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MAP Kinase Phosphatase-1 and Septic Shock

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Abstract

Mitogen-activated protein (MAP)[§] kinase cascades are crucial signal transduction pathways in the biosynthesis of proinflammatory cytokines. MAP kinase phosphatase (MKP)-1, an archetypal member of the MKP family, plays a pivotal role in the feedback control of p38 and JNK. *In vitro* studies using cultured macrophages have provided strong evidence for a critical role of MKP-1 in the restraint of pro-inflammatory cytokine biosynthesis. Recently, a number of studies conducted using MKP-1 knockout mice have verified the importance of MKP-1 in the regulation of p38 and JNK and in the regulation of pro-inflammatory cytokine synthesis. Upon lipopolysaccharide challenge MKP-1 knockout mice produced dramatically greater amounts of inflammatory cytokines, developed severe hypotension, and multi-organ failure, and exhibited a remarkable increase in mortality. These studies demonstrate that MKP-1 is an essential feedback regulator of the innate immune response, and that it plays a critical role in preventing septic shock and multi-organ dysfunction during pathogenic infection.

Sepsis represents a serious challenge to public health. Sepsis in the United States accounts for approximately 750,000 hospitalizations and 215,000 deaths, and costs nearly \$17 billion annually (1, 2). In spite of advancement in disease prevention and treatment, the incidence of sepsis is rising at an astonishing rate of 8.7% per year (2, 3). The Centers for Disease Control estimated that there was an incidence of 73.6 per 100,000 population in 1979, which increased to 175.9 per 100,000 in 1989 (4). The overall mortality rate is approximately 30% for all adults, rising to 40% in the elderly, and is greater than 52% in adults with septic shock (1). Sepsis and septic shock are an equally important cause of morbidity and mortality in children and neonates. Epidemiologic studies of these populations have documented an incidence that ranges from 20 to 50 per 100,000 in children aged 1-15 years, to over 500 per 100,000 in neonates and infants less than 1 year of age (5, 6). Sepsis and septic shock are the third leading cause of death in both the neonatal and the pediatric patient populations, accounting for over 42,000 deaths per year in the United States (5, 6). Sepsis-related mortality rates average 10% in children and increase to 17% in patients with severe sepsis or septic shock (5, 6).

The specific pathogenic causes of sepsis have changed over recent decades. In the past, Gram-negative organisms possessing endotoxin in their cell wall were the most common cause of sepsis. However, Gram-positive bacteria have become the predominant microorganisms in sepsis cases since 1987 and have accounted for over 52% of all cases of sepsis in 2000. *Staphylococcus aureus* is a leading cause of nosocomial pneumonia and wound infections, and represents one of the bacteria most commonly isolated from patients

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with sepsis (2, 3). For decades, Group B *Streptococcus* has been the single most frequent cause of sepsis in newborns, and it remains a primary cause of neonatal morbidity and mortality (7, 8). *Streptococcus pneumoniae* is the leading agent causing invasive bacterial infections in children, and is among the most common causes of meningitis in children and young adults (9).

Septic shock is associated with abnormal coagulation, profound and unresponsive hypotension, vasodilatory shock, and multi-organ failure. Sepsis describes a complex clinical syndrome that results from a harmful or damaging host response to microbial infection due to a dysregulated innate immune reaction, which is characterized by an excessive production of pro-inflammatory cytokines, such as TNF- α and IL-1 β (10, 11). Mononuclear cells play a key role in the synthesis of pro-inflammatory cytokines particularly TNF- α , IL-1 β , and IL-6, as well as an array of other inflammatory mediators. These pro-inflammatory cytokines can in turn trigger secondary inflammatory cascades, including the production of cytokines, lipid mediators, and reactive oxygen species, as well as the up-regulation of cell adhesion molecules that facilitate the migration of inflammatory cells into tissues. By promoting the expression of inducible nitric oxide synthase (iNOS) and augmenting the production of nitric oxide (NO), pro-inflammatory cytokines can decrease systemic vascular resistance, resulting in profound hypotension that is most commonly observed in adult septic shock (~90% of cases). In contrast, children afflicted with septic shock have a hemodynamic profile that is most often characterized by decreased cardiac output (~80% of cases) with either normal or even elevated systemic vascular resistance (12). It is important to note that over-production of NO due to iNOS induction also appears to be a critical contributor of myocardial dysfunction in sepsis (13). In addition to these important hemodynamic effects, these cytokines also stimulate the coagulation pathway, leading to thrombosis of the microvasculature that further impairs tissue perfusion. The combination of hypotension and microvascular occlusion results in tissue ischemia, and ultimately leads to multi-organ failure (14).

The production of proinflammatory cytokines in response to pathogenic microorganisms, plays a critical role in the host defense against opportunistic microbial infections (15, 16). Cytokines and other inflammatory mediators are required for the recruitment of leukocytes to the site of infection, for the containment of the infection and ultimate eradication of the invading pathogen. Upon microbial infection, the innate immunity serves as the frontline defense against the invading pathogenic organisms. The host responds rapidly by producing anti-microbial peptides and proteins, as well as triggering phagocytosis mediated by macrophages and neutrophils (17). Interaction of innate immune cells with microbial components leads to the activation of a multitude of signaling cascades, including MAP kinases and NF- κ B transcription factor, which ultimately result in the biosynthesis of a group of proinflammatory cytokines (15, 18). MAP kinases, including extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38, play crucial roles in this process (19). These kinases not only participate in the transcription of many pro-inflammatory cytokine genes, but also participate in the transport, stabilization, and translation of cytokine transcripts (Fig. 1). In mammalian cells, MAP kinases are primarily inactivated by a group of dual specificity protein phosphatases through dephosphorylation of the critical tyrosine and threonine residues of activated MAP kinases (20). Thus, it is plausible that this group of protein phosphatases may serve as pivotal feedback control regulators in the innate immune response during microbial infection and thus, play a significant role in the resolution of sepsis pathobiology. Supporting this idea, a number of recent studies using knockout mice have demonstrated that MAP kinase phosphatase (MKP)-1 plays an essential role in the protection of host against endotoxic shock (21-24). In this review, we summarize recent progress in our understanding of the function and regulation of MKP-1 in the innate immune response to microbial challenge. We discuss the

roles of MKP-1 in the anti-inflammatory mechanisms of corticosteroids and speculate on the potential role in immunoparalysis seen in patients with sepsis.

MAP Kinase Phosphatase (MKP)-1

The function and Regulation of MAP Kinases

MAP kinases are a group of highly conserved serine/threonine protein kinases in eukaryotic species. These kinases play a pivotal role in a variety of cellular processes including cell proliferation, differentiation, stress response, apoptosis, and host immune defense. In innate immune cells, MAP kinases are crucial for the syntheses of numerous cytokines, chemokines, and other inflammatory mediators that mobilize the immune system to combat pathogenic infections (19). In adaptive immune cells, MAP kinases also serve as critical regulators in the clonal expansion of effector T- and B-lymphocytes through modulation of cytokine production, cell proliferation, and survival (19).

There are three well defined MAP kinase subfamilies: ERK, JNK, and p38 (25). The MAP kinase pathway is activated through a cascade of sequential phosphorylation events, beginning with the activation of MAP kinase kinase kinase. MAP kinase kinase kinase activates MAP kinase kinase by phosphorylating two serine residues. MAP kinase kinase in turn interacts with and phosphorylates MAP kinase at the adjacent threonine and tyrosine residues in the conserved TXY motif located in a regulatory loop between the kinase subdomains VII and VIII. Activated MAP kinases can phosphorylate a wide array of downstream targets, including protein kinases and transcription factors that facilitate the transcription of MAP kinase-regulated genes (25). MAP kinases can regulate gene expression through modulation of chromatin structure and enhancement of the activities of numerous transcription factors, including AP-1 (25). In addition to transcriptional regulation, MAP kinases can also regulate protein expression by altering stability, transport, and translation of mRNA species that contain an AU-rich element (ARE), AUUUA (19). It has been demonstrated that tristetraprolin (TTP) binds to the AREs of several cytokine mRNAs and promotes the deadenylation and destabilization of these ARE-containing mRNAs (26). However, when TTP is phosphorylated by MAP kinase-activated protein kinase (MK)-2 (27), a downstream target of p38 MAP kinase, TTP-mediated degradation of ARE-containing transcripts is inhibited (28)(Fig. 1). Many pro-inflammatory cytokine transcripts, including TNF- α , IL-1 β , IL-6, granulocyte macrophage colony stimulating factor (GM-CSF), and IL-2, contain ARE(s) in their mRNA and are targets of TTP-mediated mRNA decay (29).

Since MAP kinase pathways are activated through phosphorylation, dephosphorylation of MAP kinases mediated by phosphatases is likely to be one of the most efficient modes of kinase deactivation. Indeed, a number of protein phosphatases are known to deactivate MAP kinases, including tyrosine, serine/threonine, and dual specificity phosphatases (20). In mammalian cells, the dual specificity protein phosphatases are the primary phosphatases responsible for dephosphorylation/deactivation of MAP kinases (20). Therefore, these phosphatases are often referred to as MAP kinase phosphatases (MKPs). To date, at least 10 MKPs have been identified in mammalian cells (20).

The Structure, Function, and Regulation of MKP-1

The mouse MKP-1 cDNA was initially identified over 20 years ago as an immediate-early gene induced by serum through differential hybridization screening of a BALB/c 3T3 cDNA library (30). The cDNA clone was initially referred to as 3CH134, which encodes a protein of ~40 kDa. The DNA sequence of 3CH134 was first reported in early 1992 (31), and a human homolog (CL100) was identified as a tyrosine phosphatase gene induced by oxidative stress shortly after (32). Structurally, both 3CH134 and its human homolog CL100

contain a (I/V)HCXAGXXR(S/T)AG signature motif characteristic of the catalytic domain of the tyrosine phosphatases (Fig. 2). They also share considerable homology with the dual specificity phosphatase of vaccinia virus, VH1, particularly at the catalytic site. The 3CH134 protein or its human homologue exhibited highly specific phosphatase activity towards the ERK MAP kinase, both *in vitro* and in cultured cells (33-36). Because this was the first protein phosphatase specific for the MAP kinases, selectively targeting their phosphotyrosine and phosphothreonine residues, it was designated as MAP kinase phosphatase (MKP)-1 (36). Since MKP-1 deactivates MAP kinases and is robustly induced by mitogen stimulation that also activates MAP kinases, MKP-1 is regarded as an important feedback control mechanism governing the MAP kinases (Fig. 2). Although MKP-1 was initially thought to be a phosphatase specific for the ERK MAP kinases, subsequent studies demonstrated that MKP-1 also efficiently inactivates the stress-activated JNK and p38 MAP kinases (37, 38). The fact that MKP-1 was robustly induced by genotoxic stress that potently activated JNK and p38 but had little effect on ERK, suggested that MKP-1 might play an important role in the feedback control of these MAP kinase subfamilies (37). To address the substrate preference of MKP-1, Franklin and Kraft established a U937 cell line that conditionally expresses MKP-1. By titrating the levels of MKP-1 expression, Franklin and Kraft found that p38 and JNK were much more sensitive to inhibition by MKP-1 than ERK (39). Several recent studies conducted using MKP-1 knockout cells further support the conclusion that p38 and JNK, but not ERK, are the preferred substrates of MKP-1 (21, 22, 24). However, these studies do not exclude the possibility that MKP-1 may also participate in the inactivation of ERK, particularly when it is expressed in high levels.

The activity of MKP-1 can be regulated at multiple levels. First, MKP-1 expression can be robustly induced by growth factors and stress (20). Moreover, the induction of MKP-1 by extracellular stimuli occurs in a fashion independent of de novo protein synthesis (30). In response to extracellular stimulation, MKP-1 mRNA levels are usually increased by 10-100 fold within 15-60 minutes. Since the stability of MKP-1 mRNA does not significantly change (40), it is very likely that the induction of MKP-1 expression is due to enhanced gene transcription. Second, the stability of MKP-1 can be altered by phosphorylation mediated by MAP kinases. MKP-1 protein is degraded by the ubiquitin-directed proteasome complex (41). It has been reported that MKP-1 can be phosphorylated by both ERK and JNK (41, 42). Phosphorylation by ERK inhibits ubiquitin-mediated degradation, thus enhancing MKP-1 stability (41). While JNK also phosphorylates MKP-1, such phosphorylation actually stimulates the degradation of MKP-1 (42). Finally, in addition to induction of MKP-1 transcription and to increased protein stability, the catalytic activity of MKP-1 protein can be regulated by interaction with its substrate MAP kinases (43, 44). We have shown that binding of substrate MAP kinases, including ERK, JNK, and p38, with recombinant MKP-1 protein results in a 6-8 fold increase in MKP-1 activity (43). Analysis of the crystal structure of a related phosphatase, MKP-3, has suggested that binding of MKP-1 with its substrate MAP kinases may enable MKP-1 to adapt a more efficient conformational configuration at the catalytic site (45). Subsequent studies have demonstrated that the interaction between MAP kinases and MKPs is dependent on a kinase-interaction domain at the amino terminus of the phosphatases and an acidic domain at the carboxyl terminus of the kinases. The kinase-interaction domain contains the consensus sequence of $\psi\psi\text{XRR}\psi\text{XXG}$ (where ψ represents a hydrophobic residue and X is any amino acid), which is flanked by two Cdc25-homology domains (20).

The Role of MKP-1 in the Regulation of Microbial-Induced Host Inflammatory Response

Given the critical role of MAP kinases in the regulation of innate immune responses, it was long suspected that MKP-1 might play an important role in innate immune regulation. This

notion was supported by the observation that upon exposure to *Listeria monocytogenes*, a Gram-positive bacterium, immortalized murine macrophages underwent a robust MKP-1 induction (46). Over-expression of MKP-1 in immortalized macrophages significantly attenuated the phagocytosis of *L. monocytogenes*, suggesting that MKP-1 may negatively regulate innate immune function (47). Using bone marrow-derived murine macrophages, Valledor *et al.* demonstrated that MKP-1 was highly induced by LPS through a transcriptional mechanism mediated by protein kinase C ϵ and a tyrosine kinase(s) (48). They found that MKP-1 induction appears to coincide with ERK inactivation, raising the possibility that MKP-1 might play a significant role in modulating ERK MAP kinases in this system (48). To understand the negative regulation of cytokine biosynthesis in innate immune cells during bacterial infection, we studied the role of MKP-1 using RAW 264.7 macrophages. We found that stimulation of RAW264.7 macrophages with LPS resulted in a robust, yet transient activation of JNK and p38 (49). The activities of these MAP kinases reached peak levels within 15 min, and their activities returned to nearly basal levels within 60 min. The kinetics of p38 and JNK deactivation correlated closely with the accumulation of MKP-1 protein. Unlike JNK and p38, ERK was potently activated in response to LPS stimulation, and its activity did not diminish with the accumulation of MKP-1 protein. Likewise, stimulation of RAW264.7 macrophages with peptidoglycan also elicited a transient activation of JNK and p38, followed by MAP kinase inactivation associated with MKP-1 induction (50). The importance of MKP-1 in the inactivation of p38 and JNK was demonstrated by pharmacological inhibition of MKP-1 expression by triptolide, a diterpenoid triepoxide. Triptolide blocked MKP-1 induction in LPS-stimulated macrophages, prolonged p38 and JNK activation, but had little effect on ERK activity (49, 50), illustrating the critical role of MKP-1 in the deactivation of p38 and JNK in these cells. In contrast, a modest increase on MKP-1 expression in RAW264.7 cells shortened the window of p38 and JNK activation in LPS-stimulated cells, and markedly attenuated the production of both TNF- α and IL-6 (49-51). These studies established the concept that MKP-1 is an important negative regulator of the innate immune response. The very low basal level of MKP-1 in quiescent innate immune cells allows for a window of robust inflammatory response for cytokine production. Yet the rapid induction of MKP-1 allows the system to tune down the inflammatory response, thus achieving homeostasis. Thus, by modulating the window of p38 and JNK activation, MKP-1 limits the strength and duration of the important inflammatory signals that dictate the production of inflammatory cytokines. In other words, MKP-1 serves as a restraining mechanism to prevent the overreaction of the innate immune system (Fig. 3).

To define the physiological function of MKP-1 in microbial infection, several laboratories, including ours, have studied the effects of MKP-1 knockout on host immune responses (21-24, 51). Compared to primary macrophages isolated from wild type mice, macrophages originated from MKP-1 knockout mice exhibited prolonged p38 and JNK activation after stimulation with microbial components. The kinetics of ERK activation was not altered by MKP-1 deficiency. These results confirmed the observation made in immortalized macrophages, and firmly established MKP-1 as a primary phosphatase for p38 and JNK in innate immune cells. Compared to wild type macrophages, macrophages isolated from MKP-1-deficient mice produced substantially greater quantities of pro-inflammatory cytokines, including TNF- α and IL-6. Reflecting the profound exaggeration in the host inflammatory responses, MKP-1 knockout macrophages also synthesized considerably more chemokines, including macrophage inflammatory protein (MIP)-1 α , MIP-1 β , and MIP-2, than their wild type counterparts (21-24). It is important to note that MKP-1 deficiency not only substantially altered the dynamics of production of pro-inflammatory cytokines and chemokines, but also profoundly enhanced the production of a powerful anti-inflammatory cytokine, IL-10, in LPS-stimulated macrophages, splenocytes, and bone marrow-derived dendritic cells (21, 24). The augmented inflammatory responses in MKP-1-deficient innate

immune cells are not restricted to the responses to LPS, but are seen in cells exposed to other microbial components, including ligands for TLR2, TLR3, TLR5, TLR7 and TLR9 (24, 50). However, the expression of two classic T_H-1 cytokines, IL-12 and interferon (IFN)- γ , was decreased in MKP-1-deficient splenocytes and dendritic cells (21), suggesting a shift in cytokine production profiles. To understand the function of MKP-1 in the regulation of inflammation, Hammer *et al.* challenged wild type and MKP-1 knockout mice with LPS, and compared the gene expression profiles in the spleens between these two strains of mice using microarray (22). Their analysis demonstrated that in the wild type mice approximately 160 genes exhibited >2-fold increase in expression levels while in MKP-1 knockout mice approximately 480 genes exhibited >2-fold increase in expression levels.

Consistent with the finding that MKP-1 deficiency leads to hyper-responsiveness to LPS stimulation, MKP-1-deficient mice also produced substantially greater amounts of TNF- α , IL-1 β , monocyte chemoattractant protein (MCP)-1/CCL2, GM-CSF, IL-6, IL-10, and IL-12p70, than did wild type mice upon LPS challenge (21-24). The excessive production of the pro-inflammatory cytokines and chemokines was associated with a marked increase in physiological sensitivity to endotoxemia. Compared to wild type mice, mice deficient in MKP-1 more readily succumbed to LPS challenge, as indicated by increased incidence and severity of multi-organ failure and a higher rate of mortality (21-24). Histological analyses of the lungs of the LPS-challenged MKP-1 knockout mice revealed severe lung edema associated with massive neutrophil infiltration (21). Reflecting the hepatic damage in the LPS-challenged MKP-1 knockout mice, higher blood alanine aminotransferase activities were observed in these mice (21). Histological analysis of the livers revealed a marked increase in leukocyte infiltration in the vicinity of the bile ducts in LPS-challenged MKP-1 knockout mice, but not in similarly treated wild type mice. Moreover, glycogen contents were lower in the livers of LPS-challenged MKP-1 knockout mice than in similarly treated wild type mice, which likely reflects the dramatic decrease in food intake due to severe distress by these mice (Wang and Liu, unpublished observations). Kidney function was also impaired in the MKP-1 knockout mice challenged with LPS, whereas similarly treated wild type mice exhibited normal kidney function (21). Severe hypotension is a clinical characteristic of sepsis and plays a direct role in the development of shock and multi-organ dysfunction syndrome (11). Comparison of the vasculature function between wild type and MKP-1 knockout mice revealed a marked difference in the dynamics of systemic blood pressure change after LPS challenge between the two strains of mice (21). While LPS challenge at a dose of 1.5 mg/kg body weight did not significantly change the systemic blood pressure in wild type mice, the same challenge caused a substantial and long-lasting decrease in systemic blood pressure in MKP-1 knockout mice. Underlying the severe decrease in blood pressure in MKP-1 knockout mice, a marked increase in circulating NO was observed in these mice (21). Analysis of the lungs and livers of the mice have indicated that iNOS expression levels were substantially increased in LPS-challenged MKP-1 knockout mice relative to similarly treated wild type mice (Zhao and Liu, unpublished observations). Additionally, in response to either cell wall components isolated from *Staphylococcus aureus* or heat-killed *Staphylococcus aureus*, a strain of Gram-positive bacteria, MKP-1 knockout mice also exhibited more severe inflammatory injuries and elevated mortality relative to similarly treated wild type mice (Wang and Liu, unpublished observations). These observations strongly support the idea that MKP-1 functions as a critical negative regulator during bacterial infection. By limiting the strength and duration of the inflammatory signals, MKP-1 serves to constrain the host inflammatory responses and prevents septic shock (Fig. 3).

Paradoxically, MKP-1-deficient mice also produced substantially more IL-10 than did wild type mice after LPS challenge. Unlike wild type mice, which exhibited a transient increase in serum IL-10 levels that plummeted to basal levels within 2-3 h after LPS administration,

MKP-1-deficient mice displayed a very robust and lasting increase in serum IL-10 levels (21, 22). This observation suggests that like pro-inflammatory cytokines, such as TNF- α , the anti-inflammatory cytokine IL-10 is also controlled in a negative manner by the MKP-1-mediated pathways. Previously it has been shown that either prophylactic administration of IL-10 or administration of IL-10 shortly after LPS challenge can prevent the lethal endotoxic shock syndrome (52, 53). The fact that a substantial increase in serum IL-10 levels in MKP-1-deficient mice did not prevent endotoxic shock and mortality raises two possibilities. First, IL-10 may not be produced early enough, or in sufficient amounts, to suppress the devastating effects of the excessive pro-inflammatory cytokines (52, 53). Alternatively, the anti-inflammatory function of IL-10 may actually depend on a functional MKP-1 gene. Supporting the later postulation, Hammer *et al.* have found that IL-10 boosts MKP-1 expression in LPS-stimulated macrophages, leading to an accelerated and long-lasting p38 deactivation (54). In the absence of MKP-1, the inhibitory function of IL-10 on the host inflammatory responses may be substantially compromised, thus unable to prevent the onset of the sprawling cytokine storms responsible for endotoxic shock.

The Regulation of MKP-1 during Innate Immune Responses

The expression of MKP-1 is primarily regulated through a transcriptional mechanism. MKP-1 mRNA can be detected within minutes after exposure of macrophages to bacterial components, with maximal mRNA levels > 100-fold above basal levels reached within 1 hour after exposure. In RAW264.7 macrophages, the transcriptional induction of MKP-1 by LPS was substantially inhibited by the MEK1/2 inhibitor U0126, indicating that ERK plays a primary role in the induction of MKP-1 mRNA. The fact that U0126 did not completely block MKP-1 induction strongly suggests that other pathways also contribute to MKP-1 induction (49). To determine the upstream signals responsible for the induction of MKP-1, Chi *et al.* examined the expression of MKP-1 in primary macrophages lacking MyD88 and TRIF (24). They found that the induction of MKP-1 by LPS was reduced in both the MyD88 knockout and the TRIF-deficient cells, as compared with wild type cells, indicating that both MyD88 and TRIF are required for optimal LPS-induced MKP-1 expression. In response to ligands of TLR9 and TLR2, which only signal through MyD88, MKP-1 induction was completely ablated in MyD88^{-/-} macrophages, but was normal in TRIF-deficient cells. Conversely, loss of TRIF, but not of MyD88 function, eliminated MKP-1 expression induced by poly(I-C), a TLR3 ligand that signals only through TRIF. Together, these results demonstrate that MKP-1 is induced through MyD88 and TRIF-dependent pathways in response to various TLR ligands.

In addition to the induction of MKP-1 mRNA, MKP-1 protein became markedly more stable upon LPS stimulation, with a four-fold increase in half-life (49). The increase in MKP-1 stability in response to LPS is abolished by a pharmacological inhibitor of the ERK pathway, supporting an additional role of ERK in the regulation of MKP-1. Perhaps it is not a surprise that ERK-mediated stabilization of MKP-1 protein in response to LPS was largely abolished by deletion of 53 amino acids from the carboxyl terminus of MKP-1 (49). This carboxyl terminal region contains an ERK-docking site (55) and two serine residues phosphorylated by ERK (41). Previously, Brondello *et al.* showed that phosphorylation of MKP-1 by ERK attenuates MKP-1 degradation, a process mediated by the ubiquitin-directed proteasome complex (41).

MKP-1 and Immunomodulatory Agents

The fact that MKP-1 serves as an important negative regulator of the inflammatory responses raises an interesting question of whether MKP-1 plays a significant role in the action of immunomodulatory agents. We have screened a panel of commonly used anti-

inflammatory and anti-rheumatic drugs for induction of MKP-1, and found that MKP-1 is significantly induced by dexamethasone in RAW264.7 macrophages (49). Such induction explains an earlier observation by Swantek *et al.* that JNK activation in response to LPS is inhibited by dexamethasone (56). To understand the mechanism underlying the inhibitory effect of dexamethasone on cyclooxygenase (COX) 2 expression, Lasa *et al.* examined the effect of dexamethasone on MKP-1 expression in HeLa cells (57, 58). They found that dexamethasone induced MKP-1 expression in HeLa cells, and this induction was responsible for the inhibition of p38 and decreased expression of COX2 (59). An earlier investigation by Kassel *et al.* showed that dexamethasone induced MKP-1 in mast cells and such an induction was responsible for the inhibitor effects of glucocorticoids on ERK activity (60). To delineate the role of MKP-1 in the anti-inflammatory function of glucocorticoids, our laboratory compared a group of synthetic corticosteroids with different anti-inflammatory potencies with regard to their induction of MKP-1 in RAW264.7 macrophages. We found that the ability of these synthetic glucocorticoids to induce MKP-1 were proportional with their relative anti-inflammatory potencies (51). Very recently, using macrophages isolated from MKP-1 knockout mice, Abraham *et al.* demonstrated that p38 and JNK activation, in response to LPS stimulation, was no longer inhibited by dexamethasone in MKP-1-deficient cells (61). Accordingly, many of the inflammatory genes, including cytokines, are less sensitive to dexamethasone in MKP-1-deficient cells. Moreover, we found that dexamethasone was unable to prevent endotoxic shock in MKP-1 knockout mice while it effectively protects wild type mice from endotoxin-induced mortality (Wang and Liu, unpublished observations). These studies suggest that MKP-1 induction constitutes an important part in the anti-inflammatory mechanism of glucocorticoids. Since glucocorticoids are immunosuppressive substances released endogeneously upon exposure to stress, MKP-1 induction by corticosteroids may represent a potential mechanism underlying the immunosuppressant property of stress.

IL-10 also enhances MKP-1 expression induced by LPS, although IL-10 alone does not significantly increase MKP-1 expression (54). Through microarray analysis, Hammer *et al.* found that several MKP genes were induced in macrophages by LPS (54). They further demonstrated that up-regulation of MKP-1 mRNA was transient after stimulation with LPS alone, and IL-10 enhanced and prolonged LPS-induced MKP-1 expression. IL-10 also synergized with dexamethasone in the induction of MKP-1 and in the inhibition of IL-6 and IL-12 production. Up-regulation of MKP-1 by IL-10 in LPS-stimulated macrophages was correlated with a faster down-regulation of p38 activity, suggesting that induction of MKP-1 may constitute an important part of the anti-inflammatory mechanism of IL-10 (54).

Since MKP-1 acts to restrain the inflammatory responses, it is not surprising that cytokines known to enhance inflammation can inhibit MKP-1 expression. IFN- γ is a T_H-1 cytokine that enhances macrophage antimicrobial activity. It has been demonstrated that priming resident peritoneal macrophages with IFN- γ dramatically increases the production of inflammatory cytokines and NO upon stimulation with LPS (62, 63). We found that priming of peritoneal macrophages with IFN- γ significantly attenuates the MKP-1 expression induced by LPS, which is associated with prolonged activation of p38 and JNK (21). Interestingly, while LPS does not significantly induce iNOS expression in wild type resident macrophages without IFN- γ priming, LPS in the absence of IFN- γ induces a substantial iNOS expression in MKP-1-deficient resident macrophages (Zhao and Liu, unpublished observations). These observations suggest that inhibition of MKP-1 by IFN- γ may be an important part of the mechanism underlying the immune-enhancing properties of IFN- γ .

Macrophage migration inhibitory factor (MIF) is a powerful pro-inflammatory cytokine that enhances the expression of other pro-inflammatory cytokines in macrophages. MIF is tightly linked to lethality associated with both Gram-positive and Gram-negative bacterial sepsis in

experimental models. Either knockout of the MIF gene or immunodepletion of MIF protein confers protection against septic shock (64, 65). Interestingly, MIF was identified as a physiological counter-regulator of the immunosuppressive effects of glucocorticoids (66). However, the mechanisms whereby MIF exerts its counter-balancing effect remain largely unknown. Recently, Roger *et al.* reported that MKP-1 is a critical mediator in the MIF-glucocorticoid crosstalk (67). They demonstrated that recombinant MIF antagonized the dexamethasone effect in activated macrophages. They found that MIF inhibited the induction of MKP-1 by LPS and dexamethasone. MIF rescued the production of both TNF- α and IL-8 from dexamethasone-treated macrophages. In contrast, attenuation of MIF expression augmented MKP-1 induction by dexamethasone, leading to decreased TNF- α production. Their studies demonstrate that endogenous MIF acts through attenuating MKP-1 expression to override inhibition of glucocorticoids on cytokine production in innate immune cells. Taken together, it appears that a number of immunomodulatory factors exert their function, at least in part, through modulation of MKP-1 expression, thereby affecting the MAP kinase-mediated inflammatory responses (Fig. 4).

The Potential Role of MKP-1 in Immunoparalysis

Activation of innate immunity is a fundamental component of the host response to a variety of pathogenic challenges. Perhaps no clinical scenario exemplifies this better than the human response to bacterial pathogens, which manifests as severe sepsis or septic shock. Several characteristics of immunological function have been observed during the course of sepsis. Early on, activation of immune cells such as monocytes and macrophages via TLR engagement results in well described signal transduction events. These gene expression pathways lead to production of cytokines, chemokines and adhesion molecules that are necessary for mediating neutrophil recruitment directed to pathogen clearance. Importantly, late sepsis has been increasingly associated with different alterations in immune cell function. Notably, the immune system has been often characterized as “anergic” or hyporesponsive with a key feature including the so-called “deactivation” of circulating monocytes.

Characteristics of the state of immunological deactivation in late sepsis include decreased expression of cell surface markers of activation (e.g. the MHC-II molecule, HLA-DR), decreased antigen presentation, and decreased production of cytokines following stimulation with a variety of agonists. A canonical example of this last feature is decreased LPS-induced biosynthesis of TNF- α . In some circles, investigators have used the term “immunoparalysis” to describe the phenomena of decreased HLA-DR expression (less than 30% of normal level) and impaired *ex vivo* production of TNF- α in isolated peripheral blood monocytes following LPS-stimulation (68). Interestingly, this latter feature is remarkably reminiscent of the biological response displayed by endotoxin “tolerant” cells. The term “endotoxin tolerance” describes the phenomenon that pre-exposure of cells to a sub-activating dose of LPS significantly attenuates cytokine biosynthesis to a subsequent LPS challenge (69, 70). The close clinical association between development of infections and poor outcomes in late sepsis associated with immunoparalysis highlights the need for further understanding of the related underlying biological mechanisms.

The molecular mechanisms responsible for immunoparalysis and endotoxin tolerance have been investigated extensively. Alterations in a number of signaling pathways responsible for cytokine production and cellular activation have been documented. Since p38 and JNK MAP kinase pathways play pivotal roles in both transcriptional and post-transcriptional regulation of cytokine gene expression, it is not surprising that these pathways have been targets of extensive investigation. In light of the myriad of signal transduction pathways whose activities have been reported to decrease in endotoxin tolerance and

immunoparalysis, we and others have hypothesized that global regulators of kinase-dependent phosphorylation, such as phosphatases, may play a role in this biology.

To that end, we hypothesized that MKP-1, a transcriptionally regulated phosphatase, is induced during establishment of tolerance, resulting in suppression of TNF- α expression via inhibition of p38 activity. To test this hypothesis, we used an established model of endotoxin tolerance that relies on stimulation of a human monocyte cell line *in vitro* by a sub-activation dose of endotoxin (71). We found that there is a tight correlation between decreased p38 activation and MKP-1 induction. Over-expression of constitutively active MKP-1 through adenoviral-mediated gene transfer led to decreased p38 activity and decreased TNF- α production, suggesting a direct causal relationship between MKP-1 over-expression and endotoxin tolerance (71). In contrast, macrophages isolated from MKP-1-deficient mice were only partially tolerized to endotoxin compared to peritoneal macrophages obtained from wild type mice. Taken together, these preclinical data suggest that MKP-1 is partially responsible for endotoxin tolerance or immunoparalysis.

The relevance of this biology in the clinical arena is currently under investigation. Studies in both adults and children have shown an association between immunoparalysis and poor outcome (72-74). In a related manner, patients undergoing the more predictable systemic “insult” of cardiopulmonary bypass demonstrate substantially longer hospital courses and worse outcomes when developing post-operative infections (75). It has long been hypothesized that the development of such infectious complications is associated with a down-regulation of the innate immune response. The premise is that a prior insult, e.g. infection, trauma, cardiopulmonary bypass, etc. triggers systemic activation of the innate immune system, including a number of “stress-related” pathways. Subsequently, this activation process triggers increased expression of counter-regulating signaling proteins, including MKP-1. Interestingly, recent work has linked stress-related pathways to induction of MKP-1. For example, application of a thermal stress to cells in the form of heat shock induced expression of MKP-1 and diminished LPS-induced TNF- α expression in monocytes (76). Furthermore, this heat shock induction of MKP-1 was dependent on the presence of putative heat shock elements within the promoter of MKP-1 (77). More recently, extracellular heat shock protein (HSP)-70 was shown to induce endotoxin tolerance in cells, though whether this was mediated via MKP-1 induction was not examined (78). Together, these data support the notion that activation of stress activated pathways (for example, the heat shock response) may induce tolerance via MKP-1 induction.

One of the few predictable systemic triggers of inflammation is cardiopulmonary bypass. As stated earlier, cardiopulmonary bypass is associated with both decreased HLA-DR expression and *ex vivo* LPS hyporesponsiveness characteristic of acquired immunosuppression (79). This characteristic response is associated with a higher incidence of infectious-related sepsis and longer lengths of hospital stay (75). Investigations into potential molecular mechanisms have suggested a potential role attributed to increased IL-10 expression (80). However, additional agents may contribute to this immune phenotype. For example, pre- and peri-operative steroids are routinely used by cardiothoracic surgeons to ameliorate the global inflammatory response associated with cardiopulmonary bypass. Thus, it is interesting to consider that two agents, IL-10 and steroids, that are known to increase the expression of MKP-1 are often present in the context of cardiopulmonary bypass. Therefore, it is possible that the cardiopulmonary bypass-related immune suppression may be mediated by MKP-1 induction, but whether this is in fact the case is currently being examined.

It is tempting to speculate that in the clinical context long lasting expression of MKP-1 is a “bad” thing associated with immunoparalysis and a heightened risk of subsequent infection.

Its induction by stress (e.g. heat shock or extracellular HSP) or pathogen products (e.g. endotoxin) results in substantial down-regulation of inflammatory cell signaling (notably the MAPK pathways) with resultant compromise in pathogen clearance. On the other hand, absence of MKP-1 is associated with a substantially increased risk of morbidity and mortality after microbial infection. Therefore, the real challenging is how to achieve an optimal MKP-1 expression to avert catastrophic overshooting of inflammatory responses while avoiding the repercussion of immunoparalysis. Clearly, diagnostic determination of expression of MKP-1 and related phosphatases should provide greater clarity regarding the immune phenotype of any given patient at a point in time. Specific modulators targeting MKP-1 expression or activity are needed for future therapeutic strategies to address inflammatory diseases.

Concluding Remarks

The balance between activation and subsequent deactivation of the immunological responses is critical in the host defences against opportunistic infection. While activation of the signal transduction cascades are critical for mounting an aggressive immune response to eliminate invading pathogens, deactivation of the signaling pathways limits the potentially devastating actions of the immunological system on the host, thus preventing self destruction. A variety of negative regulators operate at various steps in the critical signal transduction pathways downstream of TLRs to moderate immunological responses. These negative regulators limit the strength and duration of the transduced signals and control the production of inflammatory cytokines (81). It has been shown that TLR4 is briefly down-regulated upon exposure to endotoxin (82). In addition to negative regulation at the receptor level, a number of anti-inflammatory proteins are induced with the expression of effector pro-inflammatory cytokines. These anti-inflammatory proteins include IRAK-M, suppressor of cytokine-signaling-1, I κ B, MKP-1, and anti-inflammatory cytokines, such as IL-10 (81). These inhibitory proteins switch off downstream signaling events, thus not only stopping the production of pro-inflammatory cytokines, but also restoring the homeostasis in the innate immune cells. Therefore, a timely “switch-off” of the signaling events is crucial, as it not only prevents the over-production of the potentially harmful cytokines, but also prepares the cells for responding to subsequent pathogenic infection. The discovery of MKP-1 as a crucial negative regulator of the innate immune response, both *in vivo* and *in vitro*, places it in the center of the complex negative regulatory mechanism dictating endotoxin tolerance. In principle, modulation of MKP-1 activity in the future could be an important avenue for the treatment of sepsis and other inflammatory diseases.

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§Abbreviations used

MKP	MAP Kinase Phosphatase
MAP	mitogen-activated protein
JNK	c-Jun N-terminal kinase
ERK	extracellular signal-regulated kinase
MK-2	MAP kinase-activated protein kinase-2
IKK	I κ B kinase

IRAK	IL-1 receptor-associated kinase
LPS	lipopolysaccharides
TNF	tumor necrosis factor
IL	interleukin
IFN	interferon
GM-CSF	granulocyte macrophage colony stimulating factor
MIF	macrophage migration inhibitory factor
MIP	macrophage inflammatory protein
MCP	monocyte chemoattractant protein
NO	nitric oxide
iNOS	inducible nitric oxide synthase
COX	cyclooxygenase
NF-κB	nuclear factor- κ B
I-κB	inhibitor- κ B
AP-1	activating protein-1
ARE	AU-rich element
TTP	tristetraprolin
TLR	toll-like receptor
MyD88	myeloid differentiation factor 88
TIR	TLR/IL-1 receptor domain
TRIF	Toll-IL-1 receptor domain containing adaptor inducing IFN- β
TRAM	TRIF-related adaptor molecule
TRAF	TNF receptor-associated factor
MHC	major histocompatibility complex
HLA	human lymphocyte antigen
HSP	heat shock protein

References

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med.* 2001; 29:1303–1310. [PubMed: 11445675]
2. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med.* 2003; 348:1546–1554. [PubMed: 12700374]
3. Wang JE, Dahle MK, Yndestad A, Bauer I, McDonald MC, Aukrust P, et al. Peptidoglycan of *Staphylococcus aureus* causes inflammation and organ injury in the rat. *Crit Care Med.* 2004; 32:546–552. [PubMed: 14758177]
4. Centers for Disease Control (CDC). Increase in National Hospital Discharge Survey rates for septicemia--United States, 1979-1987. *MMWR Morb Mortal Wkly Rep.* 1990; 39:31–34. [PubMed: 2104656]

5. Watson RS, Carcillo JA. Scope and epidemiology of pediatric sepsis. *Pediatr Crit Care Med*. 2005; 6:S3–S5. [PubMed: 15857554]
6. Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, Angus DC. The epidemiology of severe sepsis in children in the United States. *Am J Respir Crit Care Med*. 2003; 167:695–701. [PubMed: 12433670]
7. Schrag SJ, Zywicki S, Farley MM, Reingold AL, Harrison LH, Lefkowitz LB, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med*. 2000; 342:15–20. [PubMed: 10620644]
8. Pearlman M. Prevention of early-onset group B streptococcal disease in newborns. *Obstet Gynecol*. 2003; 102:414–415. [PubMed: 12907122]
9. Hoffman JA, Mason EO, Schutze GE, Tan TQ, Barson WJ, Givner LB, et al. Streptococcus pneumoniae infections in the neonate. *Pediatrics*. 2003; 112:1095–1102. [PubMed: 14595052]
10. Cohen J. The immunopathogenesis of sepsis. *Nature*. 2002; 420:885–891. [PubMed: 12490963]
11. Parrillo JE. Pathogenetic mechanisms of septic shock. *N Engl J Med*. 1993; 328:1471–1477. [PubMed: 8479467]
12. Ceneviva G, Paschall JA, Maffei F, Carcillo JA. Hemodynamic support in fluid-refractory pediatric septic shock. *Pediatrics*. 1998; 102:e19. [PubMed: 9685464]
13. Kumar A, Brar R, Wang P, Dee L, Skorupa G, Khadour F, et al. Role of nitric oxide and cGMP in human septic serum-induced depression of cardiac myocyte contractility. *Am J Physiol*. 1999; 276:R265–R276. [PubMed: 9887205]
14. Levi M. Disseminated intravascular coagulation: What's new? *Crit Care Clin*. 2005; 21:449–467. [PubMed: 15992667]
15. Beutler B, Kruys V. Lipopolysaccharide signal transduction, regulation of tumor necrosis factor biosynthesis, and signaling by tumor necrosis factor itself. *J Cardiovasc Pharmacol*. 1995; 25(Suppl 2):S1–S8. [PubMed: 8699845]
16. Akira S. Mammalian Toll-like receptors. *Curr Opin Immunol*. 2003; 15:5–11. [PubMed: 12495726]
17. Janeway, CAJ.; Travers, P.; Walport, M.; Shlomchik, MJ. *Immunobiology : the immune system in health and disease*. 5th ed. Garland Publishing; New York: 2001.
18. Ono K, Han J. The p38 signal transduction pathway: activation and function. *Cell Signal*. 2000; 12:1–13. [PubMed: 10676842]
19. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol*. 2002; 20:55–72. [PubMed: 11861597]
20. Keyse SM. Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. *Curr Opin Cell Biol*. 2000; 12:186–192. [PubMed: 10712927]
21. Zhao Q, Wang X, Nelin LD, Yao Y, Matta R, Manson ME, et al. MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxic shock. *J Exp Med*. 2006; 203:131–140. [PubMed: 16380513]
22. Hammer M, Mages J, Dietrich H, Servatius A, Howells N, Cato AC, et al. Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. *J Exp Med*. 2006; 203:15–20. [PubMed: 16380512]
23. Salojin KV, Owusu IB, Millerchip KA, Potter M, Platt KA, Oravec T. Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. *J Immunol*. 2006; 176:1899–1907. [PubMed: 16424221]
24. Chi H, Barry SP, Roth RJ, Wu JJ, Jones EA, Bennett AM, et al. Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. *Proc Natl Acad Sci U S A*. 2006; 103:2274–2279. [PubMed: 16461893]
25. Whitmarsh AJ, Davis RJ. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J Mol Med*. 1996; 74:589–607. [PubMed: 8912180]
26. Carballo E, Lai WS, Blakeshear PJ. Feedback Inhibition of Macrophage Tumor Necrosis Factor- α Production by Tristetraprolin. *Science*. 1998; 281:1001–1005. [PubMed: 9703499]
27. Mahtani KR, Brook M, Dean JL, Sully G, Saklatvala J, Clark AR. Mitogen-activated protein kinase p38 controls the expression and posttranslational modification of tristetraprolin, a regulator

- of tumor necrosis factor alpha mRNA stability. *Mol Cell Biol.* 2001; 21:6461–6469. [PubMed: 11533235]
28. Stoecklin G, Stubbs T, Kedersha N, Wax S, Rigby WF, Blackwell TK, et al. MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. *EMBO J.* 2004; 23:1313–1324. [PubMed: 15014438]
 29. Lai WS, Parker JS, Grissom SF, Stumpo DJ, Blackshear PJ. Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts. *Mol Cell Biol.* 2006
 30. Lau LF, Nathans D. Identification of a set of genes expressed during the G0/G1 transition of cultured mouse cells. *EMBO J.* 1985; 4:3145–3151. [PubMed: 3841511]
 31. Charles CH, Abler AS, Lau LF. cDNA sequence of a growth factor-inducible immediate early gene and characterization of its encoded protein. *Oncogene.* 1992; 7:187–190. [PubMed: 1741163]
 32. Keyse SM, Emslie EA. Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. *Nature.* 1992; 359:644–647. [PubMed: 1406996]
 33. Charles CH, Sun H, Lau LF, Tonks NK. The growth factor-inducible immediate-early gene 3CH134 encodes a protein-tyrosine-phosphatase. *Proc Natl Acad Sci U S A.* 1993; 90:5292–5296. [PubMed: 8389479]
 34. Alessi DR, Smythe C, Keyse SM. The human CL100 gene encodes a Tyr/Thr-protein phosphatase which potently and specifically inactivates MAP kinase and suppresses its activation by oncogenic ras in *Xenopus* oocyte extracts. *Oncogene.* 1993; 8:2015–2020. [PubMed: 8390041]
 35. Zheng CF, Guan KL. Dephosphorylation and inactivation of the mitogen-activated protein kinase by a mitogen-induced Thr/Tyr protein phosphatase. *J Biol Chem.* 1993; 268:16116–16119. [PubMed: 8344896]
 36. Sun H, Charles CH, Lau LF, Tonks NK. MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. *Cell.* 1993; 75:487–493. [PubMed: 8221888]
 37. Liu Y, Gorospe M, Yang C, Holbrook NJ. Role of mitogen-activated protein kinase phosphatase during the cellular response to genotoxic stress. Inhibition of c-Jun N-terminal kinase activity and AP-1-dependent gene activation. *J Biol Chem.* 1995; 270:8377–8380. [PubMed: 7721728]
 38. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, et al. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem.* 1995; 270:7420–7426. [PubMed: 7535770]
 39. Franklin CC, Kraft AS. Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. *J Biol Chem.* 1997; 272:16917–16923. [PubMed: 9202001]
 40. Laderoute KR, Mendonca HL, Calaoagan JM, Knapp AM, Giaccia AJ, Stork PJ. Mitogen-activated protein kinase phosphatase-1 (MKP-1) expression is induced by low oxygen conditions found in solid tumor microenvironments. A candidate MKP for the inactivation of hypoxia-inducible stress-activated protein kinase/c-Jun N-terminal protein kinase activity. *J Biol Chem.* 1999; 274:12890–12897. [PubMed: 10212278]
 41. Brondello JM, Pouyssegur J, McKenzie FR. Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. *Science.* 1999; 286:2514–2517. [PubMed: 10617468]
 42. Sohaskey ML, Ferrell JE Jr. Activation of p42 mitogen-activated protein kinase (MAPK), but not c-Jun NH(2)-terminal kinase, induces phosphorylation and stabilization of MAPK phosphatase XCL100 in *Xenopus* oocytes. *Mol Biol Cell.* 2002; 13:454–468. [PubMed: 11854404]
 43. Hutter D, Chen P, Barnes J, Liu Y. Catalytic activation of mitogen-activated protein (MAP) kinase phosphatase-1 by binding to p38 MAP kinase: critical role of the p38 C-terminal domain in its negative regulation. *Biochem J.* 2000; 352(Pt 1):155–163. [PubMed: 11062068]
 44. Slack DN, Seternes OM, Gabrielsen M, Keyse SM. Distinct binding determinants for erk2/p38alpha and jnk map kinases mediate catalytic activation and substrate selectivity of map kinase phosphatase-1. *J Biol Chem.* 2001; 276:16491–16500. [PubMed: 11278799]

45. Stewart AE, Dowd S, Keyse SM, McDonald NQ. Crystal structure of the MAPK phosphatase Pyst1 catalytic domain and implications for regulated activation. *Nat Struct Biol.* 1999; 6:174–181. [PubMed: 10048930]
46. Schwan WR, Kugler S, Schuller S, Kopecko DJ, Goebel W. Detection and characterization by differential PCR of host eukaryotic cell genes differentially transcribed following uptake of intracellular bacteria. *Infect Immun.* 1996; 64:91–99. [PubMed: 8557379]
47. Kugler S, Schuller S, Goebel W. Involvement of MAP-kinases and -phosphatases in uptake and intracellular replication of *Listeria monocytogenes* in J774 macrophage cells. *FEMS Microbiol Lett.* 1997; 157:131–136. [PubMed: 9418248]
48. Valledor AF, Xaus J, Comalada M, Soler C, Celada A. Protein kinase C epsilon is required for the induction of mitogen-activated protein kinase phosphatase-1 in lipopolysaccharide-stimulated macrophages. *J Immunol.* 2000; 164:29–37. [PubMed: 10604989]
49. Chen P, Li J, Barnes J, Kokkonen GC, Lee JC, Liu Y. Restraint of proinflammatory cytokine biosynthesis by mitogen-activated protein kinase phosphatase-1 in lipopolysaccharide-stimulated macrophages. *J Immunol.* 2002; 169:6408–6416. [PubMed: 12444149]
50. Shepherd EG, Zhao Q, Welty SE, Hansen TN, Smith CV, Liu Y. The function of mitogen-activated protein kinase phosphatase-1 in peptidoglycan-stimulated macrophages. *J Biol Chem.* 2004; 279:54023–54031. [PubMed: 15485842]
51. Zhao Q, Shepherd EG, Manson ME, Nelin LD, Sorokin A, Liu Y. The role of mitogen-activated protein kinase phosphatase-1 in the response of alveolar macrophages to lipopolysaccharide: Attenuation of proinflammatory cytokine biosynthesis via feedback control of p38. *J Biol Chem.* 2005; 280:8101–8108. [PubMed: 15590669]
52. Howard M, Muchamuel T, Andrade S, Menon S. Interleukin 10 protects mice from lethal endotoxemia. *J Exp Med.* 1993; 177:1205–1208. [PubMed: 8459215]
53. Gerard C, Bruyins C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, et al. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J Exp Med.* 1993; 177:547–550. [PubMed: 8426124]
54. Hammer M, Mages J, Dietrich H, Schmitz F, Striebel F, Murray PJ, et al. Control of dual-specificity phosphatase-1 expression in activated macrophages by IL-10. *Eur J Immunol.* 2005; 35:2991–3001. [PubMed: 16184516]
55. Jacobs D, Glossip D, Xing H, Muslin AJ, Kornfeld K. Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev.* 1999; 13:163–175. [PubMed: 9925641]
56. Swantek JL, Cobb MH, Geppert TD. Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor alpha (TNF-alpha) translation: glucocorticoids inhibit TNF-alpha translation by blocking JNK/SAPK. *Mol Cell Biol.* 1997; 17:6274–6282. [PubMed: 9343388]
57. Lasa M, Mahtani KR, Finch A, Brewer G, Saklatvala J, Clark AR. Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. *Mol Cell Biol.* 2000; 20:4265–4274. [PubMed: 10825190]
58. Lasa M, Brook M, Saklatvala J, Clark AR. Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. *Mol Cell Biol.* 2001; 21:771–780. [PubMed: 11154265]
59. Lasa M, Abraham SM, Boucheron C, Saklatvala J, Clark AR. Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38. *Mol Cell Biol.* 2002; 22:7802–7811. [PubMed: 12391149]
60. Kassel O, Sancono A, Kratzschmar J, Kreft B, Stassen M, Cato AC. Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. *EMBO J.* 2001; 20:7108–7116. [PubMed: 11742987]
61. Abraham SM, Lawrence T, Kleiman A, Warden P, Medghalchi M, Tuckermann J, et al. Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. *J Exp Med.* 2006; 203:1883–1889. [PubMed: 16880258]
62. Collart MA, Belin D, Vassalli JD, de Kossodo S, Vassalli P. Gamma interferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase

- genes, which are controlled by short-lived repressors. *J Exp Med*. 1986; 164:2113–2118. [PubMed: 3097240]
63. Gordon S. The macrophage. *Bioessays*. 1995; 17:977–986. [PubMed: 8526892]
 64. Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hultner L, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. *Nat Med*. 2000; 6:164–170. [PubMed: 10655104]
 65. Roger T, David J, Glauser MP, Calandra T. MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature*. 2001; 414:920–924. [PubMed: 11780066]
 66. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, et al. MIF as a glucocorticoid-induced modulator of cytokine production. *Nature*. 1995; 377:68–71. [PubMed: 7659164]
 67. Roger T, Chanson AL, Knaup-Reymond M, Calandra T. Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1. *Eur J Immunol*. 2005; 35:3405–3413. [PubMed: 16224818]
 68. Volk HD, Reinke P, Krausch D, Zuckermann H, Asadullah K, Muller JM, et al. Monocyte deactivation--rationale for a new therapeutic strategy in sepsis. *Intensive Care Med*. 1996; 22(Suppl 4):S474–S481. [PubMed: 8923092]
 69. West MA, Heagy W. Endotoxin tolerance: A review. *Crit Care Med*. 2002; 30:S64–S73.
 70. Cross AS. Endotoxin tolerance-current concepts in historical perspective. *J Endotoxin Res*. 2002; 8:83–98. [PubMed: 12028748]
 71. Nimah M, Zhao B, Denenberg AG, Bueno O, Molkenin J, Wong HR, et al. Contribution of MKP-1 regulation of p38 to endotoxin tolerance. *Shock*. 2005; 23:80–87. [PubMed: 15614136]
 72. Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, et al. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. *Nat Med*. 1997; 3:678–681. [PubMed: 9176497]
 73. Volk HD, Thieme M, Heym S, Docke WD, Ruppe U, Tausch W, et al. Alterations in function and phenotype of monocytes from patients with septic disease--predictive value and new therapeutic strategies. *Behring Inst Mitt*. 1991; 208:215.
 74. Kobold, AC Muller; Tulleken, JE.; Zijlstra, JG.; Sluiter, W.; Hermans, J.; Kallenberg, CG., et al. Leukocyte activation in sepsis; correlations with disease state and mortality. *Intensive Care Med*. 2000; 26:883–892. [PubMed: 10990102]
 75. Allen ML, Peters MJ, Goldman A, Elliott M, James I, Callard R, et al. Early postoperative monocyte deactivation predicts systemic inflammation and prolonged stay in pediatric cardiac intensive care. *Crit Care Med*. 2002; 30:1140–1145. [PubMed: 12006816]
 76. Sanlorenzo L, Zhao B, Spight D, Denenberg AG, Page K, Wong HR, et al. Heat shock inhibition of lipopolysaccharide-mediated tumor necrosis factor expression is associated with nuclear induction of MKP-1 and inhibition of mitogen-activated protein kinase activation. *Crit Care Med*. 2004; 32:2284–2292. [PubMed: 15640643]
 77. Wong HR, Dunsmore KE, Page K, Shanley TP. Heat shock-mediated regulation of MKP-1. *Am J Physiol Cell Physiol*. 2005; 289:C1152–C1158. [PubMed: 15958526]
 78. Aneja R, Odoms K, Dunsmore K, Shanley TP, Wong HR. Extracellular heat shock protein-70 induces endotoxin tolerance in THP-1 cells. *J Immunol*. 2006; 177:7184–7192. [PubMed: 17082636]
 79. Dehoux MS, Hernot S, Asehnoune K, Boutten A, Paquin S, Lecon-Malas V, et al. Cardiopulmonary bypass decreases cytokine production in lipopolysaccharide-stimulated whole blood cells: roles of interleukin-10 and the extracorporeal circuit. *Crit Care Med*. 2000; 28:1721–1727. [PubMed: 10890609]
 80. Allen ML, Hoschitzky JA, Peters MJ, Elliott M, Goldman A, James I, et al. Interleukin-10 and its role in clinical immunoparalysis following pediatric cardiac surgery. *Crit Care Med*. 2006; 34:2658–2665. [PubMed: 16932228]
 81. Fan H, Cook JA. Molecular mechanisms of endotoxin tolerance. *J Endotoxin Res*. 2004; 10:71–84. [PubMed: 15119998]

82. Nomura F, Akashi S, Sakao Y, Sato S, Kawai T, Matsumoto M, et al. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. *J Immunol.* 2000; 164:3476–3479. [PubMed: 10725699]

Figure 1. Diagram of the signal transduction pathways initiated at TLRs by microbial components

Binding of microbial components (ligands) to TLRs triggers conformational changes that lead to the recruitment of IRAK and TRAF-6 mediated by adaptor proteins MyD88 and TRIF. TRAF-6 can activate both the NF-κB and MAP kinase pathways. NF-κB is critical for the transcription of inflammatory response genes, including genes of various cytokines and chemokines. MAP kinases, including ERK, JNK, and p38, also regulate the expression of many inflammatory genes. MAP kinases can activate AP-1 transcription factor, thus enhancing gene transcription. MAP kinases, p38 in particular, also enhance cytokine production through post-transcriptional mechanisms. p38 phosphorylates/activates MK-2, which in turn phosphorylates TTP, leading to both enhanced cytokine mRNA stability and accelerated cytokine mRNA translation. DD, death domain.

Figure 2. Diagram of the structure and function of MKP-1

(**A**) The primary structure of MKP-1. MKP-1 has an amino terminal domain responsible for interaction with MAP kinases. The catalytic domain is located at the carboxyl terminus. (**B**) Feedback control of MAP kinases by MKP-1. Extracellular stimulation triggers the activation of MAP kinases. Upon activation, MAP kinases translocate to nucleus where they phosphorylate and activate transcription factors, leading to altered gene transcription. Among the genes activated by MAP kinases is MKP-1. MKP-1 protein can dephosphorylate MAP kinases, thus terminating MAP kinase-regulated gene transcription. By phosphorylating MKP-1 protein, MAP kinases can regulate the stability of MKP-1 protein.

Figure 3. Restraint of pro-inflammatory cytokine biosynthesis by MKP-1

In response to microbial infection, the TLRs initiate a series of signal transduction pathways, including NF- κ B and MAP kinase cascades, leading to production of pro-inflammatory cytokines. Simultaneously, the signals initiated at the TLRs also induce MKP-1 gene transcription. ERK regulates MKP-1 expression by two mechanisms. ERK enhances MKP-1 gene transcription. ERK also increases MKP-1 protein stability by phosphorylating MKP-1 and slowing down its degradation. The MKP-1 protein in turn dephosphorylates JNK and p38, thus stopping the perpetuation of the inflammatory cascades and terminating cytokine production.

Figure 4. Regulation of MKP-1 expression by immunomodulatory agents

Anti-inflammatory/ immunosuppressive agents, such as glucocorticoids and IL-10, induce/ augment MKP-1 expression, leading to inhibition of the p38 and JNK cascades and attenuation of inflammatory response. In contrast, pro-inflammatory cytokines, such as IFN- γ and MIF, inhibit MKP-1 expression, thereby perpetuating the p38 and JNK signalling pathways and enhancing the inflammatory response.