Review

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What goes up must come down: molecular basis of MAPKAP kinase 2/3-dependent regulation of the inflammatory response and its inhibition

Abstract: Inflammation is normally a fast and transient response to microbial invaders or sterile damage and has to be stringently controlled. The closely-related mitogen-activated protein kinase-activated protein kinases MK2 and MK3 are involved in both up- and down-regulation of inflammation in mammals and govern the inflammatory response at different regulatory levels of gene expression and with different kinetics. In conjunction with their activator MAP kinase p38, MK2 and MK3 stimulate the transcription of immediate-early genes including that of the mRNA-binding protein tristetraprolin (TTP). TTP competes with the constitutively expressed protein human antigen R in binding to the mRNA destabilizing adenylyl-uridylate-rich element. MK2 and MK3 also regulate the activity of TTP by direct phosphorylation, determine stability and stimulate the translation of cytokine mRNAs. In addition, TTP controls its own re-synthesis via stability and translation of its mRNA in a phosphorylation-dependent manner. This results in a complex scenario of gene expression and guarantees fast up-regulation and intrinsic feedback control of the inflammatory response of macrophages. Inhibition of MK2/3 by small-molecule pharmaceutical inhibitors is an emerging strategy to manipulate the inflammatory response as a therapeutic option. This strategy could display advantages over the direct inhibition of MAP kinase p38.

Keywords: mitogen-activated protein kinase-activated protein kinase (MAPKAP); mitogen-activated protein kinase-activated protein kinases 2/3 (MK2/3); protein kinase inhibitor; protein phosphorylation.

Introduction

The role of the p38 MAP kinases in inflammation and innate immunity was first revealed when the targets of the parent anti-inflammatory compound SB203580, which suppresses lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF) production in monocytes and inhibits septic and endotoxic shock in mice, were identified as the protein kinases p38α/β (Lee et al., 1994). Later, upstream and downstream components of p38 could be added and a linear pathway of Toll-like receptor 4-TAK1-MKK3/6-p38 MAPK emerged that explains the LPS-induced activation of p38 (Han et al., 1994; Moriguchi et al., 1996). A more recent discovery was the fact that the other two isoforms of p38 MAPK – p38γ/δ – that are not blocked by the pharmacological inhibitor SB203580, are also essential for the inflammatory response. Similar to pharmacological inhibition of p38α/β, mice with double-deletion of p38γ/δ display strongly reduced TNF production and are more resistant to endotoxic shock (Risco et al., 2012). Hence, all four isoforms of p38 MAPK perform a complex role in inflammatory response.

Interestingly, the production of the master cytokine TNF by macrophages cannot only be blocked by pharmacological inhibition of p38α/β but also by inhibition of classical MAPKs (extracellular signal-regulated kinases or ERKs). This could recently be explained by the finding that p38γ/δ are essential for LPS-induced activation of ERKs by stabilizing the TPL-2/ABIN-2/p105 complex (Risco et al., 2012), which acts as a MAP3K for ERK1/2 (Dumitru et al., 2000; Waterfield et al., 2003). This stabilization is thought to require the direct phosphorylation of components of the protein complex by p38γ/δ (Risco et al., 2012). Hence, p38 signaling is responsible for LPS-induced TNF production via at least two synergistic pathways: the SB203580-dependent activation of p38α/β and the SB203580-insensitive activation of ERK1/2 via p38γ/δ.
Downstream signaling of p38 is extremely diverse, resulting in the phosphorylation of more than 60 different substrate proteins (Trempelec et al., 2013). Unexpectedly, for the SB203580-dependent pathway of regulation of TNF biosynthesis, it transpired that the MAPK-activated protein kinases MK2 and MK3 (reviewed in: Gaestel, 2006) are the main and essential downstream targets of p38. Genetic deletion of MK2 in mice dramatically reduced TNF production and increased resistance against endotoxic shock, and these phenotypes were further enhanced in mice deleted for both MK2 and MK3 (Kotlyarov et al., 1999; Ronkina et al., 2007). In these genetic models, the dominant role of MK2 over MK3 is attributed exclusively to higher expression and activity of MK2 in most cell types.

The role of MAPK-activated protein kinases, including MK2 and MK3, in inflammation has recently been extensively described in an excellent review (Moens et al., 2013). Therefore, this review will solely focus on some emerging points of interest namely on the molecular mechanism by which MK2 and 3 regulate TNF biosynthesis, and on the state of the art in the development and use of MK2 and 3 inhibitors.

MK2/3 in inflammation and some underlying mechanisms

MK2/3 in diseases with inflammatory components

MK2 and MK3 are major regulators of the TNF production of macrophages. Hence, it is not surprising that MK2 and MK2/3 knockout mice display modified outcomes in various disease models with inflammatory components. This not only concerns the impressive reduction in the disease score in the model of collagen-induced arthritis (Hegen et al., 2006), but also the development of cerulein-induced pancreatitis (Tietz et al., 2006), skin inflammation (Funding et al., 2009; Fyhrquist et al., 2010; Schottelius et al., 2010), inflammatory promotion of papilloma formation (Johansen et al., 2009), angiogenesis II-induced vascular inflammation (Ebrahimian et al., 2011), inflammatory bone turnover (Li et al., 2011; Braun et al., 2013), acute proliferative glomerulonephritis (Guess et al., 2013) and dextran sodium sulfate-induced mouse colitis (Li et al., 2013). In addition, deletion of MK2 in mice has dramatic effects in an infection model using Listeria monocytogenes (Lehner et al., 2002). Furthermore, Plasmodium falciparum glycosylphosphatidylinositol-induced production of TNFα (Zhu et al., 2009), shiga toxin-induced inflammatory response (Saenz et al., 2010) and Clostridium difficile-associated inflammation (Bobo et al., 2013) depend on MK2/3, implying that these protein kinases also have a role in diseases like malaria, hemolytic uremic syndrome and gastrointestinal diseases ranging from mild diarrhea to toxic megacolon sepsis. Interestingly, the kaposin B protein and the viral G-protein-coupled receptor of the Kaposi’s sarcoma-associated herpes virus (KSHV) specifically induce MK2 activity and specific mRNA stabilization (McCormick and Ganem, 2005; Corcoran et al., 2012), probably to prevent the shutoff of certain pathogenetically-important host genes. Conversely, Mycobacterium tuberculosis lipomannan and anthrax lethal toxin inhibit MK2 activation in macrophages and endothelial cells, leading to increased virulence and disruption of the endothelial barrier (Rajaram et al., 2011; Liu et al., 2012).

Translational regulation of TNF by MK2/3 and tristetraprolin

Due to the wide-ranging effects of MK2/3-dependent production of inflammatory cytokines, such as the master cytokine TNFα, the molecular mechanisms of action of MK2/3 are of great interest and their understanding could open novel therapeutic options for inflammatory diseases. Here, I will focus on the mechanism of MK2/3-dependent TNF biosynthesis in mice. Since in spleen cells and macrophages of mice the LPS-induced increase in the TNF mRNA level is almost independent of the presence of MK2/3 (Kotlyarov et al., 1999; Neininger et al., 2002; Tiedje et al., 2012), it can be assumed that MK2 and 3 do not regulate TNF biosynthesis at the level of transcription or transcript stability, but at later stages of the regulatory gene expression program. In addition, genetic evidence has been obtained in mice that the adenylate-uridylate-rich element (ARE) of the TNF mRNA and the ARE-binding protein tristetraprolin (TTP) are involved in the MK2-dependent regulation (Neininger et al., 2002; Hitti et al., 2006).

TTP is one of the mRNA-binding proteins mainly responsible for destabilization of its target mRNAs. Deletion of TTP in mice leads to symptoms of cachexia that can be reverted by anti-TNF antibodies, indicating a regulatory role of TTP in TNF biosynthesis (Taylor et al., 1996). Indeed, direct TTP binding to the mRNA of TNF followed by its destabilization could be detected as a mechanism of feedback inhibition of TNF biosynthesis (Carballo, 1998). Apart of the defined role of the p38-MK2/3-TTP axis for an ARE-dependent stabilization of various cytokine mRNAs (Anderson, 2008; Venigalla and Turner, 2012), increasing
MK2/3 regulate protein occupation of the tumor necrosis factor adenylate-uridylate-rich element

MK2 and MK3, which are activated upon LPS-treatment, phosphorylate murine TTP at the two different serine residues S52 and S178 (Chrestensen et al., 2004; Stoecklin et al., 2004). Polysome profiling of LPS-stimulated primary macrophages from MK2-deficient and MK2/3-double deficient mice, however, revealed a clear MK2/3-dependent polysomal recruitment of TNF mRNA (Tiedje et al., 2010). Interestingly, this polysomal recruitment of TNF mRNA was also achieved in MK2/3-deficient macrophages when treated with siRNA against TTP. Hence, MK2 and MK3 are obviously able to inactivate the repressive function of TTP on TNF-mRNA translation.

How does this work? Using various pull-down approaches, it was rather controversial whether phosphorylation changes TTP’s binding affinity for the ARE (Hitti et al., 2006; Clement et al., 2011; Zhao et al., 2011). Recently, in vitro titration experiments combined with RNA electrophoretic mobility shift assay using phosphorylated and non-phosphorylated TTP revealed a change in the Kd value for ARE-binding from 5×10^-7 (non-phospho-TTP) to 7×10^-4 (phospho-TTP) (Tiedje et al., 2012).

Is this shift sufficient to explain the regulation of TNF mRNA? It probably needs an additional important observation: the MK2/3-dependent translational de-repression of TNF mRNA in mouse macrophages depends on the presence of another ARE-binding protein – the constitutively ARE-binding protein human antigen R (HuR). Knockdown of HuR completely abolishes the MK2/3-dependent translational activation of TNF mRNA (Tiedje et al., 2012). In RNA electrophoretic mobility shift assays, HuR displays an MK2/3-independent ARE-affinity with a Kd of about 6×10^-7. Since TTP and HuR compete to bind to the same ARE mRNA element, the phosphorylation-dependent change in TTP’s affinity for the ARE can lead to an almost complete replacement of TTP by HuR at the ARE, and – after dephosphorylation of TTP – a vice versa displacement of HuR (Tiedje et al., 2012). Of course, changes in the cytoplasmic concentrations of TTP and HuR can further modulate the ARE-binding equilibrium between both proteins. While TTP recruits the translational repressor p54/RCK or could interfere with the function of PABP1, HuR is known to stimulate cap-dependent translation (Kawai et al., 2006) while inhibiting internal ribosomal entry site-dependent translation (Kullmann et al., 2002). Therefore, translational regulation of TNF mRNA can be explained by the regulated exchange of TTP and HuR at its ARE (Figure 1C).
Figure 1  Mechanisms of translational control of tumor necrosis factor (TNF) mRNA by tristetraprolin (TTP) and MK2.(A) The interaction between TTP and the poly(A)-binding protein 1 (PABP1, Marchese et al., 2010) may interfere with productive mRNA circularization via the cap-binding eukaryotic initiation factor (eIF)4E and the bridging factor eIF4G. Phosphorylation of TTP (Chrestensen et al., 2004) and PABP1 (Bollig et al., 2003) could weaken this interference. (B) TTP recruits the polyadenylases carbon catabolite repressor 4 (CCR4) and CCR4-associated factor 1 (CAF1) via the scaffold protein negative regulator of transcription 1 (NOT1) to TNF mRNA. Phosphorylation of TTP weakens this recruitment (Marchese et al., 2010; Clement et al., 2011; Sandler et al., 2011), possibly by interfering with TTP-NOT1 interaction (Frank et al., 2013). (C) MK2/3-driven exchange between TTP and human antigen R (HuR) at adenylate-uridylate-rich element (ARE)-containing mRNAs. The ARE-binding equilibrium between TTP and HuR is regulated via phosphorylation of TTP. Non-phospho-TTP displays high affinity for the ARE (left). TTP interacts with the RNA helicase/translational repressor p54/RCK (RCK) (Coller and Parker, 2005; Qi et al., 2011), which could explain the translational arrest of the ARE-containing mRNA. Since phospho-TTP shows lower affinity to the ARE, increased binding of the translational activator HuR can occur in the presence of the active TTP-kinases MK2 and MK3 (right). TTP also interacts with the E3 ubiquitin ligase scaffold protein Cullin 4b (Cul4B) (Pfeiffer and Brooks, 2012). By directing E3 ligases towards TTP, Cul4B could be involved in a further inhibitory ubiquitinylation of TTP (Schichl et al., 2011). In this scheme, TNF-mRNA also acts as a representative for similarly regulated mRNAs, such as TTP mRNA (Tiedje et al., 2012).
Increasing complexity

Besides the ARE, the 3′UTR of TNF mRNA also carries other regulatory elements, such as the 2-aminopurine response element (Osman et al., 1999) and the constitutive decay element (Stoecklin et al., 2003). Recently, it has been demonstrated that the constitutive decay element-binding proteins Roquin 1/2 (Leppik et al., 2013) suppress TNF biosynthesis and TNF-mediated pathology in mice (Pratama et al., 2013). It remains to be elucidated whether crosstalk between these different mRNA elements, its binding proteins and their regulation adds further levels of complexity to the regulation of TNF.

Translation of TNF mRNA proceeds as pre-pro-TNF at the rough endoplasmic reticulum (ER) and leads to insertion of the precursor pro-TNF as a type II membrane protein into the ER membrane (Kriegler et al., 1988). Pro-TNF is transported to the trans-Golgi network and subsequently exits this network for delivery to the cell surface. After cleavage of pro-TNF by the TNFα-converting enzyme (metalloprotease tumor necrosis factor-α-converting enzyme), mature TNF is then shed as a freely diffusible trimeric protein into the extracellular space. It is interesting to note that in mouse macrophages and human monocytes, TNF shedding and tumor necrosis factor-α-converting enzyme activation are also regulated by ERK- and p38-signaling, respectively (Rousseau et al., 2008; Scott et al., 2011). Together with the fact that NOGO-B, also known as reticulon 4, a protein involved in ER/trans-Golgi network transition (Yang and Strittmatter, 2007), is one of the substrates of MK2 (Rousseau et al., 2005), this opens the intriguing possibility that MK2 and MK3 are also involved in the regulation of pro-TNF transport and shedding.

Intrinsic feedback regulation

Interestingly, TTP is an immediate early gene that is transcriptionally stimulated via phosphorylation of serum response factor by MK2/3 (Ronkina et al., 2011). TTP’s own transcript also contains an ARE in the 3′UTR (Tchen et al., 2004) and translation of TTP is MK2- and HuR-dependent (Tiedje et al., 2012). Hence, MK2 and MK3 not only inactivate the repressive function of pre-existing and early-synthesized TTP by phosphorylation, but subsequently also stimulate the de novo synthesis of repressive non-phospho-TTP. This probably contributes to an intrinsic feedback control of TNF biosynthesis and limits acute inflammation by the scenario depicted in Table 1.

The down-regulation of the LPS-induced inflammatory response is further guaranteed by forward signaling to the anti-inflammatory cytokine IL-10 (Ehlting et al., 2007), to phosphorylation-driven internalization of the common cytokine receptor subunit gp130 (Radtke et al.,

Table 1  TTP in the complex scenario of lipopolysaccharide (LPS)-induced inflammation (early phase) and its resolving (late phase).

<table>
<thead>
<tr>
<th>Process</th>
<th>Resting macrophages</th>
<th>LPS-stimulated (early)</th>
<th>Switch</th>
<th>LPS-stimulated (late)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p38-mediated MK2/3 activity</td>
<td>MK2/3 inactive</td>
<td>MK2/3 active</td>
<td>Dephosphorylation of p38/MK2/3</td>
<td>MK2/3 inactive</td>
</tr>
<tr>
<td>Nuclear factor kappa beta, p38-, ERK-dependent transcription TTP status</td>
<td>Basal transcription of cytokine and TTP genes</td>
<td>Induced transcription of cytokine and immediate early genes (TTP)</td>
<td>TTP mRNA induced and phospho-TTP generated</td>
<td>Lower phospho-TTP-affinity to ARE, HuR replaces TTP at pre-existing AREs and decorates newly synthesized mRNA-AREs</td>
</tr>
<tr>
<td>TTP ARE-binding</td>
<td>Low levels of non-phospho-TTP</td>
<td>High TTP-affinity to ARE</td>
<td>HuR-TTP-exchange</td>
<td>ARE status</td>
</tr>
<tr>
<td>ARE status</td>
<td>Some TTP-mRNA-ARE complexes</td>
<td>HuR-mRNA-ARE complexes</td>
<td>TTP-mRNA-ARE complexes</td>
<td>Cytokine expression</td>
</tr>
<tr>
<td>Cytokine expression</td>
<td>Basal cytokine mRNAs unstable and/or translational inactive</td>
<td>High: induced cytokine mRNAs stable and translated</td>
<td>Cytokine mRNAs translational inactive and/or destabilized for decay</td>
<td></td>
</tr>
<tr>
<td>TTP biosynthesis</td>
<td>Low: TTP biosynthesis blocked by auto-inhibition of TTP translation</td>
<td>High: TTP mRNA stable and translated</td>
<td>Increase in newly synthesized non-phospho-TTP</td>
<td>Low: TTP mRNA translational inactive and/or destabilized</td>
</tr>
</tbody>
</table>

Key: ARE, adenylate-uridylate-rich element; HuR, human antigen R; TTP, tristetraprolin.
MK2/3 in the development of cells involved in the innate immune response

The molecular mechanisms illustrated above do not preclude the possibility that MK2 and MK3 play an additional role in the development of cytokine-producing subsets of monocytes or macrophages, since activation of MK2/3 is observed in LPS-stimulated hematopoietic stem cells, common myeloid precursors, monoblasts and monocytes (Bendall et al., 2011; Qiu et al., 2011) and MK2 and MK3 are known modulators of the chromatin-remodeling polycomb complex (Voncken et al., 2005; Schwermann et al., 2009; Prickaerts et al., 2012). The polycomb group protein B lymphoma insertion region 1 (Bmi1) was demonstrated to be directly phosphorylated and regulated in its chromatin binding properties by MK3 (Voncken et al., 2005). Recently, it was shown that Bmi1 protein expression is rapidly induced upon LPS-treatment in macrophages (Sienerth et al., 2011). Hence, Bmi1 induction and phosphorylation can cause transcriptional reprogramming upon LPS-stimulation and, therefore, also contribute to the innate immune response. This transcriptional reprogramming could, for example, determine developmental shifts to specific monocyte/macrophage subsets, like Gr-1<sup>high</sup> monocytes. Interestingly, such Gr-1<sup>high</sup> monocytes display higher activation of the p38–MK2/3 axis and express higher levels of TNF and other key inflammatory mediators (O’Dea et al., 2011).

Apart from this, there is an established role of MK2 and MK3 and their substrate leucocyte-specific protein 1 in the cell migration of neutrophils (Hannigan et al., 2001; Wu et al., 2004, 2007; Kahle et al., 2013) and a specific function of MK2 and MK3 in the activation of p90 ribosomal S6-kinase during dendritic macro-pinocytosis (Zaru et al., 2007). These additional, cell type-specific functions of MK2/3 could also contribute to the innate immune response.

Inhibition of the p38-MK2/3 pathway

Small molecule inhibitors against p38

Inhibition of the p38-MK2/3 axis is a therapeutic strategy against chronic inflammation, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, lupus erythematoses, and asthma (Cohen, 2009; Gaestel et al., 2009). Since the identification of the anti-inflammatory
### Table 2  Properties of some prominent MK2 inhibitors.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Developer</th>
<th>Structure</th>
<th>ATP-comp.</th>
<th>IC50 (nm)</th>
<th>Reference/three-dimensional structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK2α inhibitor CMPD1 (MK2-specific p38 inhibitor)</td>
<td>Boehringer</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>-</td>
<td>330</td>
<td>(Davidson et al., 2004)</td>
</tr>
<tr>
<td>PHA-781089 (PH089)Compound 23</td>
<td>Pharmacia (Pfizer)</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>+</td>
<td>126</td>
<td>1100 210 4800 (Anderson et al., 2007) MK2: 2P3G</td>
</tr>
<tr>
<td>Benzothiophene Compound 35 (4-methyl-3-pyrimidyl-benzothiophene)</td>
<td>Pfizer</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>+</td>
<td>5.0</td>
<td>150 (Anderson et al., 2009a, Anderson et al., 2009b MK2: 3FY)</td>
</tr>
<tr>
<td>MK2 Inhibitor III (2-(2-Quinolin-3-ylpyridin-4-yl)1,5,6,7-tetrahydro-4H-pyrrolo-[3,2-c]pyridin-4-one)</td>
<td>Pfizer</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>+</td>
<td>8.5</td>
<td>210 81 4400 (Cheng et al., 2010) MK3: 3FHR Hillig et al., 2007 MK2: 2JBO</td>
</tr>
<tr>
<td>PF-3644022 [(10R)-10-methyl-3-(6-methylpyridin-3-yl)-9,10,11,12-tetrahydro-8H-[1,4]diazepino[5′,6′:4,5]thieno[3,2-f] quinolin-8-one]</td>
<td>Pfizer</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>+</td>
<td>5.2</td>
<td>53 5 159 (Mourey et al., 2010)</td>
</tr>
<tr>
<td>Squarate Compound 50</td>
<td>Wyeth</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>+</td>
<td>670</td>
<td>1100 (Lovering et al., 2009) MK2: 3FPM</td>
</tr>
<tr>
<td>Diaminopyrimidine Compound 31b</td>
<td>Abbott</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>+</td>
<td>35</td>
<td>86 (Harris et al., 2010 Argiriadi et al., 2010 MK2: 3KA0, 3KC3</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>Developer</td>
<td>Structure</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (nm)</td>
<td>Reference</td>
<td>Three-dimensional structure</td>
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<tr>
<td>Pypripyridione Compound 16</td>
<td>Novartis</td>
<td>+</td>
<td>51</td>
<td>24</td>
<td>110</td>
</tr>
<tr>
<td>3-aminopyrazoles Compound 14e</td>
<td>Novartis</td>
<td>+</td>
<td>61</td>
<td>2500</td>
<td>MK2 3KGA (Velcicky et al., 2010)</td>
</tr>
<tr>
<td>Pypripyridinone Compound 5b</td>
<td>Merck</td>
<td>+</td>
<td>4</td>
<td>6?</td>
<td>980</td>
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<tr>
<td>Pypripyridinone Compound 5b –m3b</td>
<td>Merck</td>
<td>+</td>
<td>1</td>
<td>230</td>
<td>MK3: 3R1N (Oubrie et al., 2011)</td>
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<tr>
<td>5-(4-Chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-(pyridin-2-ylmethyl)furan-2-carboxamide</td>
<td>Merck</td>
<td>O</td>
<td>-</td>
<td>110</td>
<td>MK2 inhibitor IV; H-N-[4-(piperazin-1-yl)phenyl]-N-(pyridin-2-methyl)fur-2-carboxamide</td>
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<tr>
<td>Inhibitor</td>
<td>Developer</td>
<td>Structure</td>
<td>ATP-comp.</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (nm)</td>
<td>Reference/three-dimensional structure</td>
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<tr>
<td>Tricyclic lactam</td>
<td>Merck</td>
<td></td>
<td>-</td>
<td>1.9</td>
<td>(Xiao et al., 2013)</td>
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<tr>
<td>Compound 2s</td>
<td></td>
<td>![Image](...</td>
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<tr>
<td>Pyrazolopyrimidine</td>
<td>Teijin Institute</td>
<td>+</td>
<td>54</td>
<td>8400</td>
<td>(Kosugi et al., 2012)</td>
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<tr>
<td>Compound 64</td>
<td></td>
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<td></td>
<td>(Fujino et al., 2010)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MK2: 3A2C</td>
</tr>
<tr>
<td>Peptide MMI-0100</td>
<td>Moerae matrix</td>
<td>-</td>
<td>740</td>
<td></td>
<td>(Zu et al., 1996; Hayess and Benndorf, 1997; Ward et al., 2009; Brugnano et al., 2011)</td>
</tr>
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</table>

Inhibitory concentration 50 (IC<sub>50</sub>) is given for MK2 and also MK3 or MK5, if available. IC<sub>50</sub> for tumor necrosis factor (TNF) indicates the concentration for inhibition of TNF production of primary monocytes or monocytic cell lines in culture. The three-dimensional structure of MK2/3 together with the inhibitor is given by Protein Data Bank entry identifier. Further MK2 inhibitors published only in patents are summarized in an excellent review (Schlapbach and Huppertz, 2009).
compound SB203580 as a potent p38α/β inhibitor, many improved p38 inhibitors have been generated and tested in clinical phase I and II trials. Unfortunately, most p38 inhibitors display a lack of efficacy and/or have side effects ranging from skin rash to liver toxicity. Therefore, none of these inhibitors have successfully entered phase III clinical trials to date (Dominguez et al., 2005; Genovese et al., 2011; Dullos et al., 2013). The failure of p38 inhibitors can be explained by the fact that inhibition of p38 also blocks its feedback control of the upstream protein kinases TAK1 and MLK2/3, which activates not only p38 but also c-Jun N-terminal kinases (JNKs) and nuclear factor kappa-beta, and stops its forward anti-inflammatory signaling to mitogen- and stress-activated kinases MSK1/2, which is necessary for biosynthesis of inflammatory cytokines, such as interleukin-10 (reviewed in: Cohen, 2009; Gaestel et al., 2009). Hence, as direct downstream targets of p38α/β, MK2 and MK3 became increasingly attractive as alternative targets in inflammation, especially since the anti-inflammatory phenotype of the MK2 knockout mouse was elucidated. Very recently, the effects of p38 (SB203580, Vertex745) and MK2 (PH089) inhibitors have been compared on TNF-stimulated JNK activation in THP1 cells, primary macrophages and fibroblast-like synoviocytes from osteoarthritis and rheumatoid arthritis patients (Dullos et al., 2013). All three inhibitors decrease phosphorylation of the MK2 substrate Hsp27, indicating their ability to inhibit the p38-MK2/3 pathway. In contrast, the p38 inhibitors induce an increased and sustained JNK activation, which the MK2 inhibitor does not. This result again supports the findings that p38 is involved in feedback control of inflammation.

**(MK inhibitors keep up and trespass)**

During the past few years various MK2 inhibitors have been generated (Table 2) that could also be of general use for basic research to probe the involvement of MK2, 3 and 5 in cellular systems different from mouse, for which MK2/3/5 knockout cells are available. Three-dimensional structures of MK2 (see Table 2) have revealed that the ATP-binding pocket of MK2 is rather narrow and deep, only allowing planar small inhibitors to enter and bind. The development of MK2-inhibitors was therefore slow and difficult, especially since typical approaches of optimizing selectivity by appending substituents out of the binding plane were not readily applicable (Mourey et al., 2010). However, potent and selective, oral inhibitors have since been generated that display low nanomolar inhibitory concentration 50 (IC_{50}) of in vitro inhibition of recombinant MK2 and often also of MK5/p38 regulated and activated kinase, while inhibition of MK3 is only moderate.

Of note, for most MK2 inhibitors the IC_{50} determined for TNF production of monocytes is much (10–100 fold) higher than the IC_{50} of inhibitor binding in vitro (see Table 2). Hence, the biochemical efficiencies of the MK2 inhibitors generated, defined as the ratios of binding affinity to target versus cellular activity, are rather low. This can be explained by the relatively high ATP concentrations in living cells together with the relatively high affinity of non-active MK2 for ATP compared to other protein kinases that display high ATP affinity only in their activated state (Mourey et al., 2010).

The low biochemical efficiency of existing MK2 inhibitors (0.01–0.1) means these inhibitors are unlikely to emerge as suitable drugs, since 76 per cent of the drugs on the market today have biochemical efficiencies higher than 0.4 (Swinney, 2004). Only the statins, inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and cholesterin synthesis, display similarly low biochemical efficiency values of 0.003–0.025. Since low biochemical efficiency means that higher doses of the inhibitor will have to be applied, the inhibitors’ suitability as drugs will strongly depend on their low toxicity and rare off-target effects. The first non-ATP-competitive MK2 inhibitors have recently been identified (Huang et al., 2012) and it remains to be seen whether these inhibitors can be developed towards a higher biochemical efficiency.

**Conclusion**

This review demonstrates a clear involvement of MK2 and MK3 in the up- and down-regulation of the inflammatory response. Inhibition of MK2/3 as a strategy for blocking inflammation seems reasonable, since the anti-inflammatory role of MK2/3 is only relevant in cases where inflammation was first up-regulated. The identification of small-molecule MK2/3 inhibitors is feasible and these inhibitors could gain therapeutic relevance in the future.

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