Macrophage migration inhibitory factor: molecular, cellular and genetic aspects of a key neuroendocrine molecule

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Abstract

The immunological and neuroendocrine properties of macrophage migration inhibitory factor (MIF) are diverse. In this article we review the known cellular, molecular and genetic properties of MIF that place it as a key regulatory cytokine, acting within both the innate and adaptive immune responses.

The unexpected and paradoxical induction of MIF secretion by low concentrations of glucocorticoids is explored. The role of MIF as a locally acting modulator of glucocorticoid sensitivity within foci of inflammation is also discussed. MIF has no homology with any other pro-inflammatory cytokine and until recently lacked a recognised transmembrane receptor. MIF has also been shown to be directly taken up into target cells and to interact with intracellular signalling molecules, including the Jun activation domain-binding protein Jab-1.

Comprehensive analysis of the MIF gene has identified important functional polymorphisms and a series of genetic studies has revealed both association and linkage of MIF with inflammatory diseases. Altered MIF regulation may therefore be pivotal to acquiring chronic inflammation following an innate immune response.

Background

Many aspects of macrophage migration inhibitory factor (MIF) have proved intriguing to researchers over the course of the past 40 years. MIF was originally identified, in 1966, by two groups of researchers studying delayed-type hypersensitivity, as an activity isolated from guinea pig T lymphocytes that was capable of inhibiting the random migration of macrophages (Bloom & Bennett 1966, David 1966). However, these early experiments used crude conditioned media contaminated with other factors known to affect macrophage movement (interleukin (IL)–4 and interferon–\(\gamma\)).

The human MIF cDNA was finally cloned in 1989 (Weiser \textit{et al}. 1989), and its genomic localisation to chromosome 22q11·2 later mapped (Budarf \textit{et al}. 1997). Studies using pure recombinant human MIF and specific neutralising antibodies have shown MIF to be a potent pro-inflammatory cytokine, and a key modulator of immune and inflammatory responses.

An endocrine identity for MIF was proposed when MIF was found in pituitary corticotroph cells, appeared to be in the same dense-core neurosecretory granules as adrenocorticotropic hormone (ACTH), and was proposed to be co-secreted with ACTH (Nishino \textit{et al}. 1995). It was proposed that the circulating pool of MIF was derived, to a significant degree, by pituitary secretion, under the control of hypothalamic corticotrophin-releasing hormone. MIF expression was also found in pituitary thyrotroph cells. In addition, it was reported that MIF secretion from macrophages was induced by very low concentrations of glucocorticoid (dexamethasone or hydrocortisone at 10\(^{-14}\) M) in a rodent cell line (Calandra \textit{et al}. 1995). As MIF was shown to oppose glucocorticoid inhibition of pro-inflammatory cytokine secretion, MIF was proposed to function both as a systemically acting, ‘endocrine’ cytokine, and also as part of a locally acting, short-feedback loop limiting the anti-inflammatory actions of glucocorticoids.

More recently, careful studies in human volunteers have failed to demonstrate the same mode of regulation as proposed from the rodent studies. ACTH secretogogues did not alter serum MIF concentrations, and neither did exogenous glucocorticoids (Isidori \textit{et al}. 2002).
The human MIF gene

The genomic organisation of the human MIF gene was reported by Paralkar & Wistow in 1994. They described a ‘remarkably small’ gene of less than 1 kb, identified from the screening of a human placenta genomic library. The human MIF gene has three exons of 205, 173 and 183 bp. These are separated by two introns of 189 and 95 bp (EMBL ID: Homo Sapiens Macrophage Migration Inhibitory Factor (HSMIF) http://www.ebi.ac.uk/cgi-bin/emblfetch/).

Only a single functional MIF gene exists in humans, unlike in mice where several processed (intronless) pseudogenes have been described (Bozza et al. 1995, Kozak et al. 1995, Mitchell et al. 1995). The MIF gene of other mammals is similarly short and present in one expressed copy per haploid genome.

The clones sequenced by Paralkar & Wistow (1994) identified 250 bp of 3' untranslated region and 1 kb of 5' flanking region. Primer extension and 5' rapid amplification of cDNA ends were used to map the transcription start site, and identified a single site 97 bp upstream from the initiator methionine, in a TATA-less promoter (Paralkar & Wistow 1994). These approaches cannot exclude the presence of additional transcription start sites, and the anatomy of the MIF promoter, consisting of abundant GC content, and no TATA box, would suggest the presence of multiple transcription start sites. However, analysis of mRNA harvested from multiple tissues, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas, by Northern blot analysis identified a single transcript (of approximately 800 nucleotides). Certain tissues, such as kidney and brain, showed high MIF expression, whilst others, including muscle and pancreas, had relatively low expression (Paralkar & Wistow 1994).

Polymorphism of the human MIF gene

The presence of raised MIF protein concentrations within the serum, plasma or tissue in several diseases of an endocrine or chronic inflammatory basis (Niino et al. 2000, Yabunaka et al. 2000, de Jong et al. 2001, Matsumoto & Kannatusu 2001, Murakami et al. 2001, Donn et al. 2002, Meazza et al. 2002, Morand et al. 2002, Sakai et al. 2003), suggests a role for MIF in pathogenesis. Alternatively, increased expression of MIF in these sites could be consequential, rather than causative. In an attempt to address this conundrum, variation of the MIF gene was determined. The identification of any polymorphic variants of MIF would allow future comparisons of specific combinations of these genetic changes (or genotypes) with diseased and non-diseased individuals to be undertaken. If a genetic basis to altered endogenous MIF protein production were found to underlie the disease states this would be important evidence for a central contributory role of MIF in the pathogenesis of the disease.

Donn et al. (2002) used denaturing high performance liquid chromatography (WAVE machine, Transgenomic, Crewe, UK) to look for variations across the whole of the MIF gene and 1 kb of the 5' flanking region. This screen was performed in 32 healthy, normal UK Caucasian individuals and in 96 children with juvenile idiopathic arthritis (JIA), a chronic inflammatory disease in which raised MIF protein concentrations had been described (Meazza et al. 2002). Four polymorphic positions were found. These were all seen both in the healthy volunteers and subjects with JIA. The CATT repeat element, originally documented by Paralkar & Wistow (1994), was found to be polymorphic with five to eight alleles existing. The eight-allele repeat is found at only very low allele frequency in UK Caucasians. Also, within the 5' flanking region, a single nucleotide polymorphism (SNP) at nucleotide position −173(G to C) (relative to HSMIF) was seen. Two intronic polymorphisms at nucleotide positions+254(T to C) and at +656(C to G) were also identified. These polymorphic positions and the allele frequencies taken from the study of 342 UK healthy controls are shown in Fig. 1. Baugh et al. (2002), having sequenced the 5' flanking region of MIF in six normal individuals and six rheumatoid arthritis patients, also described the tetranucleotide CATT repeat element which begins at position −794.

Linkage disequilibrium and haplotype tagging of MIF SNPs in UK Caucasians

Where more than one SNP has been defined in a gene it is important to study the extent of linkage disequilibrium (allelic association) that exists. This can be informative as it potentially allows the study of haplotypes, or chromosomal segments that are transmitted en bloc. This should be determined within a control population initially and then reassessed for any disease group studied.

Furthermore, this determines how informative particular SNPs to be informative by predicting a haplotype containing SNPs at other positions. This results in less genotyping, so reducing both the cost and the through-put time. The SNPs that allow the largest number of haplotypes to be captured by the minimum number of SNPs are called haplotype tagging SNPs or htSNPs (Johnson et al. 2001).

Evidence for strong linkage disequilibrium was found for the four polymorphic positions of MIF in the UK Caucasian population investigated by Donn et al. (2002), specifically for the common alleles at each polymorphic position.

htSNPs of MIF have been determined in the UK Caucasian controls and are shown in Table 1. This indicates that, in any study using UK Caucasian as controls, 93.1% of the MIF haplotypes occurring at a frequency of >1% can be determined by genotyping just the CATT and the +656 polymorphisms.
Genetic polymorphism of MIF and association with disease

Following the description of genetic polymorphism of MIF a number of disease association studies have been described. Baugh et al. (2002) showed that the short CATT repeat (CATT5) was associated with less severe rheumatoid arthritis in a cohort of hospital-derived patients from Wichita, KA, USA.

Barton et al. (2003) found an association of a specific MIF promoter haplotype composed of CATT7-MIF-173*C and susceptibility to adult inflammatory polyarthritis. The majority of this cohort developed rheumatoid arthritis. When outcome measures of disease severity were considered however, no association with MIF polymorphisms were seen (Barton et al. 2003). These differences may be explained by variation in case ascertainment but, taken together, a pathogenic role for MIF is supported. The same promoter haplotype (CATT7-MIF-173*C) has been shown to be both linked and associated with JIA (Donn et al. 2004). JIA is the commonest chronic arthritis to commence before the age of 16 years. It encompasses seven clinically distinct subgroups, all of which share the feature of chronic synovitis (Petty et al. 1998).

The MIF-173*C polymorphism has been shown to be positively associated with sarcoidosis in biopsy-proven erythema nodosum in a Spanish population (Amoli et al.

Table 1 MIF haplotype frequencies and haplotype tagging SNPs in UK Caucasian controls

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<tr>
<th>CATT*</th>
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<th>MIF+254</th>
<th>MIF+656* n</th>
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*htSNPs. htSNPs were investigated in 342 normal Caucasian individuals. Only haplotypes occurring at a frequency of >1% in this population were considered (n=318). Of these 93.1% can be assigned by genotyping for both the CATT repeat and the MIF+656 polymorphism only. The haplotypes captured are shown in bold.
2002). This same promoter polymorphism of MIF has also been shown to be predictive of disease outcome in patients with systemic onset JIA, a particularly severe onset type of JIA (De Benedetti et al. 2003a). More specifically, De Benedetti et al. (2003a) found that carriage of the MIF-173*C polymorphism was correlated with raised serum and synovial fluid levels of MIF protein, and to be predictive of the duration of response to intra-articular injection of triamcinolone hexacetonide (TXA). Those individuals with a mutant allele at position −173 (MIF-173*C) relapsed more quickly than individuals with the MIF-173*G wild-type genotype (De Benedetti et al. 2003a). This effect has now also been demonstrated for the response to intra-articular TXA injection in patients with an oligoarticular phenotype (≤ four joints affected at presentation), the commonest presentation of JIA. Again, the duration of clinical response to the steroid treatment (months with no clinical evidence of synovitis) was significantly shorter in patients carrying a MIF-173*G allele (median 6 months; range 1–39) than in the MIF-173*GG homoygotes (median 9 months, range 2–62) (De Benedetti et al. 2003b).

Promoter activity of MIF and the significance of the known polymorphic variants

Two groups (Baugh et al. 2002, Donn et al. 2002, 2004) have confirmed the putative promoter identified by Paralkar & Wistow (1994) in several cell lines. Baugh et al. (2002) described variation in reporter gene luciferase activity for the different CATT alleles, with the CATT5 allele being shown to have the lowest level of basal and stimulated MIF-promoter activity in human lung epithelial (A549) and fibroblast cell lines. Donn et al. (2002) have shown that the MIF-173G/C polymorphism regulates promoter activity and that higher reporter gene luciferase activity is found for the MIF-173*C allele in the human T lymphoblast cell line (CEMC7A), whilst in human lung epithelial cells (A549) MIF-173*G allele has higher luciferase promoter activity. Importantly, there is a functional interaction between the CATT repeats and the −173 SNP. Constructs of MIF-173*C with CATT5, 6 and 7 length repeats and, similarly, a series of constructs with MIF-173*G together with CATT5, 6 and 7 length repeats were studied. Here, again, a cell type-specific difference was found. In a T lymphoblast cell line increasing CATT repeat with the MIF-173*C polymorphism significantly increased the luciferase promoter activity. No effect of CATT repeat length with the MIF-173*G was seen. In the A549 cells again increasing CATT repeat length significantly increased luciferase activity of the MIF-173*C constructs. However, increasing CATT repeat length reduced the luciferase activity of the MIF-173*G constructs (Donn et al. 2004). This work suggests that different transcription factors, occurring within different cell types by binding to the CATT and MIF-173*G/C polymorphic sites, alter MIF promoter activity (Fig. 2). Such cell type-specific interactions with naturally occurring gene promoter polymorphisms are well described, for example in prolactin, IL-10 and IL-6, and likely reflect the differential expression of transcription factors, and co-factors between cell types (Fishman et al. 1998, Stevens et al. 2001, Kay et al. 2002).

In addition to basal promoter activity, MIF promoter regulation has also been studied. There are sequences within the proximal promoter with resemblance to cAMP response elements, and indeed forskolin has been shown to stimulate the activity of both endogenous and transfected MIF genes in the mouse pituitary cell line, AtT20 (Waebier et al. 1998). However, analysis of the regulatory elements required for tissue-specific, and appropriately regulated MIF transcription, and the influence of gene polymorphism on such expression is still lacking. In particular, the peculiar mode of glucocorticoid up-regulation of MIF expression requires explanation, and is of major clinical importance as glucocorticoids are widely used to treat human inflammatory disease.

It is increasingly recognised that important gene regulatory elements can map at distant sites both 5′ and 3′ to the coding region of the gene (Nobrega et al. 2003, Ho et al. 2004). Furthermore, the chromatin environment of different cell types could dramatically influence the amount of MIF protein produced. It will therefore be necessary to look comprehensively at the genomic region surrounding MIF, for such regulatory sites, and to study them within a native chromatin environment.

MIF protein

The product of the MIF gene is a small 115 amino acid protein of molecular mass 12.5 kDa. The protein is highly conserved and homologues exist in the plant Arabidopsis thaliana, nematodes and vertebrates (Esumi et al. 1998). The sequence homology between human and mouse MIF is 89%. MIF has no significant sequence homology to any
other protein. Also, the three-dimensional structure of MIF is unlike any other cytokine or pituitary hormone. The only proteins that share partial structural similarity with MIF are the enzymes D-dopachrome tautomerase (Sugimoto et al. 1999), found in humans (and mapping close to MIF on chromosome 22q11·2), and 4-oxalocrotonate tautomerase, 5-carboxymethyl-2-hydroxymuconate isomerase and chorismate mutase, of microbial origin (Chook et al. 1994, Subramanya et al. 1996).

The crystal structure of MIF, to 1·8 A resolution, showed MIF to exist as a trimer (Sugimoto et al. 1996, Sun et al. 1996, Suzuki et al. 1996, Taylor et al. 1999). However, it is currently not known whether this represents the true, physiological state of MIF protein, as studies have suggested bioactive MIF monomers or dimers (Mischke et al. 1998). Each monomer of MIF is known to be composed of the following: two antiparallel α-helices (α1 and α2) and six β strands (β1–β6). Four of the six β strands (β1, β2, β4, β5) form a β-pleated sheet above which the two α-helices rise. This composition is very similar to the peptide-binding domain of a major histocompatibility complex molecule (Bjorkman et al. 1987). The remaining two β strands have been shown to interact with the β sheets of adjacent MIF subunits in a trimer. When formed as a trimERIC complex the three β sheets form a barrel containing a solvent-accessible channel which runs through the centre of the protein.

Mechanisms of MIF regulation and action

Glucocorticoid regulation of MIF secretion

The initial report of glucocorticoid induction of MIF secretion was exciting, as this was an extremely unusual mode of regulation for a pro-inflammatory cytokine (Calandra et al. 1995). A number of anti-inflammatory cytokines are known to be up-regulated by glucocorticoid, notably IL-10, but pro-inflammatory cytokines, e.g. tumour necrosis factor-α (TNFα) and IL-1 are typically suppressed (Elenkov & Chrousos 2002). Further analysis in rodents has suggested that organ MIF protein content was suppressed (Elenkov & Chrousos 2002). Further analysis in rodents has suggested that organ MIF protein content was suppressed (Elenkov & Chrousos 2002). Careful studies in human volunteers have confirmed the regulation of circulating MIF by glucocorticoids (Isidori et al. 2002), and this phenomenon requires further study to determine if it extends across the species barrier.

MIF appears to circulate in healthy volunteers at relatively high serum concentrations (2–6 ng/ml) and at such concentrations a direct effect of MIF on immune cells, or on glucocorticoid activity, might be expected. Intriguingly, a circadian rhythm of MIF has been described in humans, with a late morning peak (0700 h) coincidental with that of cortisol (Petrovsky et al. 2003). Much higher circulating concentrations of MIF are found in inflammatory diseases, and in response to experimental endotoxaemia or inflammation in animal models (reviewed in Fingerle-Rowson & Bucala 2001, Baugh & Bucala 2002, Gregersen & Bucala 2003).

MIF effects on glucocorticoid action

MIF is a pleiotropic molecule. It is a pro-inflammatory cytokine, an anterior pituitary hormone, and it possesses enzymatic activity. Therefore, several molecular pathways are likely to mediate its actions.

A number of studies have addressed the interaction between MIF and glucocorticoid action. Glucocorticoids suppress the increase in cytokine secretion from macrophages induced by lipopolysaccharide exposure, and exposure to MIF prevents this effect (Bernhagen et al. 1993, Calandra et al. 2000). In addition, MIF opposes the anti-proliferative effects of glucocorticoids on T lymphocytes, and similarly the glucocorticoid suppression of IL-2 from the same cells (Bacher et al. 1996). In vivo studies have shown that MIF opposes the anti-arthritic effects of glucocorticoids in animal models of arthritis (Mikulowska et al. 1997, Leech et al. 1998, 2000, Santos et al. 2001). The underlying mechanism for such antagonism is not known, but MIF is known not to affect the nerve factor kB (NFkB) signalling pathway. However, MIF does act to cause prolonged activation of ERK and p38 MAP kinases, which may result in molecular antagonism of the glucocorticoid receptor (GR), although no change in GR expression or affinity have been found (Daun & Cannon 2000).

In an attempt to identify intracellular proteins that interact with MIF, Kleemann et al. (2000) used full-length human MIF cDNA as a bait in a yeast two-hybrid screen against a human brain cDNA library. This identified the Jun activation domain-binding protein 1 (JAB-1). Co-immunoprecipitation and pull-down experiments confirmed the specific MIF–JAB-1 association. JAB-1 was originally described as a co-activator of the transcription factor AP-1. Subsequently, multiple actions of JAB-1 have been documented, including regulation of p21Kip1 degradation, binding to glucocorticoid and progesterone receptors and interaction with p53. Kleeman et al. (2000) used luciferase reporter and gel shift assays to show that endogenous and exogenous MIF inhibited JAB-1–induced AP-1 transcriptional activity, but did not interfere with NFkB activity. Similarly, recombinant MIF inhibited JAB-1-stimulated and TNF-induced MAP
kinase 8 activity. This action of MIF is counter-intuitive, as up-regulation of AP-1 activity is typically seen in response to pro-inflammatory cytokine action (Kleemann et al. 2000).

The MIF–JAB-1 complex was found to localise within the cytosol, near the peripheral plasma membrane. This complex was also found to occur following uptake of MIF into target cells by non-receptor-mediated pathways.

Mechanisms of MIF actions in monocytes/macrophages

MIF is known to lack a classic N-terminal leader sequence or an internal signal sequence for import into the endoplasmic reticulum. Furthermore, Flieger et al. (2003), using endotoxin-stimulated THP-1 monocytes, showed that MIF does not enter the endoplasmic reticulum and that MIF secretion occurs via a non-classical export route. Glyburide and probenicid, but no other typical inhibitors of the non-classical protein export, strongly inhibit MIF secretion. This indicates that the export of MIF involves an ABCA1 transporter (Flieger et al. 2003).

As the actions of MIF appear to involve interactions with intracellular proteins, recent studies identifying a transmembrane receptor for MIF are interesting. Leng et al. (2003), however, using expression cloning and functional analysis found CD74 (antigen-associated invariant chain II) to be a high-affinity membrane-binding protein for MIF. MIF was shown to bind to amino acids 109–149 of a recombinant soluble form of CD74 with a dissociation constant of $\sim 9 \times 10^{-9}$ kDa, as measured by surface plasmon resonance (BIAcore analysis). The binding appears necessary for the MIF-induced activation of the extracellular MAP kinase cascade, cell proliferation and prostaglandin E2 production (Leng et al. 2003).

Monocytes and macrophages constitutively express large quantities of MIF. This is unusual for a cytokine. When microbial pathogens bind to their recognition receptors, macrophages become activated and release a panoply of different cytokines that initiate the host’s innate and adaptive immune responses (Fig. 3). In an elegant series of experiments, Roger et al. (2001) showed that mouse macrophages transfected with antisense MIF mRNA, and macrophages from MIF$^{-/-}$ mice, were hyporesponsive to endotoxin (lipopolysaccharide) and gram-negative bacteria, as measured by TNF$\alpha$ and IL-6 production. The MIF-deficient cells were found to have reduced mRNA and protein expression of a specific toll-like receptor, toll-like receptor 4 (TLR4). Electromobility shift assays and promoter truncation/mutation analysis found that the lack of MIF expression impaired basal PU.1 transcription factor binding to the mouse TLR4 gene. This in turn reduced TLR4 protein expression and resulted in hypo-responsiveness to lipopolysaccharide and gram-negative bacteria. Inhibition of MIF activity may, therefore, be of therapeutic benefit for individuals with gram-negative septic shock.

**Figure 3** Schematic representation of MIF as a key mediator of the immune response.
Conclusions

MIF has several unusual properties for a pro-inflammatory cytokine. It circulates in healthy individuals at relatively high concentrations (Petrovsky et al. 2003), and is expressed in many normal tissues, including the pituitary (Paralkar & Wistow 1994, Nishino et al. 1995). Expression of MIF is augmented in inflammation, influenced by neuroendocrine mechanisms acting on the pituitary and also, paradoxically, is increased by exposure to low concentrations of glucocorticoids. The actions of MIF seem to be mediated by activation of cellular ERK activity, and JAB-1, but it remains unclear whether a transmembrane receptor or a cellular uptake mechanism is the primary means of signal transduction across the membrane. Although the MIF gene structure is highly conserved there appear to be differences between rodents and humans in terms of glucocorticoid induction of MIF secretion, perhaps implying that this mechanism is not conserved across species.

MIF is an important mediator of the innate immune response (Roger et al. 2001). Local MIF production may influence recruitment of elements of the acquired immune response (Bacher et al. 1996, Santos et al. 2001). Such a ‘gatekeeper’ role may explain why genetic variation of MIF is beginning to be found to be important in susceptibility to several complex inflammatory diseases. Understanding MIF biology may lead to new insights into the aetiology of many chronic, disabling human disorders and, also, to the development of novel therapeutic approaches to treat such conditions.

References


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