REPORT

Mitogen activated protein kinases as targets for development of novel anti-inflammatory drugs

M Karin

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Given the prevalence and debilitating nature of chronic inflammatory diseases there is a never ending quest for identification of novel targets for the rational development of anti-inflammatory drugs. Although the major signalling pathway that controls inflammation associated gene expression is the one leading to activation of transcription factor NF-κB, considerable attention has also been given to mitogen activated protein kinases (MAPKs) as likely targets for development of novel anti-inflammatory therapeutics. Indeed, inhibitors targeting these pathways have been developed and preliminary preclinical data suggest that they exhibit anti-inflammatory activity. This report focuses on the possible mechanisms through which such inhibitors may interfere with inflammation and discusses the pros and cons of targeting MAPKs in the treatment of chronic inflammatory disease.

Chronic inflammatory diseases, such as rheumatoid arthritis (RA) and psoriatic arthritis, are highly debilitating diseases affecting a large segment (up to 10%) of the population. Recently, it has become apparent that even metabolic diseases, such as type II diabetes, and cardiovascular disease, such as atherosclerosis, should also be considered to be inflammatory in nature. Thus it is not surprising that inflammation seems to be at the root of almost all chronic diseases (cancer notwithstanding) and that huge efforts and resources are constantly focused on the development of anti-inflammatory drugs. However, due to its very nature—being a chronic disorder that in its initial and even more advanced stages is frequently not life threatening—chronic inflammation presents a difficult challenge as one needs to develop drugs relatively free of side effects that can be used over a long period of time. Therefore there is a never ending quest for new targets for the development of anti-inflammatory drugs that hopefully will be highly specific and free of side effects. The logical identification of such targets is obviously dependent on better understanding of the signalling pathways involved in the initiation and maintenance of inflammation and the availability of target validation technology, such as targeted mutagenesis of mice and small interfering (si)RNA mediated gene silencing.

Considerable evidence indicates that the primary signalling pathway involved in the initiation and amplification of inflammatory responses is the one that leads to activation of nuclear factor (NF)-κB transcription factors.[1 2] Although many different receptors can lead to activation (that is, nuclear translocation) of NF-κB, they all rely on two major signalling pathways known as the classical (or canonical) and the alternative NF-κB signalling pathways.[3] While the first pathway mostly affects diverse NF-κB dimers, such as the most common RelA(p65):p50 heterodimer, through phosphorylation induced proteolysis of the inhibitors of NF-κB (IκBs), the alternative pathway only affects the activation of RelB:p52 dimers through phosphorylation induced processing of the p100 precursor protein.[4] Ample evidence suggests that it is the classical pathway, which relies on the IKKβ catalytic subunit and IKKγ regulatory subunit of the IκB kinase (IKK) complex, that is most important for the initiation and propagation of inflammatory responses.[3 5] The alternative pathway, in contrast, is most important for secondary lymphoid organ development and adaptive immunity.[6]

Despite its central role, it is unlikely that the mere activation of NF-κB is sufficient for transcriptional activation or induction of any single NF-κB target gene that is involved in the initiation of inflammatory responses. On most promoters that have been critically analysed, for instance the interferon β promoter,[7] NF-κB requires assistance from other sequence specific transcription factors. Quite often the activity of such transcription factors, for instance members of the activator protein (AP)-1 family,[8] is dependent on mitogen activated protein kinase (MAPK) signalling pathways. A classic MAPK cascade is composed of a MAPK, which is activated through phosphorylation on serine and tyrosine residues by a MAPK kinase (MKK or MAP2K), the MKK and the kinase responsible for its activation the MKK kinase (MEKK or MAP3K). The MAP3Ks are activated through a variety of mechanisms, most of which are not entirely clear, in response to engagement of cell surface receptors. Thus the MAP3Ks provide the stimulus specificity, whereas the MAPKs carry out the effector functions of each cascade, either though direct phosphorylation of effector proteins or via the activation of subordinate kinases, called MAPK activated kinases (MAPKAK).

Several distinct MAPK cascades have been identified in mammals including humans, and the three most common ones are the extracellular regulating kinase (ERK), the c-Jun-N-terminal kinase (JNK), and the p38 MAPK cascades. Each cascade leads to activation of several closely related MAPK enzymes, for instance ERK1 and ERK2 or JNK1 through JNK3, which can be activated by two MKKS, for instance MEK1 and MEK2 for the ERK cascade. The MKKs, however, can be activated by a myriad of MAP3Ks. Gene disruption experiments, reviewed by Chang and Karin[9] indicate that each of the MAPK cascades has a distinct function, although a given stimulus, for instance tumour necrosis factor α (TNFα) or lipopolysaccharide (LPS), can activate to variable extents all three MAPK cascades. Gene disruption experiments also reveal that the response specificity (that is, the type of stimulus that activates any given cascade) is determined by the MAP3K.[10]

Abbreviations: AP-1, activator protein-1; ERK, extracellular regulating kinase; JNK, c-Jun-N-terminal kinase; IκBs, inhibitors of NF-κB; IKK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; NF, nuclear factor; RA, rheumatoid arthritis; TNF, tumour necrosis factor

The following discussion will focus on the role of individual MAPK pathways in the control of inflammation and their suitability for therapeutic intervention.

EXTRACELLULAR REGULATING KINASE

Compared with the other two MAPK cascades leading to p38 MAPK and JNK activation, there is relatively little information on the role of the ERK cascade in inflammation. However, a recent publication indicates that the MAP3K Tpl2/Cot is responsible for ERK activation in response to bacterial endotoxin (LPS) in macrophages.[11] The knockout of Tpl2 abolished ERK activation by LPS, an agonist of toll-like receptor 4 (TLR4)[12] and most importantly inhibited the induction of TNFα release.[11] Further investigation revealed that the inhibition of ERK activation or the deletion of Tpl2 prevented the transport of TNFα mRNA from the nucleus to the cytoplasm but had no effect on transcription of the *Tnfa* gene or the stabilisation of TNFα mRNA.[11] Although the exact substrate for ERK that controls the nuclear export of TNFα mRNA remains to be identified, these interesting findings suggest that inhibition of ERK activity may offer a unique strategy for inhibition of TNFα production. Since TNFα is a major mediator of chronic inflammation, the inhibition of ERK offers a new way for therapeutic intervention.

Although no direct ERK inhibitors have been reported as yet, several inhibitors that interfere with the activity of the MKKs that act upstream to the ERKs and downstream to the MAP3Ks, MEK1 and MEK2 have been described. These compounds, including PD 98059[13] and U0126,[14] are quite effective inhibitors of ERK activation. In the light of the results discussed above it would be worthwhile testing their effectiveness in animal models of RA. More recently, it has been found that the activation of Tpl2 and the subsequent activation of ERK in LPS treated macrophages is dependent on the activation of IKK (S C Sun, personal communication). It was observed that activation of Tpl2 depends on its dissociation from p105, the precursor for the NF-κB subunit p50 (S C Sun, personal communication). As the processing of p105 to p50 is IKK dependent, IKK inhibitors can also inhibit the activation of Tpl2 and the subsequent activation of ERK by LPS. It would therefore be of interest to evaluate which of the therapeutic effects of IKK inhibitors in mouse models of RA[15] are due to inhibition of NF-κB activation and which are due to inhibition of ERK activation.

C-JUN-N-TERMINAL KINASE

In comparison with ERK, JNK activity is more strongly induced in response to proinflammatory stimuli, and there is preliminary evidence that inhibition of JNK activity can retard or even prevent tissue damage in animal models of RA.[16]

JNKs were first identified by their ability to phosphorylate and thereby activate the transcriptional potential of c-Jun, a critical component of the AP-1 transcription factor.[17] However, it is quite obvious that in addition to c-Jun, JNKs can stimulate the activity of other transcription factors[18 19] and various other proteins.[10 20] Although not as critical as NF-κB, AP-1 and related transcription factors, such as ATF2 (whose activity is also stimulated by JNK mediated phosphorylation) play an important role in the inflammatory response through their ability to contribute to the indication of important cytokine genes, such as those that code for TNFα and interferon β.[8] In addition, AP-1 activity is required for the induction of matrix degrading enzymes, such as collagenase.[21] In addition to being involved in the induction of TNFα, AP-1 activity is induced by TNFα[22] and JNK activity is required for this activation.[16]

Several different JNK inhibitors have been recently identified through high throughput screening and at least one of them, SP6000125, has been tested in a rat model of RA where it was found effective not only in reducing inflammation (paw swelling) but also in prevention of tissue damage.[16] The reduction in tissue damage was attributed to inhibition of collagenase and stromalysin expression, an effect that correlates with reduced c-Jun-N-terminal phosphorylation and TNFα induced AP-1 activity.[16] Similar findings were obtained using mice efficient in JNK1, the major JNK isoform responsible for c-Jun phosphorylation and AP-1 activation,[16] thereby validating the results obtained with the low molecular weight JNK inhibitor. Interestingly, JNK1 deficient mice are also resistant to obesity induced insulin resistance, a metabolic disorder thought to be caused by low grade inflammation elicited by fat deposition.[23]

More recently, we examined how many of the LPS inducible genes in macrophages are JNK dependent. While most LPS inducible genes are dependent on IKKβ only a small percentage of them are sensitive to inhibition of JNK (M G Ruocco and J M Park, unpublished results). Importantly, however, the JNK dependent genes include TNFα and several other members of the TNF family, such as Fas ligand (FasL), an important death inducing cytokine (M G Ruocco and J M Park, unpublished results).

P38 MAPK

p38 MAPK was first identified as an interleukin (IL)-1[24] and LPS[25] activated kinase. Therefore, from the very beginning it was expected to play an important role in inflammation. Indeed, early support for the critical role of p38 in inflammation was derived from studies that identified p38 as a critical target for a group of novel anti-inflammatory drugs, a prototype of which is SB202190.[26]

Importantly, the p38 inhibitors are potent inhibitors of LPS mediated TNFα production by macrophages.[26] However, the mechanism through which p38 contributes to TNFα production is not entirely clear. It is almost certain that p38 activity is not required for transcriptional activation of the *Tnfa* gene. Yet, being a very important and central mediator of inflammation, TNFα synthesis is subject to intricate control. In addition to transcriptional activation, the mRNA for TNFα which is inherently short lived in non-stimulated cells becomes stabilised in response to cell stimulation, but p38 activity does not appear to be required for this process either. In addition, p38 does not seem to be involved in the transport of TNFα mRNA from the nucleus to the cytoplasm, a process that depends on ERK activity instead. Most likely p38 activity is required for initiation of TNFα mRNA translation.[26] However, the mechanism through which p38 contributes to activation of TNFα mRNA translation is not known. Nevertheless, the ability of p38 inhibitors to block TNFα synthesis can be exploited in the treatment of inflammatory diseases and p38 inhibitors have been shown to inhibit the development of RA in small animal models.[27] It is not entirely clear, however, whether the therapeutic effect of such inhibitors is solely dependent on inhibition of TNFα production or whether p38 also contributes to other important processes. For instance, we found that inhibition of p38 activity prevents the transcriptional activation of both the *Il-1a* and *Il-1b* genes coding for IL-1 in LPS stimulated macrophages.[28]

PITFALLS AND BENEFITS

In addition to their role in expression of cytokines and other genes involved in inflammation and tissue remodelling, MAPK cascades have other important biological functions, which can either complicate the outcome of their inhibition or generate additional benefits. Most importantly, MAPK cascades are involved in the control of cell survival, especially in macrophages which are central regulators and effectors of

inflammatory responses. We found that inhibition of p38 activity greatly increases the susceptibility of macrophages to the induction of apoptosis in response to activation of TLR4.[28 29] While in some cases induction of macrophage apoptosis can accelerate the resolution of inflammation, we found that massive or systemic macrophage apoptosis can strongly potentiate inflammation because it leads to the processing and release of mature IL-1β, a major mediator of inflammation. It is well established that the processing of the IL-1β precursor requires the activation of caspase 1, and that without caspase 1 activation no mature IL-1β can be released.[30–32] As caspase 1 activation is linked to apoptosis, any condition that increases macrophage apoptosis, including p38 inhibition, potentiates IL-1β release. Such problems, however, if encountered, can be addressed through the use of IL-1 inhibitors, such as IL-1 receptor antagonist (IL-1ra).

In contrast with p38, JNK activation can promote apoptosis.[33] Therefore, inhibition of JNK, in addition to inhibition of inflammation, may also be used in conjunction with IKK inhibition. Due to the central role of NF-κB in the suppression of apoptosis,[34] one potential side affect of anti-IKK therapy is an unwanted increase in the susceptibility of various cells to proapoptotic stimuli. Such a problem, however, may be solved, as recently shown, through JNK inhibition.[33] Anyhow, without further experimentation with actual IKK, p38, and JNK inhibitors, it may be difficult to extrapolate from gene knockout studies in which the activity of a given kinase is completely inhibited to drug therapy where a partial inhibition may be sufficient for achieving the desired therapeutic effect.

Correspondence to: M Karin, Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology, School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093–0723, USA; karinoffice@ucsd.edu

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