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D-dopachrome tautomerase 1 (D-DT or MIF-2): Doubling the MIF cytokine family

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Abstract

D-dopachrome tautomerase (D-DT) is a newly described cytokine and a member of the macrophage migration inhibitory factor (MIF) protein superfamily. MIF is a broadly expressed pro-inflammatory cytokine that regulates both the innate and the adaptive immune response. MIF activates the MAP kinase cascade, modulates cell migration, and counter-acts the immunosuppressive effects of glucocorticoids. For many cell types, MIF also acts as an important survival or anti-apoptotic factor. Circulating MIF levels are elevated in the serum in different infectious and autoimmune diseases, and neutralization of the MIF protein via antibodies or small molecule antagonists improves the outcome in numerous animal models of human disease. Recently, a detailed investigation of the biological role of the closely homologous protein D-DT, which is encoded by a gene adjacent to MIF, revealed an overlapping functional spectrum with MIF. The D-DT protein also is present in most tissues and circulates in serum at similar concentrations as MIF. D-DT binds the MIF cell surface receptor complex, CD74/CD44, with high affinity and induces similar cell signaling and effector functions. Furthermore, an analysis of the signaling properties of the two proteins showed that they work cooperatively, and that neutralization of D-DT *in vivo* significantly decreases inflammation. In this review, we highlight the similarities and differences between MIF and D-DT, which we propose to designate “MIF-2” and discuss the implication of D-DT/MIF-2 expression in MIF-based therapies.

Keywords

MIF; CD74; sepsis; cancer; biomarker

Introduction

MIF is one of the first cytokines to be described [1, 2] and has a pivotal role in the immune response [3]. Historically, T cells were regarded as the major source of circulating MIF [4, 5], but studies in the last two decades have shown that MIF is released from numerous other

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cell types upon their activation [6, 7] and acts then in an autocrine and paracrine manner. Cells that are activated by MIF include immune cells, epithelial and endothelial cells, different parenchymal cells, and cancer cells. In addition to induction by inflammatory stimuli, macrophages and T cells secrete MIF after stimulation with low doses of glucocorticoids, and MIF also counter-regulates their immunosuppressive effects [8–10]. Via this mechanism, MIF sustains inflammation, which is clinically detrimental for patients with autoimmune and chronic inflammatory diseases. In the cytokine cascade, MIF is localized upstream of tumor necrosis factor (TNF) α , interleukin (IL)1 β , interferon (IFN) γ , and other effector cytokines, in large part because it is released initially from pre-formed cytoplasmic pools [11]. In an inflammatory setting, for example after LPS challenge, circulating levels of these effector cytokines are reduced when the MIF protein is neutralized or genetically deleted [12–15]. The molecular mechanism underlying MIF action has become better understood since the discovery of its cell surface receptors: CD74, which signals via regulated intramembrane cleavage or by co-activating CD44 [16–18] and the chemokine receptors CXCR2 and CXCR4 [19, 20]. The MIF receptor, CD74, is widely expressed on different cell types, including monocytes/macrophages, B cells, fibroblasts, and endothelial, epithelial, and stromal cells. In monocytes, there is an intracellular form of CD74, the invariant chain, that also functions in the transport of class II proteins from the endoplasmic reticulum to the Golgi. Approximately 2–5% of CD74 is expressed on the monocyte cell surface independently of class II, and after binding MIF, the MIF/CD74 complex is rapidly internalized [16]. MIF initiates the ERK1/2 MAP kinase pathway by binding to the extracellular domain of CD74 and recruiting the co-receptor and signaling component CD44 to induce cell proliferation and inhibit apoptosis [17]. Via the non-cognate receptors CXCR2 and CXCR4, MIF modulates the migration of immune cells [20].

Since the discovery of the MIF receptor, CD74, several reports have described the observation that the deletion or neutralization of this transmembrane protein produces a similar phenotype as the immunoneutralization or genetic deletion of MIF, but the measured effect is about 2-fold more pronounced in receptor-deficient cells [20–23]. This observation led to the hypothesis that there might be a second ligand for the MIF receptor. Within the mammalian genome, there is only a single gene homologous with MIF, which encodes a protein called *D*-dopachrome tautomerase (D-DT). *D*-dopachrome tautomerase appears in the literature for the first time in 1993 as an enzyme detectable in the cytoplasm of human melanoma, human liver and rat organs that converts *D*-dopachrome to 5,6-dihydroxyindole [24]. In 1998, Sugimoto *et al.* reported the first structural study of D-DT, revealing a significant three-dimensional homology with MIF [25]. A few descriptive reports regarding D-DT were published during this period. In 2003, for example, it was shown that the enzymatic activity of D-DT is detectable in the epidermis of the skin and is increased after UV irradiation [26]. Other studies employing proteomics demonstrated that D-DT is elevated in the rat liver [27] and in the urine in a model of carbon tetrachloride-induced hepatic fibrosis [28], and that its expression increased after selenite induced apoptosis in HeLa cancer cells [29]. Only in the last four years studies have appeared that focused on the biological role of D-DT [30–32]. In this review, we summarize recent biological studies of D-DT and highlight the similarities and differences between the D-DT and MIF function.

Gene Structure

In the human genome, the *DDT* and *MIF* genes are located in close proximity (~80 kb apart) on chromosome 22. In both mouse and human genomes, the genes are clustered with two theta-class glutathione S-transferase genes, suggesting that an early duplication event led to the present overall gene structure. This hypothesis is further supported by the organization of the *DDT* and *MIF* genes. Both genes consist of three exons of almost identical size (*DDT*: exon 1: 108 bp, exon 2: 176 bp, exon 3: 70 bp vs. *MIF*: exon 1: 107 bp, exon 2: 172 bp,

exon 3: 66 bp), and only the non-coding introns have different lengths (*DDT*: intron 1: 363 bp, intron 2: 2144 bp vs. *MIF*: intron 1: 190 bp, intron 2: 96 bp). In addition, the promoter regions of both genes share sequences for the predicted binding of transcription factors such as SP-1 and CREB, and the mRNA of D-DT and MIF are almost 50% identical. Genetic analysis of the genes for D-DT and MIF in other species revealed comparable results. In mice, for example, the *Ddt* and *Mif* genes are located on chromosome 10, clustered with two theta-class glutathione S-transferases. The two genes also consist of three exons and the identity between the mRNA is ~40%.

MIF expression is not only regulated by transcription factors, but also by two distinct polymorphisms in its promoter region, a single nucleotide polymorphism at position -173 (guanine-to-cytosine), and a 5–8 CATT tetranucleotide repeat at position -794 [33]. Gene reporter assays [34] as well as human genetic studies [35–37] have shown a correlation between transcription rate and number of tetranucleotide repeats. Furthermore, clinical studies demonstrated an association between the functional polymorphism and the severity of different inflammatory diseases [14, 35–42]. To date, no polymorphic sites have been reported for the *DDT* gene.

Protein Structure

On the protein level, the amino acid sequence of D-DT and MIF shows 34% sequence identity in humans and 27% in mice. The investigation of the tertiary and quaternary structure of the two proteins by X-ray crystallography revealed a highly conserved structure, but also demonstrated distinct differences (Fig. 1) [25, 43, 44]. Both D-DT and MIF possess the characteristic N-terminal proline-1 (after cleavage of the initiating methionine) which is the basis of their enzymatic tautomerase activities. Although both family members tautomerize the model substrate *D*-dopachrome, their products are different; MIF catalyzes a pure tautomerization to generate 5,6-dihydroxyindole-2-carboxylic acid whereas D-DT catalysis results in an additional de-carboxylation to produce 5,6-dihydroxyindole. Furthermore, a comparison of the enzymatic activity of the two proteins showed that human MIF is about 10-times more active than the human D-DT protein. A possible explanation for this discrepancy may lie in the region surrounding the active pocket of the two proteins [25, 43, 44]. The MIF protein is positively charged both in the active site and the surrounding area, whereas the D-DT protein is positively charged in the active site pocket while the surrounding area is negatively charged [25]. Furthermore, in human MIF, five distinct amino acids are implicated in the binding and catalysis of its substrate (Pro-1, Lys-32, Ile-64, Tyr-95 and Asn-97) [45]. In comparison, human D-DT possesses only three of these five substrate-binding amino acids (Pro-1, Lys-32 and Ile-64), whereas Tyr-95 and Asn-97 are substituted by Leu-95 and Arg-97, respectively. The structural differences in the active site between MIF and D-DT may also influence biological activity, although this would most likely not occur by differences in enzymatic activity but because the active site region engages the MIF receptor. In the case of MIF, this distinction was demonstrated in a genetic *knock-in* mouse in which the endogenous gene for MIF was replaced by a catalytically inactive, mutant MIF (Pro1→Gly1). Cells expressing the tautomerase-null, P1G-MIF protein showed reduced proliferative capacity, and MIF^{P1G/P1G} mice showed a reduced development in benzo[*a*]pyrene-induced skin tumors. Furthermore, the tautomerase-null protein showed reduced binding affinity to the receptors CD74 and CXCR2, and an impaired ability to induce ERK1/2 MAP kinase activation [46]. MIF's catalytic activity thus is not essential for biologic function but the catalytic residue (Pro1) has a structural role in MIF binding to its receptor. Notably, the tautomerization of the physiologic isomer, *L*-dopachrome, mediates melanotic encapsulation, which is a primitive invertebrate defense pathway, leading to the suggestion that the non-physiologic catalytic activities of MIF or D-

DT with respect to *D*-dopachrome may reflect a vestigial property of these proteins originating from their ancestral position in invertebrate immunity.

All known mammalian MIF proteins have three conserved cysteines (Cys-56, Cys-59, Cys-80), but D-DT possesses only one (Cys-56). The CXXC motif (Cys-56 and Cys-59) in MIF has been implicated in its ability to regulate cellular redox homeostasis, inhibit apoptosis, mediate monocyte/macrophage activation and possibly modulate the binding of proteins [47]. Furthermore, D-DT also lacks the *pseudo-(E)LR* (Arg¹¹, Asp⁴⁴) motif that mediates MIF's binding with the non-canonical, chemokine receptor CXCR2 [19]. To date, the question of whether D-DT interacts with particular chemokine receptors has not been addressed.

D-DT conservation across species

The MIF protein is highly conserved across species. The protein is found not only in mammals, but also in fish, nematodes, and protozoa including *Leishmania* and *Plasmodium* (Fig. 2A) [48–52]. Notably, there are no MIF-like genes in *Drosophila* and yeast. The level of conservation ranges from 100% sequence identity between human and primate MIF down to ~20% sequence identity between human MIF and its orthologs in protozoa. D-DT shows a high level of conservation across species, albeit with a lower alignment score than MIF (alignment score: 7557 vs. 8587 for D-DT and MIF, respectively) (Fig. 2B). In mammals, the sequence identity in reference to human D-DT ranges from 100–70%. Interestingly, many nematodes and protozoa express two or more MIF-like proteins [48, 51, 53]. Vermiere *et al.* analyzed all known nematode MIF-like amino acid sequences and described the common occurrence of two structurally related proteins: MIF-type-1 and MIF-type-2 [54]. In light of recent information about the biological function of D-DT, these findings can be interpreted as the existence of the *MIF* and *DDT* genes.

Expression Pattern

MIF is constitutively expressed in organs such as lung, liver, heart, bowel, kidney, spleen, and skin [32, 55] as well as in tissues of the endocrine system [6, 56]. After stimulation, MIF is released from cells of the immune system including, but not limited to, T and B cells, macrophages, dendritic cells, eosinophils, and neutrophils. In addition, MIF is released constitutively, albeit at a much lower rate. In contrast to most other cytokines, MIF exists in cells in preformed pools, and no *de novo* synthesis is necessary prior to its release, which is effectuated via a non-conventional pathway excluding the endoplasmic reticulum and the Golgi [11]. Similarly, D-DT is ubiquitously expressed and possesses no N-terminal leader peptide. D-DT mRNA is detectable in all organs with the highest level measured in the liver [57]. Western blot analysis and immunohistochemistry confirmed these results, demonstrating expression of D-DT in all analyzed organs with the highest levels detected in liver and testis. Notably, the testis is the only organ among those studied with a significant difference in the expression level of D-DT and MIF [32]. Immunohistochemistry has demonstrated that D-DT, like MIF, is localized in the cytoplasm and the rapid release of these proteins from damaged or necrotic cells suggests that they may subserve functions of alarmins or damage-associated molecular patterns (*i.e.* DAMPs).

Function – Macrophages

In 1994, Calandra *et al.* reported that not only are activated T lymphocytes major producers of MIF, but that monocytes and macrophages, which were considered historically to be the main targets of MIF action, also produce MIF. They observed that MIF protein exists in preformed pools in resting cells, and that LPS or TNF α stimulation leads to its rapid release. Furthermore, MIF is essential for the production of pro-inflammatory mediators such as

TNF α , IL-1 β , PGE₂ and nitric oxide by macrophages [58]. The expression of MIF correlates with macrophage functions such as adherence and phagocytosis and it reduces the activation-induced apoptosis in macrophages and other cell types by inhibiting the p53 tumor suppressor [59]. An important signaling cascade activated by MIF in monocytes/macrophages is the MAP kinase pathway, ERK1/2. The transient (induction and decay within 90 mins) or sustained (>90 mins) activation of target cells can lead to a proliferative response and prostaglandin production [60, 61]. The ability of MIF to activate this signaling cascade is strictly dependent on the presence of the receptor complex CD74/CD44. Deletion of either the binding receptor CD74 or the signaling transducer CD44 prevents activation by MIF [16, 17]. Glucocorticoids are potent anti-inflammatory mediators with a strong immunosuppressive effect. MIF is induced by low levels of glucocorticoids *in vitro* and *in vivo* [62, 63]. While these effects initially appeared difficult to reconcile with the pro-inflammatory functions of MIF, MIF has the unique ability to counter-regulate the immunosuppressive actions of glucocorticoids on inflammatory cytokine secretion [63]. Lastly, as its name indicates, MIF inhibits the migration of macrophages. Although MIF first was associated with inhibition of the random migration of macrophages [5], it was later determined that MIF also inhibits directed migration of monocytes to chemokines such as monocyte chemoattractant protein 1 (MCP-1) [64].

Similar to MIF, D-DT exists in preformed pools in human and murine macrophages and LPS stimulation leads to its rapid release with peak levels measured after 16 hrs. MIF mRNA levels are only modestly upregulated after LPS stimulation, and no data exist regarding the effects on D-DT mRNA expression [11, 32]. LPS-stimulated macrophages appear to produce 20-fold more MIF than D-DT however. It further was shown by siRNA-mediated depletion that D-DT does not regulate MIF expression and vice versa. In macrophages, it also was demonstrated that D-DT recapitulates all important actions of MIF, *i.e.* stimulation of the ERK1/2 MAP kinase pathway, inhibition of macrophage migration, and counter-regulation of the immunosuppressive effects of glucocorticoids [32]. Like MIF, D-DT's ability to induce the ERK1/2 MAP kinase cascade also is strictly dependent on the presence of the receptor complex CD74/CD44. These results were supported by surface plasmon resonance measurements. D-DT binds the MIF receptor CD74 with high affinity (K_D of 5.42×10^{-9} M), albeit with a somewhat lower binding constant than MIF ($K_D = 1.54 \times 10^{-9}$ M) [16, 32]. Interestingly, D-DT has an 11-fold higher dissociation rate than MIF with respect to CD74 but it associates about 3-times faster with CD74 than MIF. These differences in binding kinetics may influence signal transduction and explain differences in the dose-response profiles between MIF and D-DT. Detailed analysis of D-DT function in macrophages demonstrated that D-DT action is significantly reduced at low concentrations when compared MIF, *i.e.* D-DT inhibition of macrophage migration or its ability to counter-regulate the immunosuppressive effects of glucocorticoids requires a significantly higher concentration than MIF.

Function – Cancer cells

Multiple studies investigating gene deletion, immuno-neutralization and small molecule antagonism of MIF in *in vivo* tumor models have found encouraging - albeit modest - reductions in tumor burden and disease survival (reviewed in [65, 66]). The two most prevalent causes of reduced tumor burden associated with genetic deletion or inhibition of MIF are: 1) reduced tumor-associated angiogenesis [67–70], and 2) increased p53-dependent apoptosis and cell cycle inhibition [71–73]. Recent studies investigating the biology of D-DT reveal that there is a significant degree of overlap between activities, expression patterns and phenotypic influences between these two proteins [30, 31] in malignant cells.

MIF and D-DT were recently found to additively induce CXCL8 and VEGF expression and secretion from lung adenocarcinoma cells [30]. Several prior studies have demonstrated an important contribution of MIF to CXCL8/VEGF expression and maintenance of angiogenic phenotypes in malignant cells and tissue [18, 67, 74–76]. The observed effect in lung carcinomas was the first demonstration of a functional overlap between MIF and its only known homolog, D-DT. Both MIF and D-DT were shown to be necessary for maximal c-jun-N-terminal kinase (JNK)-dependent AP-1 transactivation and subsequent CXCL8 and VEGF transcription in human lung adenocarcinoma cells. Perhaps more importantly, the cognate MIF receptor, CD74, was found to be necessary for CXCL8 expression and maximal JNK and c-jun phosphorylation induction by both family members. These findings are consistent with a study demonstrating that CD74 is necessary for MIF-dependent contributions to prostatic adenocarcinoma cell invasion, anchorage independence and tumor-associated neo-vascularization [22]. It is less clear how MIF and D-DT activate JNK through CD74 engagement. An earlier study revealed that MIF functionally regulates Rac1 effector binding by stabilizing cholesterol-enriched membrane microdomains [77]. Although JNK is a well known effector of Rac1, there is no evidence, as yet, that the defective JNK observed with loss of MIF is linked to Rac1. However, a recent study demonstrates that MIF-induced JNK activation requires the presence of the alternate MIF receptor, CXCR4, in conjunction with cell surface-associated CD74 [78]. Importantly, this study also revealed that JNK activation through the CXCR4/CD74 receptor complex requires both c-Src and phosphatidylinositol-3-kinase (PI 3-kinase) activities. Because PI 3-kinase is a well known activator of Rac1 [79, 80], it is not unreasonable to speculate that MIF – and likely D-DT – induce JNK activation in a PI 3-kinase → Rac1-dependent fashion. Although results indicate that AP-1 activity is important for MIF and D-DT contributions to CXCL8 and VEGF expression, it is possible that other signaling pathways may be involved. Of note, CD74 signaling induced by MIF ligation has recently been suggested to modulate CXCL8 expression in an NF- κ B-dependent manner [18].

Recent studies from the Mitchell laboratory indicate that D-DT is also induced by hypoxia and actively suppresses p53 stabilization and transcriptional activity in p53 wildtype tumor cell lines (unpublished observations – E. Brock, D. Xin and R.A. Mitchell) suggesting an additional compensatory role for D-DT in MIF-dependent tumor promotion. D-DT's ability to compensate for MIF – and *vice versa* – in p53 antagonism is consistent with a study demonstrating a functional requirement for MIF and D-DT in maintaining cyclooxygenase-2 (COX-2) expression in human colorectal adenocarcinoma cell lines [31]. Similar to the signaling pathway involved in MIF and D-DT-dependent VEGF and CXCL8 expression in NSCLC cell lines [30], JNK and subsequent c-jun phosphorylation were found to be necessary for D-DT-dependent COX-2 transcription. Interestingly, a functional role for β -catenin-dependent transcription also was identified to be necessary for maximal COX-2 expression in CRC cell lines indicating the convergence of two signaling pathways in MIF and D-DT-dependent COX-2 transcription – the JNK/c-jun pathway and the β -catenin/TCF pathway. Intriguingly, the mechanism governing D-DT-dependent β -catenin transcription was found to be through stabilization of β -catenin protein thus increasing cellular β -catenin levels and subsequent transcription [31].

Studies involving simultaneous gene-targeted deletions of MIF and D-DT will allow for greater clarity and understanding as to the extent of overlap and compensation between these two family members in malignant diseases. If extensive MIF vs. D-DT compensation is confirmed, it would follow that therapeutic targeting of MIF and D-DT simultaneously would have improved anti-cancer clinical potential than targeting either individually.

Disease and Therapy

MIF's role in endotoxemia and sepsis is well documented [6, 12, 81–86]. In the blood of mice with endotoxemia or bacterial peritonitis high levels of MIF were detected, peaking at 16–20 hrs after challenge. MIF is considered both an immune cytokine and an endocrine hormone, and the rise in its serum levels during endotoxic stress is concomitant with a dramatic fall in its pituitary content [12]. Systemic administration of recombinant MIF alone does not induce shock, but when co-administered with LPS significantly increases lethality. Correspondingly, *Mif*^{-/-} mice showed a significantly better survival rate than wildtype animals in models of endotoxemia and sepsis, and MIF-deficient animals had a reduced production of circulating pro-inflammatory effector cytokines such as TNF α , IL-1 β , and INF γ . In accordance, the administration of neutralizing anti-MIF antibody significantly improved the survival rate in models of endotoxemia and sepsis and was accompanied by reduced levels of pro-inflammatory cytokines in the serum. In models of peritonitis, neutralization of MIF reduced bacterial counts and improved survival. Notably, in mouse models of lethal infection, anti-MIF therapy is one of only three immunologic interventions where delayed administration remains efficacious after endotoxin administration or bacterial dissemination; the others being anti-HMGB1 and anti-TREM-1 [12, 87, 88]. Delayed intervention is highly desirable because it increases the potential clinical utility of MIF-directed therapies in settings where MIF overexpression may be deleterious.

The clinical potential for therapeutic inhibition of MIF in septic shock and sepsis must be reconsidered however in the light of human genetic data indicating that high expression MIF alleles both reduce susceptibility to and improve survival from community-acquired pneumonia, which is the most common etiology for lethal sepsis [40]. In the recently published GenIMS (Genetic and Inflammatory Markers of Sepsis) Study, which is the largest study in septic shock published to date, only polymorphisms at the *MIF* locus among the twelve candidate immune response genes that were fully analyzed were clinically significant with respect to influencing sepsis outcome. While the progression from sepsis to septic shock has long been considered to result from an exaggerated inflammatory response, it is clear from this study that at least in certain infections, interference with MIF action may not be desirable.

We recently performed the first measurements of D-DT in experimental endotoxemia and in human sepsis. LPS challenge of mice led to a significant increase of D-DT levels in the serum, peaking at 16 hrs, and the kinetics observed for the rise of D-DT in the serum were comparable to the kinetics measured for MIF [32]. Moreover, both proteins were detectable in the serum at similar concentrations. At baseline, measured levels were ~6 ng/ml for D-DT and ~2 ng/ml for MIF. At peak concentrations, the levels rose to ~30 ng/ml for D-DT and ~40 ng/ml for MIF. Circulating MIF and D-DT levels appear comparable under conditions of endotoxemia, which is noteworthy given that cultured macrophages produce 20-fold higher levels of MIF than D-DT. These data suggest that cells other than macrophages are an important source of the D-DT that is expressed *in vivo* during systemic inflammation.

As mentioned above, it was reported almost 20 years ago that immuno-neutralization of MIF protects mice from endotoxemia. In an analogous experiment, it was shown that the neutralization of D-DT with a specific polyclonal antibody also protected animals from lethal endotoxemia. The protection from LPS shock is accompanied by a reduced level of pro-inflammatory effector cytokines such as TNF α , IL-1 β , INF γ , and IL-12p70, but not of IL-6. Interestingly, the neutralization of MIF also decreases the concentration of TNF α , IL-1 β , INF γ and has no effect on IL-6 levels. Both the inhibition of D-DT or MIF, respectively, resulted in a 60% better survival rate compared to the control treated animals.

Better characterization of MIF versus D-DT dependent responses may be afforded by the study of *Ddt*^{-/-} and *Mif*^{-/-} *Ddt*^{-/-} mice, which are currently in development.

In an examination of septic patients, there was a statistically significant increase in circulating D-DT protein in patients with sepsis when compared to healthy controls (sepsis patients=55.5±61.3 ng/ml, control group= 5.9±3.9 ng/ml, P<0.0001) (Fig. 6A). MIF levels also were elevated (sepsis patients =111.0±69.0 ng/ml, control group=6.3±6.2 ng/ml, P<0.0001). Receiver operator characteristic (ROC) analysis revealed an area under the curve of 0.99 for MIF or D-DT, indicating that both proteins show excellent sensitivity and specificity for the diagnosis of sepsis. These measurements further revealed that serum levels of D-DT, like MIF [84, 89], correlate with disease severity as determined by APACHE II clinical severity scores.

In human tumors, patients with ovarian cancer show an overexpression of D-DT. Similarly, MIF levels are also elevated in the serum of patients with ovarian cancer. Of note, there is a significant correlation between D-DT and MIF concentrations in patients with ovarian cancer with a coefficient of determination over 0.9. In comparison, D-DT and MIF levels in the serum of healthy donors also demonstrate some correlation, but the coefficient of determination is only 0.3.

Anti-cytokine therapies, *i.e.* the targeting of cytokines with antibodies, small molecule inhibitors, or soluble receptors, are a notable success story for the treatment of inflammatory diseases. Prominent examples are drugs like Infliximab and Etanercept that target TNF α and have shown exceptional results in the treatment of rheumatoid arthritis or inflammatory bowel disease. MIF is a promising target, and MIF-based therapies in pre-clinical development include humanized monoclonal antibodies and small molecule inhibitors targeting the MIF/MIF-receptor interaction site [90, 91]. The success of these approaches in pre-clinical studies in the context of inflammatory disease is summarized in Table 1. Targeting of MIF improves disease development significantly; however, there are limitations to efficacy that may be obviated by simultaneously targeting D-DT. Moreover, there may be pathologic circumstances in which the preferential targeting of MIF or D-DT may be preferred.

There are several approaches to concurrently target D-DT and MIF. Two specific antibodies, one targeting MIF the other D-DT, may be combined. Another approach could be the design of a bispecific antibody that neutralizes both MIF and D-DT. Due to the structural similarity of the two proteins, it also may be feasible to generate a single cross-reactive antibody or a small molecule inhibitor targeting the receptor binding domain of both proteins. Alternatively, one might also use soluble CD74 to prevent the binding of D-DT and MIF to their receptor, and it is known that a soluble CD74 ectodomain is biologically neutralizing [16]. A humanized anti-MIF receptor (CD74) antibody also is presently in clinical evaluation for B cell malignancies [92], and it can be argued that anti-MIF receptor strategies would show greater efficacy than therapies targeting MIF or D-DT alone.

Conclusion

MIF's upstream regulatory role in the innate and adaptive immune response positions it as an important mediator in diseases such as rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, and different cancers. Numerous studies employing *Mif*^{-/-} animals, neutralizing MIF antibodies and small molecule inhibitors have demonstrated that targeting MIF holds much promise for therapy. Attention is now turning toward the MIF structural homolog D-DT, which has been understudied, if not overlooked, for almost two decades after its discovery. Studies in macrophages and cancer cells have demonstrated an

overlapping functional spectrum of action of the two proteins. Furthermore, *in vivo* studies showed that neutralization of D-DT via antibodies protects animals from lethal endotoxemia by reducing expression of downstream effector cytokines, as also seen for MIF. Finally, analysis of serum samples showed that circulating levels of D-DT are highly elevated in patients with sepsis and cancer, and they positively correlate with disease severity. Although to date, no studies addressed the question whether the neutralization of both MIF and D-DT *in vivo* will have an added benefit for the host, experiments employing siRNA-mediated knockdown of D-DT and MIF in cancer cells revealed an additive action of the two proteins. These data suggest that the combined therapeutic targeting of D-DT and MIF will have an added benefit for existing MIF-based treatments that are in advanced pre-clinical development.

Highlights

- D-dopachrome tautomerase (D-DT) is a MIF-like pro-inflammatory cytokine
- D-DT binds and signals via the MIF receptor CD74 and initiates similar signaling pathways
- D-DT is a highly sensitive biomarker for sepsis and ovarian cancer

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References

1. George M, Vaughan JH. In vitro cell migration as a model for delayed hypersensitivity. *Proc Soc Exp Biol Med*. 1962; 111:514–521. [PubMed: 13947220]
2. Rich AR, Lewis MR. The nature of allergy in tuberculosis as revealed by tissue culture studies. *Bull Johns Hopkins Hosp*. 1932; 50:115–131.
3. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol*. 2003; 3:791–800. [PubMed: 14502271]
4. Bloom BR, Bennett B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science*. 1966; 153:80–82. [PubMed: 5938421]
5. David JR. Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc Natl Acad Sci U S A*. 1966; 56:72–77. [PubMed: 5229858]
6. Bernhagen J, Calandra T, Mitchell RA, Martin SB, Tracey KJ, Voelter W, et al. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. *Nature*. 1993; 365:756–759. [PubMed: 8413654]
7. Calandra T, Bernhagen J, Mitchell RA, Bucala R. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J Exp Med*. 1994; 179:1895–1902. [PubMed: 8195715]
8. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, et al. MIF as a glucocorticoid-induced modulator of cytokine production. *Nature*. 1995; 377:68–71. [PubMed: 7659164]
9. Leng L, Wang W, Roger T, Merk M, Wuttke M, Calandra T, et al. Glucocorticoid-induced MIF expression by human CEM T cells. *Cytokine*. 2009; 48:177–185. [PubMed: 19646897]
10. Flaster H, Bernhagen J, Calandra T, Bucala R. The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. *Molecular endocrinology* (Baltimore Md. 2007; 21:1267–1280.

11. Merk M, Baugh J, Zierow S, Leng L, Pal U, Lee SJ, et al. The Golgi-associated protein p115 mediates the secretion of macrophage migration inhibitory factor. *J Immunol.* 2009; 182:6896–6906. [PubMed: 19454686]
12. Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hultner L, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. *Nat Med.* 2000; 6:164–170. [PubMed: 10655104]
13. Arjona A, Foellmer HG, Town T, Leng L, McDonald C, Wang T, et al. Abrogation of macrophage migration inhibitory factor decreases West Nile virus lethality by limiting viral neuroinvasion. *J Clin Invest.* 2007; 117:3059–3066. [PubMed: 17909632]
14. Mizue Y, Ghani S, Leng L, McDonald C, Kong P, Baugh J, et al. Role for macrophage migration inhibitory factor in asthma. *Proc Natl Acad Sci U S A.* 2005; 102:14410–14415. [PubMed: 16186482]
15. Santos LL, Dacumos A, Yamana J, Sharma L, Morand EF. Reduced arthritis in MIF deficient mice is associated with reduced T cell activation: down-regulation of ERK MAP kinase phosphorylation. *Clin Exp Immunol.* 2008; 152:372–380. [PubMed: 18341611]
16. Leng L, Metz CN, Fang Y, Xu J, Donnelly S, Baugh J, et al. MIF signal transduction initiated by binding to CD74. *J Exp Med.* 2003; 197:1467–1476. [PubMed: 12782713]
17. Shi X, Leng L, Wang T, Wang W, Du X, Li J, et al. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. *Immunity.* 2006; 25:595–606. [PubMed: 17045821]
18. Binsky I, Haran M, Starlets D, Gore Y, Lantner F, Harpaz N, et al. IL-8 secreted in a macrophage migration-inhibitory factor- and CD74-dependent manner regulates B cell chronic lymphocytic leukemia survival. *Proc Natl Acad Sci U S A.* 2007; 104:13408–13413. [PubMed: 17686984]
19. Weber C, Kraemer S, Drechsler M, Lue H, Koenen RR, Kapurniotu A, et al. Structural determinants of MIF functions in CXCR2-mediated inflammatory and atherogenic leukocyte recruitment. *Proc Natl Acad Sci U S A.* 2008; 105:16278–16283. [PubMed: 18852457]
20. Bernhagen J, Krohn R, Lue H, Gregory JL, Zerneck A, Koenen RR, et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat Med.* 2007; 13:587–596. [PubMed: 17435771]
21. Gore Y, Starlets D, Maharshak N, Becker-Herman S, Kaneyuki U, Leng L, et al. Macrophage migration inhibitory factor induces B cell survival by activation of a CD74-CD44 receptor complex. *J Biol Chem.* 2008; 283:2784–2792. [PubMed: 18056708]
22. Meyer-Siegler KL, Iczkowski KA, Leng L, Bucala R, Vera PL. Inhibition of macrophage migration inhibitory factor or its receptor (CD74) attenuates growth and invasion of DU-145 prostate cancer cells. *J Immunol.* 2006; 177:8730–8739. [PubMed: 17142775]
23. Heinrichs D, Knaul M, Offermanns C, Berres ML, Nellen A, Leng L, et al. Macrophage migration inhibitory factor (MIF) exerts antifibrotic effects in experimental liver fibrosis via CD74. *Proc Natl Acad Sci U S A.* 2011; 108:17444–17449. [PubMed: 21969590]
24. Odh G, Hindemith A, Rosengren AM, Rosengren E, Rorsman H. Isolation of a new tautomerase monitored by the conversion of D-dopachrome to 5,6-dihydroxyindole. *Biochem Biophys Res Commun.* 1993; 197:619–624. [PubMed: 8267597]
25. Sugimoto H, Taniguchi M, Nakagawa A, Tanaka I, Suzuki M, Nishihira J. Crystal structure of human D-dopachrome tautomerase, a homologue of macrophage migration inhibitory factor, at 1.54 Å resolution. *Biochemistry.* 1999; 38:3268–3279. [PubMed: 10079069]
26. Sonesson B, Rosengren E, Hansson AS, Hansson C. UVB-induced inflammation gives increased d-dopachrome tautomerase activity in blister fluid which correlates with macrophage migration inhibitory factor. *Exp Dermatol.* 2003; 12:278–282. [PubMed: 12823441]
27. Hiyoshi M, Konishi H, Uemura H, Matsuzaki H, Tsukamoto H, Sugimoto R, et al. D-Dopachrome tautomerase is a candidate for key proteins to protect the rat liver damaged by carbon tetrachloride. *Toxicology.* 2009; 255:6–14. [PubMed: 18930782]
28. Smyth R, Lane CS, Ashiq R, Turton JA, Clarke CJ, Dare TO, et al. Proteomic investigation of urinary markers of carbon-tetrachloride-induced hepatic fibrosis in the Hanover Wistar rat. *Cell Biol Toxicol.* 2009; 25:499–512. [PubMed: 18777138]

29. Fu L, Liu Q, Shen L, Wang Y. Proteomic study on sodium selenite-induced apoptosis of human cervical cancer HeLa cells. *J Trace Elem Med Biol.* 2011; 25:130–137. [PubMed: 21767938]
30. Coleman AM, Rendon BE, Zhao M, Qian MW, Bucala R, Xin D, et al. Cooperative regulation of non-small cell lung carcinoma angiogenic potential by macrophage migration inhibitory factor and its homolog, D-dopachrome tautomerase. *J Immunol.* 2008; 181:2330–2337. [PubMed: 18684922]
31. Xin D, Rendon BE, Zhao M, Winner M, McGhee Coleman A, Mitchell RA. The MIF homologue D-dopachrome tautomerase promotes COX-2 expression through beta-catenin-dependent and -independent mechanisms. *Mol Cancer Res.* 2010; 8:1601–1609. [PubMed: 21071513]
32. Merk M, Zierow S, Leng L, Das R, Du X, Schulte W, et al. The D-dopachrome tautomerase (DDT) gene product is a cytokine and functional homolog of macrophage migration inhibitory factor (MIF). *Proc Natl Acad Sci U S A.* 2011; 108:E577–E585. [PubMed: 21817065]
33. Bucala R. MIF and the Genetic Basis of Macrophage Responsiveness. *Current Immunol Revs.* 2006; 2:217–223.
34. Baugh JA, Chitnis S, Donnelly SC, Monteiro J, Lin X, Plant BJ, et al. A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. *Genes Immun.* 2002; 3:170–176. [PubMed: 12070782]
35. Radstake TR, Sweep FC, Welsing P, Franke B, Vermeulen SH, Geurts-Moespot A, et al. Correlation of rheumatoid arthritis severity with the genetic functional variants and circulating levels of macrophage migration inhibitory factor. *Arthritis Rheum.* 2005; 52:3020–3029. [PubMed: 16200611]
36. Sreih A, Ezzeddine R, Leng L, LaChance A, Yu G, Mizue Y, et al. Dual effect of the macrophage migration inhibitory factor gene on the development and severity of human systemic lupus erythematosus. *Arthritis Rheum.* 2011; 63:3942–3951. [PubMed: 22127710]
37. Renner P, Roger T, Bochud PY, Sprong T, Sweep FC, Bochud M, et al. A functional microsatellite of the macrophage migration inhibitory factor gene associated with meningococcal disease. *Faseb J.* 2011
38. Meyer-Siegler KL, Vera PL, Iczkowski KA, Bifulco C, Lee A, Gregersen PK, et al. Macrophage migration inhibitory factor (MIF) gene polymorphisms are associated with increased prostate cancer incidence. *Genes Immun.* 2007; 8:646–652. [PubMed: 17728788]
39. Wu SP, Leng L, Feng Z, Liu N, Zhao H, McDonald C, et al. Macrophage migration inhibitory factor promoter polymorphisms and the clinical expression of scleroderma. *Arthritis Rheum.* 2006; 54:3661–3669. [PubMed: 17075815]
40. Yende S, Angus DC, Kong L, Kellum JA, Weissfeld L, Ferrell R, et al. The influence of macrophage migration inhibitory factor gene polymorphisms on outcome from community-acquired pneumonia. *Faseb J.* 2009; 23:2403–2411. [PubMed: 19346297]
41. Doernberg S, Schaaf B, Dalhoff K, Leng L, Beitin A, Quagliarello V, et al. Association of macrophage migration inhibitory factor (MIF) polymorphisms with risk of meningitis from *Streptococcus pneumoniae*. *Cytokine.* 2011; 53:292–294. [PubMed: 21208809]
42. Grigorenko EL, Han SS, Yrigollen CM, Leng L, Mizue Y, Anderson GM, et al. Macrophage migration inhibitory factor and autism spectrum disorders. *Pediatrics.* 2008; 122:e438–e445. [PubMed: 18676531]
43. Sun HW, Bernhagen J, Bucala R, Lolis E. Crystal structure at 2.6-Å resolution of human macrophage migration inhibitory factor. *Proc Natl Acad Sci U S A.* 1996; 93:5191–5196. [PubMed: 8643551]
44. Suzuki M, Sugimoto H, Nakagawa A, Tanaka I, Nishihira J, Sakai M. Crystal structure of the macrophage migration inhibitory factor from rat liver. *Nat Struct Biol.* 1996; 3:259–266. [PubMed: 8605628]
45. Lubetsky JB, Dios A, Han J, Aljabari B, Ruzsicska B, Mitchell R, et al. The tautomerase active site of macrophage migration inhibitory factor is a potential target for discovery of novel anti-inflammatory agents. *J Biol Chem.* 2002; 277:24976–24982. [PubMed: 11997397]
46. Fingerle-Rowson G, Kaleswarapu DR, Schlender C, Kabgani N, Brocks T, Reinart N, et al. A tautomerase-null macrophage migration-inhibitory factor (MIF) gene knock-in mouse model reveals that protein interactions and not enzymatic activity mediate MIF-dependent growth regulation. *Mol Cell Biol.* 2009; 29:1922–1932. [PubMed: 19188446]

47. Thiele M, Bernhagen J. Link between macrophage migration inhibitory factor and cellular redox regulation. *Antioxid Redox Signal*. 2005; 7:1234–1248. [PubMed: 16115028]
48. Kamir D, Zierow S, Leng L, Cho Y, Diaz Y, Griffith J, et al. A leishmania ortholog of macrophage migration inhibitory factor modulates host macrophage responses. *J Immunol*. 2008; 180:8250–8261. [PubMed: 18523291]
49. Sato A, Uinuk-ool TS, Kuroda N, Mayer WE, Takezaki N, Dongak R, et al. Macrophage migration inhibitory factor (MIF) of jawed and jawless fishes: implications for its evolutionary origin. *Dev Comp Immunol*. 2003; 27:401–412. [PubMed: 12631522]
50. Cho Y, Jones BF, Vermeire JJ, Leng L, DiFedele L, Harrison LM, et al. Structural and functional characterization of a secreted hookworm Macrophage Migration Inhibitory Factor (MIF) that interacts with the human MIF receptor CD74. *J Biol Chem*. 2007; 282:23447–23456. [PubMed: 17567581]
51. Falcone FH, Loke P, Zang X, MacDonald AS, Maizels RM, Allen JE. A *Brugia malayi* homolog of macrophage migration inhibitory factor reveals an important link between macrophages and eosinophil recruitment during nematode infection. *J Immunol*. 2001; 167:5348–5354. [PubMed: 11673551]
52. Augustijn KD, Kleemann R, Thompson J, Kooistra T, Crawford CE, Reece SE, et al. Functional characterization of the *Plasmodium falciparum* and *P. berghei* homologues of macrophage migration inhibitory factor. *Infect Immun*. 2007; 75:1116–1128. [PubMed: 17158894]
53. Marson AL, Tarr DE, Scott AL. Macrophage migration inhibitory factor (mif) transcription is significantly elevated in *Caenorhabditis elegans* dauer larvae. *Gene*. 2001; 278:53–62. [PubMed: 11707322]
54. Vermeire JJ, Cho Y, Lolis E, Bucala R, Cappello M. Orthologs of macrophage migration inhibitory factor from parasitic nematodes. *Trends in parasitology*. 2008; 24:355–363. [PubMed: 18603473]
55. Bacher M, Meinhardt A, Lan HY, Mu W, Metz CN, Chesney JA, et al. Migration inhibitory factor expression in experimentally induced endotoxemia. *Am J Pathol*. 1997; 150:235–246. [PubMed: 9006339]
56. Fingerle-Rowson G, Koch P, Bikoff R, Lin X, Metz CN, Dhabhar FS, et al. Regulation of macrophage migration inhibitory factor expression by glucocorticoids in vivo. *Am J Pathol*. 2003; 162:47–56. [PubMed: 12507889]
57. Esumi N, Budarf M, Ciccarelli L, Sellinger B, Kozak CA, Wistow G. Conserved gene structure and genomic linkage for D-dopachrome tautomerase (DDT) and MIF. *Mamm Genome*. 1998; 9:753–757. [PubMed: 9716662]
58. Mitchell RA, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. *Proc Natl Acad Sci U S A*. 2002; 99:345–350. [PubMed: 11756671]
59. Hudson JD, Shoaibi MA, Maestro R, Carnero A, Hannon GJ, Beach DH. A proinflammatory cytokine inhibits p53 tumor suppressor activity. *J Exp Med*. 1999; 190:1375–1382. [PubMed: 10562313]
60. Mitchell RA, Metz CN, Peng T, Bucala R. Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF). Regulatory role in cell proliferation and glucocorticoid action. *J Biol Chem*. 1999; 274:18100–18106. [PubMed: 10364264]
61. Lue H, Kapurniotu A, Fingerle-Rowson G, Roger T, Leng L, Thiele M, et al. Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. *Cell Signal*. 2006; 18:688–703. [PubMed: 16122907]
62. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, et al. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc Natl Acad Sci U S A*. 1996; 93:7849–7854. [PubMed: 8755565]

63. Calandra T, Bucala R. Macrophage migration inhibitory factor: a counter-regulator of glucocorticoid action and critical mediator of septic shock. *J Inflamm.* 1995; 47:39–51. [PubMed: 8913928]
64. Hermanowski-Vosatka A, Mundt SS, Ayala JM, Goyal S, Hanlon WA, Czerwinski RM, et al. Enzymatically inactive macrophage migration inhibitory factor inhibits monocyte chemotaxis and random migration. *Biochemistry.* 1999; 38:12841–12849. [PubMed: 10504254]
65. Bifulco C, McDaniel K, Leng L, Bucala R. Tumor growth-promoting properties of macrophage migration inhibitory factor. *Current pharmaceutical design.* 2008; 14:3790–3801. [PubMed: 19128232]
66. Rendon BE, Willer SS, Zundel W, Mitchell RA. Mechanisms of macrophage migration inhibitory factor (MIF)-dependent tumor microenvironmental adaptation. *Exp Mol Pathol.* 2009; 86:180–185. [PubMed: 19186177]
67. Xu X, Wang B, Ye C, Yao C, Lin Y, Huang X, et al. Overexpression of macrophage migration inhibitory factor induces angiogenesis in human breast cancer. *Cancer Lett.* 2008; 261:147–157. [PubMed: 18171602]
68. Hagemann T, Robinson SC, Thompson RG, Charles K, Kulbe H, Balkwill FR. Ovarian cancer cell-derived migration inhibitory factor enhances tumor growth, progression, and angiogenesis. *Mol Cancer Ther.* 2007; 6:1993–2002. [PubMed: 17620429]
69. Wilson JM, Coletta PL, Cuthbert RJ, Scott N, MacLennan K, Hawcroft G, et al. Macrophage migration inhibitory factor promotes intestinal tumorigenesis. *Gastroenterology.* 2005; 129:1485–1503. [PubMed: 16285950]
70. Hira E, Ono T, Dhar DK, El-Assal ON, Hishikawa Y, Yamanoi A, et al. Overexpression of macrophage migration inhibitory factor induces angiogenesis and deteriorates prognosis after radical resection for hepatocellular carcinoma. *Cancer.* 2005; 103:588–598. [PubMed: 15612021]
71. Fingerle-Rowson G, Petrenko O, Metz CN, Forsthuber TG, Mitchell R, Huss R, et al. The p53-dependent effects of macrophage migration inhibitory factor revealed by gene targeting. *Proc Natl Acad Sci U S A.* 2003; 100:9354–9359. [PubMed: 12878730]
72. Welford SM, Bedogni B, Gradin K, Poellinger L, Broome Powell M, Giaccia AJ. HIF1alpha delays premature senescence through the activation of MIF. *Genes Dev.* 2006; 20:3366–3371. [PubMed: 17142669]
73. Martin J, Duncan FJ, Keiser T, Shin S, Kusewitt DF, Oberyzy T, et al. Macrophage migration inhibitory factor (MIF) plays a critical role in pathogenesis of ultraviolet-B (UVB) - induced nonmelanoma skin cancer (NMSC). *Faseb J.* 2009; 23:720–730. [PubMed: 18952710]
74. Ren Y, Law S, Huang X, Lee PY, Bacher M, Srivastava G, et al. Macrophage migration inhibitory factor stimulates angiogenic factor expression and correlates with differentiation and lymph node status in patients with esophageal squamous cell carcinoma. *Ann Surg.* 2005; 242:55–63. [PubMed: 15973102]
75. Ren Y, Chan HM, Li Z, Lin C, Nicholls J, Chen CF, et al. Upregulation of macrophage migration inhibitory factor contributes to induced N-Myc expression by the activation of ERK signaling pathway and increased expression of interleukin-8 and VEGF in neuroblastoma. *Oncogene.* 2004; 23:4146–4154. [PubMed: 15064733]
76. White ES, Flaherty KR, Carskadon S, Brant A, Iannettoni MD, Yee J, et al. Macrophage migration inhibitory factor and CXC chemokine expression in non-small cell lung cancer: role in angiogenesis and prognosis. *Clin Cancer Res.* 2003; 9:853–860. [PubMed: 12576459]
77. Rendon BE, Roger T, Teneng I, Zhao M, Al-Abed Y, Calandra T, et al. Regulation of human lung adenocarcinoma cell migration and invasion by macrophage migration inhibitory factor. *J Biol Chem.* 2007; 282:29910–29918. [PubMed: 17709373]
78. Lue H, Dewor M, Leng L, Bucala R, Bernhagen J. Activation of the JNK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on CXCR4 and CD74. *Cell Signal.* 2011; 23:135–144. [PubMed: 20807568]
79. Hawkins PT, Eguinoa A, Qiu RG, Stokoe D, Cooke FT, Walters R, et al. PDGF stimulates an increase in GTP-Rac via activation of phosphoinositide 3-kinase. *Curr Biol.* 1995; 5:393–403. [PubMed: 7627555]

80. Clark EA, King WG, Brugge JS, Symons M, Hynes RO. Integrin-mediated signals regulated by members of the rho family of GTPases. *J Cell Biol.* 1998; 142:573–586. [PubMed: 9679153]
81. Tohyama S, Onodera S, Tohyama H, Yasuda K, Nishihira J, Mizue Y, et al. A novel DNA vaccine-targeting macrophage migration inhibitory factor improves the survival of mice with sepsis. *Gene Ther.* 2008; 15:1513–1522. [PubMed: 18650852]
82. Chuang CC, Wang ST, Chen WC, Chen CC, Hor LI, Chuang AY. Increases in serum macrophage migration inhibitory factor in patients with severe sepsis predict early mortality. *Shock.* 2007; 27:503–506. [PubMed: 17438455]
83. Sprong T, Pickkers P, Geurts-Moespot A, van der Ven-Jongekrijg J, Neeleman C, Knaup M, et al. Macrophage migration inhibitory factor (MIF) in meningococcal septic shock and experimental human endotoxemia. *Shock.* 2007; 27:482–487. [PubMed: 17438452]
84. Bozza FA, Gomes RN, Japiassu AM, Soares M, Castro-Faria-Neto HC, Bozza PT, et al. Macrophage migration inhibitory factor levels correlate with fatal outcome in sepsis. *Shock.* 2004; 22:309–313. [PubMed: 15377884]
85. Bozza M, Satoskar AR, Lin G, Lu B, Humbles AA, Gerard C, et al. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. *J Exp Med.* 1999; 189:341–346. [PubMed: 9892616]
86. Al-Abed Y, Dabideen D, Aljabari B, Valster A, Messmer D, Ochani M, et al. ISO-1 binding to the tautomerase active site of MIF inhibits its pro-inflammatory activity and increases survival in severe sepsis. *J Biol Chem.* 2005; 280:36541–36544. [PubMed: 16115897]
87. Bouchon A, Facchetti F, Weigand MA, Colonna M. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. *Nature.* 2001; 410:1103–7. [PubMed: 11323674]
88. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science.* 1999; 285:248–251. [PubMed: 10398600]
89. Emonts M, Sweep FC, Grebenchtchikov N, Geurts-Moespot A, Knaup M, Chanson AL, et al. Association between high levels of blood macrophage migration inhibitory factor, inappropriate adrenal response, and early death in patients with severe sepsis. *Clin Infect Dis.* 2007; 44:1321–1328. [PubMed: 17443469]
90. Kerschbaumer RJ, Rieger M, Volkel D, Le Roy D, Roger T, Garbaraviciene J, et al. Neutralization of macrophage migration inhibitory factor (MIF) by fully human antibodies correlates with their specificity for the beta-sheet structure of MIF. *J Biol Chem.* 2012
91. Leng L, Chen L, Fan J, Greven D, Arjona A, Du X, et al. A small-molecule macrophage migration inhibitory factor antagonist protects against glomerulonephritis in lupus-prone NZB/NZW F1 and MRL/lpr mice. *J Immunol.* 2011; 186:527–538. [PubMed: 21106847]
92. Berkova Z, Tao RH, Samaniego F. Milatuzumab - a promising new immunotherapeutic agent. *Expert Opin Investig Drugs.* 2010; 19:141–149.
93. Chen PF, Luo YL, Wang W, Wang JX, Lai WY, Hu SM, et al. ISO-1, a macrophage migration inhibitory factor antagonist, inhibits airway remodeling in a murine model of chronic asthma. *Mol Med.* 2010; 16:400–408. [PubMed: 20485865]
94. Amano T, Nishihira J, Miki I. Blockade of macrophage migration inhibitory factor (MIF) prevents the antigen-induced response in a murine model of allergic airway inflammation. *Inflamm Res.* 2007; 56:24–31. [PubMed: 17334667]
95. Kobayashi M, Nasuhara Y, Kamachi A, Tanino Y, Betsuyaku T, Yamaguchi E, et al. Role of macrophage migration inhibitory factor in ovalbumin-induced airway inflammation in rats. *Eur Respir J.* 2006; 27:726–734. [PubMed: 16455830]
96. Yamaguchi E, Nishihira J, Shimizu T, Takahashi T, Kitashiro N, Hizawa N, et al. Macrophage migration inhibitory factor (MIF) in bronchial asthma. *Clin Exp Allergy.* 2000; 30:1244–1249. [PubMed: 10971470]
97. Burger-Kentischer A, Gobel H, Kleemann R, Zernecke A, Bucala R, Leng L, et al. Reduction of the aortic inflammatory response in spontaneous atherosclerosis by blockade of macrophage migration inhibitory factor (MIF). *Atherosclerosis.* 2006; 184:28–38. [PubMed: 15921687]
98. Chen Z, Sakuma M, Zago AC, Zhang X, Shi C, Leng L, et al. Evidence for a role of macrophage migration inhibitory factor in vascular disease. *Arterioscler Thromb Vasc Biol.* 2004; 24:709–714. [PubMed: 14751814]

99. Pan JH, Sukhova GK, Yang JT, Wang B, Xie T, Fu H, et al. Macrophage migration inhibitory factor deficiency impairs atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation*. 2004; 109:3149–3153. [PubMed: 15197138]
100. Burger-Kentischer A, Goebel H, Seiler R, Fraedrich G, Schaefer HE, Dimmeler S, et al. Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. *Circulation*. 2002; 105:1561–1566. [PubMed: 11927523]
101. He XX, Chen K, Yang J, Li XY, Gan HY, Liu CY, et al. Macrophage migration inhibitory factor promotes colorectal cancer. *Mol Med*. 2009; 15:1–10. [PubMed: 19009023]
102. Lee H, Rhee H, Kang HJ, Kim HS, Min BS, Kim NK, et al. Macrophage migration inhibitory factor may be used as an early diagnostic marker in colorectal carcinomas. *Am J Clin Pathol*. 2008; 129:772–779. [PubMed: 18426738]
103. Hoi AY, Hickey MJ, Hall P, Yamana J, O'Sullivan KM, Santos LL, et al. Macrophage migration inhibitory factor deficiency attenuates macrophage recruitment, glomerulonephritis, and lethality in MRL/lpr mice. *J Immunol*. 2006; 177:5687–5696. [PubMed: 17015758]
104. Foote A, Briganti EM, Kipen Y, Santos L, Leech M, Morand EF. Macrophage migration inhibitory factor in systemic lupus erythematosus. *J Rheumatol*. 2004; 31:268–273. [PubMed: 14760795]
105. Nicoletti F, Creange A, Orlikowski D, Bolgert F, Mangano K, Metz C, et al. Macrophage migration inhibitory factor (MIF) seems crucially involved in Guillain-Barre syndrome and experimental allergic neuritis. *J Neuroimmunol*. 2005; 168:168–174. [PubMed: 16171874]
106. Dagia NM, Kamath DV, Bhatt P, Gupte RD, Dadarkar SS, Fonseca L, et al. A fluorinated analog of ISO-1 blocks the recognition and biological function of MIF and is orally efficacious in a murine model of colitis. *Eur J Pharmacol*. 2009; 607:201–212. [PubMed: 19239912]
107. de Jong YP, Abadia-Molina AC, Satoskar AR, Clarke K, Rietdijk ST, Faubion WA, et al. Development of chronic colitis is dependent on the cytokine MIF. *Nature immunology*. 2001; 2:1061–1066. [PubMed: 11668338]
108. Ohkawara T, Koyama Y, Onodera S, Takeda H, Kato M, Asaka M, et al. DNA vaccination targeting macrophage migration inhibitory factor prevents murine experimental colitis. *Clin Exp Immunol*. 2011; 163:113–122. [PubMed: 21062270]
109. Ohkawara T, Nishihira J, Takeda H, Hige S, Kato M, Sugiyama T, et al. Amelioration of dextran sulfate sodium-induced colitis by anti-macrophage migration inhibitory factor antibody in mice. *Gastroenterology*. 2002; 123:256–270. [PubMed: 12105854]
110. Oliver J, Marquez A, Gomez-Garcia M, Martinez A, Mendoza JL, Vilchez JR, et al. Association of the macrophage migration inhibitory factor gene polymorphisms with inflammatory bowel disease. *Gut*. 2007; 56:150–151. [PubMed: 17172590]
111. Kithcart AP, Cox GM, Sielecki T, Short A, Pruitt J, Papenfuss T, et al. A small-molecule inhibitor of macrophage migration inhibitory factor for the treatment of inflammatory disease. *Faseb J*. 2010; 24:4459–4466. [PubMed: 20624927]
112. Denkinger CM, Denkinger M, Kort JJ, Metz C, Forsthuber TG. In vivo blockade of macrophage migration inhibitory factor ameliorates acute experimental autoimmune encephalomyelitis by impairing the homing of encephalitogenic T cells to the central nervous system. *J Immunol*. 2003; 170:1274–1282. [PubMed: 12538686]
113. Akcali A, Pehlivan S, Pehlivan M, Sever T, Neyal M. Association of macrophage migration inhibitory factor gene promoter polymorphisms with multiple sclerosis in Turkish patients. *J Int Med Res*. 2010; 38:69–77. [PubMed: 20233515]
114. Niino M, Ogata A, Kikuchi S, Tashiro K, Nishihira J. Macrophage migration inhibitory factor in the cerebrospinal fluid of patients with conventional and optic-spinal forms of multiple sclerosis and neuro-Behcet's disease. *J Neurol Sci*. 2000; 179:127–131. [PubMed: 11054496]
115. Mikulowska A, Metz CN, Bucala R, Holmdahl R. Macrophage migration inhibitory factor is involved in the pathogenesis of collagen type II-induced arthritis in mice. *J Immunol*. 1997; 158:5514–5517. [PubMed: 9164975]
116. Leech M, Metz C, Santos L, Peng T, Holdsworth SR, Bucala R, et al. Involvement of macrophage migration inhibitory factor in the evolution of rat adjuvant arthritis. *Arthritis Rheum*. 1998; 41:910–917. [PubMed: 9588744]

117. Lehmann LE, Book M, Hartmann W, Weber SU, Schewe JC, Klaschik S, et al. A MIF haplotype is associated with the outcome of patients with severe sepsis: a case control study. *J Transl Med.* 2009; 7:100. [PubMed: 19941661]

Fig. 1. X-Ray Structure of Human D-DT and MIF

A) Three dimensional structure of D-DT. *Left panel:* Human D-DT monomer. *Right:* Human D-DT trimer. Structures are drawn from PDB entry 1DPT using PyMOL. **B)** Three dimensional structure of MIF. *Left panel:* Human MIF monomer. *Right:* Human MIF trimer. Structures are drawn from PDB entry 1MIF using PyMOL.

Fig. 2. Sequence alignment of selected D-DT or MIF proteins

A) Sequence alignment of selected D-DT proteins. The accession numbers are: *H. sapiens* CAG30317.1, *M. mulatta* XP_001087658.1, *B. taurus* NP_001092620.1, *M. musculus* NP_034157.1, *G. gallus* NP_001025838.1, *D. rerio* NP_001002147.1 **B)** Sequence alignment of selected MIF proteins. The accession numbers are: *H. sapiens* CAG30406.1, *M. mulatta* AAT74528.2, *B. taurus* DAA20377.1, *M. musculus* NP_034928.1, *G. gallus* AAA48939.1, *D. rerio* NP_001036786.1.

Table 1

Function of MIF in Different Disease Models

Disease	Experimental Model	Treatment/ Study with	Overexpression/ Polymorphism	Ref.
Asthma	Airway inflammation via OVA sensitization	Anti-MIF antibody ISO-1 <i>Mif</i> ^{-/-} mice	+/+	[14, 93–96]
Atherosclerosis	ApoE ^{-/-} mice	Anti-MIF antibody --- <i>Mif</i> ^{-/-} mice	+/-	[97–100]
Colorectal Cancer	Subcutaneous tumor model	Anti-MIF antibody ISO-1 <i>Mif</i> ^{-/-} mice	+/-	[69, 101, 102]
Glomerulonephritis/SLE	NZB/NZW F1 and MRL/lpr mouse strains	Anti-MIF antibody ISO-1 <i>Mif</i> ^{-/-} mice	+/+	[36, 91, 103, 104]
Guillain-Barre syndrome	Experimental allergic neuritis	Anti-MIF antibody ISO-1 ---	-/-	[105]
Inflammatory Bowel Disease	Dextran-sulfate sodium-induced colitis TNBS model CD45RB ^{hi} transfer model	Anti-MIF antibody ISO-F <i>Mif</i> ^{-/-} mice	+/+	[106–110]
Multiple Sclerosis	Experimental autoimmune encephalomyelitis	Anti-MIF antibody CPSI-1306/CPSI-2705 <i>Mif</i> ^{-/-} mice	+/+	[111–114]
Rheumatoid Arthritis	Collagen induced arthritis Adjuvant-induced arthritis Antigen-induced arthritis	Anti-MIF antibody --- <i>Mif</i> ^{-/-} mice	+/+	[15, 34, 35, 115, 116]
Sepsis/Endotoxemia	LPS Gram-positive bacteria Gram-negative bacteria	Anti-MIF antibody ISO-1 <i>Mif</i> ^{-/-} mice	+/+	[12, 85, 86, 117]

Treatment/study with anti-MIF antibody, small molecule MIF antagonist, or *Mif*^{-/-} mice showed significant alleviation of disease. Overexpression indicates an increased MIF concentration in serum/analyzed tissue, and polymorphism indicates significant association between a functional *MIF* promoter polymorphism (*rs5844572*) and disease.