

SUPPLEMENT ARTICLE

Macrophage Migration Inhibitory Factor and Host Innate Immune Defenses against Bacterial Sepsis

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Macrophages are essential effector cells of innate immunity that play a pivotal role in the recognition and elimination of invasive microorganisms. Mediators released by activated macrophages orchestrate innate and adaptive immune host responses. The cytokine macrophage migration inhibitory factor (MIF) is an integral mediator of the innate immune system. Monocytes and macrophages constitutively express large amounts of MIF, which is rapidly released after exposure to bacterial toxins and cytokines. MIF exerts potent proinflammatory activities and is an important cytokine of septic shock. Recent investigations of the mechanisms by which MIF regulates innate immune responses to endotoxin and gram-negative bacteria indicate that MIF acts by modulating the expression of Toll-like receptor 4, the signal-transducing molecule of the lipopolysaccharide receptor complex. Given its role in innate immune responses to bacterial infections, MIF is a novel target for therapeutic intervention in patients with septic shock.

The innate immune system is the first line of host defense against infection [1–3]. Detection of invasive microorganisms by the innate immune system is mediated by soluble factors, such as the lipopolysaccharide (LPS)–binding protein (LBP) and by pattern-recognition receptors expressed on immune cells (e.g., CD14 and the Toll-like receptors [TLRs]). Binding of pathogens or microbial toxins to these receptors activates the signal transduction pathways and transcription of immune genes, resulting in the release of multiple effector molecules in the extracellular milieu, including cytokines. Cytokines promote inflammation and orchestrate the host's cellular and humoral responses that

are aimed at the elimination or walling off of invasive pathogens.

The cytokine macrophage migration inhibitory factor (MIF) was discovered nearly 40 years ago during studies of delayed-type hypersensitivity reactions. MIF was identified as a soluble nondialyzable factor released by activated lymphocytes that inhibited the random migration of peritoneal exudate cells—hence its name [4, 5]. Over the ensuing 20 years, MIF was found to exert macrophage activation functions, such as cell adhesion, phagocytosis, and killing of tumor cells and intracellular parasites [6]. However, the biologic activities attributed to MIF remained questionable, because all studies were done with cell culture supernatants of activated T cells containing numerous effector molecules in addition to MIF. A human MIF cDNA was cloned in 1989 [7], and shortly thereafter MIF was rediscovered as a protein released in a hormone-like fashion by anterior pituitary cells stimulated with endotoxin (i.e., LPS) [8]. Invigorated by this observation, investigations of MIF's biochemical properties and biologic activities progressed rapidly once purified mouse

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and human recombinant MIF proteins and neutralizing anti-MIF antibodies became available [8, 9].

MIF GENE AND PROTEIN: STRUCTURE, HOMOLOGIES, AND BIOLOGIC ACTIVITIES

The human *Mif* gene is located on chromosome 22 (22q11.2) and is composed of 3 exons separated by small introns [7, 9–11]. In all tissues examined by Northern blotting, a single MIF mRNA was identified. Baseline MIF mRNA levels are elevated in several organs including kidney, liver, and brain. In the mouse, the *Mif* gene has been mapped to chromosome 10 [12]. More than 9 *Mif* pseudogenes have been identified in the mouse genome [13, 14]. In contrast, there are no *Mif* pseudogenes in the human genome. Analyses of the sequences of the exons of mouse and human *Mif* show a high degree of homology (70%–86%). Until now, *D-dopachrome tautomerase* has been the only other human gene found to share some degree of homology (~30%) with *Mif* [15]. This suggests that MIF does not belong to any of the well-characterized cytokine superfamilies.

At the amino acid level, mouse and human MIF are 90% identical. There is also >80% homology between mouse, rat, gerbil, chicken, calf, and human MIF proteins, indicating that MIF is highly conserved across species. Of note, MIF is identical to a protein known as glycosylation-inhibiting factor, which suppresses IgE synthesis by inhibiting the *N*-glycosylation of IgE-binding factors and is associated with antigen-specific suppressor activity [16]. As indicated, MIF shares a 33% aa sequence homology with *D-dopachrome tautomerase* [17]. Bioinformatic searches for MIF homologues in genome databases have revealed the presence of MIF-like molecules in *Caenorhabditis elegans* and *Arabidopsis thaliana* and in filarial parasites such as *Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus*.

Crystallographic analyses of human and rat MIF strongly suggest that MIF is a trimer with structural similarity to dopachrome tautomerase and 3 microbial enzymes: 4-oxalocrotonate tautomerase, 5-carboxymethyl-2-hydroxyruconate isomerase, and chorismate mutase [17–21]. In agreement with these observations, MIF exerts tautomerase activity and catalyzes the conversion of 2-carboxy-2,3-dihydroindole-5,6-quinone (dopachrome) into 5,6-dihydroxyindole-2-carboxylic acid [22, 23].

Other catalytic properties have been ascribed to MIF, including the enolization of phenylpyruvate and the ketonization of *p*-hydroxyphenylpyruvate [23] and a thiol-protein oxidoreductase activity linked to a conserved Cys57-Ala-Leu-Cys60 (CALC) motif [24]. Although these findings suggest that MIF is an enzyme, the physiologic relevance of these MIF enzymatic activities remains unclear. Deletion or replacement of the N-terminal proline of MIF abolished the tautomerase activity [25] but not the capacity of MIF to inhibit monocyte migration

[26]. However, insertion of an alanine between the Pro-1 and Met-2 of MIF abolished both the catalytic dopachrome tautomerase activity and the glucocorticoid overriding activity of MIF [27]. In contrast, the first 6 N-terminal residues were not essential for the insulin-reducing oxidoreductase activity assay, yet appeared to be required for full expression of 2-hydroxyethyl disulfide activity, another assay for testing oxidoreductase activity [28]. Chromosomal mapping indicates that the *Mif* and *dopachrome tautomerase* genes are located in close proximity, which suggests the possibility that both genes are duplications of a common ancestral gene and have evolved to exert different biologic functions.

CELLULAR SOURCES AND TISSUE DISTRIBUTION OF MIF

MIF mRNA and protein are constitutively expressed by many organs and cells, including brain (neurons of the cortex and cerebellum, hypothalamus, hippocampus, pons, glial cells, and ependyma), pituitary gland (corticotrophic and thyrotrophic cells), kidneys (mesangial and epithelial cells of the proximal tubules, collecting ducts, glomeruli, and Bowman capsule), lungs (alveolar macrophages and epithelial cells of the bronchi), liver (Kupffer cells, hepatocytes, and endothelium of the central venules), spleen (white and red pulp), adrenal glands (zona glomerulosa and zona fasciculata), skin (keratinocytes, sebaceous gland, and outer root sheet of the hair follicle), endothelial cells, and fibroblasts [29].

MIF is also found in β cells of the islets of Langerhans of the pancreas, where its production is regulated by glucose in time- and concentration-dependent manners [30]. In turn, MIF potentiates insulin secretion induced by glucose. Within the immune system, MIF has been detected in monocytes, macrophages, dendritic cells, T and B lymphocytes, eosinophils, mast cells, basophils, and neutrophils. After administration of LPS to rats, preformed MIF was rapidly released from all tissues examined. The initial depletion phase was followed by the strong induction of MIF mRNA, de novo synthesis of MIF protein, and reconstitution of the intracellular MIF pool [29].

MIF AND INNATE IMMUNITY

Although MIF was first described as a T cell cytokine of the adaptive immune system, it serves important functions within the innate immune system. For >2 decades, the macrophage was considered a major target of MIF action; therefore, it was surprising to discover that monocytes and macrophages constitutively express large amounts of MIF mRNA and protein [31, 32]. Macrophage MIF is released after stimulation with microbial products that include bacterial endotoxin (LPS) and exotoxins (toxic shock syndrome toxin [TSST]–1 and strep-

tococcal pyrogenic exotoxin A [SPEA]), malaria pigment, and after exposure to gram-negative and gram-positive bacteria, mycobacteria, and proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and interferon- γ [31, 33, 34]. Many of these stimuli induce a typical “bell-shaped” release of MIF by macrophages with minimal production at the far end of the dose-response curve.

Another unusual feature of MIF is that its secretion is induced rather than inhibited by glucocorticoid hormones, as with most cytokines [32, 35]. The dose-response curve of macrophage MIF production induced by dexamethasone or cortisol is also bell shaped, with the greatest release occurring at very low glucocorticoid concentrations (10^{-12} – 10^{-14} M). Other investigators have made similar observations with synovial cells [36]. At first, this observation appeared to contradict the proinflammatory properties of MIF. However, MIF was subsequently found to override the immunosuppressive effects of glucocorticoids [32]. In vitro, MIF reverses glucocorticoid-induced inhibition of TNF- α and interleukin (IL)-1, -6, and -8 synthesis by peripheral blood mononuclear cells and of cytosolic phospholipase A2 activity and arachidonic acid release by fibroblasts [37]. Likewise, in vivo, recombinant MIF reverses the protective effects mediated by glucocorticoids in an experimental model of lethal endotoxemia [32]. Supported by a rapidly growing body of data [32, 37], a concept has emerged that MIF acts as a physiologic antagonist of glucocorticoid activity and that MIF and glucocorticoids operate in concert to regulate innate and acquired immune responses.

During investigations into the role played by MIF in the activation of the arachidonic acid–prostaglandins-leukotrienes pathway in acute inflammation and sepsis, Mitchell et al. [37, 38] observed that the proinflammatory function and viability of LPS-stimulated MIF-deficient macrophages was impaired, compared with wild-type macrophages. In the absence of MIF, macrophage survival was reduced as a result of the activation of p53-mediated apoptosis, a finding in agreement with the observation by Hudson et al. [39] of an inhibition of the tumor suppressor p53 by MIF. MIF-mediated inhibition of p53 in macrophages was found to be dependent on the induction of arachidonic acid metabolism and cyclooxygenase-2 expression. Thus, excessive production and release of MIF during inflammation and sepsis may help sustain inflammation by prolonging the lifespan of macrophages that would otherwise go into apoptosis.

Studies conducted with antisense MIF macrophages and macrophages from MIF knockout (MIF^{-/-}) mice revealed an important role for MIF in host responses to LPS and gram-negative bacteria [40]. Compared with wild-type cells, MIF-deficient macrophages were hyporesponsive to endotoxin and gram-negative bacteria, as shown by a profound reduction of NF- κ B activity and cytokine production. In contrast, MIF-de-

ficient macrophages exhibit normal responses to gram-positive bacteria (group A streptococci, *Staphylococcus aureus*, or *Streptococcus mitis*), peptidoglycan, the yeast particle zymosan, and phorbol 12-myristate 13-acetate plus calcium ionophore, which indicates that the defective response is restricted to LPS and gram-negative bacteria.

Impairment of the host defense capacity of MIF-deficient macrophages is linked to a down-regulation of TLR4, the signal-transducing molecule of the LPS receptor complex. In contrast, expression of TLR2 is preserved in MIF antisense macrophages, a finding that is congruous with normal responses to gram-positive bacteria and yeast particles. Studies of the molecular mechanism by which MIF modulates TLR4 expression indicate that reduced expression of MIF in macrophages impairs basal activity of members of the Ets family of transcription factors, which are critical for the transcription of the mouse *TLR4* gene, and results in reduced expression of TLR4 protein and defective innate immune response to LPS and gram-negative bacteria. The results also provide a mechanism whereby MIF^{-/-} mice are resistant to endotoxic shock and a rationale for the intriguing observation that MIF is expressed constitutively by macrophages and by tissues in close proximity with our natural environment. As shown in figure 1, by up-regulating the basal expression of TLR4 in resting macrophages, MIF facilitates the recognition of endotoxin-containing bacteria and enables innate immune cells to rapidly release proinflammatory cytokines that are essential for mounting the host defensive response.

MIF IN SEPSIS

The fact that MIF exerted potent proinflammatory activities and served an important function in innate immune responses of macrophages to microbial products strongly suggested a role for MIF in sepsis. This hypothesis was verified in experimental models of septic shock and in humans with bacterial sepsis. Although the administration of large quantities of recombinant MIF protein was not lethal when given alone, coinjection of MIF and LPS increased mortality, compared with LPS alone [8]. In contrast, neutralization of MIF activity with anti-MIF antibodies attenuated TNF- α production and saved mice from endotoxic shock. Similar results were obtained with MIF^{-/-} mice [41].

Resistance to lethal endotoxemia was associated with reduced circulating concentrations of TNF- α and delayed appearance of blood IL-12. MIF is also implicated in the pathogenesis of gram-positive toxic shock syndromes. Low concentrations of TSST-1 or SPEA stimulated MIF production from macrophages, and prophylactic administration of anti-MIF antibodies prevented death from toxic shock syndrome caused by the administration of TSST-1 to D-galactosamine-sensitized mice

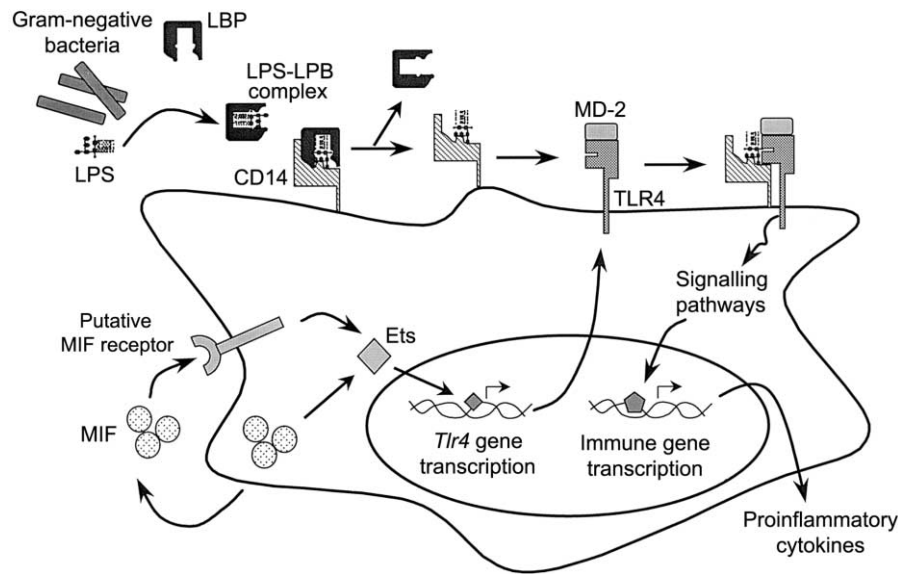


Figure 1. Role of the cytokine migration inhibitory factor (MIF) in recognition of endotoxin and gram-negative bacteria by macrophages. MIF, a trimeric molecule by crystallography, promotes the recognition of endotoxin-containing elements by up-regulating the basal expression of Toll-like receptor (TLR) 4 mRNA and protein. MIF is constitutively expressed by quiescent macrophages and may exert its effects via a direct intracellular pathway or by being first released from macrophages to then act in an autocrine fashion by binding to an unidentified putative MIF receptor. TLR4 is a signal-transducing molecule of the lipopolysaccharide (LPS; a gram-negative bacteria) receptor complex. MIF effects on *Tlr4* gene transcription are mediated by transcription factors of the Ets family. CD14, surface antigen expressed on myeloid cells that binds LPS-LBP complexes; LBP, LPS-binding protein; MD-2, protein associated with TLR4 extracellular domain that amplifies responses to LPS

[33]. Likewise, compared with wild-type mice, MIF^{-/-} mice were resistant to exposure to lethal doses of staphylococcal enterotoxin B [41]. Taken together, these results show that MIF is an important mediator of the pathogenesis of toxic shock syndrome induced by gram-negative endotoxin and gram-positive exotoxins.

MIF contributes to the pathogenesis of experimental sepsis induced by live bacteria. MIF concentrations were increased in the peritoneal fluid and in the systemic circulation of mice infected with *Escherichia coli*, and polymicrobial peritonitis and neutralization of MIF activity with anti-MIF antibodies protected mice from septic death [42]. Of note, in the cecal ligation and puncture (CLP) model, mice were protected even when treatment was started 8 h after the onset of bacterial peritonitis. The fact that mice could be rescued despite delayed administration of anti-MIF therapy is an important finding if one contemplates the possibility of using anti-MIF treatment strategies in humans. Indeed, antisepsis therapies will always be initiated after the onset of infection in patients who are admitted with severe sepsis and septic shock.

In previous studies, mice with compromised innate immune responses (e.g., those treated with anti-LBP, anti-CD14 antibodies, or with deletion of the TNF- α gene or type 1 TNF receptor) had increased susceptibility to bacterial sepsis [43–47]. As anticipated, TNF- α knockout mice were very sensitive

to CLP and succumbed quickly to uncontrolled infection. Remarkably, treatment with a single dose of anti-MIF monoclonal antibodies given after CLP protected mice from death and increased survival from none to 62%. Thus, blocking MIF activity saved these severely immunocompromised mice from lethal septic shock. Treatment with anti-MIF antibodies was associated with a reduction of plasma TNF- α concentrations and circulating bacterial counts in the *E. coli* peritonitis model. However, TNF- α and *E. coli* were not primary targets of anti-MIF therapy in these sepsis models. Indeed, anti-MIF therapy protected mice from death, even in the absence of TNF- α , and the possibility of a direct causal relationship between blockade of MIF activity and reduction of circulating bacteria was ruled out by *in vitro* studies [42].

Similar to findings in the endotoxic shock model, increasing the levels of MIF with a bolus injection of recombinant MIF at the onset of infection increased the mortality in the *E. coli* peritonitis model (21% in mice exposed to *E. coli* alone vs. 62% in mice coinjected with recombinant MIF and *E. coli*; $P = .008$). Thus, elevated tissue or circulating MIF concentrations is harmful during the course of an acute infection.

Consistent with this hypothesis, high MIF concentrations have been detected in the circulation of patients with severe sepsis or septic shock caused by gram-negative and -positive bacteria. Moreover, sicker patients had the highest MIF plasma

concentrations. Median plasma MIF levels were 17.8 ng/mL (range, 6.6–154.4 ng/mL) in patients with septic shock and 12.2 ng/mL in patients with severe sepsis (range, 6.2–141.8 ng/mL), compared with only 3 ng/mL in healthy persons (range, 1.9–5.4 ng/mL; $P = .001$) [42]. Similarly, MIF serum levels measured on admission to an intensive care unit were higher in patients with septic shock (mean \pm SD) (14.3 ± 4.5 ng/mL) than in trauma patients (3.1 ± 1.7 ng/mL) or control patients (2.5 ± 2.1 ng/mL; $P < .01$) [48]. In patients with septic shock, MIF concentrations were 18.4 ± 4.8 ng/mL in nonsurvivors and 10.2 ± 4.2 ng/mL in survivors ($P = .001$). Likewise, MIF concentrations were higher in patients with adult respiratory distress syndrome (ARDS) than in those without ARDS (19.4 ± 4.7 vs. 9.2 ± 4.2 ng/mL, respectively; $P = .12$). By logistic regression analysis, the serum concentrations of MIF are a better parameter than ARDS for predicting a patient's outcome. In addition, in patients with sepsis, MIF levels are correlated with cortisol or IL-6 [48], a finding that is in agreement with our previous observations of parallel increases of MIF and glucocorticoids in the systemic circulation during inflammation, infection, and stress [32, 42].

CONCLUSIONS

As anticipated because of its potent proinflammatory properties, MIF is an important mediator of sepsis in experimental models of infection and in humans with severe sepsis or septic shock. Immunoneutralization of MIF or deletion of the *Mif* gene protected mice against lethal endotoxemia, gram-positive toxic shock syndromes, and experimental bacterial peritonitis; however, administration of recombinant MIF increased the mortality of endotoxemia and bacterial sepsis. Likewise, high concentrations of MIF were detected in the systemic circulation of patients with severe sepsis and septic shock and were associated with patient outcome. During sepsis, MIF may endanger life when expressed in large quantities. Anti-MIF strategies, which interfere either with MIF production or with MIF activity once it is released in the extracellular milieu, may have a role in the care of patients with septic shock.

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