MIF

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SUMMARY

Although first described as a soluble activity produced by activated T cells nearly four decades ago, interest in macrophage migration inhibitory factor (MIF) was rekindled when the mouse homolog of this protein was identified to be secreted from the anterior pituitary gland in a hormone-like fashion (Bernhagen *et al*., 1993). Initially, MIF was shown to play a critical role in the host inflammatory response to endotoxin. Further studies showed that the inflammatory activity of MIF was mediated by its ability to promote proinflammatory cytokine release (Calandra *et al*., 1994) and to suppress the anti-inflammatory effects of glucocorticoids (Calandra *et al*., 1995; Bacher *et al*., 1996). Since the identification of MIF as a unique proinflammatory molecule and the development of neutralizing monoclonal antibodies, several reports have been published describing the role of MIF in inflammatory diseases, including *arthritis*, *glomerulonephritis*, *peritonitis*, and the *delayed-type hypersensitivity* reaction. Clinical evidence demonstrating increased MIF expression during inflammatory disease pathogenesis further supports the potential role of MIF in inflammation. In addition to its role in the inflammatory response, MIF has been shown to exhibit growth-promoting activities. Recent investigations by two independent laboratories have revealed that immunoneutralization of MIF can inhibit tumor growth and angiogenesis (Chesney *et al*., 1999; Shimizu *et al*., 1999b). Further-more, MIF has been shown to inactivate p53, a potent tumor suppressor molecule (Hudson *et al*., 1999) supporting the role of MIF in cellular proliferation. Several chronic inflammatory conditions are associated with the development of tumors. Therefore, the identification of MIF as a suppressor of p53 activity might provide a mechanistic link between inflammation and tumorigenesis.

BACKGROUND

Discovery

Macrophage migration inhibitor factor (MIF) is considered to have been the earliest cytokine 'activity' identified, and it was recognized as far back as the late 1950s to be associated with immune cell activation (for reviews, see Metz and Bucala, 1997 and Swope and Lolis, 1999). By 1966, Barry Bloom and John David independently characterized this activity to be a soluble factor produced by activated T lymphocytes that could inhibit the random migration of macrophages (Bloom and Bennett, 1966; David, 1966). This aroused considerable interest among immunologists as MIF became one of the first soluble, non-immunoglobulin factors amenable to study *in vitro*. Over the next 20 years, MIF was found to correlate with general macrophage-activation functions including adherence, spreading, phagocytosis, and enhanced tumoricidal activity. There was little progress made with respect to the molecular characterization of MIF and interest in MIF as a discrete factor declined when the proteins such as IFNγ and IL-4 were found also to exhibit macrophage migration inhibitory activity. In 1989, a unique cDNA for MIF was cloned and a molecular analysis of the biological, biochemical, and biophysical properties of MIF could be approached. Unfortunately, the MIF protein used in these early studies was in the form of an unpurified COS cell supernatant that was later found to have also contained significant quantities of a mitogen, phytohemagglutinin.

Several years ago, we initiated studies to identify potentially new mediators that might counterregulate, at the systemic level, the suppressive effects of glucocorticoids on the immune system. As part of this program, we cloned an apparently novel 12.5 kDa protein that was released in abundant quantities by

LPS stimulation of the mouse corticotropic pituitary cell line AtT-20. This protein was found to share 90% identity with the recently identified human MIF protein sequence. In addition to being secreted from the pituitary in a hormone-like fashion, MIF was released from immune cells as a consequence of glucocorticoid stimulation. Recombinant MIF (rMIF) was shown to 'override' or counter-regulate the immunosuppressive effects of glucocorticoids on immune cells and an emerging body of data indicates that rMIF can act in concert with glucocorticoids to control the 'set point' of the immune and inflammatory response. More recent studies have shown MIF to be widely expressed, and to be secreted in response to a variety of proinflammatory or mitogenic stimuli.

Alternative names

Glycosylation inhibitory factor (GIF), a protein associated with T cell immunosuppressive factors, has the same amino acid sequence as MIF (Mikayama *et al*., 1993). There is evidence that GIF inhibits the *N*-glycosylation of IgE-binding factors.

Structure

The aberrant chromatographic behavior of MIF on various gel filtration media hampered the biochemical analysis of its native molecular weight. X-ray crystallographic analyses of MIF (human and rat) have identified the protein to be a trimer of identical subunits of approximately 12.5 kDa each (Sun *et al*., 1996; Suzuki *et al*., 1996). Several hydrogen-binding sites between the monomers and a hydrophobic core act to stabilize the MIF trimer.

Main activities and pathophysiological roles

Originally, MIF was described as a lymphokine that inhibited the random migration of macrophages. However, over the past several years MIF has been shown to exhibit a number of pleiotropic effects, as summarized in **Table 1**.

GENE AND GENE REGULATION

Accession numbers

Human cDNA: Z23063
Mouse cDNA: Z23048
Rat cDNA: U62326
Bovine cDNA: P80177
Chicken cDNA: M95776
Gerbil cDNA: F045740
B. *malayi* cDNA: AF002699

Chromosome location

The mouse MIF gene has been mapped to chromosome 10 between the Bcr and S100b loci (Kozak *et al*., 1995; Mitchell *et al*., 1995). Numerous pseudogenes for MIF have been identified in the mouse genome (Bozza *et al*., 1995; Kozak *et al*., 1995). By contrast, only a single human MIF gene sequence has been found, and this has been localized to chromosome 22q11.2. This region is known to be in syntenic conservation with the MIF-containing region of chromosome 10 in the mouse (Budarf *et al*., 1997).

Relevant linkages

The mouse MIF gene maps to a position coincident with several recessive mutations, including the gray lethal (gl), mocha (mh), and grizzled (gr) mutations (Mitchell *et al*., 1995). Currently, there is no indication that mutations of the MIF gene result in a known mouse defect or disease. The gene for D-dopochrome tautomerase, which has approximately 30% homology in its coding region to the MIF gene and shares an identical exon structure to MIF, is closely linked on chromosome 10 (mouse) and 22 (human) (Esumi *et al*., 1998).

Regulatory sites and corresponding transcription factors

Several consensus sequences that may be involved in the transcriptional regulation of the mouse MIF gene have been identified (Mitchell *et al*., 1995). These enhancer/regulatory binding domains within the promoter regions include a sequence motif implicated in the basal expression of the proto-oncogene c-*fos*, an SP-1 site, a cAMP-responsive element (CRE), an AP-2 site, and a potential 'negative' glucocorticoid-responsive element (nGRE). All are located within 1 kb of the RNA transcription start site. A cytokine 1 (CK-1) site and a nuclear factor κB (NFκB) site were also identified further upstream on the minus DNA strand. A recent functional analysis of the murine MIF promoter region using the AtT-20 pituitary cell line demonstrated that CREB is the mediator of

Table 1 *In vitro* and *in vivo* activities of MIF

	References
In vitro	
Phagocytosis of particles	Onodera *et al*., 1997
Glucocorticoid counter-regulator	Calandra *et al*., 1995; Bacher *et al*., 1996
Promotes NO and TNFα release from macrophages	Calandra *et al*., 1994
Mediator of T cell activation and antigen-specific immunity	Bacher *et al*., 1996
Promotes insulin release from pancreatic β cells	Waeber *et al*., 1997
Suppression of inhibin release from Leydig cells	Meinhardt *et al*., 1996
Tautomerase activity	Rosengren *et al*., 1996
Suppression of erythroid progenitor development	Martiney *et al*., 1996, 2000
Regulator of phosphofructokinase-2 (PFK-2) activity	Benigni *et al*., in preparation
Mitogen for NIH 3T3 cells	Mitchell *et al*., 1999
Promotes tumor cell proliferation	Takahashi *et al*., 1998
Inhibits p53 functional activity	Hudson *et al*., 1999
Mitogen for primary fibroblasts	Hudson *et al*., 1999
Promotes endothelial cell proliferation	Chesney *et al*., 1999
***In vivo*: disease progression/pathologies (experimental animal models)**	
Endotoxemia and *exotoxemia*	Bernhagen *et al*., 1993; Calandra *et al*., 1998; Bozza *et al*., 1999
Peritonitis	Calandra *et al*., 2000
Delayed-type hypersensitivity reaction (DTH)	Bernhagen *et al*., 1996
Antigen-dependent T cell activation	Bacher *et al*., 1996
Collagen-induced arthritis and *adjuvant-induced arthritis*	Mikulowska *et al*., 1997; Leech *et al*., 1998
Glomerulonephritis	Lan *et al*., 1997
Hepatic liver failure	Kobayashi *et al*., 1999
Tumor growth and angiogenesis	Chesney *et al*., 1999; Shimizu *et al*., 1999b
Malaria anemia	Martiney *et al*., 2000

corticotropin-releasing factor (CRF)-induced MIF gene transcription in these cells (Waeber *et al*., 1998). The CRE site is located 41 bases upstream of the transcription start site.

Cells and tissues that express the gene

As detailed in Table 2, MIF protein has been detected in a wide variety of tissues and cell types. Most cell and tissue sources of MIF protein identified to date also express the MIF mRNA.

PROTEIN

Accession numbers

Human MIF: 312334
Mouse MIF: 312221
Rat MIF: 1432169

Gerbil MIF: 2854224
Bovine MIF: 730025
Chicken MIF: 212258

Sequence

See **Figure 1**.

Description of protein

Mouse and human MIF show greater than 90% identity in their primary amino acid sequence (115 amino acids) (Figure 1). The mammalian MIF proteins also have three invariant cysteines at positions 57, 60, and 81. All MIF protein sequences described to date lack a classical N-terminal leader sequence and appear to be released by a specialized pathway and all (except that which is found in rice) are distinguished by an invariant proline at the mature N-terminus (Figure 3).

Discussion of crystal structure

The human and rat MIF protein structures, which differ only in the positioning of 11 C-terminal residues, have been solved by X-ray crystallography (Sun *et al*., 1996; Suzuki *et al*., 1996). In addition, solution conformation data have been obtained by two-dimensional NMR (Muhlhahn *et al*., 1996). As shown in **Figure 2**, human MIF is a homotrimer of approximately $35\,\text{Å} \times 50\,\text{Å} \times 50\,\text{Å}$. This structure defines a new protein 'fold' and structural superfamily. While the tertiary structure of the MIF monomer may resemble that of the IL-8 dimer and major histocompatibility complex (MHC) structures, the folding of MIF is unique. It forms an α/β structure consisting of three β sheets surrounded by six α helices to form a solvent-accessible channel that runs the length of the molecule. The β sheet is also unusual in that the strands have a parallel rather than an antiparallel orientation. Several hydrogen bonding sites between the monomers, and a hydrophobic core act to stabilize the MIF trimer. The C-terminal domain is believed to be important for stable trimer formation (Bendrat *et al*., 1997). While this region was well resolved in the crystal structure of human MIF, it could not be resolved in rat structure suggesting the existence of a more flexible confirmation.

MIF displays significant three-dimensional structural homology but no sequence homology with two bacterial isomerases, 4-oxalocrotonate tautomerase (4-OT) and 5-carboxymethyl-2-hydroxymuconate isomerase (CHMI) (Subramanya *et al*., 1996). The structural similarity between MIF and 4-OT or CHMI also extends to the enzymatic active site (described below). Each protein has an N-terminal proline with an unusually low pK_a that acts to facilitate proton transfer in the substrate (Stamps *et al*., 1998). Purified rMIF tautomerizes unnatural substrates, such as the D-isomer of dopachrome and its methyl esters (Rosengren *et al*., 1996), as well as the aromatic amino acid metabolites, α-hydroxyphenylpyruvate and phenylpyruvate (Rosengren *et al*., 1997). Whether MIF mediates any of its biological properties via an enzymatic reaction, or by interaction with a low molecular weight substrate remains to be determined.

Figure 1 Amino acid sequences for human and mouse MIF.

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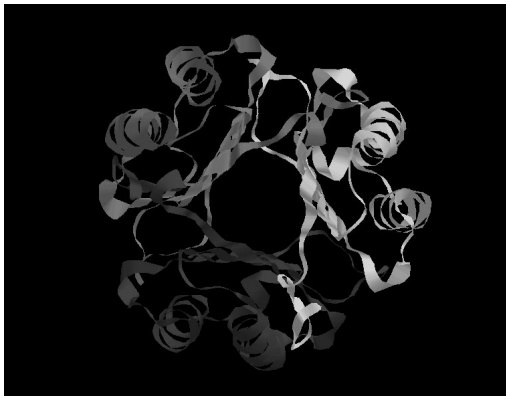
Human MIF:
1   MPMFIVNTNV PRASVPDGFL SELTQQLAQA TGKPPQYIAV
    HVVPDQLMAF GGSSEPCALC SLHSIGKIGG AQNRSYSKLL
    CGLLAERLRI SPDRVYINYY DMNAANVGWN NSTFA
Mouse MIF:
1   MPMFIVNTNV PRASVPEGFL SELTQQLAQA TGKPAQYIAV
    HVVPDQLMTF SGTNDPCALC SLHSIGKIGG AQNRNYSKLL
    CGLLSDRLHI SPDRVYINYY DMNAANVGWN GSTFA
```

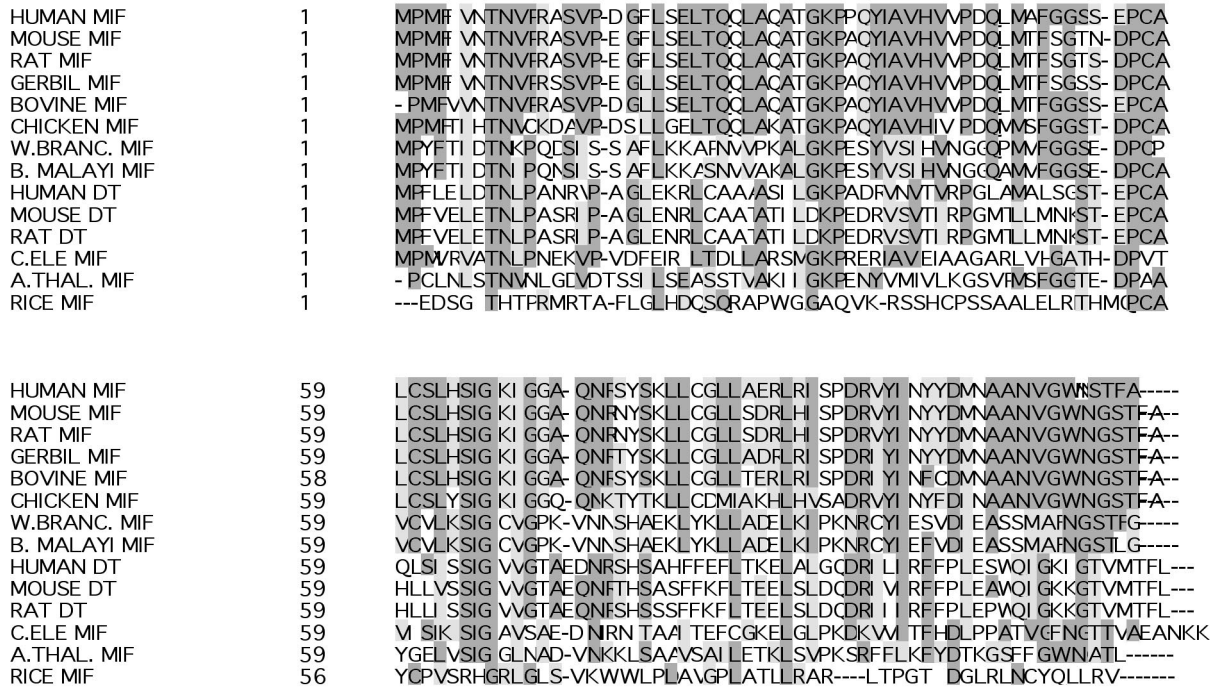
Figure 2 Crystal structure of the human MIF trimer.



Important homologies

Mouse and human MIF show greater than 90% identity in their primary amino acid sequence (115 amino acids), the highest known homology described to date for a human/mouse 'cytokine' pair. MIF homologs have been identified in the parasites *Wuchereria bancrofti and Brugia malayi*, as well as in *Caenorhabditis elegans and Arabidopsis thaliana* and rice (**Figure 3**). While there is only 27% primary

Figure 3 Amino acid sequence of several MIF homologs.



sequence identity between human MIF and rat D-dopachrome tautomerase (DT) (Zhang *et al*., 1995), there is a high degree of three-dimensional structure homology (J. Nishihira, personal communication).

Posttranslational modifications

There are two consensus sites for *N*-glycosylation in the primary sequence of MIF, however no detectable posttranslational modifications have been identified by mass spectroscopy (MS) or by enzymatic deglycosylation studies of native MIF purified from rat liver. MIF purified from bovine brain has been reported to exist as two species with different isoelectric points (9.4 and 9.5) (Galat *et al*., 1993). The molecular basis of this difference is not clear, but it may be due to the adventitious oxidation of free sulfhydryl groups.

CELLULAR SOURCES AND TISSUE EXPRESSION

Cellular sources that produce

Although MIF activity was originally identified as a secreted product of activated T cells, significant quantities of MIF protein have been detected in a number of different tissues and cell types (**Table 2**). In many cases, MIF expression is regulated by pro-inflammatory or mitogenic stimuli.

RECEPTOR UTILIZATION

A classical receptor for MIF has not yet been identified, however there have been preliminary reports of putative binding proteins/factors that may mediate the activity of crude MIF preparations.

Over 10 years ago, Liu and coworkers (1985) reported findings that implicated a glycolipid 'receptor' for MIF activity on the surface of human monocytes. Pretreatment of monocytes with fucosidase or neuraminidase, an exoglycosidase specific for sialic acid, eliminated the inhibition of migration activity by crude MIF-containing extracts. This work predated the molecular cloning of MIF and no further characterization of the molecules involved in this effect has been reported.

Several reports indicate that MIF specifically interacts with the sialic acid-binding protein sarcolectin (Kayser and Zeilinger, 1993; Zeng *et al*., 1994). Sarcolectin has been determined since to be indistinguishable from a subfraction of human albumin.

Table 2 Tissue/cellular distribution of MIF protein

Cell type	Stimuli	References
Anterior pituitary		
Corticotropic cells	CRF, LPS	Bernhagen *et al.*, 1993; Nishino *et al.*, 1995
Immune system		
Monocytes/macrophages	LPS, TNFα, IFNγ, glucocorticoids	Calandra *et al.*, 1994
	TSST-1, exotoxin A	Calandra *et al.*, 1998
T cells (TH2> TH1), mast cells	αCD3, PMA/ionomycin, PHA	Bacher *et al.*, 1996; Chen *et al.*, 1998
Eosinophils	PMA, C5a, IL-5	Rossi *et al.*, 1998
HL-60, myelomonocytic	LPS	Nishihira *et al.*, 1996
Adrenal gland		
Cortex–zona glomerulosa, zona fasciculata	LPS	Bacher *et al.*, 1997
Lung		
Bronchial epithelium	LPS	Bacher *et al.*, 1997
Alveolar macrophages		Donnelly *et al.*, 1997
Kidney		
Tubule epithelial cells, proximal tubules	LPS	Imamura, 1996
Glomerular epithelial cells, endothelium, Kupffer cells	LPS	Lan *et al.*, 1996
Tubular epithelial cells	LPS	Lan *et al.*, 1998
Mesangial cells	LPS, PDGF-AB, IFNγ	Tesch *et al.*, 1998
Liver		
Hepatocytes surrounding central veins, Kupffer cells	LPS	Bacher *et al.*, 1997
Skin		
Keratinocytes, sebaceous glands, outer root sheath of hair follicle, epidermal layer, endothelial cells	LPS, croton oil	Shimizu *et al.*, 1996, 1999a
	UV B	Shimizu *et al.*, 1999
	Acute inflammation	Shimizu *et al.*, 1997
	Acute inflammation	Gomez *et al.*, 1990; Goebler *et al.*, 1991
Testes		
Leydig cells		Meinhardt *et al.*, 1996, 1998
Pancreas		
Islet β cells	Glucose	Waeber *et al.*, 1997
Eye		
Corneal epithelial cells		Wistow *et al.*, 1993
Endothelial cells, lens		Matsuda *et al.*, 1996a
Iris, ciliary epithelium		Matsuda *et al.*, 1996b
Brain		
Cortex, hypothalamus	LPS	Bacher *et al.*, 1998

Table 2 (*Continued*)

Cell type	Stimuli	References
Cerebellum–neurons		Nishibori *et al*., 1996
Glial cells, ependyma, astrocytes		Suzuki *et al*., 1999
Telecephalon		
Bone		
Neonatal calvaries and osteoblasts, cancellous bone	LPS	Onodera *et al*., 1996
Fat tissue		
3T3L1 adipocytes	TNFα	Hirokawa *et al*., 1997, 1998
Prostate		
Epithelial cells		Frenette *et al*., 1998; Meyer-Siegler, 1998
Vasculature		
Endothelial cells	LPS	Nishihira *et al*., 1998; Chesney *et al*., 1999

Active investigations are presently underway to isolate and characterize the cell surface (or soluble) binding proteins and/or receptors involved in MIF actions. Given that MIF defines a new structural superfamily, it is possible that the cell surface receptor will also be of a new class.

IN VITRO ACTIVITIES

In vitro findings

See Table 1.

Macrophage Activity

MIF was identified historically as a soluble lymphocyte factor that inhibited the random migration of macrophages. This activity has been confirmed with purified recombinant human and mouse MIF. The effect on macrophages follows a bell-shaped dose response with peak activity at ~ 100 ng/mL. MIF also promotes nitric oxide (NO) release from IFNγ-primed macrophages (Calandra *et al*., 1994), and enhances phagocytosis (Onodera *et al*., 1997) and killing of intracellular pathogens, such as Leishmania (Juttner *et al*., 1998).

Glucocorticoid Regulation: Monocytes/ Macrophages and T Cells

Many of the proinflammatory effects of MIF appear to be due to MIF's unique ability to 'override' or counter-regulate glucocorticoid inhibition of immune cell activation and proinflammatory cytokine production. This response follows a bell-shaped dose response profile with respect to glucocorticoid concentrations, and high, anti-inflammatory levels of glucocorticoids ($\geq 10^{-6}$ M) suppress MIF release. Of importance, glucocorticoids in physiological amounts induce the release of MIF from macrophages and T cells. The observation that glucocorticoids induce the release of a 'proinflammatory' factor led to the hypothesis that the primary role of MIF might be to regulate the anti-inflammatory effects of glucocorticoids. This was verified experimentally by Calandra and coworkers (1994), who demonstrated that the addition of rMIF to cultured monocytes could override in a dose-dependent manner, glucocorticoid inhibition of TNFα, IL-1β, IL-6, and IL-8 secretion.

Similarly, when primed human T cells were incubated with either mitogen or antigen plus glucocorticoid, MIF was found to override in a dose-dependent manner the glucocorticoid-mediated suppression of T cell proliferation and cytokine (IL-2) release (Bacher *et al*., 1996). The counter-regulatory activity of MIF in glucocorticoid-mediated immunosuppression for both macrophages and T cells has been verified *in vivo* (see *In vivo* biological activities).

Cell Proliferation

The role of MIF on cell proliferation *in vitro* has been examined by several groups using different cell types/ lines, including colon 26 cells (Takahashi *et al*., 1998), G361 melanoma cells (Shimizu *et al*., 1999b), primary

microvascular endothelial cells (Chesney *et al*., 1999), and NIH 3T3 fibroblast cells (Mitchell *et al*., 1999). In both colon 26 and G361 melanoma cells, transfection with an MIF antisense plasmid resulted in a significant decrease in cell proliferation when compared to cells transfected with empty vector. Chesney and coworkers showed that primary endothelial cells exhibited decreased proliferation after treatment with either anti-MIF antibody or MIF antisense transfection (Chesney *et al*., 1999). In these two model systems, the mechanism by which MIF regulates cell proliferation is not known. However, the molecular pathway by which MIF controls cell proliferation was recently explored using the NIH 3T3 cell line. Both endogenously secreted (as shown by neutralizing MIF antibody addition) and exogenously added rMIF stimulate the proliferation of NIH 3T3 cells (Mitchell *et al*., 1999) and this response was associated with the sustained activation of the p42/p44 extracellular signal-regulated (ERK) MAP kinases. This MIF-induced activation was dependent upon protein kinase A activity.

Recently, Hudson and coworkers (1999) revealed that MIF could inhibit the transcriptional activity of p53, a potent tumor suppressor that normally functions to prevent proliferation of tumor cells. Further studies showed that treatment of primary mouse fibroblasts with recombinant MIF significantly prolonged their lifespan.

Enzymatic Activities

Probably one of the most unusual properties uncovered for MIF is its ability to catalyze keto-enol isomerization (i.e. tautomerization) reactions. While investigating enzymes that could effect the tautomerization of L-dopachrome (2-carboxy-2,3-dihydroindole-5,6-quinone), a melanin precursor, Rorsman and coworkers identified a protein that catalyzed the tautomerization of the non-natural D-isomer (D-dopachrome) to 5,6-dihydroindole-2-carboxylic acid (Rosengren *et al*., 1996). N-terminal sequencing of this protein suggested it to be the bovine homolog of MIF. This isomerization activity was subsequently confirmed by testing human rMIF in the D-dopachrome tautomerization assay. Around the same time, analysis of the MIF crystal structure revealed structural homology with two bacterial isomerases, 4-oxalocrotonate tautomerase (4-OT) and carboxymethyl-2-hydroxymuconate isomerase (CHMI) (Subramanya *et al*., 1996). This has presented the possibility that certain biological actions of MIF may occur via an enzymatic reaction. Further efforts to identify a physiological substrate for MIF revealed that it could catalyze the enolization of phenylpyruvate and the ketonization of *p*-hydroxyphenylpyruvate (Rosengren *et al*., 1997). These molecules are products of phenylalanine and tyrosine degradation, however the K_m values for these substrates suggest that this reaction is unlikely to occur physiologically. The possibility remains that a naturally occurring substrate(s) exists, but is yet unidentified. Bernhagen and coworkers also have reported that MIF, which contains three cysteine residues, may act as an oxidoreductase to reduce disulfide bonds in model substrates such as insulin (Kleemann *et al*., 1998).

Bioassays used

Several assays have been developed used to measure MIF 'bioactivity' (*see* **Table 3**).

IN VIVO BIOLOGICAL ACTIVITIES OF LIGANDS IN EXPERMENTAL ANIMAL MODELS

Normal physiological roles

With the molecular cloning of MIF, its preparation in pure, bioactive form, and the development of neutralizing monoclonal antibodies, a number of

Table 3 Bioassays for MIF

Bioassay	References
Macrophage migration	George and Vaughn, 1962; Bernhagen *et al*., 1994
Glucocorticoid overriding – macrophages and T cells	Calandra *et al*., 1995; Bacher *et al*., 1996
Induction of phosphofructokinase 2 (PFK2) activity	Begnini *et al*., in preparation
Proliferation of NIH 3T3 cells	Mitchell *et al*., 1999

regulatory activities for MIF have been uncovered *in vivo* using animal models.

Circulating Mediator Secreted in Response to Inflammatory Stimuli and Hypothalamic-Pituitary-Adrenal (HPA) Axis Activation

The first *in vivo* studies utilizing purified MIF protein and neutralizing antibodies in rodents showed that the pituitary release of MIF is an integral part of the host's systemic stress response. Rodents subjected to the physiological stress of handling showed an increase in circulating MIF levels, which coincided with increases in plasma ACTH and corticosterone. When mice received an intraperitoneal injection of endotoxin, there was a dramatic decrease in the pituitary content of MIF protein, a concomitant rise in plasma MIF levels and a slower, time-dependent increase in the expression of pituitary MIF mRNA (Bernhagen *et al*., 1993; Calandra *et al*., 1995). Purified rMIF showed proinflammatory activities. MIF was found to potentate the toxic response to endotoxin when coinjected with LPS into mice. Conversely, neutralizing anti-MIF antibodies protected mice from a lethal dose of LPS, demonstrating that MIF, like TNFα, IL-1, and IFNγ, plays a critical role in the inflammatory network leading to endotoxic shock and death. These results were confirmed by using MIF knockout mice. MIF$-/-$ mice were resistant to the lethal effects of high-dose bacterial LPS, and of enterotoxin B after D-galactosamine-sensitization (Bozza *et al*., 1999).

In addition to its role in host response to gram-negative (LPS) (Bernhagen *et al*., 1993) and gram-positive (TSST-1 and SPEA) (Calandra *et al*., 1995), MIF has been shown to mediate inflammation during the development of experimental *peritonitis* (Calandra *et al*., 2000). This recent study has shown that administration of anti-MIF antibodies protected TNFα knockout and wild-type mice from lethal peritonitis, whereas recombinant MIF markedly enhanced lethality.

MIF: Glucocorticoid Counter-regulator

The counter-regulatory activity of MIF in glucocorticoid-mediated immunosuppression also was examined *in vivo*. These studies demonstrated that the administration of rMIF together with dexamethasone in mice completely blocked the protective effects of dexamethasone on LPS lethality (Calandra *et al*., 1995).

Later studies established that T cells also secrete MIF in response to glucocorticoids and that once released, MIF can override the immunosuppressive effects of steroids on T cell activation and cytokine (IL-2, IFNα) production (Bacher *et al*., 1996). The role of MIF/glucocorticoid regulatory system on T cell responses *in vivo* was shown by administering neutralizing MIF antibodies to mice at the time of immunization with soluble antigen. Anti-MIF significantly inhibited the development of both antigen-specific T cells and the primary antibody response – an effect that was attributed to the increased immunosuppressive effects of endogenous glucocorticoids after neutralization of endogenous MIF.

Delayed-type Hypersensitivity (DTH)

By the 1960s, MIF 'activity' was believed to be expressed by T cells and to mediate macrophage accumulation in the *delayed-type hypersensitivity* (DTH) reaction (Bloom and Bennett, 1966; David, 1966). The central role of MIF in DTH was recently verified in a study of tuberculin-DTH in mice, where anti-MIF was found to specifically block this response (Bernhagen *et al*., 1996). Interestingly, the macrophage was found to be a significant source of the MIF expressed in these lesions.

Glomerulonephritis (Anti-Glomerular Basement Membrane Disease; αGBM)

Macrophage activation and accumulation in renal tissue contributes to the inflammatory pathology of *glomerulonephritis*, frequently leading to the hallmark lesion of the glomerular crescent. The expression of MIF by the intrinsic kidney cells and the endothelium was found to increase during the development of rat *antiglomerular basement membrane glomerulonephritis* (αGBM), a well-characterized animal model of macrophage-mediated renal injury (Lan *et al*., 1996). Macrophage accumulation was observed in the areas of highest MIF expression (Bowman's space) and was associated with crescent formation. Glomerular MIF expression during the progression of αGBM glomerulonephritis also correlated with increased urinary protein excretion and decreased creatinine clearance. Further experiments have examined the role of neutralizing anti-MIF antibodies in rat *glomerulonephritis* (Lan *et al*., 1997). Animals treated with a neutralizing anti-MIF monoclonal antibody had significantly reduced proteinurea, enhanced renal function, reduced histological renal damage, and substantially decreased renal leukocytic infiltration and activation compared to animals treated with an isotypic control antibody. The role of MIF in suppressing rat crescentic glomerulonephritis appeared to be related to its ability to inhibit cell-based mechanisms of tissue injury.

Arthritis

In joints obtained from rodents with *collagen-induced arthritis* (Mikulowska *et al*., 1997) and *adjuvant-induced arthritis* (Leech *et al*., 1998) MIF expression was increased in the synovial lining cells when compared to control animals. MIF was detected in the infiltrating macrophages (and to a lesser extent in T cells), as well as in endothelial cells surrounding the areas of inflammation. Depletion of MIF by the administration of neutralizing anti-MIF antibodies in rodents led to a dramatic delay in the time of *arthritis* onset and markedly reduced the severity of the disease, as determined by various immunopathology scores (Mikulowska *et al*., 1997; Leech *et al*., 1998).

Tumor Growth/Angiogenesis

Neutralizing anti-MIF antibodies have been found to reduce significantly the growth and the vascularization of the mouse 38C13 B cell lymphoma *in vivo* (Chesney *et al*., 1999). MIF was expressed predominantly in the tumor-associated neovasculature. Cultured microvascular endothelial cells, but not 38C13 B cells, were observed both to produce MIF and to require its activity for proliferation *in vitro*. In addition, the administration of anti-MIF antibody to mice was found to significantly inhibit the neovascularization response elicited by Matrigel implantation, a model of new blood vessel formation *in vivo*. Additional investigations by Shimizu and coworkers (1999b) have shown that immunoneutralization of MIF also inhibited tumor-associated angiogenesis using the G361 melanoma model in mice. These data indicate that MIF plays an important role in tumor angiogenesis and suggest a potential new target for the development of antineoplastic agents that inhibit tumor neovascularization.

Malaria

Macrophages secrete large quantities of MIF protein after phagocytosis of parasitized erythrocytes, or *malaria* pigment (hemazoin). In the *Plasmodium chabaudi* model of malaria infection in mice, increased levels of circulating MIF coincide which red blood cell parasitemia and disease severity (Martiney *et al*., 1996, 2000). Recent studies indicate that rMIF has a direct inhibitory effect on the formation of erythrocyte progenitors *in vitro*. MIF may play an important role in the defective red blood cell production that frequently accompanies malaria infection. In fact, MIF fulfils the biochemical criteria for the long sought after circulating factor that mediates bone marrow suppression in this disease (Martiney *et al*., 2000).

Species differences

No functional differences in MIF bioactivity have been reported between human and rodent MIF in different experimental systems.

Knockout mouse phenotypes

An MIF knockout mouse (MIF$-/-$) was successfully produced in 1999 (Bozza *et al*., 1999). Analysis of the role of MIF during experimental *septic shock* showed that the MIF$-/-$ mice were resistant to the lethal effects of high-dose bacterial LPS, or of enterotoxin B in D-galactosamine-sensitized mice. In addition, the MIF$-/-$ mice exhibited lower plasma levels of TNFα and NO (but not IL-6 and IL-12) compared to wild-type animals. MIF$-/-$ mice also cleared pulmonary *Pseudomonas aeruginosa* that had been instilled into their tracheas more efficiently and exhibited lower neutrophil accumulation in bronchoalveolar fluids when compared to the wild-type mice.

Interactions with cytokine network

The discovery that the macrophage is a significant source of MIF protein *in vivo* prompted investigations into the regulation of MIF expression by this cell type. Interestingly, the amount of LPS required to induce MIF protein release and increased MIF mRNA expression was found to be 10–100-fold less than the amount required to induce TNFα expression. MIF secretion from macrophages was also induced by TNFα and IFNγ, but not by IL-1β or IL-6. MIF promotes TNFα and NO production by macrophages. These data suggest that TNFα and MIF might act in a mutually stimulatory re-entrant loop to propagate the inflammatory cascade. In T cells, MIF expression is required for the production of IL-2 and IFNγ.

Endogenous inhibitors and enhancers

No endogenous inhibitors or enhancers have yet been identified; however, MIF does have appreciable binding activity toward long-chain fatty acids (Bendrat *et al*., 1997).

PATHOPHYSIOLOGICAL ROLES IN NORMAL HUMANS AND DISEASE STATES AND DIAGNOSTIC UTILITY

Normal levels and effects

Circulating MIF levels in normal individuals or rodents vary between 2 and 6 ng/mL. These levels increase significantly as a consequence of systemic inflammatory stimulation. Normal circulating levels of MIF exhibit glucocorticoid counter-regulating activity *in vitro*, and normal circulating glucocorticoid levels induce MIF secretion from immune cells. These findings suggest that the baseline state of the MIF/glucocorticoid dyad is an active balance between 'proinflammation' and 'anti-inflammation'. The ensuing course of the inflammatory or immune response thus would be the result of the interaction between MIF and glucocorticoids that are present in a lymph node, inflammatory lesion, or site of tissue invasion.

Role in experiments of nature and disease states

Adult Respiratory Distress Syndrome (ARDS)

ARDS is often considered to be an archetype for a clinical response in which the dynamic balance within the immune response shifts toward excessive inflammation and tissue destruction. MIF is expressed in both type II alveolar cells and infiltrating cells. MIF levels in the broncheoalveolar lavage fluids (BALs) of ARDS patients were found to be significantly elevated when compared with those from control subjects (Donnelly *et al*., 1997). Human rMIF enhances both TNFα and IL-8 secretion from ARDS alveolar macrophages (*ex vivo*) when compared to control cells. Pretreatment of these cells with anti-MIF antibodies significantly decreased TNFα and IL-8 production from ARDS alveolar cells. Moreover, rMIF was found to override, in a concentration-dependent fashion, glucocorticoid-mediated inhibition of cytokine secretion in ARDS macrophages. These were the first data to indicate that the MIF/glucocorticoid dyad is active in cells that had undergone proinflammatory activation *in vivo* during human disease. Significantly elevated levels of alveolar MIF were found in those at-risk patients who progressed to ARDS compared to those who did not. MIF may act as an important mediator to promote and sustain the pulmonary inflammatory response in ARDS. Its prominent expression in ARDS may explain the fulminate course of this disease and perhaps why glucocorticoid treatment has proven disappointing in established cases of this disease.

Based on the observation that MIF levels were elevated in the BALs of ARDS patients when compared with controls, further studies examined MIF levels in BALs obtained from asthmatic patients. Significantly elevated alveolar MIF levels were found in the BAL fluids of asthmatics when compared to controls (Rossi *et al*., 1998).

Arthritis

Synovial fluid obtained from the joints of patients with *rheumatoid arthritis* contain significantly greater levels of MIF than those obtained from patients with osteoarthritis or normal control subjects (Metz and Bucala, 1997; Onodera *et al*., 1999). As revealed by immunohistochemical staining methods, the synovial lining (Leech *et al*., 1999) and infiltrating mononuclear cells within the human arthritic joint are the primary source of MIF (Onodera *et al*., 1999).

Eye Disease

The average MIF levels in the sera of *uveitis* patients are elevated, when compared with control subjects (Kitaichi *et al*., 1999). Serum MIF levels are particularly high in patients with *Behçet's disease* at the ocular exacerbation stage and patients with *sarcoidosis* at the severe uveitis stage (Kitaichi *et al*., 1999). In addition, significantly elevated levels of MIF were reported in the vitreous fluid of patients with both proliferative vitreo-retinopathy and rhegmatogenous retinal detachment when compared with control patients (macular hole or idiopathic epiretinal membrane) (Mitamura *et al*., 1999).

Atopic Dermatitis

Atopic dermatitis is a chronic pruritic inflammatory skin disorder. Its pathogenesis, in part, is thought to be due to dysregulated cytokine production by peripheral mononuclear cells. In lesions from patients with atopic dermatitis, MIF protein is diffusely distributed throughout the entire epidermal layer with increased expression by keratinocytes (Shimizu *et al*., 1996). In normal skin, MIF has mainly been localized to human epidermal ketatinocytes. Serum MIF levels of atopic dermatitis patients were 6-fold higher than those of control subjects. In addition, serum MIF levels in atopic dermatitis patients decreased as clinical features improved, suggesting that MIF may play a pivotal role in the inflammatory response in the skin during atopic dermatitis.

IN THERAPY

Preclinical – How does it affect disease models in animals?

Neutralizing anti-MIF antibodies have been found to be extremely effective in numerous animal models of inflammatory and autoimmune diseases. Interestingly, anti-MIF is effective in models considered to be primarily TNFα (macrophage) or IL-2 (T cell) driven. These situations include *gram-negative shock*, *gram-positive shock*, *delayed-type hypersensitivity*, *anti-rat GBM disease*, *collagen arthritis*, *adjuvant arthritis*, *lupus* (NZB/NZW mice), and tumor angiogenesis.

Anti-MIF

A humanized, neutralizing anti-MIF antibody is presently in preclinical development by IDEC Pharmaceuticals (San Diego, CA, USA) and Cytokine Networks Inc. (Seattle, WA, USA). It is anticipated that MIF neutralization could have broad clinical utility in autoimmune and inflammatory diseases, particularly in those associated with resistance to steroid therapy. Neutralizing the endogenous MIF response and MIF's counter-regulatory effects on steroids should allow the endogenous adrenal glucocorticoid response to be more efficacious in suppressing the host's inflammatory and immune responses. Accordingly, this approach could be highly effective in decreasing or eliminating the pharmacological requirement for steroid therapy, and reducing the often toxic effects of glucocorticoids.

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