Mitogen activated protein (MAP) kinase signal transduction pathways and novel anti-inflammatory targets

D W Hommes, M P Peppelenbosch, S J H van Deventer

Over the last decade important advances have been made in our understanding of the molecular events underlying cellular responses to extracellular signals. Increased understanding of signal transduction mechanisms and gene regulation involved in immune responses has created opportunities for the discovery of novel therapeutic compounds useful in treating inflammatory disorders. One of the best studied signalling routes is the mitogen activated protein (MAP) kinase signal transduction pathway which plays a crucial role in many aspects of immune mediated inflammatory responses. Here, our current understanding of the MAP kinase pathway is reviewed, as well as recent advances in the design of novel agents that are able to modulate the activity of these signalling cascades.

SUMMARY
Proper regulation of genes in all forms of cellular life is dependent on intracellular regulatory circuits or signal transduction pathways. Among eukaryotic cells, phosphorylation of intracellular factors followed by specific gene transcription is a universal outcome of such signal transduction pathways, and the major elements of such pathways are similar in species as different as humans, fungi, and plants. In mammalian species, these ancient regulatory circuits maintain the balanced gene transcription necessary for correct cell growth, differentiation, and death. Scientific interest in these information highways from the cell surface to the nucleus has exploded over the past years, and understanding of the biology of these signalling cascades has progressed dramatically. One of the best studied signalling routes is the mitogen activated protein (MAP) kinase signal transduction pathway which plays a crucial role in many aspects of immune mediated inflammatory responses. Hence members of this family of kinases have come to be appreciated as key cellular signal transducers and attractive targets for drug development. This has led to current initiation of clinical trials in inflammatory disease states evaluating small molecule inhibitors of MAP kinase proteins and encouraging results have been obtained. Because it is anticipated that several small MAP kinase inhibitors will be evaluated for efficacy in inflammatory diseases, here we review current knowledge of the MAP kinase signalling pathways as well as potential inhibitory drugs.

INTRODUCTION
During the last decade important advances have been made in our understanding of the molecular events underlying cellular responses to extracellular signals. This scientific progress has set the stage for an exciting new field of research: pharmacological modulation of signal transduction pathways to control gene expression. Altered gene expression plays a key role in the pathogenesis of many inflammatory diseases such as pancreatitis, rheumatoid arthritis, hepatitis, psoriasis, and inflammatory bowel disease. It is only in recent years that signal transduction mechanisms responsible for inducing inflammatory gene expression have been identified. These mechanisms seem fundamental in the initiation of inflammatory responses. Products of induced inflammatory genes include cytokines, chemokines, and adhesion molecules that serve to promote recruitment of immunocompetent cells from the circulation to the affected site which results in an inflammatory injury. It is for this reason that these intracellular pathways have come to be appreciated as attractive targets for drug development.

The aim of this review is to present the current understanding of the MAP kinase pathway, a key mechanism of inflammatory signal transduction in eukaryotic cells. Furthermore, we will review recent advances in the design of novel agents that are able to modulate the activity of these signalling cascades.

SIGNAL TRANSDUCTION AND CONTROL OF GENE EXPRESSION.
There are two major routes from extracellular signalling proteins to changes in gene transcription (fig 1). Firstly, several intracellular self propagating phosphorylation cascades have been identified that activate different resident nuclear transcription factors. Secondly, a number of latent cytoplasmic transcription factors are activated after cell surface receptor-ligand interactions that

Abbreviations: AP-1, activator protein 1; ATF-2, activating transcription factor 2; ERK, extracellular signal regulated kinase; IFN, interferon; IL, interleukin; JAK, Janus kinase; JNK, c-Jun NH2 terminal kinase; LPS, lipopolysaccharide; MAP, mitogen activated protein; MAPK, MAP kinase; MAPKAP, MAP kinase activated protein kinase; MAPKK, MAP kinase kinase; MAPKKK, MAP kinase kinase kinase; MEF, myocyte enhance factor; MEK, MAP/ERK kinase; MKK, MAP kinase kinase; NF, nuclear factor; STAT, signal transducers and activators of transcription; TGF, transforming growth factor; TNF, tumour necrosis factor.

See end of article for authors’ affiliations

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Accepted for publication 3 September 2002

Gut 2003;52:144–151

www.gutjnl.com
accumulate in the nucleus to drive transcription. Recently, a classification system for transcription factors was proposed to structure this still increasing repertoire of intracellular proteins regulating cell development and cell specialisation.

MAP kinases, which belong to a large family of serine/threonine kinases, constitute major inflammatory signalling highways from the cell surface to the nucleus. Other kinase cascades include those that are activated after increases in intracellular second messengers (including CAMP and Ca\(^{2+}\)) and ligand interaction with G coupled cell surface receptor proteins and receptor tyrosine kinases. Usually such pathways result in the phosphorylation and subsequent activation of transcription factors, producing altered gene transcription.

Eight principal pathways for activation of latent cytoplasmic transcription factors are now recognised. The two pathways that are directly activated at the cell surface receptor are the family of SMAD molecules that are involved in signal transduction of the transforming growth factor \(\beta\) (TGF-\(\beta\)) superfamily of ligands, and the STAT family (signal transducers and activators of transcription) which are activated by more than 20 cytokines sharing Janus kinase (JAK) associated receptors that phosphorylate the respective receptors on tyrosine residues to initiate signal transduction. In short, inactive JAK enzymes are loosely attached to the cytoplasmic domains of specific cytokine receptors (including interferon (IFN)-\(\alpha/\beta/\gamma\), interleukin (IL)-2, IL-4, IL-6, IL-10, and IL-12). After ligand binding, the receptor associated JAKs become active through phosphorylation, and in their turn phosphorylate tyrosine residues in the cytoplasmic portions of the clustered receptors. Subsequently, cytosolic STAT proteins recognise these phosphoryrosine moieties and are phosphorylated after attachment to the receptors by receptor associated JAKs. After binding of two STAT proteins, they dissociate from the receptor and migrate to the nucleus where they bind to DNA sequences in the promoter regions of cytokine responsive genes and activate gene transcription. STAT activation may have important consequences. For instance, in T helper cell differentiation, STAT4 activation is sufficient to confer a T helper 1 cell phenotype whereas STAT6 activation causes transition from a precursor cell to a T helper 2 cell.

“The Wnt signalling pathway is pivotal in normal and malignant development”

Four other important pathways that can activate latent transcription factors require cytoplasmic serine phosphorylation and/or proteolysis. This group includes the Rel/nuclear factor \(\kappa\)B (NF\(\kappa\)B) family which is activated by, and essential for, correct response to a wide variety of extracellular stimuli such as tumour necrosis factor \(\alpha\) (TNF-\(\alpha\)), IL-1, and viral and bacterial products. This group also includes the Wnt pathway and the Notch pathway. The Wnt signalling pathway is pivotal in normal and malignant development. For example, adenomatous polyposis coli protein has been thought to function as a tumour suppressor through its involvement in the Wnt/\(\beta\)catenin signalling pathway. The Notch signalling pathway has been implicated in lymphopoiesis: recent data suggest that Notch activity plays a pivotal role in T cell lineage commitment from the common lymphoid progenitor. Furthermore, Notch signalling seems crucial in the developing nervous system; its function determines cell fate from the first segregation of neuronal precursors to the terminal specification of cells as neurones and glia.

Finally, fluctuations in cytoplasmic second messengers (Ca\(^{2+}\) and phosphoinositide concentrations) can activate latent cytoplasmic transcription factors such as nuclear factors in activated T cells (NFAT) and Tubby, a product of a gene which is associated with obesity. A detailed analysis of the aforementioned signalling mechanisms lies beyond the aim of this review, and we will focus on the most ancient and conserved signalling pathway that is pivotal during immune responses: the MAP kinase pathway.

**MAP KINASE PATHWAY**

MAP kinases are an evolutionarily conserved family of enzymes that form a highly integrated network required to achieve specialised cell functions controlling cell differentiation, cell proliferation, and cell death. These cytoplasmic proteins can modulate the activities of other intracellular proteins by adding phosphate groups to their serine/threonine amino acids (fig 2). To date, over 20 MAP kinase isoforms have been reported, which are summarised in table 1. Activation of MAP kinase enzymes themselves is unusual in that they require phosphorylation on both a threonine (Thr) and tyrosine (Tyr) residue and thus need the activity of dual specificity kinases which are known as MAP/ERK kinases (MEKs) or MAP

![Diagram](http://group.bmj.com/)

**Figure 1** Different routes from extracellular signalling proteins to changes in gene transcription. Left: Intracellular phosphorylation cascades activate nuclear transcription factors. Right: Latent cytoplasmic transcription factors are activated on receptor-ligand interaction.
kinase kinases (the phosphorylating potential of all other mammalian kinase families is restricted to either phosphorylation of serine/threonine residues or tyrosine residues). The specificity of MEK for MAP kinase is context dependent: all MEK enzymes exert their dual phosphorylation on Thr-Xxx-Tyr amino acid sequence motifs but the X confers specificity. The amino acid X is glutamic acid (Glu) for extracellular regulated protein kinase (ERK), proline (Pro) for c-Jun NH2 terminal kinase (JNK), and glycine (Gly) for p38 MAP kinase. ERK MAP kinases are activated by MAP kinase kinase (MKK) 1 and MKK2, JNK by MKK4 and MKK7, and p38 MAP kinase by MKK3, MKK4, and MKK6 (fig 3). Hence activation of a specific MEK will result in a defined set of further cellular consequences.

The activity of MEKs is in turn controlled by phosphorylation, phosphorylated MEKs being enzymatically active. This phosphorylation status is under the critical control of so-called MAP kinase kinase kinases (MAPKKK, MKKK, or MEKK), a family of proteins of which the c-Raf proto-oncogene is the most prominent member. The control of this family of enzymes and their substrate specificity is still only partially understood.

On activation of MAP kinases, transcription factors present in the cytoplasm or nucleus are phosphorylated and activated, leading to expression of certain target genes resulting in a biological response. The multiple interactions between the different MAP kinase cascades serve to integrate responses and to moderate outputs. Indeed, it has been demonstrated that MAP kinases have overlapping substrate specificities and phosphorylation of regulatory sites is shared among multiple protein kinases.

As noted above, three major groups of distinctly regulated groups of MAP kinase cascades are known in humans that lead to altered gene expression: ERK1/2, JNK, and p38 MAP kinase.

**Table 1** Mitogen activated protein (MAP) kinases

<table>
<thead>
<tr>
<th>MAP kinase Isoform</th>
<th>Alternative name</th>
<th>Substrate</th>
<th>Phenotypes of MAP kinase knockout mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERK 1</td>
<td>p44 MAPK</td>
<td>MAPKAP-K1, MNKs, MSKs, Elk1</td>
<td>Defective T cell development</td>
</tr>
<tr>
<td>ERK 2</td>
<td>p42 MAPK</td>
<td>MAPKAP-K1, MNKs, MSKs, Elk1</td>
<td></td>
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<tr>
<td>ERK 3α</td>
<td>p63 MAPK</td>
<td>MNKs, MSKs</td>
<td></td>
</tr>
<tr>
<td>ERK 3β</td>
<td>human ERK3</td>
<td>MNKs, MSKs</td>
<td></td>
</tr>
<tr>
<td>ERK 4</td>
<td>ERK1b</td>
<td>MNKs, MSKs</td>
<td></td>
</tr>
<tr>
<td>ERK 5</td>
<td></td>
<td>MNKs, MSKs</td>
<td></td>
</tr>
<tr>
<td>ERK 7</td>
<td></td>
<td>MNKs, MSKs</td>
<td></td>
</tr>
<tr>
<td>JNK 1</td>
<td>SAPKγ</td>
<td>c-Jun, JunD, ATF-2, Elk1</td>
<td>Defective T cell differentiation to Th2 cells</td>
</tr>
<tr>
<td>JNK 2</td>
<td>SAPKα</td>
<td>c-Jun, JunD, ATF-2, Elk1</td>
<td>Defective T cell differentiation to Th1 cells</td>
</tr>
<tr>
<td>JNK 3</td>
<td>SAPKβ</td>
<td>c-Jun, JunD, ATF-2, Elk1</td>
<td>Resistance to excitotoxic neuronal cell death</td>
</tr>
<tr>
<td>p38α</td>
<td>CSBP, SAPK2, MPK2, RK, Mxi2</td>
<td>MAPKAP-K2/3, MSKs, ATF-2, Elk1, MEF2c</td>
<td>Placental defect, insufficient production of erythropoietin</td>
</tr>
<tr>
<td>p38β</td>
<td>p38-2, p38ββ</td>
<td>MAPKAP-K2/3, MSKs, ATF-2</td>
<td></td>
</tr>
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<td>p38γ</td>
<td>ERK6, SAPK3</td>
<td>ATF2</td>
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</tr>
<tr>
<td>p38δ</td>
<td>SAPK4</td>
<td>ATF2</td>
<td></td>
</tr>
</tbody>
</table>

ATF-2, activating transcription factor 2; ERK, extracellular signal regulated kinase; JNK, c-Jun NH2 terminal kinase; MAP, mitogen activated protein; MAPK, MAP kinase; MAPKAP, MAP kinase activated protein kinase; MEF, myocyte enhance factor; SAPK, stress activated protein kinase; MNKs, MARK activating protein kinases; MSKs, mitogen and stress activated protein kinases; CSBP, cytokine suppressive anti-inflammatory binding protein; RK, reactivating kinase.
The ERK1/2 signal transduction pathway

The ERK signalling module was the first MAP kinase cascade to be characterised, being a vital mediator of a number of cellular fates including growth, proliferation, and survival. There are two ERK isoforms that are ubiquitously expressed, ERK1 and ERK2, and these are often referred to as p42/p44 MAP kinases. In this cascade, MEK1 and MEK2 function as upstream MAPKK and the Raf proteins as MAPKKK (fig 3). Duration of ERK1/2 activation depends on regulating mechanisms: removal of one or both phosphates by tyrosine, serine/threonine, or phosphatases radically decreases this MAP kinase activity. Furthermore, the specificity of these phosphatases is dependent on their intracellular localisation. ERK1/2 as well as other MAP kinases target not only transcription factors but also membrane proteins (that is, phospholipase A2) and cytoplasmic proteins (that is, downstream kinases). Results from gene knockout experiments have illustrated the importance of the ERK1/2 pathway. Disruption of one of the three Raf proteins known to activate ERK1/2 is invariably fatal in mice. Also, when MEK1 was genetically targeted, embryonic death was observed with signs of tissue necrosis. Precisely how ERK1/2 affects cellular physiology in vivo is poorly understood. Most often an important role is attributed to ERK1/2 dependent regulation of the activating protein 1 (AP-1) family of transcription factors. Members of this family that are phosphorylated by ERK1/2 include c-Jun, c-Fos, and activating transcription factor 2 (ATF-2) but the in vivo relevance of this phosphorylation is not yet clear.

“ERK1/2 as well as other MAP kinases target not only transcription factors but also membrane proteins”

Although the primary role of ERK1/2 mediated signalling has long been thought to be restricted to cell growth and proliferation, it has become clear that several inflammatory processes involve ERK1/2 activation. Erk-1 deficient mice were normal and fertile but showed defective thymocyte maturation and reduced expression of α and β chains of the T cell receptor. This indicates that ERK activation is crucial for T cell activation, and this was reported to be mediated by the AP-1 family of transcription factors.

The JNK signal transduction pathway

The JNK signalling pathway is a cell stress activated pathway that is involved in the regulation of cell proliferation and apoptosis. JNK protein kinases are encoded by the three genes JNK1, JNK-2, and JNK-3, which are alternatively spliced to form the JNK isoforms (table 1). In parallel with ERK1/2, JNK appears to be essential for AP-1 activation caused by stress and exposure to various cytokines. The two MAPKK proteins that act as upstream JNK activators are MKK7, which is primarily activated by cytokines (TNF, IL-1), and MKK4, primarily activated by environmental stress (fig 3). MAPKKK activators of JNK are composed of a large group of more than 12 intracellular proteins, including those of the MEKK group (MEKK 1–4), although it is not clear which are relevant to specific physiological stimuli. Duration and amplitude of JNK activation represents the balance between external stimuli and intracellular inactivation mechanisms. Four negative JNK regulatory factors have now been identified: MAP kinase phosphatase MKP7, heat shock protein 72, EvI oncoprotein, and nitric oxide. The two primary AP-1 transcription components that are phosphorylated by JNK are c-Jun and ATF-2.

“The JNK signalling pathway is a cell stress activated pathway that is involved in the regulation of cell proliferation and apoptosis”

The three genes JNK1–3 have been disrupted in mice; this results in defects in immune response and apoptosis. Isolated embryonic fibroblasts demonstrated defective AP-1 transcription activity, decreased proliferation, and resistance to stress induced apoptosis. Gene knockout studies targeting MKK7...
showed that TNF activates JNK through MKK7 whereas basal activity of MKK4 is required for full activation of JNK in response to TNF. JNK2 was shown to have a crucial role in Th cell differentiation and cytokine production. JNK2−/− Th2 cells produced markedly reduced amounts of IFN-γ, a key cytokine for Th1 cells. The JNK signalling pathway has been implicated in a large variety of pathological conditions, including cancer, stroke, ischaemic heart disease, and inflammatory disorders, and has therefore been appreciated as an attractive candidate for drug development.

The p38 MAP kinase signal transduction pathway

The p38 MAP kinase pathway shares many similarities with the other MAP kinase cascades being associated with inflammation, cell growth, cell differentiation, and cell death. Murine p38 was first identified in 1994 as a kinase activated in response to bacterial lipopolysaccharide (LPS). To date, four p38 MAP kinase isoforms have been identified sharing about 60% homology (table 1), and two isoforms (p38α, p38β) are ubiquitously expressed. p38γ is predominantly expressed in skeletal muscle whereas p38δ gene expression is found in the lungs, kidneys, testis, pancreas, and small intestine. In most inflammatory cells, p38α is the major isoform that is activated. Extracellular stimuli of the p38 MAP kinase pathway include a variety of cytokines (IL-1, IL-2, IL-7, IL-17, IL-18, TGF-β, and TNF-α) and a number of pathogens that activate p38 through the different Toll receptors, including LPS, staphylococcal peptidoglycan, staphylococcal enterotoxin B, echovirus 1, and herpes simplex virus 1. Moreover, several growth factors (that is, granulocyte macrophage-colony stimulating factor, colony stimulating factor 1, erythropoietin) are capable of inducing p38 as well as environmental factors such as heat shock, changes in osmolarity, ultraviolet, oxygen radicals, and hypoxic states.

"p38 MAP kinase probably plays a central role in the regulation of a wide range of immunological responses"

MK3, MKK4, and MKK6 serve as upstream MAPK kinases responsible for p38 activation, although MKK3 activates mainly p38β, p38γ, and p38δ (fig 1). A large group of proteins, acting as MAPKK kinases, activate the MKK/p38 pathway, which explains why this cascade can be activated by such a variety of stimuli. Downregulation of p38 activation is achieved by specific phosphatases capable of dephosphorylating not only activated p38 MAP kinases but also ERK and JNK. The downstream targets of p38 are either other kinases or transcription factors such as ATF-2 and MEF2. ATP-2 can form heterodimers with Jun transcription factors and thus associates with AP-1. p38 has also been associated with activation of NFκB, as a p38 inhibitor (SB203580) has been shown to attenuate NFκB dependent transcription. MAPKAP kinase 2 (MK2) is one of several kinases that are regulated through direct phosphorylation by p38 MAP kinase. Targeting the MK2 gene, it has been reported that mice lacking MK2 show increased stress resistance and survive LPS induced endotoxic shock due to a reduction in the production of TNF-α by approximately 90%. Thus this seems to be a post-transcriptional event as TNF-α mRNA is not reduced and TNF-α secretion is not affected.

The main biological response of p38 activation involves the production and activation of inflammatory mediators to initiate leukocyte recruitment and activation. For example, TNF-α induced upregulation of E-selectin, expressed on the surface of endothelial cells during selectin mediated rolling of leucocytes, is partly regulated through p38. Furthermore, it was demonstrated that LPS stimulation of neutrophils resulted in p38 activation, leading to cell adhesion and regulation of TNF-α synthesis. Also, p38 can regulate TNF-α induced expression of vascular cell adhesion molecule 1 in endothelial cells, and chemotactants such as N-formyl-methionyl-leucyl-phenylalanine, platelet activating factor, and TGF-β can induce neutrophil chemotaxis through p38 activation. Thus p38 MAP kinase probably plays a central role in the regulation of a wide range of immunological responses, as seen in inflammatory disorders, but there is a considerable overlap with other signalling routes such as ERK and JNK. In inflammatory bowel disease, it was recently reported that p38α expression was increased in gut lamina propria macrophages and neutrophils.

CROSSTALK BETWEEN MAP KINASES

Although MAP kinases generally function as autonomous signalling modules, crosstalk between the different pathways exist. This crosstalk localises to two different levels: upstream and downstream activators. The most prominent example is GTPase p21Rac that is activated by a variety of proinflammatory mediators, in particular colony stimulating factor 1 and TNF-α. In turn, Rac activates the MEK kinase p65PAK, at least in myeloid and lymphoid cells, which in turn activates MKK3, 4, 6, and 7, and thus is responsible for simultaneous activation of the p38 MAP kinase and JNK pathways, a general feature observed in most proinflammatory reactions. Also, downstream of MAP kinases scope exists for crosstalk. Activation of MAP kinases causes activation of so-called dual specificity phosphatases, dephosphorylating MAP kinases and thus responsible for the temporal limitations in MAP kinase signalling. As the specificity of these phosphatases is limited, activation of one MAP kinase will result in the deactivation of other MAP kinases. Thus crosstalk can result in both cooperative interaction (activation of one MAP kinase leading to activation of another isoform) as well as lateral inhibition (inhibition of MAP kinases by isoforms). The actual outcome of these interactions is of course highly dependent on the cellular context.

TARGETING MAP KINASE PATHWAYS FOR ANTI-INFLAMMATORY DRUG DEVELOPMENT

The MAP kinase signalling pathways are new hunting grounds for pharmaceutical companies. Developing drugs that interfere with signalling routes and gene expression, aiming at attenuating the proinflammatory response, could prove to be a promising avenue. Most of the protein kinase inhibitors are small molecules that either interfere with phosphorylation or bind (competitive) in the ATP binding site, an area within the activation loop of the MAP kinase in which the dual phosphorylation takes place.

Inhibition of MEK1/2

Although no data are available on the use of MEK inhibitors in inflammatory models, we will discuss several compounds briefly. Two companies have developed inhibitors of MEK1/2 which are commercially available and are currently being developed as therapeutic agents against cancer (fig 4). Both inhibitors, PD98059 and U0126, appear to block MEK1 phosphorylation and subsequent activation of ERK1/2. A second generation MEK inhibitor, PD184352, has recently been shown to inhibit elevated ERK activity in colon carcinoma cells, thereby inhibiting tumour growth, and is now being tested in phase I clinical oncology trials.

"Two companies have developed inhibitors of MEK1/2 which are commercially available and are currently being developed as therapeutic agents against cancer"

Roche identified a selective in vitro MEK1 inhibitor (Ro 092210) in fermented microbial extracts, which was able to block anti-CD3 induced peripheral blood T cell activation and...
antigen induced IL-2 secretion. Antagonically, Merck reported that a resorcylic acid lactone, L783277, also isolated from microbial extracts, inhibits MEK effectively in vitro. In models of traumatic brain injury, MEK inhibitors were beneficial. In a rat model of global brain ischaemic tolerance, inhibition of ERK activation after preconditioning ischaemia by PD98059 significantly prevented the inhibitory effects of preconditioning ischaemia on ischaemic injury and reduced inflammatory responses to Helicobacter pylori LPS in rats. Therefore it should be interesting to observe the effects of such inhibitors in human disease.

Inhibition of JNK
No specific JNK inhibitor has entered clinical evaluation. SP600125, developed by Celgene, is an inhibitor of JNK2 that does not interfere with the ERK or p38 MAP kinase pathway and reduces paw swelling in a rat model of inflammatory arthritis. CEP1347, a compound that inhibits members of the so-called mixed lineage kinases which are upstream activators of the JNK pathway, is a potent anti-inflammatory compound in experimental arthritis. Moreover, in acute experimental cerulein pancreatitis, treatment with CEP1347 resulted in significant amelioration and reduction of pancreatic edema. A membrane permeable peptide inhibitor of JNK (JNKi) has not been tested in vivo but inhibits AP-1 activation in alveolar macrophages. Thus pharmacological JNK inhibition has important anti-inflammatory consequences and emerges as a promising approach for combatting inflammatory disease in human pathology.

Inhibition of p38 MAP kinase
Signalling through p38 is required for synthesis of several proinflammatory cytokines, and several more or less selective p38 inhibitors have been developed (fig 4). Elucidation of the structural aspects of inhibitor/kinase interaction by x ray crystallography, mutagenesis, and mechanistic enzymology has improved the potency and selectivity of these compounds. The anti-inflammatory agent pyridinylimidazole and its analogues (SB (SmithKline Beecham) compounds) were shown to suppress proinflammatory cytokines through inhibition of p38α and p38β in several animal models of inflammation. In vitro studies as well as models of gene disruption demonstrated that SB203580, a widely used p38 inhibitor, can regulate gene transcription of IL-12 and IFN-γ and thus also the Th1 type immune responses. In a murine model of endotoxin shock, SB203580 reduced mortality. Furthermore, SB203580 showed beneficial effects in collagen induced arthritis in mice. In TNBS induced colitis, SB203580 did not ameliorate inflammation but suppressed the proinflammatory cytokines IL-12 and IFN-γ.

A potential anti-inflammatory drug is CNI-1493, a synthetic guanylishydrazine which inhibits the phosphorylation of both p38 MAP kinase and JNK. CNI-1493 can suppress macrophage activation and the production of several proinflammatory cytokines, including TNF-α, IL-1, IL-6, macrophage inflammatory protein 1α, and macrophage inflammatory protein 1β. In animal models of endotoxic shock, adult respiratory distress syndrome, pancreatitis, and rheumatoid arthritis CNI-1493 showed protective effects. In a pilot study in Crohn’s disease patients, CNI-1493 administration resulted in a significant decrease in Crohn’s disease activity index as well as mucosal healing. This study suggests that JNK is the more relevant target for CNI-1493 treatment as JNK phosphorylation was inhibited more potently than p38 in LPS stimulated peripheral blood mononuclear cells in vitro, and in mucosal inflammatory cells in vivo. This hypothesis was further supported by recent findings in TNBS induced colitis in mice which revealed that although the p38 MAPK inhibitor SB203580 effectively inhibited p38 MAPK enzymatic activity in these mice, no attenuation of disease progression was observed. A large controlled dose finding multicentre study with CNI-1493 is underway.

“...and in mucosal inflammatory cells in vivo. This hypothesis was further supported by recent findings in TNBS induced colitis in mice which revealed that although the p38 MAPK inhibitor SB203580 effectively inhibited p38 MAPK enzymatic activity in these mice, no attenuation of disease progression was observed. A large controlled dose finding multicentre study with CNI-1493 is underway.”
In rheumatoid arthritis, all three MAP kinase kinases are expressed in rheumatoid synovial tissues, and p38 was targeted for the development of several oral inhibitors: SB220025 was tested in a murine model of collagen-induced arthritis and prevented progression of disease.\(^1\) SB242235, another member of the pyridinyl imidazole class, potently inhibits TNF-\(\alpha\) and showed protective effects in antigen induced arthritis.\(^2\) Finally, two recently developed compounds VX-745 and VX-702 are now under clinical investigation for rheumatoid arthritis.

**TARGETING MAP KINASES FOR THE DEVELOPMENT OF ANTI-ONCOGENIC DRUGS**

Various MAP kinase pathways have been linked to processes relevant for oncogenesis. More specifically, ERK activation is considered essential for entry into the cell cycle and thus mitogenesis. Inhibition of ERK may therefore be useful in inhibiting tumour growth. Activation of the JNK pathway is associated in various ways with the apoptosis processes, and modulators of the JNK pathway may become useful for induction of cancer cell death or sensitising cancer cells to apoptosis on radiation therapy or chemotherapy. p38 MAP kinase, with its obvious role in inflammation, is often considered a target with respect to leukemia. Nevertheless, in vivo data as to the efficacy of such inhibitors in patients or even in animal models of oncogenic disease are still relatively sparse. No in vivo data, either in humans or in rodent models of oncogenic disease, have as yet been reported with respect to either the JNK or p38 MAP kinase pathway. An important study by Sebott-Leopold et al described the discovery of an orally active MEK inhibitor with substantial in vivo bioactivity in mice with colon carcinomas of both mouse and human origin.\(^3\) Thus MEK inhibitors are currently undergoing clinical trials but not much data have, as yet, entered the public domain. Various inhibitors of Raf, which act upstream of MEK, have also been shown to reduce tumour growth in xenograft models encompassing tumours of the colon, lung, and pancreas but human data are still not available.\(^4\) The clinical effects of farnesyl transferase inhibitors, which target different variants of the even more upstream Ras proteins, have until now been somewhat disappointing.\(^5\) Hence the actual usefulness of targeting MAP kinase pathways for combating human cancer remains unproved.

**CONCLUSIONS**

Increased understanding of signal transduction mechanisms and gene regulation involved in immune responses has created opportunities for the discovery of novel therapeutic compounds useful in treating inflammatory disorders. These new “design drugs” are promising because they are capable not only of reducing a spectrum of proinflammatory cytokines but many are small molecules that can be administered orally. Although MAP kinases are necessary for life, the reported clinical side effects of MAP kinase inhibitors have not precluded further clinical development. Moreover, MAP kinase inhibitors have been used in combination with other immunosuppressive drugs, including biological therapy. Finally, production of oral MAP kinase inhibitors will not be as costly as, for example, antibody based therapies.

**REFERENCES**

15. Fukushima R, Hunter T. MKK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. EMBO J 1997; 16:1921–33
36. Pietersma M, Tilly BC, Gaestel M, et al. p38 mitogen activated protein kinase regulates endothelial VCAM-1 expression at the
Signal transduction


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Gut 2003 52: 144-151
doi: 10.1136/gut.52.1.144

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