Inflammation Research Association

11th National Conference

Sunday, October 6 – Thursday, October 10, 2002, 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 variety of serious diseases with inflammatory processes.

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Welcome

October 6, 2002

Dear Colleague:

Welcome to the Inflammation Research Association’s 11th National Conference. We are pleased to gather for what promises to be a most exciting biennial meeting featuring symposia, mini-symposia and posters addressing key areas of current drug- and disease-mechanism research using molecular and cellular biology, pharmacology, and medicinal chemistry in the search for an improved understanding of inflammatory mechanisms in human disease and strategies for therapeutic intervention.

The organizing committee selected The Sagamore in Bolton Landing, New York, as a new venue for this, our 11th, biennial meeting bringing scientists of all levels together to discuss inflammation in an inviting and comfortable resort setting. The Sagamore’s facilities appear to ideally match our organization’s traditional needs for a healthy mix of science, recreation, networking and fellowship. We welcome your feedback on this as a possible site for future biennial meetings.

The program committee has made every effort to minimize scientific session overlap and maximize registrants’ opportunities to attend the fullest possible complement of scientific presentations. Our meeting features no fewer than six symposia, including Sunday afternoon’s “Osteoarthritis: Markers and Targets” and Monday evening’s “Airway Remodeling in Chronic Pulmonary Diseases,” sponsored by the Pulmonary Research Group, our guest society. The Conference Keynote address:

“Rheumatoid Arthritis: Have we won the war? A view from the front lines”
by Michael E. Weinblatt, M.D.

is scheduled for Sunday evening. It will provide both a clinical perspective on currently available treatments for rheumatoid arthritis as well as a point of reference for our Monday-Thursday morning symposia. They will focus on recent advances in our understanding of the glucocorticoid receptor, medicinal chemistry of current drug discovery targets, molecular signaling in chronic inflammatory diseases, and our now traditional New Drugs session, emphasizing new therapeutic agents in or about to enter clinical trials. We have modified our former workshops into mini-symposia to be held Monday and Wednesday afternoons, following the poster sessions, and hope this scheduling will enhance the review and discussion of the experimental design, data and conclusions presented to support the submitted abstracts. You are especially encouraged to attend the presentations by competitors for the C. Gordon Van Arnum Scholarship Awards, which are also scheduled for Monday afternoon.

Finally, our meeting would not be complete without the planned social activities and banquet. Ample space and facilities are available for informal interactions throughout the duration of the meeting, and the opening reception following the plenary lecture Sunday evening as well as the Tuesday dinner cruise on Lake George should provide opportunities to renew old acquaintances and make new ones. A number of friendly competitions, including defense of the Flatfoot Trophy in volleyball, are planned and appropriate awards will be delivered along with comments from the outgoing and incoming IRA presidents at the banquet Wednesday evening.

I look forward to seeing you during the course of the meeting and hope you will join me in thanking all contributing IRA members who have worked to plan and deliver a meeting that will be of high scientific quality at a beautiful and comfortable venue.

Sincerely yours,

Richard D. Dyer

Richard D. Dyer, Ph.D.
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Symposia abstracts
Osteoarthritis: Markers and targets

Co-chairpersons: Anthony Manning, PhD (Roche Biosciences)
                Steven Abramson, MD (New York University)

Osteoarthritis is a group of overlapping distinct diseases that may have different etiologies, but with similar biologic, morphologic and clinical outcomes. The disease process not only affects articular cartilage, but the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane, and peri-articular muscles. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration and full thickness loss of the joint surface. Disease management today is aimed exclusively at the reduction of joint pain and improvement in function and quality of life. Based on an expanded knowledge of the molecular mechanisms of cartilage degeneration, clinical trials of several disease-modifying drugs for OA have been initiated in humans. This symposium will provide an overview of current research aimed at the development of disease-modifying drugs for OA.
S1 Regulation of intracellular signaling pathways in chondrocytes by nitric oxide and prostaglandins

Robert Clancy, Paul Gomez, Michael Pillingier and Steven B Abramson
NYU-Hospital for Joint Diseases, New York, NY

Activation of the NFκB and MAPK/kinase signal transduction pathways following cytokine stimulation results in a catalytic phenotype of chondrocytes, leading to metalloproteinase production and decreased matrix synthesis, as well as the induction of inflammatory proteins such as iNos and COX-2. We examined the effects of the products of iNos and COX-2 on these events. The addition of exogenous peroxynitrite (ONOO-), the free radical reaction product of nitric oxide (NO) and superoxide anion (O2·-), augmented NFκB (p65 Rel A) nuclear translocation in cultured chondrocytes. In contrast, the stable S-nitrosothiol S-nitrosocysteine (SNO) inhibited this effect, illustrating that the effect of nitric oxide may be either pro- or anti-inflammatory, depending upon the predominant redox end product generated in tissue. We sought to determine whether ONOO- was produced by cytokine activated bovine chondrocytes by measuring nitrotyrosine (stable product of ONOO- and tyrosine). In addition, we sought to determine whether the properties of cytokine elicited nitric oxide in activated chondrocytes on NFκB translocation resembled ONOO- or SNO. Exposure of bovine chondrocytes to IL-1β + TNFα induced the formation of intracellular nitrotyrosine (IF) consistent with ONOO- formation. EPR measurements demonstrated concomitant production of O2.-. Cytokine stimulation induced the nuclear localization of the p65Rel A subunit (IF), which was 40% complete by 5 minutes, maximal at 15 minutes, sustained at 60h and showed to decline by 24 hr. In contrast, MAP kinase Erk did not translocate to the nucleus in cytokine-stimulated cells (6 hr). To determine whether cytokine-stimulated nitrotyrosine production and NFκB translocation were mediated by induced nitric oxide production, chondrocytes were stimulated in the presence or absence of the NOS inhibitor, L-NMMA. L-NMMA, which reduced measurable nitric oxide production (Greiss) by 90%, prevented nitrotyrosine production in cytokine-treated chondrocytes, as expected. This inhibition of presumed ONOO- was associated with a significant inhibition of cytokine-induced maximal NFκB translocation at 6 hr, although no inhibitory effect was observed at the earlier time points, as expected. The MeK (preximal activator of Erk) inhibitors PD98059 and U0126 also inhibited NFκB nuclear translocation. In addition, MeK inhibitors, attenuated IL-1β + TNFα-stimulation of MMP-1, PGE2 and PGE2, but not PGE2, also inhibited IL-1β + TNFα-stimulated Erk activation and MMP-1 production. In contrast, both non-selective and selective NSAIIDs inhibitors enhanced ERK activation and MMP-1 production, presumably via reversal of Erk inhibition by COX-2 derived PGE2. Taken together, these data indicate that in chondrocytes: 1) ONOO- is a predominant redox species produced following exposure to IL-1β + TNFα; 2) nitric oxide derivatives (particularly ONOO-) augment matrix synthesis via cytokine-dependent NFκB translocation; 3) activation of ERK promotes NFκB translocation and MMP-1 production; and 4) ERK activation is inhibited by COX-2 derived prostaglandins and enhanced by NSAIIDs. These data provide insight into the mechanism by which nitric oxide and prostaglandins regulate the actions of catabolic cytokines and modulate the perpetuation of cartilage degeneration in osteoarthritis.

Even if articular tissue destruction characterizes the condition of osteoarthritis, synovial inflammation is of fundamental importance in the progression of cartilage lesions in this disease. The inflamed synovium secretes mediators, such as proinflammatory cytokines, which have an impact on cartilage matrix by altering chondrocyte metabolism. Several soluble mediators have been identified in articular tissues from various arthritic diseases. Findings point to the importance of proinflammatory cytokines, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), in the catabolic process in osteoarthritis. IL-1β is the prime cytokine involved. Osteoarthritis also involves changes in the surrounding bone, and subchondral bone sclerosis is a well-recognized manifestation in human osteoarthritis. Recent data underlines the concept that abnormal subchondral bone cell functions may contribute to the osteopenization of osteoarthritis. Recent work also suggests that very early in this disease process, biological and morphological disturbances occur in the subchondral bone and may have a role in the modulation of articular cartilage metabolism. Indeed, evidence indicates that altered subchondral bone metabolism in osteoarthritis is possibly caused by abnormal osteoblast behavior. This includes altered expression of biomarkers (alkaline phosphatase, osteocalcin), two systems (IGF-1 and IGF-1/IGF-1 receptor) involved in the promotion of osteoblast growth and remodeling, and factors such as cytokines/inflammatory mediators (IL-6, PGE2) that regulate subchondral bone remodeling/resorption.

In summary, it is now clearer that the progression of osteoarthritis is associated with the influence of tissue cross-talking. It appears that there is a movement of factors and cytokines from the different joint tissues to the cartilage. In this regard, the cytokines appear to be an interesting link in tissue cross-talking in osteoarthritis as they are responsible for important structural changes in joint articular tissues.

S3 Therapeutic targets for disease modifying osteoarthritis drugs: how to select the most promising molecules

Jean-Pierre Pelletier, MD, Professor of Medicine
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Head, Arthritis Division and Director, Osteoarthritis Research Unit
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Over the last decade, there have been several interesting advances in the treatment of osteoarthritis (OA). A clearer understanding of the pathophysiology of this disease has facilitated the development of new approaches for treatments aimed at specifically and effectively retarding the disease progression. Also, new classes of molecules that inhibit one or more OA catabolic processes are under evaluation.

One of the most attractive recent findings is the data pointing to an association between inflammation and disease appearance and progression. There are a number of pathways linked to synovial inflammation, which represent the most interesting targets. For instance, cytokines, such as IL-1α, appear responsible for the OA conditions. There exist a number of ways by which the reduction of the production or the activity of this cytokine could be managed, and this will be presented.

Among the different catabolic pathways that are activated by inflammation, nitric oxide (NO) and the eicosanoids are interesting targets in this disease. Indeed, our data showed that reducing inducible nitric oxide synthase (NOS) excess production may not only reduce the symptoms but also the progression of disease. Findings in the literature on the effects of eicosanoid overproduction in the metabolism of joint tissues reveal a variety of catabolic activities. New developments on these factors will be discussed.

Although several pharmacological agents are under investigation to treat OA, the tools to study such effects in humans remain unsatisfactory. Recent studies indicated that magnetic resonance imaging (MRI) is the most promising tool for investigating human knee OA. We have developed a novel imaging system assessing cartilage volume/thickness using MRI of the knee. Preliminary data revealed that statistically significant changes in the volume and thickness of OA knee cartilage were detectable at 12 months. This technology should significantly improve the investigation of new drugs and their potential to modify the progression of OA.

S2 New insights into the major pathophysiological process responsible for the development of osteoarthritis

Johanne Martel-Pelletier, PhD, Professor of Medicine
Director, Osteoarthritis Research Unit
Centre Hospitalier de l’Université de Montréal - Hôpital Notre-Dame
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Osteoarthritis (OA) could be defined as a complex or interactive degradative and repair process in cartilage and subchondral bone with secondary components of synovial membrane inflammation. The etiopathogenic processes involve various factors including mechanical, biochemical, genetic, etc. In the course of time, the chondrocytes react to the injuries by elaborating degradative enzymes and developing inappropriate repair responses. Much recent research implicates proinflammatory cytokines activity as a key feature of the disease progression.

The main developments over the last few decades have been in the change in the basic concepts concerning the pathophysiology of the disease, which have moved from a fairly mechanical hypothesis of wear and tear to include a number of interactive pathways explaining the structural changes. Moreover, it is now known that there is global cross-talking among the three major tissues involved in this disease: cartilage, subchondral bone and synovial membrane.

The extracellular matrix structure plays an integral role in the function of cartilage. In this tissue, matrix homeostasis is controlled by the chondrocytes through a balanced regulation of synthesis and the rate of new matrix production being equal to the rate of matrix degradation. Both processes are controlled by a variety of extracellular messenger proteins termed growth factors and/or cytokines. Disease progression or accelerated growth factors may compromise the macromolecule synthesis and degradation pattern, and therefore are responsible for the development of pathological conditions such as osteoarthritis.
A small molecule inhibitor of IkB kinase β (IKK-β) blocks inflammation and protects joint integrity in in vivo models of arthritis

GlaxoSmithKline, King of Prussia, PA 19406

Nuclear Factor-κB (NF-κB) is a key transcriptional regulator of many pro-inflammatory mediators (e.g. TNFα, IL-1β), IL-6 and activated NF-κB has been observed in several debilitating inflammatory disorders, including rheumatoid arthritis and osteoarthritis. The IkB kinase β (IKK-β) is required for the phosphorylation of IkBα and subsequent NF-κB activation by the IkB kinase complex (IKKα, IKKβ, IKKe) in response to pro-inflammatory stimuli. We have designed and synthesized a series of potent and selective inhibitors of IKK-β kinase activity. Treatment of human monocytes with an inhibitor from this series resulted in a concentration-dependent inhibition of LPS-induced TNF-γ production (IC50=150 nM). Similarly, exposure to the analog caused a concentration-related reduction in IL-1β-induced IL-8 and IL-6 production (IC50=130 nM and ~100 nM, respectively) from human primary synovial fibroblasts. Both prophylactic and therapeutic oral administration of this analog resulted in a profound reduction in inflammation as measured by paw volume in both a murine collagen-induced arthritis and a rat adjuvant-induced arthritis model. Evaluation of paw tissue cytokine production indicated a dose-dependent reduction in pro-inflammatory mediator levels. Consistent with this finding, immunoblot analysis of cytosols from paw tissue showed a blockade of IkB degradation and evaluation of nuclear fractions confirmed a reduction in NF-κB nuclear levels. Together, these data support the development of selective inhibitors of IKK-β as novel anti-inflammatory agents for the treatment of chronic joint disease.
On defining the mechanism of action for GR modulators with improved safety

Co-Chairpersons: Leonard Buckbinder, PhD (Pfizer Global R&D)  
Jean Shearin, PhD (GlaxoSmithKline)

Glucocorticoids are highly effective anti-inflammatory agents, but serious side effects limit their use. The complexity of Glucocorticoid Receptor actions results from diverse functions including DNA-binding and protein-interactions. A "safer-steroid" has been rationalized based on the premise of developing modulators with selective receptor activities. This symposium will explore mechanisms of GR action and the activity of preclinical compounds exploiting the selective strategy.
Role of accessory factors in the assembly of the glucocorticoid response unit on the PEPCK gene promoter

Daryl K. Granner, M.D., Vanderbilt University Medical School

Glucocorticoid induction of the phosphoethanolamine carboxyl kinase (PEPCK) gene requires a glucocorticoid response unit (GRU) comprised of two non-consensus, weak glucocorticoid receptor (GR) binding sites, GR1 and GR2, that are themselves not functional, and at least four accessory factor elements (gA1-F-3 and the CRE). These elements bind the transcription factors COUP-TF/NF4, HNF3, COUP-TF and CEBP, respectively. The intact GRU fully supports transcription, however, mutation of any one of the accessory elements reduces the glucocorticoid response of the PEPCK gene. Mutations in the accessory response. DNA-binding accessory proteins are commonly required for the regulation of genes whose products play an important role in metabolism, development, and a variety of host defense responses, but little is known about why they are necessary. Quantitative, real-time assays of cooperative protein-DNA interactions in complex media (e.g., nuclear extracts) had not previously been reported. We performed quantitative, real-time equilibrium and stopped-flow fluorescence anisotropy measurements of protein-DNA interactions in nuclear extracts to demonstrate that GR binds to the GR1-GR2 elements poorly (30x less avidly) as compared to a palindromic or consensus GRE. Inclusion of either the gA1-F or gA2-F element with GR1-GR2, however, creates a high-affinity binding environment for GR, and restores binding affinity to that noted with a palindromic or consensus GRE. GR can undergo multiple rounds of binding and dissociation to the palindromic GRE in less than 100 nM concentrations. However, the dissociation rate of GR is dramatically slowed by the gA1-F or gA2-F elements that bind two functionally distinct accessory factors, COUP-TF/NF4 and HNF3 respectively. Dissociation is very slow and incomplete in the presence of NF3. Dissociation is more complete, but still not as efficient as from the consensus GRE, in the presence of gA1-F/NF4/COUP-TF. Chromatin immunoprecipitation (ChIP) experiments were performed to analyze how GR binds to the PEPCK gene promoter, and how this binding affects the assembly of a productive transcription complex. The four accessory factors are always bound to the promoter, but GR binds only when complexed with ligand. The association of the ligand-GR complex results in increased association of CREB-binding protein (CBP) and polymerase II (polII) to the PEPCK gene promoter.

These studies show that one role of accessory factors is to convert the GR1-GR2 elements into high affinity binding sites for GR. These accessory factors also affect the dissociation of GR from these elements. These combined effects result in a productive CUG. The association of GR results in the assembly of CBP and polII to the promoter.

The dynamics of nuclear receptor interactions with gene targets

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Activation of transcription in eucaryotic systems is associated in current models with the formation of a stable preinitiation complex. We previously reported the direct observation of glucocorticoid receptor (GR) binding to the mouse mammary tumor virus (MMTV) promoter in living cells. We unexpectedly found that the glucocorticoid receptor (GR) exchanges rapidly with regulatory elements in the continued presence of ligand [Science 287:1262 (2000)]. We have now examined the dynamic behavior of several transcription factors at the MMTV tandem array. A coactivator, (GRIP1) shows the same rapid exchange as observed for GR. In contrast, the large subunit of RNA polymerase II (RPB1) shows a residence time of approximately thirteen minutes. We have also studied the interaction of purified GR with chromatin templates assembled in vitro. We have reconstituted the GR induced nucleoprotein transition with chromatin assembled on MMTV DNA. The remodeling event is ATP-dependent, and requires either a nuclear extract from HeLa cells or purified human SWI/SNF. Through the use of a direct interaction assay (magnetic bead "pull-down"), we demonstrate recruitment of human SWI/SNF to MMTV chromatin by GR. We find that GR is actively displaced from the chromatin template during the remodeling process. Displacement requires the presence of ATP, can be reversed by the addition of apyrase, and is specific to chromatin templates. The disengagement reaction can also be induced with purified human SWI/SNF. These in vitro and in vivo results are inconsistent with the presence of a long-lived preinitiation complex on regulated promoters. These findings support, rather, a dynamic model in which the receptors initiate chromatin remodeling and secondary factor recruitment, but are ejected rapidly from the template during nucleoprotein reorganization.

Structure and function of the glucocorticoid receptor: tools for identification of novel ligands

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Widespread clinical use of oral corticosteroids is limited by a number of side effects ranging from increased bone loss, growth retardation, and suppression of the hypothalamic-pituitary-adrenal axis. Discovery of a glucocorticoid receptor (GR) agonist that retains the beneficial anti-inflammatory activities without displaying undesired side effects is the subject of intense pharmaceutical effort. We have undertaken a multi-faceted approach for discovery of a selective glucocorticoid receptor ligand. One of these efforts has led to the successful expression, purification, and crystalization of the ligand-binding domain (LBD). Specific features of the GR LBD structure and structure-based functional studies will be discussed. Another tool being utilized is a novel cofactor-peptide interaction assay that uses multiplexed fluorescent beads. These major advances have greatly improved our understanding of the molecular basis of ligand-induced receptor activation and will provide insights for new ligand design.

A safer glucocorticoid receptor-dependent anti-inflammatory agent: The identification of a selective glucocorticoid receptor modulator

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Glucocorticoids are commonly used to treat inflammatory disease, but unfortunately, the long-term use of these steroids leads to a large number of debilitating side effects. The anti-inflammatory effects of glucocorticoids are believed to reside in the ability of the glucocorticoid receptor (GR) to inhibit expression of pro-inflammatory genes. In contrast, side effects are most likely due to metabolic dysregulation of other regulated GR target genes. As an yet undescribed pharmacological goal is the development of a compound capable of separating detrimental side effects from anti-inflammatory activity in vivo. This presentation will describe the discovery and characterization of A-224817, a nonsteroidal glucocorticoid receptor ligand that exhibits an altered gene regulation profile, able to repress and activate only a subset of the genes normally regulated by glucocorticoids. When evaluated in vivo, A-224817 retains anti-inflammatory efficacy and potency of steroids, but has fewer side effects at equivalent anti-inflammatory doses. In contrast to the steroid prednisolone, A-224817 has little or no effect on glucose metabolism. Furthermore, A-224817 is capable of antagonizing stress-induced hyperglycemia. The mechanism underlying this selective in vivo and in vitro activity may be the result of differential cofactor recruitment in response to ligand. A-224817 reduces the interaction between the glucocorticoid receptor and PGC-1, a cofactor critical for steroid-mediated glucose upregulation, while maintaining normal interactions with GRIP-1, a coactivator associated with both activation and repression by GR. In chronic in vivo models of glucocorticoid-mediated bone metabolism and osteoporosis, A-224817 exhibits reduced bone remodeling side effects compared to an equally efficacious dose of prednisolone. This class of compounds serves as a prototype for a unique, nonsteroidal alternative to conventional glucocorticoids in treating inflammatory disease.
Airway remodeling in chronic pulmonary diseases

Co-Chairpersons: Nansie A. McHugh, PhD (Schering-Plough Research Institute)
James Hogg, MD, PhD (University of British Columbia)

Airway remodeling is the term used to describe the many alterations in lung structure that occur in patients with asthma and COPD that compromise normal lung function. Depending on the severity and type of disease, these remodeling changes can include: subepithelial fibrosis and thickened basement membrane, smooth muscle cell hyperplasia/ hypertrophy, increased collagen deposition, goblet cell hyperplasia and activation of myofibroblasts. Although Asthma and COPD are inflammatory diseases of the lung, the inflammatory mediators involved are different and the exact contribution of inflammation to the remodeling process is not known. The epithelial cells and fibroblasts are rich sources of many cytokines and the actions of growth factors on myofibroblast precursors may explain the broad range of structural changes from collagen deposition in the subepithelium to the changes observed in the submucosa and smooth muscle. This symposium will explore the role of the fibroblast, smooth muscle and cytokines in the remodeling process and the use of transgenic mice as a tool for airway remodeling research.
Airway remodeling in asthma and COPD

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Asthma and chronic obstructive lung disease (COPD) are both inflammatory conditions of the lung associated with structural "remodelling" inappropriate to the maintenance of normal lung function. The clinically observed distinctions between asthma and COPD are reflected by differences in the remodelling process, the patterns of inflammatory cells and cytokines and also the predominant anatomical site at which these alterations occur. In asthma the epithelium appears to be more fragile than that of COPD, the epithelial reticular basement membrane (RBM) is significantly thicker, there is marked enlargement of the mass of bronchial smooth muscle and emphysema does not occur in the asthmatic non-smoker. In COPD, there is epithelial mucous metaplasia, wall fibrosis and inflammation associated with loss of surrounding alveolar attachments to the outer wall of small Airways: bronchial smooth muscle is increased also. Emphysema is a feature of severe COPD; in spite of the destructive process, alveolar wall thickening and focal fibrosis may be detected. The hypertrophy of submucosal mucus-secreting glands is similar in extent in asthma and COPD. The number of bronchial vessels and the area of the wall occupied by them increases in severe corticosteroid-dependent asthma: it is likely that these increases also occur in severe COPD as they do in bronchiectasis. Pulmonary vasculature is remodeled in COPD. In asthma several of these structural alterations begin very early in the disease process, even in the child. In COPD the changes begin later in life. The associated inflammatory responses differ in asthma and COPD but whether or not remodelling is dependent on the prior development of the distinct patterns of chronic inflammation is unknown. Thus, asthma and COPD differ in a number of important respects, at least if the patients are taken for study at the extreme ends of the spectrum of reversibility. Future studies of the areas of clinical overlap will yield interesting new data.

The role of lung fibroblasts and the CD40/CD40 ligand system in inflammation and remodeling

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Lung injury, scarring (fibrosis) and vascular damage can occur as a consequence of many etiologies including trauma, treatment for cancer, infection and connective tissue disease. The lung fibroblast is the key effector cell responsible for lung fibrosis. We have been investigating the concept that lung fibroblasts act not only as late stage effector cells, but also as "inflammatory sentinels" that become activated early following injury and synthesize cytokines, chemokines and lipid mediators that recruit white blood cells to the lung. We have identified a receptor called CD40 that is constitutively present on human lung fibroblasts and permits their rapid activation after stimulation by CD40 ligand (L1CD154). These activated human lung fibroblasts then synthesize a panel of pro-inflammatory mediators including cyclooxygenase-2 and prostaglandins, IL-1, IL-6, IL-8 and MCP-1. These mediators serve to recruit white blood cells and to enhance their activation. The major source of CD40L in humans is believed to be the activated T lymphocyte. Recent results showed that activated platelets were shown to contain large amounts of preformed CD40L, that is rapidly released following their activation. We evaluated whether stimulated human platelets and their released CD40L could activate human lung fibroblasts that naturally express CD40. Activated platelets and supernatants from these activated platelets containing CD40L powerfully stimulate lung fibroblasts to produce mediators of acute inflammation (IL-6, prostaglandins, etc.). The induction of these proinflammatory mediators was blocked by a neutralizing anti-CD40L antibody demonstrating the dependence of lung fibroblast activation on the CD40L/CD40L system. These findings support the concept that platelets and their released CD40L are crucial elements in inducing lung inflammation that may play a key role in lung scarring and in airway and parenchymal remodeling. Platelets should now be considered as key elements in inciting regional pulmonary inflammation.

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The role of cytokines in remodeling

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The involvement of various cytokines in the initiation and maintenance of chronic lung disease with fibrosis may represent the results of a sequence of host responses which have gone awry. Under a normal host defense paradigm, it is likely that the initial cell-mediated reaction involves the expression of gamma interferon and mediators that would fall under the rubric of a type I response. This immune response involving high gamma interferon response, is efficient in activating the phagocytosis and killing activity of neutrophils, monocytes, and macrophages, as well as inducing MHC class II expression on antigen presenting cells (APC). This immune interferon also serves a key role in the regulation of fibroblast activation. The ability of gamma interferon to suppress fibroblast activity has long been recognized as an important role of this type I cytokine. However, if the initiating factor or pathogen is not cleared by the initial immune response, the host enters a transition phase, which is characterized by the expression of a different cytokine phenotype. The significance of this new mix of mediators to the host defense is that a different type of immune response is now available to aid in clearing the antigen. The switch to a more sophisticated immune response allows the host to mount a continued response with renewed vigor. It is also recognized that specific type 2 cytokines can induce fibroblast proliferation and collagen deposition. Thus, if the antigen continues to persist and escapes the grasp of the type-2 directed response, the cytokine phenotype is now in place to induce the fibroblasts to proliferate and lay down collagen to wall off the inciting agent. This scenario could serve as one of the underpinnings for end-stage disease.

IL-13 is one of the type-2 cytokines that appears to be able to increase the fibrotic response by stimulating fibroblasts to increase collagen expression via networks which involve the expression of mediators such as the chemokine, monocyte chemotactic protein-1 (MCP-1), or TGF-beta. One of the central roles of IL-13 to the fibrotic process makes this cytokine an ideal target to develop new therapies to regulate the pathologic response of end-stage disease. One therapeutic approach for the treatment of fibrosis is the use of an IL-13 fusion protein, which is IL-13 tagged with a derivative of Pseudomonas exo toxin. Once bound to the cell expressing the receptor, the IL-13 fusion exo toxin can destroy the targeted cell. Recent in vivo studies have shown that IL-13 responsive cells were targeted via an intranasal administration of the IL-13 fusion protein in an experimental model of peripheral fibrosis. Experimental animals receiving as little as 200 ng of the construct exhibited a significant decrease in collagen deposition with a concomitant decrease in lymphocytes in the bronchoalveolar lavage. There is little doubt that the development of novel therapeutic strategies for pulmonary fibrosis should target the fibroblast as a key component of this progressive process. Data now underscores the effector role of the fibroblast, as a cell involved in the active recruitment and activation of leukocytes into the lung. The fibroblast can no longer be viewed as a passive, bystander cell, but a true player in the evolving lung response. Furthermore, the balance between normal tissue repair and excessive collagen deposition appears to be dictated by key communication loops involving both immune cells and lung fibroblasts during progressive chronic disease.

Activation of smooth muscle oxidant signaling mechanisms by physiological stimuli

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Our laboratory has identified multiple oxidant and redox regulated mechanisms that influence smooth muscle function, generally from studies in isolated endothelium-removed porcine pulmonary and coronary arteries. Changes in oxygen tension modulates the endogenous production of superoxide derived from a NADH oxidase whose activity is controlled by cytosolic NADH(H) redox. Post-hypoxic reoxygenteion appears to activate a hydrogen peroxide-mediated relaxation through mechanisms including stimulating soluble guanylate cyclase to produce cGMP. The control of NAD(H) redox by the pentose phosphate pathway appears to regulate a novel vasoconstrictor mechanism activated by decreases in NADPH, and this mechanism may involve a process controlled by the influence of glutathione on calcium transport mechanisms. Peroxide derived from NADH oxidase also appears to be involved with ERK MAP kinase-mediated increases in force development in responses to vasoconstrictor stimuli stretch in bovine pulmonary and coronary arteries. The control of NAD(P)H redox by lactate and pyruvate also regulates the ERK MAP-kinase pathway. The sensitivity of smooth muscle to relaxation by nitric oxide also seems to be regulated by NAD(P)H redox controlling the inhibitory effect of superoxide production and through the influence of NAD(P)H redox on a methemoprotein reductase which maintains the home of soluble guanylate cyclase (Gsy). The elevation in guanylate stimulation by nitric oxide. Thus, NAD(P)H linked-redox systems control multiple redox-regulated systems that potentially regulate smooth muscle function and remodeling through mechanisms that are dependent and/or independent of oxidant species.
Pathogenesis of airway remodeling and therapeutic implications
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Mechanisms of airway remodeling that develop in chronic asthma are unknown, although airway inflammation and abnormal tissue repair are involved. An imbalance between matrix metalloproteinases (MMPs) and their inhibitors or ineffective fibrillogenesis may cause abnormal tissue repair. Plasmaegeen activator inhibitor (PAI)-1 has the ability to inhibit MMP activity and fibrillogenesis.

PAI-1 is a member of the serine protease inhibitor super family and inhibits uPA and tPA, resulting in the accumulation of ECM and fibrosis. Recent experimental evidence indicates that PAI-1 is essential for the development of pulmonary fibrosis in vivo by controlling the MMP and fibrillogenesis systems. Mice with a targeted deletion of the PAI-1 gene (PAI-1-/- mice) are protected against ECM accumulation and fibrosis in the lung after bleomycin challenge or hypersensitivity, whereas PAI-1 overexpressing mice suffer from these fibrotic reactions. However, whether PAI-1 promotes airway remodeling remains unknown.

We demonstrated that mast cells are an active source of PAI-1 in asthmatic airways and secrete an abundant amount of functionally active PAI-1 upon stimulation by IgE receptor cross-linking. Activated mast cell-derived PAI-1 completely suppresses tPA activity and converts a fibrinolytic environment to a fibrosis-dominant condition. We also have showed that the 4G allele of the PAI-1 gene, which is associated with elevated plasma PAI-1 level, may contribute to the development of asthma in humans.

PAI-1 production is greater in lung tissue and bronchovascular lavage fluids (BALF) in ovalbumin (OVA)-sensitized mice after inhalation challenge with OVA for 4 weeks. Collagen accumulation was considerably less in lung tissue from PAI-1 (+/-) mice than wild type (WT) mice after OVA challenge. MMP-9 activity was approximately 3-fold higher in lung tissue and BALF from PAI-1 (+/-) mice than WT mice. Irreversible fibroblast expansion was 4-fold less in the airways and surrounding lung parenchymal tissue from PAI-1 (+/-) mice when compared to WT mice after OVA challenge. These results suggest that PAI-1 promotes ECM deposition in the airways of these mice by regulating MMP-9 activity and fibrillogenesis and that PAI-1 could play an important role in structural changes in the airways of asthmatics.

S14
Transgenic modeling of airway remodeling
Zhou Zhu, Tao Zheng, Chun G. Lee, Robert J. Homer, and Jack A. Elias
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Airway remodeling refers to the non-inflammatory structural alterations in the asthmatic airway, such as airway wall thickening, subepithelial fibrosis, myocyte hypertrophy and hyperplasia, myofibroblast hyperplasia, mucous metaplasia, and vascular alterations. Airway remodeling may result from the chronic inflammation characteristic of asthma. On the other hand, the structural alterations may contribute to the clinic and pathophysiological features of the asthmatic disorder. However, the pathogenesis of these alterations, the role of airway remodeling in generating the asthma phenotype, and the natural history of airway remodeling have not been adequately defined. Since exaggerated cytokine production has been known to be a characteristic feature of the asthmatic airway, we utilized the constitutive and inducible overexpression transgenic systems to investigate the possible contributions that interleukin 11 (IL-11) and IL-13, asthma-relevant cytokines, might make to airway remodeling responses.

In the constitutive system, a lung-specific promoter such as the Clara cell 10-kDa protein (CC10) promoter is used to target the gene of interest directly to the lung. This system is simple and convenient but has a number of limitations. It cannot model waxing and waning disease processes and it does not allow clear differentiation of the tissue responses to the transgene in developmental stage from those in adult stage. To overcome these deficiencies, we developed an inducible overexpression system in the lung that provides a temporal control of the transgene expression. This is based on two transgenic constructs. The first one is the CC10-driven reverse tetracycline trans-activator (rtTA), which is a fusion protein of mutated tet repressor and the herpesvirus VP-16 trans-activator. The second construct contains the tetracycline operator (tet-O) and a CMV minimal promoter-controlled gene of interest. Under normal circumstances, the CC10 promoter constitutively directs the expression of rtTA in the lung. Without induction agent doxycycline (dox), rtTA does not bind or binds weakly to the tet-O. Therefore no or minimum level of the transgene is expressed. When the mice are given dox in the drinking water, rtTA binds to the tet-O and activates the minimal CMV promoter, which in turn initiates the transgene expression. Thus the inducible system allows the investigator to regulate the transgene expression in mice by simply adding dox to or withdrawing dox from the drinking water.

Our studies demonstrated that transgenic overexpression of IL-11 causes a phenotype that includes airway wall thickening, subepithelial fibrosis, the enhanced deposition of types I and III, but not type IV, collagen, the enhanced accumulation of fibroblasts, myofibroblasts, and myocytes, baseline airway obstruction and AHR upon methacholine challenge, and enlarged airway. Constitutive transgenic overexpression of IL-13 also elicits a phenotype that manifests many features of airway remodeling, including prominent peribronchial inflammation with enhanced numbers of eosinophils, macrophages, and lymphocytes; epithelial hypertrophy; mucous metaplasia; and subepithelial fibrosis. Further studies using inducible overexpression system confirmed that all the responses described above can occur in adult mice when transgenic IL-13 production is induced by dox in the drinking water.

In summary, transgenic overexpression modeling is a powerful tool for studying the in vivo effector functions of asthma-relevant cytokines. The inducible overexpression system is suitable to model complex disease processes such as airway remodeling responses in asthma. These systems will prove invaluable in facilitating investigators in tackling questions such as reversibility of airway remodeling and identification of therapeutic targets for asthma.
Progress in the preclinical medicinal chemistry of new anti-inflammatory drugs

Co-Chairpersons: Jerauld Skotnicki, PhD (Wyeth Research)  Ronald Magolda, PhD (Wyeth Research)

There is exciting growth in the area of new targets in inflammation pathways. This has presented chemists with new challenges for the discovery of NCEs to treat inflammatory diseases and to treat non-classical inflammatory diseases now known to have inflammation-like pathways. To capture these new endeavors, this symposium will focus on the recent advances in medicinal chemistry of inflammatory diseases. There will be a diverse set of lectures with the common theme of the discovery of new molecules. A description of the chemistry, biology and pre-clinical pharmacology of these new molecules will be presented for a general audience interested in inflammatory processes in disease.
S15

Discovery and structure-activity relationship of N-(Ureidoalkyl)-Benzyl-piperidines as potent small molecule CC chemokine receptor-3 (CCR3) antagonists

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Eosinophilia has been correlated with disease symptoms in allergic asthma. CC chemokine receptor 3 (CCR3) is the dominant chemokine receptor expressed on eosinophils, and is activated by numerous pro-inflammatory CC chemokines, including eotaxin and RANTES. Thus, a small molecule CCR3 antagonist may be useful for the treatment of allergic asthma. Screening our in-house library of compounds led to the identification of compounds 1, 2, and 3 as weak CCR3 antagonists. In addition, compound 4—which is related to the published Belex CCR1 antagonists—also showed CCR3 binding affinity similar to the screening hits 1, 2, and 3. All of these compounds had some structural features in common as outlined with the bold bonds. Since the compound 4 had the additional ural functionality that the other compounds lacked, we concentrated our efforts on disecting the structure of 4 to determine the effect on binding and selectivity for CCR3 vs CCR1.

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We found that 5 could be further simplified to give 6 and 7 while maintaining binding affinity for the CCR3 receptor. An examination of a variety of substituted piperidines to replace the common 4-hydroxy-4-phenylpiperidine motif revealed that most changes gave less potent analogues. However, replacement of the 4-hydroxy-4-phenylpiperidine with a 4-benzylpiperidine gave compounds that were not only equivalent, but also selective for binding CCR3 relative to binding CCR1 (compare 5 and 8). Notably, the simplified N-benzyl analog 9 also retained binding affinity for the CCR3 receptor, and this affinity could be improved ~10-fold through the m- introduction of the urea functionality (see 10, CCR3 IC50 = 0.9 µM).

Further structure-activity studies were carried out to optimize the series exemplified by 10, and several potent and specific CCR3 antagonists were obtained (see 11–13, CCR3 IC50 < 0.01 µM). These antagonists were found to have greater than 100-fold selectivity for binding CCR3 relative to binding several other G protein-coupled receptors, including the CC chemokine receptors 1, 2, and 5. The structure activity relationship profile, oral bioavailability, and the functional activity of this series of compounds will be described in this presentation.

S16

Design and synthesis of VLA-4 antagonists

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The adhesion molecule, VLA-4, plays a key role in the initiation and maintenance of the inflammatory process. Its interaction with its native receptor VCAM-1, an endothelial cell surface protein, leads to inflammatory cell recruitment. The disruption of the VLA-4/VCAM interaction represents a new therapeutic approach to inflammatory diseases such as asthma, atherosclerosis, colitis, multiple sclerosis, and rheumatoid arthritis. We have previously identified small molecules based on tosyl-proline-phenylalanine which inhibit the interaction between VLA-4 and its counter-receptor, VCAM-1. The genesis of these compounds will be discussed, as will their evolution to compounds exhibiting potent, and selective VLA-4 antagonism.

S17

Discovery of novel LFA-1 antagonists


Boehringer Ingelheim Pharmaceuticals, Inc., Research and Development Center, 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877 USA

Lymphocyte Function-Associated Antigen-1 (LFA-1) is a heterodimeric cell adhesion molecule expressed on all hematopoietic cells and essential for their proper trafficking and activation. Inhibition of the binding of LFA-1 to its ligands, the Intercellular Adhesion Molecules (ICAMs), has been shown to suppress immune function in several animal models of disease.

We have previously reported the discovery and characterization of BIRT0377, a small molecule antagonist of LFA-1/ICAM interactions. This molecule binds to an allosteric site on LFA-1 and prevents the protein from accessing its ligand-binding conformation.

This presentation will focus on the second-generation antagonists from our program. In particular, efforts directed at improving in vitro potency and pharmacokinetic properties will be discussed.

S18

Cathespin S inhibitors: modulators of antigen presentation

Dennis S. Yamashita,* Xiaoyang Dong, Ren Xie, Scott K Thompson, Atiq Rahman, Robert W Marquis, Yu Ru, Daniel P Veber, Mika K Lindvall, Thadeus A Tomaszczyk, Michael S McQueney, Enoch Gao, Diana G Chalapoutsou, Elizabeth A Capper, Josephine H Fox, Andrea Petrone, Ruth J Mayer, and Patricia I Podolín

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Antigen-specific immune responses are mediated by presentation of peptide antigens by MHC class II on the surface of dendritic cells, B-cells, and macrophages to CD4+ T cells. Cathespin S (Cat S) is a cysteine protease that has been characterized to cleave the invariant chain, a modulator of peptide antigen binding to MHC class II. Inhibition of Cat S is hypothesized to diminish presentation of peptide antigens including self-peptides and, therefore, may be useful to treat disease states such as rheumatoid arthritis caused by aberrant activation of autoreactive CD4+ T cells.

We have previously disclosed a class of novel azepaneal cysteine protease Cathepsin K inhibitors. Herein, we present the identification of selective, nanomolar azepane analogues of Cat S. We have demonstrated that these Cat S inhibitors prevent the processing of SLIP to CLIP in mouse spleen cells at micromolar concentrations. These inhibitors also demonstrate inhibition of T-cell activation in a tetanus recall response assay at micromolar concentrations.
The discovery and development of selective inhibitors of p38 MAP kinase from distinct chemical classes

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Tumor necrosis factor (TNF) and interleukin (IL)-1 are clinically validated targets for the treatment of rheumatoid arthritis (RA). p38 mitogen activated protein (MAP) kinase is an intracellular enzyme involved in the regulation of cytokine biosynthesis and signaling. The inhibition of p38 MAP kinase suppresses TNF and IL-1, as well as cyclooxygenase (COX) expression, suggesting that inhibitors are likely to be efficacious in RA and other inflammatory diseases. The development of inhibitors of protein kinases, in general, has been hampered by the difficulty in the identification of ATP-competitive molecules with adequate therapeutic margins. The high attrition rate of kinase inhibitors is presumably related to the unknown absolute selectivity of these agents versus the large number of protein kinases in the human genome. In order to optimize the chances for discovering small molecule inhibitors of p38 with acceptable safety, we committed to the development of several inhibitors from unrelated structural templates. This presentation will report on the discovery and optimization of orally active inhibitors of p38 MAP kinase from four structurally distinct chemical classes; 4-azaindoles, 5-amino-1-phenyl-pyrazoles, 7-oxopyridopyrimidines and oxopyrimidopyrimidines. The use of crystallographic information of the molecules bound in the active site of the enzyme to optimize both the selectivity and potency of these inhibitors will be highlighted.
Molecular signaling pathways in chronic inflammatory disease

Co-Chairpersons: Ann Welton, PhD (Celera)
William Selig, PhD (UCB Research, Inc.)

Our current understanding of the molecular processes and enzymatic pathways regulating the cellular events mediating chronic inflammatory diseases is rapidly expanding. This information is allowing many new drug discovery strategies for anti-inflammatory and/or immunomodulatory therapies to be pursued. This symposium will focus on presentations that will elaborate both kinase- and protease-mediated cellular events that have the potential to lead to new therapies for chronic inflammatory conditions.
The discovery of modulators of NFBβ and AP-1 for the treatment of immunoinflammatory diseases

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The induction of proinflammatory genes often results from the increased activity of NFBβ and AP-1, two transcription factors that have become attractive targets for the development of novel anti-inflammatory drugs. NFBβ coordinates the expression of numerous soluble proinflammatory mediators including cytokines (IL-1, TNF-α, IL-6), chemokines, adhesion molecules as well as inducible enzymes (COX2, iNOS) (1). Our understanding of the NFBβ signaling pathway has generated numerous targets that are amenable to drug discovery efforts identifying small molecular weight inhibitors that will block the translocation of NFBβ to the nucleus and prevent inflammatory gene expression. These include members of the Signalosome, a large molecular weight complex consisting of IκB proteins (IKK1, IKK2) and adaptor proteins (IKKγ, IκKAP or NEMO), inadible kinases such as JNK1 or JNK2, and an IκB ligase selective for phosphorylated IκB. IKK2 inhibitors including SP600125 have been shown to be effective both in vitro and in models of arthritis (adjuvant, CIA) and are being developed clinically to treat RA, MS and cancer (2).

Several miogen-activated protein kinase (MAPK) cascades are involved in inflammation and joint destruction. The MAPK referred to as Jun N-terminal kinase (JNK) activates the transcription factors c-Jun and ATF2 and other members of the Jun family that are components of the AP-1 transcription factor complex (3). The JNK signaling pathway is involved with cell stress response, growth, differentiation, and apoptosis. The upstream pathway prior to JNK activation is complex. MKK4 and MKK7 activate JNKs, but in turn are activated by numerous additional kinases. Consequently, the pathway also offers many targets for drug discovery initiatives.

We have identified several classes of selective JNK inhibitors, including SP600125, and anapryrazole which demonstrated significant inhibition of JNK1, -2 and -3 (4). SP600125 is a reversible ATP-competitive inhibitor with >20-fold selectivity vs. a range of kinases and enzymes tested. In cells, SP600125 dose dependently inhibited the phosphorylation of c-Jun, the expression of inflammatory genes COX-2, IL-2, IFN-γ, TNF-α, and prevented the activation and differentiation of primary human CD4 T cell cultures.

In vivo, JNK inhibitors effectively block TNFα production induced by LPS, allergen induced asthma and adjuvant arthritis (5). They are also effective against experimental ischemia-reperfusion injury in the rat.

Pharmacologic modulation of both the NFBβ and AP-1 pathways offer existing new approaches to develop small molecule drugs to treat a broad range of immunoinflammatory diseases including RA, asthma, IBD and multiple sclerosis.


S22

Adult blood derived mast cells for the identification of novel targets for allergic disease

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 Mast cells (MC) are an important cellular target in allergic disease, particularly due to the diversity of mediators that they release which play essential roles in early and late stages of allergic responses. We have developed a novel system for the culture of human MC from normal and atopic donors from peripheral blood progenitors. In contrast to rodent or human cord blood derived MC, these cells possess many properties that have been documented for tissue MC that make them an excellent model for the discovery of new therapeutic targets. An important difference between human and rodent MC is that adult blood derived MC are highly functional and express a high level of the high affinity IgE receptor without a requirement for treatment with IL-4 or exposure to IgE. Sufficient numbers (5 × 10⁷ to 10⁷) of mature MC have been generated after 4-6 weeks of culture from 100-200 c of blood of all individuals that have been cultured to date. We have combined transcriptional profiling with a variety of experimental conditions to identify pathways as well as specific targets that are differentially regulated or show mast cell restricted expression.

A model of chronic antigen exposure was developed using this culture system which revealed significant differences from MC triggered once versus MC triggered multiple times. A time course of activation of mature 6 week old MC revealed that transcription for many chemokines are induced at 3-6 hours following exposure of the high affinity IgE receptor to naive MC activated for the first time. In contrast, chronically stimulated MC were activated at week 6, 7 and 8, have a much reduced induction of chemokines at 3 hours following the final activation, while other inflammatory mediators, such as GM-CSF are induced to a high level. The spectrum of chemokines that are produced from naive MC is consistent with the role that MC may play in innate immunity that has been proposed from rodent systems; however, the chronic stimulation model demonstrates how their function may change during chronic allergen exposure. In addition to a discussion of this model, the use of this system for the identification and evaluation of potential therapeutic targets will be presented.

The role of cation S in physiology and disease

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MHC class II molecules display antigenic peptides on the surface of APC for recognition by CD4+ T cells. Class II molecules associate in the endoplasmic reticulum with the aid of invariant chain (II), a chaperone protein that not only directs delivery of the class II molecules into the endocytic pathway but also prevents premature occupancy of the peptide-binding groove. Ii chain associated with class II molecules undergoes progressive proteolytic degradation within the endosomal/luminal compartment to generate a fragment of approximately 10 kDa (IiD). This fragment corresponds to a region of the II chain extending from the N-terminus of the molecule to the C-terminus of CLIP, the 3 kDa fragment which is exchanged for antigenic peptides. Cathepsin S has been identified as a key enzyme in the degradation of invariant chain (II). Published in vivo studies using the irreversible inhibitor, LiVHS, or the catS knockout mouse (catS-/-) have demonstrated accumulation of MHC class II-associated Ii complexes and lack of degradation of IiD (Reise, et al., Nakagawa, et al.). Studies presented were undertaken to determine if reversible catS inhibitors can elicit the same functional impact both in vivo and in vivo that results from catD deficiency or treatment with LiVHS.

B cells and dendritic cells from catS-/- failed to present OVA to OVA-specific T cells and hyperresponsiveness as measured by ear swelling, et al.). Similarly, in vitro, conducted in-house with reversible catS inhibitors, demonstrated a dose-dependent inhibition of IL-2 secretion from D0.11.10.1 C E following OVA presentation by A20 B cells (EcA20). In vivo, anti-catS antibodies sensitized, antigen-responsive T cells and the proliferation of Ig secreting B cells in LN germinal centers. Generation of antigen-responsive T cells and germinal center expansion are both suppressed in catS-/- mice (Shi, et al.). Oral treatment of C57Bl/6 mice with reversible catS inhibitors (3.5 mg/kg b.i.d.) prior to and during the course of antigen sensitization resulted in dose-dependent suppression of immunization-induced increases in T cells and B cells. LN germinal center expansion in immunized mice was also significantly reduced following oral treatment with reversible catS inhibitors. Additionally, cells harvested from these LN, 1) demonstrated a time-dependent accumulation of IiD and, 2) failed to secrete IL-2 in an ex vivo antigen- recall assay suggesting an inhibition of II chain degradation and attenuated in vivo sensitization of T cells.

Collagen-induced arthritis (CIA) was elicited in genetically susceptible DBA/1 mice by immunization with type II collagen (CII). Treatment with a reversible catS inhibitor (10 or 30 mg/kg b.i.d.) initiated at the time of CIA immunization significantly decreased the incidence and severity of CIA and levels of anti-CII antibodies. Histological examination of vehicle-treated mice revealed marked destructive arthritis with pannus formation, loss of articular cartilage and erosion of subchondral bone. Inflammatory cell influx, as well as cartilage and bone destruction, was inhibited by oral treatment with a catS inhibitor (10 or 30 mg/kg b.i.d.). Those results demonstrate that treatment of CIA mice with an orally-bioavailable, reversible catS inhibitor can inhibit arthritis both clinically and histologically.

Cathepsin S has a critical role in the late stages of Ii chain degradation. Inhibition of intracellular catS proteolytic activity influences not only peptide loading of MHC class II molecules but also the repertoire of peptides presented by MHC class II. Extracellular cathepsin S can degrade all major matrix components. Reversible inhibitors of catS are, therefore, attractive candidates for new disease modifying drugs for treatment of inflammatory joint diseases.

S23

The role of p38 MAP kinase in non-stress signaling pathways suggests novel therapeutic uses for p38 inhibitors

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p38 MAP kinase was originally implicated as part of a signaling cascade triggered by stress inducing conditions such as osmotic shock, toxic agents (asparagine), UV light and inflammatory mediators such as bacterial LPS, TNF and IL-1. The latter role of p38 kinase in inflammatory responses has led to the development of selective inhibitors of p38 kinase as potential anti-inflammatory agents. Indeed, the potential therapeutic utility of selective p38 kinase inhibitors has been demonstrated in several clinical studies including our own. However, it has become increasingly evident that p38 kinase plays a critical role in non-stress related cellular signaling. For example, we have provided evidence, using selective p38B kinase inhibitors as pharmacological probes, that p38 kinase is critical for the generation of TH2-type responses from human T cells. These findings indicate that p38 kinase inhibitors may be useful in the treatment of allergic disease in addition to other novel findings which expand the utility of p38 kinase inhibitors will be discussed.
Minisymposia and poster session abstracts
A1

Reversible cathepsin S (cAT) inhibitors block invariant chain processing in vitro and reduce the severity of collagen-induced arthritis (CIA) in vivo
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The cysteine protease, catS, has been identified as a key enzyme involved in the degradation of invariant chain (Ii). Inhibition of II proteolysis leads to an accumulation of NiII class II-associated Ii complexes that inhibit antigen presentation. Published in vivo studies using LHV5s or the catS knock out mouse (catS-/-) show accumulation of an Ii fragment of approximately 10 kDa (Iip10). Studies were undertaken to determine if reversible catS inhibitors could elicit the functional impact both in vitro and in vivo that result from depletion of catS or treatment with the irreversible catS inhibitor, Me-Lue-HomoPhe-tyrosylamine (LHV5). C57BL6 mice receiving a single oral dose (100–150 mg/kg) of either 3 reversible inhibitors or LHV5 demonstrated diminished II processing with Iip10 accumulation in both splenocytes and mesenteric lymph nodes (MLN). DBA/1 mice, reported to be less dependent on catS processing, demonstrated Iip10 accumulation in splenocytes, but not MLN, with either LHV5 or reversible inhibitors. DBA/1 mice immunized with chick collagen type II (CII) develop CIA, an inflammatory joint disease with many similarities to human rheumatoid arthritis (RA). Oral treatment (30 or 10 mg/kg, b.i.d.) with reversible catS inhibitors resulted in decreased levels of anti-cII antibodies and diminished disease as measured by both joint inflammation and histological analysis of joint tissue. Our data demonstrate that reversible catS inhibitors, like irreversible inhibitors, can affect in vivo II processing and the development of CIA.

A2

The use of thylakoid extract (PureCell Complex) as a bio-active ingredient to modulate inflammation
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The modulating activity of PureCell Complex on the inflammatory process was investigated. Lipopolysaccharides (LPS) stimulated alveolar macrophages were treated with various concentrations of PureCell Complex alone and in combination with commercial anti-inflammatory ingredients, such as budesonide, and pro- and anti-inflammatory cytokine levels, tumor necrosis factor (TNF) and interleukin-10 (IL-10) respectively, were measured in cell free supernatant at different time after the treatment. Results demonstrated that PureCell Complex modulates the synthesis of cytokinases in a dose-dependent manner. Furthermore, PureCell Complex stimulated the release of IL-10 when given in pre- and post-treatment to LPS and a synergistic effect was observed in combination with budesonide. In contrast, TNF release was inhibited by pre-treatment with PureCell Complex and/or budesonide. These exciting data suggest that PureCell Complex possesses some anti-inflammatory properties and could potentiate the effect of other anti-inflammatory agents.

A3

Effect of naproxen and vioxx in a rat model of osteoarthritis-associated pain
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Osteoarthritis (OA) is a degenerative joint disease that is characterized by joint discomfort and a progressive loss of articular cartilage. The objective of this study was to describe an in vivo model whereby a single injection of mono-iodoacetic acid (MIA; 1 mg/joint) into the right hind knee promotes the development of OA-like symptoms including joint discomfort. Changes in hind paw weight distribution between the right (articular) and left (contralateral control) limbs were utilized as an index of pain. Two articular, orally administered analgesics, Naproxen and Vioxx, were examined for their ability to decrease MIA-induced joint pain. Both compounds demonstrated the capacity to significantly (p<0.05) decrease joint pain in a dose-dependent fashion, indicating that the pain associated with MIA injection is susceptible to pharmacological intervention. In addition, histologic and morphologic changes to the cartilage and subchondral bone were similar to that noted for human OA. It is concluded that the rat MIA model of OA is a technically straightforward, reproducible model that closely mimics the behavioral, pathologic and pharmacologic features associated with human OA.

A4

Development of joint pain in a murine model of osteoarthritis
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Osteoarthritis (OA) is characterized by structural changes to the joint and joint pain. Studies to elucidate the pathophysiology of OA would benefit greatly from the use of transgenic animals that lack or over express relevant genes. These endeavors, however, have been hampered by the lack of a rapid, reproducible animal model that mimics both the histopathology and pain associated with human OA. Here we describe a rapid model of OA pain in the mouse in which a single injection of mono-iodoacetic acid (MIA), an inhibitor of glycosylation, into the right knee promotes the rapid development of OA-like joint pain. Changes in hind paw weight distribution were utilized as an index of pain in the arthritic knee. A mouse strain survey demonstrated significant phenotypic differences between strains in response to MIA. Although histologic changes consistent with that noted in human OA were observed in all strains, the degree of joint discomfort (pain) differed between strains. In responders, both Naproxen and Vioxx significantly (p<0.05) decreased pain, indicating that the pain noted after MIA injection was susceptible to pharmacologic intervention. It is concluded that the mouse MIA model of OA is an effective, short-term model for evaluating therapeutic approaches designed to attenuate OA-mediated pain.

A5

Modulation of experimental autoimmune encephalomyelitis (EAE) by a non-peptide inhibitor of cathepsin S
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EAE is a model of the human autoimmune disease multiple sclerosis (MS), which can be induced in Lewis rats by immunization with myelin basic protein (MBP). Neuroinflammation in EAE and MS is the result of the presentation of myelin sheath antigens by the immune system. The cysteine protease cathepsin S is responsible for the cleavage of invariant chain (CLIP) into CLIP, necessary for myelin peptide loading on the MHC-II complex. We evaluated the effects of cathepsin S inhibition on EAE using the cathepsin S inhibitor, S-(2-((S)-1-(2-(4-((S)-3-(2-pyridin-2-yl)ethyl)-acetyl)azepan-4-yl)aminobutyryl)butyryl)amide (compound 1), with Ki against human and rat cathepsin S of 8.3 and 6.5 μM, respectively. Compound 1 inhibited constitutive surface expression of CLIP by Daudi cells in a concentration-dependent manner as measured by flow cytometry, with an ED50 of 4.6 μM. Compound 1 inhibited antigen-induced proliferation and TNFα production by lymph node cells harvested from MBP-immunized rats in a concentration-dependent manner. In vivo, compound 1 significantly delayed the onset of EAE in Lewis rats. Lymph node cells from compound 1-treated rats exhibited significantly reduced proliferation and IFNγ production in response to antigen ex vivo than did control cells. In a preliminary experiment, cells cultured with an analog of compound 1 were unable to passively induce EAE in the SJL mouse. These results demonstrate that cathepsin S inhibition could be a beneficial therapy in the treatment of autoimmune diseases, such as MS.

A6

Pharmacological effect of CCR1 blockade on murine models of acute and chronic inflammation
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The involvement of chemokines in the recruitment and activation of leucocytes during an inflammatory process is well established. Chemokines act by binding to G protein coupled receptors, an attractive pharmacological target. Most of the small molecule antagonists of chemokine receptors reported up to date have not been tested in animal models due to their lack of crossreactivity between species. Recently, a CCR1 antagonist that recognizes both human and mouse receptors has been disclosed. Our aim was to evaluate the effect of CCR1 blockade using this compound in two acute and chronic inflammatory models of inflammation.

In the murine air pouch model challenged with carrageenan, the pretreatment of mice with the CCR1 antagonist led to the inhibition of cell recruitment (54%) together with a significant reduction in the levels of TNFα, CCL2, CCL3 and CCR5 in the inflammatory exudate. No effect on the CXC chemokines, CXCL1 and CXCL2, was found. The inhibition of CCL2 (30%) was intriguing since it was hardly observed (14%) in animals treated with an anti-CXCR2 antibody, despite its superior inhibition on cell recruitment (74%). These results suggest that the target cells of CCL2 were not only infiltrating leucocytes, but also maintaining extravascular immune infiltrates. These findings were confirmed in mice challenged intraperitoneally with LPS. In this model, resident liver macrophages (Kupffer cells) are believed to be the main source of cytokines and chemokines. We have found that the G protein expressing system (cells expressing the CCR1 antibody), when compared to vehicle-treated mice, did not significantly reduce the levels of TNFα, an increase in L10 and a significant decrease in CCL2 levels in plasma compared to vehicle treated mice. Again, these effects were not observed in animals treated with the anti-CXCR2 antibody. When the compound was tested in the collagen-induced arthritis model, either prophylactically or therapeutically for 12 days (10 mg/kg, once daily), it inhibited the pain response about 60%.

To our knowledge, this is the first time that the effect of a small molecule antagonist for CCR1 is reported in rodent models of inflammation and suggests a potential role for CCR1 as an inflammatory mediator, evidenced in the acute models, together with the efficacy demonstrated in the disease model suggest that CCR1 is a potential target for the treatment of this disease.
A7  Beneficial effects of estrogen treatment in the HLA-B27 rat model of inflammatory bowel disease  
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A well-established model of chronic bowel inflammation is the HLA-B27 rat that exhibits a spontaneous disease phenotype resulting in chronic diarrhea caused by immune cell activation. Estrogens have previously been shown to modulate the immune system and both estrogen receptors (ER) and ERE are present in the intestine and cells of the immune system. Therefore, the ability of estrogen to ameliorate disease progression in the HLA-B27 rat was determined. Twenty-two-week old HLA-B27 rats with chronic diarrhea were treated with 10 μg/kg/day of 17-ethynylestradiol (EE) for 5 days. EE treatment resulted in a dramatic improvement of stool scores after 3 days. Since immune cell infiltration into the colon is thought to be involved in the development and progression of inflammatory bowel disease (IBD), myeloperoxidase activity (MPO) was assessed as an assessment of neutrophil infiltration. MPO levels were reduced by 80% by EE treatment. Histological scoring of the colon also demonstrated dramatic improvements by EE treatment. A significant improvement in the degree of ulceration, inflammatory cell infiltration, fibrosis and lesion depth was observed. Co-treatment with the pure ER antagonist ICI182,780 blocked the effect of EE on stool character, MPO activity and the histology scores strongly suggesting that the activity of EE is mediated through one of the ERα. To determine the mechanism of EE action, gene chip analysis was performed with RNA isolated from vehicle and EE treated colons. A number of cell cycle specific genes were upregulated. Therefore, the EE treatment reduced mRNA expression of EE to block cell mass activation could explain the inhibition of neutrophil infiltration and tissue destruction observed in the colons of EE-treated HLA-B27 rats.

A8  Anti-inflammatory effect of an orally administered hyperimmune egg product, in the rat established adjuvant arthritis model  
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Hyperimmune® eggs (or “immune” eggs) are obtained from chickens repeatedly inoculated with 26 killed human enteric bacterial pathogens. These eggs were pasteurized and spray dried, to obtain an egg powder that is rich in immunoglobulins of the IgY class directed against the human pathogen, and several immunoregulatory factors. Recently, we have defeated the hyperimmune egg product using an acidic extraction procedure and retained the antibody activity and immunoregulatory factors. The whole egg powder and the egg powder was used in the established model of adjuvant arthritis model, the rats were gavaged at various doses once a day from Day-29. At 100 mg/rat and 400mg/rat dose, the whole egg showed anti-inflammatory activity, and reduced paw edema ranging from 3-19% at Days 16, 23 and 30. The 200mg/rat dose however, the whole egg significantly reduced inflammation by 51%, 33%, and 51% on days 16, 23 and 30 respectively. Some positive correlation’s, especially at days 16 and 23 at the time of early and progressive joint inflammation were observed regarding paw edema, plasma fibrogen levels and joint histopathology of the uninjected paw. The positive standard Indomethacin, at 1mg/kg produced significant changes (70-80% inhibition of inflammation) using a daily oral regimen from Day-16 to 29. The hyperimmune egg may be useful in playing a major role in the treatment of chronic rheumatoid arthritis or other chronic inflammatory diseases by its antiinflammatory activity and a safer GI profile, as evidenced by histopathological observations of the Peyer’s patches.

A9  Inhibition of osteoarthritis induced by surgical meniscal tear in the rat by matrix metalloproteinase inhibitors  
The objective of this study was to characterize a model of osteoarthritis induced by a surgically transecting the medial collateral ligament and meniscus (RMT) model, and to evaluate the effectiveness of matrix metalloproteinase inhibitors (MMPI). A time dependent increase in the degeneration of the surgical knee was observed that demonstrated histological features characteristic of human OA. Two MMPIs were potent (low nm) inhibitors of MMPs 2, 3, 4, 8, 9, and 13 and relatively weak inhibitors of MMPs 1 and 7 were administered orally twice daily (b.i.d.) at 25 mg/kg to rats in the RMT model. Administration of PGE-494887 resulted in significant (P < 0.05) inhibition of cartilage degradation and osteophyte formation (total joint histology score) of 41%, 32%, 26% and 56% in 4 separate experiments. Similarly, PGE-491292 at 25 mg/kg b.i.d. inhibited joint damage in a single experiment by 22% (P < 0.05). These results suggest the RMT model of osteoarthritis is suitable for evaluating MMPIs and support a potential therapeutic role for MMP inhibition in the treatment of human osteoarthritis.

A10  Reduction of streptococcal cell wall (SCW) induced arthritis in mice lacking IL-18  
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Interleukin-18 is a pleiotropic cytokine and it is well known that IL-18 promotes Th1-mediated immune responses. In contrast, the direct proinflammatory role of IL-18 is not fully established yet. We investigated the role of IL-18 in a model for acute inflammatory arthritis. Wild type C57Bl6 and IL-18 deficient mice (Il18−/− background) were intraperitoneally injected with 25 μg SCW fragments. Arthritis was monitored by measurement of joint swelling, cartilage chondrocyte function and histology. IL-18 deficient mice showed significantly reduced joint swelling at days 1, 2 and 4 after induction of arthritis. Measurement of chondrocyte function revealed that lacking of IL-18 did not prevent inhibition of chondrocyte PG synthesis during the early phase of arthritis. Interestingly, complete restoration of chondrocyte proline/cysteine synthesis was found in the IL-18 deficient mice at day 4. Examination of local cytokine levels revealed that both TNFα and IL-10 were reduced in Il18−/− animals. Histology at day 7 confirmed that deletion of IL-18 gene resulted in reduction of joint pathology. These data indicate that IL-18 plays a pivotal role in acute inflammatory joint diseases. Targeting of IL-18 may be a novel therapy for both acute and chronic inflammatory joint diseases.

A11  CXCXR3 gene plays a minor role in atherosclerotic plaque formation in the LDLR−/− mice  
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Recent evidence suggests that innate and acquired immunity participate in atherosclerosis. Macrophages and NK cells, as well as T lymphocytes exist in the atherosclerotic lesions. Interestingly, the chemokine receptor CXCXR3, which binds IP-10 (IFN-γ-inducible protein 10), MIG (monokine induced by IFN-γ) and ITAC (IFN- inducible T-cell alpha chemoattractant) and is expressed in activated T cells, is observed within human atherosclerotic lesions. To verify the role of CXCXR3 in atherosclerosis, low-density lipoprotein (LDL) receptor-deficient (LDLR−/−) mice were fed and a high cholesterol diet. There were no statistically significant differences between LDLR−/− mice and LDLR/CXCXR3 double knockout mice in the pathological lesions. The potential role of CXCXR3 in atherosclerosis thus requires further study.

A12  Blocking endogenous IL-17 during murine collagen arthritis prevents bone destruction and downregulates synovial RANKL and IL-1 expression  
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T cell IL-17 displays pro-inflammatory properties and is expressed in the synovium of rheumatoid arthritis patients. Its contribution to the destructive arthritis process has not been fully identified. Here, we report prevention of joint destruction after blocking endogenous IL-17 during established collagen-induced arthritis (CIA) using a soluble murine IL-17 receptor fusion protein (sIL-17R:Fc). Treatment with sIL-17R:Fc after onset of CIA did not reduce the clinical arthritis score compared with the control group. However, 8 and 14 days after onset, radiographic analysis revealed significant reduction of bone erosion after blocking endogenous IL-17. No difference in T cell responses to murine collagen peptides and IgG1 and IgG2a anti-collagen antibody levels were detected between the sIL-17R:Fc treated group and the control group. Interestingly, 14 days after onset reduced mRNAs expression levels of IL-1beta and RANKL were detected in the synovium after sIL-17R:Fc treatment compared with the control group. Our data suggest IL-17 is a novel target for the treatment of destructive arthritis and imply the T cell factor is an important upstream mediator of key catabolic cytokines in arthritis.
Cytokine antagonists in antigen presentation and inflammation

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MHC Class II invariant chain (Ii) processing is mediated by cytokine antagonists, leading to the hypothesis that modulation of this proteolytic activity may be a useful therapeutic approach to regulating antigen-mediated inflammatory diseases. Irreversible cytokine protease inhibitors block antigen presentation in cell-based systems and lead to the intracellular accumulation of the p10 fragment of Ii. Here we show that Mu-Leu5-Asp5-Val-y-Val-Asp5-Pro-y-Val5-IIH5S, when administered in the rat adjuvant-induced arthritis model, following either a prophylactic or therapeutic dosing regimen, resulted in decreased swelling, erythema, and joint destruction. In cell-based studies with new more selective and reversible inhibitors of Cathepsin S, we have also demonstrated inhibition of Ii processing and antigen presentation in cell systems.

PMA-induced skin hyperplasia and inflammation using new, quantitative methods

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Multiple, topical applications of phorbol ester to mouse ear skin induces a robust inflammatory response in the skin and a pronounced tissue hyperplasia, similar to conditions in psoriasis. However, no quantitative method for clinically assessing skin hyperplasia and no account of the pro-inflammatory mediators present in the skin have been described for this model. Here, we assessed the proliferative events in the skin using new, quantitative, radiological and histological methods not previously employed in this model. A radiolabeled DNA precursor[3H]-Ido-desoxyuridine (2 μCi/mouse), was optimized for use in this system to track DNA synthesis in the mouse ear tissue. Peak incorporation levels of 0.058 ± 0.0272% were observed 72 hours after two treatments of PMA (1μg/ml at T=0 and 48 h). Next, the PCNA index of epidermal cells from mouse ear tissue sections treated with PMA (90.6 ± 1.94%) and without PMA (32.5 ± 2.92%) were determined histologically. To provide a broader characterization of the pro-inflammatory mediators involved in eliciting the effects of PMA, time course evaluations of IL-1β, KC, TNF-α, IL-6, PGE2, and LTB4, levels in the ear were completed. Finally, to validate this model and these methods as a reasonable system for the pharmacological assessment of novel compounds, topical dexamethasone (0.13 mg/ear) was evaluated and found to significantly inhibit (~90%) both the proliferative and inflammatory aspects of this model.

Administration of antiRANKL antibody to overreactivated swiss-webster mice maintains trabecular bone mineral density and increases cortical area with a concurrent increase in biomechanical strength

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Normal bone metabolism depends on a delicate balance between bone resorption and bone formation. The receptor activator of NF-κB (RANK) and its ligand (RANKL) have been shown to be central players in this balance, as the binding of RANKL to the RANK receptor is necessary for the formation, activation, fusion and survival of the osteoclast. In this study, we blocked the actions of endogenous RANKL with a rat anti-mouse RANKL antibody (76mg/kg/wk) in the overreactivated Swiss-Webster mouse. The antiRANKL-treated mice had a significant attenuation of bone loss (p<0.001) compared to the overreactivated and sham-operated groups and bone mineral density (BMD) was significantly higher compared to the group treated with the standard bisphosphonate, alendronate sodium (p=0.004, positive control). A repeat study using three doses of antiRANKL (0.76, 7.6 and 76mg/kg/wk) showed a dose response effect. The two highest doses were as effective as alendronate sodium in maintaining bone mass. Interestingly, the antiRANKL-treated mice also had a significantly higher cortical bone density and cortical area was maintained; an effect that is not observed with standard bisphosphonate therapies. Biomechanical testing demonstrated that the lumbar (L5/L6) vertebrae in the group treated with the highest dose of antiRANKL were able to absorb 13% more load than did other groups (p=0.002). These data suggest that antiRANKL therapy may have an anabolic affect on cortical bone and further studies are warranted to determine the effects on senescence-induced cortical bone loss.

Humanization of the CCR3 gene in mice: generation of an animal model in which to evaluate the utility of human CCR3 antagonists


The chemokine receptor CCR3 has been demonstrated to be expressed on eosinophils (eos), mast cells, basophils, airway epithelial cells, and a subset of T helper cells. The predominance of CCR3 on cells intimately involved in allergic inflammation has led to the hypothesis that blocking this chemokine receptor might provide a new therapeutic approach to allergic disorders. The availability of CCR3 in small animal models has been hindered by lack of potent cross-reactivity of candidate compounds to the mouse receptor. The generation of mice expressing the human, rather than the murine CCR3 gene product would allow for in vivo evaluation of human CCR3 antagonists in pharmacodynamic and airway disease models. To this end, the mouse CCR3 coding region was replaced with human CCR3 sequences by targeted gene replacement in ES cells. After crossing the resultant hCCCR3 Knock-In mice to an IL2 transgenic line, expression of the human CCR3 on eos derived from these mice was confirmed by flow cytometry and TaqMan analysis. Murine eos expressing the human CCR3 gene were evaluated for their ability to respond to CCR3 ligands in chemotaxis assays, and for sensitivity to human CCR3 selective antagonists.

Role of activating FcrRII and inhibiting FcRRIIB during immune complex mediated arthritis

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We investigated the role of activating FcRRII and inhibiting FcRRIIB in inflammation and cartilage damage during immune complex arthritis (ICA). ICA was induced in knee joints of FcRRII and FcRRIIB knockout mice. Joint swelling at day 1 and 3 after ICA induction was significantly reduced in FcRRIIB-/- compared to wildtype controls. Histology of inflamed and exsudate showed a recruitment of respectively 50 and 50% in joint inflammation at day 3 after ICA induction. Moreover also proteoglycan depletion and matrix metalloproteinases (MMP)-mediated cartilage destruction were respectively 70 and 100% lower. In FcRRII+, an increase of 75-100% of inflammatory cell mass was found. Proteoglycan depletion and MMP-mediated destruction were both elevated with 40% at day 3 and 80% at day 7 compared to controls. These results indicate that coordinate expression of activating FcRRII and inhibiting FcRRIIB determines joint inflammation and cartilage destruction during arthritis induced by merely immune complexes.

Kinin B1 receptor antagonists: a novel approach in the treatment of diabetic hyperalgesia

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Insulin-dependent diabetes mellitus is an inflammatory autoimmune disease associated with vascular permeability changes leading to many complications. The B1 kinin receptors were recently found to be upregulated during the development of the diabetes and to be involved in its complications. In the present study, we studied the effect of the selective kinin B1 receptor antagonist DBK (des-Arg9-BK) and antagonists R-715 (Ac-Lys3-D-Asp9-NH2, des-Arg9-BK) and R-954 (Ac-Ome1-[Lys3, D-Asp9] des-Arg9-BK) on diabetic hyperalgesia. Diabetes was induced in male CD-1 mice using streptozotocin and the nociception was assessed using the hot plate test. Our results showed that induction of diabetes provoked a marked hyperalgesia in diabetic mice. Following acute treatment with R-715 (400 μg/Kg, s.p.) and R-954 (200 μg/Kg, s.p.), the hot plate latencies of diabetic mice returned to the normal level. In addition, acute administration of DBK (400 μg/Kg, s.p.) significantly potentiated diabetes-induced hyperalgesia, an effect that was totally reversed by both R-715 (2.5 mg/Kg, s.p.) and R-954 (1.6 mg/Kg, i.p.). These data provide evidence for the implication of the kinin B1 receptors in the development of hyperalgesia associated with diabetes and suggest a novel approach in the treatment of diabetic complications using kinin B1 receptor antagonists. (Supported by the CIHR).
A20 Systemic gene-therapy with the soluble form of IL-1RAp ameliorates collagen-induced arthritis (CIA)  
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IL-1 receptor accessory protein (IL-1RAp) is an essential component of the IL-1 receptor complex. The murine liver expresses a soluble form (sIL-1RAp), derived from alternative splicing of the gene, with unknown function. We developed an adenovirus with RGD modified short hairpin for the expression of the sIL-1RAp (Ad5/full-RGDD) to evaluate its effect on CIA and compared it with IL-1Ra (+) control and IL-1Ra (-) control. Viruses (3x106 PFU) were injected intravenously 3 days after the booster and 14 days thereafter mice were scored for CIA. 

<table>
<thead>
<tr>
<th>Activity score/Incidence</th>
<th>Ad5CMLV-1Ra</th>
<th>Ad5/full-RGDD</th>
<th>Ad5CMLV-1Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hind paw</td>
<td>0.28 ± 0.25</td>
<td>0.45 ± 0.26</td>
<td>0.33 ± 0.19</td>
</tr>
<tr>
<td>55%</td>
<td>44%</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td>Knee joint</td>
<td>0.69 ± 0.15</td>
<td>0.25 ± 0.12</td>
<td>0.13 ± 0.09</td>
</tr>
<tr>
<td>81%</td>
<td>22%</td>
<td>17%</td>
<td></td>
</tr>
</tbody>
</table>

No differences were found in humoral immunity against collagen-type II between the groups. This showed that sIL-1RAp can act as an inhibitor in experimental arthritis, almost as good as IL-1Ra, and it suggests that modulating IL-1 activity in the biological function of sIL-1RAp in vivo.

A23 Blood levels of type II collagen cross-linked peptides can monitor joint protection in the adjuvant arthritis rat  
Simon Blake1, Barbara Swift1, Lynne Atley2, David Eyre3, Shing Mei Hwang3, Peter Liang3, Lauren Darr1, George Strop3, Alison Badger3, Michael Lask3  
1GlaxoSmithKline Pharmaceuticals, King of Prussia, PA; 2Osteopathic Research Labs, University of Washington, Seattle, WA  
Matrix breakdown and loss of articular cartilage are hallmarks of the chronic joint diseases rheumatoid arthritis (RA) and osteoarthritis (OA). Validated biochemical markers are needed that can monitor this process and aid in prognosis and provide a surrogate index of response to therapy. One candidate marker is the C-telopeptide cross-linking domain of type II collagen (ColIICTX). Using a specific ELISA for ColIICTX we first demonstrated significantly elevated circulating levels of this marker during the chronic inflammatory phase of adjuvant-induced arthritis (AA) in the rat. Administration of the p38 mitogen activated protein kinase inhibitor SB-242235, 1,4-piperazinediyl-4-[4-(fluorophenyl)-5-[2-methoxy-4-pyrimidinyl]imidazol, resulted in a significant reduction of circulating ColIICTX in arthritic rats. A protective effect of the inhibitor on cartilage matrix was confirmed by histology. These data support the utility of measuring ColIICTX levels to monitor disease activity and assess therapeutic effectiveness in diseases in which cartilage matrix is destroyed.

A24 A selective inhibitor of IκB kinase shows efficacy in a mouse model of collagen-induced arthritis  
Kathleen M. Gillooly, David J. Shuster, Yuping Qiu, Christopher Zusi, Donna M. Dambach, Kim W. McIntyre, James Burke  
We have identified a novel, potent, and selective inhibitor of IκB kinase in vitro. Compound 1 was assessed in a mouse model of collagen-induced arthritis (CIA), a model for human rheumatoid arthritis. DIIA/LacI mice were immunized with bovine type II collagen at the base of the tail on day 0 and on day 40. Compound 1 was administered in a "preventative" mode, starting on day 0 through day 42 of the study. After day 21, the mice were assessed visually 3 times a week for clinical signs of disease. Compared to vehicle, compound 1 demonstrated a significant reduction in both disease incidence as well as disease severity. In addition, mice treated with compound 1 maintained body weight throughout the study. Weekly caliper measurements were taken of all experimental groups beginning on day 21. Mice treated with compound 1 showed a significant reduction in paw thickness. Therefore, in a "preventative" dosing mode in CIA, compound 1 demonstrated significant efficacy. Compound 1 was then evaluated in an "established" mode of CIA. In this study, mice were monitored for symptoms of disease and randomized into treatment groups when swelling in one paw was evident. Treatment continued for 14 days following disease onset. Mice treated with compound 1 showed a significant decrease in paw thickness as well as in number of paws pawed compared to mice treated with vehicle. Furthermore, compound 1-treated mice displayed a significant decrease in paw thickness while maintaining body weight during treatment period. In conclusion, these results demonstrate that a novel, potent, and selective inhibitor of IκB kinase is efficacious in a chronic model of arthritis disease in the mouse.
A25
Identification of a disease modifying IKK2 Inhibitor in rat adjuvant arthritis

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The transcription factor NF-B regulates the expression of various target genes such as cytokines, chemokines, cell adhesion molecules, and proinflammatory enzymes. Many of these target genes have been implicated in the progression of rheumatoid arthritis. A small molecule, SPC39, has been identified as an ATP-competitive inhibitor of IKK2 with an IC50=62 nM in vitro. SPC39 has a greater than 10 fold selectivity against a panel of protein kinases and dose-dependently inhibits ICAM-1 and VCAM expression in HUVeC cells with an IC50=1.5 nM. In stimulated Jurkat or THP-1 cells, SPC39 decreased secreted TNF-alpha levels, IL-1α, IL-2, and IL-8 levels in a dose-dependent manner. Furthermore, LPS-stimulated plasma TNF-alpha levels were reduced by 89% after oral administration of SPC39 (30 mg/kg) to rats in a non-bleachable assay. In a rat model of rheumatoid arthritis (developed adjuvant arthritis), SPC39 was shown to be disease modifying. Oral dosing of SPC39 resulted in a reduced mean paw edema of 48% and 73% at 30 mg/kg/day and 100 mg/kg/day, respectively, by day 28. Radiographic analysis revealed a near complete protection of bone erosion at the 100 mg/kg/day dose level. Histological evaluation of the contralateral paws supported the radiographic observations. The compound was well tolerated throughout the course of dosing (days 4-28). These data suggest that SPC39 or related IKK2 inhibitors, may have therapeutic benefit in rheumatoid arthritis.

A26
Characterization of nuclear factor eB(NF-eB) Activation in the Marine Adapative Transfer Model of Inflammatory Bowel Disease

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Inflammatory Bowel Disease (IBD) is characterized by inflammatory cell infiltration of the gut and pro-inflammatory cytokine production. NF-eB is a transcriptional regulator of many chemokine and cytokine genes which is activated in biopsies of IBD patients. To study the role of NF-eB in colitis we performed time course experiments in a murine adaptive transfer model of IBD. Immunocompromised C57BL/6 SCID mice were injected i.v. with naïve T cells from B10.D2 donors followed by i.p. injection of staphylococcal enterotoxin B 24 hours later. Mice were evaluated for weight change and absolute granulocyte number, as well as NF-eB activation and cytokine production. The number of cells. The number of cells. The number of cells. The number of cells. The number of cells. The number of cells. The number of cells. The number of cells. The number of cells.

A27
Pharmacological profile of DPC 333, a selective tissue kinase

Richard Liu*, Ron Magolda, Robert Newton, James Daun, Kris Vaidi, Tom Madhusuki, Mingxin Qian, Robert Collins, Tracy Tayer, John Giannaras, Sherrill Nurnberg, Paul Strzemieniak, Maryanne Covington, Steven Friedman, Carl Decicco, and James Traasjord

Inflammatory Diseases Research, Bristol-Myers Squibb Company, Wilmington, DE 19880

Tumor necrosis factor-alpha converting enzyme (TACE) is believed to play an important role in inflammatory arthritis disorders. In this report, we describe the in vitro and in vivo characterization of a TACE inhibitor, DPC 333. This molecule possesses selectivity of >100 fold for TACE over several other MMPs including MMP-1, -2, -7, -8, -9, -10, -13, -14, -15, -16 as well as ADAM family members including ADAM-9, ADAM-10, ADAM-TS1, and ADAM-TS5. DPC 333 is a competitive inhibitor for TACE with a Ki for the enzyme-inhibitor complex of 1 x 10⁻⁸ M. First released orally, DPC 333 inhibits soluble TNF-alpha secretion that follows LPS challenge in mice with an ED50 of 5 mg/kg. Treatment of collagen-induced arthritis mice with DPC 333 resulted in a significant reduction in the clinical signs of arthritis in a manner similar to that achieved by the soluble TNF-alpha receptor, taceasept.

A29
Cyclic urea inhibitors of p38 kinase: design, synthesis, and pharmacology of 2-nox-3,4-dihydroxylurea, and 3,4-dihydro-1H-pyrimidine[4,5-d]pyrimidin-2-one inhibitors of p38 MAP kinase

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Four new classes of potent p38 inhibitors exemplified by structures A, B, C, and D were designed and prepared in succession (A→B→C→D). An iterative approach, involving structure based design, synthesis, biological assay, and structure refinement afforded significant improvements in potency and pharmacological profile of the successor inhibitor classes.

A30
A highly-selective inhibitor of IκB kinase (IKK) with potent anti-inflammatory and immunosuppressive activities

James Burke*, Mark Pattoli, Violetta Iotova, Kurt Gregor, John MacMaster, David Shuster, Kathleen Gilliozzy, Kim McIntyre, Donna Dambach, Laura Miller, Karen Barry, Jennifer Forstlaker, Robert Townsend, Patrick Brasili, Valerie Bidgal, Yaping Qiu, and Christopher Zari

Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543 and Wallingford, CT 06492

NF-κB regulates the transcription of numerous pro-inflammatory genes including cytokines, cell adhesion molecules, and enzymes. An essential step in the receptor-stimulated activation of NF-κB is the IκB kinase (IKK)-catalyzed phosphorylation of IκB. Compound 1 is a potent and highly-selective inhibitor of IκK and shows 100% oral bioavailability in A549. Consistent with the central role of NF-κB-dependent proteins in immunological and inflammatory conditions, compound 1 showed striking activity in preventing signs of inflammation, both clinically and histologically, in models of arthritis, inflammatory bowel disease, and lung inflammation. In addition, the compound was active in a heart transplant model when given in combination with other agents.
Identification of a small molecule inhibitor of IkB kinase $\beta$ (IKK-$\beta$)
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GlaxoSmithKline, King of Prussia, PA 19406

Nuclear factor $\kappa B$ (NF-$\kappa B$) is a key transcriptional regulator of several pro-
inflammatory mediators associated with acute and chronic inflammatory disorders. IkB kinase $\beta$ (IKK-$\beta$) is responsible for phosphorylation of IkBa, and subsequent NF-$\kappa B$ activation by the IkB kinase signalling cascade, in response to pro-inflammatory stimuli. Using a kinase scintillation proximity assay, we have identified a 2-aminothio-
ephene analog as an inhibitor of IKK-$\beta$ (IC$\text{_{50}}$=1$\mu$M). Exposure of human primary synovial fibroblasts to the analog, resulted in a concentration-dependent inhibition of IL-1$\beta$ induced IL-8 (IC$\text{_{50}}$=2.6$\mu$M) and PGF2$\alpha$ (IC$\text{_{50}}$=3$\mu$M) production. In addition, treatment with the 2-aminothioephene analog resulted in a dose-dependent inhibition of carrageenan-induced paw edema, and a corresponding decrease in tissue cytokine (IL-6, TNF$\alpha$) levels. Western analysis of cytosols from paw tissue showed a blockade of IkBa degradation, and gel-shift assays of nuclear fractions, demonstrated a reduction in NF-$\kappa B$ nuclear binding. Together, these data provide further rationale for the development of IKK-$\beta$ inhibitors as novel anti-inflammatory agents.

Synthesis and biological evaluation of BIRR 796 analogs as potent inhibitors of p38 MAP kinase
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Departments of Medicinal Chemistry and Biology, Boehringer Ingelheim Pharmaceuticals, Inc., Research and Development Center, 900 Ridgebury Rd. Ridgefield, CT 06877

BIRR 796.1-(3-tert-Butyl-2-p-tolyth-2H-pyrazol-3-yli)-3-[4-(2-morpholin-4-y-ethyl)oxy]-naphthalene-1-sulfonic acid, is a potent, selective and orally active inhibitor of p38 MAP kinase and TNF$\alpha$ production, is currently in Phase II clinical trials for the treatment of rheumatoid arthritis, Crohn’s disease and psoriasis. The compound inhibits p38 by occupying a novel binding site, as well as by occupying the ATP and kinase specificity pockets. In the ATP pocket, a key hydrogen bond is established between the oxygen atom of the morpholine and NH of Met109. The synthesis and structure-activity relationship for a series of BIRR 796 analogs is presented. Changes to the hydrogen-bond acceptor heterocycle, the nature and length of its linker to the naphthalene core, and the nature of the groups appended to the pyrazole moiety, are investigated with an aim to improve potency and physicochemical properties.

A role for Bcl-3, an I$\kappa$B family member, in inflammatory disease
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Dept. Med., Pharm. and Toxic., Dartmouth Medical School, Hanover, NH

Objective: To study the role of Bcl-3 in chondrocytes to determine if this regulator of NF-$\kappa B$ function alters expression of matrix metalloproteases (MMP) and the risk of cartilage degradation enzymes implicated in the development of rheumatoid arthritis.

Materials and Methods: MMP-1 mRNA expression was assayed in SW-1353 chondrocytes that were stimulated with IL-1$\beta$ or were constitutively expressing Bcl-3. In SW-1353 cells, Bcl-3 expression was blocked with antisense oligonucleotides and IL-1 induced MMP-1 mRNA was assayed. MMP-1 and KB promoter constructs were transfected into SW-1353 cells, along with a Bcl-3 expression construct. Results: Microarray and real time RT-PCR indicated that IL-1 induces Bcl-3 in SW-1353 cells. Exogenous expression of Bcl-3 stimulated the endogenous MMP-1 gene, and antisense to Bcl-3 decreased IL-1$\beta$ induced MMP-1 expression. Co-transfection of Bcl-3 with KB and Bcl-3 promoter constructs indicated that Bcl-3 works, partly, through the NF-$\kappa B$ pathway.

Conclusion: Our findings implicate Bcl-3 as an important contributor to chronic inflammatory disease states, such as rheumatoid arthritis, through the induction of MMP-1.

Efficacy of a novel JNK inhibitor in reducing lung inflammation
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Sercorino Pharmaceutical Research Institute
14, Chemin des Aulx 1228, Plan-les-Ouates, Geneva, Switzerland

The c-Jun N-terminal Kinase (JNK) signalling pathway plays a key role in the activation of transcription factors such as c-Jun and ATF2 in various cell types, which in turn regulate diverse biological processes. A new, potent and selective JNK inhibitor (SNK3, K=50nM) has been discovered which shows a broad activity in treatment of diverse inflammatory diseases. In vitro this low molecular weight molecule presents an effective anti-inflammatory profile. The activity of this compound has been further investigated in vivo, in animal models of lung inflammation. The results that will be presented demonstrate the efficacy of the inhibitor in reducing the production of proinflammatory cytokines, the recruitment of inflammatory cells in bronchoalveolar lavage fluid and the development of lung histopathology evaluated by different markers. Altogether, these data establish the therapeutic potential of this JNK inhibitor in the treatment of inflammatory lung diseases.

Gene expression analysis as a tool to define the mechanism of action of novel steroids
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Steroids define a class of structurally related compounds with known pharmacologies and assayable cellular effects. We have studied the relationship between the biological actions of a steroid and its effect upon cellular gene expression. Our aim was to assess the concept of functionally categorising steroidal molecules by their gene expression pattern. To understand the full range of compound effects and to obtain a comprehensive fingerprint, gene expression was studied at multiple concentrations and at multiple time points after compound addition using fluorescence-based microarrays.

We describe the microarray technology, steroid pharmacologies and statistical analysis of the data. We demonstrate that using compound-treated cell lines, a characteristic and reproducible gene expression 'fingerprint' can be identified that is indicative of glucocorticoid receptor-mediated transcriptional modulation. In addition, we show that gene expression profiles can be used to discriminate between steroids that have different pharmacological effects. The potential application of this technology to drug discovery is discussed.

Control of the p38 pathway with small-molecule inhibitors of the upstream activators MKK3 and MKK6
Cleveland Signal Research Division, San Diego, CA 92121

The activity of the p38 MAP kinase is greatly increased in response to a wide variety of inflammatory stimuli and is implicated in a number of inflammatory conditions. However, the physiological role of the p38 MAP kinase signalling pathway is not yet completely understood. The p38 signalling cascade includes MKK3 and MKK6, which are responsible for activating p38, the target of the small molecule inhibitors. Since several p38 inhibitors have encountered obstacles in the clinic, we have undertaken an alternative strategy to discover inhibitors of these upstream kinases. High-throughput screening led to the identification of structurally and functionally distinct small-molecule inhibitors of MKK3 and MKK6. In particular, we have identified compounds which inhibit both MKK3 and MKK6 as well as compounds which inhibit only MKK3 or MKK6. Some of these compounds are competitive with ATP substrate, as expected, whereas other compounds are not ATP-competitive. Kinetic data indicate that some of the compounds behave as competitive inhibitors with respect to protein (p38) substrate. We have optimized the potency and selectivity of these series and have achieved activity in vitro against MKK inhibition in the nM range. Compounds are active in inhibiting TNF production in cell models for inflammatory responses, and testing of selected compounds in animal models of acute inflammation is underway.
A37

The NF-kappaB pathway: 2 sides to every story

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Laboratory of Gene Regulation and Signal Transduction, Dept. of Pharmacology, University of California San Diego, USA.

Aims. The NF-kappaB signalling pathway has a pivotal role in the regulation of immune and inflammatory responses and has attracted much interest as a novel target in inflammatory disease. Data shown here describes a role for NF-kappaB in the resolution of inflammation through the promotion of leukocyte apoptosis. Methods. We have used models of acute resolving inflammation to study the role of NF-kappaB in the initiation and progression of inflammation in vivo. Pharmacological agents were used to modulate NF-kappaB activation and determine effects on the cellular kinetics and mediators of the inflammatory response. Results. NF-kappaB activation in leukocytes recruited in the inflammatory response in vivo is associated with the resolution of inflammation and leukocyte apoptosis. Prophylactic inhibition of NF-kappaB attenuated the inflammatory response and pro-inflammatory gene expression, however therapeutic inhibition of NF-kappaB prevented the proper resolution of inflammation and delayed leukocyte apoptosis. Conversely, this anti-inflammatory NF-kappaB pathway was promoted by cyclopentenone prostanoids (cycPGs). Conclusions. NF-kappaB may have a novel anti-inflammatory role in the resolution of inflammation through the promotion of leukocyte apoptosis. CycPGs and their derivatives may represent novel selective agents for the inhibition of pro-inflammatory NF-kappaB activation that promote resolution of inflammation in vivo.

A40

Mechanism of inhibition of P38 map kinase by BIRB 796, a new clinical candidate

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The p38 MAP kinase plays a crucial role in regulating the production of several important pro-inflammatory cytokines (e.g., TNF-α and IL-1β). Blocking p38 MAP kinase may offer a novel and attractive therapy for treating arthritis and other inflammatory diseases. Here we report the identification of a new class of pyrazole naphthyl urea based compounds that block p38 kinase activity as well as p38 activation. These compounds demonstrate potency in cell-based assays of cytokine production and efficacy in animal models of cytokine production and arthritis. The mechanism and biological profile of a clinical development candidate, BIRB 796, will be described in detail.

A41

Anti-inflammatory properties of integrin-linked kinase inhibitors

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Integrin-linked kinase (ILK) is a ser/thr kinase coupling integrins and growth factor receptors to signaling pathways involved in cell survival and migration. Via its downstream targets protein kinase B (PKB/Akt) and glycogen synthase kinase-3β (GSK-3β), overexpression of ILK causes anchorage independent cell growth, increased cell cycle progression, and increased transcriptional activation of NFκB. NFκB mediates the production of pro-inflammatory agents IL-6, TNF-α, and nitric oxide (NO). Thus, we predict that inhibitors of ILK could have anti-inflammatory applications.

Kinetics has discovered a number of potent and selective ILK inhibitors and evaluated them in cell models of inflammation. In activated macrophages ILK inhibitors block the activation of NFκB in response to LPS and IFNγ. These effects are mediated by decreased activation of NFκB and not cell toxicity. ILK inhibitors also suppress production of NO by decreasing expression of inducible nitric oxide synthase (iNOS). ILK inhibitors also reduce macrophage production of IL-6 and TNF-α and decrease T-cell and endothelial cell proliferation. These findings suggest a role for ILK inhibitors as anti-inflammatory agents.

A42

Epstein-barr virus immediate-early protein BZLF-1 interfere with transcription factors NF-κB and cREB to suppress PGE2 synthesis

Marin Savaste*, Louis Flamand2 and Jean Gosselin
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Impairment of phagocytes functions is a major strategy used by viral and bacterial pathogens to weaken the host's first line of defense and promote the spread of infection. We previously demonstrated that Epstein-Barr virus (EBV) infects and modulates several cellular functions of monocytes such as cytokines synthesis and eicosanoids production. Further investigations revealed that EBV immediate-early protein BZLF-1 is able to interfere with the activity of the transcription factors NF-κB and CREB. In this study, we postulated that this EBV immediate-early protein BZLF-1 may interfere with the activity of the transcription factors NF-κB and CREB. Our results showed that there is a decrease in the expression of these transcription factors in response to BZLF-1.
A43 Evaluating the NF-κB pathway as an important therapeutic target for rheumatoid arthritis
Anneli Savinainen*, Kyla Egger, Danyi Wen, Laurie Salib, Jennifer Morgan, Yajuan Xia, Nicole Avishal, Rebecca Mosher, Karen Anderson, Bruce Jaffe, and Lisa Schoepf
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Signaling through NFκB plays a central role in the regulation of many immune and inflammatory processes including rheumatoid arthritis (RA). NFκB has been shown to be highly activated in the synovium of patients with RA. We now have confirmed that this activation occurs in the joints of rats using both adjuvant- and streptococcal cell wall-induced arthritis models. We have also detected the expression of NFκB regulated genes such as TNFα, IL-6, IL-1β and ICAM in these arthritic joints. The mRNA levels of these genes were reduced when arthritic rats are treated with methotrexate, a drug commonly used for the treatment of RA. This result suggests the importance of NFκB regulation in this disease and in its treatment. In addition, we evaluated the histology of joints from arthritic rats every third day over the course of 3 weeks to determine when bone and cartilage erosion begins and to characterize the severity of inflammation and synovioocyte hyperplasia. We also purified nuclear extracts from joint tissue over time and determined that NFκB activation precedes swelling. Furthermore, our kinetic analysis included cytokine expression profiling in the joint and plasma, draining lymph node, and spleen. We now are examining novel therapies for the treatment of RA, which specifically target the NFκB pathway.

A44 Upregulation of the dual-specificity phosphatase MKP-7 mRNA in TNFα-stimulated lung microvascular endothelial cells (HMVEC)
Lung Microvascular Endothelial Cells (HMVEC) Gordon Toddendorf*, Dana Banas and Suzanne J. Suchard
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Endothelial cells orchestrate the transmigration of leukocytes into tissue in response to injury or inflammation. The endothelium is exquisitely sensitive to activation by TNFα, a mediator associated with inflammation. To identify TNFα-responsive HMVEC genes, we constructed subtraction libraries using RNA from HMVEC exposed to TNFα for varying times. One of the genes identified encodes for the dual-specificity phosphatase MKP-7. The increased expression of MKP-7 mRNA closely paralleled the expression of E-selectin mRNA. Specifically, MKP-7 mRNA levels increased within 1 h of TNFα treatment, with peak expression by 6 h and elevated expression still observed at 24 h. We have expressed this protein and demonstrated phosphatase activity both in a full-length and truncated construct. MKP-7 mRNA is expressed in adenoid gland, testis, bone marrow, thymus, liver and osteoblasts, and protein is highly expressed in myeloid cells, lung tissue and bone articular chondrocytes, as well as osteoblasts found in degenerative arthritis. Published data suggests that this phosphatase acts on JNK and p38 but its role in inflammation is currently unresolved.

A45 Receptor density dictates the behavior of a subset of steroid ligands in glucocorticoid receptor-mediated transpression
QiHong Zhao*, Jian Pang, Margaret Favata, and James M. Traske
Bristol-Myers Squibb Company, Inflammatory Biology, Wilmington, DE 19880
To study the effects of receptor density on the behavior of steroids in glucocorticoid receptor (GR)-mediated transpression, we coexpressed GR with several luciferase reporters (e.g. TNFα (-1311)-promoter luciferase) in GR-deficient Cos-7 cells. Our results show that changes in both potency and efficacy for GR full agonists (e.g. dexamethasone) in transpression are proportional to the changes of GR expression levels (density). Intriguingly, receptor density dramatically influenced the behavior of the GR agonist RU486 and GR agonist medroxyprogesterone acetate (MPA). At high receptor density, both MPA and RU486 behaved as full agonists in transpression. Reducing GR density, however, resulted in conversion of these ligands from full agonist to full antagonists in transpression. Notably, this effect appears to be stimulus- and promoter context-independent. In contrast, varying GR density could not convert cortisol and budesonide from GR agonists to antagonists. These results have clearly demonstrated, for the first time, an effect of receptor density on the agonist and antagonist properties of RU486 and MPA in GR-mediated transpression.

A46 Differential effects of PR-A versus PR-B on the pharmacological properties of progesterins and antiprogestins
QiHong Zhao*, Jian Pang, Margaret Favata, and James M. Traskos
Bristol-Myers Squibb, Inflammatory Biology, Wilmington, DE 19880
The mechanisms governing tissue- and/or cell type-dependent pharmacological properties of progesterins (R5020 and progesterone) and antiprogestins (RU486) are poorly understood. To investigate the effects of PR-A versus PR-B on progesterin or anti-progesterin-dependent transactivation and transpression, we coexpressed either PR-A or PR-B with several luciferase reporters in Cos-7 cells. In this report, we show that, although only PR-B can mediate progesterin-dependent transactivation, both PR-A and PR-B can mediate progesterin-dependent transpression. Interestingly, progesterins appeared to exhibit higher efficiency and lower potency in transpression mediated by PR-A than that by PR-B. Surprisingly, the anti-progesterin RU486 is far more potent than progesterin in PR-A-mediated transpression, but not so in PR-B-mediated transactivation. This suggests that PR-A and PR-B differentially affect the pharmacological properties of progesterins and anti-progesterins not only in transactivation but also in transpression. Therefore, these results account, at least in part, for the tissue- and/or cell type-dependent pharmacological behavior of progesterins and antiprogestins.

A47 Development of a rapid assay to assess the effects of glucocorticoid analogs on the differentiation of human osteoblasts
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Glucocorticoids (GC) are used to treat inflammatory diseases, however, many serious side effects limit chronic use. The osteoblast (Ob) is a major target for bone side effects. One hypothesis is that GC stimulate differentiation and shrink the precusor Ob pool, leading to osteoporosis. To better describe this phenomenon we have studied effect of GC on the differentiation of cultured primary human Ob. Nodule formation and mineral deposition were assessed after 28 days of GC treatment in confluent Ob cultures by micrographic analysis with von Kossa or alkaline phosphatase staining. Cells treated with GC showed marked dose-dependent increase in mineral deposition. To facilitate assessment of GCs and new analogs, we developed a novel 96-well colorimetric assay to detect alkaline phosphatase activity in cultures after only 7 to 10 days of treatment and demonstrated correlation with the 28 day micrographic results. We have established a facile method predictive for Ob differentiation and, we propose, bone safety.

A48 Regulation of Sox9 in SW1353 chondrosarcoma cells
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Sox9 is a transcription factor that is a critical determinant of chondrogenesis, regulating the expression of Col2, Col11, and aggrecan. Mutations in a single Sox9 allele cause camptomelic dysplasia, a lethal syndrome affecting all cartilage derived tissues. To understand the role of Sox9 in cartilage maintenance and disease, we sought to identify a human cell line that models the Sox9 regulatory pathways identified in mice. Here we characterize the activity of Sox9 in SW1353 cells. Basal expression of a Col2 enhancer reporter construct was detectable and could be increased further by co-expression of a Sox9. As with primary murine chondrocytes, the basal expression of the Col2 enhancer could be stimulated by treating SW1353 cells with FGF-1 and -2 but not FGF-7. We make the novel observation that FGF-9 also regulates enhancer activity. We demonstrate that IL-1 and TNFα repress Col2 enhancer expression. We attribute the FGF and cytokine responses to an effect on Sox9 mRNA levels. These studies demonstrate the relevance of SW1353 cells for studying Sox9 signaling pathways.
A49 Characterization of SB-239063, a p38 MAP kinase inhibitor, in both T cell mediated and monocyte driven murine models of inflammation

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Cytokine Suppressing Anti-Inflammatory Drugs (CSAIDs) are a class of compounds shown to block stress-activated p38 MAP kinase activity in vitro and in vivo. CSAIDs inhibit production of proinflammatory cytokines such as IL-1β and TNF-α by affecting a stress induced kinase. In this study, we compared the activity of SB239063 (trans-1-(4-hydroxyxycyclohexyl)-4-cyclohexyl-5-oxo-2-thioxopyrimidine-4-ylimidazole), a p38 MAP kinase inhibitor, against dexamethasone, anti-murine-TNF-α monoclonal antibodies (mAb), and Enbrel, in both monocyte and T cell driven murine models of inflammation. SB-239063 significantly reduced TNF-α levels in serum and conferred complete protection against mortality in the LPS shock model, but was less effective in the T cell driven SEB shock model. Anti-TNF-α mAbs treatment was equally effective in LPS and SEB shock models, while Enbrel was slightly less effective. SB-239063 was able to inhibit ear edema and cytokine production in PMA induced ear inflammation, but not in the oxazolone DTH model, and anti-TNF-α mAbs were less effective in both models. In summary, as expected dexamethasone was highly effective in all animal models, while TNF-α mAbs was more effective in the shock models. Interestingly, SB-239063 worked best in the LPS and PMA models suggesting that p38 MAP kinase inhibitors may be more effective for disorders in which monocytes and/or TNF-α play a predominant role.

A50 cAMP-mediated inhibition of leukotriene and PAF biosynthesis in human neutrophils: reversal by arachidonic acid and lyso-PAF

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Leukotrienes (LT) and PAF are inflammatory lipid mediators involved in diseases such as asthma and rheumatoid arthritis. LT and PAF biosynthesis is initiated by the release of arachidonic acid (AA) and lyso-PAF by the cPLA₂. The incubation of human neutrophils (PMN) with arachidonic acid leads to the intracellular cAMP concentration ([cAMP]) such as adenosine, histamine and PGE₂ leads to the inhibition of both LT and PAF biosynthesis. The inhibitory effect of elevated [cAMP] on PAF and LT biosynthesis was prevented by inhibitors of PKA and was reversed by the addition of exogenous substances (AA and PAF). The inhibitory effect of elevated [cAMP] on lipid mediator biosynthesis also correlated with a decrease in the phosphorylation of p38 in activated PMN. These data suggest an inhibitory effect of PKA on p38 activation and on the signaling cascade involved in the activation of cPLA₂ leading to the inhibition of LT and PAF biosynthesis in PMA. Supported by the CIHR and the Arthritis Society of Canada

A51 Interferon-γ-inducible protein –10 induces interleukin-8 expression in PMBCs

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Interferon-γ-inducible protein-10 (IP-10) is a potent chemotactant for Th1/Tc1 T-cells, monocytes, and NK cells. These cells express the IP-10 receptor CXCR3. Recently, IP-10 has been shown to induce interferon-γ (IFN-γ) expression by peripheral blood mononuclear cells (PBMCs) (J Immunol 2001;166(4): 2750). This finding leads to the hypothesis that there is a greater role for IP-10, in addition to its chemotactic properties, in the regulation of the inflammatory response. Thus, we asked whether IP-10 could regulate the expression of other chemokines in PBMCs. PBMCs were isolated from healthy human donors and stimulated with IP-10 (1μg/mL). After 24 hours the supernatants were collected and tested via enzyme linked immunosorbent assay to determine the presence of a panel of cytokines and chemokines. A dose dependent increase in interleukin 8 (IL-8) was observed in biologically relevant quantities, however, the remainder of the panel was not significantly changed. These data suggest that IP-10 has a therapeutic role in the regulation of expression of chemokinin molecules in the inflammatory response to insult.

A52 Gingival tissue cyclooxygenase-2 gene expression is elevated in patients with chronic periodontitis

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The inducible isoform of cyclooxygenase (COX-2) may play an important role in the pathogenesis of chronic periodontitis. Objectives: The goal of this prospective case-control study was to investigate the role of COX-2 in periodontal inflammation. Methods: Clinical measures, crevicular fluid, and gingival biopsies were obtained from patients (n=16) with untreated moderate or severe chronic periodontitis (CP) and healthy volunteers without periodontitis (n=8). Biopsies from CP patients were from visibly inflamed sites (gingival index score (GI) of 2 or 3), while biopsy sites from healthy volunteers displayed little or no inflammation (GI of 0 or 1). COX-2 expression in gingival tissue was determined by reverse transcription-PCR (RT-PCR) and immunoblotting. Results: COX-2 mRNA was significantly elevated in CP tissue compared with that of healthy subjects (99 fg/μg vs. 28 fg/μg of total RNA; p<0.01). Immunoblotting of gingival tissue lysates from patients with CP revealed detectable COX-2 protein compared to undetectable levels of COX-2 protein in lysates from healthy gingiva. Also, crevicular fluid IL-1β was elevated nearly 3-fold in the CP versus non-diseased group (p<0.05). Conclusions: Taken together, these results suggest that COX-2 expression is elevated in gingival tissue and may play an important role in the treatment and/or prevention of periodontitis; and 3) measurement of COX-2 expression is a useful biomarker of the state of gingival inflammation.

A53 Leukotriene B4 (LTB4) plays an important role in the development of esophageal adenocarcinoma (EAC)

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Five-lipoxygenase (5-Lox) and leukotriene A4 hydrolase (LTA4H) were overexpressed in human EAC. Leukotriene B4 (LTB4), the arachidonic acid (AA) metabolite of the 5-Lox/LTA4H pathway, increased in the tumor samples versus the adjacent pathologically normal tissues. One LTB4 receptor, BLT1, was also overexpressed in EAC. We then studied the effect of LTB4, on a human EAC cell line (SEG-1) that expresses the two LTB4 receptors, BLT1 and BLT2. LTB4 (0.01-10μM) exerts the growth-stimulatory effect on the SEG-1 cells in a time- and dose-dependent manner. BLT1-specific agonist blocked the effect of LTB4. Kinase profiling indicated the activation of the ERK, JNK, p38 and p38 MAPK. In conclusion, these studies suggested that 5-Lox-mediated AA metabolism pathway may play an important role in the development of EAC, and could be a target for chemoprevention of EAC.

A54 Overexpression of 15-lipoxygenase in endothelial cells produces oxidative stress and apoptosis

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Linoleic acid (LA) induces oxidative stress, inflammatory responses and apoptosis in endothelial cells. 15-lipoxygenase (15-LO) acts on unsaturated fatty acids including LA to produce lipid hydroperoxides. We stably transfected ECV-304 cells with 15-LO (ECV-LO) to examine its effects on these responses. ECV-LO cells produced significant amounts of enzymatic activity, immunoreactive protein and mRNA in contrast to the parental line. LA exposure resulted in oxidative stress in ECV-LO cells, but not in the parental line or in the presence of non-15-LO substrate fatty acids. Inhibition of lipoxygenase activity inhibited oxidative stress. Exposure to LA also resulted in cell death only in ECV-LO cells. LA-induced cell death was time- and concentration-dependent, and was not observed with non-15-LO substrate fatty acids. The LA 15-LO reaction product 13-hydroperoxyoctadecadienoate and 13-hydroxyoctadecadienoate did not induce cell death in ECV-304 cells suggesting that intracellular production of the products is an important cellular effect. Demonstration of PARP cleavage and protection from cell death by zVAD-fmk propose a role for apoptosis in this process. We conclude that activation of 15-LO in endothelial-like cells promotes oxidative stress and apoptosis, which may impact atherogenesis.
A55 Role of leukotrienes in antimicrobial defense in pulmonary histoplasmosis

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Aim: To investigate the contribution of leukotrienes (LTs) to the protective immune response in pulmonary histoplasmosis. The lungs of mice infected with Histoplasma capsulatum yeast (5×10^3) and/or 100 μg of a histoplasma cell wall component, Lipid I (Lc) or Histoplasma (Hc) was evaluated. LT production was measured using the LT assay. The LTs were determined using the LT assay.

Results: LT production was significantly increased in the lungs of mice infected with Histoplasma capsulatum yeast (5×10^3) and/or 100 μg of a histoplasma cell wall component, Lipid I (Lc) or Histoplasma (Hc). The LT production was significantly increased in the lungs of mice infected with Histoplasma capsulatum yeast (5×10^3) and/or 100 μg of a histoplasma cell wall component, Lipid I (Lc) or Histoplasma (Hc).

Conclusion: LT production was significantly increased in the lungs of mice infected with Histoplasma capsulatum yeast (5×10^3) and/or 100 μg of a histoplasma cell wall component, Lipid I (Lc) or Histoplasma (Hc). The LT production was significantly increased in the lungs of mice infected with Histoplasma capsulatum yeast (5×10^3) and/or 100 μg of a histoplasma cell wall component, Lipid I (Lc) or Histoplasma (Hc).

A56 The discovery of potent human CCR5 antagonists based on a cyclopentane scaffold

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Since the discovery of CCR5, a 7-transmembrane receptor for the chemokines MIP-1α, MIP-1β, and RANTES, is a primary co-receptor with CD4 for cell entry of macrophage-tropic HIV-1 strains, CCR5 has also been implicated in asthma, rheumatoid arthritis, multiple sclerosis and transplant tissue rejection. Two years ago this laboratory disclosed the discovery of (2S)-1-(N-methyl-N-phenylisobutyryl)-2-methyl-2-phenyl-4-(4-(N-allyl-N-4-nitrobenzoyloxy)-2-benzylamino)pyperidin-1-ylbutane (1) having a CCR5 inhibitory activity of an IC50 of 1 μM in a 125I-MIP-1α binding assay and an IC50 of 3 μM in an HIV-1 infectivity assay. Cyclization of the butane chain of 1 into a cyclopentane scaffold and further modification of the 1-amino and piperidine substituents afforded compound 2 having a CCR5 IC50 binding affinity of 0.45 nM, good selectivity over other chemokine receptors, an IC50 <0.01 μM in the HIV-1 infectivity assay, and good oral absorption in three species. Based on these and other results, compound 2 was selected for further evaluation as a CCR5 antagonist safety assessment candidate.

Laura Meurer, Malcolm MacCoss,


A57 A role for cathepsin in atherosclerosis

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Cathepsin S (Cat S) is a cysteine protease known to be involved in extracellular matrix degradation and may serve a role in the progression of atherosclerotic lesions. Most cells also express Cathepin C (Cys C) which binds to and inhibits the activity of Cat S. Here, we show human macrophages exposed to oxidized LDL (Ox-LDL) increase intracellular levels of Cat S protein and mRNA without changing intracellular protein concentrations of Cys C. Exposure to Ox-LDL, but not acetylated-LDL, increases the ratio of mature Cat S to pro-Cat S secreted into the foam-cell conditioned media in a time and concentration dependent manner. Western blot analysis reveals an increased expression of Cat S in atherosclerotic lesions of Apo E-/- mice, and immunohistochemistry localizes Cat S expression around the shoulder of an atherosclerotic plaque and to vascular smooth muscle cells adjacent to and within the plaque. Oil Red O staining of serial sections suggests expression of Cat S is upregulated in the foam cells within the lesion. We also show that blockage of PDGF-stimulated human coronary artery smooth muscle cells from Matrigel. The collective data suggest that increased expression of Cat S in foam cells derived mature Cat S may aid in the remodeling of smooth muscle cells at the site of lesion while simultaneously contributing to the weakening of atherosclerotic plaques.

A58 N-desulfated non-anticoagulant heparin inhibits inflammation in vitro and in vivo

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Heparin, a highly sulfated proteoglycan, is known to have strong anticoagulant and anti-inflammatory activities. Here we report that compared to heparin, an N-desulfated heparin derivative had a 158-fold reduction of activated partial thromboplastin time. It inhibited activation of human procoagulant HL-60 cells to the stimulated human umbilical vein endothelial cells (HUVECs) under flow and prevented the transmigration of human neutrophils through the monolayers of the stimulated HUVECs. Further, intravenous administration of this compound attenuated the peritoneal infiltration of neutrophils in a mouse model of acute peritonitis. It also reduced tissue edema and leukocyte deposition in a rabbit ear model of ischemia and reperfusion injury. It is our best knowledge that this derivative of N-desulfated heparin has the lowest anticoagulant activity among low molecular weight heparin and chemically modified heparin derivatives while preserving a potent anti-inflammatory activity. These combined properties appear to suggest it as a safer medicine for treatment of inflammation.


A59 In vivo cartilage degradation induced by IL-18 gene transfer is mediated by IL-1

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Interleukin-18 is a member of the IL-1 family of proteins that exerts proinflammatory effects and induces cartilage destruction in vitro. The goal of the present study was to investigate whether IL-18 mediates joint destruction in vivo. To investigate whether IL-18 mediates joint destruction in vivo. To investigate whether IL-18 mediates joint destruction in vivo.

Histology, examined by day 4, 7, and 14 and revealed that local overexpression of IL-18 resulted in increased joint inflammation and cartilage destruction in C57BI/6 mice. Of high interest, IL-18 gene transfer in IL-18 mice did not show cartilage damage at the different time points, although joint inflammation was similar to wild type animals. Endogenous TNF was not involved in IL-18 induced joint pathology. The present study demonstrated that IL-18 induces joint inflammation independently of IL-1 and TNF. In addition, we showed that IL-1 generation, due to IL-18 expression was essential for marked cartilage degradation. These findings implicated that IL-18 contributed to cartilage destruction by induction of IL-1.

A 60 Effect of the marine manxamines, potent thromboxane B2 inhibitors, on human platelet thromboxane synthase

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The Manxamines (MZ) are marine alkaloids isolated from the marine Halichondria sp. sponges. Our study on the effect of 6 MZ analogs, namely MZa, MZb, MZc, MZd, MZe and MZf on thromboxane B2 generation by activated rat brain microglia demonstrated that MZa strongly inhibited TXB2 (IC50 = 0.1 μM) and O2(3P < 0.1 μM) with concomitant very low toxicity (LD50 >30 μM) ( Mayer et al. Immunology Res 55(5): S207, 2001). The purpose of the current investigation was to initiate mechanism of action studies with the MZ by determining the effect of MZa, MZb, MZc, MZd on PGH2-induced TXB2 generation in human platelets, a process catalyzed by the thromboxane A synthase. The release of TXB2 was determined by ELA. Although Fupeca (3,5-dipyridylmethyl)-2-benzofuranonocaproate, a thromboxane synthase inhibitor strongly inhibited PGH2-induced human platelet TXB2 generation, neither MZa, nor MZb, MZc or MZd were inhibitory. Our data thus appears to indicate that the mechanism by which the marine MZ potently inhibit brain microglia TXB2 and O2 does not involve inhibition of the thromboxane synthase. Furthermore, these results suggest extending our mechanism of action studies with the marine MZ to other enzymes involved in arachidonate release and metabolism in human platelets. Supported by Harbor Branch Oceanographic Institution and Midwestern University. U.S. and international patents filed 5/23/2000 and 3/24/2000, respectively.
The effects of cathepsin S inhibition on antigen presentation in human CD14/-
derived dendritic cells
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Cathespin S (cat S) is a member of the papain family of cysteine proteases, and has been shown to be expressed in resting or activated human antigen (Ag) presenting cells. It has been shown to be responsible minimally for the the final step of the proteolytic processing of invariant chain (Ii) of the MHC class II complex to the fragment CLIP, which is then released from class II. The consequences of cat S inhibition on HLA-DR expression at the cell surface and occupancy by CLIP were evaluated in CD14/-derived dendritic cells. HLA-DR surface expression was unaffected, but surface expression of CLIP-HLA-DR complexes was effectively inhibited by a representative compound. In addition, cat S inhibition reduced the ability of dendritic cells to effectively present Ag and stimulate autologous T cells, resulting in reduced T cell proliferation. IL-12, IL-2 and IFN-gamma production, T cell proliferation in tetanus toxoid stimulated PHMC and surface CLIP expression were all concomitantly reduced in the presence of a cat S inhibitor, consistent with the hypothesis that cat S plays a key role in the loading and presentation of Ag in human dendritic cells.

MIF regulation of proliferation, apoptosis and p53 in arthritis
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An imbalance between proliferation and apoptosis results in hyperplasia and joint damage in rheumatoid arthritis (RA). The tumour suppressor protein p53 acts as a cell cycle checkpoint in fibroblast-like synoviocytes (FLS), but factors regulating p53 in RA have not been identified. We investigated the role of macrophage migration inhibitory factor (MIF), a cytokine known to be involved in inflammatory signalling in RA, in regulating synovial hyperplasia and p53.

p53 mice exhibited an increase in severity of antigen-induced arthritis, associated with increased synovial apoptosis and increased synovial p53. FLS proliferation ex vivo was significantly reduced in p53-/mouse, p53 levels were increased in MIF-/- cells in vivo, and spontaneous and Na nitroprusside-induced apoptosis were significantly increased in p53-/fibroblasts. pMIF inhibited p53 expression, increased proliferation and inhibited apoptosis in RA FLS.

These results indicate a role for MIF in the regulation of synovial p53 expression and p53-mediated events including proliferation and apoptosis. Given that disease progression in RA depends on hyperplasia, small molecule antagonists of MIF (under development) could influence RA inflammation and also disease progression.

AMD-3100 and high-affinity SDF-1 peptide mutants: selective and potent CXCR4 antagonists in human lymphocytes and monocyes
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SDF-1o is highly expressed in synovial fibroblasts of patients with rheumatoid arthritis. A recent study using the CXCR4 antagonist, AMD-3100, supports an involvement of SDF-1 in a murine model of collagen-induced arthritis. Because of the basic nature of AMD-3100 and little data on its effects on inflammatory cells, we set out to determine its potency and selectivity in human leukocytes. In addition, two SDF-1 peptide mutants (aa5-14) from the literature were assessed. The affinity of these agents was measured in a Jurkat cell binding assay. The biological properties were determined in an SDF-1o-induced actin polymerization (AP) assay in isolated human lymphocytes, monocytes and neutrophils. AMD-3100, SDF-1o nonmonic mutant (L5YRYWFCR) and the dimer (dissulf-linker) inhibited [35S]SDF-1 binding with IC50's of 0.3, 0.7 and 0.16 uM, respectively, which are 10 - 50X less potent than SDF-1o's IC50 value. The three agents blocked SDF-1o (EC95)-induced AP responses with IC50's of 0.4, 0.7 and 0.14 uM, respectively. However, none of the agents antagonized the responses brought about by either LTBB, FMLP or MCP-1 (tested at EC95 concentration, IC50's =10 uM). Furthermore, we found no evidence of agonistic activities of the peptide mutant in the AP test. Similar potencies of these agents demonstrated in the binding and AP assay suggest their actions are mediated via CXCR4. We conclude that AMD-3100 and the peptide mutants are potent and selective antagonists of CXCR4.

The distinct effect of IL-1 and TNF-a on chondrocyte interleukin-1β and matrix metalloproteinase production
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Objective: To investigate the roles of IL-1 and TNF-a in chondrocyte IL-1β and MMP gene expression. Method: Human chondrosarcoma (SW1353) cells were stimulated with IL-1α, TNF-a, phorbol ester (PMA), IL-1a+PMA, or TNF-α+PMA. Intracellular chondrocyte IL-1β was measured by metabolic labeling or Western blot analysis. Extracellular chondrocyte IL-1β was measured by ELISA. MMP-1 and MMP-13 were detected using specific ELISA kits from R&D Systems. Results: (1) Chondrocyte IL-1β expression was induced by IL-1α, but not TNF-α; (2) Chondrocytes incubated simultaneously with PMA and TNF-α or IL-1α produced larger amounts (synergism) of prol-1β than from cells stimulated with PMA or IL-1a alone. (3) TNF-αand IL-1 were equally potent in inducing MMP-1 and MMP-13 production by SW1353 cells. However, only the induction of MMP-1 gene expression was synergistically enhanced by PMA. Conclusion and Discussion: Although IL-1 and TNF-a bind to distinct cellular receptors, both are potent activators of many known chondrocyte signaling pathways, including NFκB, AP-1, and p38. An unexpected finding is that even though both TNF-α and IL-1 can induce MMP-1 and MMP-13 gene expression in human chondrocytes, only IL-1 induces chondrocyte IL-1β production. Therefore, IL-1 may act in an autocrine and paracrine fashion to exacerbate cartilage destruction. IL-1 may be more important than TNF-a in the pathogenesis of rheumatoid arthritis and osteoarthritis.

A study of the effects of inhibiting cathepsin S (Cat S) in both mouse and human in vitro systems
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Cat S plays an important role in antigen presentation through cleavage of the invariant chain. Cat S is therefore a potential therapeutic target for inhibition of antigen presentation in the arthritic lung. We have studied the expression of Cat S and the effects of a Cat S inhibitor, 5-2-Morpholin-4-ylethoxybenzofuran-2-carboxylic Acid (3S)-3-Methyl-1-(3S)-3-cyclo-2-[2-(3-pyridin-2-yl)phenyl]-carboxylic acid (mesan-4-ylcarboxyloxy) butyamide (compound 1) in asthma relevant tissues and cells. By IHC, Cat S expression was found to be upregulated in asthmatic versus normal human lung sections, and in ova challenged versus normal mouse lung sections. Cat S expression was also detected in human blood derived APCs and mouse bone marrow derived DCs (BMDCs). Incubation of Mouse BMDCs in vitro with compound 1 resulted in the accumulation of SLIP, the substrate of Cat S. This correlated with downstream inhibition of T-cell proliferation. In an adoptive transfer model of allergic inflammation, treatment with compound 1 resulted in SLIP accumulation in cells of the spleen and lymph nodes, and a reduction in eosinophilia and lymph node CD4 counts. In human in vitro systems, as for mouse, we detected accumulation of SLIP when human blood derived APCs were incubated with compound 1. In conclusion, we are able to measure in vitro, inhibition of Cat S activity in both mouse and human cell based assays, and also in vivo mouse models.

A soluble form of the IL-1R accessory protein (ACp) enhances the ability of soluble IL-1R-II to inhibit IL-1
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Human IL-1β is able to bind a soluble form of human IL-1R-II with nanomolar affinity. The resulting ligand neutralization that occurs is an important component of the IL-1 system’s self-regulation and is also the basis for therapeutic intervention in inflammatory disease with recombinant soluble IL-1R-II. Pre-clinical studies in primates have shown that recombinant human soluble IL-1R-II is efficacious in an acute model of inflammation. Surprisingly, in vitro binding studies with primate IL-1β (reusus and cynomolgous species were used) revealed that they bind soluble IL-1R-II with an affinity three orders of magnitude lower than human IL-1β. These findings led to the hypothesis and subsequent discovery that a soluble form of ACp is able to form a ternary complex with, and to enhance the binding affinity between, IL-1 and soluble IL-1R-II. Soluble ACp is required to achieve functional inhibition of monkey IL-1β with soluble IL-1R-II. Additionally, the expression of soluble IL-1R-II for human IL-1β and IL-1α is significantly augmented in the presence of soluble ACp. We present evidence that a soluble form of the ACp protein exists at significant levels in the circulation of humans, mice, and monkeys (IL-1R-II values will be presented). A known splice variant appears to be the primary mechanism for its generation. Among the conclusions drawn from this study is the realization that soluble IL-1R-II may be a more thorough antagonist of the IL-1 system than previously believed, as a consequence of the enhanced binding affinity for human IL-1α that is provided by soluble ACp.
A 67

Design and synthesis of novel cathepsin S inhibitors
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The specificity of the immune response relies on processing of foreign proteins and presentation of antigenic peptides at the cell surface. Inhibition of antigen presentation, and the subsequent activation of T-cells, should, in theory, modulate the immune response. The cysteine protease cathepsin S provides a key step in antigen presentation and therefore represents an attractive target for inhibition. Herein, we report a series of potent and reversible cathepsin S inhibitors. These inhibitors show nanomolar inhibition of the target enzyme as well as cellular potency in a human B cell line. The X-ray crystal structure of a reversible inhibitor co-crystallized with Cathepsin S is also reported.

A 68

Identification of a potent and orally active non-peptide Cts receptor antagonist
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The asialoglycoprotein Cts is a potent chemoattractant factor for neutrophils and other leukocytes and functions as an important inflammatory mediator. Through a high capacity screening followed by chemical optimization, we identified a novel non-peptide Cts receptor antagonist, N[4-(4-

-dimethylaminoethyl)methyl]-N-[4-azepylphosphoryl]-7-methoxy-12,14-

tetrahydrocarboline-1-carboxamide (Compound 1). Compound 1 inhibited the binding of 3H-labeled Cts to human neutrophils with an IC50 value of 2.2 mM. Compound 1 also inhibited Cts-induced intracellular Ca2+ mobilizations, chemotaxis, and the generation of reactive super oxide species in human neutrophils with IC50 values of 3.1, 2.7, and 1.6 mM, respectively. In Cts-induced intracellular Ca2+ mobilization assays with human neutrophils, compound 1 did not show agonistic activity at up to 10 µM, and shifted rightward the concentration-response curves to Cts without depressing the maximal responses. Examination on the species specificity of compound 1 revealed that it was able to inhibit Cts-induced intracellular Ca2+ mobilizations in neutrophils of cynomolgus monkeys and Mongolian gerbils but not mice, rats, guinea pigs, rabbits, and dogs. In gerbils, compound 1 (3 to 30 mg/kg, p.o.) inhibited Cts-induced neutropenia in a dose-dependent manner. This is the first description of an orally active non-

peptide Cts receptor antagonist that could contribute to the treatment for inflammatory diseases mediated by Cts.

A 69

Mechanism of inactivation of progestinid H2 synthase 1 (PGH2-S-1) by retinoid and its analogs
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An in depth analysis of the inhibition of PGH2-S-1 by 3,5-dihydroxy-cane-stibene (retinoid) identified it as a mechanism based inhibitor. The irreversible loss of cyclooxygenase (cox) and peroxidase (perox) activity was accompanied by the oxidation of retinoid to yield the inactivator and had an absolute requirement for a peroxide substrate. Structure Activity Relationship (SAR) studies performed with two methoxy (OME) retinoid analogs identified that the 3 and 5 hydroxy groups were essential for inactivation (see Table). (Supported by AHA postdoctoral fellowship to L. Szewczuk)

Oxidation of IC50 perox IC50 cox Kp Compound

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<thead>
<tr>
<th>Retinoid</th>
<th>(µM)</th>
<th>(µM)</th>
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<tr>
<td>retinoid</td>
<td>2.8</td>
<td>67</td>
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<tr>
<td>4'-OME-retinoid</td>
<td>5.1</td>
<td>30</td>
</tr>
<tr>
<td>3,5-di-OME-retinoid</td>
<td>&gt;100</td>
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</tr>
</tbody>
</table>

Values are elevated because peroxide (PO2) must be enzyme generated

Presence of 300 µM hydrogen peroxide

A 70

Protein binding impacts the ability of small molecule MMP inhibitors to penetrate cartilage
J Tillier*, N Mollova, E Collins, M Geck, J Brooks, S Glasson, E Morris

Wyeth Research, Cambridge, Massachusetts

*We evaluated the ability of exogenous protein to decrease the potency of an MMP inhibitor in vitro and compared this to inhibitor concentrations within cultured bovine articular cartilage (BAC).

Methods: In vitro: Fluorescence resonance energy transfer (FRET) assays were carried out to determine IC50 values of the inhibitor against MMP-13 in the absence or presence of 25% human serum (HS) or fetal bovine serum (FBS) or 25µg/ml bovine serum albumin (BSA). Protein binding of the inhibitor was determined using a Biacore system. In vivo: FRET ICR was used to determine IC50 in HS, BSA, or BSA respectively when compared to media alone. Biacore analysis found the inhibitor 99% protein bound. Ex viv: Cartilage levels of inhibitor decreased 7.2 fold upon the addition of the inhibitor.

Conclusions: Protein binding limits the amount of inhibitor available for in vitro efficacy assays and for cartilage extracellular matrix penetration.

A 71

Differential pharmacology of the chemokine receptor, CXC4R4, in primary human mononuclear leukocytes, and in lymphoblastic cell lines

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2Discovery Technology Center, Cambridge, MA

To select a cell line with a CXC4R4 pharmacology most similar to that of primary human mononuclear leukocytes (HMLN), we have evaluated CXC4R4 expression and ligand affinity in Jurkat and Raji cells by conducting quantitative PCR, FACs analysis of PE-labeled anti-CXC4R4 (12G5) binding, and 125I-SDF-1 binding experiments. Tagman RT-PCR demonstrated the presence of mRNA for CXC4R4 and its splice variant, CXC4R4-3' in both Jurkat and Raji cell lines. Whereas PE-labeled 12G5 bound to both cell lines, Jurkat cells expressed a higher mean fluorescence intensity than Raji cells. To assess additional differences in CXC4R4 pharmacology, 125I-SDF-1 binding was used to evaluate receptor expression, chemokine selectivity, and ligand affinity. 125I-SDF-1 bound to both HMLN, and to human lymphoblastic cell lines with affinity that are capable of binding in Jurkat and Raji cells. Although both Jurkat and Raji cells have a similar density of 125I-SDF-1 binding sites, there were significant differences in Kd values (15 vs. 2 µM), inhibitory activity by 125I (Ki 6.4 vs. > 300 µM), chemokine selectivity (KC, lymphotactin, MCP-4, RANTES 1.2 µM in Jurkat, vs. inactive in Raji), and K for SDF-derived peptides. A correlation analysis using the Kd values for these agents suggests that binding activity in Jurkat cells may be more predictive of activity in HMLN than binding in Raji cells. In addition, characterization of 125I-SDF-1 binding on human primary lymphoblastic cell lines demonstrated differences in receptor binding affinity and selectivity that were not due to receptor variant expression.

A 72

Mapping enzymes and mediators in the inflammatory pain pathway
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St. Louis, MO 63167

Arthritis is a disease characterized by chronic inflammation of the joints and adjacent tissue inducing pain, fever and joint destruction. It has been shown that prostaglandins produced by COX-2 are important contributors to the signs and symptoms of arthritis. Recent studies in models of peripheral inflammatory pain indicate that central nervous system prostaglandin formation may participate in the development of the pain process. We utilized a model of chronic pain, adjuvant induced arthritis in the rat, to evaluate central and peripheral prostaglandin production, enzymes involved in their biosynthesis and the effect of COX-2 inhibitors. In this model, injection of Freund’s adjuvant into one paw results in severe arthritis in the contralateral paw commencing approximately 14 days later. A lowered threshold for mechanical pain (hyperalgesia) was present in the injected paw 1 day after adjuvant injection and was constant through the development of arthritis in the contralateral paw. In the non-injected paw, hyperalgesia was measurable after 14 days when the joint swelling was first observed. One day after adjuvant injection, marked increases in CSF levels of PGE2, PGI2, and 6keto-PGI2 were observed. After 14 days when arthritis was evident in the contralateral paw, the main prostaglandins in the CSF were PGE2, with small or no increase in PGD2, PGF2a, TXB2, and 6keto-PGF2a levels observed in all strains. Peaks of PG production were observed 1 day after arthritis in both mid-brain and spinal cord, correlating with increases in COX-2 and PGE2 mRNA levels and prostaglandins in these regions; no changes in COX-1 and PGD2 mRNA levels were found at any time point. Administration of COX inhibitors such as indomethacin and celecoxib to arthritic rats completely reversed hyperalgesia in the contralateral paw with an associated reduction of PG levels in the CNS. In the adjuvant arthritis model, arthritic rats for seven days with COX inhibitors or dexamethasone partially down regulated COX-2 and PGE2 mRNA levels in CNS tissue. These results further support a role for PGs at multiple points in the inflammatory pain pathway.
A 73
Characterization of cysteinyl leukotriene receptors
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Merk Research Laboratories, West Point, Pennsylvania 19486

The CysLT1 and CysLT2 receptors are 7-trans-membrane spanning proteins of 337 and 346 amino acids, respectively, that functionally couple through activation of calcium mobilization. We have identified a novel dual antagonist, termed DUO-LT, that blocks CysLT1-induced calcium mobilization through both the CysLT1 and CysLT2 receptors. The tissue distribution on the CysLT1 and CysLT2 receptors is very different. The CysLT1 receptor shows highest expression in spleen and peripheral blood leukocytes, while the CysLT2 receptor is most highly expressed in the heart, placenta and adrenal medulla. In situ and immunohistochemical analyses of the distribution of the CysLT1 receptor showed expression in normal lung smooth muscle and interstitial macrophages and in peripheral blood eosinophils. In nasal lavage cells from patients with seasonal allergic rhinitis both the CysLT1 and CysLT2 receptors and 5-lipoxygenase were shown to be expressed in eosinophils, monocytes and macrophages and mast cells, and in addition CysLT1 receptors were expressed on neutrophils. These studies emphasize the expression of CysLT1 synthetic and signaling pathways in the key pathological cells in asthma and allergic rhinitis.

A 74
Substance P up-regulates macrophage inflammatory protein-1β expression in human T lymphocytes
Chang-jiang Guo*, Jian-ping Lai, Ji-bong Yang, Hong-mei Luo, Steven D. Douglas and Wen-zhe Ho
Joseph Stokes Jr. Res. Institute of The Children’s Hospital of Philadelphia, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104

Substance P (SP) is an important modulator of neuroimmunoregulation. We have investigated whether SP stimulates synthesis of macrophage inflammatory protein-1β (MIP-1β) in human T lymphocytes. SP significantly enhanced MIP-1β expression in a human T cell line (Jurkat-SPR, 3-SPR) that contains SP receptor gene at both mRNA and protein levels as determined by real-time RT-PCR and ELISA assays. The supernatants from SP-stimulated 3-SPR cells enhanced T lymphocyte chemotaxis in vitro indicating that SP-induced MIP-1β is functional. In addition, SP augmented secretion of MIP-1β from primary culture of peripheral blood lymphocytes (PBL) isolated from some of the donors. This viability is due to differential expression of neurokinin-1 receptor (NK-1R), the primary SP receptor on the PBLs from different donors. Our further experiments indicate that SP-induced MIP-1β expression could be abrogated by a specific SP receptor antagonist (CP-99,994). These data provide a potential mechanism by which SP selectively influences such cellular immune response as α-chemokine expression in human T lymphocytes through NK-1R, which may have an important in vivo implication in inflammatory diseases.

A 76
Cathespin S inhibitors block invariant chain processing and antigen-induced proinflammatory human collagen-induced arthritis (CIA) and rat ovalbumin-induced asthma in vivo
GlaxoSmithKline, King of Prussia, PA 19406

Cathespin S is a cysteine protease that catalyzes the proteolytic cleavage of II, enabling lii to dissociate from, and antigenic peptide to bind to, MHC class II. Cathespin S was found to be constitutively expressed in human peripheral blood monocytes and dendritic cells, but not T cells. Treatment of human and mouse cells with the cathespin S inhibitor 5-(2-morpholinyl-4-ethylnonyl)benzofuran-2-carboxylic acid (S)-3-methyl-1-[(S)-3-oxo-1-[2-(3-pyridin-2-ylphenyl)-acetyl]-azepan-4-ylcarboxy]butylamide (compound 1) (Ki vs. human and mouse cathespin S = 8.3 and 6.7 nM, respectively) resulted in the concentration-dependent inhibition of II processing and antigen-induced proliferation. In the CIA model, mice treated with compound 1 exhibited significantly reduced mean clinical score and mean paw thickness, as well as paw TNF-α and IL-1β levels. In the rat ovalbumin-induced asthma model, cathespin S inhibition resulted in significantly reduced eosinophil and leukocyte accumulation in the bronchoalveolar lavage fluid.

A 77
Adenoviral-mediated gene-transfer of IL-18BP to knees ameliorates collagen-induced arthritis

IL-18 plays an important role in innate and acquired immunity. Murine IL-18 binding protein isoform-c (IL-18BPc) potently inhibits IL-18 biological activities. The objective of this study was to elucidate the local role of IL-18 in collagen-induced arthritis (CIA). For this, we developed a first generation adenovirus for the gene-transfer of murine IL-18BP cDNA (AdCMV-IL-18BPc). A single dose of 1x10⁵ PFU of AdCMV-MurIL-18BPc virus or AdCMV-Luc as a control vector was injected into the knee joint cavity of DBA/1 mice bilaterally at day 21 of immunization and 7 days later the study was ended. IL-18BPc over-expression significantly reduced joint swelling in CIA with 60% (p<0.001) in the knees and approx. 50% (p<0.01) in the hind leg paws. Furthermore, local IL-18BPc treatment reduced IL-6 serum levels by 64% (p<0.001) but showed normal IgG levels of anti-collagen type II. Moreover, IL-18BPc reduced synovial TNFα levels by 70% and almost completely prevented joint inflammation, cartilage and bone destruction. This study demonstrated that local IL-18BPc gene-therapy is efficacious in CIA and that IL-18 plays an important pro-inflammatory role.

A 78
The chondrogenic potential of human osteoarthritic infrapatellar adipose tissue
Merin L. Boehm, Pfizer, Inc., MS 8220-2345, Eastern Point RD, Groton, CT 6340, USA

Human cell lines used in the study of cartilage biology do not typically express and synthesize high levels of aggrecan and type II collagen, hallmarks of the articular cartilage chondrocyte phenotype. Primary articular chondrocytes rapidly lose phenotype with passage and primary cells lose the expression of collagen and aggrecan after several weeks in culture. It would be ideal to have a readily available pool of human cells that exhibit a normal chondrocyte phenotype. It is well known that bone marrow-derived mesenchyal stem cells can be induced by different growth factors to commit to numerous particular cell lineages. Interestingly, several abstracts presented that adipose tissue-derived stem cells from human lipolysis or rat inguinal fat pads can be induced to exhibit a chondrogenic phenotype in culture via treatment with TGF-β1 and/or demethasone. We wished to determine if aged human osteoarthritic synovial adipose tissue would serve as a possible source of adipose derived stem cells with chondrogenic potential. We examined the TGF-β1 +/− demethasone induction of chondrocyte differentiation of these cells by measuring the ability of adipocyte cell pellet cultures to incorporate and/or synthesize collagen and proteoglycans. We also attempted to determine whether AT has an anabolic effect in this adipocyte chondrogenic culture system, and whether the addition of a sole anabolic factor can cause the same response as treatment with serum.
Role of alveolar macrophages in airway responsiveness

Eric Careau* and Elise Bissonnette
Centre de recherche, Hôpital Laval, Institut universitaire de cardiologie et de pneumologie du Québec, Laval, Canada

Airway hyperresponsiveness to a wide variety of stimuli is a characteristic feature of asthma and is closely related to its severity. Alveolar macrophages (AM) are well known to suppress T cell activation and antigen presentation activities of dendritic cells but less is known about their role in airway responsiveness. We have investigated the role of AM in allergic response and airway hyperresponsiveness development. AM were depleted using liposomes containing clodronate and airway responsiveness was measured. Furthermore, ovalbumin (OA)-sensitized Brown Norway and Sprague Dawley rats with or without AM were challenged with OA and airway responsiveness was measured. AM depleted rats developed respiratory distress during OA challenge and they showed airway hyperresponsiveness 24 h post challenge. Intranasal sensitized Brown Norway rats depleted without AM developed airway hyperresponsiveness after OA challenge, whereas Sprague Dawley rats had normal airway responsiveness. These data suggested that AM functions may be altered in Brown Norway rats. Thus, AM from Sprague Dawley rats were transferred into Brown Norway rats, without AM. These rats did not show respiratory distress during OA challenge and their airway responsiveness returned to normal level. These new and interesting data show that AM may protect against development of early response and airway hyperresponsiveness.

Oxidant stress causes pulmonary inflammation and activation of the p38 kinase pathway in BALB/c mouse

COPD Biology, Respiratory, Inflammation and Respiratory Pathogens Centre of Excellence for Drug Discovery, GlaxoSmithKline, Philadelphia, PA, USA

Oxidant stress has been implicated as a pro-inflammatory stimulus in pulmonary diseases such as chronic obstructive pulmonary disease (COPD). We used ozone exposure to induce oxidative stress in the lungs of Balb/c mice to investigate the role of the p38 MAP kinase pathway in pro-inflammatory cytokine signaling. Following ozone exposure at 3.0 ppm, Western blot analysis of lung tissue homogenates revealed p38 MAP kinase activation by 30 minutes which was maximal following a 2 hour exposure. Two direct downstream substrates of p38, MAPKAP kinase-2 (MK2) and MAPKAP kinase-3 (MK3), were also activated at this time-point with the ratio of activity being 5:1 in favor of MK2. Western blot analysis of lung homogenates revealed that the heat shock protein 27 (HSP27), a substrate of both MK2 and MK3, was also phosphorylated 2 hours post-exposure. Following ozone exposure (3.0 ppm, 6h) a time-dependent increase in the pro-inflammatory cytokine IL-6 and the neutrophil chemokine KC was detected in bronchoalveolar lavage (BAL) fluid, peaking at 6 hours post-ozone. BAL fluid analysis also revealed time- and dose-dependent increases in neutrophil recruitment which was maximal 24 hours post-ozone. Treatment of mice with the potent and selective p38 MAP kinase inhibitor SB239663 [1-(4-hydroxyoctoate)-4-(4-fluorophenyl)-5-(3-methoxyphenylimidazo-4-yl)imidazole] dose-dependently inhibited KC levels in BAL fluid and caused an inhibition in neutrophil recruitment which was maximal using a 50 mg/kg (p.o., b.i.d.). These data demonstrate activation of the p38 MAP kinase pathway following ozone-induced oxidative stress and suggest that inhibitors of this pathway may be of therapeutic benefit in chronic inflammatory lung diseases.

Inhibition of inflammatory cell recruitment into the bronchial airways of staphylococcus enterotoxin B-treated mice using the selective PDE4 inhibitor cilomilast

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COPD Biology, Respiratory, Inflammation and Respiratory Pathogens Centre of Excellence for Drug Discovery, GlaxoSmithKline, Philadelphia, PA, USA

Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory disease characterized by increased macrophages, CD8 T-lymphocytes and neutrophils in the lungs. In the present study, we have characterized a pulmonary inflammation model using staphylococcus enterotoxin B (SEB) in MRL mice, and investigated the utility of selective PDE4 inhibition. Following intra-tracheal administration, time and dose-dependent increases in macrophages and neutrophils in bronchoalveolar lavage (BAL) fluid were observed, peaking on day 3 post-SEB exposure. Flow cytometric analysis of BAL cells from SEB-treated mice revealed an 8.1 ratio in favor of CD4+ over CD8+ T-cells. Stimulation of cells from SEB-treated mice with concanavalin-A significantly increased the levels of the Th1 cytokine INF-γ detected in the culture medium compared to PBS controls. Administration of the selective PDE4 inhibitor, Cilomilast (Atoril® 2 mg/kg, i.p.) for 3 days, to SEB-treated mice inhibited lymphocyte recruitment in BAL fluid from 25.0 x 10⁷ ± 2.0 cells in vehicle-treated mice to 14.1 x 10⁷ ± 0.4 cells (p<0.05). Neutrophil recruitment was also inhibited from 15.8 x 10⁷ ± 2.1 cells in vehicle-treated mice to 11.6 x 10⁷ ± 0.3 cells following Cilomilast treatment (p<0.05). Administration of the corticosteroid predniason (2 mg/kg, i. p., i.d.) however, had no effect on attenuating lymphocyte or neutrophil recruitment into the lungs of SEB-treated mice. These data suggest that cyclic AMP elevation by selective PDE4 inhibition may have therapeutic utility in pulmonary diseases such as COPD.

Airway remodeling in a non-human primate model of asthma

E.C. Martin*, S.L.Kunkel and M.A. Nedelman
1 Charles River Laboratories and 2 University of Michigan Medical School

This study sought to further characterize the model of Ascaris suum-induced pulmonary bronchoconstriction in cynomolgus monkeys by histologic evaluation of lung tissue for evidence of remodeling comparable to what is seen in humans. Lung tissue samples were obtained from a colony of asthmatic macaques that had undergone chronic aerosol challenges with As. suum antigen over a period of 7-17 etha. Samples were collected either within 24 hours of an antigen challenge from symptomatic animals (n=4, acute phase) or from animals that had not been challenged for at least 3 weeks, and showed no clinical signs of asthma (n=5). Acute inflammation was limited in the acute samples and included significant inflammatory cell infiltrate within the alveolar space and tissue, pulmonary edema and hemorrhage. However, despite the lack of clinical signs in the animals that had undergone a recovery period, significant pulmonary remodeling attributed to chronic exposure to antigen was seen in these animals as well as in those exhibiting clinical signs. Evidence of pulmonary remodeling included goblet cell and smooth muscle hypertrophy, collagen deposition, epithelial cell lining sloughing, disorganization of the epithelium and thickening of the basement membrane. Marked submucosal and bronchial inflammatory cell infiltrates accompanied by expansion of the bronchial associated lymphoid tissue were also present. Vascular remodeling was also evident in these animals and included intimal hyperplasia, smooth muscle cell hypertrophy and collagen deposition. Thus, multiple antigen exposures used in this primate model of allergic asthma result in airway remodeling consistent with the pathologic changes seen in human asthma and would be a valuable tool for evaluation of efficacy of novel therapeutics.

Kinin involvement in antigen-induced airway inflammation in ovalbumin (OA)-sensitized mice

J. Erić* and P. Siroć
Institute of Pharmacology of Serbia, School of Medicine, University of Serbia, Belgrade, Serbia, PO. Box, Canada, J11 5N4

The physiological and pathophysiological effects of kinins on the lungs are still not well defined. The aim of the present study was to investigate, through the use of selective B1 and B2 receptor antagonists, the contribution of bradykinin B1 receptor in a murine model of allergic lung inflammation. The B1 agonist, desArg9-Bk (Bk, 10- 100 pg/kg) administered intratracheally (i.t.) to normal mice had no effect on the cells recovered in the bronchoalveolar lavage fluid (BALF) and on plasma extravasation whereas the B2 selective agonist, bradykinin (BK, 20 μg/kg, i.t.) stimulated mononuclear cell migration, neutrophilia and plasma extravasation. The selective B2 antagonist, icatibant (HOE-140, 10 μg/kg) completely inhibited the effects elicited by BK. HOE-140 (1, 10 and 100 μg/kg) injected intravenously 5 min prior to aerosol OA challenge decreased mononuclear cell and eosinophil infiltration in BALF of OA-sensitized mice and prevented the airway hyperreactivity (AIRH) to carbachol. B2 antagonists, R-715 (Ac-Asp-Lys [D-Nal4] [D-Ile10] [D-Bk]) and R-954 (Ac-Orn [D-Nal4] [I-Ile10] [D-Bk]) decreased by about 50 % the lung eosinophilia but did not reduce AIRH in OA-sensitized mice. Our results suggest that the airway inflammatory response induced by antigen challenge in mice is mediated by stimulation of bradykinin B1 and B2 receptors. (Supported by the MRC).

Anti-inflammatory role of pre-elafin, an elastase specific inhibitor, in lung inflammation

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Pre-elafin, also known as trappin-2, is a 10.5 kDa serine protease inhibitor present in the lung. In our effort to delineate the role of pre-elafin as an effective regulatory element in controlling lung tissue damage and inflammation, we produced recombinant human full-length pre-elafin and showed that it inhibits neutrophil elastase-induced lung hemorrhage, as well as lipopolysaccharide (LPS) - and silica-induced lung inflammation. Pre-elafin significantly reduced leukocyte accumulation in the lung, the alveolar spaces and the associated gelatinase burden, and down-regulated the expression of pro-inflammatory cytokines and chemokines. Pre-elafin did not significantly bind to either LPS or LPS-binding protein. Thus, pre-elafin is a potent anti-inflammatory molecule, which probably acts directly on alveolar macrophages and/or other cells. This study was supported by the Bayer - Canadian Blood Services - Héma-Québec Partnership Fund.
A 55
IFN-γ-inducible protein 10 induces neutrophil infiltration into the mouse lung
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GlaxoSmithKline, Respiratory, Inflammation and Resp. Path., CEDD, King of Prussia, PA 19406.
IFN-γ-inducible protein 10 (IP-10) is a chemokine which is chemotactic for activated T cells that express its receptor, CXCR3. It is known to be produced by activated monocytes, lymphocytes, keratinocytes, endothelial cells and neutrophils. In vivo studies were initiated to evaluate the potential for murine IP-10 to attract lymphocytes into the lung. Balb/c mice were given murine IP-10 (1, 10 and 100 ng/mouse, i.n.) in a 50 µl volume. After 24h, the lungs were washed with PBS and the cell numbers and differentials counted. Paradoxically, IP-10 at doses of 1, 10 and 100 ng/mouse, i.n. (5×6 for each) produced a significant infiltration of neutrophils (1747%, 1769% and 3128%, respectively) with no increase in lymphocyte numbers. Therefore, the bronchoalveolar lavage fluid was assayed for cytokine levels. The murine IL-8 orthologue, KC and MIP-2, were significantly elevated in a dose-related manner. It appears that murine IP-10 introduced into the mouse lung intranasally has the ability to attract neutrophils by elevating murine KC and MIP-2 which are associated with an acute inflammatory response.

A 86
Effects of a novel p38 MAPK inhibitor on LPS-induced TNFα production in mouse lung tissue
Clare S. Zimmitt*, Jeffrey B. Madwed and Thomas C. Noonan
Department of Pharmacology, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA.
p38 MAPK is an integral enzyme involved in intracellular signaling and cytokine production. We investigated lung specific pharmacologic properties of a novel and selective p38 MAPK inhibitor, BIX-983 using a technique that maintains integrity of the lung microenvironment. In our model, mouse lungs were removed at different time points after oral dosing and sliced into ~0.5 mm³ pieces by a tissue chopper. Lung tissue aliquots (40-60 mg) were stimulated with LPS (10 ng/ml) for 3 hours. Supernatant TNFα was measured by ELISA. Results of BIX-983 along with Dexamethasone and Prednisolone are summarized as follows:

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<tr>
<th>Ex vivo</th>
<th>ΔΔCt values</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>Dexamethasone</td>
<td>0.3 - 1 µg/kg</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>&gt; 10 µg/kg</td>
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BIX-983 inhibits TNFα production from mouse lung tissue with a long duration of action, and its inhibitory effect is comparable to Dexamethasone and Prednisolone. These results suggest that BIX-983 may provide protective effect in the lung after oral dosing and may be a potential therapy for inflammatory mediated pulmonary disease.

A 87
Identification of metabolic pathways of 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) responsible for the downregulation of alveolar macrophage function
Léa-Isabelle Proulx*, André Castonguay, and Elyse Y. Bissonnette
Centre de recherche, Hôpital Laval, Institut universitaire de cardiologie et de pneumologie de l’Université Laval, Québec, Canada.
Tobacco smoke contains about 20 identified carcinogens specific to pulmonary tissues. One of the most abundant is the nicotine derived NNK. NNK can be metabolized by 2 major pathways which also lead to the formation of keto alcohol (KAL) and keto acid (KA), two non-carcinogenic components. We have previously demonstrated that NNK inhibits alveolar macrophage (AM) function by reducing interleukine-12 (IL-12), tumor necrosis factor (TNF) and nitric oxide (NO) production and by stimulating IL-10 production. However, the metabolic pathway implicated in these modulations was not identified. We used precursors such as 4-(acectoxymethyl)-nitrosamino)-1-(3-pyridyl)-1-butanone (NNKOAc) and N-nitro(acectoxymethyl)methylamine (NDMAOAc) that allow studying each pathway individually. Stimulated AM were treated with different concentrations of NNKOAc and NDMAOAc (12, 25 and 50 µM). Culture supernatants were assessed for cytokine and NO levels. NNKOAc significantly inhibited the release of IL-10, IL-12, TNF and NO whereas NDMAOAc did not modulate cytokine production by AM. Furthermore, KAL significantly down-regulated the production of TNF and IL-12 whereas KA did not modulate cytokine production by AM. Thus, in addition of being responsible for NNK, NNKOAc oxidation and formation of KAL, this pathway may also be implicated in the immunosuppressation mediated by NNK.
Van Arman Award competition abstracts
VI

Lentivirus-mediated gene delivery to joints: long-term intra-articular expression at biologically relevant levels.

Elvire Gouze1, Robert Pawlick1,2, Jean-Noël Gouze1,2, Carmencita Pilapiñ1, Christina Fleer1, Glyn D. Palmer1, Christopher H. Evans1, Philippe Leboulch2,3, and Steven C. Ghivizzani1

1Center for Molecular Orthopaedics, and 2Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston MA; 3Genetix Pharmaceuticals Inc., Cambridge MA; 4Division of Health Sciences and Technology, and 5Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge MA

Gene therapy for arthritis shows considerable promise, but requires a vector capable of achieving both in vivo delivery and long-term transgene expression to become clinically useful. Lentiviral vectors transduce rat synovium efficiently enabling extremely high levels of transgene expression. Here, we report the effects of intra-articular injection of a recombinant lentiviral vector encoding the human interleukin-1 receptor antagonist (LV-HIL-1RA) in a model of arthritis driven by human interleukin-1β (hIL-1β). Twenty-four hours after unilateral injection of 5x105 iu LV-hIL-1Ra, arthritis was induced by injection into both knees of fibroblasts engineered to overexpress hIL-1β. Increasing doses of cells were injected in different groups of animals, allowing a range in the severity of joint pathology. Injection of LV-hIL-1RA strongly prevented knee joint swelling in the treated joints, even in the most severely grouped. In histological analyses, cellular infiltration, cartilage degradation, and invasiveness of inflamed synovium were dramatically inhibited by LV-hIL-1Ra in both treated and contralateral knees. A systemic beneficial effect was observed. Lentiviral-mediated transgene expression increased dose dependently with the number of IL-1β cells injected. In atrophic nude rats, lentivirus-mediated expression persisted for at least 6 months. These results indicate that lentiviral vectors are suitable both for in vivo gene delivery to synovium and for achieving long-term transgene expression at biologically relevant levels.

VII

The synthetic triterpenoid CDDO inhibits MMPI-13 gene expression independently of PPARγ

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The over-expression of matrix metalloproteinases (MMPs) during arthritis progression leads to irreparable destruction of joint cartilage. The collagenases, MMP-1, and MMP-13, are chiefly responsible for degradation of fibrillar collagens within cartilage, therefore reducing the levels of these enzymes is a viable strategy for inhibiting cartilage destruction. We have previously characterized a novel triterpenoid compound, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO), for its ability to inhibit the transcription of MMP-1 and MMP-13 in human SW-1353 chondrosarcoma cells (Arthritis Rheum, 2001; 44:1096). In this study, we investigate the role of peroxisome proliferator-activated receptor-γ (PPAR-γ), the only known receptor for CDDO, in the inhibition of MMP-13. We demonstrate that PPAR-γ protein is expressed and transcriptionally responsive to CDDO in SW-1353 human chondrosarcoma cells, thus verifying PPAR-γ as a receptor for CDDO. Primary mouse embryonic fibroblasts (MEFs) deficient in PPAR-γ were used to study the regulation of MMP-13 in response to CDDO by RT-PCR. Interestingly, we found that CDDO inhibits the induction of MMP-13 independently of its only known receptor, PPAR-γ, suggesting a novel mechanism of action for CDDO.

VIII


Bispey H. Gabar1 and Pierre Sinis2

1Institute of Pharmacology of Sherbrooke, School of Medicine, University of Sherbrooke, Sherbrooke, PQ, Canada, J1H 3N4

Insulin-dependent diabetes mellitus is an inflammatory autoimmune disease associated with vascular permeability changes leading to many complications. The B2 kanin receptors were recently found to be upregulated during the development of the diabetic and to be involved in its complications. In the present study, we studied the effect of the selective kanin B2 receptor agonist BBK (des-Arg11-BBK) and antagonist R-715 (Ac-Arg11-Des-Arg11-Nal11, Nle12-Arg12-BBK) and R-954 (Ac-Arg11-Des-Arg11-Nal11, Nle12-Arg12-BBB) on diabetic hyperglycemia. Diabetes was induced in male CD-1 mice using streptozotocin and the necropsy was assessed using the hot plate test. Our results showed that induction of diabetes provoked a marked hyperglycemia in diabetic mice. Following systemic administration of R-715 (200 μg/kg, i.p.) and R-954 (200 μg/kg, i.p.), the hot plate latios of diabetic mice returned to the normal level. In addition, acute treatment of DBK (400 μg/kg, i.p.) significantly pointuated diabetes-induced hyperglycemia, an effect that was totally reversed by both R-715 (2.4 mg/kg, i.p.) and R-954 (1.6 mg/kg, i.p.). These data provide evidence for the implication of the B2 kanin receptors in the development of hyperglycemia associated with diabetes and suggest a novel approach in the treatment of diabetic complications using kanin B2 receptor agonists. (Supported by the CIHR).

IX

The Prostaglandin D2 metabolite 15d,e-epoxy-12Z,14Z-PGJ2 promotes RelA (p65) cleavage and leukocyte apoptosis during the resolution of inflammation in vivo.

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Aims. Cyclopentenone prostaglandins (cPGs) are putative endogenous anti-inflammatory mediators. Here we describe how the cPG 15d,e-epoxy-12Z,14Z-PGJ2 promotes the resolution of inflammation in vivo through the induction of leukocyte apoptosis. Methods. We have used the rat carrageenin pleurisy as a model of acute resolving inflammation. Pharmacological agents were used to modulate cPG production and determine effects on the cellular kinetics of the inflammatory response. Results. 15d,e-epoxy-12Z,14Z-PGJ2 production is associated with the resolution of inflammation and leukocyte apoptosis. Therapeutic administration of the selective COX2 inhibitor NS398 prevented the proper resolution of inflammation and delayed leukocyte apoptosis. Furthermore, this was reversed by the simultaneous administration of exogenous 15d,e-epoxy-12Z,14Z-PGJ2. NS398 also prevented cleavage of RelA and the inhibition of NF-6B target gene expression, which was again reversed by administration of 15d,e-epoxy-12Z,14Z-PGJ2. Conclusions. 15d,e-epoxy-12Z,14Z-PGJ2 promotes leukocyte apoptosis during the resolution of inflammation in vivo and inhibits NF-6B dependent gene expression. cPGs and their derivatives may represent novel selective agents for the inhibition of pro-inflammatory NF-6B activation that promote resolution of inflammation in vivo.

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OA is characterized by osteophyte formation and cartilage damage and TGF-β has been suggested to play a dualistic role in OA. TGF-β can increase cartilage PG synthesis and content but TGF-β also induces fibrosis and osteophytes. The objective of this study was to determine the natural role of TGF-β during OA. As a TGF-β antagonist we used the soluble extracellular part of TGF-β type II receptor (solRⅡ), since it represents a binding of high affinity. solRⅡ protein was produced by us in high amounts and mice with experimentally induced OA were systemically treated with solRⅡ for 7 or 14 days. TGF-β neutralization dramatically reduced osteophyte size by >75%, whereas it significantly increased PG loss in articular cartilage. We conclude that endogenous TGF-β plays a direct role in osteophyte development and blocking endogenous TGF-β has an important protective role in the regulation of cartilage PG content during experimental OA.
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