

REVIEW ARTICLE

Mitogen-activated protein kinase phosphatase-1 (MKP-1): a critical regulator of innate immune responses

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Abstract

Innate immune responses mediated by macrophages and dendritic cells through Toll-like receptors (TLRs) play a central role in sensing and eliminating microbial pathogens. However, excessive innate immune responses can result in sepsis, autoimmunity, and chronic inflammation. Cells have evolved multiple mechanisms to prevent deleterious TLR activation, including transcriptional induction of intracellular negative regulators. Mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) is a nuclear-localized dual-specificity phosphatase that is induced by TLR stimulation in macrophages. MKP-1 preferentially dephosphorylates p38 MAPK and c-Jun N-terminal kinase, resulting in the attenuation of TLR-triggered production of pro-inflammatory cytokines and other inflammatory mediators. MKP-1 deficiency in mice leads to a markedly elevated susceptibility to endotoxemic shock (a murine model of sepsis) and autoimmune arthritis, highlighting a key role for MKP-1 in regulating innate immunity. Herein we discuss biochemical activities and physiological functions of MKP-1 and the regulation of its expression in TLR-mediated innate immune responses.

Key words: *Mitogen-activated protein kinase phosphatase-1, cytokine expression, innate immune responses, sepsis regulation*

Introduction

The mitogen-activated protein kinase (MAPK) group of serine/threonine protein kinases mediates the response of cells to many extracellular stimuli, such as cytokines and growth factors. Activation of the MAPKs is mediated by a core kinase module comprised of MAP3K, MAP2K and MAPK through sequential protein phosphorylation (1). Negative regulation of MAPK activity is effected primarily by MAPK phosphatases (MKPs), a group of 11 dual-specificity phosphatases that dephosphorylate the MAPKs on their regulatory threonine and tyrosine residues (2–5). MKP-1, the archetypal member of this family, was initially identified as an immediate-early response gene induced by growth factors and stress (6–8). MKP-1 localizes to the nucleus through its amino terminus (9) and preferentially dephosphorylates activated p38 MAPK and c-Jun N-terminal kinase (JNK) relative to extracellular signal-regulated kinase (ERK) (10). Although MKP-1 has been extensively studied in terms of the

regulation of growth factor signaling, its role in innate immune responses mediated by Toll-like receptors (TLRs) is only just beginning to be understood. In recent studies of mice deficient in the expression of MKP-1 (MKP-1^{-/-}), a pivotal function was revealed for MKP-1 in modulating TLR signaling and innate immune responses. In this review, we will discuss the functions of MKP-1 in innate immune regulation, focusing on the biochemical activities of MKP-1 and its physiological roles in cytokine expression and sepsis regulation. We will also address the regulation of MKP-1 expression by TLRs and other immunomodulatory agents in innate immune cells.

TLR-mediated innate immunity

The primary function of the immune system is to protect the organism from invading pathogens. To perform this function, the mammalian immune system has developed two components: innate and

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adaptive immunity. Both arms of immunity recognize invading pathogens as non-self, although they utilize different receptor systems. In adaptive immunity, T and B lymphocytes recognize non-self through antigen-specific receptors, such as T-cell receptors and immunoglobulins. These receptors are generated by gene rearrangement, which allows the recognition of a vast number of different antigens. The mechanism of gene rearrangement is evolutionarily a recent event and thus adaptive immunity is present only in vertebrates. Also, adaptive immune responses are slow to develop and therefore generally mediate protection only several days or more post-infection. In contrast, innate immune cells, including macrophages and dendritic cells, utilize an evolutionarily conserved receptor system of pattern recognition (11). Such pattern-recognition receptors (PRRs) are germline-encoded and do not undergo gene rearrangement. Cells expressing these receptors recognize pathogens within hours rather than days of infection and therefore provide the first line of defense against invading pathogens. Among the best-known PRRs are the TLRs, which bind to highly conserved sequences expressed by microorganisms. TLR4, the first cloned mammalian TLR, recognizes lipopolysaccharide (LPS) or endotoxin, major components of Gram-negative bacterial outer membranes. Among other TLRs, TLR3, TLR7, and TLR9 are activated by double-stranded RNA, single-stranded RNA, and bacterial CpG DNA, respectively (12). TLRs are evolutionarily conserved from the worm *Caenorhabditis elegans* to mammals; currently, >10 TLRs have been cloned in mammalian cells. TLRs recognize pathogens at either the cell surface or inside lysosome/endosome membranes, whereas TLR-independent PRRs, such as the NOD-LRR proteins and the CARD-helicase proteins, are responsible for the detection of pathogens that have invaded the cytosol (11).

The engagement of TLRs by microbial components results in a potent inflammatory response characterized by the release of pro-inflammatory cytokines, including tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , -6, -12 and -18 (12). These pro-inflammatory cytokines are important for subsequent activation of adaptive immunity and clearance of infectious organisms (13). However, exuberant production of pro-inflammatory cytokines leads to severe immune-mediated diseases, such as sepsis, autoimmunity, and chronic inflammation. In order to prevent deleterious TLR activation, a number of signaling mechanisms are evoked. These include the downregulation of surface TLR expression, transcriptional induction of negative regulators such as IL-1 receptor-associated kinase-M (IRAK-M), Suppressor of cytokine signaling 1 (SOCS1),

and SH2-containing inositol phosphatase (SHIP), and production of anti-inflammatory cytokines, mainly IL-10 and transforming growth factor (TGF)- β (14). Compared with the release of pro-inflammatory mediators, which occurs rapidly after TLR stimulation, production of these negative regulators is considerably slower, thus assuring proper regulation of the pro- and anti-inflammatory balance at the appropriate time (14).

Regulation of MAPK activities in TLR signaling

Stimulation of TLRs on innate immune cells activates MAPKs which, together with the nuclear factor (NF)- κ B pathway, transduce extracellular signals to cellular responses (Fig. 1) (12). The three major subfamilies of MAPKs include ERK1/2, JNK1/2/3, and p38 MAPK (p38 MAPK $\alpha/\beta/\gamma/\delta$). MAPKs contain the signature sequence -TXY-, where T and Y are threonine and tyrosine, respectively and X is glutamate, proline, or glycine, in ERK, JNK, or p38 MAPK, respectively (1). Phosphorylation of both the threonine and tyrosine within this signature sequence is required for MAPK activation. Phosphorylation of MAPKs is achieved via a signaling cascade involving a MAPK kinase (MAPKK or MAP2K) which is responsible for phosphorylation of the appropriate MAPK, and a MAPK kinase kinase (MAPKKK or MAP3K) which phosphorylates and activates MAPKK (1). There are a total of ≈ 20 MAP3Ks in

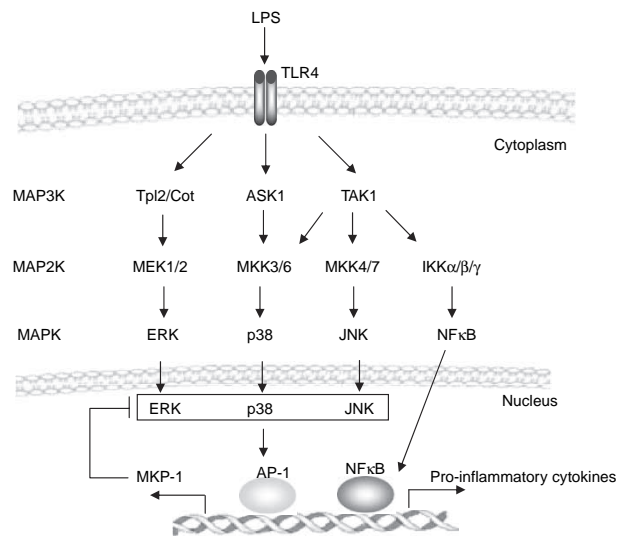


Fig. 1. Regulation of MAPKs and NF- κ B in TLR signaling. Activation of MAPKs is mediated by a cytoplasmic signaling cascade comprised of upstream MAP3Ks and MAP2Ks. TAK1 is a major MAP3K for JNK/p38 MAPK activation, although MEKK3 and ASK1 have also been implicated. Activated MAPKs translocate to the nucleus where they phosphorylate AP-1 and other transcription factors, leading to initiation of inflammatory responses. As a feedback control, MKP-1 induced by TLR activation switches off MAPK signaling in the nucleus.

mammalian cells, each of which receives and integrates specific upstream signals (15). Following TLR stimulation, Tpl2/Cot is a critical MAP3K inducing the activation of MEK1/MEK2 and the downstream ERK pathway (16). Another MAP3K, TAK1, integrates upstream TLR signals leading to the activation of JNK, p38 MAPK, and the I κ B kinase (IKK)/NF- κ B pathways (17,18). In addition, MEKK3 and ASK1 have also been implicated in JNK/p38 MAPK activation in TLR pathways (19,20). The main MAP2Ks mediating JNK activation are MKK4 and MKK7, whereas p38 MAPK can be activated by MKK3 and MKK6, as well as by MAP2K-independent pathways such as TAB1 and ZAP70 (21). Upon activation, MAPKs regulate key cellular events in the cytoplasm by phosphorylation of membrane-associated and cytoplasmic proteins, including other kinases and cytoskeletal elements. Activated MAPKs also translocate to the nucleus to phosphorylate transcription factors such as c-Jun, c-Fos, Elk-1, and c-Myc. Specific transcription factors activated by the MAPKs coordinately induce expression of important cytokines and cellular proteins (1).

Negative regulation of MAPK activities is effected primarily by MKPs, a group of 11 dual-specificity phosphatases that dephosphorylate the MAPKs on both the threonine and tyrosine residues in the signature sequence -TXY- (2-5). These MKPs have unique and overlapping substrate specificity toward MAPKs, and localize to either cytoplasm or nucleus or both (2-5). Some of the MKPs are ubiquitously expressed whereas others are more restricted. Macrophages, an innate immune cell type involved in the phagocytosis of infectious agents and the production of inflammatory mediators, express several MKPs, including MKP-1 (DUSP1), MKP-5 (DUSP10), and PAC-1 (DUSP2) (22,23).

MKP-1 inactivates TLR-triggered JNK and p38 MAPK activation

MKP-1, the archetypal member of the MKP family, was initially identified as an immediate-early response gene induced by growth factors and stress (6-8). MKP-1 is composed of two cdc25 homology domains, designated CH2A and CH2B, within the NH₂ terminus and a phosphatase domain on the COOH terminus. MKP-1 localizes to the nucleus through its NH₂ terminus (9). Although MKP-1 was initially characterized as an ERK-specific phosphatase (8), it is also capable of dephosphorylating all three MAPK subfamilies when overexpressed. Subsequent analysis has shown that MKP-1 prefers p38 MAPK and JNK as substrates relative to ERK (10). Accordingly, mouse embryonic fibroblasts (MEFs) deficient in MKP-1 show unaltered ERK activation

in response to serum (24,25) but exhibit substantial hyperactivation of p38 MAPK and JNK and an increased rate of apoptosis following stimulation with serum and various stresses, including anisomycin and osmotic and oxidative stresses (25,26). Consistent with the nuclear localization of MKP-1, MAPKs are inactivated by MKP-1 in the nucleus, whereas in the cytoplasm they are regulated by an MKP-1-independent pathway (27).

In macrophages, treatment with triptolide, a diterpenoid triepoxide that potently blocks MKP-1 induction by LPS, prolongs LPS-induced p38 MAPK and JNK activation (28,29). These pharmacological approaches suggested the involvement of MKP-1 in LPS-mediated MAPK signaling. More definitive support for the requirement for MKP-1 in the attenuation of MAPK activation in innate immunity was recently established using genetic ablation approaches. Macrophages lacking MKP-1 respond to LPS stimulation with augmented and more sustained activities of p38 MAPK and JNK compared with wild-type cells, indicating an essential requirement for MKP-1 in the inactivation of these MAPKs in TLR signaling (29-33). Accordingly, one of the nuclear targets of activated MAPKs, the activator protein-1 (AP-1) transcription factors, show enhanced DNA binding activity in MKP-1^{-/-} macrophages following LPS stimulation (30). Although macrophages express other MKPs, these findings clearly demonstrate that MKP-1 plays a non-redundant role in attenuating TLR-induced p38 MAPK and JNK activities.

MKP-1 regulates the TLR-induced gene expression program

Given the importance of JNK and p38 MAPK to gene expression in the innate immune system (34), it is not surprising that MKP-1 plays a reciprocal role in modulating a number of target genes downstream of TLR activation (Table I).

Regulation of the pro-inflammatory cytokines TNF- α and IL-6

TNF- α and IL-6 are potent inflammatory cytokines, and their excessive production initiates widespread tissue injury and organ dysfunction. The role of MKP-1 in the regulation of TNF- α and IL-6 in macrophages was initially examined using overexpression approaches. Overexpression of MKP-1 in macrophage cell lines and primary macrophages resulted in the suppression of secretion and mRNA expression of TNF- α and IL-6 after LPS or peptidoglycan treatments (28,29,35). Although such results demonstrated that MKP-1 was capable of ameliorating TNF- α and IL-6 production, it remained unclear

Table 1. Altered inflammatory mediators in MKP-1^{-/-} innate immune cells stimulated with LPS in vitro and in MKP-1^{-/-} mice during endotoxic shock in vivo.

Inflammatory mediators	Changes in vitro ^a	Changes in vivo ^a
Pro-inflammatory cytokines		
TNF- α	++	+++
IL-6	++	+++
IL-1 β	ND	+
IL-12	-	+
IFN- γ	--	+
Anti-inflammatory cytokines		
IL-10	+++	+++
Pro-inflammatory chemokines		
CCL2	ND	+
CCL3	ND	+
CCL4	ND	+
CXCL2	ND	+
CXCL10	ND	+/-
Lipid mediators		
PGE2	ND	+/-
Oxygen radicals		
Nitric oxide	ND	+
Surface activation molecules		
CD40	+	ND
CD86	+	ND

^aAs the magnitude of the change varies with the time point, the maximal differences observed among all time points are indicated here. + = <3-fold increase; ++ = 3–5-fold increase; +++ = >5-fold increase; - = <3-fold decrease; -- = 3–5-fold decrease; +/- = no statistical difference observed; ND = not determined.

as to whether MKP-1 was physiologically important for these functions. The most definitive evidence for an essential role for MKP-1 in cytokine expression was derived from recent analyses of mice deficient in MKP-1. Compared with wild-type cells, MKP-1^{-/-} macrophages produced greater levels of TNF- α and IL-6, especially at the earlier time points following TLR stimulation which correlated with increased activities of p38 MAPK and JNK (30–33). The increased TNF- α production by LPS-stimulated MKP-1^{-/-} macrophages was inhibited by SB-203580, a p38 MAPK-specific inhibitor. Hence, it appears that MKP-1 exerts its negative regulatory role on the production of TNF- α by inactivating p38 MAPK (32). In addition, dendritic cells and splenocytes also utilize MKP-1 for the negative regulation of TNF- α and IL-6 (33). Finally, MKP-1^{-/-} mice injected with LPS produced increased levels of TNF- α and IL-6 in serum compared with wild-type controls (30–33). Collectively, these studies establish a critical role for MKP-1 in negatively regulating TNF- α and IL-6 production.

Regulation of IL-10, an important anti-inflammatory cytokine

IL-10, a potent anti-inflammatory regulator of innate immune responses, is produced by macrophages,

certain types of T cells (Th2 and regulatory T cells), B cells, and cell types of non-hematopoietic lineage (36). Upstream signals leading to IL-10 production in activated macrophages are not fully understood, although IL-10 synthesis appears to be mediated by adaptor molecules distinct from those involved in the production of pro-inflammatory cytokines (37). When stimulated with LPS, MKP-1^{-/-} mice exhibit more than a fivefold higher level of IL-10 production in macrophages and substantially higher levels of IL-10 in serum (30–33). Notably, loss of one allele of MKP-1 is sufficient to cause overproduction of IL-10 in macrophages (30). These studies demonstrate that MKP-1 plays an essential function in the negative regulation of IL-10.

A subset of IL-10-induced genes, including *Bcl3*, *SOCS3*, *NFIL3*, *Ndr1*, and *Gadd45 γ* , show increased expression in LPS-stimulated MKP-1^{-/-} macrophages as compared with wild-type cells (30,31). This probably reflects the secondary effects of IL-10 overproduction. Conversely, IL-10 overproduction downregulates TNF- α mRNA levels at the later stage (5 h) of LPS stimulation in MKP-1^{-/-} macrophages (30). Although MKP-1^{-/-} macrophages show increased p38 MAPK and JNK activation, blocking p38 MAPK activity with SB203580 alone completely abolishes the increased IL-10 production, indicating an important role for MKP-1 in providing a negative

signal for p38 MAPK activation to regulate TLR-induced IL-10 production (30). This is consistent with previous observations (38) that p38 MAPK activity is crucial for IL-10 production during LPS stimulation.

Regulation of other cytokines and inflammatory mediators

IL-12, a pro-inflammatory cytokine produced by macrophages and dendritic cells, has a central role in driving the differentiation of naive T cells into Th1 cells. Deficiency of MKP-1 reduces LPS-induced IL-12 production in these innate cells, which is associated with decreased RNA levels of both subunits of IL-12: IL-12p35 and IL-12p40 (33). Therefore, MKP-1 plays a positive role in the expression of IL-12. Interferon (IFN)- γ , another cytokine important for Th1 cell differentiation and also for macrophage activation, is found to be similarly reduced in MKP-1^{-/-} splenocytes following LPS treatment (33). However, in MKP-1^{-/-} mice challenged with LPS, IL-12 and IFN- γ levels in the serum are more abundant compared with those in wild-type mice (32). The reasons for these discrepancies are unclear, but they further highlight the complex mechanisms by which MKP-1 regulates innate immunity. Moreover, MKP-1 modulates a subset of LPS-induced chemokines and surface molecules. Several pro-inflammatory chemokines, including CC-chemokine ligand 3 (CCL3), CCL4 and CXC-chemokine ligand 2 (CXCL2), are more abundant in the spleen and serum from MKP-1^{-/-} mice after LPS challenge. In contrast, another chemokine, CXCL10 (IP-10), is not affected by the absence of MKP-1 at the mRNA or protein level (31). Macrophage activation is associated with upregulation of surface molecules, including CD86 and CD40. CD86 is a costimulatory ligand for T-cell activation via its interaction with CD28, whereas CD40 and its ligand play a critical role in stimulation of pro-inflammatory cytokine production by macrophages. Macrophages that are positive for the expression of CD86 and CD40 molecules are found at higher frequency in TLR-activated MKP-1^{-/-} cells than in their wild-type counterparts, suggesting that MKP-1 negatively regulates expression of these surface activation molecules (32).

Regulation of endotoxin tolerance

Endotoxin tolerance is defined as a reduced responsiveness to an LPS challenge following a first encounter with LPS. It is an important mechanism to protect against a lethal challenge of LPS and to prevent infection and ischemia–reperfusion damage.

Endotoxin tolerance is characterized by a reduction in pro-inflammatory cytokines, in particular TNF- α , and attenuated activation of MAPKs (39). Tolerization of macrophages induces MKP-1 expression (40,41). The role of MKP-1 in regulating endotoxin tolerance was examined using overexpression and loss-of-function systems. Overexpression of MKP-1 in THP-1 cells, a monocyte cell line, suppresses TNF- α production and mimics the induction of LPS hyporesponsiveness. Moreover, MKP-1-deficient macrophages cannot be fully tolerized. Tolerized wild-type macrophages show 72% reduction in response to secondary LPS challenge, whereas MKP-1^{-/-} macrophages exhibit only 24% reduction (40). These results suggest that MKP-1 is a potential mediator of endotoxin tolerance.

MKP-1 suppresses endotoxic shock and autoimmune arthritis in vivo

Sepsis is a clinical disorder resulting from systemic inflammatory responses to severe infection. Approximately 40% of patients with sepsis progress to septic shock, a condition characterized by severe and irreversible hypotension, reduced perfusion, thrombosis, and multiple organ failure (42,43). Sepsis is responsible for 10% of total deaths registered in the world. Approximately 150 000 people die annually in Europe and >200 000 die annually in the USA from sepsis (43,44). Many of the components of the innate immune response that are normally concerned with host defenses against infection can contribute to sepsis. A commonly used mouse model for sepsis is endotoxic shock, which involves the administration of LPS to mice to induce inflammation and tissue damage. Using this model, four research groups have independently found that MKP-1^{-/-} mice are considerably more susceptible to LPS-induced death than wild-type mice (30–33). Prior to death, MKP-1^{-/-} mice exhibit hallmarks of sepsis, including multiple organ failure characterized by severe impairment of renal, hepatic, and pulmonary functions. In addition, LPS challenge results in severe hypotension and increased nitric oxide production in MKP-1^{-/-} mice (33). These studies show that the MKP-1-mediated pathway plays a central role in suppressing the endotoxic shock response in vivo.

Septic shock is mediated by the overproduction of pro-inflammatory cytokines. Mononuclear cells (macrophages and monocytes) play key roles in this process, releasing the classic pro-inflammatory cytokines TNF- α , IL-6, and IL-1 β , and other cytokines and inflammatory mediators (42,43). The amounts of TNF- α , IL-6, and IL-1 β are substantially increased in the serum of LPS-challenged MKP-1^{-/-}

mice (Table I). These findings are consistent with the role of MKP-1 in the negative regulation of these cytokines in macrophages observed *in vitro*. Given the pivotal function of these cytokines in mediating pathological changes in sepsis, their overproduction by MKP-1^{-/-} mice is most likely to be responsible for the increased susceptibility. The anti-inflammatory cytokine IL-10 is also markedly elevated in MKP-1^{-/-} mice challenged with LPS. Although administration of IL-10 can effectively rescue mice from endotoxic shock (36), the increased levels of IL-10 in MKP-1^{-/-} mice may not be produced early enough, or in sufficient quantities, to suppress the detrimental effects of pro-inflammatory cytokines. Another feature of the host response in sepsis is overproduction of pro-inflammatory chemokines, which induces infiltration of leukocyte populations, including neutrophils and monocytes, into tissues and target organs, further augmenting inflammation. In MKP-1^{-/-} mice challenged with LPS, the chemokines CCL2 (monocyte chemoattractant protein-1), CCL3, CCL4, and CXCL2 are elevated to higher levels in the serum as compared with wild-type mice (Table I) (31,32). These results indicate that MKP-1 plays a key role in suppressing the endotoxic shock response by negatively regulating the expression of pro-inflammatory cytokines, chemokines, and other inflammatory mediators.

The role of MKP-1 in immune regulation *in vivo* has been investigated further in murine collagen-induced arthritis, a model of rheumatoid arthritis (RA) (45). RA is a systemic inflammatory autoimmune disease characterized by inflammation in the synovium, which leads to destruction of cartilage and the underlying bone. Pro-inflammatory cytokines produced by infiltrating cells play a critical role in the pathogenesis of RA by increasing osteoclast activity in the joints. Following immunization with collagen, the development of the arthritic disease and joint inflammation is markedly accelerated in MKP-1^{-/-} mice compared with wild-type mice. In addition, serum levels of TNF- α and IL-6 are significantly higher in MKP-1^{-/-} mice. These findings indicate that uncontrolled upregulation of pro-inflammatory cytokines results in exacerbated inflammation in MKP-1^{-/-} mice immunized with collagen, and implicate MKP-1 as a negative regulator of autoimmunity and susceptibility to arthritis.

TLR stimulation induces a dynamic pattern of MKP-1 expression

The expression of MKP-1 can be induced by a number of growth factors and stresses in multiple cell types. In macrophages responding to LPS stimulation, there is a strong and rapid induction

of MKP-1 mRNA and protein expression, which reaches a peak at 1 h (28–30, 33,41). The induction of MKP-1 correlates with the reduction of JNK and p38 MAPK activities, consistent with a role for MKP-1 in the inactivation of these MAPKs. Upstream signals leading to LPS-induced MKP-1 expression are beginning to be defined. LPS activates two distinct pathways that are mediated by MyD88 and TRIF, respectively (12). LPS-induced MKP-1 expression is reduced in mice lacking either MyD88 or TRIF, suggesting that MKP-1 is induced through MyD88- and TRIF-dependent pathways in response to LPS (30). In addition, a signaling pathway comprised of Raf-1 and protein kinase C- ϵ is required for LPS-induced MKP-1 expression in macrophages (46,47). Furthermore, both ERK and p38 MAPK play a role in LPS-induced MKP-1 expression (28,41). Chromatin remodeling has been shown to facilitate stress-induced MKP-1 expression in fibroblasts (48), and whether a similar mechanism regulates TLR-induced MKP-1 expression is unknown. Notably, MKP-1 expression in LPS-stimulated macrophages is biphasic and, at 3–4 h after LPS stimulation, MKP-1 protein expression returns to basal levels (30). MKP-1 protein is known to undergo ubiquitin-mediated proteasomal degradation that could either be positively or negatively regulated by ERK activation in a cellular context-dependent manner (49,50). It will be interesting to identify the molecular pathways that downmodulate MKP-1 expression during the late phase of TLR activation.

What is the physiological significance of such dynamic changes in MKP-1 expression in relation to cytokine regulation in LPS-stimulated macrophages? Cytokines are released in a sequential manner, resulting in a “cytokine cascade”. In the initial phase of macrophage activation, pro-inflammatory cytokines, in particular TNF- α , are rapidly synthesized and released, and this is partly mediated by MAPKs (32). The second phase of macrophage activation involves the delayed and more gradual production of IL-10, an immunosuppressive cytokine (51). IL-10 production is also dependent on the activities of MAPKs, in particular p38 MAPK (30). We propose that the induction of MKP-1 is required to suppress the initial burst of LPS-induced pro-inflammatory cytokines but is rapidly downregulated to allow production of IL-10 by the remaining active MAPKs. To summarize, MKP-1 expression is regulated in a temporal manner in order to appropriately coordinate the production of pro- and then anti-inflammatory cytokines following TLR stimulation.

MKP-1 expression integrates signals from multiple immunomodulatory agents to regulate innate immune responses

MKP-1 is induced in TLR-induced macrophages as a feedback mechanism to restrain excessive activation of MAPK and overt inflammation. MKP-1 has also been found to be induced by multiple immunosuppressive agents, including glucocorticoids and anti-inflammatory cytokines, and this induction partially mediates the negative effects of these agents on MAPK activation and inflammation (52). Conversely, several pro-inflammatory agents, such as IFN- γ , attenuate MKP-1 expression to promote their positive effects on MAPK activation (33). Therefore, it appears that the induction of MKP-1 represents a common mechanism by which immunomodulatory agents “fine tune” innate immune responses (Fig. 2).

Induction of MKP-1 by glucocorticoids

Glucocorticoids potentially inhibit the expression of pro-inflammatory genes and are hence extensively used in the treatment of inflammatory diseases. The precise mechanisms by which glucocorticoids suppress pro-inflammatory gene expression remain incompletely understood. Several groups have found that glucocorticoid treatment increases MKP-1 expression levels that involve a combination of both increased transcription and reduced protein degradation (28,29,53,54). Importantly, glucocorticoid-dependent inhibition of LPS-induced JNK and p38 MAPK is abolished in MKP-1^{-/-} macrophages (52). In addition, glucocorticoid-mediated suppression of several pro-inflammatory genes (*TNF- α* , *cyclooxygenase-2*, and *IL-1*) is impaired in MKP-1^{-/-} mouse

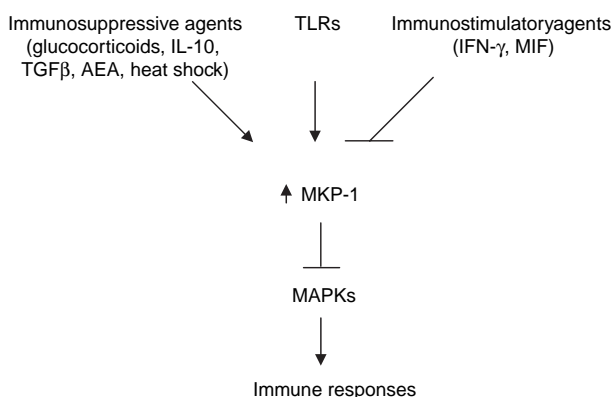


Fig. 2. MKP-1 expression integrates multiple signals from upstream stimuli to modulate MAPK activity. TLR induces MKP-1 as a feedback mechanism to switch off MAPK activation. Immunoregulatory agents induce MKP-1 to inhibit MAPK activation, whereas immunostimulatory agents suppress MKP-1 expression to promote MAPK activation.

macrophages, whereas other pro-inflammatory genes are inhibited by glucocorticoids in a MKP-1-independent manner. Moreover, in vivo immunosuppressive effects of glucocorticoids on zymosan-induced inflammation are impaired in MKP-1^{-/-} mice. Therefore, induction of MKP-1 by glucocorticoids is required for the suppressive effects of glucocorticoids on pro-inflammatory signaling pathways in macrophages and in vivo (52).

Induction of MKP-1 by the anti-inflammatory cytokines IL-10 and TGF- β

MKP-1 has been recently identified as an IL-10-induced gene in activated macrophages (55). Increased levels of MKP-1 in IL-10-treated macrophages, especially in combination with glucocorticoids, correlate with inhibition of cytokine production and downregulation of p38 MAPK activation. Therefore, MKP-1 is likely to be a mediator of IL-10-induced macrophage deactivation. In contrast to glucocorticoids, IL-10 increases MKP-1 expression only in activated, but not resting, macrophages, suggesting that different mechanisms are operative (55). TGF- β , another potent anti-inflammatory cytokine, is also capable of inducing MKP-1 expression in macrophages (56). Although induction of MKP-1 by IL-10 and TGF- β has been suggested to mediate the immunosuppressive effects of these cytokines, a direct proof of this hypothesis using MKP-1^{-/-} mice has yet to be provided.

Induction of MKP-1 by the endocannabinoid anandamide in microglia during central nervous system injury

Microglia, which share similar characteristics of macrophages, are resident innate immune cells of the brain. Microglia are important for providing constant immune surveillance in the brain. Endocannabinoid anandamide (AEA), a small lipid molecule, is released during central nervous system inflammation, as seen in patients with multiple sclerosis. AEA acts on microglial cells and induces MKP-1 expression, which subsequently inactivates ERK signaling in LPS-activated microglia (the effects on JNK and p38 MAPK were not analyzed in the study). This in turn abolishes nitric oxide release and suppresses attack of microglial cells on neurons (57). Therefore, during brain injury, MKP-1 induction in microglia by AEA interferes with LPS signaling and limits brain damage. However, release of TNF- α is not affected by MKP-1 in microglia, which may reflect the differential regulation of TNF- α by MKP-1 between microglia and macrophages (57).

Induction of MKP-1 by heat shock

Application of heat shock before an inflammatory stimulus often results in an attenuated response to that stimulus. In macrophages, prior heat shock treatment leads to a reduction of LPS-triggered p38 MAPK and ERK activation, and TNF- α expression. Heat shock treatment induces MKP-1 expression (58). In cells lacking MKP-1, the suppressive effects of heat shock on LPS-induced p38 MAPK and ERK activation and TNF- α expression are impaired (58). Therefore, induction of MKP-1 mediates the effects of heat shock in decreasing TNF- α production in response to LPS (58).

Attenuation of MKP-1 expression by the pro-inflammatory cytokines IFN- γ and macrophage migration inhibitory factor

Whereas MKP-1 induction appears to be a common mechanism by which anti-inflammatory agents suppress innate immunity, recent findings also demonstrate that MKP-1 expression can be suppressed by the pro-inflammatory cytokines IFN- γ and macrophage migration inhibitory factor (MIF). IFN- γ can boost the antimicrobial activity of macrophages and substantially enhance the secretion of TNF- α by LPS-activated macrophages. IFN- γ treatment suppresses LPS-induced MKP-1 expression, and this correlates with prolonged activation of JNK and p38 MAPK. Therefore, inhibition of MKP-1 expression may contribute to the biological activities of IFN- γ , although direct evidence has not yet been provided (33). Another pro-inflammatory cytokine, MIF, acts as a physiological counter-regulator of the immunosuppressive effects of glucocorticoids. MIF inhibits glucocorticoid-induced MKP-1 upregulation in macrophages and this is hypothesized to mediate the effects of MIF to override the suppressive effects of glucocorticoids on TNF- α expression (59). These results identify MKP-1 as a molecular target of MIF–glucocorticoid crosstalk and provide a molecular basis for the control of macrophage responses by a pair of physiological regulators of innate immunity.

MKP-1 is functionally unique in modulating innate immunity: a comparison of immune phenotypes of MKP-1^{-/-}, MKP-5^{-/-}, and PAC-1^{-/-} mice

Macrophages express several MKPs, including MKP-1, MKP-5, and PAC-1, and their expression can be strongly induced by LPS stimulation (22,23). Genetic ablation of MKP-1, MKP-5, and PAC-1 in mice leads to distinct phenotypes in macrophage

activation and function, highlighting their non-redundant function in innate immunity. MKP-5-deficient mice show defects in both innate and adaptive immunity. Macrophages deficient in MKP-5 exhibit increased JNK activity, but unaltered ERK or p38 MAPK activity, after LPS challenge. These cells produce twofold higher levels of TNF- α and IL-6 in response to LPS when compared with wild-type counterparts. Injection of LPS into MKP-5^{-/-} mice also results in twofold higher levels of TNF- α in the serum. In addition, MKP-5^{-/-} innate immune cells have enhanced function to promote T-cell proliferation and cytokine production. These results show that MKP-5 is a negative regulator of innate immunity. However, the fold increases in TNF- α and IL-6 levels in LPS-challenged MKP-5^{-/-} mice appear to be less profound than those in MKP-1^{-/-} mice, probably reflecting the limited substrate specificity of MKP-5 on JNK, but not on p38 MAPK or ERK (22).

In contrast to MKP-1^{-/-} and MKP-5^{-/-} cells, PAC-1^{-/-} innate immune cells show reduced function. LPS-activated PAC-1^{-/-} macrophages show reduced TNF- α , IL-6, and IL-12 production and decreased prostaglandin E2 (PGE2) and nitric oxide levels. Moreover, PAC-1^{-/-} mice show considerably reduced inflammatory responses in a serum transfer model of RA. PAC-1 deficiency results in increased JNK activity but, surprisingly, decreased ERK and p38 MAPK activities in LPS-activated macrophages. This is associated with impaired transcriptional activities of Elk-1 and nuclear factor of activated T cells (NFAT) AP-1, two main nuclear targets of the MAPK pathways. It is proposed that in the absence of PAC-1, the enhanced JNK activity mediates inhibition of ERK, as the reduced activation of ERK in PAC-1-deficient cells can be reversed by pharmacological inhibition of JNK. Indeed, there is precedent for crosstalk amongst the MAPKs, as both p38 MAPK and JNK can negatively regulate ERK (60,61). Therefore, PAC-1 appears to suppress JNK-mediated inhibition of ERK signaling in macrophages to positively regulate innate immunity (23).

Summary and future perspectives

Since its identification in the early 1990s, MKP-1 has been extensively studied in the context of regulating a variety of physiological processes, including cell proliferation, apoptosis, and inflammation. Initial studies on MKP-1^{-/-} mice by Dorfman et al. (24) in 1996 demonstrated that MKP-1 is not required for either development or growth factor-mediated ERK activation. Subsequently, a number of groups have addressed the post-developmental role of MKP-1 using this MKP-1-deficient mouse model. From the

collective conclusions reached on the role of MKP-1 in the innate immune system, we are now beginning to understand the critical role of MKP-1 in inactivating p38 MAPK and JNK in TLR signaling and in suppressing endotoxic shock and autoimmune arthritis. However, the fact that MKP-1 regulates both pro- and anti-inflammatory cytokine expression suggests that MKP-1-mediated regulation of innate immunity is more complex than previously appreciated. Future work is clearly warranted to further dissect the role of MKP-1 in innate immune regulation. First, it will be important to dissect the molecular mechanisms involved in the regulation of MKP-1 expression. In response to TLR stimulation, the expression of MKP-1 is rapid and transient, therefore providing an important feedback regulatory mechanism. MKP-1 expression also appears to integrate signals from a number of immunomodulatory agents to regulate innate immune responses. Identification of the molecular pathways affecting MKP-1 induction and downregulation will facilitate our understanding of innate immune regulation. Second, given a critical role of MKP-1 in suppressing endotoxic shock and autoimmune arthritis, further work is required to define the cell types in which MKP-1 plays a critical function. Most of the *in vitro* studies have utilized macrophages to investigate MKP-1 function. Whether MKP-1 is functionally important in other innate immune cells, such as dendritic cells and neutrophils, is less clear. In addition, whether MKP-1 regulates adaptive immunity requires further investigation.

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