Chapter 4

MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF): A PRO-INFLAMMATORY MEDIATOR OF SEPSIS

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Introduction

Over the last ten years, the protein known as macrophage migration inhibitory factor (MIF) has emerged to be a central cytokine of the innate immune system and was found to play an important part in the control of inflammatory responses. MIF is considered to be one of the first cytokine activities described. Investigations of the mechanisms of delayed-type hypersensitivity reactions conducted in the late 1960s have led to the recognition of an activity that inhibited the random migration of guinea pig peritoneal exudate cells. In 1966, Bloom and David associated this macrophage migration inhibitory activity to a soluble, non-dialyzable factor released by sensitized lymphocytes (1,2). These reports stimulated the curiosity of immunologists as MIF was one of the first lymphokines to be described. Over the next 20 years, MIF was found to enhance a broad-spectrum of macrophage functions, including adherence, phagocytosis, spreading, and tumoricidal activity (reviewed in reference 3). The biological activities ascribed to MIF remained uncertain as cytokines such IFNγ and IL-4 also were observed to inhibit macrophage migration. The cloning of a human MIF cDNA in 1989 was a milestone in the investigations of the biochemical and biological properties of MIF (4). However, initial studies

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were performed with unpurified recombinant MIF protein that was later found to contain a mitogenic contaminant.

In the early 1990s, MIF was re-discovered as a pituitary peptide during investigations of factors that may serve to control inflammatory responses induced by sepsis. LPS-stimulated AtT-20 anterior pituitary cells were found to release copious amounts of a 12.5-kDa protein (5). N-terminal sequencing of the protein revealed that this protein was the mouse homologue of human MIF and shared 90% identity with human MIF. Cloning and sequencing of an AtT-20 MIF cDNA confirmed these results. Additional experiments indicated that MIF was secreted from the pituitary gland and from the adrenal cortex in a hormone-like fashion after LPS stimulation (6,7). These initial observations provided the impetus to clone the mouse and human *Mif* genes, to express recombinant proteins and produce antibodies to study the biological activities of MIF.

In recent years, several comprehensive reviews of the biochemical and biological properties of MIF have been published (3,8,9). The objective of the present review is to focus on the role played by MIF in host responses against infection.

MIF GENE AND GENE REGULATION

Cloning and Characterization

The human and mouse *Mif* genes have been cloned (10,11). The mouse *Mif* gene has been mapped to chromosome 10 and lies between the Bcr and S100b loci (11,12). At least nine *Mif* pseudogenes have been identified in the mouse genome (12,13). A mouse *Mif* gene isolated from the liver was shown to be composed of three exons and two short introns of 201 and 145 nucleotides (11). The position of mouse *Mif* gene on chromosome 10 is coincident with several recessive mutations, including the gray lethal (gl), mocha (mh), and grizzled (gr) (11). However, there is no indication that mutations of the *Mif* gene result in any known mouse defect or disease. In fact, MIF knockout mice exhibit a normal phenotype and were reported to be healthy (14). In contrast to mouse *Mif*, human *Mif* was reported to exist as a single functional gene without pseudogenes (10). It was mapped to a region of chromosome 22 (22q11.2) known to be in syntenic conservation with a section of mouse chromosome 10 containing the *Mif* gene (15). The genomic structure of human *Mif* is analogous to mouse *Mif* and contains three exons spanning less than 1 kb that are separated by small introns (189 and 95 bp). Predicted coding sequence of the *Mif* gene was identical to sequences of the *Mif* cDNA of the human eye lens (16) and the Jurkat human

T-cell line (17). A single MIF mRNA band of approximately 0.8 kb was found in all human tissues examined by Northern blotting. Like in the mouse, many human organs, including the kidney, brain and liver, expressed high MIF mRNA levels at baseline. The mouse and human *Mif* genes exhibit a high degree of homology within each exon (70.4%, 86.4% and 67.5% for exons 1, 2 and 3, respectively). Except for D-dopachrome tautomerase that shares about 30% homology with *Mif*, no noteworthy homology have been identified between *Mif* and any other DNA sequences (18).

Promoter Elements

Analysis of the 5'-regulatory region of the mouse *Mif* gene revealed the presence of several consensus sequences for transcription factors. The promoter region comprises sequence motifs implicated in the basal expression of the proto-oncogene *c-fos*, an Sp-1 site, a c-AMP responsive element (CRE), an AP-2 site, and an alleged negative glucocorticoid responsive element (nGRE) (11). All have been identified within 1 kb of the RNA transcription start, except for a cytokine-1 (CK-1) and a nuclear factor-κB (NF-κB) site located further upstream on the minus DNA strand. Functional studies of the mouse MIF promoter showed that CRE binding protein (CREB) mediated corticotrophin releasing factor (CRF)-induced *Mif* gene transcription in pituitary cells (19). Sequence analyses of the 5' region flanking the human MIF gene also revealed the presence of CRE and multiple Sp-1 sites (10).

STRUCTURE OF THE MIF PROTEIN

Mouse and human MIF display 90% identity of their amino acid sequences. Overall, the MIF proteins exhibit more than 80% homology across the species examined (mouse, rat, gerbil, chicken, calf, and human) and comprise an invariant proline residue at the N-terminus. For example, mouse and rat MIF differ only by one amino acid residue. MIF is identical to a protein known as glycosylation-inhibiting factor (GIF). GIF has been shown to suppress IgE synthesis and be associated with antigen-specific suppressor activity (20). Homologues of the MIF protein have been reported in *Caenorhabditis elegans*, *Arabidopsis thaliana* and in filarial parasites (*Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus*). MIF shares a 33% amino acid sequence homology with D-dopachrome tautomerase (21). No significant sequence homology have been found between MIF and other cytokines, indicating that it does not belong to any cytokine superfamily. MIF and D-dopachrome tautomerase may be two

members of a new protein superfamily. Like IL-1, basic FGF, and a secreted form of cyclophilin, MIF lack a classical N-terminal leader sequence and is thus released from cells by a nonconventional protein secretion pathway. Two potential N-glycosylation sites have been identified at positions 75 and 110, but structural analysis of native MIF by mass spectrometry or enzymatic studies have ruled out any detectable, post-translational -glycosylation.

Crystal Structure

The crystal structure of human and rat MIF have been solved (22,23). It has revealed that MIF is a trimer with dimensions of 35 x 50 x 50 Å. MIF monomer consists of two antiparallel α-helices and six β-strands. The secondary and tertiary structure of MIF display a pseudo-twofold symmetry with a βαββββαββ sequence. The pseudo-twofold symmetry axis is perpendicular to the β-sheet. The β1, β2, and β4 sheets form the inner part of the trimer and wrap around so that the trimer has the form of a barrel with a solvent-accessible channel. Despite no sequence homology, significant three-dimensional structural homology have been found between MIF and two bacterial isomerases, 4-oxalocrotonate tautomerase (4-OT) and 5-carboxymethyl-2-hydroxymuconate isomerase (CHMI) (24). Readers interested in a more detailed discussion of the three-dimensional structure of MIF should refer to an excellent review article on this topic (9).

Enzymatic Activity

Purified recombinant MIF protein was found recently to exhibit a tautomerase activity catalyzing the conversion of the melanogenic substrate 2-carboxy-2,3-dihydroindole-5,6-quinone (dopachrome) into 5,6-dihydroxyindole-2-carboxylic acid (DHICA) (25,26). These observations have suggested the intriguing possibility that MIF may exert some of its biological effects by catalyzing an enzymatic reaction. However, *D*-dopachrome is not a naturally-occurring product and natural MIF substrates thus remain to be identified. Interestingly, the fact that MIF displays an isomerization activity bears some resemblance with the situation described for cyclophilin. Cyclophilin, the target for the immunosuppressant drug cyclosporin A, has been shown to act intracellularly as a peptidyl-prolyl *cis-trans* isomerase, and very much like MIF was found to be released from cells in response to pro-inflammatory stimuli. The catalytic activity of MIF was linked to the N-terminal proline of the protein as deletion or replacement of this amino acid residue abolished the enzymatic activity (27). Other enzymatic properties have been ascribed to MIF. These include the

enolization of phenylpyruvate and the ketonization of p-hydroxyphenylpyruvate (26). However, the physiological significance of the enzymatic activity linked to MIF remains unclear. Substitution of the N-terminal proline by a serine abrogated the tautomerization activity of MIF, but not its glucocorticoid counter-regulatory activity (27). Consistent with these initial observations, it was recently observed that MIF mutants in which the N-terminal proline was replaced either with a serine or with a phenylalanine exhibited reduced or no phenylpyruvate tautomerase activity, yet they remain capable of inhibiting monocyte chemotaxis and random migration (28). These data strongly suggest that the immunological or migration inhibiting properties of MIF are not mediated by an enzymatic activity. Chromosomal mapping of the *Mif* and dopachrome tautomerase genes have shown that they are in close proximity. It is therefore conceivable that both genes arose by duplication of a common ancestral gene and have evolved to exhibit different functions. MIF was reported to exert a thiol-protein oxidoreductase activity (29). Work is in progress to elucidate the biological relevance of this finding.

CELLULAR SOURCES OF MIF WITHIN THE IMMUNE SYSTEM

As shown in Table 1, the MIF protein displays a wide tissue distribution. We will focus our attention on the cellular sources of MIF within the immune system as the objective of the present article is to review the role played by MIF in host responses against infection.

Monocytes and Macrophages

Originally, T cells were thought to be the main cellular source of MIF and macrophages considered to be the main cellular target of MIF released by T-cells. However, in 1994 cells of the monocyte/macrophage lineage were identified to be an abundant source of MIF (6). Unlike other cytokines, high baseline levels of MIF mRNA and MIF protein were detected in resting macrophages. Macrophages release MIF when stimulated with microbial products [LPS, toxic shock syndrome toxin-1 (TSST-1), streptococcal pyrogenic exotoxin A (SPEA) and malaria pigment], bacteria (Gram-negative and Gram-positive, *M. tuberculosis*) and cytokines (TNFα and IFNγ) (6,30-32) (Oddo M et al., unpublished). Of note, macrophage MIF secretion is induced by very low concentrations of LPS, TSST-1 or SPEA. Production of MIF by macrophages follows a classical bell-shaped curve that may protect the host from the detrimental effect of excessive concentrations of this powerful pro-inflammatory cytokine. MIF induces TNFα secretion by macrophages and synergizes with IFNγ to augment nitric

Table 1. Tissue Distribution and Cellular Sources of MIF

Immune system:
Monocytes/macrophages, T cells, B cells
Eosinophils, mast cells, basophils
Neutrophils
Spleen (white pulp, red pulp)
Endocrine system:
Pituitary gland (corticotrophic cells)
Adrenal cortex (zona glomerulosa, zona fasciculata)
Pancreas (β cells of the islet)
Brain:
Cortex (neurons), hypothalamus, cerebellum (neurons), hippocampus, pons, glial cells, ependyma
Kidney:
Epithelial cells (proximal tubules, collecting ducts, glomeruli Bowman's capsule), mesangial cells
Lung:
Alveolar macrophages, epithelial cells (bronchi)
Liver:
Kuppfer cells, hepatocytes, endothelium (central venules)
Gastrointestinal tract:
Epithelial cells (esophagus, stomach, small and large intestines)
Neurons (myenteric and submucosal plexi)
Skin:
Keratinocytes, sebaceous gland, hair follicle (outer root sheet) endothelial cells, fibroblasts
Eye:
Lens, epithelial cells (cornea, iris, ciliary body), endothelial cells, retina (epithelium, Müller cells, astrocytes)
Testis:
Leydig cells
Prostate:
Epithelial cells
Ovary:
Granulosa cells of follicles
Bone:
Osteoblasts
Fat tissue:
Adipocytes
Vasculature:
Endothelial cells

oxide production, and thus works in concert with these two cytokines to intensify pro-inflammatory responses of macrophages (6,17). Unexpectedly, glucocorticoid hormones were found to induce, rather than to inhibit MIF secretion (33). As observed with bacterial toxins, glucocorticoid-induced macrophage MIF production also showed a bell-shaped dose response curve. Peak MIF release occurred at concentrations of dexamethasone in the range of 10^{-12}-10^{-14} M. Similar results were obtained when macrophages were stimulated with cortisol. The implication of the paradoxical observation that glucocorticoids stimulate the release of a pro-inflammatory cytokine is discussed below.

T Cells

Even though MIF was discovered originally as a factor released by lymphocytes, relatively little is known about the effect of MIF on either T cells or B cells, presumably because the macrophage was thought to be the main cellular target of MIF. As observed for macrophages, pre-formed MIF protein was detected in resting T cells (34). T cells purified from mouse spleen or human peripheral blood expressed MIF mRNA and MIF protein, which is released after stimulation with mitogen, anti-CD3 antibody, and specific antigen (34). MIF was found to be produced predominantly by the T_H2 subset of helper T cells. Subsequent experiments showed that the staphylococcal superantigen TSST-1 was a very powerful inducer of MIF secretion from mouse splenocytes (30). MIF secretion was induced by concentrations of TSST-1 that were much lower than those required to trigger IL-2 or IFNγ secretion (10 pg/ml versus 1 ng/ml). Subsequent studies established that MIF contributes to T cell activation *in vivo*. Anti-MIF antibodies inhibited T cell proliferation and IL-2 production *in vitro* (34). In agreement with the observation that MIF is released by T_H2 lymphocytes, anti-MIF antibody also suppressed antigen-driven T cell activation and antibody production *in vivo*. Moreover, pretreatment of mice with neutralizing anti-MIF antibody prior to injection of the superantigenic staphylococcal exotoxin TSST-1 prevented spleen enlargement and reduced by half the proliferation of splenocytes measured *ex vivo* 72 hr after TSST-1 challenge (30). These results therefore indicate that MIF exerts autocrine effects on T cells and contributes to the mitogenic response of lymphocytes after activation with staphylococcal exotoxins, tetanus toxoid or anti-CD3 antibodies. Glucocorticoids also induced MIF secretion by T cells in a concentration-dependent manner (34). Work is in progress to characterize the role played by MIF in T-cell mediated immunity.

B Cells

Previous studies have suggested a role for MIF in activation of B cells. Anti-MIF antibody were shown to inhibit antibody production by B cells (34). More recently, MIF protein was reported to be released in copious amounts by an Epstein-Barr virus-transformed human B-cell line (35). Interestingly, MIF was observed to bind to HLA-DRB1*0405 via the amino acid sequence Y36IAV39, confirming a part for MIF in B cell activation. MIF protein was also detected in the WEHI 231 B-cell line (36). In contrast to wild-type WEHI 231 B-cells or cells transfected with a control vector, cells transfected with a MIF antisense plasmid (WaM2), that decreased the levels of endogenous MIF protein, displayed reduced growth arrest and apoptosis after crosslinking of IgM. This was associated with a reduced proportion of cells in G1 phase and an increased proportion of cells in S phase. These experiments therefore suggest a role for MIF in the control of cell cycle progression and apoptosis in B cells.

Neutrophils

Together with complement, neutrophils are the first line of host defenses against invading micro-organisms and as such are a critical element of the innate immune system. MIF protein is expressed in neutrophils (37). However, it is expressed in much lower quantities than in macrophages or T cells, as shown by the fact that initial Western blotting analysis of the MIF content of human neutrophils with anti-mouse MIF antibodies yielded negative results, while macrophages and T cells showed strongly positive signals (6). Pretreatment of neutrophils with recombinant MIF resulted in an augmentation of superoxide production after stimulation with FMLP (37), suggesting that MIF might exert a priming effect on neutrophils. Recombinant MIF was found to augment chemoattractant-induced luminol- and lucigenin-amplified neutrophil chemiluminescence, but inhibited phorbol myristate acetate (PMA) –induced neutrophil luminol-amplified chemiluminescence. (M. Markert and T. Calandra, unpublished). Administration of anti-MIF antibody to rats decreased LPS-induced influx of neutrophils into the lungs and reduced the myeloperoxidase activity in lung tissue (38). Compared to heterozygous or wild-type mice, MIF knockout mice had lower neutrophils counts in the BAL after the instillation of *Pseudomonas aeruginosa* into the trachea and enhanced clearance of the bacteria (14).

Eosinophils, Mast Cells and Basophils

Allergic reactions often follow a bimodal course with an early and a late phase. Mast cells and basophils play an important role in the early phase, while eosinophils are the main cellular component of the late phase. Given the important contribution of MIF in inflammatory responses and the role played by eosinophils, mast cells and basophils in the pathogenesis of allergic diseases, researchers measured the concentrations of MIF in the broncho-alveolar lavage (BAL) of asthmatic patients and looked for the cellular sources of MIF. As anticipated, elevated concentrations of MIF were detected in the BAL of these patients (39). Like monocytes, macrophages, T cells and B cells, unstimulated eosinophils were described to also be an abundant cellular source of MIF (39). Stimulation of human eosinophils with C5a, IL-5 and phorbol myristate acetate (PMA) induced an immediate release of MIF protein. The finding of elevated concentrations of MIF in the BAL of asthmatic patients may have potential therapeutic implications. Indeed, anti-MIF strategy might help to control excessive airway inflammation, which is a key feature of allergic pulmonary reactions. Furthermore, MIF mRNA and protein were identified in mast cells isolated from the rat peritoneum (40). The human HMC-1 immature mast cell line and in the KU812 basophil cell line constitutively express and secrete high amounts of MIF (40).

Natural Killer Cells

Together with monocytes, macrophages and neutrophils, natural killer (NK) cells contribute to innate immune responses. During the course of studies that examined the mechanisms sustaining immune privilege in the anterior chamber of the rabbit eye, a factor sharing more than 90% homology with residues 95-108 of human MIF was shown to inhibit the NK-mediated lysis of corneal endothelial cells (41). Of note, recombinant mouse MIF inhibited NK activity and neutralizing anti-MIF restored it. Yet, MIF was not directly toxic to NK cells, but exerted its effects by interfering with the release of cytolytic perforin granules from NK cells. Interestingly, MIF did not affect perforin release by allospecific cytotoxic T lymphocytes (CTL) and did not inhibit CTL-mediated cytolysis of allogeneic target cells. It is unclear whether NK cells express MIF protein. Yet, the eye lens, the iris and the cornea and tissue macrophages are well-known sources of MIF that may act locally on NK cells (16,42).

BIOLOGICAL ACTIVITIES OF MIF

The main properties and biological activities of MIF are reported in Table 2.

Table 2. Properties and Biological Activities of MIF

Innate Immunity and Inflammation	Inhibition of macrophage migration Pituitary peptide, macrophage and T cell cytokine secreted in response to pro- and anti-inflammatory stimuli (endotoxins;exotoxins: TSST-1, SPEA; PPD; malaria pigment; cytokines: TNFα, IFNγ ; glucocorticoids) Activation of macrophages (promotes cytokine and NO production, phagocytosis of particles and killing of micro-organisms) Critical mediator of septic shock (endotoxemia, TSST-1-induced shock, *E. coli* peritonitis, cecal ligation and puncture peritonitis) Counter-regulator of the anti-inflammatory effects of glucocorticoids (macrophages) Involved in the pathogenesis of glomerulonephritis and arthritis Involved in the pathophysiology of malaria anemia
Acquired immunity	Required for antigen-dependent T cell proliferation, antibody production by B cells and delayed-type hypersensitivity reaction Counter-regulator of the immunosuppressive effects of glucocorticoids (T cells)
Glucose metabolism	Promotes insulin release from cells of the islet of Langerhans Regulates glycolysis by inducing expression of 6-phosphofructo-2-kinase/fructose-2,6-bisphophatase (PFK-2)
Angiogenesis	Promotes proliferation of endothelial cells
Cell growth	Mitogen for fibroblasts Promotes proliferation of tumor cells
Enzymatic activity	Tautomerase activity Oxidoreductase activity

MIF as a Mediator of Sepsis

The fact that MIF was rediscovered in the early 1990s as a pituitary and macrophage mediator induced by microbial products (LPS, TSST-1, SPEA) and cytokines (TNFα and IFNγ) suggested a role for MIF in host defenses

against infection (5,6,30). Subsequent studies have confirmed a critical part for MIF in innate immunity. Immunohistochemistry, Western blotting and *in situ* hybridization analyses have revealed that MIF is also expressed constitutively in several cell types of numerous organs, including lung, liver, spleen, kidney, pituitary gland, adrenal gland and skin (6,7). Administration of LPS in the rat was accompanied within 6 hours by a release of pre-formed MIF protein from nearly all tissues examined. This was followed by a strong induction of MIF mRNA resulting in restoration of supranormal levels of intracellular MIF at 24 hours (7). Similar to TNFα and IL-1, MIF is a critical mediator of experimental endotoxemia, even though it does not induce hypotension when injected intravenously in rodents. Recombinant MIF aggravates lethal endotoxemia when co-injected with LPS into mice. On the contrary, neutralizing anti-MIF antibodies fully protect mice from endotoxic shock (5). Increased survival was associated with a 50% reduction of the circulating levels of TNFα (Bernhagen et al., unpublished). Confirming the initial results obtained with anti-MIF antibodies, mice with deletion of the MIF gene were reported to be resistant to endotoxic shock (14). Of note, serum levels of TNFα, but not of IL-6 or IL-10, were lower in MIF knockout mice than in wild-type endotoxemic mice.

MIF also is implicated in the immune responses induced by staphylococcal and streptococcal exotoxins. Low concentrations of TSST-1 and SPEA were found to induce the release of MIF from macrophages and anti-MIF antibodies prevented death in a mouse model of toxic shock syndrome caused by the staphylococcal exotoxin TSST-1 (30). Moreover, anti-MIF antibodies also decreased lymphocyte proliferation stimulated by the superantigenic TSST-1. MIF knockout mice also were resistant to shock induced by staphylococcal enterotoxin B in galactosamine-sensitized mice (14). Overall these studies showed that Gram-negative endotoxins and Gram-positive exotoxins are very potent inducers of MIF secretion by immune cells and established an important role for MIF in the pathogenesis of toxic shock syndromes.

To assess the role of MIF in the pathogenesis of septic shock caused by live bacteria, experiments were performed in two models of bacterial peritonitis that mimic one of the most frequent causes of septic shock in humans (31). Infection was elicited either by an intraperitoneal injection of *E. coli* or by cecal ligation and puncture (CLP). Development of bacterial peritonitis increased the concentrations of MIF in the peritoneum and in the blood. Anti-MIF antibody protected mice from lethal peritonitis induced by CLP and by *E. coli*, even when treatment was started as late as 8 hours after the onset of CLP (Figures 1 and 2) (31). Noteworthy, anti-MIF treatment also protected TNF knockout mice, which are exquisitely sensitive to CLP due to the inability to mount an immune response in the absence of this

pivotal pro-inflammatory cytokine. The latter finding provided a direct evidence for an intrinsic contribution of MIF to the pathogenesis of sepsis. As was observed in experimental endotoxemia, anti-MIF therapy reduced plasma levels of TNFα, and to a lesser extent of IL-6, of mice with *E. coli* peritonitis. However, TNFα is probably not the main target of MIF-mediated effects in sepsis as treatment with anti-MIF antibody protected TNFαknockout mice from death. Survival advantage achieved with neutralization of MIF activity was associated with lower blood counts of *E. coli*. However, there was no evidence of a direct cause and effect relationship between anti-MIF therapy and bacterial counts. rMIF did not promote the growth of *E. coli in vitro*. Moreover, in contrast to the results obtained with anti-TNF agents, anti-MIF therapy did not increase mortality in mice challenged with an low inoculum of *E. coli* (31). Compared to wild-type mice, MIF knockout mice have been shown to clear *Pseudomonas aeruginosa* instilled into the trachea better than wild-type mice and had lower neutrophil counts in their broncho-alveolar fluid. Taken together, these results suggest that bacterial clearance is an early marker of improved outcome rather than a primary target of anti-MIF-mediated effects. Furthermore, mice treated with anti-MIF IgG had similar serum levels of corticosterone than those treated with control IgG, as measured 12 hours after the onset of *E. coli* peritonitis (T. Calandra, unpublished). Thus, neutralization of MIF activity did not increase the circulating levels of glucocorticoids, suggesting that the protective effects of MIF were not mediated by an augmentation of endogenous glucocorticoid hormones. What could then be the mechanism by which MIF plays a detrimental role in septic shock? MIF was shown recently to stimulate cytosolic phospholipase A_2 activity and arachidonic acid release by fibroblasts (43). Activation of the arachidonic acid-prostaglandins-leukotrienes pathway might be an important component of MIF-mediated effect in sepsis. Experiments carried out with macrophages expressing an antisense MIF cDNA plasmid have revealed an important role for MIF in LPS signaling (T. Roger, manuscript in preparation). On the other hand, lethality of peritonitis was increased when mice were co-injected with rMIF and *E. coli*, thus supporting the concept that elevated tissue or blood levels of MIF are harmful. In fact, high levels of MIF were detected in the plasma of patients with severe sepsis or septic shock caused by Gram-negative or by Gram-positive bacteria (31). Median concentrations of MIF were 12.2 ng/ml (range: 6.2 to 141.8 ng/ml) and 17.8 ng/ml (range: 6.6 to 154.4 ng/ml) in patients with severe sepsis and septic shock, respectively, compared with 3 ng/ml (range: 1.9-5.4 ng/ml) in healthy controls (P values were 0.001 and 0.0004 for controls versus patients with severe sepsis or septic shock, respectively, and 0.3 for patients with severe sepsis versus septic shock) (Figure 3).

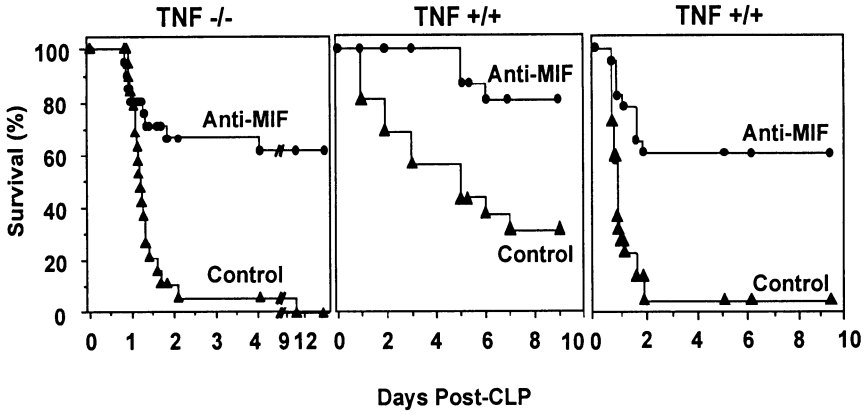

Figure 1. Anti-MIF monoclonal antibodies (mAb) protect TNF knockout (TNF -/-) and wild-type (TNF +/+) mice from septic shock induced by cecal ligation and puncture. Left panel : Survival : Anti-MIF mAb : 62% , control antibody : 0%, P=0.00002. Middle panel : Anti-MIF mAb was given at the time of surgery (t=0h), survival : Anti-MIF mAb : 81%, control antibody : 31% , P=0.01. Right panel : Anti-MIF mAb was given 4.5 h after surgery, survival : Anti-MIF mAb 61%, control antibody : 5%, P=0.00008. Adapted from reference #31 with permission.

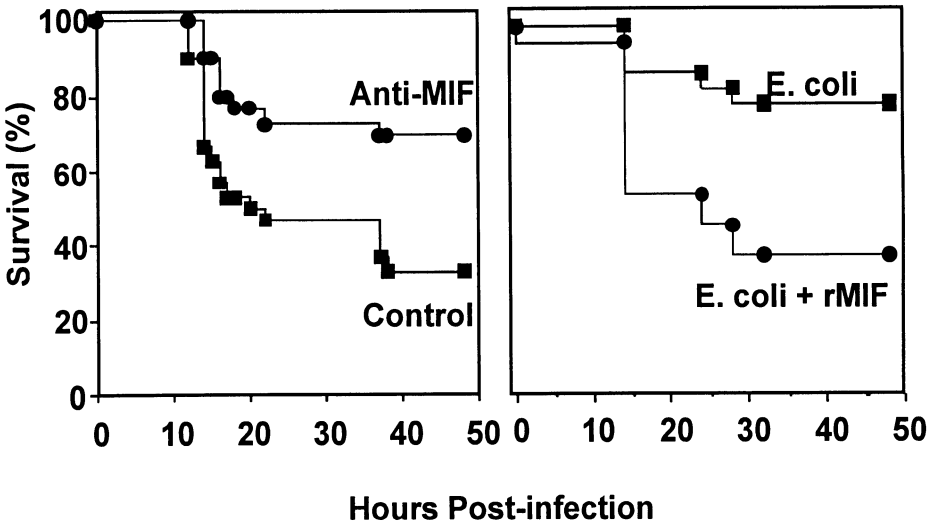

Figure 2. Anti-MIF IgG protects against lethal *E. coli* peritonitis (left panel). Survival : anti-MIF IgG : 70%, control antibody : 33%, P=0.009. MIF potentiates lethality of *E. coli* peritonitis (right panel). Mortality : *E. coli* alone: 21%, *E. coli* + rMIF : 62 %, P=0.008. Adapted from reference #31 with permission.

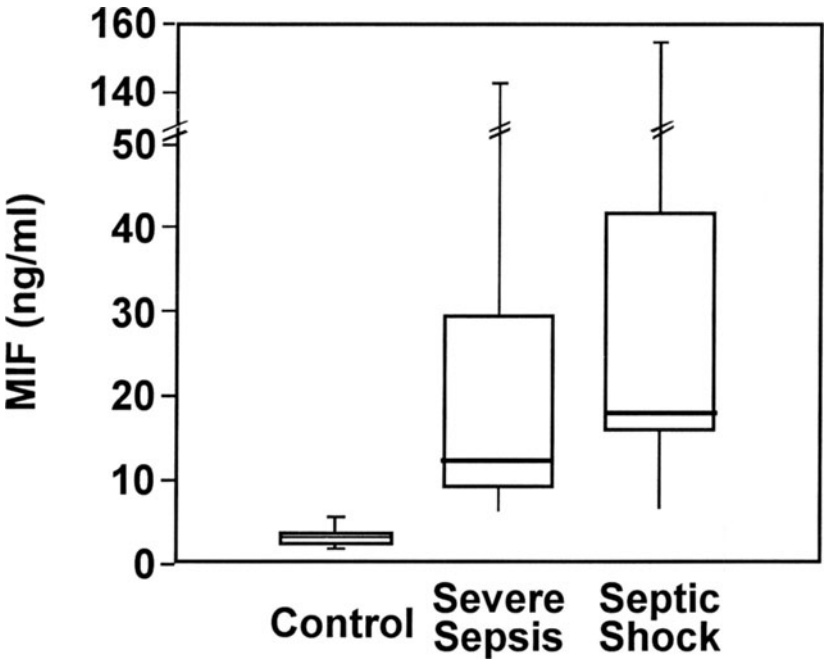

Figure 3. Boxplots of the plasma concentrations of MIF of healthy individuals (controls) (n=6) and of patients with either severe sepsis (n=7) or septic shock (n=9). The bottom, median and top lines of the box mark the 25th, 50th and 75th percentiles, respectively. The vertical line shows the range of values comprised between the 5th and 95th percentiles. Reproduced from reference #31 with permission.

In addition to septic shock, MIF was shown to be involved in the pathophysiology of malaria and leishmaniasis. Ingestion of erythrocytes infected with *Plasmadia chabaudi* or malarial pigment (hemozoin) by macrophages stimulated the release of MIF (32). Interestingly, MIF was found to inhibit erythroid, multipotential, and granulocyte-macrophage progenitor-derived colony formation, suggesting that MIF could be implicated in the pathophysiology of malaria anemia. MIF mRNA and protein have been detected in the lymph nodes of mice infected with *Leishmania major* (44). Consistent with the macrophage activating properties of MIF, recombinant MIF increased the killing of *Leishmania major* by macrophages in a TNFα- and nitric oxide-dependent fashion. Furthermore, MIF delivered orally by a *Salmonella* strain engineered to express cytokines reduced the severity of infection in an experimental

Leishmania model (45). Taken together these studies define a critical role for MIF in the pathogenesis of septic shock and in innate immune responses induced by a wide range of microorganisms. They also identify MIF as a potential novel target for therapeutic intervention in patients with severe sepsis and possibly also with other infectious diseases.

Pathophysiological Role in Other Inflammatory Diseases

Elevated concentrations of MIF were detected in alveolar airspaces of patients with the adult respiratory distress syndrome (ARDS) (46). Recombinant MIF was found to augment IL-8 and TNFα secretion by alveolar cells of ARDS patients, whereas incubation of cells in the presence of anti-MIF antibodies decreased the production of both cytokines. Expression of MIF is up-regulated in patients with rheumatoid arthritis (RA). MIF mRNA and protein were detected in the synovium of patients with RA. Infiltrating mononuclear cells (T cells and macrophages) and fibroblast-like synoviocytes were identified to be the primary sources of MIF (47,48). Synovial fluid levels of MIF were higher in patients with RA than in patients with osteoarthritis (49). Stimulation of synoviocytes with pro-inflammatory cytokines (IL-1β, TNFα and IFNγ) did not augment MIF production (48). Depending upon the dose, dexamethasone exerted either stimulatory (10^{-10}-10^{-12}M) or inhibitory (10^{-7}M) effect on synoviocyte MIF expression (48). Patients with atopic dermatitis have been reported to have increased expression of MIF in keratinocytes and high circulating concentrations of MIF (50). Anti-MIF antibodies also reduced inflammation in experimental models of glomerulonephritis, and allograft rejection, confirming a role for MIF in the regulation of inflammatory responses.

MIF – Glucocorticoids Interactions

Inflammation is an essential component of host defenses against infectious agents. The cytokines released by leukocytes play a pivotal role in innate and acquired immune responses and act to coordinate the cellular and humoral responses that contribute to eradicate the infection or wall-off the invasive micro-organism. Because it is an essential feature of host defenses against infection, inflammatory responses need to be kept under tight control. On one hand, overwhelming inflammatory reactions are detrimental to the host and may even be lethal as seen in septic shock. On the other hand, the lack of inflammatory response typically encountered in immunocompromised patients may also be harmful by promoting life-

threatening infections. The release of glucocorticoid hormones is a central feature of the physiological stress response induced by inflammation and infection. Glucocorticoids are potent anti-inflammatory and immunosuppressive agents and act as a sentinel to keep inflammatory responses in check. To control the development of the immune response, the host needs to balance the opposing effects of immunostimulatory and immunosuppressive molecules. By analogy with other physiological systems, it is reasonable to postulate that counter-regulatory systems should also exist within the immune system to maintain the equilibrium between pro-inflammatory and anti-inflammatory stimuli.

Recent data have suggested that MIF and glucocorticoids may function as a physiological counter-regulatory dyad to control host inflammatory and immune responses. As discussed above, MIF is produced by many cells of the immune system, including macrophages and the T lymphocytes (6,34). MIF is also stored in copious amounts in the pituitary gland and in the adrenal cortex, and is released in the systemic circulation during stress and infection (5,7,51). MIF exerts important functions within both the innate and acquired immune system. MIF has been shown to be a critical mediator of the host responses to a broad-spectrum of micro-organisms or microbial products (5-7,30,31,33). It also plays an important role in antigen-specific T cell activation and in the development of a humoral antibody response (34). MIF was reported to be an essential mediator of tuberculin-induced delayed-type hypersensitivity (52). A puzzling observation made during investigations of the biological activities of MIF was that physiological concentrations of glucocorticoids (dexamethasone or cortisol) induced MIF release from macrophages and T cells (33,34). Of note, glucocorticoid-induced macrophage MIF production followed a bell-shaped dose response curve with maximal release induced by concentrations of steroids in the range of 10^{-10}-10^{-14}M. However, at higher concentrations (10^{-6} M) dexamethasone was observed to inhibit MIF release. Moreover, the intravenous injection of dexamethasone increased the circulating concentrations of MIF in the rat (33). At first glance, these results appeared paradoxical and difficult to reconcile with the pro-inflammatory properties of MIF (5,6). Yet, this observation provided the impetus that led to the discovery that MIF functions as a counter-regulator of the anti-inflammatory effects of glucocorticoids. This intriguing property of MIF was revealed by the fact that adding recombinant MIF to human monocytes overcame in a dose-dependent fashion dexamethasone-induced inhibition of cytokine production (TNFα, IL-1β, IL-6 and IL-8) (33). MIF also was found to override dexamethasone-mediated suppression of T cell proliferation and cytokine production (IL-2 and IFNγ) stimulated by anti-CD3 antibodies (34).

Likewise, MIF overcame the anti-inflammatory effects of glucocorticoids on alveolar cells isolated from patients with ARDS (46).

In vivo, stress-induced activation of the HPA axis led to a rise of the serum MIF levels concomitant with the elevation of the blood concentrations of the stress-hormones ACTH and corticosterone (33). The counter-regulatory effect of MIF was confirmed in a mouse model of endotoxemia. Administration of MIF suppressed the protection conferred by glucocorticoids pre-treatment (33). Furthermore, median plasma concentrations of MIF are 3 ng/ml (i.e. 0.25 nM) (range: 1.9-5.4 ng/ml) at baseline (31), which is within the range of circulating levels of cortisol (10 nM). As noted for glucocorticoids, circulating concentrations of MIF also increase during inflammation, infection, or stress and thus may also exert modulatory activities during the acute phase of inflammatory or infectious diseases (31).

The observations that macrophage MIF production induced by microbial products and by glucocorticoids follows a bell-shaped curve and that its overriding capacity is reduced at high concentration of steroids imply the existence of numerous check points for MIF-glucocorticoid interactions. Taken together, these observations support the concept that MIF and glucocorticoids function as a physiological counter-regulatory dyad that controls host inflammatory and immune responses in the periphery and in the systemic circulation.

Glucocorticoids are known to inhibit cytokine production via an inhibition of gene transcription. The molecular mechanisms by which glucocorticoids exert these effects include the direct interaction between activated glucocorticoid receptors and the transcription NF-κB, AP-1 or CREB. Dexamethasone was reported to induce the transcription of the IkBα gene (53,54), thereby increasing IkBα protein concentrations in the cytoplasm and decreasing the amount of NF-κB translocating to the nucleus, leading to a reduction in cytokine production. Glucocorticoid-mediated effect on chromatin structure also may account for part of the anti-inflammatory effects of steroids. Glucocorticoids may bind to several transcription co-repressor molecules involved in histone deacetylase activity. The net effect is a tighter coiling of DNA that reduced the access of transcription factors to their specific binding sites, and thus result in the suppression of gene expression. Glucocorticoids may also reduce the stability of mRNA by binding to AU rich sequences in the 3' untranslated region of cytokine genes.

Little is known about the mechanisms underlying MIF overriding effects. However, two recent observations have shed some light on the mechanisms by which MIF may inhibit glucocorticoid-mediated effects. MIF was found to stimulate cytosolic phospholipase A_2 ($cPLA_2$) activity and

arachidonic acid release by NIH-3T3 fibroblasts via a protein kinase A and p44/p42 extracellular signal-regulated (ERK) mitogen-activated protein kinase pathway (43). Given that $cPLA_2$ is the an important target of the anti-inflammatory action of glucocorticoids, the authors rightfully reasoned that $cPLA_2$ might be at the crossroad of opposing effects of MIF and glucocorticoids. In agreement with this hypothesis, recombinant MIF completely reversed the inhibitory effect of dexamethasone on arachidonic acid production by fibroblasts, providing a very plausible explanation as to how MIF may antagonize glucocorticoid action. Additional studies of the molecular basis of MIF-glucocorticoid interactions in the macrophage have revealed that MIF inhibits both transcriptional and post-transcriptional glucocorticoid-mediated suppression of pro-inflammatory gene expression (IL-8 and TNF) (T. Roger, manuscript in preparation).

The fact that MIF is located both centrally and in tissues in direct contact with the environment is consistent with its critical role in innate immune responses. In the periphery, the MIF produced by immune and epithelial cells in response to infectious stimuli would serve, in concert with other cytokines, to promote inflammatory and immune reactions to eliminate the invasive pathogen. By counteracting the anti-inflammatory and immunosuppressive effects of stress-induced augmentation of glucocorticoids, MIF specifically facilitates the development of primary host defenses. The release of pituitary MIF into the bloodstream also contributes to promote systemic innate and acquired immune responses.

CONCLUSIONS

Over the last decade, macrophage migration inhibitory factor (MIF) has emerged to be an important mediator of the innate immune system. Originally discovered as a T-cell cytokine, MIF was identified recently to be released by a vast array of cells, including immune (monocytes/macrophages, T cells, B cells, eosinophils, mast cells), endocrine (pituitary and adrenal cells) and epithelial cells in response to infection and stress. One important characteristic of MIF is the fact that it exists as a pre-formed intracellular protein, and is thus ready to be rapidly released when cells are activated. Bacteria, microbial toxins (LPS, TSST-1, SPEA) and cytokines (TNFα, IFNγ) were found to be powerful inducers of MIF secretion by monocytes and macrophages. High blood levels of MIF were measured in mice with bacterial peritonitis and in humans with severe sepsis and septic shock. Studies of experimental septic shock provided a direct evidence for an intrinsic contribution of MIF to the pathogenesis of sepsis. Immunoneutralization of MIF or deletion of the MIF gene protected mice against lethal endotoxemia, staphylococcal toxic shock and peritonitis

induced by *E. coli* or cecal ligation and puncture. Noteworthy, anti-MIF antibodies were found to also protect TNFα knockout mice from lethal septic shock. Conversely, administration of recombinant MIF at the onset of endotoxemia or bacterial sepsis increased lethality, confirming the detrimental effects of excessive systemic levels of this pro-inflammatory mediator. MIF stimulates the expression of pro-inflammatory mediators by immune cells, and thus vigorously promotes inflammatory and immune responses. The surprising observations that MIF release was induced by glucocorticoids and that MIF had the unique feature to override the anti-inflammatory and immunosuppressive effects of glucocorticoids on macrophages and T-cells functions further emphasized the critical regulatory role played by MIF within the immune system. MIF and glucocorticoids appear to function as a physiological counter-regulatory dyad that modulates host inflammatory and immune responses. Given the central place of MIF in innate immune responses against infection and in the regulation of inflammatory responses, pharmacological modulation of MIF production or neutralization of MIF activity could have broad clinical applications and may offer new treatment options for the management of patients with severe sepsis, inflammatory and auto-immune diseases.

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