

# MIF: a new cytokine link between rheumatoid arthritis and atherosclerosis

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**Abstract** | Macrophage migration inhibitory factor (MIF) is well established as a key cytokine in immuno-inflammatory diseases such as rheumatoid arthritis. Inflammation is now also recognized as having a crucial role in atherosclerosis, and recent evidence indicates that MIF could also be important in this disease. Here, we review the role of MIF in rheumatoid arthritis and atherosclerosis, discuss the ways in which MIF and its relationship with glucocorticoids could link these diseases, and consider the potential of MIF as a new therapeutic target for small-molecule and antibody-based anti-cytokine drugs.

## Glucocorticoids

Commonly used anti-inflammatory drugs (often referred to as steroids in this context) originally derived from adrenal hormones.

## Cytokine

A soluble product that enables cell–cell communication through its interaction with a specific receptor.

**Matrix metalloproteinases (MMPs).** Degradative enzymes involved in connective tissue damage.

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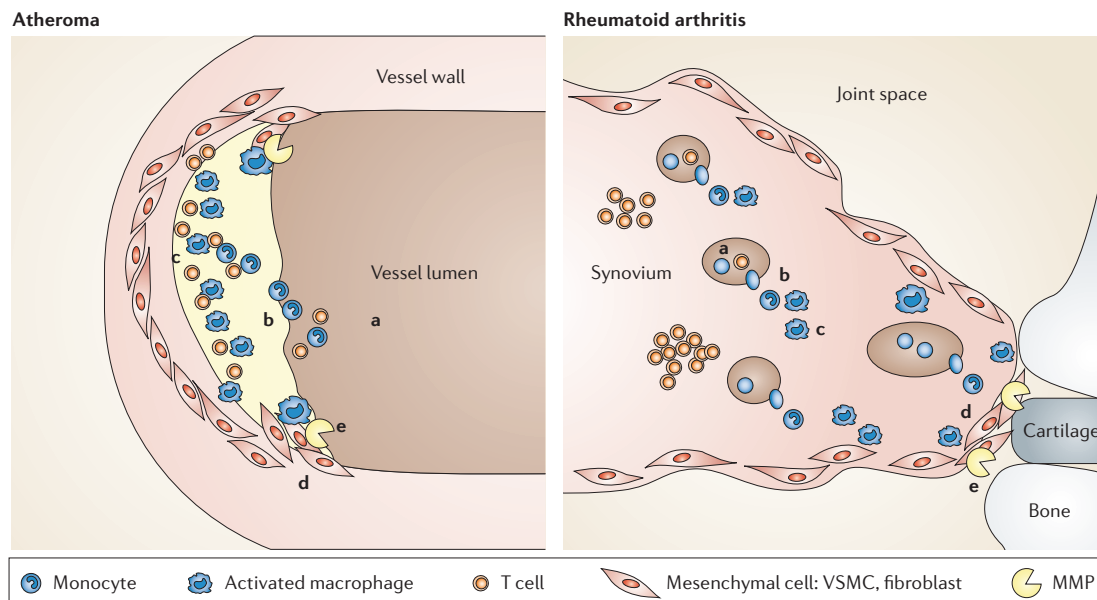
Cytokines have been shown to be important contributors to the pathology of many human acute and chronic inflammatory diseases. It has recently become clear that the most common cause of vascular disease — atherosclerosis — shares many features with more ‘typical’ chronic inflammatory diseases such as **rheumatoid arthritis (RA)** (FIG. 1). Indeed, the prevalence of atherosclerosis is increased in many chronic inflammatory diseases, including RA; this increase is potentially related to shared mechanisms, circulating mediators that affect the vasculature or the effects of treatment. Many molecules, such as pro-inflammatory cytokines and chemokines, are potential contributors to the link between inflammatory disease and atherosclerosis<sup>1</sup>. The cytokine macrophage migration inhibitory factor (**MIF**) has been shown to have pro-inflammatory effects in a wide range of diseases, including RA and atherosclerosis. MIF was originally described as a soluble factor expressed by T cells in delayed-type hypersensitivity responses (DTH)<sup>2</sup> and was later shown to be expressed by activated T cells under various conditions<sup>3</sup>. As a cytokine that is detectable in the circulation, as well as in inflamed sites, it is a candidate to provide a mechanistic link between chronic inflammatory diseases such as RA and accelerated atherosclerosis. Moreover, as MIF is unique among pro-inflammatory cytokines in being inducible by glucocorticoids, it could be implicated in the further acceleration of atherosclerosis that is associated with glucocorticoid therapy in inflammatory disease. In this article, we review the role of MIF in the pathogenesis of RA and atheroma, propose mechanisms by which it could connect these diseases, as well as relate the treatment of inflammatory

disease with the prevalence of atherosclerosis, and outline means by which therapeutic targeting of MIF could potentially be achieved.

## MIF in rheumatoid arthritis and atherosclerosis

**Rheumatoid arthritis.** RA affects about 1% of adults, and is characterized clinically by chronic inflammatory destructive polyarthritis. There are a number of key pathological features of RA: a marked expansion of the normally hypocellular synovium by infiltrating leukocytes (especially monocytes/macrophages and T<sub>H</sub>1 T lymphocytes), which is mediated by the effects of chemokines, such as monocyte chemoattractant protein 1 (**MCPI**); the expansion and disordered apoptosis of resident cells; and the participation of cytokines such as tumour-necrosis factor (TNF), granulocyte-macrophage-colony-stimulating factor (**GM-CSF**) and the interleukins **IL-1**, **IL-6**, **IL-8**, **IL-15** and **IL-17** (REF. 4). Cytokines contribute to pain and swelling via the upregulation of mediators (for example, prostaglandin E<sub>2</sub>), and to joint damage through activation of matrix metalloproteinases (MMPs), aggrecanases and proteins involved in osteoclast activation such as **RANKL** (receptor activator of nuclear factor-κB (NF-κB) ligand). MIF has been shown to be involved in almost all these phenomena, which suggests a role for this molecule in RA (BOX 1).

Mikulowska *et al.*<sup>5</sup> first explored the role of MIF in RA in the murine collagen-induced arthritis (CIA) model. In this study, treatment with neutralizing anti-MIF antibodies before immunization with type II collagen led to delayed onset and lowered frequency of arthritis, and decreased immunoglobulin G2a (IgG2a) responses to type II collagen. Subsequently, Leech



**Figure 1 | Shared cellular mechanisms in the pathogenesis of rheumatoid arthritis and atherosclerosis.** Atherosclerosis and rheumatoid arthritis (RA) are characterized by a similar profile of inflammatory events that are regulated by cytokines such as macrophage migration inhibitory factor (MIF). Initially, leukocytes such as monocytes and T cells in vessel lumens (a) actively traffic through the endothelium into the lesion (b) — that is, the vessel wall (sub-endothelial layer or intima) in atheroma or synovial tissue in RA — where they accumulate and are activated (c). Prolonged survival of lesional macrophages and T cells is a hallmark of both conditions. Increased survival and/or proliferation, as well as activation of resident mesenchymal cells (vascular smooth muscle cells (VSMCs) or synovial fibroblasts), is also observed (d). Local production of matrix metalloproteinases (MMPs) is pivotal to the mediation of plaque instability in atheroma lesions and to the damage of cartilage in RA (e).

*et al.*<sup>6</sup> reported a role for MIF in the evolution of a more severe model of RA, rat adjuvant-induced arthritis. MIF was increased in the synovial tissues and serum of rats with arthritis. Anti-MIF monoclonal antibody (mAb) administration during the evolution of adjuvant arthritis led to a dramatic reduction in clinical and histological disease parameters. Rat adjuvant-induced arthritis can be further exacerbated by adrenalectomy, thereby removing the suppressive effects of endogenous glucocorticoids. Anti-MIF mAb treatment of adrenalectomized rats with adjuvant-induced arthritis not only reduced arthritis severity, but also increased the survival rate of the rats to 100% from the 80% lethality observed in untreated adrenalectomized arthritic rats<sup>7</sup>. In another model of RA, antigen-induced arthritis, progression of RA was also profoundly inhibited by anti-MIF mAb administration<sup>8</sup>. This study also confirmed inhibition of DTH by anti-MIF mAb treatment<sup>9</sup>. In mice, anti-MIF mAb administration has also been shown to suppress arthritis in a passive-transfer model of CIA<sup>10</sup>.

*Mif*<sup>-/-</sup> mice have been recently used to confirm the role of MIF in RA. Two studies demonstrated the suppression of CIA in *Mif*<sup>-/-</sup> mice<sup>11</sup>, and, in the antigen-induced arthritis model, the severity of histological arthritis was decreased in *Mif*<sup>-/-</sup> mice, including evidence of reduced cartilage damage<sup>12</sup>. The latter study also showed reduced proliferation, and increased p53 expression and apoptosis of synoviocytes in the absence of MIF (synoviocyte expansion contributes significantly

to the development of joint damage in RA by facilitating the invasion of synovium into cartilage and bone). Studies using *Mif*<sup>-/-</sup> mice also showed a key role for MIF in the regulation of leukocyte recruitment in response to stimuli such as endotoxin and TNF, as well as directly demonstrating a requirement for MIF for leukocyte recruitment into the joint<sup>13</sup>, which indicates that MIF contributes to the hypercellularity of RA synovial lesions through its effects on leukocyte recruitment and proliferation, as well as survival.

In terms of human correlates of these animal studies, the overexpression of MIF in the cells and tissues of patients with RA compared with controls was reported in 1999 (REF. 14); MIF is also elevated in the serum of patients with RA to concentrations that are sufficient to induce leukocyte activation *in vitro*<sup>14</sup>. MIF is localized to macrophages, endothelial cells and fibroblast-like synoviocytes in human RA synovial tissue, and is less abundant in CD3-positive synovial lymphoid aggregates<sup>14,15</sup> (these are all cell types implicated in the pathogenesis of RA). In addition, it was shown that MIF mediated induction of monocyte TNF by RA synovial fibroblast-conditioned medium<sup>14</sup>. An association between RA disease activity — as measured by serum C-reactive protein (CRP) concentrations — and synovial MIF content in patients with RA was also recently reported<sup>16</sup>. Significantly increased synovial MIF was also detected in synovial biopsies from patients with active RA compared with paired samples obtained following successful treatment<sup>16</sup>.

**p53**  
A tumour-suppressor protein involved in the regulation of cell-cycle events, including apoptosis.

**Synoviocyte**  
A cell that resides in the tissues that line the joints.

**Box 1 | Actions of MIF in RA and atheroma**

Macrophage migration inhibitory factor (MIF) induces a range of actions *in vitro* and *in vivo* that coincide with the pathology of both RA and atheroma, suggesting that MIF could participate in these pathological events in both diseases:

- Leukocyte adhesion and infiltration<sup>13,18,43</sup>
- T-cell activation<sup>8,126</sup>
- Cytokine expression<sup>18,66,127</sup>
- Inflammatory mediator expression<sup>17,18,22</sup>
- Mitogen-activated protein kinase activation<sup>18,21,121,128</sup>
- Expression of matrix metalloproteinases<sup>19,20</sup>
- Modulation of p53 expression and inhibition of apoptosis<sup>12,22</sup>
- Resident-cell proliferation<sup>129,130</sup>

A role for MIF in human RA synovioyte activation has been demonstrated, with reports of upregulated expression of phospholipase A<sub>2</sub> (PLA<sub>2</sub>), cyclooxygenase 2 (COX2)<sup>17</sup>, IL-6, IL-8 (REFS 10, 18), MMP1 and MMP3 (REF. 19). Onodera *et al.*<sup>20</sup> also reported the induction of MMP9 and MMP13 in rat osteoblasts, which could be relevant to bone destruction and osteoporosis in RA. MIF induces the proliferation of human RA synovioytes and inhibits p53 expression and apoptosis in these cells<sup>12,21</sup>, which also suggests that MIF inhibition could provide a desirable pro-apoptotic signal to these hyperplastic cells. Inhibition of p53 expression and apoptosis by MIF, which prolongs cell survival, has also been described in macrophages<sup>22</sup>.

Finally, MIF promoter polymorphisms that are associated with increased systemic MIF expression have been linked to increased clinical disease severity<sup>23</sup> and increased risk of joint erosions and damage in adult patients with RA<sup>24</sup>. This follows demonstrations of overrepresentation of abnormal MIF genotypes in populations of patients with diseases such as juvenile arthritis, psoriasis, colitis and sarcoidosis<sup>25–28</sup>.

Together, these data suggest that MIF inhibition could have significant protective effects in RA, as MIF is implicated in leukocyte recruitment, activation, proliferation and survival, as well as the production of pro-inflammatory cytokines and mediators, and mechanisms of bone and cartilage injury, all of which contribute to the pathology of RA. In addition, the observation that MIF participates in the regulation of TNF and IL-6 expression suggests that MIF inhibition could block the expression of cytokines that have already been shown to be valid therapeutic targets in RA.

**Atherosclerosis.** Although instigated by ‘early’ proatherogenic risk factors such as hypercholesterolaemia which lead to vascular endothelial activation and damage, inflammation is now recognized as a crucial driving force in the initiation and progression of atherosclerotic lesion formation<sup>29–32</sup>. A key clinical correlate of this is the association of vascular lesion progression with clinical markers of inflammation such as CRP<sup>33</sup>. Inflammatory mechanisms involved in atheroma include a predominant

mononuclear cell contribution, with lesions bearing increased numbers of monocyte-derived macrophages and macrophage-derived foam cells, and, to a lesser extent, T<sub>H</sub>1 T cells. Chemokines such as MCP1 and IL-8, and inflammatory cytokines, such as TNF, GM-CSF and IL-12, are also pivotal in driving both monocyte recruitment and intimal macrophage/foam-cell activation in both native and injury-induced intimal lesion and neointimal lesion formation<sup>29,34,35</sup>. This profile of cells and mediators bears a striking similarity to that observed in RA (BOX 1). Given the role of MIF in these events in RA, the potential involvement of MIF in atheroma is strongly suggested.

Indeed, numerous recent studies now indicate a role for MIF in atherogenesis, atheroma formation and vascular disease. The expression of MIF in human atheroma lesions was first reported in 2002, with the demonstration of increasing MIF expression associated with increasing lesion area and disease progression. Overexpression of macrophage and foam cell MIF was particularly apparent in advanced carotid plaques of patients with atherosclerosis compared with tissue-matched control samples<sup>36</sup>. MIF expression also closely correlates with atherosclerotic disease severity in a rabbit model and in apolipoprotein E (APOE)-deficient (*ApoE*<sup>-/-</sup>) and low-density lipoprotein (LDL)-receptor-deficient (*Ldlr*<sup>-/-</sup>) mouse models of native atherogenesis<sup>37–39</sup>.

Importantly, MIF is also functionally linked to atherosclerosis lesion development. A study in atheroma-prone *Ldlr*<sup>-/-</sup> mice crossed with *Mif*<sup>-/-</sup> mice showed significant reductions in atheroma lesions in the absence of MIF<sup>39</sup>. In a study using neutralizing anti-MIF antibody, peripheral MIF depletion in *ApoE*<sup>-/-</sup> mice led to marked reductions in the inflammatory response associated with atherosclerosis development, including reductions in concentrations of circulating and lesional inflammatory cytokines, lesional adhesion molecules and MMPs, and expression of inflammatory transcription factors<sup>37</sup>. In none of the studies was the effects of anti-MIF treatment or MIF deficiency significantly related to alterations of plasma cholesterol or triglyceride concentrations, indicating that MIF does not prominently affect the regulation of lipid metabolism during atherogenesis. Neointimal lesion formation and repair, such as that encountered during restenotic responses that follow angioplasty and other forms of coronary intervention, is associated with various related inflammatory processes<sup>35,40,41</sup>. Chen *et al.*<sup>42</sup> showed an inhibitory role for anti-MIF mAb in a murine vascular injury model — atheroma-prone *Ldlr*<sup>-/-</sup> mice subjected to experimental angioplasty had improved inflammatory and healing responses. Similarly, a protective effect of anti-MIF mAb was observed in a separate study that used wire-induced vascular injury in *ApoE*<sup>-/-</sup> atheroma-prone mice<sup>43</sup>. These data support a potential role for MIF inhibition in post-angioplasty restenosis prevention.

Furthermore, MIF’s association with atherogenic lesion formation is not only observed in the proximal aorta, the heart valve and carotid vessels, but also in abdominal aortic aneurysm (AAA), as serum MIF was significantly correlated with initial AAA size and the annual expansion rate in a human AAA study<sup>44</sup>.

**Foam cells**

Fat-laden macrophages present in atherosclerotic lesions.

**Neointimal lesion**

A lesion formed as a result of the response of the vascular lining to damage that involves the production of new vessel lining.

Macrophage uptake and handling of lipids, such as LDL, and the activation of macrophages by this process is a key early event in atheroma lesion formation<sup>30</sup>. In *in vitro* studies using macrophage cell lines, MIF mRNA and protein expression were markedly upregulated in the presence of oxidized LDL. Conversely, MIF enhanced both the uptake and degradation of oxidized LDL by macrophages<sup>36,45</sup>. This is a key observation as, unlike the formation of RA lesions in which an autoimmune disease initiation is generally proposed<sup>4</sup>, atheroma lesions show

inflammation without evidence of a break of immunological tolerance. This suggests that lipid-mediated induction of inflammatory proteins such as MIF is capable of directly inducing the inflammatory response.

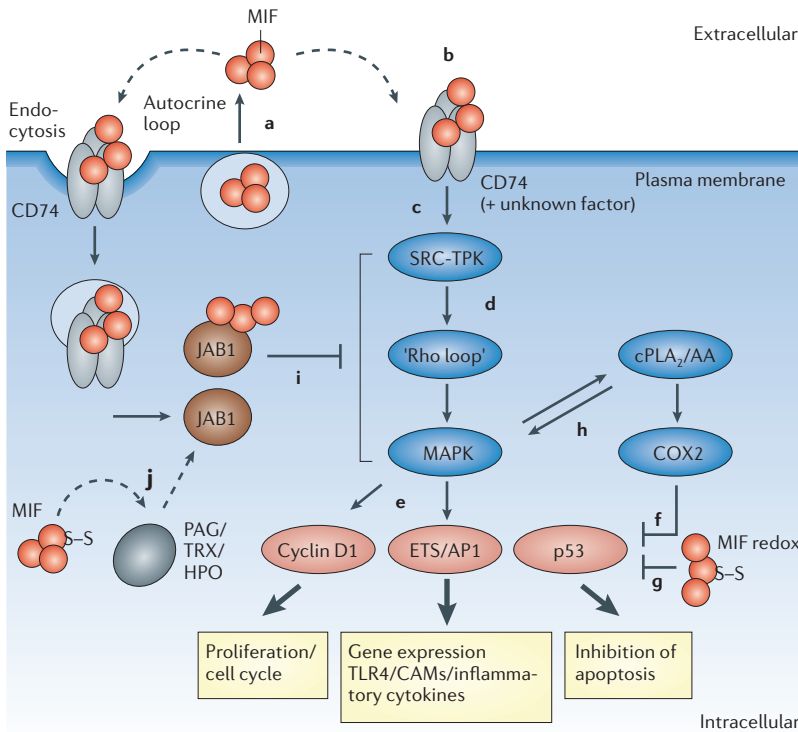
MIF could also be relevant to the phenomena of the unstable atherosclerotic plaque and the dilatation and destabilization of the arterial wall in AAA. MIF expression in plaque lesions is associated with upregulation of MMP1, MMP9 and MMP12, and downregulation of tissue inhibitors of MMPs (TIMPs)<sup>37,46</sup>. Moreover, MIF directly induced MMP1 and MMP9 expression in vascular smooth muscle cells and macrophages, respectively<sup>47,48</sup>. MIF blockade by anti-MIF mAb *in vivo* led to a reduction in vascular MMP2 expression<sup>37</sup>. Plaque instability is associated with an increased intimal macrophage and foam-cell content and, inversely, a decreased vascular smooth-muscle-cell content and collagen index. This is at least in part due to an increased MIF-mediated monocyte arrest in the endothelium, suggesting a crucial role for MIF in leukocyte recruitment in atherogenesis<sup>43</sup> similar to that shown in a model of RA<sup>13</sup>.

The existing data, therefore, suggest that MIF is induced by lipid, which is the main instigator of atheroma lesion formation, and in turn regulates key events in lesion formation, inflammatory cell activation, plaque instability and neointimal responses. Together with the overlap between other known actions of MIF, such as inhibition of p53 expression and activation of mitogen-activated protein kinases (MAPKs) this suggests that MIF participates in all the major events of atherosclerosis and that targeting MIF could, therefore, have significant benefits in this disease.

**Overlapping mechanisms and glucocorticoids**

*Overlapping actions of MIF in RA and atherosclerosis.* The available data suggest that MIF participates in mechanistic and functional events in atherosclerosis development that are highly reminiscent of its effects in RA (BOX 1). Although the mechanism of action of MIF is not completely understood, MIF is known to activate MAPKs, including extracellular signal-regulated kinase (ERK) and p38 MAPK in synoviocytes<sup>10,18</sup>, similar to other pro-inflammatory cytokines (FIG. 2). MAPK activation is well-described as a feature of atherosclerotic inflammation<sup>49</sup>, although the involvement of MIF in this has yet to be investigated. A further potential mechanism for the overlapping involvement of MIF in RA and atheroma relates to the unique effects of MIF on p53. MIF is the only cytokine known to directly down-regulate p53 expression and function<sup>12,21,50,51</sup>, in particular under cell stress (M. Thiele and J.B., unpublished observations) (FIG. 2). It has been shown that deficiency of p53 is associated with increased severity in models of both RA and atheroma<sup>52,53</sup>. This effect of MIF is therefore consistent with the hypothesis that the functions of MIF in RA could be equally relevant to atheroma.

In addition to this mechanistic overlap, the effects elicited by MIF could also provide an explanation for the increased prevalence of atherosclerosis in inflammatory disease. Premature coronary artery disease has emerged as a major cause of morbidity and mortality in



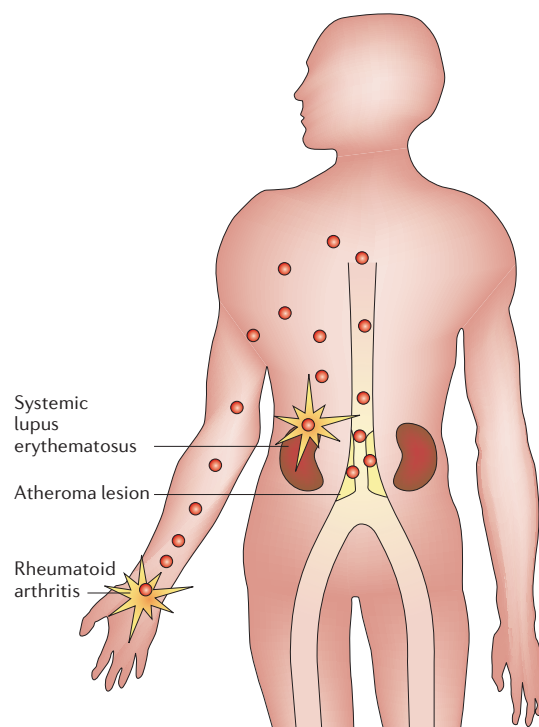
**Figure 2 | Molecular mode of action of MIF.** Macrophage migration inhibitory factor (MIF) regulates cell activation through extracellular, receptor-mediated signalling pathways, and intracellular interactions. Extracellular MIF, which includes MIF bound from intracellular stores in an autocrine fashion (a) interacts with cell surface CD74 (b). MIF activates the extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (MAPK) pathways in a CD74-dependent manner (b,c). Intermediate protein regulators between the MIF-CD74 interaction and subsequent intracellular events have yet to be characterized (c). Downstream events include a SRC-family-type tyrosine kinase and RhoGTPase-Rho kinase-myosin light chain kinase (MLCK)-integrin-focal adhesion kinase (FAK) activation loop (d). This results in the activation of cyclin D1, ETS domain-containing transcription factors (ETS) and activator protein 1 (AP1) transcription factors, leading to downstream effects on cell cycle and gene expression including cell-adhesion molecules (CAM) and Toll-like receptor 4 (TLR4) (e). p53-dependent inhibition of apoptosis by MIF can also be initiated through the MIF-CD74 interaction, which involves the downstream activation of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>), generation of arachidonic acid (AA), and activation of cyclooxygenase 2 (COX2) (f). Apoptosis induced under conditions of pro-oxidative stress is further inhibited by MIF's antioxidant activity, which depends on intramolecular disulphide (S-S) bonds (g). Arachidonic acid can in turn lead to MAPK activation and AP1-regulated gene expression (h). Certain intracellular proteins directly interact with MIF. High concentrations of endocytosed MIF bind to c-Jun activation domain binding protein 1 (JAB1) and negatively regulate MIF signalling through MAPKs (i). Intracellular MIF also possibly regulates JAB1 and other cell functions through enzymatic regulation via peroxiredoxin 1 (PAG), thioredoxin (TRX) or hepatopietin (HPO) (j). In addition, MIF can bind to MLCK, and constitutive photomorphogenesis 9 signalosome subunit 6.

RA and systemic lupus erythematosus (SLE)<sup>54–57</sup>, both of which are associated with increases in circulating MIF concentrations<sup>14,58</sup>. MIF concentrations are elevated in the circulation of patients with inflammatory disease (FIG. 3). The concentrations of circulating MIF observed in patients with inflammatory disease are similar to those required for activation of the cellular events of atherogenesis. Increased circulating concentrations of MIF in inflammatory disease states could therefore activate cellular events, such as monocyte/macrophage adhesion to endothelium and activation of macrophages/foam cells, that are pivotal to atherosclerosis development. Other inflammatory mediators produced in RA could also participate by inducing the local overexpression of MIF in the developing sub-endothelial lesions.

**MIF: a unique relationship with glucocorticoids.**

Glucocorticoids are among the most effective anti-inflammatory substances known, acting through various mechanisms to inhibit inflammation at many levels. Known effects of glucocorticoids include inhibition of leukocyte recruitment, T-cell activation, antibody production, macrophage activation, and cytokine and mediator expression. Glucocorticoids are endogenously expressed, both tonically and in response to inflammation, and form a major part of the feedback inhibitory control of the immune system. Glucocorticoids are also widely used therapeutically, with some studies suggesting that up to 1% of the adult population is taking glucocorticoids at any given time<sup>59</sup>. In the case of inflammatory diseases such as RA, even in the era of biological anticytokine therapies, up to 70% of patients regularly use glucocorticoids<sup>60</sup>. Molecules involved in the effects of glucocorticoids on inflammation include the anti-inflammatory protein annexin 1 (REF. 61), the endogenous inhibitor of the transcription factor NF- $\kappa$ B (I $\kappa$ B)<sup>62</sup>, and histone deacetylase 2 (HDAC2), which has recently been reported to also influence NF- $\kappa$ B pathway activation<sup>63</sup>. Intriguingly, recent data suggests that HDACs could also be important in atherogenesis<sup>64</sup>.

A unique relationship between MIF and glucocorticoids exists that is also potentially relevant to links between inflammatory disease and atherosclerosis. Like many hormones that exert broad physiological effects, the actions of glucocorticoids are tightly regulated. Although other cytokines can be induced by glucocorticoids under certain conditions<sup>65</sup>, MIF was identified as a crucial component of the glucocorticoid counter-regulatory system when it was shown to be both inducible by, and capable of reversing the effects of, glucocorticoids<sup>66</sup>. Subsequent human and animal studies *in vivo* and *in vitro* have confirmed that this is the case<sup>7,8,66</sup>, and MIF is now viewed as an endogenous antagonist of the effects of glucocorticoids on the immune system. The cellular mechanism via which MIF reverses the effects of glucocorticoids, and therefore its role in regulating glucocorticoid sensitivity, has also recently been described by two groups and depends, at least in part, on the regulation of MAPK phosphatase 1 (REFS 67,68). MIF is therefore postulated to physiologically ‘fine-tune’ the anti-inflammatory effects of glucocorticoids.

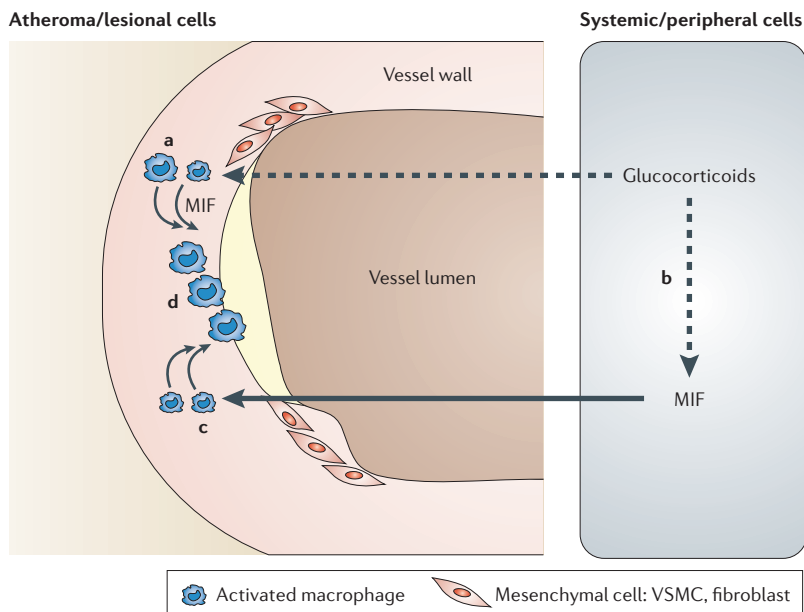


**Figure 3 | MIF as a potential connection between inflammatory disease and atherosclerosis.**

Macrophage migration inhibitory factor (MIF) produced at sites of inflammation (for example, the joint in rheumatoid arthritis or the kidney in systemic lupus erythematosus) results in increased circulating concentrations of MIF. Circulating MIF might contribute to the acceleration of atherosclerosis in inflammatory diseases by acting directly on the tissues and cells that make up the atheroma lesion.

The induction of a pro-inflammatory cytokine by glucocorticoids also serves to highlight what has been referred to as the bimodal effects of glucocorticoids. The therapeutic use of glucocorticoids for immunosuppression has led to the conceptualization of glucocorticoids as having unidirectional suppressive effects on immune function. However, normal immune and inflammatory responses proceed in the presence of physiological glucocorticoid concentrations, and the hypothesis that immune responses are supported by physiological glucocorticoids is backed by several experimental studies, as well as by observations of the outcome of sepsis in the setting of adrenocortical insufficiency<sup>69–72</sup>. Bimodal effects of glucocorticoids are particularly relevant to physiological glucocorticoid concentrations — the capacity for dynamic flux in glucocorticoid concentration can enable both a low glucocorticoid ‘immunopermissive’ state, during which normal inflammatory responses are promoted, and a high glucocorticoid ‘immunosuppressive’ state. Glucocorticoids suppress the expression and release of most pro-inflammatory molecules<sup>73</sup>. By contrast, MIF secretion by macrophages, T cells and certain endocrine cells in response to varying concentrations of glucocorticoids is bimodal: low glucocorticoid concentrations

**Systemic lupus erythematosus (SLE).** An infrequent but serious systemic inflammatory and autoimmune disease that is associated with a high risk of atherosclerosis.



**Figure 4 | MIF as a potential connection between glucocorticoids, inflammatory pathways and atheroma.** Glucocorticoids induce the expression of macrophage migration inhibitory factor (MIF) in multiple cell types. Glucocorticoid-induced MIF expression can occur locally or remotely from the site of MIF action. Here, it is proposed that MIF could be induced by glucocorticoids both in lesional cells such as atheroma lesion macrophages (a) and systemically in other tissues (b), for example arthritic joints or inflamed kidneys (as shown in FIG. 3), and subsequently act on atheroma lesions via an endocrine effect (c). Glucocorticoid-induced MIF can participate in cellular activation and survival events involved in atherosclerosis (d). VSMC, vascular smooth muscle cell.

induce MIF secretion, whereas higher concentrations do not, which closely mirrors bimodal physiological glucocorticoid regulation of immune function. This leads to the proposal that MIF could, in part, underpin the permissive/suppressive bimodality of physiological glucocorticoids.

**MIF: a link between glucocorticoids and atheroma?** Notwithstanding the complexity of this model, it is clear that, in contrast to other pro-inflammatory cytokines, MIF is induced by glucocorticoids and can exert its effects in the presence of glucocorticoids. This identifies MIF as a potential contributor to the accrual of atherosclerosis both in untreated patients and in the setting of glucocorticoid therapy. Evidence for the role of MIF in several pathophysiological processes relevant to the development of atherosclerosis, together with growing evidence that glucocorticoids promote atherosclerosis, leads to the intriguing possibility that MIF might not only directly promote atherosclerosis, but might also be mechanistically involved in glucocorticoid-induced atherosclerosis.

Interpretation of the evidence that glucocorticoids have a deleterious effect on vascular health has been confounded by the fact that the inflammatory processes that mandate glucocorticoid use are themselves associated with the development of atherosclerosis, as outlined above. An additional complication relates to the association of known atherosclerosis risk factors with

glucocorticoid exposure, including hypertension, impaired glucose tolerance, dyslipidaemia and abdominal obesity. However, there is increasing support for the contention that glucocorticoids can directly promote atherosclerosis regardless of the presence of inflammation or the induction of intermediate risk factors.

Perhaps the best evidence of glucocorticoid promotion of atherosclerosis comes from the recognition that patients with glucocorticoid excess — Cushing's syndrome — exhibit increased atherosclerosis, with acute myocardial infarction being the commonest cause of death in these patients in multiple case series<sup>74–76</sup>. What is striking in a number of these studies is that the duration of hypercortisolism was the strongest predictor of atherosclerotic plaque development. In addition to the induction of traditional cardiovascular risk factors by hypercortisolism, patients with Cushing's syndrome have been described to have elevated plasma concentrations of endothelin, a molecule implicated in accelerated atherosclerosis<sup>77</sup>. In a large study of healthy participants, levels of salivary cortisol were significantly associated with intima-media thickness in the common carotid artery<sup>78</sup>. Glucocorticoids have also been shown to directly influence the isoform expression and subcellular localization of coronary vascular protein kinase C (PKC), an effect postulated to promote both abnormal vascular signalling and atherogenesis<sup>79</sup>.

A remarkable recent study examines the contribution of glucocorticoids to the development of atheroma in *ApoE*<sup>-/-</sup> mice. Blockade of the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase 1 (11 $\beta$ -HSD1) leads to reduced generation of cortisol from inactive cortisone and lowers intracellular glucocorticoid concentrations. In *ApoE*<sup>-/-</sup> mice, this resulted in a dramatic reduction in the size of plaques, which, despite the glucocorticoid-lowering effect, was also associated with reduced levels of the pro-inflammatory cytokine MCP1 (REF. 80); these data also show the bimodality of the effects of glucocorticoids and suggest the glucocorticoid-inducible local production of a pro-atherogenic factor.

The existing data allow conceptualization of glucocorticoid as a promoter of atherogenesis via context-dependent pro-inflammatory mechanisms. Therefore, it can be proposed that a glucocorticoid-induced pro-atherogenic molecule, such as MIF, mediates glucocorticoid effects on vascular health, as shown in FIG. 4. This hypothesis requires further examination, but, if proven, the clinical effectiveness of MIF inhibition in patients with inflammatory disease receiving glucocorticoids could be accompanied by a specific protective effect against glucocorticoid-mediated pro-atherogenic events.

**MIF biochemistry suggests targeting strategy**

**Anticytokine therapies.** Cytokines are favoured targets for intervention in immune and inflammatory diseases as they are key regulatory proteins governing cells that drive inflammation<sup>81–83</sup>. A prominent approach to cytokine targeting has been the development of protein-based 'biological' therapeutics, such as anticytokine antibodies and soluble cytokine receptors<sup>84–86</sup>. Several biological therapeutic approaches could be considered

**Cushing's syndrome**  
The clinical syndrome resulting from overexposure to glucocorticoids.

Table 1 | Cytokine intervention strategies: targeted molecular mechanisms

Target mechanism	Selective for a specific cytokine	Directly targetable with a biological*	Directly targetable with a small molecule	Examples in use or under development
Gene transcription	Possible	Possible	Possible	siRNA
Protein synthesis	No	No	Yes	Thalidomide
Protein release/secretion	Possible	No	Yes	TACE inhibitors
Extracellular protein neutralization	Yes	Yes	Unknown	Anti-TNF mAb, sTNFR-Fc and IL-1RA
Receptor antagonists	Yes	Yes	Yes	IL-6R mAb
Signal transduction pathways initiated by or leading to the synthesis of cytokines	No	No	Yes	p38 MAPK inhibitors and NF- $\kappa$ B inhibitors

\*Biologicals include, for example, antibodies and soluble receptors. IL, interleukin; IL-1RA, IL-1 receptor antagonist; IL-6R, IL-6 receptor; mAb, monoclonal antibody; MAP, mitogen-activated protein; NF- $\kappa$ B, nuclear factor- $\kappa$ B; siRNA, small-interfering RNA; TACE, TNF $\alpha$ -converting enzyme; TNF, tumour-necrosis factor; sTNFR-Fc, soluble TNF receptor-Fc fusion protein.

for MIF inhibition. For example, an antibody approach to extracellular MIF is supported in diseases including asthma, severe infections and certain cancers, because anti-MIF antibody inhibits disease in corresponding animal models<sup>6,87–89</sup>. Alternative biological approaches could include soluble CD74 species. Although not yet independently confirmed, CD74 was recently identified as a cell-surface binding site for MIF on immune cells<sup>90,91</sup> (FIG. 2); whether CD74 acts as a receptor, binding site, or other participant in the transduction of signals induced by MIF has yet to be ascertained.

Although injectable biological agents, such as anti-cytokine antibodies or soluble cytokine receptors, have been successful treatments for RA, which affects about 1% of the community, these strategies have significant associated risks and limitations<sup>92</sup>. Moreover, the cost of their application to more common community diseases, such as atheroma, would be high. Non-protein-based anticytokine therapies are therefore being intensively sought<sup>92</sup>. Cytokines could be targeted by different molecular strategies or at different phases during their expression and action, and oral, small-molecule inhibitors could potentially be implemented in many of these strategies (TABLE 1). In the case of TNF, several small-molecule inhibitors that act indirectly are in preclinical and clinical evaluation. These include small-molecule inhibitors such as thalidomide (inhibition of TNF synthesis), p38 MAPK inhibitors (inhibition of signalling events leading to TNF synthesis), or TACE inhibitors (inhibition of the release of membrane-bound TNF)<sup>98</sup>.

However, strategies using small-molecule inhibitors could have several limitations. For example, synthesis inhibitors could target a spectrum of downstream processes and could lack specificity, and MAPK inhibitors could affect an undesirably broad range of cellular functions. Furthermore, when targeting TNF, it must be considered that retained membrane-associated TNF has significant biological activity that is independent of soluble TNF. So far, therefore, there have been no successfully developed small-molecule cytokine inhibitors. However, biochemical and structure–function analysis of MIF has laid the basis for an

unexpected small-molecule approach that is new in cytokine-targeted intervention and could be used to specifically target MIF.

### The development of small-molecule MIF inhibitors

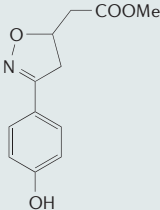
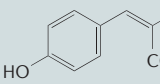
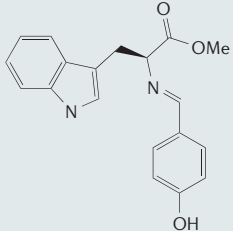
Uniquely, the MIF molecule comprises two evolutionarily conserved motifs that have otherwise only been identified in bacterial enzymes and catalyse isomerization/tautomerization and oxidation–reduction reactions<sup>93–96</sup>. *In vitro*, MIF has both catalytic isomerase/tautomerase and oxidoreductase properties<sup>94,96,97</sup>. The molecular function *in vivo* of the tautomerase catalytic properties of MIF is not clear, with results from different laboratories providing conflicting evidence for the necessity of the catalytically required residues for biological function<sup>94,97–103</sup>. Moreover, the homotrimeric tertiary structure of MIF required for assembly of the tautomerase catalytic region could be implicated in the interaction between MIF and the trimeric extracellular domain of CD74 (REFS 90,91,104,105).

Recently, small-molecule inhibitors that interact with the active tautomerase/isomerase pocket of MIF have been shown to inhibit its cytokine function. Specifically, several inhibitors have been reported that can interact with the amino (N) terminus, which incorporates a proline residue at position 2. The N terminus contains residues that are key components of the catalytic tautomerase/isomerase site of MIF<sup>102,103,106,107</sup>. As recently reviewed<sup>108</sup>, structurally, the best-characterized inhibitors comprise derivatives of hydroxycinnamate, Schiff-base tryptophan analogues or iminoquinone metabolites of acetaminophen (TABLE 2). The molecular interactions of the inhibitors with the tautomerase pocket are based on interactions with the N-terminal Pro-2 residue of MIF (which has an unusually low  $pK_a$  value of <6 and can act as a general base catalyst in isomerization reactions), hydrophobic and van der Waals interactions, as well as interactions with residues Lys-33, and Ile-65 in one subunit of the MIF trimer and with Tyr-96 and Asn-98 in an adjacent subunit<sup>106,107,109–112</sup>. So, as shown in FIG. 5, targeting the catalytic site of MIF could have a direct impact on those residues that are

#### Isomerase/tautomerase

A class of enzymes that catalyse the conversion of one isomeric/tautomeric form of a molecule to another. MIF is an isomerase/tautomerase that catalyses a reaction in which *o*-2-carboxymethylester-2,3-dihydroindole-5,6-quinone is converted to 5,6-dihydroxyindole-2-carboxymethyl ester.

Table 2 | Small-molecule MIF inhibitors

Compound	Structure	Tautomerase inhibitor	Inhibition of cellular effect of MIF	Tested in vivo	Refs
ISO-1		Yes	Yes	Yes	124,125
(E)-2-fluoro-p-hydroxycinnamate		Yes	—	—	106
L-tryptophan Schiff base		Yes	Yes	—	109

ISO-1, (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester; MIF, macrophage migration inhibitory factor.

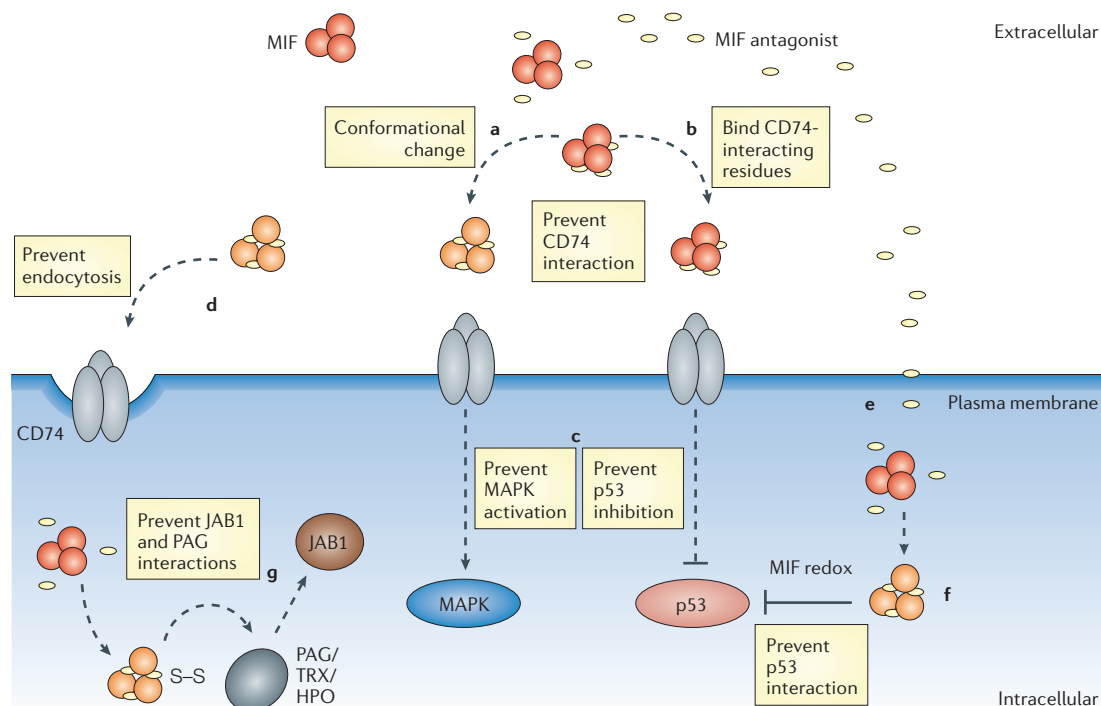
essential to the MIF-CD74 activation, or could alternatively invoke a conformational change in MIF that prevents the MIF-CD74 interaction. Furthermore, although similar structural motifs are found in bacterial enzymes, MIF is unique among cytokines in possessing these elements. Targeting the catalytic pocket of MIF with small-molecule inhibitors could, therefore, represent a new and selective anti-cytokine approach. A MIF-like redox-active peptide (MIF[50-65]) has also been reported to adopt MIF-like structural features and to mimic MIF signal transduction events<sup>113</sup>, and could serve as an alternative template for the development of small-molecule inhibitors of the MIF-CD74 interaction. Strikingly, a MIF small-molecule approach could combine the usual advantages of small-molecule inhibitors (such as oral availability, low or no antigenicity and affordable production costs) with a cytokine structure-specific activity profile.

**Intracellular MIF inhibition.** MIF protein is expressed in the cytoplasm as well as in the extracellular space, and both the extra- and intracellular fractions could contribute to MIF's pro-inflammatory activity<sup>114-116</sup> (FIG. 2). So, in addition to targeting extracellular MIF, small-molecule MIF inhibitors could readily be tailored to be membrane-penetrating and therefore to target intracellular MIF (FIG. 5). This could, in turn, disrupt the effects of MIF on intracellular proteins with which it interacts, including: the co-activator and signalosome component constitutive photomorphogenesis complex 9 (COP9) signalosome subunit 5/c-Jun activation domain binding protein 1 (CSN5/JAB1)<sup>101,117</sup>; the signalling protein

myosin light chain kinase (MLCK)<sup>118,119</sup>, and the redox regulator peroxiredoxin 1 (PAG)<sup>120</sup>. This, in turn, would interfere with the downstream signal transduction processes connected with these interactions, such as activator protein 1 (AP1), MAPK or redox signalling<sup>93,121,122</sup> (FIGS 2,5). Specific small-molecule MIF inhibitors that act on intracellular, as well as extracellular, MIF could, therefore, potentially have a broad profile of actions. This approach would be preferable to directly targeting the intracellular targets of MIF, or targeting cell signalling processes that are initiated by the MIF-CD74 interaction such as MAPK pathways, and p53- and redox stress-dependent effects on apoptosis, given the range of cellular functions of these proteins. There could also be improved selectivity over direct approaches targeting Toll-like receptor (TLR)-4 signalling, which is also regulated by MIF<sup>123</sup>. Alternatively, membrane-impermeable small-molecule MIF inhibitors could be readily devised that could be used to specifically target the extracellular site of MIF action, which might be preferable depending on the desired effect.

#### Small-molecule MIF inhibition: proof of concept

Although potentially targetable using recombinant protein technology, the unique structural characteristics of MIF render it attractive for a MIF-specific, small-molecule drug approach. Compared with biologicals, small-molecule approaches offer lower cost of manufacture and flexible delivery options, including oral administration, and could therefore represent a new, attractive approach to cytokine-specific intervention in inflammatory disease. If successfully developed,



**Figure 5 | Proposed actions of small-molecule MIF inhibitors.** **a,b** | Macrophage migration inhibitory factor (MIF) in the extracellular space interacts with a small-molecule MIF inhibitor, which prevents the interaction between MIF and CD74 either by inducing a conformational change in MIF (**a**) or by directly binding to motifs involved in the interaction (**b**). **c** | The inhibition of this interaction blocks downstream events, including mitogen-activated protein kinase (MAPK) activation and p53 inhibition. **d** | Endocytosis of MIF, which is required for interaction with certain intracellular targets, is also prevented. **e–g** | Small-molecule MIF inhibitors that are able to cross the cell membrane (**e**) could also, or alternatively, interact with intracellular MIF protein and prevent its redox-dependent effects on p53 (**f**) and/or interactions with c-Jun-activation-domain-binding protein 1 (JAB1) and peroxiredoxin 1 (PAG) (**g**). HPO, hepatopoietin; TRX, thioredoxin.

small-molecule MIF inhibitors could be the first orally available direct cytokine inhibitors.

Several groups have reported small molecules that interact specifically with structural elements of MIF. Some of these compounds have been shown to have biological activity *in vivo* in models of inflammation. One compound, (*S,R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (known as ISO-1), was the result of a structure-based design approach to the MIF catalytic site pocket and is known to inhibit both the tautomerase activity of MIF protein and its cytokine actions on  $PLA_2$  activity, and also to reverse glucocorticoid-inhibited TNF release<sup>110</sup>. More recently, this compound was reported to be active *in vivo* in models known to involve MIF, improving survival in lipopolysaccharide models of endotoxic shock- and caecal ligation and puncture-induced models of septic shock<sup>124</sup>. ISO-1 has also been reported to be active in a model of diabetes mellitus-like pancreatic islet inflammation induced by streptozotocin<sup>125</sup>. Oral activity of ISO-1 has not been reported.

Two other groups have reported orally active small-molecule MIF inhibitors in conference proceedings, although structures were not disclosed. The compounds AVP-13546 and AVP-13748, reported by Avanir, are active in an assay based on interference with recognition of MIF protein by anti-MIF mAb, implying a

conformational alteration of the MIF protein (W. Ying and J. Sircar, unpublished observations). AVP-13546 was reported to inhibit tautomerase activity and, *in vivo*, to improve endotoxic shock lethality. Importantly, AVP-13546 was also reported to be active in a passive transfer model of collagen-induced arthritis. AVP-13748 was reported to have similar *in vitro* activity and was orally active in endotoxic shock and collagen-induced arthritis. Cortical Ltd reported a small-molecule MIF inhibitor compound — COR100140 — that was derived from a structure-based design approach and is orally active in antigen-induced arthritis (E.F.M., P. Tapley, P. Hall, L. di Nezza, T. Gilbert, Y. Yang, B. Danylec and M. Iskander, unpublished observations). This compound has also been reported to be active in preventing atheroma progression in *ApoE*<sup>-/-</sup> mice when administered once-daily orally (M. Ditiatkovski, E.F.M., M. Iskander, B-H. Toh and A. Bobik, unpublished observations).

### Conclusions and future directions

A striking set of overlapping observations in RA and atherosclerosis suggest that MIF is a pivotal mediator and therapeutic target in both these diseases. The availability of circulating MIF to the vasculature also suggests a possible mechanism for the accelerated atherogenesis observed in inflammatory diseases, including RA.

Finally, support for the hypothesis that MIF mediates the acceleration of atherosclerosis during glucocorticoid therapy for the treatment of inflammatory diseases comes from the observed enhancement of both MIF expression and atherosclerosis progression on administration of glucocorticoids.

Further study is needed to test the proposal that MIF mediates the exacerbation of atheroma by glucocorticoids. Examination of an association of glucocorticoid use and MIF expression with vascular risk in high-risk populations, such as those with RA or SLE, who have increased concentrations of circulating MIF and increased atherosclerosis risk, should be investigated early; elevations in circulating MIF concentrations that are dependent on glucocorticoid exposure even after adjusting for disease activity have been reported in SLE<sup>58</sup>. The emergence of data regarding MIF-overexpression genotypes in patient populations with diseases such as RA suggests that there is value in screening for these genotypes in populations with atherosclerosis: if a relationship between MIF overexpression and

atheroma risk is established, a useful diagnostic or prognostic test could emerge.

MIF inhibition represents an opportunity to simultaneously reduce inflammation *per se* in 'typical' inflammatory diseases and atherosclerosis by addressing their shared mechanisms, and to lessen the vascular disease burden associated with inflammatory diseases and their treatment. It remains to be seen whether potential immunosuppression associated with MIF inhibition is an issue in addressing this target in patients with common diseases such as atherosclerosis; data from *Mif*<sup>-/-</sup> mice do not suggest a high risk of infection with MIF inhibition. However, if developed, small-molecule MIF inhibitors could represent a new class of orally active direct anti-cytokine drugs with potential applications not only in typical inflammatory diseases, such as RA, but also in the common community problem of atheroma, in which the use of biological anti-cytokine therapies would be very costly. It remains to be seen whether such compounds can be developed, but significant efforts are underway in several laboratories towards this goal.

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### Competing interests statement

The authors declare **competing financial interests**: see Web version for details.

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