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Macrophage migration inhibitory factor and its role in autoimmune diseases

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Summary

After several decades of research into the macrophage migration inhibitory factor (MIF), its diverse actions in the immune system are yet to be fully revealed. What has become clear is that MIF plays an important role in both innate and adaptive immunity. However, while several pathways mediating the function of MIF in the immune system have been established, its role in pathogenic states such as autoimmune diseases has remained unresolved. MIF has been implicated in different autoimmune diseases, including rheumatoid arthritis, glomerulonephritis, and multiple sclerosis, but knowledge about the underlying cellular and molecular mechanisms is just emerging. However, overall it appears that the inhibition of its proinflammatory action is likely to be a successful new therapeutic strategy for some autoimmune diseases, possibly by reducing the need for steroids. As more aspects of the role of this cytokine in the pathogenesis of autoimmune diseases are elucidated, better strategies to target it therapeutically can be expected.

Key words: **MIF • multiple sclerosis • EAE • glomerulonephritis • rheumatoid arthritis • autoimmune disease**

Abbreviations: **MS** – multiple sclerosis, **MIF** – macrophage migration inhibitory factor, **EAE** – experimental autoimmune encephalomyelitis, **LPS** – lipopolysaccharide, **CRF** – corticotropin-releasing factor, **cPLA2** – phospholipase A2, **NO** – nitric oxide, **RA** – rheumatoid arthritis, **mAb** – monoclonal antibody, **GN** – glomerulonephritis, **IP-10/CXCL10** – interferon-γ-inducible protein-10, **MIP-1a** – macrophage inflammatory protein-1a, **MCP-1** – monocyte chemoattractant protein-1, **PGE 2** – prostaglandin E2, **MMP** – matrix metalloproteases, **PAG** – proliferation-associated gene, **VCAM** – vascular cell adhesion molecule, **ICAM** – interlcellular cell adhesion molecule, **AP** – activator protein, **NF** – nuclear factor, **COX** – cyclooxygenase, **LOX** – lipoxygenase, **ERK** – extracellular signal-regulated kinase, **MAP** – mitogen-activated protein, **JAB** – Janus-kinase binding protein.

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INTRODUCTION

Cytokines are soluble proteins that act non-enzymatically in small concentrations through specific receptors to regulate cellular functions. The central role of cytokines is to control the direction, amplitude, and duration of immune responses and to allow communication within the immune system and communication with other organ systems and tissues. Individual cytokines can have pleiotropic, overlapping, and sometimes contradictory functions depending on their concentration, the cell type on which they act, and the presence of other cytokines and mediators. Because of the potent and profound biological effects of cytokines, their activities are tightly regulated. The regulation takes place at the level of production, secretion, processing, and/or receptor expression. Another form of regulation is the simultaneous action of different cytokines and hormones modulating their mutual effects. The dysregulation of inflammatory and anti-inflammatory cytokines is found in a number of autoimmune diseases, determining their outcome.

The macrophage migration inhibitory factor (MIF) was one of the first cytokines discovered in the early 60's. Its broad range of immunologic effects and its expression by a variety of cells, including T cells and macrophages, suggest a fundamental role in the regulation of immune responses. Its name is derived from the first well-known function of the protein, namely the inhibition of the migration of macrophages[41]. In 1966, Bloom and Bennett[18], and David[32] independently found that T lymphocytes represent a main source of the soluble cytokine. In the following 20 years few new discoveries regarding MIF were made. MIF was associated with macrophage activation, the induction of phagocytosis, and the enhancement of anti-tumor immunity[79, 80]. The development of specific, neutralizing antibodies[110] and cloning of a cDNA for human MIF[111] advanced research in this area decisively and permitted the exact analysis of the biological, biochemical, and biophysical characteristics and functions of the protein. MIF is among the few cytokines known to exhibit enzymatic activities[58, 91, 92] and currently it is the only cytokine known to be induced rather than inhibited by glucocorticoids. It can, in turn, act to counter-regulate the immunosuppressive effects of glucocorticoids and control the magnitude of inflammatory responses[22]. Its role in many inflammatory and autoimmune diseases, such as sepsis[15, 24], rheumatoid arthritis (RA)[62, 71, 87], glomerulonephritis (GN)[60] and multiple sclerosis (MS)[34], is under active investigation. Neutralization of MIF in animal models of these inflammatory diseases has pronounced therapeutic effects.

CELLULAR SOURCES AND SECRETION OF MIF

The mouse MIF gene has been mapped to chromosome 10 and encodes a 12.5 kDa protein of 115 amino acids[100]. Genetic polymorphisms for a single nucleotide or a tetranucleotide repeat in the promotor region of the MIF gene were associated with susceptibility to inflammatory polyarthritis[9, 10] as well as to juvenile idiopathic arthritis[36].

MIF is produced and stored in the pituitary gland and released systemically upon physiological stress[22] and the presence of endotoxin[15, 82]. Altogether it constitutes about 0.05% of the total protein amount in the pituitary gland[15, 24, 82] and it is stored in three different cell types, either alone or together with adrenocorticotropin-releasing hormone or thyroid-stimulating hormone[82]. Macrophages also store large quantities of MIF, similar to cells of the pituitary gland and T cells[18, 32], and they play an important role in the local secretion of MIF during the innate immune response[25]. In 1993, MIF secretion from macrophages after lipopolysaccharide (LPS) stimulation[4, 15] was shown. The secretion is also induced by Gram-positive exotoxins[27], tumor necrosis factor (TNF)-α, glucocorticoids, and interferon (IFN)-γ, but not by interleukin (IL)-1β or IL-6[25]. In eosinophils, secretion is promoted by IL-5 and C5a[93]. Small quantities of the protein are constitutively produced in many cells of the body, such as microglia in the central nervous system (CNS)[3, 15, 60, 83], bronchial and glomerular epithelial cells[60], hepatocytes, and Kupfer-cells[4].

MIF lacks a classical N-terminal leader sequence and its release seems to follow a non-conventional protein secretion pathway similar to the release of IL-1 or fibroblast-growth-factor[14, 16]. Details of the secretion mechanism remain to be resolved. The secretion of MIF from the pituitary gland is induced *in vitro* through corticotropin-releasing factor (CRF) in a dose-dependent manner. However, lower quantities of CRF are necessary for the secretion of MIF than for the induction of adrenocorticotropin-releasing hormone secretion[82]. CRF, moreover, promotes the transcription of MIF in murine pituitary cells[108]. The increased secretion of MIF following systemic infection or stress led to the assumption that MIF plays an important role in the hypothalamic-pituitary-adrenal axis.

THE STRUCTURE OF MIF

MIF consists of 115 amino acids combined in a unique structure[14]. Radiographic crystallography has shown that MIF is a homotrimer of identical subunits. Each monomer contains two antiparallel α he-

lices and six β strands. Three β strands are surrounded by 6 antiparallel α helices and form a central barrel structure with open ends. The structure includes a channel 4–15 Å in diameter which runs through the center of the protein along a molecular axis and consists mainly of hydrophilic, positively charged atoms, suggesting that it interacts with negatively charged molecules. Although this structure is homologous to 4-oxalocrotonate tautomerase and 5-carboxymethyl--2-hydroxymuconate isomerase, it is unique among cytokines and hormonal mediators and suggests that MIF participates in novel ligand-receptor interactions[14, 50, 100].

ENZYMATIC ACTIVITY

The 3-dimensional structure and its resemblance to prokaryotic enzymes such as the 4-oxalocrotonate tautomerase, 5-carboxymethyl-2-hydroxymuconate isomerase, and chorismate mutase from *Bacillus subtilis* led to the observation that MIF possessed enzymatic activity. Furthermore, MIF has similarities to the active site of 4-oxalocrotonate tautomerase and 5-carboxymethyl-2-hydroxymuconate isomerase such that it shares an N-terminal proline, which acts as a catalytic base[98]. Meanwhile, MIF was found to have catalytic activities similar to D-dopachrome-tautomerase[92], phenylpyruvate-keto-enol-isomerase[91] and thiol-protein-oxidoreductase[58]. Proliferation--associated gene, a thiol-specific antioxidant protein, was shown to be a potential protein substrate of the enzymatic activity of MIF[50]. To what extent these enzymatic functions have a physiological meaning remains to be seen. Previous mutation analysis could not establish a connection between the enzyme capacity of MIF and its biological function[45, 101].

MIF IN ACQUIRED IMMUNITY

Early reports associated MIF with delayed-type hypersensitivity reactions[41], and today it is believed that it has an important role in the innate immune system. Nevertheless, MIF is also an important regulator of acquired immunity.

The induction of MIF-mRNA production in T cells as well as the secretion of the MIF protein after stimulation with anti-CD3 antibodies or superantigen was demonstrated[5, 113]. Neutralization of MIF produced by T cells with an anti-MIF monoclonal antibody (mAb) prevented both the anti-CD3 and superantigen-induced IL-2 secretion. In addition, it reduced T cell proliferation by 40–60%[5]. *In vivo* anti-MIF treatment resulted in decreased T cell proliferation and a reduction in the production of antigen-specific IgG[5], indicating the effect of MIF on the priming of T cells.

An important role of MIF in the effector phase of the immune response was shown in experimental autoimmune encephalomyelitis (EAE)[34]. In this model, primed neuroantigen-specific T cells were adoptively transferred to mice pre-treated with anti-MIF mAb, leading to a delayed onset and an ameliorated course of disease in comparison with mice pre-treated with control mAb. Moreover, anti-MIF mAb treatment showed an effect in EAE when injected 11 days after immunization, i.e. at a time point when the majority of neuroantigen-specific T cells was already primed[34]. Thus, while impaired priming of naive T cells may have contributed to the lower frequencies of antigen--specific T cells, MIF blockade also influenced the effector phase of the immune response, in particular in the CNS. It was suggested that the weakened effector phase of the immune response in EAE was due to impaired homing and transmigration of antigen-specific T cells to the CNS. As a consequence, it appeared that the autoreactive T cells were deprived of T cell receptor and costimulatory molecule-mediated signals necessary for cell activation and/or survival[89]. Interestingly, vascular cell adhesion molecule (VCAM)-1 expression by the endothelium of the CNS was decreased in anti-MIF mAb-treated mice in this model. VLA-4, the ligand for VCAM-1, has been reported to function as a costimulatory molecule[31] and to protect T cells from apoptosis[113].

Certain aspects of MIF on the regulation of effector functions and migration of T cells may be dependent on the T cell subset or the disease model studied. For example, both Th1 and Th2 cells produce MIF, but mRNA production and secretion of MIF protein is predominantly increased in T cell clones of the Th2 subset after stimulation *in vitro*[5]. This observation is supported by the finding that humoral immunity was inhibited after a treatment with anti-MIF-mAbs *in vivo*[5]. However, lymph node cells from *Leishmania* antigen-stimulated MIF knockout mice show no significant change in production of IL-4 compared with cells from wild-type mice[95].

Finally, the mouse tumor model of the ovalbumin-transfected tumor cell line EL4 (EG.7) showed that splenocytes of mice primed with EG.7 exhibited higher levels of MIF after *in vitro* recall with antigen. Neutralization of MIF in splenocyte cultures with anti-MIF mAb showed a significant increase in the cytotoxic T lymphocyte response directed against EG.7 cells compared with control mAb-treated cultures[1]. In addition, the levels of IFN-γ were increased and a larger infiltration of $CD4^+$ and $CD8^+$ T cells was present by histology, as well as a higher number of apoptotic tumor cells in anti-MIF mAb-treated mice. Increased infiltration of $CD8^+$ T cells into the

tumor mass was demonstrated by the adoptive transfer of labeled cells from anti-MIF mAb-treated mice harboring an EG.7 tumor into untreated mice with the same tumor[1].

MIF IN THE INNATE IMMUNE SYSTEM

Monocytes and macrophages contain large quantities of preformed MIF that can be released upon stimulation with LPS[4, 15], glucocorticoids[5, 24], Gram-positive exotoxins[27], proinflammatory cytokines (TNF-α, IFN-γ)[5, 25], and other mediators[38]. MIF functions in a paracrine and autocrine way and promotes the activation of cells as well as the release of proinflammatory cytokines and, moreover, it counter-regulates the effect of glucocorticoids at sites of inflammation[5, 24]. Furthermore, MIF enhances macrophage phagocytosis[86] and the killing of intracellular pathogens such as *Leishmania*[51] and it has been shown to mediate macrophage accumulation in delayed-type-hypersensitivity reactions[18, 32].

MIF, administered in addition to LPS, increased the lethality of LPS-induced shock, whereas treatment with anti-MIF mAb reduced the lethality[15] and lowered the TNF-α concentrations by about 50%[26]. These results were confirmed by studies using MIF knockout mice[20]. MIF knockout mice were resistant to the lethal effects of high-dose LPS and showed diminished production of TNF-α, but normal IL-6 and IL-12. Thus, MIF plays an important role in the pathogenesis of sepsis caused by Gram-negative bacteria[26]. The administration of MIF aggravates the disease and increases the lethality, while treatment with anti-MIF mAbs up to 8 h after the infection prevents lethality. Furthermore, a significant correlation was observed between elevated MIF levels and the occurrence of death in septic shock patients[12]. In addition, Calandra et al.[26] showed that TNF-α knockout mice were protected against lethal septic shock when treated with anti-MIF mAbs, suggesting that another mechanism than TNF-α modulation was responsible for the beneficial effect of the antibody.

Neutralization of MIF showed similar protective effects in shock induced by exotoxin of Gram-positive bacteria, e.g. in staphylococcal toxic shock syndrome[27] and MIF knockout mice were resistant to injection of lethal doses of staphylococcal enterotoxin B[26].

MIF is secreted from macrophages following glucocorticoid stimulation in a bell-shaped dose-response curve (Fig. 1). Once released, MIF antagonizes glucocorticoid suppression of macrophage cytokine production (i.e. TNF-α, IL-1β, IL-6, IL-8)[24] and also T cell cytokine release (i.e. IL-2 and IFN-γ)[5]. The magnitude of these effects depends on the concentration of both glucocorticoids and MIF, suggesting that the two mediators act in a counter-regulatory manner to control cytokine production and inflammation[5, 24].

MOLECULAR MECHANISMS OF MIF EFFECTS

MIF has a plethora of immunologic and non-immunologic activities, summarized in Table 1. The multiple immunologic and non-immunologic effects of MIF prompted an investigation of its molecular

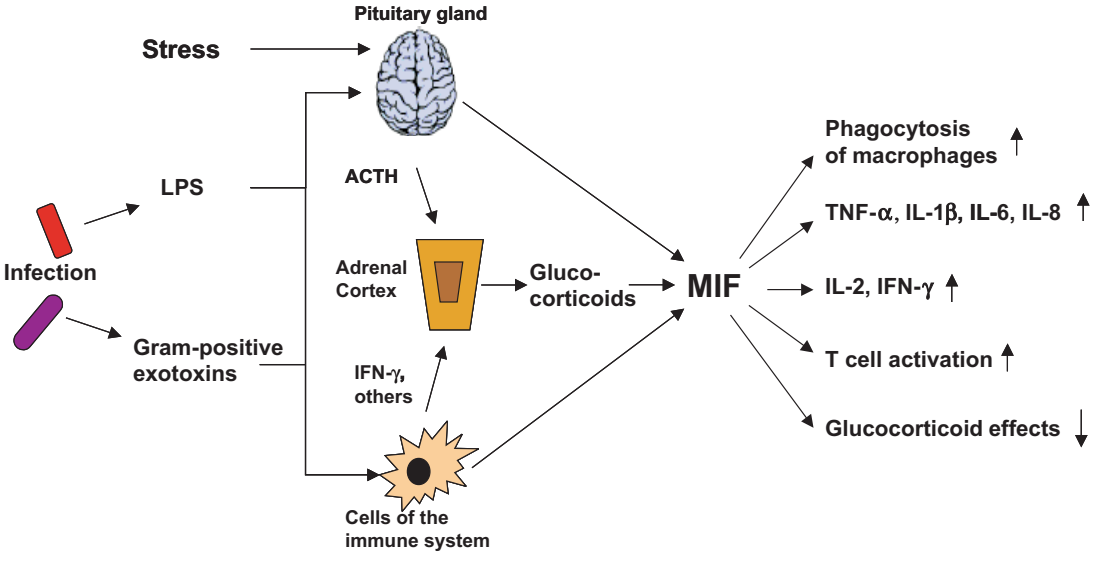

Figure 1. Glucocorticoids and the MIF. Glucocorticoids and MIF are connected in a tightly regulated balance. MIF is secreted upon glucocorticoid induction and then counterregulates glucocorticoid effects. This balance might be dysregulated in autoimmune diseases leading to an overexpression of MIF and proinflammatory cytokines.

Table 1. Effects of MIF

Effect	References
Enzymatic effects:	
D-dopachrome-tautomerase	92
Phenylpyruvate-keto-enol-isomerase	91
Thiol-protein-oxidoreductase	58
Cytokine effects:	
Macrophage migration inhibition	41
Counterregulation of glucocorticoids	24
T cell growth and activation	5
Induction of macrophage phagocytosis	86
Downregulation of VCAM-1	59
Killing of intrecellular parasites	51
TNF-α secretion	26
Inhibition of p53	72
Inhibition of Bcl-2 family in neutrophils	11

mechanism of action. The protein appears to act as an inflammatory cytokine and also as a neuroendocrine hormone and enzyme. Considering the diverse actions of the protein and the conserved gene structure[14], one can assume that MIF is an evolutionarily conserved protein, with its functions mediated through multiple pathways.

Numerous studies suggested an interaction of MIF with a receptor on the surface of the target cells[5, 13, 15, 23, 24, 45, 107]. To date, only one receptor for MIF was described. CD74, a type II transmembrane protein, was identified as the extracellular binding site for MIF. The interaction of MIF with CD74 activates the MAP kinase pathway and promotes cell proliferation and prostaglandin E2 production[65]. Additional receptors to explain the various effects of MIF remain to be identified. Non-receptor-mediated endocytosis has also been suggested to explain certain effects of MIF[56].

MIF has direct proinflammatory effects mediated by the increased expression of TNF-α[25, 26], IL-1β, IL-6[25], IL-8[13], IFN-γ[1, 5], as well as by the increase of the nitric oxide (NO) release[16] and the induction of the cyclooxygenase-II pathway[73]. Moreover, MIF indirectly promotes inflammation through the counterregulation of the anti-inflammatory effects of glucocorticoids[24]. In this tightly regulated balance, glucocorticoids induce the release of MIF, upon which MIF then counteracts the glucocorticoid-induced inhibition of inflammatory cytokines on macrophages, synovial fibroblasts. and T cells and leads to an increase of proinflammatory cytokines. The mechanisms by which MIF overrides the effects of glucocorticoids have not been fully clarified.

Further investigations showed that fibroblasts have an increased activity of the p44/p42 extracellular signal-regulated kinase (ERK) subfamily of the mitogen-activated (MAP) kinases after stimulation with MIF, induced by binding of MIF to CD74[65]. The signaling cascade of the ERK MAP kinases results in the phosphorylation and activation of a sequence of cytoplasmic proteins, such as c-*myc* and phospholipase A2 (cPLA2). cPLA2 is an important mediator of inflammatory reactions[43] and its product, arachidonic acid, is the precursor of leukotrienes and prostaglandins. Because cPLA2 is also a target protein of glucocorticoids, activation through MIF could be a possible mechanism to counteract glucocorticoids[73].

Another mechanism by which MIF could influence inflammation and oppose the effects of glucocorticoids is via the nuclear factor (NF)-κB pathway. NF-κB is an important regulator of the gene expression of inflammatory cytokines[8] and the expression of surface molecules on endothelial cells[102]. Several studies support the assumption that glucocorticoids hinder the effect of NF-κB by inducing production of IκB[2], an inhibititory subunit of NF-κB, which prevents its nuclear localization. Cannon and Daun[28] demonstrated that MIF inhibits glucocorticoid-mediated induction of IκB production in human mononuclear cells which leads to an increased activity of NF-κB. David et al.[33] showed that MIF is an important regulator of Toll-like receptor (TLR)4 on macrophages via the transcription factor PU.1[90]. MIF-deficient macrophages show down-regulation of TLR4 and are hyposensitive to LPS.

However, at high concentrations MIF can also influence the transcription activity of the activator protein (AP)-1 through an interaction with Jab-1. MIF uptake can be mediated through receptor-independent endocytosis[56]. AP-1 is a transcription factor for IL-1, IL-2 and IFN-γ, and binds DNA as a heterotrimer, together with the FOS- and Jun-oncoproteins. Jab-1 stabilizes this trimeric complex. The complex is destabilized via the interaction of MIF with Jab-1. Moreover, Jab-1 binds to p27Kip1 and promotes the degradation of this protein that functions as an inhibitor of the cell cycle. Thus, at high concentrations MIF inhibits the growth-promoting effects of Jab-1 on fibroblasts[57]. Together these data contradict the proinflammatory and growth-promoting effects of MIF described in the literature[73]. However, these findings are consistent with the notion that MIF exercises different functions when present in different concentrations as proposed by Bernhagen et al.[16], and Stavitsky and Xianli[99].

It is becoming widely accepted that steroid-resistant autoimmune and inflammatory diseases are associated with increased AP-1 and NF-κB-activity[7], which

places MIF in a decisive position to influence the activity of both endogenous and therapeutically administered glucocorticoids.

Matrix metalloproteinases (MMPs) are elevated in inflammation reactions, in particular in rheumatic and neuroinflammatory disorders, and they play an important role in the pathogenesis of these diseases[39, 54]. Glucocorticoids reduce the concentration of MMPs in RA, while MIF up-regulates mRNA of MMP-1 and MMP-3 in synoviocytes[85]. Thus, MMPs could be another target mechanism for MIF to overcome the anti-inflammatory effects of glucocorticoids.

In addition, it has been shown that adhesion-induced MIF secretion by fibroblasts promotes integrin-dependent activation of MAP kinases and cyclin D1 expression, which further downstream modulates the phosphorylation of the tumor-suppressor protein retinoblastoma and thereby plays an important role both in malignant cell transformation and counteraction of glucocorticoid effects[67].

The cell growth-promoting and anti-apoptotic properties of MIF may also be an important mechanism by which MIF contributes to the pathology of autoimmune diseases (Fig. 2). During immune (and autoimmune) responses, uncontrolled cellular expansion is prevented by apoptosis mechanisms such as Fas-FasL-induced cell death. Similarly, p53, a tumor-suppressor gene, promotes apoptosis, and thus its inhibition may enhance inflammation[78]. Therefore it has been suggested that negative regulation of p53, for example via expression of COX-2, results in decreased apoptosis in macrophages[106], and enhanced inflammation. Importantly, MIF induces cPLA2[73] and increase the expression of COX-2[72]. Moreover, MIF reduces p53 accumulation via an autocrine-regulatory pathway both *in vitro*[48] and *in vivo*[72]. This reduction could be due to the upregulation of arachidonic acid and COX-2, or due to an as yet undefined direct effect. Furthermore, the delayed cleavage of the pro-apoptotic Bcl-2 family members Bid and Bax promoted by MIF prevents the release of cytochrome c and Smac from the mitochondria[11] and inhibits apoptosis. However, under certain conditions MIF can promote macrophage apoptosis, for example via the induction of NO and its effect on the p53-pathway[70], suggesting multiple feedback loops of MIF in cell survival.

MIF IN AUTOIMMUNE DISEASES

The above-mentioned evidence led to the investigation of the role of MIF in autoimmune diseases. The

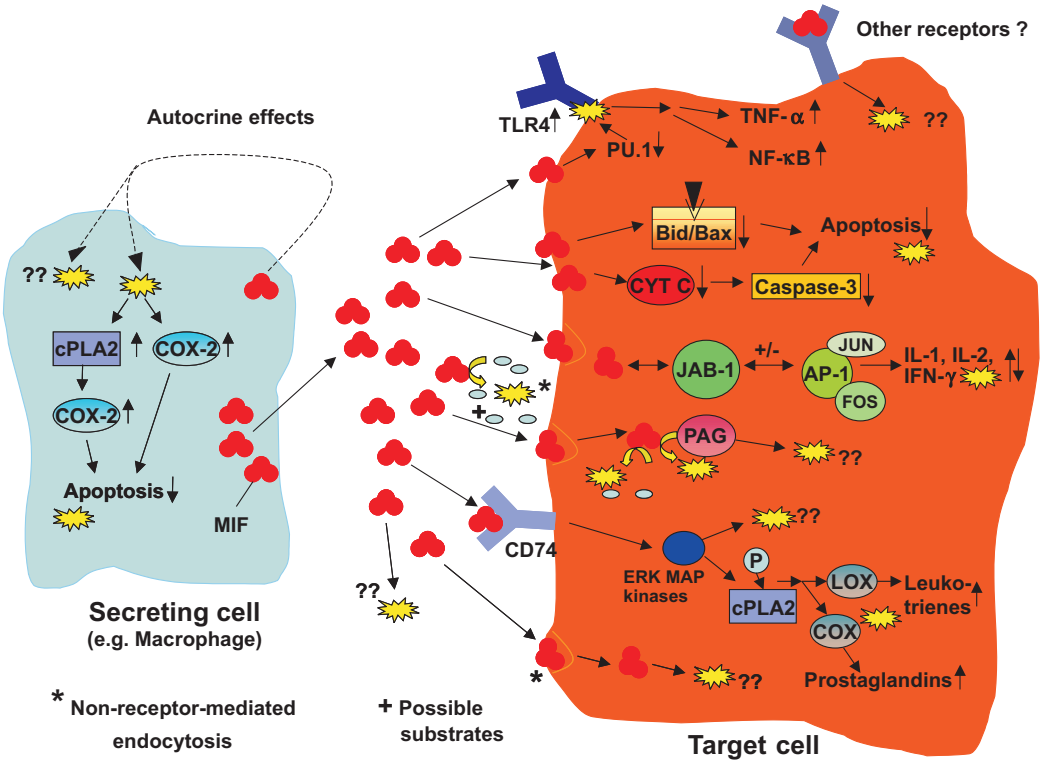

Figure 2. Hypothetic scheme of MIF signaling. Several pathways have been hypothesized in the signaling of MIF. The protein acts in an autocrine pathway on the secreting cell. On the target cell it exerts influence via a receptor-mediated pathway, through enzymatic catalysis of PAG and other substrates and/or through pathways following JAB-1 or the ERK MAP kinases. Abbreviations: +/-: dose dependent effect; ↔: protein interactions; →: signaling; ??: mechanism unknown; ⟆: enzymatic reaction; yellow star: effect.

Table 2. MIF in autoimmune diseases

Diseases	References
Rheumatoid arthritis	63, 64, 71, 76, 87
Multiple sclerosis	6, 34, 81
Glomerulonephritis	19, 59, 69, 112

data strongly support a role for MIF in the pathogenesis of autoimmune diseases and suggest that this cytokine could be a promising target for therapeutic strategies (Table 2). First, enhanced levels of MIF were observed in patients with autoimmune diseases, both systemic and within affected tissues. Specifically, increased levels of MIF were found in psoriasis[97], Wegener's granulomatosis[84], GN[46], systemic lupus erythematosus[74] and iridocyclitis[55], as well as in the synovial tissues of RA joints[87]. Moreover, an increased MIF concentration was present in the serum and in the cerebrospinal fluid of patients with MS and neuro-Behcet's disease[81].

MIF in RA was studied in adjuvant-induced arthritis in rats and in collagen type II-induced arthritis in mice[62, 64, 71, 94]. In collagen type II-induced arthritis in mice, neutralization of MIF with a blocking antibody before immunization resulted in delayed onset and decreased incidence of disease. This was accompanied by lower levels of IgG2a[71]. In another model of murine antigen-induced arthritis, disease activity was significantly inhibited by anti-MIF mAbs as shown by decreased synovial cellularity[94]. Treatment with dexamethasone inhibited the disease, but the effect was reversed when MIF was administered in addition. This finding is consistent with the counter-regulation of glucocorticoid effects by MIF[94].

In adjuvant arthritis in rats[64] the level of MIF in the serum and in the synovial lining cells was elevated, and cultured macrophages recovered from the synovia released high amounts of the cytokine. Treatment with anti-MIF mAb starting on the day of immunization led to a significant dose-dependent decrease in arthritis severity as measured by clinical score, paw swelling, and leukocyte infiltration and to a delayed onset of symptoms[64]. In this model, adrenalectomy resulted in an exacerbation of clinical disease, with higher mortality concomitant with an increased release of MIF from the pituitary gland and enhanced serum levels. The mortality was reduced when these rats were treated with an anti-MIF mAb[62]. Importantly, MIF levels were enhanced in the synovial lining of patients with RA[87]. MIF secretion from cultured synoviocytes was attenuated by dexamethasone in a dose-dependent fashion[63, 87]. Immunostaining for synovial MIF correlated strongly with disease activity measured by C-reactive protein concentration in humans, while a reduction in clinical disease parameters was accompanied by significant reduction in synovial MIF[76]. Based on this information, therapeutic approaches that target MIF are already considered for RA.

In MS, MIF protein was shown to be present in cells of the cortex and the cerebral white matter, in epithelial cells of the choroid plexus, astrocytes, ependymal cells, and cells of the pituitary gland[15, 75]. In addition, activated macrophages and T cells in the inflammatory infiltrates can secrete MIF in MS[18, 25, 32]. MIF was found to be elevated in the inflammatory infiltrates in the CNS in the Biozzi AB/H mouse model of MS[6]. Concentrations of MIF in the cerebrospinal fluid of patients with the conventional or the opticospinal form of MS were increased compared with patients with non-inflammatory neurological diseases[81]. Recently, the role of MIF was examined in mice with EAE, an animal model with many similarities to human MS[34]. The results showed that treatment of SJL mice with anti-MIF mAbs before or after the onset of clinical EAE symptoms improved the disease severity and accelerated recovery. Furthermore, the results demonstrated that MIF blockade decreased the expression of VCAM-1 in the CNS and impaired the homing of neuroantigen-specific T cells to this site. Interestingly, VLA-4, the ligand for VCAM-1, has been reported to function as a co-stimulatory molecule[31] and to protect T cells from apoptosis[113]. The VLA-4/VCAM-1-engagement also prevented T cell apoptosis in experimental autoimmune neuritis[66] and of human B cells from RA synovial tissue[44]. Thus, VCAM-1 regulation could be an important mechanism by which MIF perpetuates the disease. In addition, MIF blockade reduced the clonal size of the autoantigen-specific Th1 cells and increased their activation threshold[34]. Altogether, the results indicated an important role for MIF in the pathogenesis of EAE/MS and suggested that treatment with anti-MIF mAb or small molecular compounds that inhibit MIF could be a novel therapeutic approach for autoimmune demyelination.

GN is another disease in which MIF appears to play an important role. The early phase of GN is characterized by the deposit of antibody and complement fragments. Progression of GN is known to critically depend on the accumulation of macrophages and T cells[18, 19, 25, 32, 59]. The degree of renal impairment correlates with macrophage and T cell accumulation, arguing for an immune cell-mediated mechanism that furthers the progression of the disease. In a rat model of immunologically induced crescentic anti-glomerular basement membrane GN, up-regulation of MIF was shown during the disease in endothelial

cells, as well as in glomerular and tubular epithelial cells[60]. TNF-α up-regulated MIF locally and systemically in this model[61]. Pre-treatment of the animals with anti-MIF mAbs prevented the loss of renal function and resulted in a reduction of severe focal lesions and the formation of glomerular crescents. Furthermore, infiltration and activation of glomerular macrophages and T cells was decreased. Further studies indicated that MIF neutralization impaired glomerular and interstitial expression of intercellular adhesion molecule (ICAM)-1 and VCAM-1 and inhibited the up-regulation of IL-1β and inducible NO synthase in the infiltrating macrophages and the renal cells[59]. The down-regulation of VCAM-1 and ICAM-1 could impair the homing of T cells to sites of inflammation, as was suggested by the studies in EAE. Since interactions of VCAM-1 with VLA-4 may protect T cells from apoptosis[31], both mechanisms could account for the reduced T cell influx as suggested previously[34, 44].

Treatment with anti-MIF mAbs reversed the disease and restored renal function. It reduced histological damage even when administered seven days after the induction of disease and decreased the infiltration and activation of macrophages and T cells, and resulted in a reduction of IL-1 production[112]. The role for MIF in renal disease was corroborated by human studies showing higher levels of MIF in the urine of patients with IgA nephropathy, which significantly correlated with the degree of glomerular crescent formation and mesangial cell proliferation[14]. No increase in the levels of MIF in the urine was found in patients with minimal-change nephrotic syndrome[69].

Taken together, we suggest that MIF affects the progression and outcome of autoimmune diseases via the following mechanisms, alone or in concert: a) counter-regulation of immunosuppressive glucocorticoid effects, b) stimulation of immune-cell effector functions such as production of cytokines, c) promotion of immune-cell migration and homing via up-regulation of adhesion molecules and/or chemokines, d) increased survival and/or clonal expansion of inflammatory cells.

The glucocorticoid counter-regulatory properties of MIF on effector functions of immune cells such as cellular proliferation and cytokine production are unique amongst cytokines, and the significance of this mechanism has been demonstrated in animal models of RA and GN[62, 94, 103, 112]. Effects of MIF on adhesion molecules and cell migration were suggested by the animal studies in GN and EAE. It is clear that the migration of autoreactive T cells to sites of the autoimmune attack is critically dependent on the interaction of adhesion molecules on these cells with their ligands such as VCAM-1, ICAM-1, CD31, and leukocyte function-associated antigen-1 on tissues in the target organ[21, 29, 47, 49, 88]. MIF blockade decreased the expression of VCAM-1 both in the EAE model and in the glomerulonephritis model and impaired the homing of antigen-specific T cells to the sites of inflammation[34, 59, 112]. However, MIF blockade may in addition impair the effector functions of T cells and/or microglia/oligodendrocytes, such as the production of cytokines or chemokines. In particular, IFN-γ-inducible protein-10 (IP-10; CXCL10), macrophage inflammatory protein (MIP)-1a, and monocyte chemoattractant protein (MCP)-1 have been implicated in the migration of activated T cells into the CNS during EAE[40, 105] and into synovial joints in RA[42], as well as in the infiltration of polymorphnuclear leukocytes and monocytes/macrophages in GN[103]. Treatment with anti-MIP-1a prevented acute EAE[52], whereas anti-MCP-1 treatment decreased the severity of clinical relapses[53]. IP-10--deficient mice showed decreased recruitment of $CD4^+$ and $CD8^+$ T cells into the brain in combination with reduced levels of demyelination upon infection with a neurotropic mouse hepatitis virus, suggesting a role for IP-10 in regulating T cell trafficking into the CNS *in vivo*[37]. Furthermore, MIF induced IL-8 in monocytes and dendritic cells[77] and anti-MIF mAb treatment inhibited MCP-1-induced chemotaxis of human peripheral blood mononuclear cells[45], indicating a role for MIF in the regulation of chemokine expression.

The evidence for a role of MIF in immune cell survival is based on several observations. VLA-4, the ligand for VCAM-1, has been reported to function as a co-stimulatory molecule and to protect T cells from apoptosis[44, 66, 113]. Moreover, anti-MIF mAb treatment was found to significantly decrease the frequencies of T cells in the CNS in EAE[34] and also in the GN model[61, 112], whereas T cell frequencies in the spleen were only moderately reduced. Moreover, in the EAE model the activation threshold of proteolipid protein-reactive T cells in anti-MIF mAb treated mice was significantly decreased[34], suggesting that the treatment deleted or anergized T cells with high affinity for self antigen[104]. Adoptive transfer studies in the EAE model clearly showed that T cells from anti-MIF mAb-treated mice were less pathogenic and suggested that MIF was necessary to promote the survival of the encephalitogenic T cells. Thus, in the absence of MIF, autoreactive T cells may have been deprived of T cell receptor and/or co-stimulatory signals necessary for cell activation and/or survival[30, 89, 109]. Without these signals, the autoreactive T

cells may have become more susceptible to apoptotic cell death or induction of anergy. Overall, it is clear that more research is needed to define exactly the molecular targets for MIF and carefully dissect its role in the pathogenesis of autoimmune diseases. However, it can be assumed that MIF has great potential as a therapeutic target in autoimmune diseases.

MIF AS A THERAPEUTIC TARGET

As outlined above, MIF seems to be a promising target in the therapy of autoimmune diseases. It is the only known cytokine that can counteract the glucocorticoid-mediated inhibition of proinflammatory cytokines[5] and may therefore be the critical factor limiting the immunosuppressive effects of glucocorticoid therapy. Potentially it could play a critical role in patients that have become resistant to glucocorticoid therapy during treatment for autoimmune diseases, or in patients who are severely affected by the side effects of high-dose glucocorticoid application. Thus there is a need to determine whether the combination of MIF neutralization and glucocorticoids could overcome these clinically highly relevant problems.

Pharmacologically, the effects of MIF could be blocked either by anti-MIF mAb or by small organic compounds that block MIF directly or at the receptor level. The presence of an easily accessible catalytic site for dopachrome substrates within MIF – irrelevant for its biological actions – provides opportunity for the design of selective small organic compound inhibitors. As an additional advantage, as suggested by the EAE studies, the anti-MIF reagents would not only ameliorate glucocorticoid side effects and dosage problems, but also alleviate the need for exact knowledge of the nature of the neuroantigens targeted by the autoimmune T cells. This is a major advantage over other current attempts at antigen-specific immunotherapy, for example using altered peptide ligands.

Several pharmaceutical companies (e.g. IDEC pharmaceuticals[17] and Avanir pharmaceuticals) already follow different strategies in the development of MIF blocking reagents. In a recent study, Senter et al.[96] tested both the biological role of the substrate pocket with D-dopachrome-tautomerase-activity and possible substrates with bio-inhibitory activity and found a compound with a strong structural relationship to the analgesic drug acetaminophen. Acetaminophen was found to be a weak inhibitor of the dopachrome tautomerisation. This study also published the first small molecular inhibitor, N-acteyl-p-benzoquinone imine, that inactivates both the catalytic and biological activity of MIF, probably through a conformational change after the enzymatic reaction in the structure of the protein that disrupts a biologically important epitope. Other molecules with potential to inhibit the biological actions of MIF are isoxazolines[68] and imine conjugates created by coupling amino acids with a benzaldehyd derivative[35].

In conclusion, MIF appears to play a role in a number of autoimmune diseases. Moreover, the inhibition of its proinflammatory effects is a promising new therapeutic strategy, which may alleviate side effects and decrease problems associated with glucocorticoid treatment. Future research in the molecular immunology of this fascinating cytokine is expected to provide even more insights into its role in autoimmune diseases and novel pathways for the therapy of autoimmune diseases.

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