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MACROPHAGE MIGRATION INHIBITORY FACTOR IN RHEUMATOID ARTHRITIS

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1. ABSTRACT

Rheumatoid arthritis is characterised by the interaction of multiple mediators, among the most important of which are cytokines. In recent years, extensive data demonstrates a pivotal role for one cytokine, macrophage migration inhibitory factor (MIF), in fundamental events in innate and adaptive immunity. MIF has now been demonstrated to be involved in the pathogenesis of many diseases, but in the case of RA the evidence for a role of MIF is very strong.

MIF is abundantly expressed in the serum of RA patients, and in RA synovial tissue where it correlates with disease activity. MIF induces synoviocyte expression of key proinflammatory genes including TNF, IL-1, IL-6, IL-8, cPLA2, COX2 and MMPs. MIF also regulates the function of endothelial cells and B cells. Moreover, MIF is implicated in the control of synoviocyte proliferation and apoptosis via direct effects on the expression of the tumor suppressor protein p53. In multiple rat and mouse models of RA, anti-MIF antibodies or genetic MIF deficiency are associated with significant inhibition of disease. MIF $^{-/-}$ mice further demonstrate increases in synovial apoptosis. That the human *Mif* gene is encoded by different functional alleles in subjects with inflammatory disease also provides evidence for the role of MIF in RA.

The mechanism of action of MIF is becoming better understood. MIF appears to interact with cell surface CD74, with consequent activation of MAP kinases but possibly not NFκB intracellular signal transduction. This apparent selectivity may be implicated in the ability of MIF to antagonise the effects of glucocorticoids. As MIF expression is induced by glucocorticoids, inhibition of its antagonistic effects may permit enhanced therapeutic effect of glucocorticoids, or "steroid sparing".

To date there are no clinical trials of MIF antagonism in any disease, but exploitation of antibody, soluble receptor, or small molecule approaches enabled by the unique crystal structure of MIF, may soon lead to the ability to test in the clinic the importance of this cytokine in human RA.

2. INTRODUCTION

The importance of cytokines to the pathogenesis and clinical manifestations of rheumatoid arthritis (RA) has been strongly supported by the efficacy of specific antagonists of tumor necrosis factor (TNF) and interleukin (IL)-1(1). Despite the specific nature of such therapies, however, many patients are incompletely responsive to antagonism of TNF or IL-1, suggesting other cytokines and mediators are involved (2). Therefore, the search for important etiological factors in RA continues, both in terms of understanding the biology of the disease and creating improved treatments.

Among the candidates for therapeutic targeting in RA are other pro-inflammatory cytokines with broad spectrum effects within the immune system, such as IL-6, IL-15, IL-18 (1). Another cytokine with a strong case for involvement in the pathogenesis of RA is macrophage migration inhibitory factor (MIF). In this article we will also review body of evidence which implicates MIF in the pathogenesis of RA, validating its potential as a therapeutic target in this disease.

3. BASIC BIOLOGY OF MIF

3.1. MIF: history and discovery

In 1966, studies of delayed-type hypersensitivity identified a non-dialysable substance which inhibited the migration of normal peritoneal macrophages (3). This represented the first evidence of lymphokine activity and was termed macrophage migration inhibitory factor (MIF) (4). The discovery of lymphokines including IFN-g and IL-4 which were also capable of inhibiting macrophage migration (5) contributed to a substantial delay in characterising and cloning the MIF molecule. Human MIF was not cloned until 1989 and the mouse gene until 1993 (6, 7). This history of discovery, and the range of actions since attributed to MIF, have led some to suggest that it could be considered 'interleukin-0' (8). In common with many other pro-inflammatory cytokines, the range of functions now associated with MIF far outstrips those suggested by the original nomenclature.

3.2. Structure and biochemical properties

Mouse MIF was first successfully cloned from the cDNA of the AtT-20 anterior pituitary cell line and human MIF from the Jurkat T-cell line (9 , 10). MIF is a 12.5 kDa protein. Mouse and rat MIF exhibit 90% homology over 115 amino acids with human MIF (9). In common with cytokines like IL-1, the MIF protein in both mice and humans lacks an N-terminal leader sequence and is released from cells by a non-conventional protein secretion pathway (11).

The mouse and human MIF genes are less than 1 kB and have high homology (9). The murine MIF promoter region contains regions including a c-AMP response element (CRE), an Sp-1 site and a negative glucocorticoid response element (nGRE), a nuclear factor kB (NFkB) site and a cytokine-1 (CK-1) site (9, 12). The MIF promoter therefore contains regulatory sequences characteristic of both endocrine (CRE and nGRE) and cytokine (NF-kB) responses. Multiple Sp-1 sites and a CRE were similarly identified in the promoter region of the human MIF gene, located on chromosome 19 (13).

Human MIF mRNA is 0.8 kB and is constitutively expressed at high levels in many organs including the kidney, brain and liver (14). In the mouse, MIF mRNA is 0.6 kB and is expressed constitutively in almost all tissues. This similar to the rat, which has constitutive expression of MIF mRNA in most organs including immune and non-immune cells (15).

The X-ray crystal structure of MIF was reported in 1996 as a homotrimer. Each monomer of MIF consists of two antiparallel alpha helices and 6 beta strands (16, 17). This structure is not typical of other cytokines or hormones. The structural form of MIF in biological fluids less certain, but has recently been suggested as also being the trimeric form (18). Recombinant MIF has been shown to bind small molecules like glutathiones, gangliosides and D-dopachrome, which being negatively charged, may interact specifically with the reported positively charged central channel. The crystal structure has informed the process of identifying potential sites against which therapeutic compounds could be targeted (see subsequent sections).

3.3. Enzymatic properties of MIF

MIF was shown to have enzymatic activity during investigations into melanin biosynthesis. A cytoplasmic activity identified as MIF was responsible for catalysing the conversion of the non-naturally occurring D-dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA), a tautisomerization reaction. The catalytic activity of native MIF was confirmed in studies using purified recombinant human MIF (19). The association between this enzymatic activity and the cytokine or biological function of MIF has been extensively investigated. A number of studies utilising single amino acid mutations of MIF which inactivate the enzymatic function result in a protein with retained cytokine function, implying that the enzymatic and cytokine functions of MIF are distinct (20). In one study, insertion of an alanine between Pro-1 and Met-2 of MIF was associated with loss of some but not all cytokine activity of MIF (21). As will be discussed below, small molecule compounds designed to interact with the tautomerase site of MIF can exert inhibitory effects on MIF biological activity. MIF has also been reported to exert oxidoreductase activity, with a possible connection to cytokine function (22).

4. EXPRESSION AND FUNCTION OF MIF IN IMMUNITY AND TISSUES RELEVANT TO RA

MIF is known to be expressed by and to act upon a very wide range of cells relevant to RA, including both leukocytes and resident/tissue cells. This information is summarised in table 1.

4.1. Adaptive immunity and T cells

MIF was originally considered as a T-cell cytokine (4), and the presence of pre-formed MIF has been confirmed in resting T-cells (23). MIF mRNA is expressed in human peripheral blood and mouse splenic T cells, and these cells release MIF in response to recall antigens, mitogens and anti-CD-3 antibody (23, 24). Splenocyte MIF release is also induced by low concentrations of staphylococcal exotoxin (25). Anti-MIF mAb exposure inhibits in vitro T-cell proliferation and IL-2 production (23), and in vivo inhibits the development of classic delayed-type hypersensitivity (DTH) responses(26, 27). The involvement of MIF in mechanisms of cell mediated immunity, in models of DTH and resistance to Leishmania suggest that it contributes significantly to Th1 effects (27, 28). In keeping with this, IL-10 inhibits the synthesis of MIF and antagonises rMIF-mediated inhibition of human monocyte migration (29). In addition, it has been suggested

Table 1. Cellular actions of MIF in, or relevant to, rheumatoid arthritis

Cell	Observation	Reference
Synovial fibroblasts	Expression of MIF	54
	Induce $cPLA_2$, COX-2	81
	Induce proliferation	50
	Inhibit apoptosis and p53 expression	51
	Activate MAP kinases and AP-1 DNA binding	50, 73, 82
	Expression of MMP 1 & 3	71
	Induce IL-6, IL-8, IL-1	73, 82
Monocyte/macrophage	Expression of MIF	19
	Induction by endotoxin	12
	Induction of TNF	18, 19, 54
	Induce IL-8	30
	Inhibit apoptosis and p53 expression	63
Endothelial cells	Expression of MIF	68
	Proliferation, activation	107
	Angiogenesis	22
	Activation by thrombin	85
	Leukocyte trafficking	39
T lymphocyte	Expression of MIF	1, 6, 88
	Required for T cell activation	6
Eosinophils	Expression of MIF	80
B lymphocytes	Expression of MIF	103 99
	Growth factor	22 94
Osteoblasts	Expression of MMP 9 & 13	72

that MIF is involved in T cell receptor signalling in CD4+ T cells (30).

4.2. Innate immunity and monocyte/macrophages

Although first reported as a T lymphokine acting on macrophages, MIF is now known to be even more abundantly expressed in macrophages. MIF mRNA and protein are constitutively expressed at high levels in unstimulated macrophages, with MIF protein existing pre-formed in cytoplasmic stores (31). Endotoxins, exotoxins and cytokines including interferon (IFN)-g and tumor necrosis factor (TNF) stimulate the release of MIF by macrophages (31), and MIF in turn is able to stimulate its own synthesis by macrophages. The amount of bacterial lipopolysaccharide (LPS) required to induce MIF mRNA and protein are reported to significantly lower than those required to induce TNF-a mRNA (32). This, together with the rapid release of preformed MIF prior to the initiation of new synthesis, suggest MIF is an "early response" cytokine.

MIF is both released by and active upon macrophages. MIF induces macrophage TNF secretion and synergises with IFN-g to increase nitric oxide production (11, 33). MIF also enhances phagocytosis of foreign particles (34), and increases intracellular killing and H2O2 generation (35). MIF confers protection from experimental Leishmaniasis, mediated through increases in inducible nitric oxide in infected macrophages (28, 36). MIF has recently been shown to confer resistance to apoptosis in macrophages via regulation of p53 expression (37), a finding also confirmed in RA synovial cells and in vivo in experimental arthritis (38).

In vivo, MIF has a central role in the innate immune response to bacterial toxins and bacterial sepsis itself, a full review of which is beyond the scope of this article. It is notable, however, that endotoxic shock is significantly reduced in animals treated with anti-MIF antibodies or in MIF -/- mice (32, 39), and this protection appears to be mediated via the dependence on MIF for the expression of Toll-like receptor (TLR)-4 (40).

Clearly then, MIF has a central role in the regulation of both innate and adaptive immunity. As both pathways of immune activation are implicated in the pathogenesis of RA, the actions of MIF would predict that MIF has the potential to be important in RA.

4.3. Other cellular subsets

MIF is expressed by other cellular subsets, notably B cells, eosinophils, and endothelial cells. Purified mouse and human B cells release MIF in response to mitogenic stimulation (41), and Raji B cells proliferate in response to MIF in vitro (42). A transformed human B cell line has been described to release significant amounts of MIF (43). These B cells were additionally shown to present processed peptides derived from MIF in association with HLA DRB1 on their surface. This is the first description of cytokine-derived peptides binding to HLA molecules on human B cells. In another human B cell line, transfection of MIF antisense prevented surface immunoglobulin-mediated growth arrest and apoptosis (44).

Unstimulated circulating human eosinophils express pre-formed MIF and release significant quantities of MIF response to PMA, C5a and IL-5 (45). This stimulated release was blocked by protein synthesis inhibition using cycloheximide and by protein kinase C inhibition suggesting that protein kinase C is implicated in the regulation of MIF release. Eosinophil-associated MIF has also been reported in bronchial mucus of asthma patients (46)

Constitutive expression of MIF mRNA and protein has been identified in human vascular endothelial cells (47). Cultured microvascular endothelial cells also express MIF (48), and blockade of MIF reduces endothelial cell activation and angiogenesis (48, 49), with obvious implications for the angiogenesis present in RA pannus.

4.4. MIF and glucocorticoids

Additional interest in MIF as a potential therapeutic target in RA stems from its unique relationship with glucocorticoids. Within the immune system, MIF is secreted in response to glucocorticoid stimulation (23, 50), and we confirmed upregulation of MIF expression by low concentrations of glucocorticoids in synovial fibroblasts in vitro (51). Evidence for glucocorticoid upregulation of MIF in vivo has been obtained, in experimental arthritis (52). Others have described posttranslational regulation of MIF expression by glucocorticoids in vivo in the rat (53).

Despite being induced by glucocorticoids, MIF is able to directly antagonize their effects on the activation of immune cells. This has been shown to be the case for macrophage TNF, IL-1b, IL-6 and IL-8 secre--tion (50, 54), and for T cell proliferation and IL-2 release (23). MIF is present in healthy human serum at a concentration (3-5 ng/ml) which, in vitro, exerts this glucocorticoid-counter-regulatory activity. MIF also exhibits glucocorticoid-antagonist effect in vivo, in models involving both innate and adaptive immune responses such as endotoxic shock and antigen-induced arthritis (26, 50). While other pro-inflammatory cytokines can oppose the actions of glucocorticoids, their expression is generally suppressed by glucocorticoids, and they are therefore not present in physiological situations where glucocorticoids are acting. MIF, on the other hand, being inducible by glucocorticoids, is expressed even after exposure to glucocorticoids, and therefore is able to exert its glucocorticoid-opposing effects under physiological conditions. This suggests that MIF acts in balance with glucocorticoids to affect the "set-point" of the inflammatory and immune response (5, 50).

The mechanism through which MIF opposes the actions of glucocorticoids on immune cells is not yet well understood, but it is likely to be based on specific interactions on intracellular signal transduction pathways, as will be discussed below. It is hypothesised that therapeutic antagonism of MIF would inhibit the natural glucocorticoid antagonist role of MIF, thereby allowing glucocorticoids to prevail. This steroid augmentation effect of MIF antagonism would be the first direct example of so-called "steroid-sparing" therapy. The practical effect would be the opportunity to use lower (or zero) doses of exogenous glucocorticoids in a given context when combined with MIF antagonism, thus reducing dose-dependent steroid toxicity. In support of this, anti-MIF mAb therapy reverses the disease-exacerbating effect of adrenalectomy in rat adjuvant arthritis (52), effectively acting in place of endogenous glucocorticoids.

5. MIF IN RHEUMATOID ARTHRITIS

5.1. MIF in animal models of RA

The hypothesis that MIF is involved in the pathogenesis of RA requires testing in animal models of RA, and several studies have been reported in which a role for MIF has been clearly identified. Mikulowska et al first reported on MIF in an animal model of RA, using murine collagen-induced arthritis (55). In this study, treatment with neutralising anti-MIF Ab prior to immunisation with type II collagen delayed the onset and reduced the frequency of arthritis. Somewhat surprisingly, overall T cell proliferative responses to type II collagen were increased with anti-MIF treatment. Anti-MIF treatment was however associated with decreased IgG2a responses to type II collagen, with no differences observed in the production of IgG1, suggesting a Th1-promoting effect of MIF in this model.

We subsequently reported a role for MIF in rat adjuvant arthritis (56). MIF expression was detected in the synovial tissues and serum of rats with arthritis, at greater levels than in healthy rats. Anti-MIF mAb administration during the evolution of adjuvant arthritis was associated with a dramatic reduction in clinical and histologic disease parameters. A later study, in which this already severe model of arthritis was rendered more severe by adrenalectomy, showed not only a reduction of arthritis in anti-MIF-treated rats but also a complete protection from the lethal effects of adjuvant arthritis in glucocorticoid-deficient rats (52). A further model of RA, murine antigen-induced arthritis, was also profoundly inhibited by anti-MIF mAb administration (26), in a study also demonstrating inhibition of DTH by anti-MIF mAb treatment. The more profound effects of anti-MIF treatment detected in these studies compared to those in collagen-induced arthritis may reflect differences in the models, but more likely relate to the fact that much lower doses of neutralising mAb were used in the CIA studies (55).

Recently, MIF-/- mice were studied for the first time in an arthritis model. In the antigen-induced arthritis model, MIF -/- mice were found to exhibit significantly decreased arthritis severity compared to wild-type controls (38). This was associated with increases in synovial p53 and reductions in synovial apoptosis. MIF -/- mice have also been recently demonstrated to have reduced synovial leukocytosis in response to the non-specific irritant carrageenan (57). Finally, two recent studies have demonstrated reduced severity of inflammation in response to passive transfer of anti-type II collagen antibodies in MIF -/- mice or mice treated with anti-MIF antibodies (58, 59).

5.2. MIF and human RA inflammation

Confirmation of the role of MIF in RA requires that it be demonstrated in the synovial lesion. In 1999, we reported the expression of MIF in the serum, synovial fluid, and cultured synovial fibroblasts of patients with RA, and that MIF was overexpressed in these sites compared to healthy or osteoarthritic controls (51). MIF was abundantly expressed in fibroblast-like synoviocytes (FLS), macrophages, and in synovial tissue endothelial cells, and was less abundant in CD3-positive synovial lymphoid aggregates (51). Expression of MIF protein by RA synovial macrophages and FLS was confirmed by flow cytometry and PCR. We have also described an association between RA disease activity, as measured by serum CRP, and

synovial MIF content in patients with RA (60). Significantly increased synovial MIF was detected in synovial biopsies from patients with active RA, compared with paired samples obtained during a treatment-induced improvement in disease activity.

In addition to being expressed in RA tissues, a range of direct pro-inflammatory effects of MIF on synovial cells has been described. Conditioned media from cell cultures of RA FLS contains significant concentrations of MIF. Exposure of blood monocytes to RA FLS-conditioned media induces TNF release, which was inhibited by anti-MIF antibody treatment (51). MIF also directly induces the expression and activity of two key enzymes in prostaglandin synthesis, cytoplasmic phospholipase A2 (cPLA2) and cyclooygenase-2 (COX-2) in cultured RA FLS (61). In these studies, MIF induced activation of human RA FLS in vitro, and moreover, anti-MIF mAb prevented the activating effects of the pro-inflammatory cytokine IL-1 on cPLA2 and COX-2. We have recently demonstrated that this is associated with induction of prostaglandin E2 release by MIF (62). We and other have also reported induction by MIF of FLS IL-6, IL-8, and IL-1 expression and release (58, 62).

5.3. MIF and synovial hypercellularity

In addition to inflammatory activation, RA synovitis is characterised by significant hypercellularity. Synovial hypercellularity is believed to arise from a combination of increased leukocyte recruitment, reduced apoptosis, and possibly increased in situ proliferation. MIF has now been shown to exert specific effects on each of these phenomena.

A further novel role for MIF in the immune response, namely in the regulation of leukocyte-endothelial interactions, has very recently been demonstrated. Using intravital microscopy and MIF -/- mice, it was demonstrated that MIF is required for normal basal and inflammatory leukocyte-endothelial interactions in the synovial microcirculation (57). MIF -/- mice demonstrated reduced leukocyte rolling, adherence and transmigration in response to both LPS and TNF. These findings are consistent with the reduced leukocyte infiltrate observed in the synovium of mice treated with anti-MIF mAb or of MIF -/- mice (26, 38). The mechanism of these observations remains under investigation. This is a key discovery in terms of the contribution of MIF to inflammation, as leukocyte trafficking is a critical prerequisite of virtually all inflammatory responses.

Lacey *et al* reported that recombinant MIF induced RA FLS proliferation, and moreover that anti-MIF mAb inhibited proliferation induced by IL-1 (63). We now know this also to be the case for FLS proliferation induced by TNF (unpublished observations). It has also been demonstrated that recombinant MIF inhibits human RA FLS apoptosis in vitro (38). This study also showed that arthritic MIF -/- mice exhibited increased synovial apoptosis in situ, consistent with a regulatory role of MIF in controlling synovial apoptosis. Fibroblasts obtained from MIF -/- mice also exhibited increased basal and nitric oxide-donor-induced apoptosis in vitro. These data are consistent with the in vitro observations of Mitchell et al, who also reported an anti-apoptotic effect of MIF on cultured macrophages (37). In this study, macrophage apoptosis in response to LPS was potentiated in MIF-/- mice, and rMIF suppressed macrophage apoptosis.

5.4. MIF and joint damage

Expression and activation of matrix metalloproteinases is critical for the development of cartilage degradation in RA. Although there has been no report of MIF expression by articular chondrocytes, MIF was shown to upregulate FLS MP-1 and MMP-3 expression in vitro (64). In other cell types, particularly tumor cells, MIF has also been shown to upregulate MMP-2, and MMP-9 (65, 66) the pattern potentially associated with tumor invasion. This suggests the possibility, yet to be explored experimentally, that MIF could contribute significantly to cartilage invasion in RA. There are no data regarding any influence of MIF on aggrecanases, recently shown to be upregulated by transforming growth factor (TGF)-beta in RA FLS (67).

Bone erosion in RA requires the differentiation and activation of osteoclasts. There are no studies of the role of MIF in critical events in bone resorption and erosion, such as the regulation of osteoclast differentiation or activation, or the expression of receptor activator of NF-kB (RANK) or RANK ligand. MIF has been shown, however, to upregulate osteoblast MMP-9 and MMP-13 expression in vitro (68) and to be expressed during fracture healing in rats (69), consistent with a role in bone metabolism. As MIF participates in the upregulation of cytokines such as IL-1, IL-6 and TNF, all of which in turn play indirect roles in the activation of osteoclasts, therapeutic MIF antagonism would have the potential to favourably influence bone erosion in RA.

6. RECENT ADVANCES IN MIF BIOLOGY

Several recent developments have improved understanding of the mechanisms of action of MIF within the immune system.

6.1. MIF activation of signal transduction pathways

Although MIF is expressed in the cytoplasm in resting cells in many tissues, considerable evidence suggests that MIF is likely to signal to cells via cell-surface receptor-mediated pathways such as those used by other cytokines. The precise pathways of intracellular signal transduction utilised by MIF in RA are becoming clear, and differ significantly from those used by other pro-inflammatory cytokines. Many pro-inflammatory cytokines operative in RA activate nuclear factor kappaB (NF-kB) and mitogen-activated protein (MAP) kinase pathways (70-75). MIF induces an uniquely sustained phosphorylation of extracellular signal regulated kinase (ERK)-1/2 (76). We have also recently reported the activation by MIF of ERK and also of p38 MAP kinase in RA synovial fibroblasts (63). ERK activation is required for MIF-induced FLS proliferation, while MIF-induced p38 MAP kinase is not involved. In contrast, p38 MAP kinase mediates the

induction by MIF of COX-2 and IL-6 expression (62). Consistent with the activation of MAP kinases by MIF, several studies have documented the activation of the c-jun element of the AP-1 transcription factor by MIF (64, 68). In addition, MIF has been suggested to be involved in T cell receptor signalling in CD4+ T cells (30).

In contrast to the evidence for the activation by MIF of MAP kinases, little evidence for a direct activating effect of MIF on NF-kB has been adduced. For example, in RA FLS, despite inducing cell activation as outlined, recombinant MIF does not induce nuclear translocation of NF-kB p50 or p65 proteins at physiological or supraphysiological concentrations. Moreover, although anti-MIF mAb administration prevents IL-1-induced cell cPLA2 and COX2 expression or proliferation, it is not associated with antagonism of IL-1-induced NF-kB nuclear translocation (63). Chemical NF-kB inhibitors did not inhibit the biological effects of MIF in FLS (63). Reductions in NF-kB activation were reported in MIF -/- mice cells in response to LPS (40), but this may reflect the reduction in TLR-4 expression by these cells and does not demonstrate direct activation of NF-kB by MIF. MIF does not directly affect I-kB kinase activity, as measured by Western blotting of I-_B protein (77), and TNF induction of NF-kB reporter gene expression was identical in cells transfected with MIF antisense and control transfected cells (78). Onodera et al reported upregulation of NF-kB DNA binding by recombinant MIF in cultured human RA FLS, but only at extreme supraphysiologic concentrations (1000 ng/ml); induction of AP-1 DNA binding (which would be consistent with MAP kinase activation) was observed at a more physiological concentration (58).

The preferential utilisation by MIF of MAP kinase, as opposed to NF-kB-dependent signal transduction pathway may be relevant to the ability of MIF to antagonise the effects of glucocorticoids. Unlike the exquisitely glucocorticoid-sensitive NF-kB pathway, MAP kinase activation is relatively glucocorticoid-insensitive (79), and the glucocorticoid-glucocorticoid receptor complex has no known direct interaction with MAP kinase proteins.

In contrast to this, Daun et al reported that while MIF did not directly induce NF-_B activation, it impaired effects of glucocorticoids on the NF-_B inhibitory molecule I-kB (77). This observation is yet to be confirmed. Glucocorticoids do induce the expression of MAP kinase phosphatase (MKP)-1, a natural regulator of MAP kinase activation, thereby contributing indirectly to modulation of MAP kinases. MKP-1 has broad substrate specificity and is capable of dephosphorylating all three MAPK families, ERK, p38 and JNK(80-82). We have recently described the expression of MKP-1 in RA, and reported that its expression is induced by glucocorticoids (83), The poential for MIF to interact with the expression or function of MKP-1 is the subject of ongoing investigation.

Not all reports of MIF interaction with signal transduction pathways demonstrate a stimulatory effect. MIF has also been shown to interact with the intracellular protein Jab-1, a co-activator of AP-1 transcription (78), but the effect reported was to diminish AP-1-dependent gene transcription. It was hypothesised that high concentrations of intracellular MIF inhibit AP-1-dependent events in order to prevent undesirable overactivation of the immune system under extreme stress (84).

6.2. Identification of a putative MIF receptor

Until 2003, no information about a receptor for MIF was available, but evidence for a specific cell surface receptor for MIF has now been published (42). This work demonstrates the binding of MIF to the transmembrane domain of the MHC Class II invariant chain, CD74 (also know as Ii). Occupation of CD74 by anti-CD74 antibodies prevented cell-surface binding of MIF as measured using fluorescent labelled MIF, and prevented activation of cells by exogenous MIF. Moreover, CD74 -/- cells were non-responsive to MIF in vitro, using activation of ERK MAP kinase as a readout of cell activation, and soluble CD74 protein prevented the action of recombinant MIF on this pathway. A definitive physical association between MIF and Ii was also demonstrated using BIAcore®, and it is of considerable interest that the extracellular domain of CD74 has a predicted structure which is trimeric in form (85).

Demonstrating in vivo the role of CD74 in MIF responses in the immune system will be extremely difficult, given the involvement of Ii in the immune response generally. One approach would be to create bone marrow chimeras which express CD74 only on non-bone marrow derived cells, which in the case of models of RA would allow the examination of the effects of MIF-CD74 interactions in the absence of effects on antigen presentation.

6.3. MIF and p53

As noted above, MIF has direct inhibitory effects on apoptosis of synovial fibroblasts and macrophages. MIF was the only pro-inflammatory cytokine found capable of functionally inactivating the tumour suppressor protein, p53 (86). Relevant supportive data comes from a recently published study in which macrophage viability in the setting of endotoxaemia is maintained via an inhibitory effect of endogenous MIF on p53 (37). MIF induces COX-2 in RA FLS (61), and downstream PGE2 production may be required for MIF inhibition of p53 activity (37). The survival of primary murine embryonic fibroblasts which overexpress MIF is also extended, and this has been shown to be p53-dependent (86). We have recently shown that the anti-apoptotic effects of MIF on cultured RA FLS are associated with reductions in FLS p53 expression (38). In addition, we confirmed the increased p53 expression of cells derived from MIF -/- mice. These data suggest that MIF is unique among pro-inflammatory cytokines operative in RA in its ability to influence the expression and function of p53. Inactivation of endogenous p53 in RA has been reported to lead to enhanced FLS proliferation, anchorage-independent growth, and invasiveness into cartilage extracts, as well as impaired apoptosis (87, 88).

The downregulation of p53 expression and function by MIF also indicates hypothetical mechanisms by which MIF may contribute to the vascularity and

destructive potential of RA pannus. Fully functional wild-type p53 protein is required for basal transrepression of neovascularisation (89), and thus suppression of p53 by MIF could be involved in neovascularisation in RA synovium. This is consistent with the observation of the role of MIF in neovascularisation in B cell lymphomas (48). In addition, wild-type but not mutant p53 has been shown to be capable of transrepressing MMP13 transcription (90), and thus suppression of p53 by MIF could also be associated with increased cartilage proteoglycan degradation.

6.4. MIF polymorphisms and human disease

The potential for polymorphisms in the *MIF* gene to be associated with inflammatory illness has now been examined in a number of studies. One of the first descriptions was in relation to juvenile idiopathic arthritis (JIA) (91), with findings confirmed in larger cohorts (92, 93). The 173*C single nucleotide polymorphism (SNP) is associated with JIA with an odds ratio approaching 2, and was also associated with increased serum MIF expression suggesting a relationship between this mutation and the control of MIF expression. This association has been confirmed in a separate population of JIA patients (94). This same polymorphism has been reported in association with sarcoidosis (95). A distinct functional promoter polymorphism has been reported, namely a CATT-tetranucleotide repeat polymorphism at position -794 of the human *MIF* gene. This polymorphism is associated with alterations in in vitro MIF expression, and has been associated with adult RA disease severity, although not prevalence (96). In contrast, Barton et al reported the CATT-tetranucleotide repeat polymorphism to be associated with susceptibility to 'inflammatory polyarthritis' but not severity (97). Most recently, it has been reported that this polymorphism is in linkage disequilibrium with the 173*C SNP in JIA (93), but the functional importance of this linkage remains to be clarified. As has been the case for other cytokine gene polymorphisms studied in human RA, still-larger population studies are required in order to confirm the existing data, and the functional importance of these polymorphisms to MIF production remain to be fully understood (98).

7. PERSPECTIVE

As recently reviewed, MIF is now understood to be implicated across a very broad range of immunological and inflammatory events and a broad range of inflammatory diseases (99). The list of diseases in which MIF overexpression has been reported continues to grow, with the recent addition of systemic lupus erythematosus (100). Perhaps uniquely among diseases, RA is characterised by a set of pathological processes with an almost uncanny resemblance to the range of events in which MIF is involved, including not only inflammation and cytokine production, but also leukocyte recruitment, cell proliferation and resistance to apoptosis, p53 inhibition, glucocorticoid resistance, and angiogenesis . Most recently, the emerging role of MIF in atheroma formation suggests that MIF may also be involved in that increasingly well-recognised complication of RA, premature atherosclerosis (101-103).

Therapeutic strategies proposed for the antagonism of MIF include monoclonal antibodies, soluble receptors, small molecule drugs, and anti-sense oligonucleotides. As was the case for TNF antagonism, the real proof of therapeutic value will be found in well-controlled clinical trials of these agents if they are successfully developed. The potential for MIF antagonism to be effective across the range of intra- and extra-articular events associated with RA surely mandates that such trials should take place.

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