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The non-mammalian MIF superfamily

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Abstract

Macrophage migration inhibitory factor (MIF) was first described as a cytokine 50 years ago, and emerged in mammals as a pleiotropic protein with pro-inflammatory, chemotactic, and growth-promoting activities. In addition, MIF has gained substantial attention as a pivotal upstream mediator of innate and adaptive immune responses and with pathologic roles in several diseases. Of less importance in mammals is an intrinsic but non-physiologic enzymatic activity that points to MIF's evolution from an ancient defense molecule. Therefore, it is not surprising that *mif*-like genes also have been found across a range of different organisms including bacteria, plants, protozoa, helminths, molluscs, arthropods, fish, amphibians and birds. While Genebank analysis identifying *mif*-like genes across species is extensive, contained herein is an overview of the non-mammalian MIF-like proteins that have been most well studied experimentally. For many of these organisms, MIF contributes to an innate defense system or plays a role in development. For parasitic organisms however, MIF appears to function as a virulence factor aiding in the establishment or persistence of infection by modulating the host immune response. Consequently, a combined targeting of both parasitic and host MIF could lead to more effective treatment strategies for parasitic diseases of socioeconomic importance.

Keywords

Macrophage migration inhibitory factor (MIF); Homology; Immunity; Parasitology

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Conflict of interest

The authors declare that there are no conflicts of interest.

1. Introduction: mammalian MIF

Macrophage migration inhibitory factor (MIF) has proven to be an intriguing molecule of study for many scientists. Originally described as a cytokine over 50 years ago, MIF has been found in mammals to be a pleiotropic cytokine/chemokine with unique characteristics that have led to it being coined the “most interesting factor” (Bucala, 2000).

Immunologically, MIF has gained substantial attention as a pivotal upstream mediator of innate and adaptive immune responses (Flaster et al., 2007) and has been implicated in many infectious, inflammatory, and immune diseases including septic shock, colitis, malaria, rheumatoid arthritis, atherosclerosis, and tumorigenesis (Bucala and Donnelly, 2007; Bernhagen et al., 1993; Mikulowska et al., 1997; Bozza et al., 2012). Being present within the cytosol of most cells as preformed protein, MIF mediates several of its effects via an autocrine/paracrine signaling pathway leading to (i) the activation of ERK1/ERK2 MAP kinases, the triggering of downstream pro-inflammatory gene expression (e.g. TNF- α , IL-1 β , IL-6, IL-8 and IL-12) and production of matrix metalloproteases, cyclooxygenase 2 and prostaglandin E₂, (ii) up-regulation of TLR4 expression, (iii) suppression of p53 activity, (iv) counter-regulation of the anti-inflammatory and immunosuppressive effects of glucocorticoids, and (v) regulation of cell cycling (Bozza et al., 2012; Calandra and Roger, 2003; Lue et al., 2002; Leng and Bucala, 2006). In addition, MIF triggers calcium influx and integrin activation, and modulates lymphocyte/myeloid cell activation and trafficking as reviewed by Bernhagen et al. (Bernhagen et al., 2007). Secreted/released MIF can exert its functions via four cell surface receptor proteins. On one hand, MIF signals through CD74, which is a type II receptor protein whose intracellular form (i.e. the invariant chain, li) functions in the transport of class II proteins from the endoplasmic reticulum to the Golgi and a surface form (~2–5% of CD74) that functions independently of class II to bind extracellular MIF with nM affinity for internalization. On the other hand, MIF is also a non-cognate ligand for the CXC chemokine receptors CXCR2, CXCR4, and CXCR7 (Bernhagen et al., 2007; Leng et al., 2003; Alampour-Rajabi et al., 2015; Schröder, 2016), mediating interactions that may be facilitated by CD74.

In addition to its receptor-mediated signalling activities, i.e. inhibiting the random migration of cells and promoting downstream cytokine production, MIF harbors two evolutionally conserved catalytic activities that provide it with additional functional complexity. Mammalian MIF can be demonstrated to exhibit a thiol-protein oxidoreductase activity by virtue of a thioredoxin-like CXXC motif (Kleemann et al., 1998) and a keto-enol tautomerase activity catalyzed by an N-terminal proline that can tautomerize model substrates such as D-dopachrome, hydroxyphenylpyruvate or phenylpyruvate (Calandra and Roger, 2003; Rosengren et al., 1996, 1997). It is unclear however whether these MIF enzymatic activities have true functional relevance in mammals, but with respect to keto-enol tautomerization the N-terminal proline is strictly conserved among all known MIF proteins. The enzymatic tautomerization of the physiologic substrate L-dopachrome mediates the primitive invertebrate defense pathway known as melanotic encapsulation, however, MIF is only active against the non-physiologic stereoisomer D-dopachrome. A genetically-engineered knock-in mouse in which endogenous MIF was replaced by a catalytically-inactive MIF^{P1G} demonstrated a phenotype intermediate between that of wild type and *mif*

gene deficient mice. Given that MIF^{P1G} binds to CD74 with lower affinity than wild type MIF, these observations are consistent with the interpretation that the MIF tautomerase activity is dispensable for biologic function but that structural features imparted by Pro1 are essential for receptor binding and activation (Fingerle-Rowson et al., 2009).

Biologically active MIF exists as a homo-trimer with dimensions of 35 Å × 50 Å × 50 Å, forming an αβ structure with α-helices surrounding β-sheets that completely wrap around to form a barrel with open ends forming a solvent channel, whereby each monomer consists of a βαββαβ motif (Sun et al., 1996). This protein fold defines the MIF structural superfamily. The tautomerase active site within the MIF protein is situated at the interface between pairs of subunits (lined by amino acid residues 1, 33–34, and 64–66) and the overall substrate binding site is highly conserved among MIF homologues. In contrast, the residues necessary for the protein-thiol oxidoreductase activity, which is associated with a CXXC motif in mammalian MIF, are less conserved among invertebrate species.

Interestingly, within the mammalian genome there is a single gene that is homologous to the *mif* gene, which encodes a protein called D-dopachrome tautomerase (D-DT). While first described in literature in the early 1990s, few functional studies of D-DT were published until the last five years (Odh et al., 1993). Despite a low amino acid sequence identity between MIF and D-DT (34% in humans and 27% in mice), there is a significant three dimensional structural homology with MIF (Sugimoto et al., 1999). As reviewed by Merk et al. (Merk et al., 2012), like MIF, D-DT (sometimes also referred to as mammalian MIF-2) is present in most tissues and exists in pre-formed pools, it is released upon stimulation and also binds to the receptor complex CD74/CD44, leading to a similar signal transduction cascade as MIF. Yet, D-DT may be less biologically active than MIF: it binds CD74 with a ~3-fold higher association rate (k_a) but a ~11-fold faster dissociation rate (k_d) than MIF. This potentially lower potency of D-DT might lead to partial antagonism in circumstances where high concentrations of MIF are produced (Merk et al., 2011).

2. Non-mammalian MIF homologues identified throughout the eubacteria, animal and plant kingdoms

Given that MIF is an evolutionary ancient molecule, it is not surprising that genes encoding proteins that appear related to the mammalian MIF superfamily members (i.e. *mif* and its paralogue *d-dt*) have been found in different prokaryotes (e.g. bacterial cells) and eukaryotes (e.g. plants, vertebrates such as fish, amphibians, birds and mammals and invertebrates such as protozoa, helminths, nematodes, molluscs and arthropods). While Genbank analysis identifying *mif*-like genes across species is extensive, it should be noted that genomic databases primarily reflect sequences present in euchromatin and it remains possible that *mif*-related genes exist in heterochromatin. Contained herein is an overview of the most well studied/cloned non-mammalian homologues of MIF and D-DT (Table 1).

Regarding the role of bacterial MIF homologues, so far only in the marine Cyanobacterium *Prochlorococcus marinus* a MIF homologue has been identified, the protein crystalized, and found to have tautomerase activity. More detailed studies will be required to address whether

a MIF-like protein from a free-living bacterium possesses immunoregulatory features similar to those of mammalian MIF (Wasiel et al., 2010).

Three MIF/DDT-like (MDL) polypeptides [*Ath*-MDL-1 (At5g57170), *Ath*-MDL-2 (At5g01650) and *Ath*-MDL-3 (At3g51660)] have been identified by *in silico* analysis in the plant *Arabidopsis thaliana* and their function are currently under investigation. Given that plants lack a circulation/extracellular space-based mobile immune defense system, these plant MIF homologues most likely exert intracellular effects. Hereby, the suggested presence of a tautomerase activity might be of importance. D-dopachrome is an artificial substrate of mammalian MIF and other MDLs and is a cyclization product of D-3,4-dihydroxyphenylalanine (also known as D-DOPA), suggesting a role in the biosynthesis of melanin-type pigments. While plants lack conventional melanin, they synthesize catechol melanin, which is chemically related to L-DOPA and might serve a role as precursor of different secondary plant metabolites (melanin) (Solano, 2014; Soares et al., 2014).

With respect to different vertebrate MIF homologues, they appear universally to be involved in innate and adaptive immune responses and affect cell migration, pro-inflammatory cytokine secretion, and cell differentiation or morphogenesis (Bozza et al., 2012). In invertebrates such as molluscs and arthropods, which lack a developed adaptive response, MIF homologues are likely to play roles in innate immunity (Huang et al., 2016). Interestingly, MIF homologues in ticks (i.e. vectors for human illnesses such as monocytic ehrlichiosis and Lyme disease (Jaworski et al., 2001)) were suggested to facilitate cutaneous responses to ensure efficient uptake of a blood meal. Tick MIF is present in protein pools in the salivary glands prior to tick attachment and secreted early during the feeding process after which there is a switch to MIF being the main component of the midgut digestive cells (Bowen et al., 2010). In addition, it was found to inhibit *in vitro* the migration of human macrophages (Jaworski et al., 2001). Collectively, this effect could translate *in vivo* to the inhibition of migration of host cells (i.e. myeloid cells) toward the tick's mouthparts as it feeds or within the tick's midgut after feeding, thereby evading a potential host anti-tick response. Regardless of the mechanism, it is evident that vector MIF plays a role in blood feeding; tick MIF might on one hand increase inflammatory blood flow and on the other hand in concert with other tick products, such as an anaphylatoxin inactivator, inhibit aspects of inflammation, such as pain, to mask the presence of the tick and facilitate the tick life cycle (Jaworski et al., 2009). Of note, Aphids, i.e. sap-sucking insects attacking virtually all plant species and causing serious crop damages in agriculture (Kim et al., 2008), have also been found to encode a MIF homologue that inhibits major plant immune responses such as the expression of defense-related genes, callose deposition, and hypersensitive cell death, thereby allowing plant exploitation (Naessens et al., 2015).

Protozoa and helminths are extensively implicated in death, suffering and economic losses in both developing and developed nations. For example, malaria (*Plasmodium spp.*) is considered one of the most prevalent and debilitating diseases in developing countries adding up to 300–500 million clinical cases each year and 1–2 million deaths accounting for a reduction in economic growth of 1.3% per annum in Africa alone (Suh et al., 2004; Gallup and Sachs, 2016). Additionally, 500 million large ruminants are infected with parasitic worms resulting in billion dollar losses worldwide (Love and Hutchinson, 2003). While it is

evident herein that indeed MIF is an evolutionary intriguing protein which exerts several different effects amongst different species, perhaps the most interesting of these functions, from both a health and economic perspective, are those species, particularly parasitic in nature, that use MIF to modulate the host immune response and thereby favoring parasite invasion/maintenance (Bozza et al., 2012; Rosado et al., 2011).

One of the first invertebrate *mif*-like genes identified was that of the parasitic nematode *Brugia malayi*, *Bma-mif-1*, followed later by *Bma-mif-2* (Zang et al., 2002; Pastrana et al., 1998). *Brugia malayi* is the causative agent of lymphatic filariasis more commonly known as elephantiasis. Initial studies had revealed that Bma-MIF inhibits the random migration of macrophages (Pastrana et al., 1998). Subsequent studies revealed that, contrary to the classical pro-inflammatory activities of MIF, Bma-MIF shows marked synergy with IL-4 thereby enhancing the expression of alternative activation markers (Th2) and further induced IL-4R α expression, rendering macrophages immunosuppressive (Prieto-Lafuente et al., 2009). Such an environment may be considered to be favorable for the persistence of the parasite. Other parasitic nematodes also have been shown to use their MIF to modulate the host immune response to their advantage. For example, *Anisakis simplex*, the causative agent of anisakiasis, produces a MIF homologue (Asi-MIF) shown to modulate OVA-specific Th2 responses in the host (Cho et al., 2015), which was evidenced by (i) inhibition of the infiltration of inflammatory cells (particularly eosinophils and macrophages) into the lung, (ii) reduction in the concentration of IL-13, and an increase in the levels of TGF- β 1 and IL-10, (iii) recruitment of Treg cells (Treg cell mediated immune suppression) and (iv) antagonism of the effects of host MIF (Park et al., 2009). This is an interesting conundrum: on one hand Asi-MIF could provide a beneficial anti-inflammatory tool for allergic airway inflammation, however these same characteristics may allow maintenance of parasitism for prolonged periods of time. *Trichinella spiralis* (trichinosis) MIF (Tsp-MIF) was shown to inhibit the random migration of human peripheral blood mononuclear cells and reduce macrophage infiltration in inflamed tissues (Wu et al., 2003), thus potentially allowing more time for invasion and establishment of infection. *Strongyloides ratti* MIF (Sra-MIF) was shown to bind to the monocyte/macrophage lineage and induce the production of IL-10 rather than TNF- α (Younis et al., 2012) suggesting a more alternative type of activation. A final nematode MIF worthy of mention is *Ancylostoma ceylanicum* MIF (Ace-MIF). Ace-MIF is expressed only when the worms invade the mammalian host but not while they are present in the environment and, additionally, Ace-MIF was confirmed to bind with high affinity to the mammalian MIF receptor CD74 (Cho et al., 2007). While the exact mechanism in which Ace-MIF modulates the immune system is not known based on these parameters, one can hypothesize that *A. ceylanicum* can manipulate the host to favor disease establishment by its interaction with the host MIF receptor.

Protozoan parasites also produce the most experimentally well-characterized, MIF-like proteins, which appear exemplary in their ability to modulate host responses. *Trichomonas vaginalis*, i.e. the causative agent of the most common non-viral sexually transmitted infection (WHO, 2016), harbors a MIF (Tva-MIF) that binds to the human MIF receptor, CD74, to activate downstream (ERK)1/2 and Akt signaling pathways, leading to the stimulation of IL-8 secretion from monocytes, reduction of monocyte migration, and increase in the growth and invasiveness of prostate cancer cells (Twu et al., 2014).

Consequently, chronic *T. vaginalis* infection may result in Tva-MIF-driven inflammation and cell proliferation, thereby contributing to the promotion and progression of prostate cancer. Similarly, *Leishmania major* harbors two MIF homologues; Lma-MIF-1 is found exclusively in amastigotes (i.e. the intracellular stage responsible for mammalian disease) and Lma-MIF-2 is found in all life cycle stages (Richardson et al., 2009). Lma-MIF-1 (Lm1740MIF) was found to exhibit tautomerase activity and activate the (ERK)1/2 pathway in a CD74-dependent manner thereby inhibiting the activation-induced apoptosis of macrophages (Kamir et al., 2008), which in turn may allow parasites to persist within the macrophages and avoid immune destruction. The immunomodulatory role of the two Lma-MIF proteins was verified recently by the creation of a Lma-MIF-KO strain of *L. major* (Holowka et al., 2016). This mutant strain replicated normally but showed a 2-fold increased susceptibility to macrophage clearance, while mice infected with Lma-MIF-deficient *L. major*, when compared to the wild-type strain, also showed a 3-fold reduction in parasite burden. Notably, CD4⁺ T cells that developed during infection with this strain showed differences in markers of functional exhaustion and decreased apoptosis. Lma-MIF proteins thus promote parasite persistence by manipulating the host response to increase the exhaustion and depletion of protective CD4⁺ T cells.

The Tgo-MIF protein from *Toxoplasma gondii* (toxoplasmosis), can activate (ERK)1/2 pathways in murine bone marrow derived macrophages and elicit IL-8 production from human peripheral blood mononuclear cells (Sommerville et al., 2013). In turn, the secretion of IL-8 has been linked to the chemotaxis of neutrophils primarily (Leonard and Yoshimura, 1990). Paradoxically, while these neutrophils are important for the resolution of infections, they may also aid in the spread of *T. gondii*. Hereby, *T. gondii* stimulates neutrophils to produce CCL3, 4, 5 and 20, which are strongly chemotactic for dendritic cells (DC), that in turn are described as “Trojan horses” to facilitate *T. gondii* dissemination and thus successful completion of the parasite lifecycle (Sommerville et al., 2013). *Eimeria* spp. such as *E. acervulina*, causing avian coccidiosis, also have been shown to express and secrete a MIF homologue, i.e. Eac-MIF, that is able to inhibit chicken monocyte migration, bind to chicken macrophages via ChCD74p41 and induce expression of pro-inflammatory cytokines and chemokines (Kim et al., 2014; Miska et al., 2007). Finally, all *Plasmodium* spp. that have been genomically sequenced, including *Plasmodium falciparum*, *Plasmodium berghei* and *Plasmodium yoelii* secrete their respective MIFs (Pfa-MIF, Pbe-MIF and Pyo-MIF) during the asexual blood stage, most likely upon schizont rupture within the mammalian host, which is capable of binding to CD74 (Augustijn et al., 2007; Dobson et al., 2009; Liu et al., 2016). In addition, Pfa-MIF was found to inhibit the random migration of monocytes, reduce the chemotactic response of monocytes, and reduce the expression of TLR2 and TLR4 on monocytes (Cordery et al., 2007a). On the other hand, Pbe-MIF, which is also expressed in the insect vector stage and does not seem to affect its lifecycle but rather may have a more subtle role in parasite-host interactions, appears to play a role in reducing the blood cell pool for invasion via suppression of erythropoiesis (Augustijn et al., 2007). While seemingly counterintuitive, this could lead to the maintenance of a low level, perhaps longer lasting infection. Pbe-MIF does exert pro-inflammatory activation in vivo and in experimental infections promotes the development of a highly inflammatory effector T cell population at the expense of the memory T cells necessary for protective immunity (Sun et

al., 2012). Potentially, Plasmodium MIF contributes to immune evasion and the universal absence of sterilizing immunity in malaria infection. Secreted Plasmodium MIF also elicits the chemotaxis and the accumulation of CD11b⁺ Ly6C⁺ cells within the spleen (Liu et al., 2016). In contrast to the other *Plasmodium* spp., Pyo-MIF appears to have a role in liver stage development and thus plays an important role in completion of its lifecycle (Miller et al., 2012). Finally, though MIF was also found to be involved in African trypanosomosis-associated pathogenicity (Stijlemans et al., 2014, 2016), so far no reports are documented on the involvement of trypanosomal MIF. Yet, it is highly likely that these protozoa also harbor a homologue given their “close relationship” with Leishmania and Plasmodium.

Collectively, given that many organisms encode MIF, the *mif* gene must have undergone important structural/functional changes during the evolution of invertebrates into vertebrates. The function of these genes in primitive species thus may be quite different from those found in vertebrates (Du et al., 2006). This notion was strengthened by observations, based on the phylogenetic tree comprising the biologically active MIF homologues described herein, that protozoan MIF homologues are more closely related to that of *Prochlorococcus marinus* (i.e. most likely related to the ancestor MIF) when compared to the mammalian MIF (Fig. 1). In addition, the fact that both pathogen and host harbor MIF homologues might be of evolutionary importance to allow a well-balanced and timed pathogen-host interplay (See proposed model Fig. 2). Hereby, the pathogen-derived MIF homologues that have been studied to date appear to modulate the host innate immune response in order to optimally sculpt an environment that allows establishment of the infection. Host MIF by contrast contributes to the initiation of immune responses aimed at controlling/eliminating the pathogen. During the progression of the disease, the balance between signals orchestrated by pathogen-derived MIF and host-derived MIF will ensure on one hand survival/persistence of the infection and on the other hand limit immuno-pathogenicity from an uncontrolled immune response that might be deleterious to host and pathogen.

3. MIF as target for diagnostic and intervention strategies

Several studies have pointed to the utility of mammalian MIF as a biomarker for different diseases that have an inflammatory component; such as systemic infections and sepsis, cancer, autoimmune diseases as well as different metabolic disorders (Grieb et al., 2014). In addition, identification of functional promoter polymorphisms in the MIF gene (*mif*) and their association with the susceptibility or severity of different diseases can be used as tool to validate MIF's role in disease development as well as tool to better predict risk and outcome. With respect to parasitic infections, several reports have pointed to the reactivity of the host humoral immune system against parasite MIF. Indeed, host (patient/cow) serum following infections with *T. vaginalis*, *O. volvulus* and *S. ratti* was found to contain pathogen MIF specific IgG (Younis et al., 2012; Twu et al., 2014; Ajonina-Ekoti et al., 2013); suggesting that nematode MIF apparently functions as a target of B cell responses (Ajonina-Ekoti et al., 2013). In the same line, studies in malaria patients indicate that also here anti-Pfa-MIF antibodies were induced by the host during infection (Cordery et al., 2007b; Wang et al., 2009a). Consequently, detection of parasite MIF using specific monoclonal antibodies could be a valuable tool for diagnosis and benefit epidemiological studies (Wang et al., 2009a;

Shao et al., 2008). Hence, development of tools to detect pathogen or even vector MIF might allow diagnosis of the diseases or exposure to a vector bite.

From a therapeutic standpoint, much effort has gone into the development of mammalian MIF blocking molecules due to its predominantly characteristic pro-inflammatory mechanisms; these include antibodies and small molecule synthetic inhibitors (Calandra et al., 2000; Al-Abed et al., 2005; Rajasekaran et al., 2014; Xu et al., 2013; Cournia et al., 2009). Virtual screening and medicinal chemistry optimization has been successful in identifying ligands that bind to the tautomerase active site for human MIF. Perhaps one of the most commonly used MIF inhibitors in literature to date is the synthetic molecule ISO-1 (also known as (*S,R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester). ISO-1 was found to prevent the interaction of MIF with its CD74 receptor by binding (by design) to the catalytically active site within the MIF molecule. In addition, ISO-1 was shown to be a potent inhibitor of MIF's tautomerase activity (Al-Abed et al., 2005) and found to function in *in vivo* models including but not limited to chronic asthma, flavivirus infection, systemic lupus erythematosus, colon carcinoma and sepsis (Al-Abed et al., 2005; Chen et al., 2010; Conroy et al., 2010; Leng et al., 2011; Arjona et al., 2007). Despite remarkable structural similarities of MIF across species, the catalytic pocket is seemingly quite variable rendering ISO-1 ineffective against several MIFs including *A. simplex*, *A. ceylanicum*, *L. major* and *T. gondii* for example (Park et al., 2009; Cho et al., 2007; Kamir et al., 2008; Sommerville et al., 2013). A second small molecule inhibitor, 4-IPP, is notable as it is the first identified dual D-DT/MIF inhibitor (Rajasekaran et al., 2014). This compound binds covalently to the Pro1 residue, which is present in both MIF and D-DT, to produce a potent, albeit non-specific, inhibition of tautomerase activity (Rajasekaran et al., 2014; Kindt et al., 2013; Winner et al., 2008). It has previously been shown that 4-IPP inhibits Lma-MIF evoked chemotaxis (Kamir et al., 2008), but whether this small molecule inhibitory effect can be generalized towards all non-mammalian MIFs remains to be seen.

Consequently, there is more research needed into the development of parasite MIF targeting molecules which may be useful in blocking parasite immune evasion strategies. Moreover, given the seemingly pivotal role that parasite MIF plays in the establishment of infection it seems reasonable to think that pathogen-derived MIF might be the center of therapeutic or vaccination strategies. Vaccine approaches, which have been largely unexplored, may be of particular interest given the high conservation of MIF within particular parasite genera. There are few examples to date of parasite MIF targeting molecules (synthetic or antibodies) although Dahlgren et al. (Dahlgren et al., 2012) recently identified by virtual screening, several potent Pfa-MIF tautomerase inhibitors ($K(i)$ of ~40 nM) with low selectivity for human MIF ($K(i) > 100 \mu\text{M}$) that are also able to inhibit its interaction with the human CD74 receptor without affecting human MIF-CD74 interactions (Pantouris et al., 2014). These data provide the first proof-of-concept for the development of new therapeutics that selectively target pathogen MIF proteins and continued structural elucidation of pathogen-derived MIF combined with computationally-assisted screening for specific inhibitors might allow for the design of new and more effective therapies for parasitic infections. Moreover, it may be possible that targeting pathogen MIF on one side and host MIF on the other might be an attractive approach to interfere both with the establishment of the pathogen and the inflammatory pathologic sequelae of infection.

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Abbreviations

MIF	macrophage migrating inhibitory factor
ISO-1	(S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester
MDL	MIF/D-DT like.

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Fig. 1.

Evolutionary relationships of MIF. The depicted tree represents a consensus phylogram obtained by Bayesian analysis of the amino-acid sequences of confirmed MIF proteins of a wide range of eukaryotes, using a prokaryote (the bacterial species *Prochlorococcus marinus*) as outgroup. MIF proteins discussed in the text are indicated by their abbreviated names between brackets. Branch lengths are proportional to the estimated number of substitutions per site (see scale bar). Numbers above branches represent Bayesian posterior probabilities; only branches with values >0.95 should be regarded as highly supported. Branches with probabilities <0.5 are collapsed. The clustering of the nematode MIFs Asi-MIF and Sra-MIF with mollusc MIF may be a long-branch attraction artefact as a result of high evolutionary rates. A sequence alignment was created using Mafft 7 (Katoh and Standley, 2013) and entered into MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) for Bayesian phylogenetic inference. A mixed prior implementing multiple empirical models of amino-acid substitution was applied, in combinations with gamma-correction for among-site rate heterogeneity and an estimated proportions of invariable sites. Two parallel runs of four incrementally heated (temperature parameter = 0.2) Markov chain Monte Carlo (MCMC) chains were performed, with a length of 6,000,000 generations, a sampling frequency of 1

per 1000 generations, and a burn-in corresponding to the first 1,000,000 generations. Convergence of the parallel runs was confirmed by split frequency standard deviations (<0.01) and potential scale reduction factors (approximating 1.0) for all model parameters, as reported by MrBayes. Adequate posterior sampling was verified using Tracer 1.6 (Rambaut et al., 2014), by checking if the runs had reached effective sampling sizes >200 for all model parameters.

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Fig. 2.

Schematic overview of the role of MIF in parasitic mediated infections. Pathogen-derived MIF is mainly involved in establishing infection, while host MIF is aimed at controlling the parasite. In either case, MIF most often leads to increased pathology in host tissues.

Table 1

Overview of MIF-like proteins across species. (Baeza Garcia et al., 2010; Buonocore et al., 2010; Chauhan et al., 2015; Cui et al., 2011; Dubreuil et al., 2014; Fang et al., 2013; Furukawa et al., 2016; Kim et al., 2010; Li et al., 2011a,b; Marson et al., 2001; Miska et al., 2013; Nisbet et al., 2010; Oh et al., 2013; Panstruga et al., 2015; Parisi et al., 2012; Park et al., 2016; Qiu et al., 2013; Sharma et al., 2012; Shen et al., 2012; Suzuki et al., 2004; Tan et al., 2001; Umemiya et al., 2007; Wang et al., 2009b,c, 2013; Wistow et al., 1993; Zeng et al., 2013; Jin et al., 2007)(For interpretation of the references to color in this Table legend, the reader is referred to the web version of this article.)

Group	Species	Known homologue	Sequence identity to human MIF/DDT	Enzymatic activity	CD74 binding	Identified function	Reference
Prokaryotes							
Bacteria	Cyanobacteria, <i>Prochlorococcus marinus</i> as a model	MIF	36%	Low tautomerase but no oxidoreductase activity confirmed	n.a.	n.a.	Wasiel et al. (2010)
Eukaryotes							
Plants							
Plants	All plant taxa, <i>Arabidopsis thaliana</i> as model	3 MIF	28%, 30%, 32%	No oxidoreductase activity, presumed tautomerase activity	n.a.	Associated with stress inducible transcript accumulation and aerial expression	Panstruga et al. (2015)
Vertebrates							
Fish	<i>Oplegnathus fasciatus</i>	MIF/DDT	MIF: 80.9% DDT: 73.7%	Oxidoreductase activity of MIF and DDT confirmed	n.a.	Cytokine activation, immune defense	Oh et al. (2013)
	<i>Danio rerio</i>	MIF DDT?	MIF: 69%	n.a.	CD74 homologues identified	Neurotrophin: morphogenesis of embryos, inner ear development	Shen et al. (2012)
	<i>Tetraodon nigroviridis</i>	MIF	67.5%	n.a.	n.a.	Macrophage migration inhibition, possible immunological role	Jin et al. (2007)
	<i>Sciaenops ocellatus</i>	MIF	68%	presumed, not confirmed	n.a.	Migration of monocytes and lymphocytes, pathogen induced immune response	Qiu et al. (2013)
	<i>Branchiostoma belcheri tsingtauense</i>	2 MIF	39%	Tautomerase activity confirmed, could use dithiothreitol to reduce insulin	n.a.	Hypothesized involvement in cell differentiation and formation	Du et al. (2006)
	<i>Dicentrarchus labrax L.</i>	MIF	81.7%	Structurally presumed, not confirmed	n.a.	Increased on antigen stimulation,	Buonocore et al. (2010)

Group	Species	Known homologue	Sequence identity to human MIF/DDT	Enzymatic activity	CD74 binding	Identified function	Reference
						immunological role: unknown	
Amphibian	<i>Xenopus laevis</i>	MIF DDT	71% 66%	MIF tautomerase activity confirmed	n.a.	Morphogenesis namely the brain, eye, ear and mesodermal tissues	Suzuki et al. (2004)
	<i>Andrias davidianus</i>	MIF	70.4%	Redox and tautomerase activity confirmed	n.a.	Innate immunity, more experiments necessary	Wang et al. (2013)
Birds	<i>Gallus gallus domesticus</i>	MIF	71%	n.a.	yes	Macrophage migration Inhibited, enhanced proliferation of stimulated lymphocytes, concentration dependant enhancement of Th1 or Th2 cytokines. Correlates with cell differentiation in the developing chicken lens	Kim et al. (2014, 2010); Wistow et al. (1993)
	<i>Meleagris gallopavo</i>	MIF	71%	Structurally presumed, not confirmed	n.a.	inhibits migration of both mononuclear cells and splenocytes in a dose-dependent manner, enhancement of pro-inflammatory cytokines	Park et al. (2016)
Invertebrates							
Echinoderm	<i>Patiria (Asterina) pectinifera</i>	2 MIF	40%, 80%	n.a.	n.a.	Opposing functions of each MIF type, chemotactic inhibitory and stimulatory factors, respectively, and coordinately regulate mesenchyme cell recruitment during the immune response in starfish larvae	Furukawa et al. (2016)
	<i>Haliotis diversicolor</i>	MIF	43%	Tautomerase activity but not oxidoreductase activity presumed but not confirmed	n.a.	Upregulated upon bacterial stimulation, role in immune response	Wang et al. (2009c)
Molluscs	<i>Mytilus galloprovincialis</i>	Numerous variants of MIF	39.5%	n.a.	n.a.	Expression decreased upon antigen challenge, unknown innate immune mechanism	Parisi et al. (2012)

Group	Species	Known homologue	Sequence identity to human MIF/DDT	Enzymatic activity	CD74 binding	Identified function	Reference
Arthropods	<i>Pinctada fucata</i>	MIF	62.2%	Oxidoreductase activity confirmed, could use dithiothreitol to reduce insulin	n.a.	n.a.	Cui et al. (2011)
	<i>Chlamys farreri</i>	2 MIF	40%	n.a.	n.a.	Involved in fibroblast migration for wound healing, immune response	Wang et al. (2009b); Li et al. (2011a)
	<i>Bionphalaria galbrata</i>	MIF	31%	Tautomerase activity confirmed	n.a.	Induced cell proliferation and inhibited p53 mediated apoptosis, parasite control	Baeza Garcia et al. (2010)
	<i>Scylla paramamosain</i>	2 MIF/DDT	MIF: 35.97% DDT: 25.2-42.4%	n.a.	n.a.	MIF is a defense molecule DDT is thought to play a role in melanogenesis	Huang et al. (2016); Fang et al. (2013)
	<i>Litopenaeus vannamei</i>	MIF	39%	n.a.	n.a.	Response to viral infection, immunity	Zeng et al. (2013)
	<i>Eriocheir sinensis</i>	MIF	44%	n.a.	n.a.	Innate immune response	Li et al. (2011b)
	<i>Acyrtosiphon pisum</i>	5 MIF	39%	n.a.	n.a.	Involved in immunity: down regulated in the presence of symbiont, upregulated in the presence of parasite or gram negative bacteria	Dubreuil et al. (2014)
	<i>Amblyomma americanum</i>	MIF	40%	Tautomerase activity confirmed	n.a.	Inhibits migration of mammalian macrophages (presumably towards leishman when feeding)	Jaworski et al. (2001)
	<i>Haemaphysalis longicornis</i>	MIF	41%	n.a.	n.a.	Inhibits the random migration of human monocytes (presumably toward lesions while feeding), potential role in cell proliferation during blood meal, immunity	Umemiya et al. (2007)
	<i>Brugia malayi</i>	2 MIF	42%	Tautomerase activity confirmed	n.a.	Secreted, chemotactic for human cells, inhibits the random migration of macrophages, synergises with IL-4 to	Zang et al. (2002); Prieto-Lafuente et al. (2009);

Group	Species	Known homologue	Sequence identity to human MIF/DDIT	Enzymatic activity	CD74 binding	Identified function	Reference
						induce alternative activation of macrophages, modulation of the host immune system	Pastrana et al. (1998)
	<i>Trichinella spiralis</i>	MIF	42%	Tautomerase and oxidoreductase activity confirmed	n.a.	Inhibited migration of human peripheral-blood mononuclear cells. Proposed potential for modulation of the host immune response	Tan et al. (2001); Wu et al. (2003)
	<i>Caenorhabditis elegans</i>	4 MIF	22-35%	Tautomerase and oxidoreductase activity confirmed for 3/4 MIF's	n.a.	Suggested role for cellular maintenance during periods of adverse conditions that lead to developmental arrest	Marson et al. (2001)
	<i>Wuchereria bancrofti</i>	2 MIF	36%	Tautomerase and oxidoreductase activity confirmed	n.a.	n.a.	Sharma et al. (2012); Chauhan et al. (2015)
	<i>Anisakis simplex</i>	MIF	53%	n.a.	n.a.	Suppresses TH2 response in host, modulates host immune response, antagonizes host MIF	Park et al. (2009)
	<i>Onchocerca volvulus</i>	2 MIF	43%	Oxidoreductase activity confirmed for one MIF the other has confirmed tautomerase activity	n.a.	Invokes MIF specific lymphocyte responses	Ajonina-Ekoi et al. (2013)
	<i>Teladorsagia circumcincta</i>	MIF	40%	Tautomerase activity confirmed	n.a.	Proposed to play a role in protection of the parasite and modulation of the host immune response	Nisbet et al. (2010)
	<i>Strongyloides ratti</i>	MIF	28%	No tautomerase activity	n.a.	Secreted, host immune modulation and promotes IL-10 release from monocytes	Younis et al. (2012)
	<i>Ancylostoma ceylanicum</i>	MIF	53%	Tautomerase activity confirmed	yes	Proposed host immune modulation, virulence factor	Nisbet et al. (2010)
	<i>Trichomonas vaginalis</i>	MIF	31%	Tautomerase activity confirmed	yes	Secreted, elicits host immune responses, induces prostate cell	Twu et al. (2014)32

Group	Species	Known homologue	Sequence identity to human MIF/DDIT	Enzymatic activity	CD74 binding	Identified function	Reference
						growth and invasiveness upon human infection	
	<i>Leishmania major</i>	2 MIF	22%	Tautomerase activity but no oxidoreductase activity	yes	Stimulates monocyte migration, activated ERK 1/2 MAPK, inhibits apoptosis, modulates host immune response	Kamir et al. (2008)
	<i>Toxoplasma gondii</i>	MIF	26%	Tautomerase activity but no oxidoreductase activity	n.a.	Induces IL-8 production from human cells, proposed to help facilitate parasite dissemination into host tissues	Sommerville et al. (2013)
	<i>Eimeria acervulina</i>	MIF	53%	n.a.	yes	Inhibits chicken monocyte migration, triggers pro-inflammatory cytokines, modulation of the host immune response	Kim et al. (2014); Miska et al. (2007, 2013)
	<i>Plasmodium berghei</i>	MIF	30%	Tautomerase and oxidoreductase activity confirmed	yes	Secreted into infected erythrocytes, decreases host reticulocyte density contributing to anemia might maintain a lower level of parasitemia for long lasting infection	Dobson et al. (2009); Augustijn et al. (2007)
	<i>Plasmodium falciparum</i>	MIF	29%	Tautomerase and oxidoreductase activity confirmed	yes	Released during blood stage malaria, inhibited random migration of monocytes and reduces cell surface expression of TLR2, TLR4 and CD86, antioxidant functions for parasite	Dobson et al. (2009); Augustijn et al. (2007); Cordery et al. (2007a)
	<i>Plasmodium yoelii</i>	MIF	29%	n.a.	yes	MIF deficient parasites are defective in liver stage growth due to inability to regulate cell division, increases the secretion of pro-inflammatory factors, influences the accumulation of CD11b + Ly6C+ cells within the spleen	Miller et al. (2012); Liu et al. (2016)