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**Meeting Report**

# Towards a new understanding of inflammation-based disorders

## Highlights from the 8th World Congress on Inflammation

***Elisa Ferrer***
*Prous Science, P.O. Box 540, 08080 Barcelona, Spain*

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**Abstract**

The 8th World Congress on Inflammation took place in Copenhagen, Denmark, on June 16-20, 2007. This meeting covered a broad range of topics on inflammation research, including advances in the pathophysiology of chronic inflammatory diseases and future therapies. This article highlights relevant presentations, with especial emphasis on drug targets and novel drug entities disclosed at this year's meeting.

### Introduction

The 2007 World Congress on Inflammation, held on June 16-20 in Copenhagen, Denmark, gathered worldwide scientists from industry and academia to share new developments in inflammation research, including the latest advances in drug therapies. The pathogenic link between cancer and inflammation and potential new drug targets for chronic inflammatory diseases were also some of the topics discussed at this meeting. This article summarizes selected congress presentations.

### Highlights from plenary lectures

*Genetic and environmental risk factors in multiple sclerosis*

Dr. Lars Fugger's (The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, UK) talk revolved around the genetic and environmental risk factors in multiple sclerosis (MS) (1). MS is a chronic inflammatory disease of the central nervous system (CNS) that most commonly affects young adults. It is a clinically complex condition that may take two distinct forms, both responding differently to treatment: 1) a relapsing/remitting course, which is the most frequent; and 2) a chronic progressive form. Exogenous, environmental and lifestyle-related factors can trigger the development of MS in genetically susceptible individuals. Among the genetic causes of MS, the genes encoding major histocompatibility complex (MHC) molecules, and in particular the DR2 haplotype, are major factors predisposing to MS. The DR2 haplotype contains two alleles, DRB1*1501 and DRB5*0101, encoding the *DR2b* and *DR2a* genes, respectively. In mouse models, the expression of either one or the other allele (or both) leads to different MS phenotypes. Thus, mice expressing only DRB1*1501 show a clinical picture resembling the primary progressive form (gradual progression of the symptoms with no relapses or remitting periods), whereas expression of DRB5*0101 is not associated with any disease symptoms. Expression of both alleles has been correlated with the relapsing-remitting form of MS (2), indicating a genetic interaction or epistasis between DRB1*1501 and DRB5*0101, considered to be the effector and modifier allele, respectively. In this model, DRB5*0101 was found to induce peripheral deletion of $CD4^+$ autoreactive T-cells in blood (peripheral tolerance) by means of apoptosis induction, thus modulating the immune response to limit autoreactive reactions triggered by DRB1*1501 and attenuating an otherwise more severe phenotype. As Dr. Fugger pointed out, this allele combination appears to have been subjected to strong positive selection, as it may modulate disease severity.

Environmental risk factors are also important in the development of MS according to twin and migration studies. In fact, common infections have been associated with MS attacks, which could be due to the fact that pathogen-specific T-cells may fail to distinguish between the pathogen and the MS biomarker myelin basic protein (MBP). Thus, peptides from different pathogens, including *Staphylococcus aureus*, *Mycobacterium avium*, *Mycobacterium tuberculosis*, *Bacillus subtilis* and *Escherichia coli,* could act as mimicry peptides and trigger autoimmunity against MBP.

*Old and new players in allergic inflammation*

The honorary lecture delivered by Dr. Francesca Levi-Schaffer (The Hebrew University of Jerusalem, Israel) focused on the mast cell/eosinophil crosstalk in allergic inflammation, also known as "the allergic synapse" (3). Traditionally, the role of mast cells and eosinophils in the allergic inflammatory process was confined to the early (acute) and late (chronic) phase, respectively. However, recent research has provided evidence that mast cells also participate in the late phase, mainly by interacting with eosinophils. This interaction can take place via soluble mediators such as tryptase, inducing the release of IL-6 and IL-8, via the eosinophil major basic protein (mast cell degranulation) or via direct physical interactions through CD48 and DNAM-1 on mast cells and its ligand Nectin-2 expressed on eosinophils (4), which contributes to mast cell activation (5). Therefore, targeting the mast cell/eosinophil interface appears to be a potential therapeutic strategy in allergic inflammatory diseases such as asthma. Moreover, both cell types express activating and inhibitory receptors susceptible to immunomodulation. Thus, on eosinophils, the presence of CD48, a glycosylphosphatidylinositol-anchored protein that contains an intracellular region called ITAM (immunoreceptor tyrosine-based activation motif), has been shown to be essential for human eosinophil degranulation and its expression is particularly enhanced on eosinophils from atopic asthmatics (6). Neutralization of CD48 abrogated lung inflammation and mucus production in mouse models of allergic asthma (7). On the other hand, inhibitory receptors like CD300a (or Irp60) are expressed both in mast cells (8) and eosinophils and contain several ITIM (immunoreceptor tyrosine-based inhibitory) motifs. The activation of CD300a is known to suppress eosinophil chemotaxis, activation and survival (9), which could be therapeutically targeted to attenuate eosinophil activity in allergic disease. Dr. Levi-Schaffer also commented on bispecific antibody fragments to selectively target CD300a on mast cells and eosinophils, binding either IgE and CD300a or CCR3 and CD300a. This CCR3/CD300a bispecific antibody was found to reverse chronic established asthma in mice.

*Long-lived plasma cells in immunity and inflammation*

The role in immunity and inflammation of long-lived plasma cells was described by Dr. Andreas Radbruch (Deutsches Rheumaforschungszentrum, Germany) (10). In contrast to the general assumption that antibody-secreting cells are short-lived, being destroyed after a few days or weeks by apoptosis, plasma cells can survive for months or years in secondary lymphoid organs (spleen) or the bone marrow. The persistence of these plasma cells involves the transfer of plasmablasts or plasma cell precursors from the spleen to survival niches like the bone marrow, where they displace existing plasma cells. Chemokine receptors in plasmablasts and chemokines in survival niches are involved in this process. In particular, CXCL12 is a major factor secreted in bone marrow to promote the migration and survival of plasmablasts, which express CXCR4, the receptor for CXCL12, in response to B-cell activation. Inflamed tissues can also be survival niches for plasmablasts, to which they migrate in response to interferon gamma (IFN-$\gamma$)-induced chemokines like CXCL9 (which binds to CXCR3 in plasmablasts). These chemokines act as survival factors for plasmablasts in the survival niches (11). Thus, there is a steady turnover of "old" plasma cells being replaced by plasmablasts with new antigen specificities, which then differentiate into plasma cells, lose their migratory potential and become resident plasma cells. The existence of these long-lived plasma cells may explain resistance to immunosuppression with anti-B-cell therapies like rituximab (anti-CD20 monoclonal antibody), which destroy B-cells but do not completely abrogate plasma cells. Increased plasmablast generation has been observed during lupus flares, suggesting a role in autoimmunity. Thus, long-lived plasma cells are susceptible to being pharmacologically targeted for the treatment of autoimmune and inflammatory conditions. Currently, complete immunoablation with antithymocyte globulin, which causes depletion of thymus cells, including plasma cells, is the only method by which long-lived plasma cells can be eliminated. However, more specific strategies, such as inhibiting plasmablast migration by targeting chemokine receptors, remain to be tested.

*Endothelial dysfunction: links to metabolic syndrome*

At this year's conference, Dr. Salvador Moncada (Director of the Wolfson Institute for Biomedical Research, University College London) was awarded the International Association of Inflammation Societies (IAIS) Lifetime Achievement Award, recognizing his outstanding contribution to inflammation research. After reviewing his major discoveries, including the elucidation of the mechanism of action of aspirin and the discovery of prostacyclin and nitric oxide (NO), Dr. Moncada briefly outlined his current investigations on endothelial dysfunction and atherosclerotic disease. Interestingly, NO has been shown to be an endogenous regulator of oxygen consumption in the vascular endothelium. Thus, increased NO levels (such as in the vascular endothelium) may prevent the use of oxygen by the mitochondria, a situation known as metabolic hypoxia, where oxygen is available but cells cannot use it. This excess oxygen will be metabolized to form reactive oxygen species (ROS) and peroxynitrite, which can contribute to endothelial dysfunction (a cardiovascular disease predictor). The role that this pathway may play in metabolic syndrome and obesity is the object of future research.

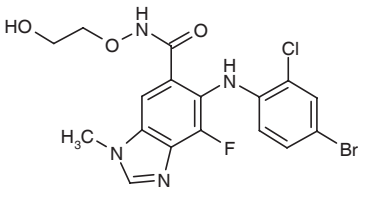
## The intersection of cancer and inflammation: new targets and new drugs

This session started with Dr. Ashlyn Eaton's (Centocor) intervention focusing on the role of IL-6 as a

potential link between inflammatory disease and cancer. It has been well described that chronic inflammation due to persistent infection or other sources can promote tumorigenesis. Thus, inflammatory bowel disease (IBD) is associated with colon cancer, and infections such as *Helicobacter pylori* and human papillomavirus (HPV) infection may result in gastric carcinoma and cervical cancer, respectively (12). In fact, inflammatory and immune cells are part of the tumor microenvironment and contribute, via cytokine release, to reducing antitumor immunity and favoring tumor progression and survival. IL-6-mediated signaling has been shown to be involved in many chronic inflammatory conditions, including rheumatoid arthritis (RA) and MS. IL-6 binds to its surface receptor complex comprised of IL-6R and glycoprotein 130 (gp130), which is responsible for signal transduction. Activation of the IL-6 receptor complex initiates the phosphorylation of JAK1 (Janus kinase 1) and STAT (signal transducer and activator of transcription) proteins (STAT1 and STAT3). Activation of IL-6/STAT3-dependent signaling is known to activate target genes involved in cell proliferation and apoptosis suppression (13). In addition, IL-6 is also produced by IL-17-secreting T-cells (or Th17), which play a pathogenic role in inflammation and autoimmunity. Hence, IL-6 supports Th17 cell development and enhances the production of inflammatory mediators via synergy with IL-17 (13). Centocor scientists have demonstrated the involvement of IL-6 in chronic inflammation in a study in which IL-6 neutralization with an anti-IL-6 monoclonal antibody (CNTO-328) suppressed nephritis, T- and B-cell activation and reduced the number of autoantibodies produced by autoreactive B-cells in a murine model of systemic lupus erythematosus (SLE) (14). Moreover, decreased STAT3 levels in T- and B-cells were also encountered. Other anti-IL-6 therapies, such as the anti-IL-6 monoclonal antibody tocilizumab (Roche, Chugai), have also been shown to reduce disease activity in refractory RA patients (15). In cells lacking the membrane-bound IL-6R, IL-6 can recognize the soluble IL-6R (sIL-6R) and trigger IL-6 trans-signaling, which has also been implicated in the development of colon cancer (13). Targeting the sIL-6R with a gp130-Fc fusion protein suppressed colitis activity and induced mucosal T-cell apoptosis in experimental Crohn's disease (16). In addition to its role in the pathogenesis of inflammatory diseases, IL-6 has also been identified as a growth and survival factor in hematological malignancies such as multiple myeloma, and Centocor is currently investigating the utility of CNTO-328 in multiple myeloma in phase II clinical trials (17).

The president and chief scientific officer of Array BioPharma, Dr. Kevin Koch, talked about the effects of targeting the mitogen-activated protein kinase (MAPK) pathway on the cancer/inflammation axis. Physiologically, the regulation of cell growth is mediated by growth factors (vascular endothelial growth factor [VEGF], epidermal growth factor [EGF], etc.) that bind their respective surface receptor tyrosine kinases and trigger a cascade of events involving the activation of the small G-protein Ras. GTP-Ras then phosphorylates Raf, which further phosphorylates the extracellular signal-regulated kinase 1/2 (ERK1/2). Phosphorylated ERK1/2 translocates to the cell nucleus, where it in turn phosphorylates a number of proteins that regulate cytoskeletal proteins, metabolism, chromatin remodeling and numerous transcription factors. Constitutive Ras/Raf/MEK/ERK activation has been implicated in aberrant cell growth and the development of many tumors (18). MEK inhibition has been suggested as an attractive strategy in cancer therapy, as MEK is the only known ERK activator. Dr. Koch's talk focused on two novel MEK1/2 inhibitors, namely ARRY-142886 (**1**) and ARRY-438162, currently under clinical development at Array for the treatment of cancer and inflammatory diseases, respectively. ARRY-142886 (also known as ARRY-886) is a highly selective, non-ATP-competitive inhibitor of MEK1/2 that showed dose-dependent tumor growth inhibition in several human tumor xenograft models in mice that correlated with a reduction in phosphorylated ERK (pERK) in tumors (19). In phase I studies, ARRY-886 has been well tolerated in more than 200 patients, with no serious adverse events, no signs of increased infections and only mechanism-based rash at the maximum tolerated dose (200 mg bi.d.). After 15 days of treatment, a notable correlation between plasma concentrations of the drug and inhibition of pERK was observed in peripheral blood mononuclear cells. Prolonged stable disease was the best clinical response in phase I studies. ARRY-886 progressed into phase II studies for several cancer indications in June 2006.

It is also well known that activation of the MEK/ERK pathway plays a pathogenic role in a number of nononcological conditions, such as pain, stroke, diabetes, chronic obstructive pulmonary disease (COPD) and inflammatory diseases. In the latter, MEK has been demonstrated to regulate the biosynthesis of certain proinflammatory cytokines, in particular tumor necrosis factor (TNF), IL-6 and IL-1. ARRY-438162 (also known as ARRY-162), a potent MEK1/2 inhibitor, is undergoing phase I investigation for the treatment of COPD and RA. In preclinical studies, ARRY-162 has shown superiority to etanercept (Enbrel®; Wyeth) in the carrageenan paw edema model. In combination with methotrexate, ARRY-162 effectively decreased the production of IL-6 in a rat RA model. Preliminary clinical results on ARRY-162 were also reported at the meeting. In a single-ascending-dose (SAD) study, healthy volunteers received oral doses of ARRY-162 ranging from 5 to 80 mg. Dose-proportional increases in drug exposure were observed with ascend-




ARRY-142886 (**1**)

ing doses. ARRY-162 demonstrated potent cytokine inhibition in *ex vivo* TPA-stimulated human whole blood samples, where IL-1β, TNF-α and IL-6 were inhibited with mean $IC_{50}$ values of 9.2, 10.0 and 9.3 ng/ml, respectively. Moreover, ARRY-162 also inhibited pERK ($IC_{50}$ = 123 ng/ml), although to a lesser extent. A pharmacokinetic/pharmacodynamic (PK/PD) correlation for IL-1β and pERK was found, with IL-1β being inhibited at lower concentrations of ARRY-162 than pERK. In the multiple-ascending-dose study, healthy subjects were given daily doses of ARRY-162 ranging from 5 to 60 mg for 14 days. Effects on cytokine inhibition and PK parameters were consistent with the SAD study. Although safety analysis is still ongoing, rash has been the most frequently reported adverse event. However, no serious adverse events have been reported and no clinically significant changes in electrocardiographic or laboratory parameters have been observed (20).

The session continued with Dr. James L. Lewis' (Surface Logix) overview on Rho-associated protein kinase 2 (ROCK2)-selective inhibitors for the treatment of cancer and fibrotic disease. ROCK1 and ROCK2 are two Rho kinases with distinct actions. ROCK1 is preferentially involved in smooth muscle contraction and vascular tone regulation, whereas ROCK2 appears to regulate the actin cytoskeleton by modulating cell proliferation, motility and migration. SLx-2119 is a selective ROCK2 inhibitor (> 100-fold selective over ROCK1) that has been shown to inhibit cell proliferation in several tumor cell lines and also in murine xenograft models. Furthermore, SLx-2119 inhibited human umbilical vein endothelial cell (HUVEC) proliferation and migration and blocked angiogenesis in Matrigel plug assays *in vivo*. In liver fibrotic disease models, SLx-2119 prevented the activation of hepatic stellate cells, the hallmark of liver fibrosis, and blocked the expression of smooth muscle α-actin. Moreover, SLx-2119 displayed liver protection in murine models of septic liver injury, where it prevented the rise in liver enzymes and increased survival (see section on Future therapies) (21). Another ROCK2 inhibitor, SLx-3060 has shown therapeutic potential in models of renal fibrosis, such as the kidney unilateral ureter-ligated obstruction (UUO) model, where treatment with SLx-3060 resulted in a marked decrease in macrophage infiltration, fibronectin and smooth muscle α-actin accumulation.

Dr. Andrew J. Dannenberg's (Cornell University, Weill Medical College, USA) presentation centered around cyclooxygenase-2 (COX-2) and its role in cancer and inflammation (22). COX-2 is overexpressed in a variety of malignant conditions (23). COX-2 expression has been found to be correlated with Ras activation status in different stages of colorectal cancer, while it is not present in normal colon tissue. Thus, overexpression of COX-2 in tumors leads to elevated levels of prostaglandin $E_2$ ($PGE_2$), which, via EP receptors, can stimulate cell proliferation and survival. In fact, $PGE_2$ effects on colon carcinogenesis are mediated via $EP_4$ receptor activation (24). Furthermore, numerous epidemiological studies have found a decreased risk of colorectal cancer with nonsteroidal antiinflammatory drug (NSAID) use, and evidence from a randomized, placebo-controlled trial established that celecoxib was effective in the prevention of colorectal adenomas (25). Dr. Dannenberg also pointed out the importance of the microsomal prostaglandin E synthase-1 (mPGES-1) enzyme as a common pathogenic mechanism in inflammatory disease and cancer. This enzyme catalyzes the conversion of $PGH_2$ to $PGE_2$ and is markedly induced by proinflammatory stimuli, downregulated by antiinflammatory glucocorticoids and functionally coupled with COX-2. mPGES overexpression has also been identified in IBD (26) and colorectal adenomas and carcinomas (27). Interestingly, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), an essential $PGE_2$-degrading enzyme, has emerged as a potential target for therapeutic intervention in both cancer and inflammation. 15-PGDH is commonly found in normal human gastrointestinal mucosa, but not in malignant colon and gastric epithelium. Restoration of 15-PGDH expression to normal colon levels has been related with marked suppression of the ability of these cells to grow aberrantly. According to these findings, 15-PGDH has been described as a COX-2 oncogene antagonist (28, 29). Moreover, the levels of 15-PGDH (protein and mRNA) are diminished in IBD, which contributes to the elevated $PGE_2$ concentration associated with this condition (30).

### Proteases in inflammation: proteinase-activated receptors (PARs) as drug targets

Recent research has implicated proteases in the pathophysiology of several inflammatory conditions, including psoriasis, atopic dermatitis and inflammatory airways disease. In addition, a role for proteases in mediating neurogenic and inflammatory pain has recently been proposed, opening a new avenue for the treatment of this incapacitating condition.

Dr. Martin Steinhoff (University Hospital Münster, Germany) opened this session examining the role of PARs in cutaneous inflammation (31). The role of endogenous serine proteases in skin biology and disease has long been known. Thus, the expression of PARs in keratinocytes, dendritic cells, endothelial cells and leukocytes has suggested that they may be conveying the signals between the skin and the immune system. In models of experimental contact dermatitis, PAR2 activation stimulates chemokine and cytokine release and induces upregulation of E-selectin and other cell adhesion molecules. In addition, knocking down the *PAR2* gene attenuates the inflammatory reaction, particularly in the early phase of skin inflammation (32). PAR2 receptors are also expressed on sensory nerve terminals, where they are thought to mediate neuroinflammation and pruritus upon activation by plasma serine proteases such as kallikreins. Further evidence has shown that tryptase and PAR2 are upregulated in sensory nerves of patients with atopic dermatitis, and PAR2 agonists have been shown to cause pruritus (33). Thus, antagonizing PAR2 receptors appears

beneficial for the treatment of inflammatory skin conditions, such as psoriasis, atopic dermatitis and pruritus.

Dr. Jason McDougall from the University of Calgary, Canada, focused his talk on another PAR family member, PAR4, and its involvement in inflammatory joint disease (34). The hallmark of the disease is the formation of the pannus, or synovial hypertrophy, which is an inflammatory tissue with increased protease activity that can attack and destroy articular cartilage. Without cartilage protection, pannus can reach the soft subchondral bone and degrade it. The signs and symptoms of joint inflammation include joint stiffness, swollen joints that are warm to touch and pain. From the four PARs that have been identified (PAR1 to PAR4), PAR1 and PAR2 have been implicated in the pathogenesis of arthritis, but data relating to other PARs have been scarce. Recent findings have delineated the role of PAR4 in inflammatory joint disease. In rodent paw inflammation models, PAR4 was shown to mediate the main features of inflammation, as its activation (via a PAR4-activating peptide) caused mouse joint hyperemia and edema, whereas the PAR4 antagonist pepducin, a bradykinin $B_2$ antagonist and plasma kallikrein inhibitor, counteracted these effects (35), suggesting a key role for PAR4 in the inflammatory response. Moreover, PAR4 also appears essential in mediating joint pain. Electrophysiological recordings of single knee joint primary afferents have revealed increased spontaneous nerve activity in response to knee noxious hyperrotation, indicating sensory nerve hyperexcitability. Injection of a PAR4-activating peptide enhanced the firing rate of knee nociceptors during normal and hyperrotation (36). Therefore, inhibition of PAR4 activity appears to be a promising strategy that may help to reduce joint pain, as well as attenuate joint destruction, due to proteolysis inhibition.

The session continued with the intervention of Dr. Nathalie Vergnolle, also from the University of Calgary, who presented additional data on PARs and signaling to sensory nerves during neurogenic inflammation and inflammatory pain (37). Three members of the PAR family (PAR1, 2 and 4) have been identified in peripheral sensory neurons, where they can be activated by different proteases. Research carried out by Dr. Vergnolle's group revealed that in sensory neurons *in vitro*, the activation of PAR1 and PAR2 causes the release of the inflammatory peptides substance P (SP) and calcitonin gene-related peptide (CGRP), while activation of PAR4 inhibits neuropeptide release, indicating the complexity of PAR-mediated signals in inflammation. Recent findings have demonstrated a role for PAR2 in the development of mechanical hyperalgesia and allodynia (exaggerated sensitivity to mildly painful or non-noxious stimuli). These effects of PAR2 activation appear to be mediated via intracellular calcium enhancement and subsequent sensitization of the transient receptor potential vanilloid 4 (TRPV4) ion channel to cause the release of nociceptive peptides (SP and CRGP) (38). Similarly, TRPV1 channels have been found to be sensitized by PAR2 receptor activation by a protein kinase C (PKC)-dependent mechanism to induce thermal hyperalgesia (39). The identification of this crosstalk between PAR and TRPV receptors has shed new light on the regulation of neurogenic and inflammatory pain signaling, and provided new clues for the development of novel analgesic/antiinflammatory drugs. Interestingly, targeting PARs may be useful in other inflammatory conditions featuring pain, such as irritable bowel syndrome (IBS). Cenac *et al.* have recently described increased protease release and proteolytic activity in IBS colonic samples, which could stimulate sensory neurons and mediate visceral hypersensitivity —the hallmark of IBS— via the activation of PAR2 receptors (40).

Dr. Vincent Lagente from the French University of Rennes reviewed the role of matrix metalloproteinases (MMPs) in the development of inflammatory airways diseases (41). Lung diseases feature excessive airways tissue remodeling due to an imbalance between the synthesis and degradation of extracellular matrix components. For instance, MMP-12 expression is increased in lung tissue of patients with COPD and contributes to the development of emphysema (42). Furthermore, MMP-12 also induces an early inflammatory response consisting of neutrophil infiltration, cytokine release and gelatinase activation (43). The selective MMP-12 inhibitor AS-111793 (Merck Serono) has been found to dose-dependently reduce neutrophil infiltration and general airways inflammation in mice exposed to cigarette smoke (Le Quément, 2007, in press). Thus, targeting MMP-12 may attenuate the acute inflammatory response. Furthermore, in a bleomycin-induced mouse model of pulmonary fibrosis, broad inhibition of MMPs with batimastat attenuated fibrosis and decreased the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) (44), suggesting that increased TIMP production may contribute to the development of fibrosis.

### *The crosstalk between atherosclerosis and chronic inflammation: macrophage migration-inhibitory factor (MIF)*

Macrophage migration-inhibitory factor (MIF) is a well-known proinflammatory cytokine that is expressed in a variety of cell types, including macrophages, T- and B-lymphocytes. It induces the expression of proinflammatory cytokines such as IL-1$\beta$, TNF-$\alpha$, IL-2, IL-6, IL-8 and IFN-$\gamma$, and is also able to increase NO production. MIF has also been implicated in the pathogenesis of different immunoinflammatory disorders, such as RA or SLE. However, evidence is emerging for MIF as a link between atherosclerosis and chronic inflammatory diseases, as Dr. Eric Morand from Monash University, Victoria, Australia, pointed out (45).

A series of cellular mechanisms are shared in atherosclerosis (A) and RA: 1) leukocyte recruitment to the vessel wall (A) and to the synovial tissue (RA); 2) prolonged survival of macrophages and T-cells, which are important pathogenic mediators; 3) increased survival and activation of vascular smooth muscle cells (A) and synovial fibroblasts (RA); and 4) local MMP secretion that may induce plaque rupture (A) and cartilage destruction (RA).

Substantial evidence has emerged to propose MIF as a mediator of most of these cellular events. About a decade ago, evidence reporting the participation of MIF in experimental models of RA started to appear (46). MIF is overexpressed in synovial tissues, as well as in the plasma of arthritic animals, where it mediates leukocyte recruitment to the joint, thus contributing to RA joint pathology. More recent evidence in humans showed that high circulating levels of MIF in RA are genetically determined by polymorphisms in the *MIF* gene and strongly correlate with more severe radiological joint damage (47). Moreover, elevated MIF levels were associated with the progression of joint destruction during the first years of disease, indicating that MIF could serve as a biomarker for RA severity. In RA, there is also a correlation between MIF and C-reactive protein (CRP), a typical inflammation marker. Recently, MIF has been found in human atheroma lesions and increased expression is associated with lesion size and progression (46). MIF was preferentially expressed on macrophages and foam cells of advanced atherosclerostic plaques. In mouse models of atherogenesis (ApoE-null mice and LDL-null mice fed a high-fat diet), MIF inhibition, either via antibody blockade or gene deletion, reduced the formation of atherosclerotic plaque (48, 49). Importantly, lipid molecules —the initiators of atheroma formation— are able to induce MIF expression, explaining the inflammatory nature of atherosclerotic lesions. As for its role in SLE, studies in lupus-prone mice have reported an improvement in disease symptoms, such as a reduction in proteinuria, interstitial inflammation and glomeruli sclerosis, with *MIF* gene deletion (50). In addition, *MIF* gene polymorphisms have been shown to confer higher susceptibility to SLE (51).

Activation of the MAPK pathway and downregulation of p53 expression and function are two known molecular mechanisms of MIF's action that could be functioning in RA, SLE and atherosclerosis. Thus, p53 deficiency has been associated with increased RA and atheroma severity in experimental models, and MAPK signaling is known to be activated in atherosclerosis, although their relationship to MIF has not yet been established (46).

In addition, there is also an augmented risk of atherosclerosis in SLE and RA patients, which, hypothetically, could be related to MIF overlapping actions in these diseases. As mentioned earlier, elevated circulating MIF levels have been identified in RA patients and also in SLE patients, which could promote inflammatory responses (macrophage/foam cell activation) important for the development of atherosclerosis. Moreover, glucocorticoids are well-characterized activators of MIF expression. Their widespread use in chronic inflammatory diseases, such as RA and SLE, could contribute to the development of atherosclerosis via MIF induction (46). Despite its upregulation by glucocorticoids, MIF actually counteracts glucocorticoid actions, functioning as an endogenous antagonist of glucocorticoid activity.

Therefore, targeting MIF is a therapeutic option currently under investigation at different laboratories for the treatment of inflammatory diseases. Details of two MIF antagonist strategies were presented at the 8th World Congress on Inflammation and are described in the Future therapies section.

### *Targeting IL-1β in inflammatory disease*

1. Pannexin-1

ATP acting on $P2X_7$ receptors ($P2X_7R$) in macrophages is one of the main physiological signals that leads to the processing and release of the proinflammatory cytokine IL-1β via caspase-1 activation, which cleaves the inactive precursor of IL-1β (Fig. 1). IL-1β is a potent inflammatory mediator released in response to infection and systemic inflammation. High concentrations of ATP, like those found at sites of injury or inflammation, are required for the activation of $P2X_7Rs$ and subsequent rapid opening of a membrane pore (52). Recent work by Dr. Pablo Pelegrín and Dr. Annmarie Surprenant at the University of Sheffield, UK, has provided new insight into the mechanism and physiological relevance of this ATP-driven $P2X_7R$-mediated pore opening. This putative $P2X_7R$-activated pore was identified as pannexin-1 (53), a member of the pannexin family of proteins related to invertebrate innexins and mammalian connexins, both responsible for the formation of intercellular channels called gap junctions. Pannexin-1 ectopic expression in oocytes has demonstrated that they can form hemichannels permeable to ATP (54).

Pannexin-1, which is highly expressed in both immune and nonimmune cell types, co-immunoprecipitated with $P2X_7Rs$ when transfected into $P2X_7$-expressing HeLa cells and localized to the plasma membrane. Knocking down pannexin-1 with short interference RNA (siRNA) or a peptide inhibitor blocked $P2X_7$-induced dye uptake (a measure of pore opening), but did not alter ATP-evoked ion currents, indicating that the $P2X_7$ receptor remained functional. Furthermore, pannexin-1 was upregulated in macrophages in response to inflammatory lipopolysaccharide (LPS) challenge and its selective inhibition in activated macrophages blocked ATP-induced release of IL-1β via inhibition of caspase-1 cleavage and processing, clearly indicating that pannexin-1 is essential for the release of mature IL-1β. The precise mechanism by which pannexin-1 pore opening in response to $P2X_7R$ activation leads to caspase-1 activation is not yet clear, although researchers have proposed that this pore pathway may be a passage for extracellular ATP to enter the cell and activate caspase-1. Interestingly, further evidence has shown that pannexin-1 is also required for IL-1β processing and release in response to nigericin and maitotoxin, two well-characterized nonphysiological stimuli that trigger IL-1β release. These two molecules appear to couple to pannexin-1-mediated IL-1β release via a mechanism independent of pore formation, which suggests the presence of an alternative pannexin-1-mediated signaling pathway that could be involved in activation of the cryopirin (or NALP3) inflammasome (see next section) (55), although this remains to be determined.

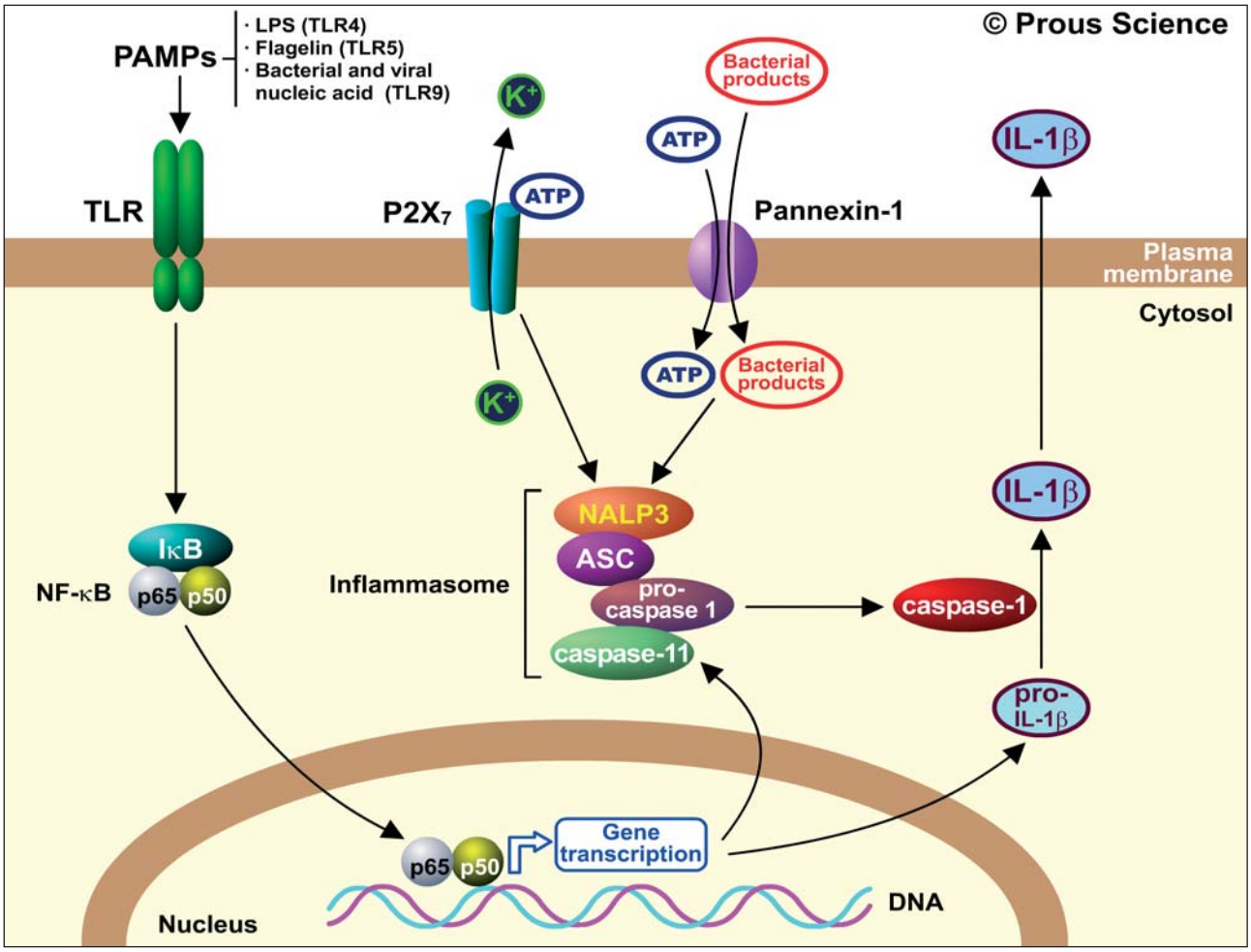
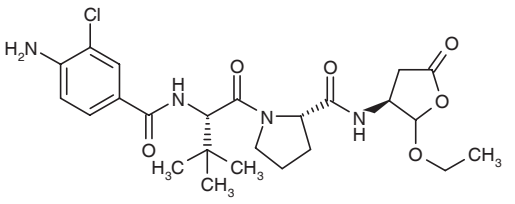



Fig.1. IL-1β release by activation of the NALP3 inflammasome. Binding of pathogen-associated molecular patterns (PAMPs) to Toll-like receptors (TLRs) triggers the activation of NF-κB and subsequent production of the IL-1β precursor (pro-IL-1β). A second signal for caspase-1 activation is activation of $P2X_7$ receptors by ATP. Bacterial products or tissue injury signals (ATP) have also been hypothesized to enter the cytosol via pannexin-1 hemichannels, which may activate the NALP3 inflammasome. The NALP3 protein together with the apoptosis-associated speck-like (ASC) protein conform the molecular scaffold for caspase-1 activation. Activated caspase-1 cleaves pro-IL-1β to release active IL-1β, which will be secreted to the extracellular space to act on its receptors in the periphery and in the CNS.

## 2. The NALP3 inflammasome

Commonly known as "the inflammasome", this term refers to a cytosolic multiprotein complex responsible for caspase-1 activation, which is essential for the processing and subsequent release of IL-1β. IL-1β is a potent proinflammatory cytokine that plays a crucial role in the immune response against infectious pathogens, but which has also been linked to a number of systemic inflammatory disorders. The NALP proteins (NALP1, 2 and 3) are the central components of the inflammasome, which is also formed by the apoptosis-associated speck-like (ASC) adaptor protein and caspase-1 (Fig. 1). Activating mutations in the *NALP3* gene (also known as *CIAS1*) have been associated with three hereditary autoinflammatory disorders: familial cold autoinflammatory syndrome, Muckle-Wells syndrome and neonatal-onset multisystem inflammatory disease (NOMID) (56). These mutations lead to increased activity of the inflammasome and excessive IL-1β production. Active IL-1β secreted by monocytes or macrophages binds to IL-1 receptors in the hypothalamus vasculature, which trigger the production of $PGE_2$, which in turn activates the thermoregulatory center to cause fever. In the periphery, active IL-1β is responsible for a broad range of manifestations from skin rash to thrombocytosis (57). The NALP3 inflammasome is sensitive to a variety of pathogen-associated molecular patterns (PAMPs) that bind to Toll-like receptors (TLRs) (56), although it has been shown that bacterial products can also activate the NALP3 inflammasome via a pannexin-1-mediated conduit independent of TLR activation (58). As mentioned earlier, injury-related signals such as elevated extracellular ATP also appear to activate the NALP3 inflammasome via a $P2X_7$/pannexin-1-dependent mechanism (55). Recently, unrelated stimuli, such as gout-associated uric acid crystals (59) and contact sensitizers (60), have been found to activate the NALP3 inflammasome,



VX-765 (**2**)

suggesting that it acts as an endogenous sensor of danger signals in different tissues (61).

Together, these findings indicate a role for the NALP3 inflammasome as an intracellular regulator of infection and inflammation and provide new clues to understand the pathogenesis of these various disorders. The development of drugs targeting components of the NALP3 inflammasome is still in the early stage. Thus, the caspase-1 inhibitor VX-765 (**2**), which is currently in phase II clinical development at Vertex for the treatment of psoriasis, has also been shown to block IL-1β secretion from monocytes of patients with familial cold autoinflammatory syndrome in preclinical studies (62). Several anti-IL-1β monoclonal antibodies (ACZ-885, Novartis; AMG-108, Amgen; XMA-005.2, Xoma) have entered the clinic for the treatment of chronic inflammation. Moreover, ACZ-885 is also being tested in patients with *NALP3* mutations (63) and Muckle-Wells syndrome (64).

## Future therapies

The final session of the meeting, together with selected poster presentations, covered the latest advances in investigational therapies for the treatment of chronic inflammatory disorders.

### *Complement inhibitors*

Activation of the complement cascade has been demonstrated in chronic inflammatory disorders such as RA. The complement component C5a binds the C5a receptor (C5aR) and facilitates leukocyte chemotaxis and the release of inflammatory mediators. Researchers at G2 Inflammation (a subsidiary of G2 Therapies) have described the generation of novel monoclonal antibodies to the C5aR using human C5aR knockin mice, in which the C5aR coding region had been replaced by the human C5aR sequence. Neutrophils from these animals were injected into wild-type mice to produce high-affinity anti-human C5aR monoclonal antibodies, such as 7F3. 7F3 was tested in the K/BxN serum transfer model of inflammatory arthritis, where it dose-dependently prevented inflammatory signs and symptoms when given prophylactically (1, 3 and 10 mg/kg i.p.) before K/BxN serum injection. Moreover, a single therapeutic i.p. dose of 7F3 5 days after serum transfer was able to dose-dependently reverse established disease, with reductions in swelling, synovial leukocyte infiltration and cartilage erosion. Established collagen-induced arthritis (CIA) was also reversed by 7F3, which suppressed clinical scores and paw size of CIA mice (65).

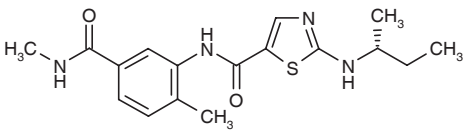
### *MIF antagonists*

MIF is a proinflammatory cytokine involved in innate and adaptive immunity that has been implicated in inflammatory diseases. Moreover, MIF inhibition has demonstrated benefit in animal models of inflammatory arthritis. COR-100140, a novel orally available molecule developed at Cortical, was tested in a mouse model of CIA with promising results. Administration of 60 mg/kg/day COR-100140 via oral gavage resulted in a significant decrease in established arthritis clinical scores compared to etanercept (25 mg/kg). In addition, COR-100140 reduced serum levels of monocyte chemotactic protein-1 (MCP-1) and TNF. In models of antigen-induced arthritis, COR-100140 (15 mg/kg/day) was able to markedly reduce histological pathology. These findings support clinical investigation with COR-100140 (66).

Sepsis is a life-threatening condition caused by an overwhelming bacterial infection. Current options for treating sepsis include corticosteroids and, more recently, drotrecogin alfa. However, these medications are not exempt of risks and adverse reactions. As MIF is an endogenous antagonist of glucocorticoids, which also induce MIF expression, therapeutic MIF inhibition will have steroid-sparing consequences. Additionally, MIF plays a role in the late stages of the sepsis cascade. Scientists at the Chinese Academy of Sciences have developed a mouse monoclonal antibody against MIF, namely 10C3, that binds MIF with high affinity ($K_d$ = 3 nM). 10C3 inhibited MIF-induced NO and TNF-α secretion in the macrophage cell line RAW 264.7. *In vivo,* 10C3 (5 mg/kg) protected mice against LPS-induced sepsis and showed synergy with dexamethasone (1 mg/kg), increasing protection by 50% (67).

### *MAPK inhibitors*

The p38 MAPK pathway regulates the release of several inflammatory mediators, including IL-1β and TNF. Researchers at LEO Pharma have discovered LEO-15520, a selective p38 MAPK inhibitor that may have potential in treating inflammatory diseases such as psoriasis and RA. LEO-15520 potently inhibits p38 MAPK ($IC_{50}$ = 1.6 nM) and has shown 99% selectivity over a panel of 80 kinases. It was also able to block LPS-induced release of TNF-α, IL-1, IL-6 and IL-8 from monocytes/macrophages with $IC_{50}$ values of 0.3, 2.3, 5.2 and 0.5 nM, respectively. *In vitro*, LEO-15520 suppressed human T-cell proliferation and IL-2 release after anti-CD3 stimulation. In murine models of CIA, LEO-15520 10 mg/kg significantly inhibited joint inflammation and bone destruction compared to vehicle and showed similar efficacy to prednisolone. Furthermore, pretreatment with LEO-15520 dose-dependently inhibited UVB-induced skin inflammation in guinea pigs after oral treatment at 10



**3**

and 30 mg/kg. At doses of 20 mg/kg, LEO-15520 normalized epidermal thickness in mice bearing psoriatic skin xenografts after 14 days of treatment, with superior efficacy compared to dexamethasone (1 mg/kg). Keratinocyte proliferation could also be inhibited by LEO-15520 at 20 mg/kg (68). LEO-15520 is currently undergoing phase I clinical studies for the treatment of inflammatory diseases.

Researchers at Bristol-Myers Squibb have reported the development of novel inhibitors of p38 MAPK. Compound **3** appeared to be a selective and potent inhibitor of p38$\alpha$ MAPK (p38$\alpha$), with an $IC_{50}$ value of 3.5 nM, and was also able to block LPS-induced TNF-$\alpha$ release by 46% ($IC_{50}$ = 2.9 nM). In rats administered an oral dose of 10 mg/kg, compound **3** exhibited an oral bioavailability of 75%, an elimination half-life of 1.8 h, a volume of distriubution of 1.8 l/kg and a clearance of 14.1 ml/min/kg. Pharmacokinetics were linear to greater than linear at higher doses, indicating adequate drug coverage. In a model of adjuvant-induced arthritis in rats, **3** demonstrated efficacy, with a 46% reduction in paw swelling at a minimum effective dose of 0.3 mg/kg once daily (69).

Scientists at Array BioPharma have reported new phase I data on ARRY-797, a potent, orally available p38 MAPK inhibitor that has shown good preclinical activity in models of RA. A single-ascending-dose phase I trial was carried out in healthy volunteers who received oral doses of ARRY-797 of 25, 50, 100, 200, 300 and 400 mg/day. Overall, ARRY-797 was well tolerated, with no serious adverse events observed. Pharmacokinetic studies revealed a linear increase in drug exposure with increasing doses. Moreover, *ex vivo* stimulation with LPS of blood samples from subjects receiving ARRY-797 resulted in > 90% inhibition of IL-1$\beta$, TNF-$\alpha$ and $PGE_2$ after a single dose of 50 mg, which persisted for more than 4 h. At the highest dose, more potent cytokine inhibition and a longer duration of action were seen. A further multiple-ascending-dose study was initiated in June 2007 (70).

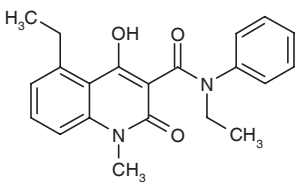
### *ROCK inhibitors*

ROCK inhibition appears to be a potentially useful strategy to inhibit septic liver injury, according to a recent study performed at Surface Logix. The potent and selective ROCK2 inhibitor SLx-2119 was tested in an LPS/D-galactosamine-induced murine model of septic liver injury. Pretreatment with SLx-2119 (100 mg/kg p.o. 2 and 20 h prechallenge) produced a > 90% reduction in liver enzyme (AST, ALT) elevation compared to vehicle- and fasudil-treated mice. Both prophylactic and therapeutic administration of SLx-2119 was effective in preventing the rise in ALT and AST levels. Prophylactic treatment with SLx-2119 given 15 min before challenge completely protected animals from death at 24 h, whereas therapeutic treatment was less effective in increasing animal survival (21).

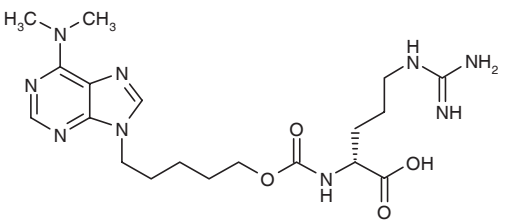
ROCK2 is essential for the regulation of actin cytoskeleton architecture by modulating cell proliferation, motility and migration. Inhibition of ROCK2 activity has been suggested to have therapeutic potential in fibrotic disease. Researchers at Surface Logix have developed SLx-3060, another selective ROCK2 inhibitor ($IC_{50}$ = 60 nM) that has shown benefit in models of renal fibrosis, such as the kidney UUO model, where treatment with SLx-3060 resulted in a marked decrease in macrophage infiltration, fibronectin and smooth muscle $\alpha$-actin accumulation. SLx-3060 reduced smooth muscle $\alpha$-actin immunostaining by 75% in the treated kidney. SLx-3060 also suppressed transforming growth factor (TGF)-$\beta$ expression, which physiologically plays a central role in tissue repair, but in excess is known to contribute to tissue fibrosis (71).

### *Other immunomodulators*

Paquinimod (ABR-215757, **4**) is a novel immunomodulating agent currently in early clinical development at Active Biotech for the oral treatment of SLE and RA. A recent study reported on the steroid-sparing effect of paquinimod in experimental lupus models. Switching from high-dose prednisolone treatment (2 mg/kg/day) to combined therapy with low-dose prednisolone (0.5 mg/kg/day) and paquinimod (0.2 mg/kg/day) resulted in a significant inhibition of hematuria and glomerulonephritis in MRL*/lpr/lpr* mice. In addition, combination therapy also reduced spleen enlargement and $CD4^+$, $CD8^+$ and $CD4^-$ $CD8^-$ T-cells. These results suggest that paquinimod may



Paquinimod (**4**)



PBI-1393 (**5**)

be useful for decreasing corticosteroid doses in SLE patients (72).

ProMetic BioSciences investigators have reported preclinical findings on PBI-1393 (**5**), which is currently undergoing phase I/II clinical studies in cancer. PBI-1393's ability to modulate Th1 cytokine production by T-lymphocytes was evaluated in human polymorphonuclear neutrophils (PMNs) stimulated with LPS. PBI-1393 reduced the production of LPS-induced TNF-$\alpha$ *in vitro* by 22% and 35%, respectively, at concentrations of 100 and 1 nM, but it had no significant effect on neutrophil infiltration. *In vivo*, PBI-1393 at 25 and 100 mg/kg also decreased TNF-$\alpha$ release by 47% and 53%, respectively, as well as LPS-stimulated $PGE_2$ production by 29% at a dose of 100 mg/kg. In LPS-treated rats, PBI-1393 reduced MCP-1 production, suggesting reduced monocyte/macrophage recruitment to the inflammation site (73).

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