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

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Activation of the Nfkb and MAPKinas signal transduction pathways following cytokine stimulation results in a catabolic phenotype of chondrocytes, leading to metalloproteinase production and decreased matrix synthesis, as well as the induction of inflammatory proteases 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 (OONO-), the free radical reaction product of nitric oxide (NO) and superoxide anion (O2-), augmented NF-kB (p65 Rel A) nuclear translocation in cultured chondrocytes. In contrast, the stable S-nitrosothiol S-nitroso-cysteine (SN0) inhibited this effect, illustrating that the effects of nitric oxide may be either pro- or anti-inflammatory, dependent upon the predominant redox product generated in tissue. We sought to determine whether OONO- was produced by cytokine activated bovine chondrocytes by measuring nitrotyrosine (stableproduct of OONO- and tyrosine). In addition, we sought to determine whether the properties of cytokine elicited nitric oxide in cartilage chondrocytes and NF-kB translocation were similar to those of OONO- or SN0. Exposure of bovine chondrocytes to IL-1b + TNF-a induced the formation of intracellular nitrotyrosine (IF) consistent with OONO- formation. EPR measurements demonstrated concomitant production of O2-. Cytokine stimulation induced the nuclear translocation of the (p65) Rel A subunit (IF), which was 46% complete by 5 minutes, maximal at 15 minutes, sustained at this level and declined to 24 hr. In contrast, MAPK kinase Erk did not translocate to the nucleus in cytokine-stimulated cells (6 hr). To determine whether cytokine-stimulated nitrotyrosine production and NF-kB translocation were mediated by induced nitric oxide production, chondrocytes were stimulated in the presence or absence of the iNos inhibitor, L-NMMA. L-NMMA, which reduced measurable nitric oxide production (Greiss) by 90%, prevented nitrotyrosine production in cytokine-activated chondrocytes as expected. This inhibition of presumed OONO- was associated with a significant inhibition of cytokine-induced maximal NF-kB translocation at 6 hrs, although no inhibitory effect was observed at the earlier time points, as expected. The MK (pro-oxidant activator of Erk) inhibitors PD98059 and U0126 also inhibited NF-kB nuclear translocation. In addition, MeK inhibitors, attenuated IL-1b/TNF-a stimulation of MMP-1, PGE2 and PGE2, but not PGE2, also inhibited IL-1b/TNF-a stimulated Erk activation and MMP-1 production. In contrast, both non-selective and selective NSAIDs inhibited enhanced Erk activation and MMP-1 production, presumably via reversal of Erk inhibition by COX-2 derived PGEs. Taken together, these data indicate that in chondrocytes: 1) OONO- is a predominant redox species produced following exposure to IL-1b/TNF-a; 2) nitric oxide derivatives (particularly OONO-) augment/sustain cytokine dependent NF-kB translocation; 3) activation of Erk promotes NF-kB translocation and MMP-1 production; and 4) Erk activation is inhibited by COX-2 derived prostaglandins and enhanced by NSAIDs. 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.

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

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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-1b, 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 that 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 (iNOS) 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.
A small molecule inhibitor of IxB kinase β (IKK-β) blocks inflammation and protects joint integrity in in vivo models of arthritis

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Nuclear Factor-xB (NF-xB) is a key transcriptional regulator of many pro-inflammatory mediators (e.g. TNFα, IL-1β, IL-6) and activated NF-xB has been observed in several debilitating inflammatory disorders, including rheumatoid arthritis and osteoarthritis. The IxB kinase β (IKK-β) is required for the phosphorylation of IxBα and subsequent NF-xB activation by the IxB kinase signalsome (IKKe, IKKβ, IKKγ) 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, immuno blot analysis of cytosols from paw tissue showed a blockade of IxB degradation and evaluation of nuclear fractions confirmed a reduction in NF-xB 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.
Glucocorticoid induction of the phosphoethanolamine carboxylase (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 factors. The GR1 element binds the transcription factors COUP-TFII/HNF4, HNF3, COUP-TF and C/EBP, respectively. The intact GRU fully supports transcription, however, mutation of any one of the accessory elements reduces the glucocorticoid response of the PEPCK gene by 40-60%. Mutation of any two elements abolishes the glucocorticoid 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 analysis 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-GK2 elements poorly (30% less avidity) as compared to a palindromic or consensus GRE. Inclusion of either the gAF1 or gAF2 element with GR1-GK2, 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 msec at nM concentrations. However, the dissociation rate of GR is differentially slowed by the gAF1 or gAF2 elements that bind two functionally distinct accessory factors, COUP-TFII/HNF4 and HNF3 respectively. Dissociation is very slow and incomplete in the presence of gAF2/HNF3. Dissociation is more complete, but still not as efficient as from the consensus GRE, in the presence of gAF1/HNF4/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 the accessory factors. 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-GK2 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 GRU. The association of GR results in the assembly of CBP and polII to the promoter.

### S5

**Role of accessory factors in the assembly of the glucocorticoid response unit on the PEPCK gene promoter**

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### S7

**The dynamics of nuclear receptor interactions with gene targets**

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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.
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 "remodeling", inappropriate to the maintenance of normal lung function. The clinically observed distinctions between asthma and COPD are reflected by differences in the remodeling process, the patterns of inflammatory cells and cytokines and also the predominant anatomic 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 epithelium does not occur in the asthmatic non-smoker. In COPD, there is epithelial mucous metaplasia, airway 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 mucous-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 remodelled 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 remodeling is dependent on the prior development of the distinct patterns of chronic inflammation is unresolved. 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 effectors but also as "tissue sentinel cells" 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 by a neutralizing anti-CD40L antibody demonstrating the dependence of lung fibroblast activation on the CD40-CD40L system. These findings support the concept that lung fibroblasts act not only as late stage effector cells, but also as "tissue sentinel cells" that become activated early following injury and synthesize cytokines, chemokines and lipid mediators that recruit white blood cells to the lung. The fibroblast can now be viewed as a passive bystander cell, but a true player in the evolving immune process, the switch to a more sophisticated immune response allows the host to mount a continued response with renewed vigor. It also is 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 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 underpins for end-stage disease.

Regulation 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, it has been shown that oxidant and redox-mediated mechanisms are involved in the regulation of smooth muscle function. Furthermore, isolated endothelium-removed bovine 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 NAD(H) redox. Post-hypoxic reoxygenation appears to activate a hydrogen peroxide-mediated relaxation through mechanisms including stimulating soluble guanylate cyclase to produce cGMP. The control of NADPH redox by the pentose phosphate pathway appears to be a key component of this 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.
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 fibrinolysis may cause abnormal tissue repair. Plasminogen activator inhibitor (PAI-1) has the ability to inhibit MMP activity and fibrinolysis. 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 crucial for the development of pulmonary fibrosis in vivo by controlling the MMP and fibrinolysis 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 hyperoxia, 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 bronchoalveolar 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 fibrin deposition 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 fibrinolysis and that PAI-1 could play an important role in structural changes in the airways of asthmatics.

Transgenic modeling of airway remodeling

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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 inflammatory 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 transgenic 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 minimum 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 minimum 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, collagens, the enhanced accumulation of fibroblasts, myofibroblasts, and myocytes, baseline airway obstruction and AHR upon methacholine challenge, and enlarged alveoli. 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 effectors 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.
Discoveries and structure-activity relationship of N-(3-hydroxylpropyl)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 Berlex CCR3 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 urea functionality that the other compounds lacked, we concentrated our efforts on dissecting the structure of 4 to determine the effect on binding and selectivity for CCR3 vs CCR1.

![Chemical structures](image)

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 analogs. However, replacement of the 4-hydroxy-4-phenylpiperidine with a 4-benzylpiperidine gave compounds that were not only equipotent, but also more 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 re-introduction of the urea functionality (see 10, CCR3 I.C.50 = 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 I.C.50 < 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.

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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, 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.

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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.
S20
The discovery of modulators of NFκB and AP-1 for the treatment of inflammatory infectious diseases
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The induction of proinflammatory genes often results from the increased activity of NFκB and AP-1, two transcription factors that have become attractive targets for the development of novel anti-inflammatory drugs. NFκB coordinates the expression of numerous soluble proinflammatory mediators including cytokines (IL-1, TNFα, IL-6) chemokines, adhesion molecules as well as inducible enzymes (COX2, iNOS) (1). Our understanding of the NFκB signalling pathway has generated numerous targets that are amenable to drug discovery efforts identifying small molecular weight inhibitors that will block the translocation of NFκB to the nucleus and prevent inflammatory gene expression. These include members of the Signalsome, a large molecular weight complex consisting of IκB κα, IKKβ and adaptor proteins (IκKa, IKKβ or NEMO), inducible kinases such as IκKα or IκKβ, and an E3 ligase selective for phosphorylated IκBα. IκKβ inhibitors including SPC39 are 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 mitogen-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 they 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 astaibatrazolone 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 the target, the expression of inflammatory genes COX-2, IL-2, IFN-γ, TNF-α, and prevented the activation and differentiation of primary human CD4 cells culture.

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

Pharmacologic modulation of both the NFκB 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.

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S21
The role of cathepsin S in physiology and disease
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MHC class II molecules display antigen peptides on the surface of APC for recognition by CD4+ T cells. Class II molecules associate in the endoplasmic reticulum with the invariant chain (Ii), a chaperone protein that not only directs the degradation of invariant chain (Ii) in the endosomal/lysosomal compartments to generate a fragment of approximately 10 kDa (Ii10). 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 exchangeable 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, LHVS, or the catS knockout mouse (cats−/−) have demonstrated accumulation of MHC class II molecules as well as reduced antigen presentation on the surface of APC. Studies presented were undertaken to determine if reversible cats inhibitors can elicit the same functional impact both in vivo and in vitro that results from cats deficiency or treatment with LHVS.

B cells and dendritic cells from cats−/− failed to present OVA to OVA-specific T cells as measured by reduced IL-2 secretion (Shi, et al.). Similar studies, conducted in-house with reversible cats inhibitors, demonstrated a dose-dependent inhibition of IL-2 secretion from D0.110 T cell following OVA presentation to A20 B cells (EC50 <150nm). In vivo antigen presentation results in the generation of sensitized, antigen-responsive T cells and the proliferation of Ig secreting B cells in vivo and in models of arthritis (adjuvant, CIA) and are being developed clinically to treat RA, MS and cancer (2).

Several mitogen-activated protein kinase (MAPK) cascades are involved in inflammation and joint destruction. The MAPK referred to as JNK (Jun N-terminal kinase) 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 they in turn are activated by numerous additional kinases. Consequently, the pathway also offers many targets for drug discovery initiatives.

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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 from mouse bone 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. Sufficent numbers (3-30 X 10^6) of mature MC have been generated after 4-6 weeks of culture from 100-200 cc 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 transcripts for many chemokines are induced 3-8 hours following costimulating the high affinity IgE receptor in naive MC activated for the first time. In contrast, chronically stimulated MC that 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 induced from naive MC is consistent with the role that MC may play in tissue 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.

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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 a part of a signaling cascade triggered by stress-inducing conditions such as osmotic shock, toxic agents (xanthosyn), UV light and other inflammatory mediators. Uptown mapping of p38 MAP kinase as was described by Iwamoto et al. (1992) from skeletal muscle of mice. The roles 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 the treatment of inflammatory diseases. For example, we have provided evidence, using selective p38 kinase inhibitors as pharmacological probes, that p38 kinase inhibition is associated with the inhibition of allergic responses from human T cells. These findings indicate that p38 kinase inhibitors may be useful in the treatment of allergic diseases. These and other novel findings which expand the utility of p38 kinase inhibitors will be discussed.

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