoclonal antibody in the SJL/PLP$_{139-151}$ disease model of relapsing/remitting EAE.

The anti-GM-CSF mAb was administered to mice either as 500μg given once IP on day of immunization or 3 weekly injections of 150μg. Both treatment regimens were able to significantly reduce the disease incidence and severity of EAE.

The mAb was then evaluated in a therapeutic dosing regimen. On day 13 when mice showed clinical disease, they were treated with a single IP injection of 500μg mAb. Anti-GM-CSF mAb reduced the severity of disease progression. Clinical scores progressed to control levels by day 30.

Finally, studies to evaluate the effect of anti-GM-CSF mAb on disease relapse were initiated. Mice were allowed to progress through the initial phase of the disease without intervention. When signs of relapse were evident, groups were treated with a single dose of 500μg IP.

A048

The Inhibition of NFkB Function by Estrogen Receptor Dependent Ligands

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(b)Chemical & Screening Sciences, Wyeth Research, CN8000, Princeton, NJ 08543

(c)ArQule, 19 Presidential Way, Woborn, MA 01741

(d)Chemical & Screening Sciences, Wyeth Research, Cambridge MA 02140

(e) Life Diagnostics Inc., West Chester, PA 19380

Inflammation is now recognized as a key component in a number of diseases such as atherosclerosis, rheumatoid arthritis and inflammatory bowel disease (IBD). The transcription factor nuclear factor-kB (NF-kB) has been shown to be involved in both the early and late stages of the inflammatory-proliferative process. We have identified non-steroidal estrogen receptor (ER) ligands that selectively inhibit NF-kB transcriptional activity but are devoid of conventional estrogenic activity. These pathway selective ligands do not promote the classic actions of estrogens such as stimulation of uterine proliferation or ER mediated gene expression but are potent anti-inflammatory agents. In this presentation, a series of compounds from a unique scaffold, inhibit IL-1β mediated NF-kB activity in cell based assays via either ERα and/or ERß.

A049

WITHDRAWN

A050

Leukotriene B4 and platelet-activating factor cooperates in regulating neutrophil trafficking at dermal inflammatory sites

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[1]Faculté de pharmacie, Université de Montreal, Québec, Canada. [2]Centre de recherche en Rhumatologie et Immunologie, Centre de recherche du CHUQ (CHUL), Sainte-Foy, Québec, Canada.

Rats were treated orally with a PAF receptor antagonist SR-27417 (SR, 0.3 mg/kg) (N-(2-dimethylaminoethyl)-N-(3-pyridinyl methyl) [4-(2,4,6-triisopropyl phenyl) thiazol-2-yl] amine) and/or with a LTB_4 receptor antagonist CP105,696 (CP, 3 mg/kg) ((+)-1-(3S,4R)-[3-(4-phenyl-benzyl)-4-hydroxy-chroman-7-yl]-cyclopentane carboxylic acid) before inducing dermal inflammation by soluble agonists. PAF-elicited PMN accumulation was inhibited by 51% after SR, whereas CP did not significantly inhibit PAF chemotaxis. Both antagonists showed additive effects (78%). Similarly, the inhibitory effect of CP on LTB_4-induced PMN accumulation (67%) was enhanced by the addition of SR (84%). Interestingly, zymosan-activated plasma (ZAP)-induced PMN migration was not modulated by either antagonist alone, whilst the combined administration of SR and CP significantly inhibited ZAP-induced PMN trafficking (75%). PAF and LTB_4 may exert a cooperative effect on PMN trafficking elicited by soluble agonists. Supported by the CIHR.

A051

EVALUATION OF MARINE AND SYNTHETIC MANZAMINES AS INHIBITORS OF BRAIN MICROGLIA THROMBOXANE B_2 AND SUPEROXIDE ANION GENERATION.

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[1]Midwestern University, Downers Grove, IL 60515, [2]Department of Pharmacognosy, The University of Mississippi, Oxford, MS 38677.

Manzamine A (MZA), a marine sponge alkaloid, is a potent inhibitor of activated brain microglia thromboxane B_2 (TXB_2) and superoxide anion (O_2^-) release (*A.M.S. Mayer et al., Soc. Neurosci. Abstr 26(2): 1346, 2000; U.S. Patents 6,387,916 & 6,602,881*). The purpose of this investigation was to continue structure-activity relationship (SAR) studies (*A.M.S. Mayer & M. T. Hamman, Inflamm. Res. (S2):S121, 2003*). We used several MZ natural and semisynthetic analogs to determine which features of these β-carboline-containing alkaloids determined the anti-inflammatory potency of MZA. TXB_2 and O_2^- generated by LPS-activated rat microglia were determined by EIA and SOD-inhibitable reduction of ferricytochrome C, respectively. Both natural and semisynthetic MZ analogs inhibited O_2^- and TXB_2 with different potency, SAR revealing that the β-carboline moiety and the 8-membered tertiary amine are essential for pharmacological activity. Additional SAR studies and lead optimization studies are currently underway in our laboratories. Supported by Midwestern University and the National Institutes of Health.

A052

INHIBITION OF LPS-PRIMED HUMAN BRAIN MICROGLIA SUPEROXIDE AND THROMBOXANE B_2 GENERATION BY THE MARINE MANZAMINES.

A.M.S. Mayer[1 *], M. Hall[1] and D.G. Walker[2].
[1]Midwestern University, Downers Grove, IL 60515, [2]Sun Health Research Institute, Sun City, AZ 85372.

Microglia (BMΦ)-generated thromboxane B_2 (TXB_2) and superoxide anion (O_2^-) have been hypothesized to play a role in neuroinflammation. We have reported that Manzamine A (MZA), an alkaloid isolated from the marine *Haliclona sp*. sponge potently inhibited thromboxane B_2 (TXB_2) and superoxide anion (O_2^-) by LPS-activated rat BMΦ *(A.M.S. Mayer et al., Soc. Neurosci. Abstr 26(2): 1346, 2000*). The purpose of this investigation was to study the effect of MZA on phorbol ester-stimulated thromboxane B_2 (TXB_2) and superoxide anion (O_2^-) generation from *E. coli* LPS-activated human BMΦ *in vitro*. O_2^- was determined by SOD-inhibitable reduction of ferricytochrome C and TXB_2 by EIA. MZA inhibited both O_2^- and TXB_2 with an apparent IC_{50} < 1 μM and low toxicity, demonstrating that MZA appears to inhibit human BMΦ O_2^- and TXB_2 generation with similar potency to that observed with rat BMΦ. This constitutes to our knowledge, the first report that MZA inhibits both TXB_2 and O_2^- in human BMΦ and thus extends the preclinical anti-inflammatory pharmacology of MZA. Supported by Midwestern University and Sun Health Research Institute.

A053

Vitamin supplementation does not reduce serum C-reactive protein in healthy subjects

Sue McKay*, Richard Draijer, Theo PJ Mulder, Danielle AW Wolvers.
Unilever Health Institute, Vlaardingen, The Netherlands

Low-grade chronic vascular inflammation is a main contributor to the progression of atherosclerosis. Prospective studies have shown that increased plasma levels of the inflammatory marker C-reactive protein (CRP) independently predicts an increased risk for future coronary events in apparently healthy subjects. Epidemiological studies indicate that high intake and high plasma levels of vitamin E and carotenoids are associated with low CRP levels. Furthermore, vitamin C and zinc have been suggested to improve immune health. We hypothesised that vitamin supplementation would decrease serum CRP in healthy subjects. A placebo group (n=35) and vitamin-treated group (n=35) from a larger randomised, double blind parallel study with healthy subjects (age 40-80) were compared for serum CRP levels. The subjects received either placebo or vitamin-enriched powdered drinks, containing 288 mg d,l-α-tocopherol, 12 mg β-carotene, 375 mg vitamin C and 15 mg zinc on a daily basis. Serum CRP was measured before and after 4 and 10 weeks of supplementation. Vitamin C, vitamin E and β-carotene plasma levels increased considerably in the vitamin-treated group by 50, 100 and 900%, respectively, but remained unchanged in the placebo-treated group. CRP levels were low and did not change significantly during the intervention in the placebo group: 1.59 +/- 1.23 (week 0), 1.93 +/- 1.45 (week 4), and 1.51 +/- 1.30 (week 10) mg/l (mean +/- STD). Vitamin supplementation did not change these rather low serum levels: 1.71 +/- 1.23 (week 0), 1.72 +/- 1.17 (week 4), and 1.73 +/- 1.30 (week 10) mg/l.
Major increases in plasma vitamin levels caused by dietary supplementation did not reduce CRP levels in healthy people. This does not exclude that these micronutrients may have anti-inflammatory properties in individuals with elevated CRP levels.

A054

Leukotrienes modulate chemokines production in a murine model of histoplasmosis. **[1]Alexandra I. Medeiros*, [1]Anderson Sá-Nunes, [1]Camila M. Peres, [2]Célio L. Silva, [1]Lúcia H. Faccioli. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto and [2]Faculdade de Medicina de Ribeirão Preto, São Paulo, Brasil. alex@rpm.fmrp.usp.br**

The production of chemokines at the site of fungal infections is critical for effective recruitment of leukocytes. In this study, we examined the pulmonary tissue expression of CC and CXC chemokines and the effects of leukotriene synthesis inhibitor MK886 (3-[1-(4-chlorobenzil)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2 dimethylpropanoic acid) using Rnase protection assay (RPA). On the 2nd, 7th and 14th day post infection (p.i) with *Histoplasma capsulatum* (*Hc*) (2.5×10^5 yeast/100 μl/i.t.) the analysis of chemokines demonstrated the increase of the expression mRNA for MIP-1α, MIP-1β, IP-10, MCP-1 on the first two days p.i.; and RANTES, Eotaxin, MIP-1α, MIP-1β, IP-10, MCP-1 on the 7th and 14th days p.i. when compared with control group. The administration of MK886 increased the expression of mRNA for MCP-1 and RANTES on the 7th day and RANTES, MIP-1α, MIP-1β, MIP-2, IP-10, MCP-1 on the 14th day p.i. in comparison to infected-only animals. Moreover, when evaluated by ELISA, the levels of MCP-1 and IP-10 in lungs of *Hc*-infected animals were greater than in the control mice. Nevertheless, the levels of these chemokines were greater in infected animals treated with MK886 than in infected-only animals (**MCP:PBS**≈51±5;**Hc**≈233±32;**Hc+MK**≈800±144;**IP-10**:**PBS**≈2400±150; **Hc**≈6172±7511; **Hc+MK**≈14860±1071pg/mLp<0.05). These results suggest that *Hc* infection induces the expression and production of different chemokines and leukotrienes synthesis inhibition induces up-regulation these chemokines in murine pulmonary histoplasmosis. Financial support: FAPESP, CNPq.

A055

Suppression of Matrix Degradation and Nitric Oxide Release in Human Cartilage Explants by a Mineral Supplement (SierraSil™) alone and in Combination with the Botanical extract, Vincaria®.

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Cartilage degradation, a hallmark of both rheumatoid arthritis and osteoarthritis, is a target for therapeutic intervention. We investigated whether a natural mineral-enriched supplement, SierraSil, ± a botanical (Vincaria – Cat's claw), could prevent IL-1β-induced human chondrocyte activation and cartilage degradation *in vitro*. Following neutral and alkaline washes, SierraSil alone was ineffective in reducing nitric oxide or glycosaminoglycan (GAG) release, although SierraSil + Vincaria was effective. Following an acid wash, IL-1β induced GAG release was reduced by SierraSil (68-73%) and SierraSil + Vincaria (58 –77%), both $P<0.01$. IL-1β induced nitric oxide production was reduced ($P<0.01$), although the SierraSil + Vincaria combination was more potent ($P<0.001$). SierraSil alone and together with Vincaria, limits human cartilage degradation and chondrocyte activation by limiting transcriptionally regulated events and offers a new approach to managing arthritis.

A056

Development of a high capacity HTRF assay for measurement of antagonists of 5-lipoxygenase activation protein (FLAP)

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The presence of FLAP on cellular nuclear membranes has been shown to be necessary for the binding and transfer of arachidonic acid to 5-lipoxygenase (5-LO) where it is converted to Leukotriene A_4. A number of potent inhibitors targeting FLAP have been described and tested in the clinic where they have been found to completely block the production of leukotrienes. One of these compounds MK591 (3-[1-(4-chlorobenzyl)-3-(*t*-butylthio)-5-(quinolin-2-ylmethoxy)indol-2-yl]-2,2-dimethylpropanoic acid), a potent inhibitor of FLAP (2 nM IC_{50} on PMN membranes), has been used to develop a sensitive homogeneous time-resolved fluorescence (HTRF) assay. FLAP was engineered with a C-terminal AviTag-FLAG tag, and expressed in baculovirus. Sf9 cells were coinfected with the FLAP virus and a virus encoding the birA gene, and the 100,000 x g membranes collected were used directly for assay. MK591 was modified at the CO_2H to couple AlexaFluor647, and the resultant FLAP-AF647 conjugate was incubated with the biotin-FLAP membranes, streptavidin Eu, and test FLAP antagonist compounds. MK591 in this assay gave an IC_{50} of 2.5 nM and comparison of a series of FLAP inhibitors showed equal potency to a PMN binding assay.

A057

Assessment of the *in vitro* free radical scavenging activity of ethanolic extract of roots of *Lantana camara* L. *var.aculeata* (Verbenaceae)

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The green parts of *L. camara Linn.var.aculeata (Verbenaceae)* are toxic but roots reportedly have been used for the treatment of various ailments such as itches, cuts, ulcers, swellings, eczema, and rheumatic disorders. The preliminary evaluation indicated that ethanolic extract of roots of *Lantana camara* at 0.1mg/ml and 1.6mg/ml concentration showed 20.0% and 97.3% DPPH (1,1-diphenyl-2-picryl-hydrazyl) scavenging respectively with IC_{50} equivalent to 0.25mg/ml. The extract (1.6mg/ml) showed 99.09% scavenging of hydrogen peroxide with IC_{50} equivalent to 0.79mg/ml. Nitric oxide radical formation from sodium nitroprusside was decreased by ethanolic root extract with an $IC_{50}$of 0.69mg/ml. 0.4mg/ml ethanolic root extract showed 25.2% scavenging and at 2.0 mg/ml concentration showed 86.4% decrease in reduction of Fe^{+3} ions with IC_{50} equivalent to 0.65mg/ml. The extract also showed a significant scavenging of superoxide radicals with IC_{50} of 1mg/ml.It is concluded that L. camara ethanolic root extract possesses some constituents which have significant free radical scavenging, antioxidant and anti-inflammatory activity

A060

The role of leukotrienes in the protective immune response in experimental pulmonary tuberculosis. [1]Camila M. Peres*, [1]Lúcia de Paula, [1]Alexandra I. Medeiros, [2]Édson G. Soares, [1]Carlos A. Sorgi, [1]Daniela Carlos, [1]Fabiani G. Frantz, [2]Célio L Silva and [1]Lúcia H. Faccioli. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto and [2]Faculdade de Medicina de Ribeirão Preto, São Paulo, Brasil. E-mail: peres@fcfrp.usp.br.

Since leukotrienes are involved in the antimicrobial host defense in many infectious diseases, we explore the possibility that these endogenous mediators modulate immune response in pulmonary tuberculosis. BALB/c mice were infected with 10^5 *Mycobacterium tuberculosis* (MTB) H37Rv in 100 μL/i.t. and daily treated or not with a leukotriene inhibitor, MK 886 (3-[1-(4-chlorobenzil)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid). On days 30 and 60 post infection (p.i.), the bacterial load was assessed as the mean CFU per whole organ and cytokines levels in lung homogenates were quantified by ELISA. The number of CFU recovered from the lungs of MTB infected-mice treated with MK 886 was dramatically higher than untreated MTB infected-mice (1.73 $\log_{10}$ on the 30th p.i. and 1.5 $\log_{10}$ on the 60th p.i. *p<0.05*). Therefore, compared with untreated and MTB-infected mice, the treatment of infected-mice with MK 886 inhibited IL-12 (47 and 32%, on the 30th and 60th p.i., respectively) and IFN-γ synthesis (50 and 28% on the 30th and 60th p.i., respectively, *p<0.05*), but it did not change IL-10 production. We conclude that leukotrienes play an essential role in the protection against MTB by modulating synthesis of cytokines involved with the protective immune response. Financial support: CNPq, FAPESP.

A061

SQT82, a Novel Serine-Threonine Kinase, is Responsive to TNF-α Stimulation and May Play a Role in a MAPK Pathway

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SQT82 was identified through microarray studies in which SQT82 mRNA was upregulated in LPS- or IFNγ stimulated THP-1 macrophages and in cytokine- and house dust mite treated human bronchial epithelial cells. We hypothesize that SQT82 is a serine-threonine kinase with an atypical ATP-binding domain and a putative catalytic triad. By TaqMan RT-PCR, SQT82 RNA expression is increased in PMNs on TNF-α stimulation, B cells on CD40L, human bronchial epithelial cells on IL-13 and TGFα stimulation, HMC-1 cells due to TGFβ stimulation, and in activated T cells. Through reporter-based pathway mapping, SQT82 was hypothesized to be involved in a MAPK pathway. Kinase activity has been measured using baculovirus expressed recombinant human SQT82 in an *in vitro* kinase assay with MBP as substrate. SQT82 is also auto-phosphorylated. In HeLa cells stimulated with TNF-α, SQT82 phosphorylation increases in a time dependent manner. Inhibitors of p38, MEK1, and JNK block the phosphorylation of SQT82. Data from recently generated SQT82 knockout mice will also be presented. Our results suggest that SQT82 is a kinase and may be novel component of a MAPK pathway. As such, SQT82 may represent a novel therapeutic target for the treatment of inflammatory disease.

A062

Cytochrome C is released from mitochondria in A549 cells in response to tobacco smoke condensate.

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Inhaled cigarette smoke has been associated with inflammation and severe health effects in the lungs. Here we have investigated the effects of tobacco smoke condensate (TSC) and some of its constituents on apoptosis in a human lung alveolar epithelial cell line, A549. Cytochrome C (Cyt C) release from the mitochondria to the cytosol is an early marker of signalling in the apoptotic pathway. Cytosolic Cyt C can bind to form Apaf, which in turn signals further apoptotic events including caspase activation, DNA fragmentation and morphological changes. Cells were treated for 4 or 24hours with TSC (80μg/ml), components of TSC (nicotine (1mM), acrolein (200μM), paraldehyde (1mM)), hydrogen peroxide (10μM), and Paclitaxel (1μM). Firstly cytotoxicity of the treatments was assayed by MTT (1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan). Levels of Cyt C were determined in mitochondrial and cytosolic extracts after treatment by ELISA and western blotting. Increased cytosolic Cyt C was found in treated cells. This indicates that early apoptotic events such as cyt C release are able to occur in response to TSC and constituents.

A063

Markers of Disease Activity in Multiple Sclerosis

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Multiple Sclerosis (MS) is an autoimmune disease mediated via the activation of T-cells against components of myelin. Many MS patients have a relapsing-remitting form in which they experience intermittent clinical exacerbations or flares of the disease. These flares may be followed by complete or partial recovery. Most patients experience a gradual deterioration in neurological function. A predictive test would allow early intervention before significant deterioration has occurred or adjustment of medications that are/are not effective. A quantitative high-precision gene expression analysis of important immune modulators, associated with flare onset and disease progression may provide the earliest diagnostic warning of exacerbations and therapeutic response. In this study, samples of whole blood from an MS patient were collected and examined for gene expression of inflammatory system mediators by high precision RT-PCR. The results were compared to a database of normal subjects to identify a panel of markers that may identify MS as a class, indicate MS severity, and/or correlate with successful therapy. The results of this study indicated several potential MS marker genes. Currently we are enrolling MS subjects in an expanded gene expression study at several clinical sites. The forthcoming results will also be presented.

A065

Peritoneal Macrophages Suppress T Cell Activation Via Indoleamine Dioxygenase Production

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Prior study of minor lymphocyte stimulatory (Mls) superantigen (SAg) presentation by B cell subsets revealed that peritoneal cavity (PerC) cells inhibit the T cell response to Mls. This study defined the cell type and possible mechanism of this anti-inflammatory response. PerC cells from severe-combined immune-defective (SCID) mice suppressed T cell activation, indicating that neither B or T lymphocytes were necessary for this effect. PerC cells depleted of natural killer cells were also suppressive. Cell depletion/enrichment studies and flow cytometric analyses revealed that F4/80$^+$ CD11b$^+$ CD45R(B220)$^-$ CD11c$^-$ PerC macrophages were the anti-inflammatory cell. PerC cells from IFNγR KO mice failed to suppress T cell proliferation. Prior work has shown that IFNγ induces idoleamine dioxygenase (IDO) production, an enzyme that catabolizes tryptophan and inhibits T cell activation. Addition of the IDO antagonist 1-methyl tryptophan (1-MT) restored T cell proliferation. Addition of the pro-inflammatory cytokines TNFα, IL-1α, or IL-6 also reversed suppression. Body cavity macrophages could serve as innate suppressor cells that dampen inflammation at body sites with a high potential for pathogen burden. Supported by NIH AREA grant R15- AG19631-01.

A066

Blood tumor necrosis factor-α (TNFα) responses after the intraperitoneal (IP) and subcutaneous (SQ) administration of lipopolysaccharide (LPS) in mice

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Although lipopolysaccharide (LPS) is commonly used experimentally to induce elevated blood levels of TNFα, the influence of the route of LPS administration on TNFα responses has not been fully characterized. In this study, blood TNFα was quantified by an ELISA technique after LPS was administered IP or SQ to mice. Doses of 1 and 0.1 mg/kg LPS produced significant elevations of blood TNFα by both SQ and IP routes of administration, but the absolute value of the response was less when LPS was given SQ. Oral dexamethasone dose dependently inhibited the production of TNFα after IP and SQ LPS, exhibiting greater potency when LPS was administered SQ. These findings indicate that the route of LPS administration significantly affects not only the magnitude of the TNFα response, but it also influences the ability of anti-inflammatory agents to modulate TNFα production.

A067

Inhibition of cellular migration to bronchoaveolar space by *Lafoensia pacari* extract and dexamethasone in helminthic infection.

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We investigated the effect of *L. pacari* (Lp) treatment in eosinophils (Eo) and mononuclear cells (Mo) recruitment to the bronchoalveolar space of *Toxocara canis*-infected mice, and compared its effects with desamethasone (Dex) treatment. Seven groups of six female Swiss mice were infected with 200 embryonated eggs of *T. canis* p.o. in saline. Infected animals were treated with Lp (200 mg/kg daily p.o.) or Dex (1 mg/kg, s.c.), in different days post infection (d.p.i.) as follow. Group 1: water, 12 to 24 d.p.i.; group 2: Lp, 12 to 18 d.p.i.; group 3: Dex, 12 to 18 d.p.i.; group 4: Lp, 18 to 24 d.p.i.; group 5: Dex, 18 to 24 d.p.i.; group 6: Lp, 12 to 24 d.p.i., group 7: Dex, 12 to 24 d.p.i. A control group received water daily p.o.. Mice were sacrificed at 18 or 24 d.p.i. and bronchoalveolar fluid were collected. There was only a reduction of recruitment Eo and Mo into the bronchoalveolar fluid by Lp treatment on the 12th-24th d.p.i., while with the Dex treatment there was a reduction in all periods analysed. The results suggest an anti-inflammatory potential of Lp to inhibit recruitment of Eo in space bronchoalveolar fluid. Financial support: CAPES, FAPESP and CNPq.

A068

Protective effect of Lantana camara against ethanol-induced ulceration in rats and evaluation of its analgesic activities in mice

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The genesis of ethanol- induced gastric lesions is a multifactorial event (depletion of gastric wall mucus content, mucosal blood flow, free radicals, lipid peroxidation etc.) Ethanol-induced gastric mucosal lesions in male albino rats were used to evaluate gastric ulcer protective effects of Lantana camara leaf extracts and analgesic activity assessment was done by studying the reduction in acetic acid induced writhings in mice.

The leaf extract has been observed to show to a dose-dependent free radical scavenging activity in 1,1-diphenyl-2-picryl-hydrazyl radical (IC_{50} 20 µg/ml)and superoxide-generated assays (IC_{50} 425µg/ml). A significant dose dependent inhibition of lipid peroxidation has also been observed with IC_{50} 300µg/ml.

Lantana leaf extract pretreatment in rats caused 63.3% and 91.1% reduction in 80% ethanol(5ml/kg, per oral) induced ulcer index at dose of 1.0 and 1.5 g of extract/kg body weight respectively. It also showed 75% decrease in Indomethacin(30mg/kg,per oral) induced ulcer index at dose of 1.5g extract/kg body weight. Similarly it also showed dose dependent significant decrease in number of writhings /10min in mice. Ibuprofen at 30mg/kg body weight. showed 34.17 % decrease in number of writhings whereas lantana leaf extract at 1.5g/kg body weight showed 47.5% decrease in number of writhings/10min. indicating a strong analgesic effect.

A069

ABX10131: a Fully Human Anti-Human TNF MAb Generated Using XenoMouse® and XenoMax® Technologies.

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ABX10131 is a fully human anti-human TNF IgG1/k MAb, generated by immunizing mice expressing human IgH and Igk genes (XenoMouse® technology) and by screening and rescuing single antigen-specific B cells with the selected lymphocyte antibody method (XenoMax® technology). ABX10131 binds to soluble and membrane-bound TNF with high affinity. ABX10131 neutralizes TNF in vitro, with inhibition of TNF-induced apoptosis of MCF-7 cells and IL-8 production by whole blood (table). In mice, ABX10131 inhibits TNF-induced IL-6 production and liver damage. ABX10131 is a fully human antibody, has higher affinity and improved in vitro potency compared to currently available TNF targeting antibodies, and may prove to be a useful anti-TNF therapeutic.

	Affinity (K_D pM)	In vitro Potency (IC_{50} pM)	
	KinExA	Apoptosis	IL-8
ABX10131	3.20	1.6 ± 1.3	131 ± 9
Infliximab	14.14	31.7 ± 20.4	546 ± 65
Adalimumab	11.65	34.5 ± 8.3	896 ± 159

A070

Role of efflux pathways in hepoxilin A3 secretion and inflammation

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Intestinal epithelial cells are induced by microorganisms to orchestrate mucosal inflammation. A recently identified pro-inflammatory PMN chemoattractant, hepoxilin A_3, (hepA_3; a metabolite of the 12-lipoxygenase pathway) is released from the apical surface of intestinal epithelia and forms a gradient across the epithelial tight junction critical for neutrophils to target the lumen of mucosal tissues at sites of intestinal inflammation. In this study we investigated the role of P-glycoprotein (P-gp) and the multidrug resistance class of proteins (MRP) for the excretion of hepA_3 and the mediation of an inflammatory response by intestinal cells to the enteric pathogen *S. typhimurium.* We found that addition of *S. typhimurium* to the apical surface of polarized T84 cell monolayers induced upregulation of MRP2 and P-gp, indicating the involvement of these transporters in the physiological action of hepA_3. Thus, our novel findings suggest the dependence upon transport proteins (i.e. P-gp and MRP2) for the initiation of an inflammatory response and propose the possibility of regulating the physiological and pathological consequences of *S. typhimurium* infection, as well as more chronic inflammatory conditions such as inflammatory bowel disease by modulating these transport systems.

A071

CR002.6.4, a fully human monoclonal antibody against PDGF-D, is a potential therapeutic for IgA nephropathy

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IgA nephritis is a mesangioproliferative disease mediated by deposition of IgA in the glomerulus. We recently identified PDGF-D as a latent, protease-activated growth factor expressed by normal and neoplastic cells. Removal of the PDGF-D CUB domain by proteases allows the growth factor domain to bind and activate cells expressing PDGFRβ, including fibroblasts, smooth muscle cells and kidney mesangial cells. PDGFRβ is known to be a crucial for mesangial cell development and function. To determine if PDGF-D has a potential role in mesangioproliferative diseases, a fully human monoclonal antibody was raised against the PDGF-D growth factor domain using XenoMouse® technology. The mAb CR002.6.4 had an affinity for PDGF-D of approximately $1.8x10^{-10}$ M and neutralized the proliferative activity of PDGF-D derived from rodents, rhesus monkeys and humans. In vivo mesangial cell proliferation was induced by OX-7 mAb in the rat anti-Thy 1.1 model. Dosing of animals on days 3 and 5 with 20 and 8 mg/kg respectively of CR002.6.4 reduced mesangial cell proliferation, and decreased mesangial inflammation. Our data indicate the potential of a specific PDGF-D antagonist for the treatment of mesangioproliferative glomerulonephritides, which include IgA nephritis.

A072

Correlation of p38 Kinase Inhibition with Anti-Inflammatory Efficacy in Cellular and *in vivo* Models

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p38 is a member of the MAP kinase family of signal transduction enzymes and is activated in response to inflammatory stimuli. While p38 kinase inhibitors block the synthesis of pro-inflammatory mediators such as TNFα and PGE2, a quantitative comparison of inhibition of these mediators and that of the target, p38 kinase, is lacking. Phosphorylation of Hsp27, a downstream substrate of the p38 pathway, is an excellent biomarker of p38 activity, however detection of p-Hsp27 using Western blot analysis is generally inadequate with respect to sensitivity, quantitation and throughput. A sensitive and quantitative fluorescence based assay using the DELFIA technology was developed in a 96-well format enabling p-Hsp27 to be quantitated in cell lines, in tissues from animal models of acute inflammation and arthritic disease, and in human blood. Results of these analyses confirm p-Hsp27 as an effective target biomarker of p38 activity and further demonstrate a direct correlation between inhibition of p38 and anti-inflammatory efficacy of inhibitors of this kinase.

A073

Cigarette smoke synergistically enhances induction of respiratory mucin MUC5AC by pro-inflammatory stimuli in human airway epithelial cells NCI-H292 and in a rat model of LPS-induced airway inflammation.

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Mucin hyperproduction is one of the hallmarks of chronic obstructive pulmonary disease (COPD). Pathogenetic factors associated with COPD such as cigarette smoke, pro-inflammatory cytokines and bacterial infections can individually induce respiratory mucins *in vitro* and *in vivo*. Because, they are co-present in airway inflammation in COPD patients we hypothesized that cigarette smoke can synergistically amplify mucin induction by bacterial exoproducts and pro-inflammatory cytokines, resulting in an uncontrollable mucin hyperproduction. We demonstrated that cigarette smoke synergistically increased gene expression and production of MUC5AC mucin, induced by lipopolysaccharide or TNF-α in human airway epithelial NCI-H292 cells. Activation of epidermal growth factor receptor (EGFR) was essential for synergy. MUC5AC mucin production induced by EGFR ligands (TGF-α, amphiregulin), as well as LPS- or TNF-α-induced gene expression and/or release of these ligands was also synergistically increased by cigarette smoke. Antioxidants blocked synergistic induction of MUC5AC mucin by cigarette smoke and hydrogen peroxide partially mimicked effects of cigarette smoke. In a rat model of LPS-induced airway inflammation, cigarette smoke synergistically increased rmuc5AC gene expression and mucous-cell metaplasia. These results suggest that cigarette smoke has potential to synergistically amplify induction of respiratory mucins by pro-inflammatory stimuli relevant to pathogenesis of COPD and contribute to pathological mucin hyperproduction observed in COPD patients.

A074

Engineering of Dominant-Negative TNF Proteins as Selective Inhibitors of Soluble TNF That Spare Transmembrane TNF

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Using structure-based protein drug design, we have created mutants of human TNF that exchange with and inactivate the native cytokine in cells and animals (*Science* 2003, 301:1895). To explore the Dominant-Negative (DN) mechanism, we expressed libraries of DN-TNF proteins in *E. coli* and identified variants that selectively abolish binding to either or both TNFR1 and TNFR2. In addition, we showed by fluorescence anisotropy that DN-TNF homotrimers rapidly exchanged subunits with native TNF, forming heterotrimers in minutes. We next assessed the ability of DN-TNFs to block signaling of soluble TNF (solTNF) while sparing transmembrane TNF (tmTNF), using caspase activation in human U937 cells as a readout. Several DN-TNFs, as well as etanercept and infliximab, blocked both solTNF and tmTNF-induced caspase activation. In contrast, other DN-TNFs selectively blocked solTNF while sparing tmTNF signaling. Soluble TNF-Selective Dominant-Negatives (STS-DNs) were active in mouse and rat collagen-induced arthritis. Evidence suggests that tmTNF plays an essential role in the immune response to infectious agents; therefore, we assessed an STS-DN in murine *Listeria* infection. Etanercept, a non-selective TNF inhibitor, increased weight loss and mortality as well as colony forming units in the spleen by >200x relative to vehicle and the STS-DN. This work suggests that inhibitors of solTNF that spare tmTNF may minimize immunosuppression associated with nonselective anti-TNF biologics currently used for treatment of inflammatory diseases.

A075

Interleukin-17C (IL-17C) protects mice from LPS-induced death

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Human IL-17C shares 29% amino acid identity with IL-17, but its function remains unclear. Administration of Interleukin-17C (IL-17C) protein protected Balb/C mice from LPS-induced (175 ug, i.v.) death. Maximum survival was observed with 50 ug IL-17C administered i.v., 60 minutes post LPS challenge. Additionally, IL-17C transgenic mice (FVB) were protected both in this LPS-challenge model and an acute liver failure model. Serum TNF-alpha and IL-6 were decreased in LPS-challenged transgenics, compared to challenged wild-type controls. However, unchallenged IL-17C transgenic mice blood cell counts and cytokine profiles were similar those of wild-type mice. In vitro, IL-17C pretreatment of HUVEC (16 hours, 15.6 – 250 ng/ml) decreased the CHX (10 ug/ml) + TNF-alpha (100 ng/ml) – induced expression of active caspase 3 protein (measured by FACS). These novel findings suggested that IL-17C could have potential to treat sepsis in part by decreasing endotoxin-induced cytokine secretion and by decreasing proinflammatory cytokine-induced endothelial cell apoptosis.

A076

Local Administration of Antisense Inhibitors of p38 alpha MAPK Suppress Allergen-induced Lung Inflammation and Airway Hyperreactivity in Mice.

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p38 alpha MAPK mediates critical cytokine and chemokine receptor signal transduction during inflammatory responses. Small molecule inhibitors of p38 alpha activity have been shown to reduce allergen – induced inflammation in murine and guinea pig models of asthma. We have developed antisense oligonucleotides (AS) which inhibit the expression of p38 alpha but not p38 beta MAPK *in vivo*. Inhalation administration of p38 alpha-AS results in the reduction of lung p38a protein levels in mice. Local treatment of ovalbumin-sensitized mice with p38 alpha-AS markedly suppresses allergen-induced Th2 cytokine and inflammatory chemokine production, airway eosinophilia, and airway mucus production and reduces airway hyperreactivity in response to methacholine provocation. These findings demonstrate the importance of p38 alpha MAPK in both the inflammatory and airway epithelial responses to allergen and support a p38 alpha-AS approach for the therapy of inflammatory lung disease.

A077

Soluble IL-1 Receptor Accessory Protein (sIL-1RacP) modulates arthritis by selectively affecting humoral immunity.

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The IL-1RAcP gene encodes for a membrane protein, crucial for IL-1 signal transduction and a soluble alternative splice variant (sIL-1RAcP). In this study we compared sIL-1RAcP with the IL-1 receptor antagonist (IL-1Ra) in collagen-induced arthritis (CIA). The murine sIL-1RAcP gene was cloned into an adenoviral vector (Ad5sIL-1RacP). DBA1/J mice received $3x10^8$ ffu of Ad5sIL-1RAcP or Ad5IL-1Ra intravenously just before clinical manifestation of CIA. IL-1Ra gene transfer arrested CIA development while sIL-1RAcP gene transfer markedly delayed the development of CIA. Both treatments reduced the circulating levels of collagen type II specific IgG2a antibodies ($p<0.05$). In contrast, IL-1Ra gene therapy inhibited mitogen and antigen-induced lymphocyte proliferation whereas sIL-1RAcP gene therapy had no such effect. Addition of recombinant protein confirmed that IL-1Ra but not sIL-1RAcP was able to inhibit lymphocyte proliferation, and IL-1 induced NF-kappaB activation in luciferase reporter EL.4NOB-1 T-lymphoma cells or splenocytes from transgenic reporter mice. This study showed that sIL-1RAcP gene therapy ameliorates CIA by reducing humoral immunity in a T cell independent way, an effect distinct from IL-1Ra action.

A078

Suppressor of Cytokine Signaling (SOCS)-3 gene transfer protects against Collagen-Induced Arthritis (CIA).

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Interleukin-6 (IL-6) is produced in the inflamed joint and plays a key role in immunity and the acute phase response. IL-6 induces gene expression via the gp130 membrane receptor causing activation of the Signal Transducer and Activator of Transcription (STAT)-3. In this study we evaluated the effect of adenoviral gene-transfer of SOCS-3, the inhibitor of STAT3 activation, on murine arthritis. Induction of zymosan-induced arthritis (ZIA) in the knee joints of mice caused a rapid (5 hr) activation of STAT3 in the liver. Intravenous injection of Ad5.mSOCS3, 24 hours before induction of ZIA clearly prevented STAT3 activation and reduced liver serum amyloid A (SAA)-1 gene expression. The expression of SOCS3 did not affect Erk activation confirming selectivity for the STAT-signaling pathway. Treatment of DBA1/J mice with Ad5.mSOCS3 virus after immunization ameliorated CIA. A 72% reduction in circulating TNFα levels was found in the Ad.SOCS3 treated group. SOCS3 treatment also reduced the circulating levels of total IgG and IgG2a anti-collagen type II antibodies by 40% and 47%, respectively. Systemic SOCS3 adenoviral gene transfer ameliorated CIA possibly by inhibiting the T-helper 1 cell response in these mice.

A079

Title: INCREASED TOLL-LIKE RECEPTOR EXPRESSION IN ACUTE EXACERBATIONS OF RA AND EFFECT OF THERAPY

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Background: There is considerable evidence that RA is a systemic disease with autoimmune characteristics and that the innate immune system plays a major role. Using human peripheral blood and synovial tissue we have defined the response of the innate immune system during an acute exacerbations and documented changes to this response after treatment.

Methods: Patients with acute flares of RA were followed longitudinally. TLR2 and TLR4 expression on CD14 +ve peripheral blood mononuclear cells (PBMCs) were measured by flow cytometry using anti-CD14 and anti-TLR2 and anti-TLR4 monoclonal antibodies. TLR expression was reassessed in 3-6 weeks. Extensive clinical data and scales of activity were administered at each visit.

Results: TLR2 expression was significantly increased in RA patients with acute exacerbations compared with quiescent disease (P=0.001) and these were elevated above that of OA patients. TLR4 expression was significantly decreased in RA patients with acute exacerbations compared with quiescent disease (P=0.01) and these were lower than that of OA patients. Patients treated with TNF- inhibitors demonstrated normalising of their TLR2 and TLR4 levels.

Conclusions: Patients with RA have a marked upregulation in TLR 2 activity on CD14+ monocytes with acute RA flares. This upregulation is ameliorated with TNF- inhibitor therapy. TLR4 is downregulated on CD14+ monocytes during flares and therapy increases TLR4 expression towards normal levels.

A081

Fluorescence Polarization Immunoassay (FPIA) for Prostaglandin E_2 Based on a Rhodamine Fluorophore

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Most immunoassays for the measurement of prostaglandin E_2 (PGE_2) utilize a solid-phase format that requires multiple incubation and washing steps. We have developed a rhodamine-based fluorescence polarization immunoassay (FPIA) for the rapid measurement of PGE_2. Fluorescence polarization (FP) assays are homogeneous, single-step assays ideal for high-throughput screening (HTS) applications. The PGE_2 FPIA-Red uses a simple mix-and-read format in which a single reagent is added to the sample/standard and the assay is read after a 60 minute incubation. The assay is robust (Z' = 0.68; %CV <10%), exhibits Δ200 mP over a range of 91 pg/ml to 200 ng/ml PGE_2, and has a detection limit of 150 pg/ml. Use of the red-shifted rhodamine label reduces interference from most sample matrices and compounds in drug libraries, thereby making the assay applicable to the identification of COX-1/-2 or PGE Synthase inhibitors using whole cell or recombinant enzyme preparations. A comparison of the PGE_2 FPIA to Cayman's PGE_2 EIA and fluorescein-based FPIA will be presented.

A082

Direct Quantification of Prostaglandin D_2 by Fluorescence Polarization Immunoassay (FPIA)

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Prostaglandin D_2 (PGD_2) is produced in large quantities by allergen-stimulated mast cells and acts as a pro-inflammatory mediator in allergic reactions. Current assays for the measurement of PGD_2 utilize a solid-phase EIA format that require multiple incubation and washing steps. Due to the inherent instability of PGD_2, conversion to a methoxylamine derivative is required prior to performing the EIA. We have developed a fluorescence polarization immunoassay (FPIA) for the rapid measurement of PGD_2 that does not require prior conversion to the methoxylamine compound. Fluorescence polarization (FP) assays are homogeneous, single-step assays ideal for high-throughput screening (HTS) applications. The PGD_2 FPIA uses a simple mix-and-read format in which a single reagent is added to the sample or standard and the assay is read after a 60 minute incubation. The assay is robust (Z' = 0.74; %CV <10%), exhibits Δ120 mP over a range of 244 pg/ml to 1000 ng/ml PGD_2, and has a detection limit of 300 pg/ml. Results from the measurement of PGD_2 from whole cell preparations in the presence of COX and PGD Synthase inhibitors using FPIA and EIA formats will be presented.

A083

Development of highly selective inhibitors for multiple targets in the p38 MAPK pathway: Progress to anti-inflammatory leads

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The p38 MAP kinase and MAPKAPK-2 have been identified as promising anti-inflammatory drug targets with differing levels of validation. Several dual inhibitors of p38-α/β have shown anti-inflammatory effects in preclinical trials. However, recent studies suggest that selectivity for the p38-α isozyme may be important to avoid undesirable metabolic side effects. Although there are many direct downstream targets for p38, the targeted deletion of MAPKAPK-2 indicates that this protein may be critical for cytokine production. There are currently no disclosed selective inhibitors for MAPKAPK-2. We have developed and implemented a program to rapidly identify and optimize potent and selective chemical series for both p38-α and MAPKAPK-2. A diverse library of purified molecules (>100K) screened in microfluidic-based enzymatic assays provided very accurate inhibition data, allowing for observation of SAR directly from the primary HTS. All compounds were screened against more than 45 enzymes (kinases, proteases and phosphatases), allowing for removal of promiscuous inhibitors. Active compounds were further tested for less desirable properties to identify those that inhibit by aggregation, irreversible binding, or time-dependent inhibition. Over 30 compounds from our p38-α lead series have K_i values below 100 nM, and selectivity over 100-fold against all other enzymes tested, including p38-β. For MAPKAPK-2, the lead series has 10-20 nM potency and selectivity over all kinases tested. Several of the p38-α compounds showed sub-μM EC_{50} values in vitro (inhibition of LPS-stimulated TNFα production in THP-1 cells), with no measurable effect on cell viability.

A084

Pharmacological characterization of MF-tricylic, a selective COX-2 inhibitor, in animal models of inflammation and pyresis.

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Preclinical testing of the selective COX-2 inhibitor rofecoxib in rat models of inflammation led to the discovery of this compound as a potent anti-inflammatory agent. In the present study, we tested a close analog of rofecoxib MF-tricyclic (MFT) in mice, guinea pigs and rabbits to identify other target species for preclinical evaluation of COX-2-dependent inflammation by utilizing three models that have been shown to be COX-2-dependent in the rat, i.e., carrageenan-induced acute paw edema, adjuvant-induced chronic paw inflammation and LPS-induced pyresis. In the carrageenan model, MFT attenuated paw swelling in rats and rabbits but not in mice and guinea pigs. By comparison, indomethacin significantly reduced the swelling in all four species. In the adjuvant model, treatment with MFT blocked adjuvant-induced increase in paw PGE2 in both rats and rabbit, but only attenuated paw swelling in rats. Finally, in the pyresis model, treatment with MFT significantly (80 to 100%) attenuated LPS-induced pyresis in all four species. Our results demonstrate that COX-2 is the major isozyme mediating LPS pyresis in various species. In contrast, the role of COX-2 in inflammation is highly species dependent.

A085

Characterization of a human cell line as a model for human articular cartilage chondrocytes

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A source of well-characterized human cells that are both readily available and easily manipulated would be of great benefit in understanding anabolic and catabolic effectors of human articular chondrocytes. Interleukin-1 (IL-1) is thought to play a significant catabolic role in chondrocyte activation, and Insulin-like Growth Factor-1 (IGF-1) combined with Osteopontin-1 (OP-1) have been reported to stimulate anabolism in cultured chondrocytes. We investigated the expression of genes that are representative of known catabolic and anabolic effectors in chondrocytes following IL-1 or IGF-1/OP-1 stimulation in the human chondrosarcoma cell line, SW1353 cells. Using quantitative RT-PCR, we report that IL-1 stimulation of these cells results in a dose responsive expression of MMP's and cytokines in a manner consistent with primary human chondrocytes. OP-1/IGF-1 treatment of the SW1353 cells yields the expression of genes reported to be important in chondrocyte catabolism. The expression of numerous other chondrocyte-associated genes were examined with these stimuli.

A086

Eutigosides from *Eurya Emarginata* Inhibit Production of the Pro-inflammatory Mediators in RAW264.7 Cells

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Eurya emarginata (Thunb.) Makino (Theaceae) is distributed in coastal areas of island. The leaves of *E. emarginata* have been traditionally used to treat ulcers or as a diuretic in the coastal areas of Jeju Island. The present study investigated the anti-inflammatory activity of the isolated constituents (eutigoside B and C) from *E. emarginata* by examining the inhibitory effects on the several inflammatory markers (TNF-α, IL-1β, IL-6, NO, iNOS and COX-2) in LPS-stimulated murine macrophage, RAW264.7 cell. The eutigoside B and C inhibited both protein contents and mRNA expression of IL-6 and TNF-α in a dose-dependent manner. The protein level of iNOS were a markedly inhibited after the treatment with the eutigoside B or C. The inhibition of iNOS was concordant with the decrease of nitrite level. Also, eutigoside B and C potentially inhibited the protein level of COX-2. These results suggest that eutigoside B and C from *E. emarginata* may have anti-inflammatory activity through the inhibition of pro-inflammatory cytokines (TNF-α and IL-6), iNOS and COX-2. [Supported by a grant of Jeju-KRIBB Joint Research Project]

A087

The Role of B-lymphocyte Chemoattractant (BLC) in the Development of Murine Autoimmune Arthritis

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In Rheumatoid arthritis (RA) patients, tissue-infiltrating T cells and B cells can form ectopic lymphoid aggregates with germinal center-like structure in joint tissue that may contribute to the pathogenesis of RA. BLC is essential for the constitutive B-cell homing into follicles in the spleen and lymph nodes and for the proper development of most lymph nodes and Peyer's patches. Expression of BLC mRNA was detected in the synovium of RA patient. To determine the effects of BLC gene deletion on collage-induced arthritis (CIA) and collagen type II antibody- induced arthritis (CAIA), we backcrossed BLC-deficient mice to CIA susceptible B10.RIII mice. $BLC^{-/-}$ mice were immunized with type II collagen (CII) or passively transferred with anti-CII antibodies. We found the $BLC^{-/-}$ mice were completely resistant to CIA. $BLC^{-/-}$ mice produced significantly lower levels of anti-collagen II IgG antibodies compared with $BLC^{+/+}$ mice. However, proliferation and cytokine production by T cells, B cells, and spleen cells from immunized mice were comparable between $BLC^{-/-}$ and $BLC^{+/+}$ mice. In CII antibody-induced arthritis, $BLC^{-/-}$ mice showed significantly less severe clinical and histological arthritis than $BLC^{+/+}$ mice. Our results demonstrate an important role of BLC in the development of arthritis, participating in the early immune responses as well as later-stage effector phase of arthritis pathogenesis.
Acknowledgments: We are grateful to UCSF for providing BLC gene knockout B6 mice.

A089

Evaluation of T0906487, a CXCR3 antagonist, in a Phase 2a Psoriasis Trial

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T0906487 (T487), a potent and selective CXCR3 antagonist, was evaluated in subjects with psoriasis in a randomized, double-blind study. The objectives were to evaluate the effect of T487 on markers of inflammation in psoriatic skin lesions and peripheral blood (PB) and to assess its safety and pharmacokinetics. Forty subjects with moderate to severe plaque psoriasis (≥5% BSA) received 50 mg or 200 mg T487 or placebo orally once daily for 28 days. T487 appears to be well tolerated in this population. Lesion biopsies are being analyzed for T-cell infiltration and cytokine, CXCR3 and CXCR3 ligand expression by immunohistochemistry and quantitative PCR. T-cell subsets, including CXCR3+ cells, are being measured in PB using flow cytometry. Clinical evaluations include Psoriasis Activity and Severity Index (PASI) and Physician Global Assessment (PGA) scores. A decrease in lesion- infiltrating CXCR3+ cells will provide mechanistic proof-of-concept.

A090

Sphingosine kinase siRNA and a sphingosine kinase antagonist Dimethylsphingosine block TNFα–induced mediators IL6 and IL8.

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Bronchoconstriction and neutrophilia are hallmarks of COPD. Sphingosine-1-phosphate (S1P) is a lipid mediator and the product of sphingosine kinase (SphK1 or SphK2) that was shown to be increased in the BAL of asthmatics and to stimulate the contraction of human airway smooth muscle cells. Furthermore, stimulation of neutrophils with TNFα induces SphK activation and increases in S1P. In this study we examined the role of SphK1 on the generation of IL6 and IL8 following TNFα stimulation. Bronchial epithelial cells (H292) were transfected with a Sphk1 small interfering RNA (siRNA). 48hrs after transfection, a 90% decrease in SphK1 mRNA was measured in the SphK1 siRNA treated cells. TNFα stimulation of these cells resulted in a 40% reduction of IL6 and a 100% reduction of IL8 production compared to control siRNA-treated cells. In H292 cells treated with 10μM dimethylshingosine (DMS) a 70% inhibiton of IL6 was observed at 6hrs post TNFα stimulation. In H292 treated with 3μM DMS 50% inhibition of IL8 was observed at 24hrs post TNFα stimulation. DMS also inhibited the production of IL6 and IL8 in neutrophil differentiated HL60 in a dose dependent manner. In contrast to the cell lines, only a modest inhibition of 15% in IL8 production was observed in primary neutrophils (2/3 donors) treated with 3μM DMS and stimulated with TNFα. These studies demonstrated the direct effect of SphK activation and IL6 and IL8 production induced by TNFα. This study provides further evidence of the important role S1P may play in lung inflammation.

A092

Metabolic Fate of the CINODs, NMI-1182 and AZD3582, in Human Blood and Tissue Fractions. E.D. Cochran*, V. Dhawan, R.A. Earl, J.L. Ellis, D.S. Garvey, D.R. Janero, S.P. Khanapure, L.G. Letts, M.G. Murty, W.M. Selig, M.J. Shumway, I.S. Zemtseva, & D.V. Young. NitroMed, Lexington MA, USA

Nitric oxide (NO) is gastro-protective in many species including humans. This has led to the development of cyclooxygenase inhibitory nitric oxide donors (CINODs), where an NSAID is linked with a NO donating moiety. NO donation should prevent the significant NSAID-induced gastrointestinal (GI) side effects. This will only hold true, if bioactive NO is delivered to the stomach and circulation. The first CINOD in the clinic, AZD3582, recently completed a Phase II trial in which it demonstrated better GI tolerability than naproxen, but still produced significant ulceration. We have compared the metabolic fate of two naproxen-based CINODs, AZD3582 and NMI-1182. In human stomach mucosal S9 fractions, AZD3582 produced little naproxen, whereas much of NMI-1182 was converted to naproxen. In human blood the amount of naproxen released by NMI-1182 was twice that of AZD3582. NOx (nitrite/nitrate) release by AZD3582 in human stomach and blood was also less than that produced by NMI-1182. In human liver fractions AZD3582 was metabolized to naproxen and nitrate (inactive NO) with little release of nitrite (source of bioactive NO). By contrast, NMI-1182 was metabolized to naproxen and nitrite. If applicable *in vivo,* then AZD3582 in humans is likely to release naproxen with little production of bioactive NO. By contrast, NMI-1182 is likely to release bioactive NO and therefore may have greater potential to be GI protective than AZD3582.

A094

Acridone based IMPDH Inhibitors: Discovery and initial SAR leading to the identification of BMS-566419 as a potent inhibitor of IMPDH. T. G. Murali Dhar*, Scott H. Watterson, Ping Chen, Yufen Zhao, Zhongqi Shen, Henry H. Gu, Zili Xiao, Catherine A. Fleener, Katherine A. Rouleau, Mary Obermeier, Kim McIntyre, David Shuster, Joel C. Barrish, Jeffrey A. Robl, Robert Townsend and Edwin J. Iwanowicz. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ-08543-4000.

Inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the *de novo* synthesis of guanosine nucleotides, catalyzes the irreversible NAD-dependent oxidation of inosine-5'-monophosphate (IMP) to xanthosine-5'-monophosphate (XMP). CellCept® (MMF), a prodrug of mycophenolic acid (MPA) has clinical utility due to its inhibition of IMPDH, for the treatment of transplant rejection. The overall clinical benefit of MMF is limited by what is generally believed to be compound-related dose limiting gastrointestinal (GI) toxicity. Thus, development of an IMPDH inhibitor with a novel structure and different pharmacokinetics may reduce the GI toxicity and allow for increased efficacy. This presentation will detail the discovery and initial SAR of acridone based IMPDH inhibitors culminating in the identification of BMS-566419 {N-[1-[6-(4-ethyl-1-piperazinyl)-3-pyridinyl]-1-methylethyl]-2-fluoro-9,10-dihydro-9-oxo-3-acridinecarboxamide}, which potently and specifically inhibits IMPDH type II and type I enzymes *in vitro* (IC50 = 17±2.2 nM and 62±5.0 nM respectively). The compound demonstrated efficacy when administered orally in a rat model of arthritis.

A095

Evaluation of T cell-restricted Serine/Threonine kinase PKC theta as a Therapeutic Target for Inflammatory Diseases

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T-cell driven autoimmunity is the basis for a number of important human diseases, including Multiple Sclerosis (MS), Inflammatory Bowel Disease (IBD), Psoriasis, and Rheumatoid Arthritis (RA). PKC theta has the unique feature of its expression being highly restricted to T cells and skeletal muscle. An essential role for this kinase has been demonstrated in T-cell receptor (TCR)-mediated cell activation and proliferation. Production of cytokines that are an essential component of the T-cell response is impaired in the absence of PKC theta. Given its unique distribution and pivotal role in T cells, PKC theta may represent a potential therapeutic target for many T-cell-mediated pathologic processes, including autoimmune inflammatory diseases and organ transplant rejection. Here, we explore the role of PKC theta using knockout mice in several models of both in vitro and in vivo T cell function, including responses to anti-CD3 and allogeneic stimulation.

A096

Global analysis of gene expression changes in cartilage from the guinea pig spontaneous model of cartilage damage

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The guinea pig spontaneous model of cartilage damage shows many pathophysiologic changes that resemble the progression of human osteoarthritis. A comprehensive analysis of gene expression in this model has been limited by a lack of necessary molecular reagents. We have generated a custom guinea pig AffyMetrix array, with associated genomic information, and used it in a global assessment of mRNA expression changes associated with cartilage damage progression in this model. The final manufactured array contains 11,408 probesets for guinea pig genes. The custom array was used to assess mRNA expression changes in cartilage isolated from two- to nine-month old animals. The data have been analyzed to identify cartilage-selective gene expression, overall patterns of expression between different age tissue, and patterns of expression related to development and cartilage damage. Further analysis is ongoing to develop a clearer picture of the complex changes in gene expression associated with cartilage damage in this animal model.

A098

Inhibition of p38 mitogen-activated protein (MAP) kinase results in suppression of LPS-induced cytokine production, prevention of collagen-induced arthritis (CIA) and mechanical and thermal hyperalgesia in rats

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Rheumatoid arthritis (RA) is characterized by production of inflammatory cytokines and joint pain. p38 is an intracellular MAP kinase that is not only critical to inflammatory cytokine production and signaling but has been shown to be implicated in inflammatory pain responses. HTS identified AVE8677, a highly potent, selective, ATP-competitive p38 inhibitor. Following oral administration to rats, AVE8677 reduced LPS-induced serum levels of TNFα (ED50=10 mg/kg) with significant inhibition being observed up to 8 hours after dosing. In rat CIA, AVE8677 (10, 30 mg/kg) produced significant, dose-dependent suppression of ankle swelling and joint pathology and reductions in serum levels of acute phase proteins. Evaluation of AVE8677 (20 mg/kg) in a rat model of zymosan-induced inflammatory hyperalgesia resulted in a significant reversal of both the mechanical and thermal hyperalgesia observed at 4 hours. AVE8677 did not, however, produce a diminution in paw swelling induced by zymosan.

A099

Marked elevation of plasma tetrahydrobiopterine, not anandamide, in the systemic circulation in a porcine endotoxemia model and septic shock patients

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To evaluate which biosynthesis plays the greater role in inducing excessive vasodilatation in septic shock, we examined the plasma tetrahydrobiopterine (BH4) and anandamide (AEA) in a porcine endotoxemia model and septic shock patients. Furthermore we evaluated a correlation between the plasma level of these mediators and systemic hemodynamics. Our results demonstrate that in septic shock patients and a porcine endotoxemia model, plasma BH4 is upregulated, and may represent a better biochemical marker of septic shock than AEA.

Topic: New Animal Models of Inflammatory Mechanisms and Diseases

A100

Compstatin: a novel anti-inflammatory compound

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We have previously identified a 13-residue cyclic peptide termed compstatin that binds complement component 3 and inhibits complement activation. Its inhibitory activities have been extensively tested in various *in vitro* and *in vivo* clinically relevant models. Since its discovery, compstatin has undergone a series of optimizations, which have led to novel compstatin analogs with improved activities. Using experimental and computational combinatorial approaches, we report here on the construction of two analogs that display 45- and 99-fold higher activity than the original compound. In an *in vitro* model of extra-corporeal circuit for cardio-pulmonary bypass surgery, these analogs were found to block complement activation at concentrations as low as 1 μM. In addition, they inhibited complement-induced upregulation of CD11b on neutrophils. The activity of these analogs are currently also being tested in an *in vivo* model of heparin-protamine complex-induced complement activation in baboons.

A101

Analysis of matrix metalloproteinase gene expression changes in the guinea pig spontaneous model of cartilage damage. Debra Kellner, Jessica Eason-Butler, Sue A. Yocum, Fred Jerva, Kenneth Kilgore, Susan Bove, Denis Schrier, Jeffrey Marine, Richard Dyer, Gang Ken Hu and Joseph Menetski; Pfizer Global Research and Development, Ann Arbor Laboratories, Michigan 48105.

Members of the matrix metalloproteinase (MMP) family have been shown to be active in osteoarthritis (OA) in humans. The mRNA expression of several of MMP family members increases dramatically in the OA state. Since the guinea pig spontaneous model of cartilage damage shows many pathophysiologic changes that resemble the progression of human osteoarthritis, we determined the mRNA expression of some MMP family members in guinea pig cartilage in order to compare the changes to human OA. The data suggest that the expression of specific MMP activity may be important in developing articular cartilage based on the expression pattern observed, while other specific MMPs are expressed during the course of growth and cartilage damage. These data suggest that each MMP may have selected functions at the age it is expressed. The comparison of changes seen in the guinea pig model of cartilage damage to the reported changes in human OA will be discussed.

A102

Characterization of a potent inhibitor of p38 in *in vitro* and *in vivo* models of inflammatory disease.

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P38 has been the target of numerous drug discovery efforts that have produced clinical candidates demonstrating profound inhibition of TNF-α and IL1-β release; many have advanced to various stages of development. However, most of these compounds have failed during development due to unacceptable safety profiles. We have adopted a strategy of decreasing adverse effects by reducing exposure to affected organs by increasing cellular potency, decreasing blood-brain barrier penetration by increasing overall molecular polar surface area (*i.e.* aqueous solubility) and improving enzyme selectivity. Here we describe the characteristics of one of our pre-clinical candidates, ARRY-p38-001. This molecule has excellent developability characteristics: MW ~460, a cLogP ~4, and solubility at pH 7.4 of > 1000 ug/ml. It is a potent inhibitor of p38 enzyme (IC_{50}=5nM) and inhibits TNF-α production in human PBMCs of ~50 nM In an acute inflammation model (rat paw edema), ARRY-p38-001 inhibited paw swelling in a dose-dependent manner, with an ED_{50} of 10 mg/kg with a maximum inhibition of 71% at a dose of 30 mg/kg (highest dose tested). In a rat model of established Type II arthritis, ARRY-p38-001 (1 mg/kg) inhibited disease progression; doses of 10 m/kg, BID, and higher, reversed the disease process. Doses up to 100 mg/kg, BID, PO of ARRY-p38-001 were well tolerated in this study. Our p38 inhibitor at 3 mg/kg, BID and 1 mg/kg, BID showed equivalent activity to indomethacin and Enbrel, respectively. In summary, we have identified p38 inhibitors with excellent potency and activity in vivo with a good preliminary safety profile in the rodent. Compounds are being advanced into more comprehensive safety studies to support further development.

A103

Identification of nuclear factor of activated T cells (NFAT) family members in synovial fluid cells

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The NFAT family of transcription factors plays diverse roles in various cells and organs. The immunosuppressive drugs cyclosporin and FK-506 act largely by interfering with the activation of NFATs, and more specific NFAT inhibitors would represent potentially novel drugs for the treatment of inflammatory disorders and transplant rejection. However, little is known about the expression of NFATs in inflamed tissues. We have begun to study the expression of NFAT family members in synovial fluid cells from patients with various inflammatory joint disorders. Synovial fluid was obtained from pediatric and adult patients with joint swelling. Using commercially available antibodies (Santa Cruz), Cytospin slides were stained by standard immunocytochemistry for NFAT1, 2, and 3. All three were detected. Medium-sized mononuclear cells with morphologic features of monocytes expressed NFAT1. Interestingly, larger mononuclear cells (likely fibroblast-like synoviocytes), expressed NFAT1 and 2, and all three NFATs were found in neutrophils. The latter two cell types were not previously known to express these factors. NFAT family members may play roles in neutrophils and in the synovial lining.

A104

Requirement of IL-17 receptor signaling in resident synoviocytes for development of full blown destructive arthritis

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IL-17 is a proinflammatory cytokine suspected to be involved in inflammatory and autoimmune diseases such as rheumatoid arthritis. Here, we reported that IL-17 receptor (IL-17R) signaling is required in resident synovial cells for full progression of chronic synovitis and bone erosion. Repeated injections of streptococcal cell wall (SCW) fragments directly into the knee joint of naive IL-17R deficient (IL-17R-/-) mice had no effect on the acute phase of arthritis but prevented progression to chronic destructive synovitis as was noted in wild type (wt) mice. Significant down-regulation of leukocyte specific chemokines, selectins, and collagenase-3 in the synovium of IL-17R-/- mice was found. Bone marrow (BM) chimeric mice revealed the need for IL-17/IL-17R signaling in resident synovial cells for the development of full blown synovitis. Chimeric mice of host wt and donor IL-17R-/- BM cells developed destructive synovitis in this chronic relapsing SCW arthritis model similar as wt/wt chimeras. In contrast, chimeric mice of host IL-17R-/- and donor wt BM cells were protected from full blown destructive arthritis similar as IL-17R-/-/IL-17R-/- chimeras. These data strongly indicates the requirement of local IL-17R signaling in resident synovial cells in turning an acute macrophage-mediated inflammation into a chronic destructive synovitis.

A108

Prophylactic Administration of Abatacept (CTLA4-Ig; BMS-188667) Prevents Disease Induction and Bone Destruction in a Rat Model of Collagen-induced Arthritis

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Rheumatoid arthritis (RA) is an autoreactive disease in which activated T cells play an important role orchestrating the autoimmune responses giving rise to the inflammatory cascade responsible for joint inflammation and bone destruction. The CD28/B7 costimulatory pathway is critical for full T cell activation and modulating this pathway has been shown to inhibit T-cell activation leading to inhibition of these immune responses. Abatacept modulates T cell activation by interfering with the engagement of CD80/86 with CD28. Abatacept has been shown to provide significant improvement in the signs and symptoms of rheumatoid arthritis in a phase II trial. Here, we examine the effect abatacept administration has on disease induction, anti-collagen antibody production and bone destruction in a rat model of collagen induced arthritis. Female DA rats were immunized s.c. on day 0 with 300 ug of bovine type II collagen in incomplete Freund's adjuvant at the base of the tail. Immunized rats were administered either 1 mg/kg abatacept or a control human IgG IP on days –1, 0, 2, 4, 6, 8 and 10. By day 16 of the study, significant paw swelling was observed in the IgG treated control animals and continued to increase throughout the study until reaching a plateau (~ 3-3.5 mls.) on day 21. Administration of abatacept abrogated paw swelling throughout the course of the study. The IgG treated rats reached 100% incidence while no incidence was observed in the abatacept treated group. Serum anti-collagen antibody levels correlated well with the paw swelling data where abatacept administration resulted in 90% inhibition of collagen specific antibodies. We also found that abatacept decreased the expression of many of the circulating cytokines and chemokines which were upregulated in diseased animals. Micro-CT analysis revealed that abatacept treatment protects the bone from destruction as the knees and ankles of these rats appear to be normal. Abatacept, a selective co-stimulation modulator significantly inhibited the onset and progression of disease in a rat CIA model. In these studies, paw swelling, collagen specific antibodies and bone destruction were all inhibited by the treatment.

A109

Rational Drug Design for Inflammatory Targets

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The present investigation involves the isolation and anti-inflammatory studies of the triterperiod fractions of *Alchornea cordifolia* leaves . The leaves of *Alchrnea cordifolia* were collected, identified, dried, and reduced to coarse powder and extracted with distilled n-hexane. The n-hexane extract (A) was fractionated into absolute ethanolic insoluble (A1) and absolute ethanolic soluble (A2) fractions. The antiinflammatory effect of the fractions were evaluated using egg-albumen-induced rat hind paw oedema as a model of inflammation. Fraction A2 which exhibited a very promising anti-inflammatory effect was subjected to column chromatographic separation on silica gel layers to afford A2I, A2II, A2III. These fractions were subjected to anti-inflammatory tests and phytochemical investigation.

Fraction A2 (100 mg /kg) gave anti-inflammatory activity which was significant ($P<0.01$) at all the observation time (1-3 h). A2I (50 mg / kg) and A2III (60 mg /kg) showed very high and significant activities with percent inhibition of oedema values of 73.85 and 49.23 at 3h respectively. These compounds possess triterpenoid structure.

In clusion, the ethnolic soluble fractions(A2) of n-hexane extract of *A cordifolia* leaves could be beneficial in the management of different inflammatory disease states. Its anti-inflammatory activity may be attributed to the triterpenoids A2I and A2III isolated.

A110

Cimetidine Effect on Nitric Oxide Synthesis on Rat Adjuvant-Induced Arthritis (AIA)

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Excessive nitric oxide (NO) synthesis by inducible NO synthase (iNOS) had been implicated in the pathogenesis of inflammatory diseases. NOS is a cytochrome P450-like (cytP450) hemoprotein. The aim of this study was to evaluate the effect of cimetidine (CIM), a specific inhibitor of cytochrome P450IIIA activity, on NO production on rat AIA. AIA was induced by footpad injection of complete Freud adjuvant at adult Wistar male rats. CIM was administrated (30 mg/100g, po) for 21 days after AIA. The effect was compared with an AIA control group, an AIA group treated with N-Nitro-L-argininmethylester (L-NAME), and an intact control group. Joint and footpad swelling were measured by a pletysmograph. NO production was quantified by measurement of its metabolites nitrite/nitrate (Griess) and the final end product L-citrulline in serum. At AIA rats CIM caused joint and footpad swelling reduction ($p<0,01$), a significant decrease of nitrite/nitrate and L-citrulline production ($p<0,001$). The effects were similar to that of L-NAME. CONCLUSIONS. These results suggest that CIM induces an inhibition of inflammation-mediated NO production in AIA rats. The effect is comparable with that of L-NAME.

A111

Effect of Vitamin K_2 on Bone Metabolism in Postmenopausal Rats

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Reactive oxygen species (ROS) are considered to be responsible for the aging process and osteoporosis, resulting from marked decreases in plasma antioxidants in aged osteoporotic women. On the other hand, high-dose vitamin K_2 supplementation has been reported to reduce ovariectomy-induced bone loss in rats and to decrease osteoporotic fracture in postmenopausal women. However, the mechanism by which vitamin K_2 prevents osteoporosis is unclear. Recently, vitamin K_2 has been suggested to preserve antioxidant activity as a novel function. Therefore, we investigated the effect of vitamin K_2 on osteoporosis of postmenopausal rats, by evaluating the relationships between serum antioxidant levels and bone metabolism. Postmenopausal rats exhibited significant decreases in the serum alkaline phosphatase activity and osteocalcin level, together with lower serum levels of antioxidants such as 17β-estradiol, macrophage migration inhibitory factor (MIF), and glutathione peroxidase (GPx) activity as compared with young female rats. On the other hand, vitamin K_2 supplementation (500 mg/kg, food intake) for 98 days led to a significantly increased serum vitamin K_2 level (3045 ± 915 ng/ml vs. 4.6 ± 3.4 ng/ml in the control; $p<0.0001$) with increased serum alkaline phosphatase activity and MIF level ($p<0.05$). Unexpectedly, however, it failed to increase serum level of antioxidant such as GPx. Nor did it affect bone formation markers such as oteocalcin and osteopontin, which were significantly lower than in the young female rats ($p<0.05$). Finally, the histomorphometric properties of the proximal tibia in femur were not altered by vitamin K_2. These results suggest that high-dose vitamin K_2 supplementation neither improves lowered antioxidant levels nor prevents bone resorption in postmenopausal rats.

A112

IKK2 Inhibition Protects against Bone and Cartilage Destruction in Rat Models of Rheumatoid Arthritis

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The IKK complex regulates NFkB activation, an important pathway implicated in the RA disease process. A consequence of the disease process in RA is cartilage and bone destruction. Using adjuvant and collagen-induced arthritis models in rats, we assessed such degradation by multiple novel techniques. We used microCT imaging methods to assess bone destruction in the joints of arthritic rats, compared to rats orally dosed with inhibitors of IKK2. We determined ED_{50} values to be approximately 10mg/kg, BID, for paw swelling, yet the same compound gave an ED_{50} value of 3mg/kg in relation to the protection of bone volume loss. We also measured bone resorption factors from the serum of these rats along with cartilage degradation products. IKK2 inhibition lead to the amelioration of bone and cartilage degradation in both of these models, and provides insights to their potential utility for the treatment of RA patients. Also, we observed that down-regulation of the NFkB pathway via IKK2 inhibition dampened the inflammatory process as measured by acute phase protein production (alpha1-acid glycoprotein and haptoglobin) in the serum of arthritic rats.

A113

IRAK-1 MEDIATED SIGNALING BY TOLL-LIKE RECEPTORS IS DEPENDENT ON A HSP90-CDC37 CHAPERONE MODULE

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The protein kinase IRAK-1 plays a prominent role in mediating the activation of macrophages by Toll-like receptors (TLR). We sought to identify novel proteins that interact with IRAK-1 as such proteins may function as regulators or downstream effectors of IRAK-1. Using a mass spectrometric-based approach we have identified a cohort of molecular chaperones, including Hsp90 and Cdc37, which bind to IRAK-1 but not IRAK-4 in 293T cells. Pharmacologic inhibition of Hsp90 led to a rapid decline in the expression level of IRAK-1, whereas over-expression of Cdc37 enhanced the folding of IRAK-1 into an active conformation. Significantly, the same Hsp90 inhibitor promoted the degradation of IRAK-1 but not IRAK-4 in primary mouse macrophages. Concomitant with the loss of IRAK-1 expression was a reduction in macrophage activation following stimulation with the TLR ligands LPS and CpG DNA. We conclude that the Hsp90-Cdc37 chaperone module contributes to TLR-mediated macrophage activation by directly regulating the stability and hence signaling capacity of IRAK-1.

A114

NMI-1182, a Gastroprotective Cyclooxygenase Inhibiting Nitric Oxide Donor (CINOD): Anti-Inflammatory Activity in the Rat.

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Nonsteroidal anti-inflammatory drugs (NSAIDs) such as naproxen are a mainstay of anti-inflammatory therapy although they are associated with adverse gastrointestinal (GI) events including bleeding and ulceration. Since nitric oxide (NO) is gastroprotective in both animals and humans, we developed a novel class of NO-donating naproxen derivatives, typified by NMI-1182, which posses the beneficial properties of NSAIDs yet exhibit better GI tolerance. Following acute dosing in the rat, naproxen, and des-NO NMI-1182 produced more stomach lesions than NMI-1182 or AZD3582 (another CINOD). Plasma levels of NO biomarkers (nitrate and nitrite) also increased after CINOD dosing. NMI-1182, AZD3582 and naproxen (1-10 mg/kg p.o. equi-molar doses) were studied in the rat carageenan-induced paw edema and air pouch inflammation models. All three compounds dose-dependently reduced paw edema and inflammation. Naproxen and NMI-1182 reduced PGE_2 content and white blood cell infiltration into the pouch to a greater degree than AZD3582. Our data demonstrate that the novel NO-donor-naproxen, NMI-1182, is an orally bioavailable, GI tolerable CINOD that is effective in two rat inflammation models. The data further illustrate the potential therapeutic utility of NO enhancement to improve the safety of existing drugs.

A115

Characterization of the IKK-2:NEMO (IKKγ) Interacting Domains

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Cytokine-induced inflammatory diseases such as rheumatoid arthritis are mediated via the NfκB pathway upon activation of the IKK signalsome. Though this signalsome is comprised of IKK-1, IKK-2, and NEMO/IKKγ, it is the interaction between IKK-2 and NEMO that is critical to formation of a functional signalsome. More specifically, this critical interaction involves the C-terminal LDWSWL of IKK-2 (called the Nemo Binding Domain (NBD)) and the N-terminus of NEMO. In an approach towards modulating inflammation, we have investigated several NBD-encoding peptides for their ability to bind NEMO and inhibit the critical IKK-2:NEMO interaction. The six residue NBD peptide, LDWSWL, did not bind NEMO and did not prevent the IKK-2:NEMO interaction, however, longer NBD-encoding peptides did bind NEMO specifically and prevented the interaction. These longer peptides may be required to give the NBD an appropriate conformation for recognition by NEMO and/or to appropriately occupy the NEMO-binding pocket thus preventing the IKK-2:NEMO interaction.

A116

LPS-induced JNK Activity and Cytokine Production in Human Monocytes Require MKK7. Sheila Sanders, Brian McEwen, Laura Michael, Heather Valik, and Jennifer Swantek*. Departments of Inflammation Molecular Sciences and Pharmacology, Pfizer Global Research and Development, Ann Arbor, MI.

The cJun-N-terminal Kinase (JNK) Mitogen Activated Protein Kinase (MAPK) pathway is primarily involved in the cell stress response and has been implicated in the pathology of rheumatoid arthritis (RA). MAPK kinase (MKK) members MKK4 and MKK7 are direct upstream activators of JNK family members, however, relative contributions of each MKK to specific ligand-induced cell events have been difficult to evaluate as both MKK4- and MKK7-deficient mice display an embryonic lethal phenotype. We assessed the role for MKK4 and MKK7 in ligand-induced JNK activity and cytokine production in the human monocyte line THP-1 utilizing an siRNA approach. We identified oligos for each MKK that were successful in achieving significant MKK knockdown. Depletion of MKK4 had no significant effect on lipopolysaccharide (LPS)-or anisomycin-induced JNK activity or on LPS-induced cytokine production. In contrast, reduction of MKK7 levels resulted in significant decreases in LPS-induced JNK activity and TNF-α and IL-8 production. Our results demonstrate that MKK4 and MKK7 play non-redundant roles in human monocyte cell functions.

A117

Pharmacological Characterization of BMS-566419, a Representative Compound of a Novel Series of Potent Inhibitors of Inosine 5'-Monophosphate Dehydrogenase.

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Inosine monophosphate dehydrogenase (IMPDH) is the rate-limiting enzyme in the *de novo* synthesis of guanosine nucleotides. Inhibition of IMPDH by mycophenolate mofetil (MMF) has proven to be clinically beneficial to organ transplant recipients by significantly extending graft and patient survival and has also demonstrated clinical benefit for patients with autoimmune diseases. We have developed a novel series of potent, orally bioavailable IMPDH inhibitors, represented by BMS-566419. BMS-566419 potently and specifically inhibits IMPDH type II and type I enzymes *in vitro* (IC50 = 17±2.2 nM and 62±5.0 nM respectively). BMS-566419 inhibits the proliferation of CEM lymphoblastoid cells (770±83 nM) and human PBMC's stimulated *in vitro* with anti-CD3 + anti-CD28 (220±49 nM) . When administered orally once a day, BMS-566419 inhibited antibody production in KLH-challenged mice (≥90%) and cynomolgus monkeys (≥60%). BMS-566419 was efficacious in the rat adjuvant arthritis model, providing significant inhibition at 10 mg/kg (PO/QD) and complete inhibition of paw swelling at 25 mg/kg (PO/QD). Additionally, BMS-566419 effectively prolonged graft survival in the rat heterotopic heart transplant model as a single agent or synergistically with cyclosporine A co-administration.

A118

The Anti-inflammatory Effects of the San Cheng-Chi Tang

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San Cheng-Chi Tang (Ta-Cheng-Chi-Tang ,TCC; Xiao-Cheng-Chi Tang, XCCT; Tiao-Wei-Cheng-Chi-Tang, TWCCT) are three traditional Chinese medicine formula used frequently in relieving gastrointestinal symptoms and signs, including fever, abdominal pain, constipation. They are composed of similar ingredients including Rhei Rheizoma, Aurantii Fructus, and Magnolieae Cortex. HPLC finger print of the three formula were first done in order to verify the major compounds in each formula. In vitro anti-inflammatory effects of San Chen-Chi-Tang on LPS –induced RAW 264.7 cells were assessed by nitrite formation assay, iNOS and COX-2 protein detection by western blot analysis, and PGE_2 measurement. TCCT exerted the most significant and dose-dependent inhibitory effect on NO production by LPS-induced RAW cells . Both TCCT and XCCT inhibited PGE_2 production . Moreover, TCCT inhibited iNOS, COX-2 protein expression in RAW 264.7 cells. However, TCCT could not inhibit iNOS activity in RAW 264.7 cells.. The above results suggest TCCT has the strongest anti-inflammatory effect among the three Cheng-Chi Tang. The cause of this stronger anti-inflammatory effect of TCCT is probably related to the higher content of rhein and magnolol within this formula. Variation in the dosage of each ingredient within the formula alters the function of the formula too.

Key word: San-Cheng-Chi-Tang, Nitric oxide, PGE_2, COX-2, iNOS

A119

Abatacept (CTLA4Ig) Modulates Human T-cell Proliferation and Cytokine Production Without Affecting LPS-induced TNFα Production in Monocytes

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Activated T cells play a central role in the inflammatory cascade leading to joint inflammation and destruction characteristic of rheumatoid arthritis (RA). The cytokines secreted by activated T cells are thought to both initiate and propagate the inflammation associated with RA. Abatacept, the first of a new class of agents that selectively modulate the co-stimulatory signal required for full T-cell activation, was evaluated for its ability to regulate human T-cell proliferation and cytokine production *in vitro*. The effect of abatacept on LPS-induced TNFα from monocytes was also evaluated to distinguish the impact on innate versus adaptive immune responses. Abatacept significantly reduced T-cell proliferation, in both primary and tetanus toxin (TT) recall responses, at concentrations between 0.3 and100 µg/ml, with maximal inhibition (~60-80%) observed at ~3-10 µg/ml. This concentration is below the serum C_{min} levels observed in patients receiving a clinically effective dose of abatacept.[1] At a concentration similar to trough plasma levels in patients (30 µg/ml), abatacept inhibited IL-2, TNFα and IFNγ secretion in both primary and TT-dependent recall responses. This is consistent with suppression of T-cell activation *in vivo*. In contrast, abatacept did not inhibit LPS-induced TNFα production in human monocytes indicating that this agent may preserve innate immune responses..

[1]Kremer JM et al. *NEJM* 2003; 349:1907-1915.

A120

Role of AVE1389, an Orphan GPCR, in Murine Models of Pulmonary Inflammation

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AVE1389 is an orphan GPCR with unknown function. It is selectively expressed in human airway epithelial and smooth muscle cells. AVE1389 mRNA is up regulated by the Th2 cytokines, IL-4 and IL-13 in normal human bronchial epithelial cells and in mouse bone marrow derived macrophages by IL-1 and TNF-alpha. Expression of AVE1389 mRNA is increased in the lungs of ovalbumin (OVA)-sensitized and challenged mice. We tested the hypothesis that AVE1389 is involved in allergic (adaptive) and innate immune responses in the lung. To investigate the potential role of AVE1389 in pathogenesis of asthma, we generated knockout mice deficient in AVE1389. Initial phenotypic characterization revealed a 30 % reduction in macrophages present in the spleen of KO mice versus WT littermate controls ($P<0.05$). Mice were sensitized to OVA on days 0 and 7, intranasally challenged with OA on days 14, 15, 16, 17 and studied 24 hrs later. AVE1389 deletion did not alter the immune cell infiltrate, Th2 cytokine profile or histology of the lung. To study the potential role of AVE1389 in innate immune responses in the lung, we challenged mice with LPS (100ug/mouse, i.n.) and examined immune cell infiltration and cytokine production in BAL fluid after 24 hours. Whilst profound neutrophilia was comparable in the airways of AVE1389 KO and WT mice, we observed a 50% reduction in the number of macrophage in the BAL of KO mice compared to wild type littermate controls ($p<0.01$). This coincided with a 50% reduction in the level of GM-CSF in the BAL fluid of KO compared to wild type littermate control mice ($p<0.01$). These findings indicate that AVE1389 plays a role in macrophage development and/or function and involved in LPS-induced pulmonary inflammation.

A121

Discovery of Novel Sulfonamides and Sulfamides as Potent and Selective Inhibitors of Mammalian 15-Lipoxygenase. *David S. Weinstein*[*1], Wen Liu[1], Khehyong Ngu[1], Charles Langevine[1], Natesan Murugesan[1], Zhengxiang Gu[1], Leena Fadnis[1], Doree Sitkoff[2], Donald W. Combs, Jeffrey A. Robl[1], John E. Macor[1], Karnail S. Atwal[1], and Cort S. Madsen[3]. (1) Discovery Chemistry, Bristol-Myers Squibb Company, P. O. Box 4000, Princeton, NJ 08543-4000, FAX: 609-252-6804, david.weinstein@bms.com, (2) Department of Computer Aided Drug Design, Bristol-Myers Squibb Company, (3) Department of Cardiovascular Research, Bristol-Myers Squibb Company*

15-Lipoxygenase (15-LO) has recently emerged as an attractive target for therapeutic intervention for several human diseases. A member of the nonheme iron-containing family of enzymes responsible for the oxidation of polyunsaturated fatty acids, 15-LO has been implicated in the progression of certain cancers, chronic obstructive pulmonary disease (COPD), and atherosclerosis. A virtual utilizing a previously reported crystal structure of rabbit 15-LO led to the identification of a dansyl sulfonamide of modest potency (IC_{50} = 4.1 uM) against isolated rabbit reticulocyte 15-LO in the presence of linoleic acid (LA) as substrate. Optimization of the early lead led to the identification of several series of highly potent (IC_{50} < 10 nM) sulfonamides and sulfamides which were found to be selective against two other significant human lipoxygenase isoforms, 5-LO and platelet-derived 12-LO. The effect of structural modifications of these small-molecule inhibitors on activity against isolated enzyme and in stably transfected chinese hamster ovary (CHO) cells overexpressing human 15-LO will be described in detail.

A122

C-reactive protein and alpha 1-acid glycoprotein levels in healthy and pregnant beagle dogs

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This study was conducted to determine the physiological C-reactive protein (CRP) and alpha 1-acid glycoprotein (AAG) levels for two groups of beagle dogs: healthy dogs of various ages and pregnant dogs. Serum CRP levels were measured by ELISA and AAG levels were measured in healthy beagles of various ages by TIA, and then separately – in pregnant beagles – by SRID. No significant sex-related differences were observed in serum CRP levels. Further, there were no significant age-related differences either. Serum CRP levels increased during pregnancy and peaked at 30 to 45 days after ovulation. Serum AAG levels were also observed without any significant sex- or age-related variation. Serum AAG levels increased in all pregnant beagles and peaked in the middle of gestation. Despite a high value of 1,210 – 1,360 μg/ml being observed for serum AAG levels in 3 pregnant beagles inoculated with *Staphylococcus aureus,* its levels in umbilical cord blood were below the detection limit of SRID (40 μg/ml). No significant sex-/-age related differences were observed in serum both CRP and AAG levels and these levels increased during pregnancy. The results of AAG levels in umbilical cords were below the detection levels suggest AAG is not transported to the placenta.

A123

Transplacental transport of α2-macroglobulin and induction of α2-macroglobulin in maternal and neonatal rats with acute inflammation

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The aims of this study were to investigate transplacental transport of α2– macroglobulin (α2M) in rats and examine the degree of α2M induction in maternal and neonatal rats with acute inflammation. Serum was collected from healthy pregnant CD (IGS) rats (16 males and 16 females), neonates of the pregnant rats and their cord blood. Additional serum samples were obtained from pregnant rats inoculated with an inflammatory agent, turpentine oil, their neonates and cord blood, and neonates inoculated with turpentine oil. The serum levels of α2M were measured by means of an enzyme-linked immunosorbent assay. The average serum levels of α2M in healthy neonates and cord blood were about 380 μg/ml. Serum α2M level in neonates inoculated with turpentine oil averaged about 580 μg/ml. Serum α2M levels in maternal rats inoculated with turpentine oil, neonates from those rats and their cord blood were elevated, the value being 2,000 μg/ml or higher. It was demonstrated that induction of α2M in neonatal rats was lower than in maternal rats when inoculated with turpentine oil These results suggest that α2M is transplacentally transported from maternal rats to fetal ones.

A124

A comparison of the concentrations of C-reactive protein and α1-acid glycoprotein in the serum of young and adult dogs with acute inflammation

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The present investigation was made to compare of the concentrations of C-reactive protein (CRP) and α1-acid glycoprotein (AAG) in sera from young and adult dogs stimulated with turpentine oil, and also those in dogs subjected to oophorohysterectomy or percutaneous gastrostomy, or inoculated with *Staphylococcus aureus* or a viral vaccine. The average CRP concentration in the sera peaked 2 days after inoculation of turpentine oil. The peak CRP concentrations in 3- and 18-month-old dogs were significantly (p<0.05) greater than those in 1-month-old dogs. The average AAG concentration in the sera peaked 4 days after inoculation of turpentine oil. No significant difference was found in AAG concentrations between any of the age groups. When experimentally inoculated with *S. aureus* or subjected to oophorohysterectomy, the CRP and AAG concentrations increased in 3-month-old dogs, but they increased little in 1-month-old dogs. The CRP and AAG in dogs inoculated with viral vacctine did not increase. In dogs with fractures or subjected to percutaneous gastrostomy, the CRP and AAG concentrations correlated with the condition of dogs.

A125

Sterolides – a new class of potent anti-inflammatory compounds.

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Steroids are the most potent anti-inflammatory drugs and are a mainstay of asthma therapy. However, their use is associated with a number of unwanted side activities like growth retardation, suppression of hypothalamic-pituitary-adrenal axis (HPA), osteoporosis etc. Several strategies have been employed in development of anti-inflammatory steroids with reduced systemic side effects. Two main approaches have been: 1) increase of corticosteroids lipophilicity by esterifying the steroid hydroxyl groups, and 2) incorporation of metabolically labile functional groups at various positions of corticosteroids which undergo a predictable biotransformation to inactive metabolites upon entry into systemic circulation ("antedrug" or "soft" drug concept). We used a novel "sterolide" concept (**stero**id-macro**lide** conjugates) where we combined property of macrolides to preferentially accumulate in immune cells, especially in phagocyte cells (peripheral blood mononuclear cells, peritoneal and alveolar macrophages) with potent anti-inflammatory activity of classic steroids. These molecules accumulate in macrophage cell line Raw264.7 and lymphoid cell line Jurkat *in vitro* with greatly prolonged efflux time. Such sterolide molecules show excellent anti-inflammatory activity in several animal models with little or no systemic effects otherwise seen with standard steroids. Lead molecule is currently in the late preclinical development stage.

A126

Laser Capture Microdissection and its application to drug discovery.

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Laser Capture Microscopy (LCM) is a very simple yet elegant process of directly 'capturing' cells from a histological sample. Coupled to the precision and detail of histology and immunohistochemistry, LCM allows us to more fully for a more full determination of e determine the levels of gene and protein expression within individual cells or areas of tissue. Regions of cellular distinction can be capturedsecured from tissue, the molecular contents isolated, and the identity of disease specific genes can be determined or confirmed. For example, target genes of interest can be followed pre- and post-therapeutic treatment, or the identity of infiltrating cells can be determined using cell specific expression markers. To determine if LCM and its derived mRNA could contribute to drug discovery programs, LCM methodologies were developed and applied to address challenges in target discovery and validation. Two examples will be discussed, the first being the validation confirmation of specific gene expression in a defined cell type: the analysis of enterocyte specific genes differentially expressed in LCM isolated jejunal vs. ileal enterocytes. The second example is that of general gene expression profiling: the comparison of pro-inflammatory molecules and cellular subsets in LCM isolated regions of RA and OA tissue. We were able to demonstrate that LCM can be a powerful tool for target identification and drug discovery, and will discuss the pros and cons of applying LCM to address specific questions or to profile complex sub-histological areas.

A128

Prostacyclin and Receptor Activation in the Human Lung

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The initiating events of inflammatory reactions are essentially of vascular origin and have been linked to the release of endogenous metabolites of arachidonic acid. These locally released mediators may contract and/or relax pulmonary vessels by activation of specific receptors. The aim of this investigation was to determine prostacyclin (PGI_2) release and receptor activation in human pulmonary vessels. PGI_2 is derived from arachidonic acid via the cyclooxygenase pathway. The quantities of this mediator were determined by measuring the stable metabolite (6-keto-$PGF_{1\alpha}$). Human pulmonary arteries (HPA) in the presence of arachidonic acid (100 µM) released prostacyclin (6-keto-$PGF_{1\alpha}$; 279±27 pg/mg tissue, n=11 lung samples) and human pulmonary veins (HPV) released significantly less (6-keto-$PGF_{1\alpha}$; 181±16 pg/mg tissue, n=11 lung samples; $P<0.05$). In both types of vascular preparations, COX-1 but not COX-2 protein was detected. PGI_2 and iloprost as well as prostaglandin E_1 (PGE_1) activated the IP receptor and induced relaxation in both HPA and HPV. These ligands also induced contraction in HPV by activating an EP_1 receptor. Activation of EP_1 receptors may reduce the vasodilatory benefits of these IP agonists in patients with pulmonary hypertension.

A129

Development of a Cell-based Assay Panel to Assess Selectivity of SRC-family kinase inhibitors.

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Small molecule inhibitors of SRC family tyrosine kinases have broad utility in a variety of diseases. However, the high degree of homology between these kinases has made identification of selective inhibitors a challenge. We have developed a panel of cell-based assays to assess selectivity of SRC family kinase inhibitors. An IL-3-dependent murine cell line, 32D, was infected with ecotropic retrovirus constructs encoding constitutively active SRC family kinase domains with an IRES-GFP to normalize expression. These cell lines proliferate in an IL-3-independent, SRC family kinase-dependent manner and have constitutively phosphorylated Stat3 and Stat5. Addition of SRC family kinase antagonists results in dose dependent inhibition of proliferation as well as Stat phosphorylation. These cell lines have been used in conjunction with enzymatic assays to compare selectivity of antagonist compounds

A130

Protection of Hepatic Ischemia-Reperfusion Injury by Acetylcholine Receptor agonist: Elahe T. Crockett*, James J. Galligan, Bruce D. Uhal, Jack Harkema and Arash Motaghi. Departments of Physiology and Pharmacology, Michigan State University. East Lansing, MI 48823

Background: Cytokine production is a critical component of ischemia/reperfusion (IR) injury. Acetylcholine, the principle neurotransmitter of the vagus nerve, binds to macrophages and inhibits the synthesis of tumor necrosis factor (TNF), through the cholinergic anti-inflammatory pathway. The aim of this study was to determine whether pharmacological stimulation of the cholinergic anti-inflammatory pathway would inhibit cytokine production and hepatic injury following hepatic IR. **Methods**: Adult C57BL/6 mice underwent 90 min of partial hepatic ischemia followed by 3 hours of reperfusion. The acetylcholine receptor (AChR) agonists, 1,1-dimethyl-4-phenyl L-pioperazinium iodide (DMPP), and nicotine were administered *i.p.* before ischemia. Plasma cytokine macrophage inflammatory protein-2 (MIP-2), and TNF-α levels were measured. Liver injury was assessed by plasma alanine transaminase (ALT) and histopathology. **Results:** Three hours of reperfusion resulted in significant hepatocellular damage as indicated by increased plasma ALT levels. The injury was associated with marked elevation of plasma TNF-α, and macrophage inflammatory protein-2 levels. Pre-ischemic treatment of mice with DMPP or nicotine significantly decreased plasma ALT (i.e., 75%, $p<0.0001$) and the cytokine levels (i.e, TNF: 73%, $p<0.0001$, and MIP-2: 24%, $p=0.0003$). **Conclusions:** The pharmacological stimulation of AChR protected liver against the reperfusion injury, which could have a potential impact on therapeutic intervention strategies in reperfusion injury.

A131

Effect of p38 and TNFα inhibitors on inflammatory responses in animal models of asthma.

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TNFα and IL-1β have been proposed to play a role in the pathogenesis of asthma and some clinical data suggests anti-TNFα therapies may be of value in severe asthma. The protein kinase p38 is involved in the generation of TNFα and IL-1β induced by inflammatory stimuli. We therefore sought to investigate the effect of p38 and TNFα inhibitors in rodent models of asthma. In balb/cJ mice sensitized and challenged with ovalbumin (OVA), Lenercept (soluble p55 TNF receptor-Fc) or p38 antagonist treatment had no inhibitory effect on inflammatory cell infiltration into the lungs. In the Brown Norway rat model of OVA sensitization and challenge, Lenercept inhibited neutrophil and eosinophil accumulation into the lungs. p38 inhibitors had similar effects with inflammatory cell recruitment inhibited by over 50% at all time points. Furthermore, p38 inhibitors had a dramatic inhibitory effect on OVA-induced increases in lung TNFα and IL-1β levels. Similar results were also obtained in a Guinea Pig model of asthma. Overall, these results support the hypothesis that p38 inhibitors may have therapeutic effects in asthma patients.

A132

Evaluation of inflammation and pain responses in mPGES-1-deficient mice

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Prostaglandin E_2 (PGE_2) is a lipid mediator of pain and inflammation produced by the isomerization of PGH_2 by multiple PGE synthase (PGES) isozymes, including the inducible microsomal PGES-1 (mPGES-1). It has been postulated that inhibitors of mPGES-1 could produce anti-inflammatory and analgesic effects. We have utilized mPGES-1-deficient mice to validate this hypothesis. While peritoneal macrophages from mPGES-1-deficient animals display a dramatic reduction of PGE_2 production in comparison to cells from WT animals, no significant differences between KO and WT animals were observed in multiple inflammatory pain models. The models examined include zymosan-induced hindpaw edema, thermal and mechanical hyperalgesia, and the formalin paw pain test. The only inflammatory nociceptive test yielding a significant difference between KO and WT mice was the acetic acid stretching test. Furthermore, mPGES-1-deficient mice were not protected from disease in the passive collagen antibody-induced arthritis model. Taken together these results suggest that the inactivation of mPGES-1 does not necessarily yield the same anti-inflammatory and analgesic effects as those reported for COX-2 inhibition.

A133

PROSTAGLANDIN E_2 ACTIVATES TUMOUR SUPPRESSOR GENE p53 THROUGH SPECIFIC SERINE 15 (SER15) PHOSPHORYLATION IN HUMAN SYNOVIAL FIBROBLASTS (HSF). Q.W. He, W.H. Faour, A. Mancini., J.A. Di Battista*. Division of Rheumatology, Department of Medicine, McGill University, Montréal (QC), Canada.

We demonstrate that Ser15 phosphorylation of p53 mediates PGE_2-dependent induction of programmed cell death (apoptosis) in HSF. PGE_2 stimulated the transactivational activity of p53 through specific p38 MAP kinase-dependent Ser15 phosphorylation as judged by the activation of a p53 Luciferase reporter plasmid in the presence or absence of mutant p53 constructs containing amino acid substitutions at Ser 15 (S15G, S15A). PGE_2-induced time and dose-dependent cell death with activation of mitochondrial cytochrome C release into the cytoplasm, cleavage of Lamin A/C (40/51 kDa), of caspase 3 (17/19 kDa), of PARP (89 kDa) and fragmentation of HSF DNA into a nucleosomal ladder. These effects were completely abrogated by transfections with the S15G and S15A mutants but mimicked by a constitutively activated form of p53 (S15 (glutamic acid)E). Over-expression of an EP4 receptor construct under PGE_2-stimulation markedly increased the sensitivity of HSF to PGE_2-induced apoptosis while tandem over-expression of EP1 markedly abrogated the response. Our results suggest that PGE_2-induced apoptosis is p53-mediated but cell-type specific, dependent on the expression profile of prostaglandin receptor isoforms in target cells.

A134

Characterization of a Model of Allergic Rhinitis in Monkeys

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This study was designed to further characterize a model of allergic rhinitis in cynomolgus monkeys. Because the immune system and nasal anatomy of the non-human primates are similar to that of humans these animals would be an appropriate model of allergic rhinitis. Thirteen male cynomolgus monkeys - ten with pulmonary sensitivity to *A. suum* antigen and three normals were studied. To determine the effect of histamine on nasal geometry, increasing doses of agonist were administered intranasally while the animals were anesthetized and intubated. A dose dependent nasal congestion was observed in both normal and allergic animals using acoustic rhinometry. *A. suum* sensitive monkeys were challenged with various doses of *A. suum* antigen using similar methods to determine the optimal dose which elicits nasal congestion. *A. suum* challenge resulted in nasal congestion with a peak response occurring 45 to 60 min post challenge. Nasal congestion was characterized by a 20-30% decrease in nasal volume and 50-70% decrease in minimal cross-section area (MCA). Presently we are evaluating the effect of an antihistamine in this model. This animal model would be a useful tool for evaluation of therapeutics aimed at the treatment of allergic rhinitis.

A135

Potent and Selective Inhibitors of PDE7

William J. Pitts*, Joseph Barbosa, Junqing Guo, James Kempson, Claude Quesnelle, Marco Dodier, Patrice Gill, Marianne Carlsen, Andrew J. Watson, Guchen Yang, Kim W. McIntyre, Robert M. Townsend, Henry H. Shen, Gary C. Starling, Peter A Kiener, Steven G. Nadler, Murray McKinnon, John H. Dodd, Joel C. Barrish

Phosphodiesterases (PDEs) hydrolyze the second messenger molecules cAMP and cGMP to affect cellular signaling. PDE's are involved in a myriad of important physiological functions and as such continue to be a major target for pharmacological intervention on the part of the pharmaceutical industry. The synthesis and structure-activity relationships (SAR) of PDE7 inhibitors with respect to potency and selectivity are presented. Some target related issues will be discussed.

A136

Cannabinoids in contrast to opioids may be cartilage protective via inhibition of cytokine-induced nitric oxide and prostaglandin E_2 production and cartilage degradation

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Cannabinoids have been reported to reduce inflammation and joint damage in animal models of arthritis. Synthetic cannabinoids, HU-210 and R-(+)-win-55,212-2 were studied to determine mechanisms through which cannabinoids reduce inflammation and joint damage. Morphine sulphate and endogenous opioids, met- and leu-enkephalin were also studied. The drugs' effects were studied on interleukin-1 alpha (IL-1α)-induced nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in primary cultures of bovine articular chondrocytes and on IL-1α-induced proteoglycan and collagen degradation in bovine nasal cartilage explant cultures. At concentrations 1-10μM, the cannabinoids inhibited NO and PGE_2 production. At 5-15μM the cannabinoids also inhibited proteoglycan and collagen degradation. Opioids had no consistent effects on the parameters studied. The results suggest that cannabinoids may protect cartilage from cytokine-induced degradation, in part, by regulating NO and PGE_2 production.

A137

Cyclo-oxygenase-3 immunoreactivity in naïve and inflammatory leucocytes, and the modulation of COX activity in vivo by acetaminophen. *Ayoub SS, Read EJ, Sawatzky D, Willougby DA, Seed MP*. Experimental Pathology, William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ, UK. m.p.seed@qmul.ac.uk*

Cyclooxygenase-3 (COX-3, an intron-1 inclusive splice variant of COX-1) mRNA is expressed in canine brain and human heart (1). We have shown COX-3 selective inhibitors inhibit a brain COX-1 derived protein (2). We aim to investigate the peripheral role of COX-3, but anti-human COX-3 antibody does not detect rat tissue COX-3 protein despite mRNA expression (3). Western blotting detected a 65kDa protein, with immuno-reactivity to anti-mouse COX-3 antibody, in rat circulating, resident & carageenan pleuritic leucocytes (LCs), as well as COX-1 and COX-2. Immunocytochemistry showed neutrophil and monocyte expression blockable by the cognate murine COX-3 peptide. Acetaminophen (ApAp, 200mg/kg p.o.) given at the acute (2-6hrs) and resolution (24, 48 & 72hrs) phases of pleurisy reduced PGE_2 at 2 hrs (22%, $p<0.01$), exudate at 6hrs (37%, $p<0.001$), and not at other times up to 72hrs. Cell influx, PGD_2 and 6kPGF1α were unaltered. ApAp reduced naïve pleural PGE_2, PGD_2 and $6kPGF_1\alpha$. COX-3 immunoreactivity is constitutive in circulating and naïve, inflammatory or resolving rat pleural washout LCs. COX-3 may: i) involve LC physiology; ii) play a minor role in exudate initiation. 1) *PNAS (2002)* 99: 13926-31; 2) Ayoub et al *PNAS* (2004) in press & unpublished; 3) Warner et al JPET (2004) 17 May ePub.

A138

Acetyl-Boswellic Acids Inhibit LPS-Mediated TNFα Induction in Monocytes by Intercepting IκB Kinase (IKK) Activity.

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In LPS-stimulated human monocytes the pentacyclic triterpenes, acetyl-boswellic acids (ABA), down-regulate the TNFα expression. ABA inhibited NF-κB signaling both in LPS-stimulated monocytes as detected by electrophoretic mobility shift assay and in a NF-κB-dependent gene reporter assay. By contrast, the gene expression driven by the interferon-stimulated response element remained unaffected implying specificity of the effect. ABA did not interfere with binding of recombinant p50/p65 and p50/c-Rel dimers to DNA binding sites as analyzed by surface plasmon resonance. Instead, ABA inhibited the LPS-induced degradation of IκBα as well as phosphorylation of p65 at Ser536 and its nuclear translocation. ABA inhibited specifically the phosphorylation of IκBα and p65 by IKKs immunoprecipitated from LPS-stimulated monocytes and by recombinant IKKα and IKKβ. Both IKKs are apparently essential for the LPS-triggered induction of TNFα in monocytes as confirmed by IKK-specific antisense oligodeoxynucleotides. These findings provide a molecular basis for the anti-inflammatory properties ascribed to ABA-containing phytopharmaceutical drugs in use in some countries.

A139

Inflammatory-Induced Osteoarthritic Pain in a Novel Rat Meniscectomy Model

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Osteoarthritis has inherently been classified as a non-inflammatory degenerative condition. However, recent studies have demonstrated an inflammatory component associated with various pro-inflammatory cytokines. Unilateral medial meniscectomies were performed on young adult rats and protein levels of TNFα, IL-1α, and IL-1β were assessed in sera, synovial fluid, and knee joint homogenates at various timepoints from 7-90 days post surgery. We determined that both TNFα and IL-1β were increased above control levels in the synovial fluid and knee joint homogenates from meniscectomized knees using ELISA assays up to 90 days post surgery. Immunohistochemical analyses were consistent with ELISA assays, indicating that TNFα and IL-1β immunostaining were present in synoviocytes, chondrocytes, and macrophages in the synovial membrane and femorotibial joint surfaces for 7- 90 days following meniscectomy. Indomethacin, administered up to 45 days post surgery, was effective in decreasing the levels of TNFα and IL-1β immunostaining in the knee joint sections; thereby, lending credence to an inflammatory component of OA in our meniscectomy model.

A140

Histone deacetylase 2 controls glucocorticoid receptor acetylation and suppression of NF-κB activity: restoration of steroid sensitivity in COPD

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Glucocorticoids are the most effective anti-inflammatory agents for the treatment of chronic inflammatory diseases, but some patients show a poor therapeutic response. Here we show that deacetylation of glucocorticoid receptor (GR) by histone deacetylase (HDAC) 2 modulates glucocorticoid-mediated inhibition of NF-κB-stimulated gene expression. Specific loss of HDAC2 by RNA interference (RNAi) resulted in reduced dexamethasone suppression of interleukin (IL)-1β-induced GM-CSF production. There was a negative correlation between the reduction of HDAC2 expression and dexamethasone efficacy. By contrast RNAi of other class I HDACs (HDAC1, 3 and 8) had no effect on glucocorticoid responsiveness. Loss of HDAC2 did not affect GR nuclear translocation or GR binding to DNA, but inhibited the interaction between GR and the activated NF-κB transcriptional complex. Dexamethasone binding of GR resulted in GR acetylation which was reversed by HDAC2. HDAC2-mediated GR deacetylation enabled GR binding to the NF-κB complex to occur. Finally, we show that overexpression of HDAC2 in steroid insensitive alveolar macrophages from patients with chronic obstructive pulmonary disease (COPD) is able to restore glucocorticoid sensitivity. Thus, reduction of HDAC2 may one of the mechanisms accounting for steroid insensitivity in this disease.

Van Arman Award Competition Abstracts

The Inflammation Research Association sponsors a competition for the encouragement of young scientists to perform exploratory and applied research in the general area of inflammation. Contestants must be candidates for advanced degrees: M.S., Ph.D., M.D., D.O., D.D.S., D.V.M., etc., or first year post-doctoral fellows. Those who have won first place in a previous year are ineligible to compete again.

These awards are in recognition of the late C. Gordon Van Arman, who had a long and distinguished career as an industrial scientist, during which he published over 100 scientific papers. The development of the drugs diphenoxylate, disopyramide, sulindac, and diflunisal can be directly attributed to his work. In 1970, Dr. Van Arman with Edward Takesue, Marvin Rosenthale, and Mary Lee Graeme, founded the Inflammation Research Association as an informal forum for bench scientists to exchange research ideas in inflammatory diseases. Through this award, the IRA wishes to develop a commitment to high quality inflammation research in young scientists.

Prior to the Conference, the Scholarship Committee selects the five finalists based on submitted mini-papers. Finalists will attend the Conference and participate in poster and oral presentations to the committee. Based on these presentations and the mini-papers, awards will be presented to the finalists.

VA01

Enhanced Indoleamine 2,3-Dioxygenase (IDO) Expression in Transgenic Mice Inhibits T Cell-Mediated Allograft Rejection.

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IDO is the rate-limiting enzyme in the kynurenine pathway of tryptophan metabolism and has been shown to have inhibitory effects on T cell responses. To evaluate the effects of cells expressing IDO on allogeneic T cell responses *in vivo*, we used mice that were transgenic for IDO (Tg-MI). In this study, we have used the H-Y (male specific) alloantigen to investigate the suppressive effect of IDO on T cell responses. Most female Tg-MI mice and normal (IDO-wildtype) mice accepted primary skin grafts from syngeneic male Tg-MI mice, whereas Tg-MI and normal females rejected syngeneic male grafts from normal mice by day 25. All female recipients received a second skin graft from non-transgenic (non-Tg) B6 male 117 days after the first to determine if enhanced IDO expression by primary graft promoted allograft tolerance. The majority of females that received primary grafts from Tg-MI mice also accepted secondary B6 male allograft while most females that received primary grafts from non-Tg donors rejected secondary B6 grafts. These outcomes suggest that enhanced IDO expression on donor skin cells suppresses T cell responses to allograft and promotes long-term tolerance.

VA02

Inhaled carbon monoxide reduces the remote intestinal inflammatory response elicited by hindlimb ischemia/reperfusion

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Heme oxygenase represents the rate-limiting enzyme in the degradation of heme into carbon monoxide (CO), iron and bilirubin. Recent evidence suggests that several of the beneficial properties of HO, may be linked to CO. The objectives of this study were to determine if inhaled CO reduces the remote intestinal inflammatory response and oxidative stress elicited by hindlimb ischemia/reperfusion (I/R). Male mice underwent 1 hr. of hindlimb ischemia, followed by 3 hrs. of reperfusion. Throughout reperfusion, mice were exposed to AIR or AIR + CO (250 ppm). Following reperfusion, the distal ileum was exteriorized to quantify leukocyte rolling velocity and leukocyte adhesion in submucosal post-capillary venules with the use of intravital video microscopy. Harvested ileum samples were also analysed for pro-inflammatory cytokine expression (TNF-□ and lipid peroxidation (malondialdehyde), with the use of ELISA and Thiobarbituric Acid Reactive Substances (TBARS) assay respectively. I/R + AIR led to a significant decrease in leukocyte rolling velocity and 6.7-fold increase in leukocyte adhesion, as compared to sham mice. This was also accompanied by a significant 1.3-fold increase in ileum malondialdehyde and 2.3-fold increase in TNF-□ expression. Treatment with AIR + CO throughout reperfusion significantly reduced leukocyte recruitment and TNF-□ expression elicited by I/R, however malondialdehyde levels remained unchanged. Our data suggests that inhaled CO reduces the small intestine inflammatory response elicited by hindlimb I/R, independent of altered intestinal lipid peroxidation. CO may represent a novel anti-inflammatory therapeutic treatment to the reduce the systemic inflammatory response following acute trauma.

VA03

Cryopyrin Regulates Tumor Necrosis Factor Signaling and Cell Death

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Dysregulation of the inflammatory response can lead to disorders of chronic inflammation, several of which have been attributed to mutations in *CIAS1* which encodes the protein termed Cryopyrin. Cryopyrin is rapidly induced by inflammatory stimulants in human monocytes and can both modulate NF-κB signaling and Interleukin 1-beta (IL-1β) secretion in certain cellular contexts. Towards understanding the role of *CIAS1* in inflammation, we have constructed and utilized recombinant adenovirus and siRNA technology to examine Cryopyrin function in monocytic and non-monocytic cells. Adenovirally-introduced Cryopyrin sensitized HeLa cells to TNFα-mediated caspase-3 activation, and induced cell death in both HeLa and THP-1 monocytic cells in a dose-dependent fashion. Interestingly the adaptor protein ASC, known to interact with Cryopyrin, was required for Cryopyrin-mediated death in THP-1 cells. These data suggest that Cryopyrin levels in myeloid cells may shift the balance between pro-inflammatory signaling and programmed cell death, with mutations in CIAS1 preventing the proper resolution of inflammation.

VA04

Identification of a natural soluble form of the IL-18 Receptor accessory protein as an immunomodulator in experimental arthritis

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In the inflammatory process preceding degenerative arthritis, Interleukin-18 (IL-18) plays an important role. IL-18 is known to regulate immune responses by stimulating Th1 maturation. The objective of this study was to determine the role of a recently described soluble form of the IL-18Receptor accessory protein (IL-18Rβ) in mice. Short IL-18Rβ mRNA was highly expressed in tissue of lymphoid origin, and no expression could be observed in immune privileged organs like testis, eye and brain, suggesting a prominent role in immune regulation. Short IL-18Rβ appeared to be disease regulated in mice suffering from collagen induced arthritis (CIA), whereas the full-length IL-18Rβ was not regulated. Splenocytes of sIL-18Rβ treated immunized mice produced significantly less IFNγ and IL-10 compared to control treated animals. Adenoviral overexpression of the sIL-18Rβ before clinical manifestation of CIA significantly aggravated arthritis, which was accompanied by a reduction of circulating IL-10, IFNγ and a significant increased anti-bovine collagen II IgG1. In this study we describe the expression and regulation of a novel short soluble IL-18Rβ in mice. Furthermore, we show that this sIL-18Rβ is an important regulator of T cell function in mice suffering from collagen-induced arthritis.

VA05

A novel role for 15 deoxy Δ^{12-14} PGJ_2 *in vivo*: immunomodulatory activity in antigen induced arthritis.

Seema Gor Trivedi*, M.P. Seed, P.R. Colville-Nash.

Chronic inflammation and the resultant joint destruction seen in Rheumatoid Arthritis may be initiated by a T cell mediated response, and maintained by an imbalance of pro- and anti-inflammatory mediators. The cyclopentenone prostaglandins are associated with the resolution of inflammation, inhibit inflammation and the activation of the transcription factor NF-κB. NF-κB plays a central role in the regulation of anti-inflammatory apoptotic and pro-inflammatory pathways. It is hypothesised that the cyclopentenone prostaglandin 15 deoxy Δ^{12-14} PGJ_2 (15d-PGJ2) may play an important role in the modulation of the immune response. The therapeutic effect of 15d-PGJ2 was demonstrated in murine antigen induced arthritis. C57/BL6 mice were sensitised to methylated bovine serum albumin (mBSA) and arthritis was induced by intra-articular challenge with mBSA. 15d-PGJ_2 led to amelioration of the disease, and was associated with the inhibition of NF-κB within the inflamed stifle joint. Treatment with 15d-PGJ2 induced profound changes in draining lymph node mBSA-induced cytokine production ex vivo. In vivo administration of 15d-PGJ2 resulted in a significant increase in antigen specific lymphocyte production of interleukin-10 and interferon-γ. Our results indicate that the therapeutic action of 15d-PGJ2 is associated with profound changes in lymph node immune responses possibly invoking anti-inflammatory regulatory cells. Therefore 15d-PGJ2 may represent a novel therapy by modulation of immune function.

Presenting Author Index