MIF, MIF Alleles, and the Regulation of the Host Response

Richard Bucala*

1. Introduction

Macrophage migration inhibitory factor (MIF) is the first cytokine activity to be discovered, although it resisted cloning and molecular characterization until relatively late in the era of cytokine discovery. Beyond its eponymous effect on macrophage mobility, MIF now is understood to be a critical upstream regulator of innate immunity that sustains activation responses by mechanisms that include counter-regulating the immunosuppressive action of glucocorticoids and inhibiting stimulus-induced apoptosis. These properties act physiologically to regulate the set-point and the magnitude of an immune response. The recent description of prevalent and functional alleles for MIF and their association with autoimmunity, infection, and cancer has focused attention not only on MIF’s regulatory role in the host response but also on the importance of innate immune pathways in the clinical expression of disease. MIF alleles show significant population stratification, which may reflect the influence of selective pressure, and they likely provide an essential level of variation in innate responsiveness within the human population. Highly homologous orthologues of MIF also have been described in parasitic organisms, and early data suggest that they regulate the host-parasite interaction. First insight into the MIF receptor complex and the structural basis for MIF signal transduction has revealed unique features that hold promise for the pharmacologic modulation of MIF-dependent pathways. Recent developments in MIF biology are reviewed herein and integrated within the concept that MIF allele specific responses influence disease development, whether of an autoimmune, infectious or oncogenic etiology.

*Corresponding author: Department of Medicine, Pathology, and Epidemiology and Public Health, Yale University School of Medicine, The Anlyan Center for Biomedical Research, SS25, 300 Cedar Street, New Haven, CT 06520-8031, USA. Email: richard.bucala@yale.edu
2. MIF Gene

The observation of an immune basis for leukocyte motility can be attributed to Arnold Rich and Margaret Lewis, who in 1932 showed that the migration of cells from the lymph nodes of an antigen-sensitized animal was impaired in the presence of antigen.\textsuperscript{1} This \textit{in vitro} demonstration of cellular immunity engendered significant interest among immunologists, especially after refinements in the quantification of cell movement were made in the 1950s.\textsuperscript{2} John David and Barry Bloom attributed migration arrest to a lymphokine,\textsuperscript{3,4} and a unique gene encoding MIF was ultimately reported in 1989.\textsuperscript{5} Recombinant MIF protein followed the cloning of MIF from corticotrophic pituitary cells, which itself was unexpected and pointed to a systemic role for MIF in the regulation of the immunologic and neuroendocrine systems.\textsuperscript{6} MIF circulates normally in plasma and its levels rise together with adrenocorticotrophic hormone (ACTH) in response to stress or invasive stimuli. ACTH serves to stimulate adrenal glucocorticoid production while MIF acts to counter-regulate the immunosuppressive action of glucocorticoids.\textsuperscript{6–8}

There is a single \textit{MIF} gene in the human genome (22q11.2) and both the exonic structure and DNA sequence of MIF are highly conserved across phylogeny. MIF transcription is constitutive in many cell types and induced transcription is regulated by proinflammatory, glucocorticoid, and hypoxic signals acting on AP-1, CREB, and HIF-1\textsubscript{α} responsive elements.\textsuperscript{9,10} A remarkable feature of the \textit{MIF} gene is the presence of a microsatellite repeat (CATT\textsubscript{n}) within the 5’ promoter region.\textsuperscript{11} This repeat unit is present in 5–8 copies and lies within a Pit-1 transcription factor binding site, which may provide for the neuroendocrine regulation of MIF expression (Fig. 1). Both gene reporter assays and human clinical studies indicate that repeat number is associated with higher MIF expression.\textsuperscript{11,12} Repeat number or a single-nucleotide polymorphism that is in strong linkage disequilibrium with CATT\textsubscript{7} has been found in genetic epidemiology studies to be associated with increased innate responses in numerous human diseases.\textsuperscript{13–23} The \textit{MIF} allelic structure also shows significant population stratification, with increasing repeat number following human migration patterns and genomic diversification.\textsuperscript{24}

3. MIF Production and Signal Transduction

Diverse activating stimuli induce the rapid release of MIF from pre-formed, cytoplasmic pools; this is followed by an upregulation in MIF mRNA expression and the replenishment of intracellular protein content\textsuperscript{25,26} within the
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Fig. 1. Upper Panel: Diagram of the human MIF gene showing its exonic structure, putative transcription factor binding sites, and the functional –794 CATT5-8 promoter polymorphism. A nearby promoter SNP (–173 G/C) also may contribute to functionality. Lower Panel: Prevalence of MIF CATT alleles in different populations superimposed on human migration patterns (5 = CATT5, >5 = CATT6, CATT7, and CATT8). From Ref. 93.

immune system. Such stimuli include Toll-like receptor (TLR) agonists, mitogens, and selected proinflammatory mediators.27–29 Ischemia,30 glucocorticoids in low concentrations,7 and corticotrophin-releasing hormone also induce MIF release from different cell types.31 MIF lacks a classical signal sequence and it is secreted by an unconventional route that requires the Golgi-associated protein, p115. The inflammatory activation of macrophages by endotoxin or by intracellular infection leads to the cytoplasmic redistribution and co-export of p115 and MIF. The genetic targeting of p115 reduces MIF secretion without affecting the stimulus-induced secretion of other innate cytokines.32

The solution of MIF’s crystal structure revealed a new structural superfamily but introduced a conundrum for the potential mechanism of MIF action.33
The three-dimensional structure of MIF showed significant homology with two prokaryotic tautomerases; this fueled speculation that MIF exerts immunoregulatory function(s) by an enzymatic reaction. Rorsman and colleagues established that MIF indeed tautomerizes model, and possibly physiologic, substrates; however, most studies as well as a recent genetic knock-in mouse model suggest that MIF's enzymatic active site is most likely vestigial but nevertheless has a structural role in protein-protein interactions.

MIF activates the extracellular signal regulated kinase (ERK) 1/2 subfamily of MAP kinases, but in contrast to most inducers of this signal transduction pathway, which phosphorylate ERK1/2 transiently, the effect of MIF in many cell types is sustained. Expression cloning of an MIF receptor revealed ERK1/2 activation to result from a high-affinity interaction between MIF and CD74, which is the cell surface form of the Class II invariant chain. MIF binding to CD74 results in the serine phosphorylation of its short intracytoplasmic domain and in modulation of the phosphorylation of its signaling co-receptor, CD44 (Fig. 2). These events lead to the activation of a Src-family tyrosine kinase and initiation of the ERK1/2 signal transduction cascade. Among the ERK1/2 effectors that are activated by CD74 are proteins responsible for MIF-dependent proliferation, survival, and regulatory responses; these include cytosolic phospholipase A2 (cPLA2), which produces the arachidonic acid precursor necessary for the synthesis of prostaglandins and leukotrienes. Intracellular arachidonic acid also activates the c-Jun N-terminal kinase (JNK) to promote the efficient translation of mRNAs for TNF and other cytokines. MIF stimulates cPLA2 in the face of immunosuppressive concentrations of glucocorticoids, which is one mechanism whereby MIF overrides glucocorticoid-mediated anti-inflammatory action. Follow-up studies have demonstrated that MIF inhibits the glucocorticoid-induced expression of MAP kinase phosphatase (MKP-1), which is an important mechanism by which glucocorticoids reduce the inflammatory responses initiated by the ERK1/2, JNK, and p38 MAPK pathways. MKP-1 also promotes the translation of short-lived proinflammatory cytokine mRNAs by stabilization of 3-AU-rich elements.

The identification of the transcriptional regulator c-Jun activation domain binding protein 1 (JAB1) as a binding partner for MIF provided an explanation for MIF's noted ability to induce sustained phase ERK1/2 activation. Intracellular JAB1 levels temporally regulate MIF-dependent ERK1/2 activation: high JAB1 expression inhibits sustained but not transient ERK1/2 phosphorylation, while low JAB1 levels are sufficient for transient activation. This effect may be due to the known role of JAB1 in the COP9 signalosome, where it regulates the degradation of signaling components.
Fig. 2. Upper Panel: Integrated scheme for activating effects of MIF signal transduction and the regulation of the glucocorticoid immunosuppression based on data obtained in monocytes/macrophages and mesenchymal cells. Lower Panel: MIF signal transduction in B lymphocytes, which additionally involves the regulated intramembrane cleavage of CD74, nuclear translocation of CD74_1–42 and the activation of a transcriptional response mediated by NF-κB p65/RelA and the transcriptional co-activator TAFII105.
MIF’s unique structure recently led to the proposition that a topologic similarity with IL-8 may explain MIF’s longstanding function as an “arrest” chemokine. Activation of the chemokine receptor CXCR2 by its cognate ligand, IL-8 (CXCL8), is mediated by an N-terminal Glu-Leu-Arg (ELR) motif. MIF has a pseudo-ELR motif comprising two nonadjacent but appropriately spaced residues (Asp and Arg) in exposed neighboring loops that mimic the structure present in ELR chemokines (Fig. 3). The biologic significance of this pathway has been affirmed by the finding that blockade of MIF but not of canonical ligands for CXCR2 or CXCR4 reduces monocyte and T-cell content in murine atherosclerotic plaques. Functional cell surface complexes form between CD74 and these two chemokine receptors, and while anti-CD74 blocks MIF induction of a CXCR4-dependent AKT survival pathway, it does not inhibit AKT phosphorylation induced by a pure CXCR4 agonist such as CXCL12.

4. Innate Immunity

Studies with MIF-KO mice have confirmed an upstream activating role for MIF in diverse inflammatory responses. Mice genetically deficient in MIF appear phenotypically normal; however, a subtle defect in lung maturation due to a delay in developmental regulation by VEGF and glucocorticoids is
revealed by premature delivery. This murine phenotype recapitulates the key pathologic findings observed in the neonatal respiratory distress syndrome of prematurity.51 MIF-KO mice manifest significant immunoregulatory defects when confronted by immunologic or invasive challenge; for instance, a reduction in the expression of innate mediators such as TNF, IL-1, IL-6, IL-12, IL-18, IFN-α, PGE₂, and NO.48,49,52–55 These effects are in accord with MIF’s role in inhibiting p53-dependent apoptosis and in stimulating AKT-dependent pathways of cell survival.49,56 The activation of monocytes/macrophages is sustained in the presence of MIF and a robust proinflammatory response ensues. MIF is also required for the optimal expression of TLR4, which is the cell surface receptor for the MD-2/LPS complex, indicating that the earliest events for pathogen recognition are reliant on adequate MIF expression. The innate responses of barrier epithelial and endothelial cell types also are augmented by MIF, which may itself be released from these cells by activating or cytotoxic stimuli.57–60 In the case of the gastrointestinal tract, for instance, MIF alone upregulates microfold (M cell)-mediated transport of antigen across the follicle-associated epithelium of intestinal Peyer’s patches.61 Additional innate immune cell types such as the neutrophil, the eosinophil, and the mast cell produce MIF and contribute to MIF-dependent inflammatory responses.62–64

Whether MIF exerts a deleterious or beneficial effect on the host varies with the nature of the invasive agent. In murine models of E. coli and

![Diagram](attachment:image.png)

**Fig. 4.** Examples of genetic association studies that have revealed the influence of MIF alleles on different inflammatory diseases. For certain infections, such as those responsible for community-acquired pneumonia, high-expression alleles appear to confer a survival benefit.22
polymicrobial sepsis, malaria, or West Nile virus infection, where MIF disrupts the blood-brain barrier, a reduction in MIF-dependent responses reduces immunopathology and promotes survival. In infections caused by intracellular *Salmonella*, the protozoans *Leishmania*, *Toxoplasma*, *Trypanosoma*, or the helminthic pathogen *Taenia crassiceps*, MIF is critical for host defense. Experimental analyses of these infections have revealed important differences in the temporal or tissue-specific expression of downstream effector pathways, and these findings emphasize the important influence of MIF on the regulation of the innate response and ultimately host survival.

Antigen presentation is initiated by the innate response and provides a segue into adaptive immunity. Deficient antigen presentation in the absence of MIF has been reported in murine allergic asthma. The precise basis for this impairment remains unknown although antigen presentation function by monocytes/macrophages and mast cells appears more profoundly affected than that of dendritic cells.

5. Adaptive Immunity

Given the central role of innate immunity in the differentiation of the adaptive response, it is not surprising that MIF influences T and B cell responses. Activated T cells produce MIF, which then acts by an autocrine pathway to enhance IL-2, IL-2R, and IFN-γ production. Immunoneutralization or genetic deficiency of MIF in mice reduces T cell priming and memory responses, T cell cytokine production (e.g., IL-2, IL-4, IL-5, IL-13, eotaxin, IL-13, IFN-γ), and T cell-dependent antibody responses. T cell responses appear reduced irrespective of whether a Th1 or Th2 T cell response is elicited, and a role for MIF in the differentiation of inflammatory Th17 cells has also been reported recently. Immunoneutralization or genetic deletion of MIF ameliorates inflammatory tissue damage in T cell-mediated disease models, such as collagen-induced arthritis, inflammatory bowel disease, and autoimmune encephalomyelitis.

As in the case of monocytes, MIF likely sustains the activation responses of cells of the adaptive system. The pathway that has been examined in greatest detail is in B cells (Fig. 2). Indeed, prior to the discovery of CD74 as the MIF receptor, CD74 had already been identified as a B cell survival factor. In response to forced overexpression or an activating antibody, CD74 undergoes regulated intramembrane proteolysis to create a cytosolic protein that translocates to the nucleus to activate a transcriptional response mediated by NF-κB p65/RelA and TAFII105. This sequence of events was shown recently.
to be recapitulated by MIF engagement, and it appears to occur downstream
of CD44, which activates the Src kinase, Syk, leading to Akt phosphorylation,
augmentation of Bcl-X\textsubscript{i} and Bcl-2 transcription, and enhanced B cell
survival. Within the bone marrow, the survival of recirculating B cell
populations has been shown to be reliant on MIF produced by resident
dendritic cells\textsuperscript{75}.

6. MIF Integrates Immune, Metabolic,
and Oncogenic Responses

Infection or tissue invasion is frequently accompanied by a derangement in
host metabolism. Protein wasting, or cachexia, has long been associated
with a systemic proinflammatory response, although the role of specific
cytokines such as TNF \textit{in vivo} has been controversial. MIF-deficient mice
show normalized blood glucose and lactate responses during endotoxic
challenge\textsuperscript{76}, and high levels of MIF have a direct catabolic effect of muscle,
independently of TNF\textsuperscript{77}. Infiltration of macrophages in white adipose tissue
is considered an important mechanism for the development of insulin resist-
ance, Type 2 diabetes, and atherosclerosis, and all of these features are
reduced in the setting of MIF deficiency\textsuperscript{78}. Plasma lipids and adiposity are
not affected, however, suggesting that the primary action of MIF is in regulat-
ing the magnitude of the systemic inflammatory response. There are emerg-
ing human data, both with respect to circulating MIF levels and MIF
genotype, to support MIF’s role in the development of insulin resistance and
Type 2 diabetes\textsuperscript{79}.

The ability of MIF to induce glucose transport in muscle recently prompted
investigations into the potential importance of this effect in ischemic myocar-
dium. Miller \textit{et al.} found that MIF is released by ischemic cardiomyocytes,
where it stimulates activation of AMP-activated protein kinase (AMPK) by
engaging the CD74/CD44 receptor complex. This cardioprotective response
is critically dependent on MIF, as MIF-KO mice suffer from impaired ischemic
signaling and larger cardiac infarctions. This protective pathway also shows
reduced activity in human cells with a low-expression MIF allele, suggesting
that MIF genotype may well predict risk in patients with coronary artery
disease\textsuperscript{30}.

Multiple lines of evidence link inflammation to the development and pro-
gression of cancer. MIF’s ability to regulate molecular pathways that are
necessary for migration and invasion, proliferation, and evasion of apoptosis
have made it a molecule of high interest for investigations of tumorigenesis.
The known pathways for MIF signal transduction that include the ability to
induce sustained ERK1/2 activation, which is reminiscent of oncogenic RAS, stimulation of the AKT pathway, and the regulation of JAB1 and p53 further support this notion. MIF is strongly upregulated by hypoxia inducible factor-1α,⁹ and MIF promotes maximal HIF-1α expression,⁸⁰ suggesting mutually reinforcing pathways for tumor progression and adaptation. The signaling component of the MIF receptor complex, CD44, has also been shown to be strongly associated with tumor cell adhesion and invasion and to promote metastasis, and it is a feature of the tumor stem cell phenotype.⁸¹

7. MIF Alleles in Human Disease

Evidence for a pathogenetic role for MIF in different inflammatory and infectious diseases emerged quickly after the observation that immunoneutralization of MIF fully protects mice from endotoxic shock. This result placed MIF in a central regulatory node with respect not only to the expression of innate immunity but to the progression of tissue injurious pathways of inflammation.⁶,²⁹ The potential clinical importance of MIF has now been underscored by epidemiologic studies that have shown associations between functional promoter alleles and diseases with an immunologic basis (Fig. 4). In the examples of autoimmune or inflammatory disorders that have been studied, such as in adult or juvenile forms of rheumatoid arthritis,¹²,¹³ ulcerative colitis,¹⁴ asthma,¹⁶ and systemic sclerosis,²⁰ the predominant impact of high-expression MIF alleles is on the severity or the clinical phenotype of disease rather than on susceptibility. In asthma, for example, the presence of a longer (and higher expression) CATT repeat was related to more severe asthma as defined by GINA (Global Initiative for Asthma) criteria,¹⁶ and in systemic sclerosis, with a more severe clinical phenotype known as diffuse cutaneous disease.²⁰ Whether the actual impact of MIF is on clinical severity or if these conclusions instead reflect a statistical limitation of these studies remains to be determined. One exception to these observations is atopic dermatitis, in which high-expression MIF alleles confer a 3.5-fold increased susceptibility to disease development and where the role of the MIF locus (22q11) was identified by linkage analysis.¹⁵ Overall, these human genetic data are consonant with experimental studies that have emphasized the amplifying effect of MIF on innate pathways of tissue and end-organ damage.

The observation that the highest prevalence of the CATT₅ allele occurs in Sub-Saharan Africa (Fig. 1) led to the hypothesis that low-expression allelic variants may provide a measure of protection from malaria, as this region
historically has suffered the greatest mortality from this infection. Lethal malaria occurs most commonly in immunologically naïve children, with death ensuing from the complications of an excessive innate immune response that produces the clinical sequela of cerebral disease, severe anemia, and a sepsis-like syndrome. Malaria is also believed responsible for the selection and evolutionary persistence of minor hemoglobin genes such as HbS (sickle hemoglobin), which confer protection against lethal malaria. Notably, the prevalence of the HbS decreases in the southern latitudes of the African continent, which suggests that additional genes such as MIF may have a role in resistance to disease. Experimental studies in MIF-deficient mice have confirmed a role for MIF in the inflammatory complications producing severe malarial anemia. Genetic epidemiology studies are now showing a relationship between longer repeats of the CATT polymorphism and the development of severe malarial anemia, which is a leading cause of death in children with malaria. Whether MIF influences the development of other parasitic or chronic infections endemic to areas with a particular prevalence of MIF alleles is also coming under investigation.

The studies that have examined the influence of MIF on autoimmune inflammatory diseases, or a disease such as malaria where inflammatory complications play a lethal role, have uniformly reported a deleterious role for high-expression allelic variants. The results of a recent study of a large cohort of patients with pneumonia at risk for septic shock is therefore noteworthy because a high-expression allele — in this study, a promoter SNP (–173 C) that is closely associated with CATT7 — was found to be associated with a 50% improvement in survival. This result was somewhat unexpected given the prevailing hypothesis that an excessive innate response underlies the immunopathogenesis of septic shock. These human genetic data, which were obtained in the largest cohort of sepsis patients yet studied, emphasize the protective and microbicidal role of innate immunity. They also lend support to the notion that MIF alleles exist in a balanced polymorphism that has been maintained by the influence of different selective pressures, presumably from infections.

There is emerging interest in examining the MIF allelic system in the development of diseases that may not be considered nosologically to be inflammatory, but in which inflammation nevertheless makes a pathogenic contribution. In prostate cancer, for instance, the presence of inflammatory cells in tissue biopsies portends a worse prognosis, and cytokine signals have been hypothesized to contribute to tumor progression. In an initial study, patients with the high-expression CATT7 allele were found to have an almost five-fold increased risk of prostate cancer recurrence. In autism, which is a
neurodevelopmental disorder of unknown etiology but associated with immune abnormalities, an association between functional polymorphisms in the promoter for MIF and autism spectrum disorder behaviors was found. Affected probands also exhibited higher circulating MIF levels than their unaffected siblings.21

Finally, the recent description of close orthologues of mammalian MIF in parasitic nematodes and in the protozoan pathogens responsible for malaria and leishmaniasis has raised the question of whether these proteins play a role in immune evasion.82 There is early evidence that parasite-encoded MIFs influence cell migration and host immunity, in part by a functional interaction with the CD74 MIF receptor.83 A *Leishmania* MIF orthologue, for instance, exhibits an antiapoptotic activity that may facilitate the intracellular persistence of the parasite within the macrophage.84

8. Therapeutic Implications and Future Directions

Unique structural features together with MIF’s apex position in the inflammatory and cell survival pathways has made it an attractive pharmacologic target. Biologically based therapies are under development and a humanized monoclonal antibody (Milatuzumab) directed against the MIF receptor is in clinical trials for the treatment of B cell chronic lymphocytic leukemia.85

MIF’s intrinsic tautomerase activity and substrate binding site have also attracted considerable interest, especially after the finding that selected inhibitors of MIF tautomerase activity inhibit biologic activity, presumably by imparting a conformational change on the protein that interferes with receptor interaction.86 There has been significant progress made in the design of specific, small molecule-based receptor antagonists. Early generation compounds, including those with oral bioavailability, have shown promise in preclinical models of disease.87,88 A recent, computationally based discovery approach examined 2.1 million compounds and uncovered several high-potency receptor antagonists.89 Interestingly, the phosphodiesterase inhibitor, ibudilast, which is used in the treatment of asthma, exhibits additional anti-inflammatory activities that may be attributed to its ability to inhibit MIF’s tautomerase, receptor binding, and biologic activities. Ibudilast was recently co-crystallized with MIF and found to occupy the MIF tautomerase site.90 Another molecule in clinical development, 4-iodo-6-phenylpyrimidine (4-IPP), was discovered by a computational modeling strategy and covalently modifies the MIF tautomerase site.91 4-IPP has the interesting property of preventing cellular MIF secretion, and it appears to target a different MIF interaction32; MIF complexed with 4-IPP exhibits altered binding to the
Golgi-associated protein p115, which may disrupt the normal pathway of MIF secretion.

Finally, the recent crystallization of parasite-encoded MIFs has opened opportunities for the design of parasite-specific MIF inhibitors. It is noteworthy that while all MIF’s identified to date have a potentially catalytically active N-terminal proline, significant differences in the dimensions and in the charge distribution within the tautomerase site exist in the MIF proteins from hookworm (*Ancyclostoma ceylonicum*)\(^92\) and leishmania (*Leishmania major*).\(^84\) Both proteins also show different profiles of catalytic inhibition when tested with prototypic small molecules inhibitors of human MIF.

Polymorphic genes constitute an important basis for variation in the host immune response, and MIF clearly occupies an apex position with respect to the regulation of the innate response. Experimental studies that have defined MIF’s regulatory role in the clinical expression of autoimmunity, different infections, and in oncogenic progression are now being verified by human genetic studies. These broad findings are within the known mechanisms of MIF action and signal transduction, which reflect features more central to growth regulation, apoptosis, and cell cycle control than to simple inflammatory activation. The precise interplay between MIF and other genes with respect to pathogenesis and the expression of different diseases remains to be better elucidated. The genetically defined variations in human MIF expression that have been defined also offer the prospect of a natural therapeutic window that may guide pharmacologic interventions aimed at regulating MIF-directed pathways.

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**References**


