

## Review

# Macrophage-migration inhibitory factor: role in inflammatory diseases and graft rejection

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**Abstract.** Macrophage migration inhibitory factor (MIF) functions as a pleiotropic protein, participating in inflammatory and immune responses. MIF was originally discovered as a lymphokine involved in delayed hypersensitivity and various macrophage functions, including production of proinflammatory cytokines, glucocorticoid-induced immunomodulator, and natural killer cell inhibitory factor (NKIF), regulation of toll-like receptor expression, adherence and phagocytosis of macrophages, as well as induction of metalloproteinase. Therefore MIF is considered as a potential target protein in many pathophysiological states. In this review, considering the protein structure and the acting mechanisms of MIF, we mainly discuss the important role of MIF in pathogenesis of inflammatory diseases and graft rejection.

**Key words:** Macrophage migratory inhibitory factor – Macrophages – Transplantation – Inflammation – Graft rejection

## Introduction

Macrophage migration inhibitory factor (MIF) was originally found to inhibit the random migration of macrophages as a T-lymphocyte-derived activity [1] and was associated with delayed-type hypersensitivity reactions, inflammatory arthritis [2], glomerulonephritis [3], allograft rejection [4] and wound healing [5]. MIF can significantly modify the activation, adherence, phagocytosis and nitric oxide (NO) production of macrophages [6, 7]. Neutralization of MIF in animal models of inflammatory diseases such as arthritis, glomerulonephritis and acute lung injury has pronounced therapeutic effects. However, molecular characterization of the protein responsible for this activity and its role in the immune response has remained elusive. In addition to its role in hypersensitivity reactions, recent studies with MIF<sup>-/-</sup> mice confirmed the paramount importance of this protein in sepsis

as the mice were more resistance to LPS-lethality than their wild-type counterparts [8].

## MIF structure and its signal pathways

As most detail pertaining to structural properties has been nicely reviewed [9], here we shall briefly summarize the structural properties of MIF. MIF gene contains 115 amino acids which reduces to 114 after processing [10]. The structure of MIF with hydroxyphenylpyruvate indicates the importance of Pro-1 which functions as a catalytic base. Mutation of Pro-1 to glycine significantly reduces the neutrophil priming activity of MIF but this mutation does not affect its inhibitory effects on the migration of monocytes. MIF has  $\alpha/\beta$  structures [10]. Each MIF monomer contains two antiparallel  $\alpha$ -helices ( $\alpha 1$  and  $\alpha 2$ ) and six  $\beta$  strands ( $\beta 1$ – $\beta 6$ ). One  $\beta$ -pleated sheet is formed by four of six  $\beta$  strands ( $\beta 1$ ,  $\beta 2$ ,  $\beta 4$ ,  $\beta 5$ ) above which two  $\alpha$ -helices rise. This represents close similarity to the peptide-binding domain of a major histocompatibility complex molecule. In MIF trimer, the remaining two  $\beta$ -strands attach to the  $\beta$  sheets of adjacent MIF subunits. Six  $\alpha$ -helices surround three  $\beta$ -sheets to form a barrel containing a solvent-accessible channel which runs through the centre of the protein. Though it is suggested that MIF action is mediated by a dimer or monomer [11], it is unclear at this moment whether MIF trimer is the physiologically occurring state of MIF or not.

A MIF membrane receptor has not yet been identified, but some studies argue in favor of a proposed receptor-mediated pathway. Another possibility for MIF to mediate its functions could be through catalytic activities. MIF exhibits tautomerase, isomerase and thiol-protein oxidoreductase activities [12, 13]. The protein substrate of the enzymatic activity of MIF has been identified [14]. Other studies showed the important role of extracellular signal-regulated (ERK1/2) subfamily of mitogen-activated protein (MAP) kinase and activator protein-1 (AP-1) pathways in MIF-mediated signaling [15–18]. A recent study showed that MIF could directly promote cell survival through activation of

the PI3K/Akt pathway and this effect is critical for tumor cell survival [19]. Furthermore, an intracellular receptor protein for MIF, i.e. co-activator c-jun activation domain binding protein-1 (JAB1), has been identified [19].

### Immunoregulatory effect of MIF

Glucocorticoids have been administered clinically to treat inflammatory and autoimmune diseases for over five decades. They exert positive and negative effects on immune responses, e.g. glucocorticoids are involved in gene modulation during priming of the innate response, while they suppress cellular (Th1) and promote humoral (Th2) immunity [20]. It is considered that glucocorticoids inhibit cytokine expression but induce MIF expression by monocytes, macrophages and T-lymphocytes. MIF was described as the first pro-inflammatory cytokine to be produced upon glucocorticoid stimulation [21]. Due to the pro-inflammatory effect of MIF and anti-inflammatory effect of glucocorticoids on immune cell activation, MIF acts as counterregulatory mediator that counteracts the immunosuppressive effects of glucocorticoids [22]. In particular MIF counteracts glucocorticoid-induced inhibition of inflammatory cytokine secretion in T cells [23]. Current data indicate that MIF may play an important role in the inflammatory cascade.

The association of glucocorticoid counter-regulating activity of MIF with its redox rather than tautomerase activity was suggested by the finding of structure-function correlations [24]. The ERK MAP kinase activation [25] causes phosphorylation and prolongs activation of cytoplasmic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>), which was later confirmed [26]. This effect may lead to glucocorticoid receptor (GR) antagonism, with no alteration in GR expression or affinity [27]. cPLA<sub>2</sub> and its products, such as arachidonic acid, play a critical role in inflammatory reactions and are involved in activation of c-Jun N-terminal (JNK)/stress activated protein kinase (SAPK) pathways [28]. The blocking of JNK/SAPK activation induces glucocorticoid inhibition of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) translation [29]. MIF ability to activate cPLA<sub>2</sub> may lead to counter-regulation of the immunosuppressive effect of glucocorticoids. The effects of MIF on MAP kinase phosphatase-1 and p38 MAP kinase may regulate the sensitivity of cells to glucocorticoid [16].

### MIF and inflammatory diseases

MIF is a ubiquitous protein performing an important role in the pathogenesis of various inflammatory disease conditions in different organs such as kidney, heart, lung, liver, skin and so on [30]. Here, we briefly discuss the role of MIF in various autoimmune diseases.

Over-expressing MIF could remarkably accelerate the progression of glomerulosclerosis and end-stage renal failure [31]. The urine MIF concentration was significantly increased in proliferative forms of glomerulonephritis (GN) and correlated with the degree of renal dysfunction, histologic damage and leukocytic infiltration. Urine MIF reflected MIF expression in the injured kidney [32]. MIF performs a regulatory role in the pathogenesis of immunologically induced kidney disease [33–35]. Anti-MIF mAbs or deficiency

of MIF significantly inhibited focal lesions and glomerular crescent formation, minimizing glomerular macrophage and T-cell infiltration and activation [36]. This treatment inhibited IL-1, glomerular, interstitial and tubular inducible nitric oxide (NO) synthase expressions.

MIF is closely related with the occurrence of rheumatoid arthritis (RA) [37]. Elevated levels of MIF were found in typical RA inflammatory sites i.e. 5 to 10 fold higher than in normal volunteers. The MIF was released by infiltrating T lymphocytes, macrophages and synovial cells in synovial fluid. Anti-MIF mAb significantly suppressed the inflammatory response in experimentally induced arthritis in mouse models [2]. The typical pathological feature of RA is the connective tissue degradation by matrix metalloproteinases (MMPs). MIF is involved via up-regulation of MMP-1 and MMP-3 mRNA levels in synovial fibroblasts [38]. MMP-1 and MMP-3 are considered to be involved in the degradation of extracellular matrix components in RA. Furthermore, MIF polymorphisms are closely related with increased clinical disease severity and increased risk of joint erosions and damage in adult patients with RA [39–41]. The proliferation of human RA synoviocytes, inhibition of p53 expression and apoptosis in these cells by MIF demonstrate the role of MIF in human RA [42, 43]. Recently, the suppression of collagen-induced arthritis (CIA) in MIF<sup>-/-</sup> mice [44] confirms the role of MIF in RA. These data suggest that MIF inhibition could have significant importance as a therapeutic target in RA.

In all stages of human atherosclerosis, an elevated MIF expression and functional co-localization with JAB1 were observed [43, 45]. MIF is up-regulated in endothelial cells (EC), smooth muscle cells (SMC) and macrophages during progression of atherosclerosis in humans and hypercholesterolemic rabbits. Activated CD68<sup>+</sup> macrophages adherent onto MIF<sup>+</sup> vascular endothelial cells increased MIF expression [46], which indicated a key role of MIF in atherosclerosis. An increased MIF-mediated monocyte arrest in the endothelium suggests a crucial role of MIF in leukocyte recruitment in atherogenesis [47]. The vascular inflammation, cellular proliferation and neointimal thickening were reduced by neutralizing MIF bioactivity after experimental angioplasty in atherosclerosis-susceptible mice [48]. The genetic deletion of MIF in LDLR<sup>-/-</sup> mice reduced lipid deposition and intimal thickening in the aorta. Neutralizing anti-MIF mAb or peripheral MIF depletion in ApoE<sup>-/-</sup> mice significantly reduced the inflammatory response associated with atherosclerosis development, including reductions in concentrations of circulating and lesional inflammatory cytokines, lesional adhesion molecules and MMPs, and expression of inflammatory transcription factors. These recent studies demonstrated that MIF expression was closely correlated with atherosclerotic disease severity [43].

A significant quantity of MIF was found in the alveolar air spaces, which indicates the potential role of MIF in acute respiratory distress syndrome (ARDS) [49]. Increased MIF expression was confirmed in ARDS patients [50]. MIF plays a role in ARDS via up regulation of the neutrophil chemoattractant macrophage inflammatory protein-2 (MIP-2). An elevated level of MIF expression was shown in both lung tissues and bronchoalveolar lavage (BAL) fluids in the development of acute injury [51]. Anti-MIF antibody significantly reduced the accumulation of inflammatory cells and also reduced TNF- $\alpha$  expression in air spaces. Furthermore, human

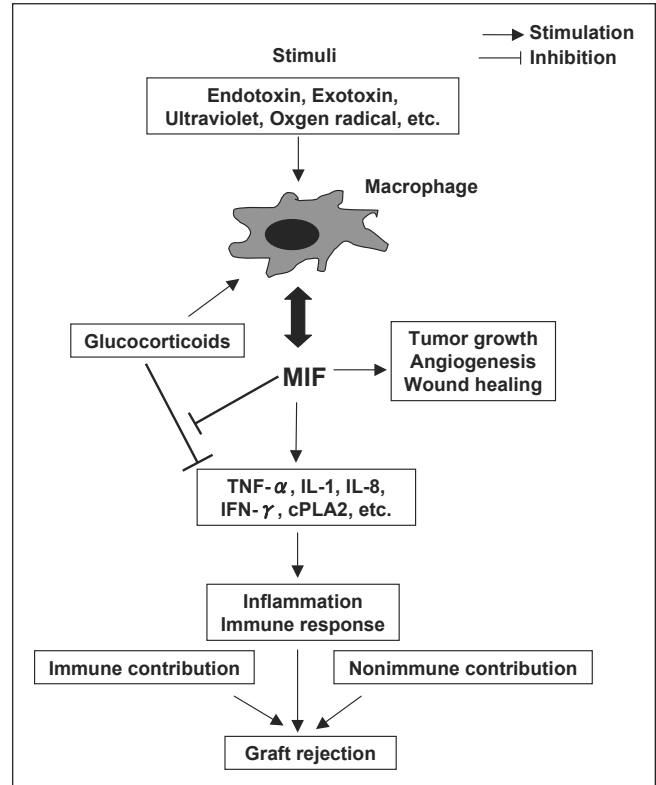
eosinophils are potent sources of MIF. Eosinophils are the key cells in the pathogenesis of allergic inflammatory diseases such as atopic dermatitis, allergic rhinitis and bronchial asthma [52]. MIF is involved in the immunopathogenesis of asthma possibly via the promotion of Th2 responses. MIF inhibition in asthma may be therapeutically beneficial and specific intervention may be guided by the MIF genotype of affected individuals [53]. However, recent studies showed that MIF is required for allergic inflammation but not for Th2 differentiation [54] and without affecting immune response [55]. These data suggest that MIF may contribute to the pulmonary inflammatory response in asthma and other allergic inflammatory conditions.

Increased level of MIF was noticed in the serum and liver of patients with hepatitis, alcoholic liver disease and cirrhosis [56, 57]. Neutralizing anti-MIF mAb significantly inhibited the severity of hepatitis by reducing the level of transaminase in sera and inhibited TNF- $\alpha$  production. In addition, acute hepatitis in mice was prevented by anti-sense MIF cDNA [58], which reduced the necrotic area in liver. Anti-mouse MIF antibody treatment reduced liver injury and inflammatory cell infiltration in the liver after injection of antigen-specific cytotoxic T lymphocytes into hepatitis B virus transgenic mice [59]. These findings suggest the therapeutic potential of MIF in hepatitis.

An experimental model of pancreatitis induced by taurocholic acid showed the involvement of MIF in the pathogenesis of pancreatitis [60]. It was observed that MIF expression was significantly increased systemically and locally in patients with pancreatitis [61, 62]. Anti-MIF antibody treatment significantly decreased the severity of pancreatitis [60].

In mice with acute gastric ulcer, macrophages were the major source of up-regulated MIF. Anti-MIF antibody significantly inhibited the up-regulation of TNF- $\alpha$  and inducible NO synthase and intercellular adhesion molecule-1 [63]. Helicobacter pylori infection induced significantly high levels of MIF protein and mRNA expressions in epithelial cells, T cells and macrophages, which suggest a role of MIF in stomach disease [64, 65]. Crohn's disease (CD) and ulcerative colitis (UC) showed enhance a MIF protein in the serum of these patients [66, 67]. In experimental colitis, MIF expression was increased during colitis and the severity of colitis was reduced by anti-MIF antibody [68], which suppressed T-helper 1-type cytokines and matrix metalloproteinase (MMP). It has been reported that MMP is overexpressed in inflammatory bowel disease (IBD) and in experimental colitis [69]. Moreover, MIF-deficient mice showed mild inflammation compared with wild-type mice [70]. Increasing expression of MIF was observed in acute neonatal necrotizing enterocolitis [71]. Colitis in acute graft-versus-host disease (GVHD) was correlated with local upregulation of MIF [72].

In addition, MIF may also be involved in intestinal tumorigenesis [73]. H. pylori induced gastritis, intestinal metaplasia and gastric cancer had progressively increased epithelial and serum MIF expression, suggesting that MIF is involved in gastric carcinogenesis and may be a valuable biomarker for the early detection of gastric cancer [74]. Various colon cancers *in vivo* and *in vitro* exhibited increased MIF level [75]. MIF expression was associated with enhanced cell proliferation. Anti-MIF antibody markedly inhibited tumor growth [75]. These studies suggest that MIF may be a possible indicator of prognosis in colorectal cancer.



**Fig. 1.** The role of MIF in inflammation and graft rejection. MIF promotes the production of inflammatory cytokines from macrophages which play important roles in immune response to allo-grafts.

### MIF and graft rejection

A potent innate immune response is initiated by ischemia-reperfusion (I/R) injury during the process of harvesting, transporting, and implanting a transplanted organ [76]. Liver I/R and surgical injury causes induction of transcripts for the cytokines including IL-10, IL-1 $\alpha$ , IL-1 $\beta$ , IL-1Ra, IL-18, IL-6, INF- $\beta$ , MIF, IL-6, INF- $\gamma$ , TGF- $\beta$ 1, RANTES, major intrinsic protein MIP-1b, MIP-1a, MIP-2, IFN- $\gamma$ -inducible protein (IP)-10, MCP-1 and TCA-3 [77, 78].

Allografts can induce macrophage accumulation and the overall macrophage accumulation promotes a rejecting immune response. The infiltration of recipient-derived macrophages was observed in the graft within 24h after surgery [79]. MIF participates in the recruitment of circulating monocytes into rejecting organs and, as a pro-inflammatory molecule, is involved in cell-mediated immunity and delayed-type hypersensitivity [3, 80]. MIF promotes the production of pro-inflammatory cytokines as activated macrophages can secrete IL-1, IL-2, IL-18, TNF- $\alpha$  and IFN- $\gamma$  [81]. These pro-inflammatory cytokines are associated with graft rejection [82, 83]. The pivotal role of MIF in inflammation and graft rejection is briefly summarized in Figure 1.

MIF may be an important mediator in the allo-immune reaction during renal transplantation. TNF- $\alpha$  involves up-regulation of local MIF expression by both infiltrating macrophages and resident kidney cells in rat crescentic glomerulonephritis. Systemic MIF production is also regulated by TNF- $\alpha$ . Thus, both TNF- $\alpha$  and MIF may participate in the

pathogenesis of immunological by induced renal disease [33]. Glomerular macrophage accumulation was reported in severe allograft rejection with worse prognosis which highlights the importance of MIF in renal transplantation [84]. As local MIF production is increased in acute renal allograft rejection, urine MIF may be used as a diagnostic tool in human renal allograft rejection [32, 85]. However, studies using MIF<sup>-/-</sup> mouse models did not support the important role of MIF in kidney or heart allo-graft rejection [86]. Though MIF blockade significantly reduced the delayed-type hypersensitivity response; neither local nor systemic MIF are required for the rejection of fully mismatched skin and renal allo-grafts [87]. It is worth noting that MIF participates in skin graft destruction after indirect recognition through an inhibition of macrophage migration and/or function [4]. Thus whether MIF plays different roles in direct and indirect antigen recognition in transplants needs to be addressed.

Pancreas transplantation offers a cure for diabetes mellitus. Some immune modulating proteins, monocyte chemoattractant protein-1(MCP-1), transforming growth factor- $\beta$  (TGF- $\beta$ ) [88] and MIF [89] are expressed in islets of Langerhans. These proteins could be involved in the development of autoimmunity in type-1 diabetes and influence intraportal islet transplantation outcome [90–92]. Isolated islets expressed several inflammatory mediators, particularly at an early stage after isolation, suggesting that a few days culture could be beneficial for outcome of islet transplantation [92].

In addition, the important role of MIF in the development of acute GVHD in a mouse model of allogeneic stem cell transplantation has been reported [93]. MIF was thus found to be one of the major cytokines involved in the rejection of the allogeneic tracheal; treatment with MIF siRNA inhibits the destruction of tracheal allografts and formation of obstructive bronchiolitis in the early phase [94].

### Closing remarks and future direction

A number of questions about the pathophysiological significance of MIF remain to be answered. It is currently known that MIF is a pro-inflammatory cytokine that plays a critical role in inflammation and cellular immunity. MIF is an important mediator in the pathogenesis of inflammatory disorders such as endotoxemia/sepsis, arthritis, glomerulonephritis, pancreatitis, inflammatory bowel disease, tumorigenesis as well as several other pathophysiologic inflammatory and immune conditions. MIF may also be closely involved in allograft rejection and dysfunction. Anti-MIF antibodies have proved to be a potent tool for effective treatment of human inflammatory diseases. MIF inhibitors may have potential therapeutic applications in patients with inflammatory diseases or allo-grafts in the clinic.

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