Regulation of IL-1 and TNF Receptor Expression and Function by Endogenous Macrophage Migration Inhibitory Factor

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Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine with a crucial role in the regulation of innate and adaptive immunity (1). MIF is implicated in multiple autoimmune and inflammatory states, including sepsis, rheumatoid arthritis (RA), tumorigensis, acute respiratory distress syndrome, glomerulonephritis, and enterocolitis (2). MIF immunoneutralization or MIF deficiency is associated with protection against endotoxic shock and lethality, and marked anti-inflammatory effects in several experimental models of autoimmune disease.

The underlying molecular mechanisms of these observed biological effects are less well-understood. MIF-induced cellular activation is mediated via MAPK and the AP-1 transcription factor. MIF is thought to signal through classical receptor-dependent activation of MAPK through binding to CD74, the cell surface form of the MHC class-II-associated invariant chain (3). In NIH3T3 fibroblasts, MIF induces sustained ERK MAPK activation associated with increased cellular proliferation and phospholipase A2 (PLA2) activity (4). In these same cells, MIF was shown to modulate adhesion-dependent sustained ERK MAPK activation and regulate cyclin D1 expression and cell cycle progression (5, 6). MIF activates MAPK and AP-1 pathways in neuroblastoma cell lines (7), osteoblasts, dermal fibroblasts (8), and RA fibroblast-like-synoviocytes (FLS) (9–11). In contrast, at supraphysiologic concentrations, MIF also signals through nonreceptor-based pathways, via intracellular binding and activation of JAB1 leading to selective down-regulation of AP-1 activation (12).

MIF, unlike other cytokines such as TNF, has a widespread tissue and cellular distribution including large preformed stores in immune cells and fibroblasts. This places MIF in an ideal position for regulation of immune and inflammatory events entrained by other factors such as endotoxin and cytokines. For example, endogenous MIF regulates TLR-4 expression to permit macrophage responses to endotoxin (13). Whereas the role of endogenous MIF in responses to endotoxin is well-documented, the role of endogenous MIF in regulating responses to proinflammatory cytokines implicated in autoimmune diseases is poorly understood. The hypothesis that endogenous MIF does play a role in regulating cellular responses to other cytokines is supported by several observations. For example, immunoneutralization of MIF with anti-MIF mAb inhibits IL-1- and TNF-induced proliferation and IL-1-induced PLA2 and cyclooxygenase-2 (COX-2) expression in RA FLS (9, 14). MIF is known to exert autocrine and paracrine effects on immune cell activation, inducing IL-1 and TNF, leading in turn to further MIF production (15, 16). We report here that endogenous MIF is required for IL-1- and TNF-induced MAPK activation and regulates the expression of receptors for these cytokines. These data provide a novel mechanism of action of MIF in the amplification of the immune response.

Materials and Methods

Cells

MIF−/− mice were provided by Dr. J. R. David (Harvard School of Public Health, Boston, MA) (17, 18), crossed for two generations with MIF wild-type (WT) C57BL/6 mice and experiments were performed with littermate MIF−/− and WT controls. Murine dermal fibroblasts (MDF) were isolated...
as described (18). Mouse embryonal fibroblasts (MEF) were generated from WT and MIF−/− embryos at day 14.5. Cells were propagated in RPMI 1640/10% FCS (ICN Biomedicals) and passages 4–6 used.

**Plasmid constructs**

The MIF coding region was amplified by PCR using the following primers: 5'-CACCATGGCTTTCTCCTGAAAAC-3' and 5'-GCCGAAA GGTTGGAGTTGTTCCC-3' (19). The MIF open reading frame was verified by sequencing and cloned into a eukaryotic expression vector pcDNA 3.1+ (Invitrogen Life Technologies). The following plasmid vectors were used: Nfkb-Luc, Ap-1-Luc, pFC-MEKK, pPUC, pFA2-Elk1, pFA2-c-jun, or pFA2-ChOP (Promega).

**Flow cytometry**

The type 1 IL-1R (IL-1R) was detected following incubation with mAb against IL-1R, or 0.3 M sorbitol (Sigma-Aldrich) were examined for MIF expression, MIF phosphorylation, and cell proliferation using Abs against IκB, MIF (Amersham Biosciences) and radioactivity was determined by liquid scintillation counting.

**ELISA**

Cells were treated with 0.1 or 5 ng/ml IL-1 for 3 h. RNA was extracted using TRIzol reagent (Invitrogen Life Technologies). One microgram of RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen Life Technologies) and PCR amplification was performed on a Rotor-Gene 3000 (Corbett Research) using LightCycler DNA Master SYBR Green 1 kit (Roche). Marine IL-6 (23), IL-1R1 (forward, 5'-TGGGGACACTAAGGAGAAA-3'; reverse, 5'-CTCTTCACATTCCAGTTCCA-3'), p55 TNFR (24), and β-actin (23) primers were used. mRNA was normalized to β-actin expression, and results were presented as the fold induction of mRNA expression relative to control samples.

**Statistical analysis**

Results are expressed as the mean ± SEM. Analysis was performed using the Student t test, and p values <0.05 were considered statistically significant. All data are the result of at least three separate experiments.

**Results**

**Endogenous MIF is required for IL-1-induced MAPK, AP-1 activation, and cell proliferation**

We next examined downstream effects on AP-1 activation, using EMSA and reporter gene assays. For EMSA, cells were treated with 0.1 or 5 ng/ml IL-1 for 1 h and nuclear protein was extracted. Specific binding was shown using a radiolabeled AP-1 probe, and nonspecific binding with a mutated (mut) probe (Fig. 1B, left panel). Supershift was demonstrated with anti-fox or anti-jun Abs. Compared with WT cells, constitutive and IL-1-induced AP-1 DNA-binding activity was significantly reduced in MIF−/− cells (Fig. 1B, right panel; p < 0.05). Similarly, AP-1 luciferase activity was reduced in MIF−/− compared with WT cells (Fig. 1C, p < 0.05).

MIF has a key role in regulation of cell growth and proliferation via activation of MAPK in various cell types (3, 4, 9). To determine the functional significance of the observed MIF-dependent alterations in cytokine-induced signal transduction, we examined cell proliferation using [3H]thymidine incorporation in WT and MIF−/− cells. Both baseline and IL-1-induced proliferation were significantly reduced in MIF−/− fibroblasts compared with WT cells (Fig. 1D; p < 0.05). Thus, endogenous MIF was required for both basal and cytokine-induced cell proliferation. To ensure that MIF was indeed expressed by these cells, MIF expression in cell lysates was measured by Western blotting (Fig. 1E). MIF protein was readily detected in WT, but not MIF−/−, cells. Expression of MIF was also confirmed in WT MEF. Confirmation of the normal regulation of MIF expression in both cell types was confirmed by the observation of increased MIF expression in response to the glucocorticoid dexamethasone in WT but not MIF−/− cells (Fig. 1E).

**Endogenous MIF is not essential for IL-1-induced NF-κB activation and IL-6 expression**

We next investigated IL-1-induced NF-κB activation by analysis of IκBα degradation by Western blotting and NF-κB EMSA. IL-1-induced IκBα degradation was similar in WT and MIF−/− cells (Fig. 2A). For EMSA, specific binding was shown using a radiolabeled NF-κB probe, and nonspecific binding with a mutated (mut) probe (Fig. 2B, left panel). Supershift was demonstrated with anti-p50, anti-p65 Abs. There was no difference detected in IL-1-induced NF-κB DNA binding between MIF−/− and WT cells (Fig. 2B, right panel).
2B, right panel). These data indicate that endogenous MIF does not influence NF-κB signaling in response to IL-1.

We next sought to determine whether endogenous MIF is required in cytokine-induced proinflammatory cytokine production such as IL-6. IL-1-induced IL-6 expression was no different in MIF+/− and WT fibroblasts at either the transcriptional (data not shown) or posttranscriptional level (Fig. 2C). In addition, IL-1-induced COX-2 expression was also comparable in MIF+/− and WT fibroblasts (data not shown). IL-1-induced IL-6 production is mediated predominantly by NF-κB, and MAPK activation has been reported to contribute (10, 25). We examined the NF-κB dependence of IL-1-induced IL-6 production in WT and MIF+/− fibroblasts using an inhibitor of IkB phosphorylation, BAY-11-70-82 (22). In the presence of BAY-11-70-82, IL-1-induced IL-6 production was significantly inhibited in both WT and MIF+/− cells (Fig. 2D). In MIF+/− cells, NF-κB inhibition was associated with a near total (96%) inhibition of IL-1-induced IL-6. These data confirmed that IL-1-induced IL-6 expression in MIF+/− cells was NF-κB dependent, and that endogenous MIF is not required for IL-1-induced NF-κB-dependent IL-6 expression.

Impairment of IL-1-induced signaling occurs upstream of MAPK activation

To determine whether hyporesponsiveness to IL-1-induced p38 phosphorylation occurred at the level of p38 MAPK or upstream in the MAPK activation cascade, we next examined IL-1-induced activation of MKK3/6, the kinase upstream of p38 (26, 27) (Fig. 3A). Compared with WT cells, MIF−/− fibroblasts were hyporesponsive to IL-1-induced MKK3/6 phosphorylation. These data demonstrated that in the absence of endogenous MIF, impairment of IL-1-induced signaling events occurred upstream of MAPK activation. To examine whether this defect was specific to cytokine receptor-induced signaling, cells were treated with the osmotic stress agent sorbitol (0.5 M) for 30 min (Fig. 3B). In contrast to the impairment of p38 phosphorylation in IL-1-treated MIF−/− cells, phosphorylation of p38 induced by sorbitol was similar in MIF−/− and WT fibroblasts. This suggested that hyporesponsiveness of MIF−/− cell MAPK activation was stimulus specific, and that a reduction in endogenous MIF may impede MAPK signaling at some point between binding of IL-1 to the IL-1R complex and upstream of MEK3/6 activation.

Decreased type 1 IL-1R expression in MIF−/− cells

To determine whether the changes in IL-1 responsiveness in MIF−/− cells related to alterations in cytokine receptor expression, expression of the IL-1R1 was analyzed by flow cytometry and real-time PCR in WT and MIF−/− cells. As measured by flow cytometric analysis of IL-1R-positive cells, cell surface expression of IL-1R1 was 4-fold lower in MIF−/− compared with WT cells (Fig. 3C; p < 0.05). Similarly, expression of IL-1R as measured by
mean fluorescence intensity (MFI), was 6.7-fold lower in MIF<sup>−/−</sup> compared with WT cells (p = 0.01) (Table I). Correspondingly, expression of the IL-1R1 mRNA was 10-fold lower in MIF<sup>−/−</sup> cells compared with WT cells (Fig. 3D; p < 0.05).

**Decreased p55 TNFR expression in MIF<sup>−/−</sup> cells**

To investigate whether these effects were restricted to the IL-1R, we examined receptor expression of TNF. Cell surface p55 TNFR expression, as measured by flow cytometric analysis of TNF-positive cells, was 3-fold lower in MIF<sup>−/−</sup> cells compared with WT cells (Fig. 3E; p < 0.05). The expression of p55 TNFR, as measured by FACS, was 2.7-fold lower in MIF<sup>−/−</sup> compared with WT cells (Table I; p = 0.0006). Expression of p55 TNFR mRNA was similarly 2.5-fold lower in MIF<sup>−/−</sup> cells compared with WT cells (Fig. 3F; p < 0.05).

**Endogenous MIF is required for TNF-induced MAPK phosphorylation and cell proliferation**

To confirm the functional significance of the observed reduction in TNF expression in the absence of MIF, we examined TNF-induced MAPK activation and proliferation. A total of 1 ng/ml TNF induced robust MAPK activation in WT fibroblasts (Fig. 4A). In contrast, TNF-induced ERK and JNK phosphorylation was impaired in MIF<sup>−/−</sup> cells, but in contrast to the results observed for IL-1 stimulation, p38 phosphorylation was intact. This indicates differential requirements for endogenous MIF in IL-1- compared with TNF-induced p38 MAPK activation. The role of endogenous MIF in MAPK-dependent TNF-induced cell activation was examined by cell proliferation. Similar to the results observed with IL-1 treatment, TNF-induced proliferation was significantly impaired in MIF<sup>−/−</sup>, compared with WT cells (Fig. 4B). Our data indicated that up-regulation of cytokine receptor expression and receptor-dependent signaling events by endogenous MIF were not specific to the IL-1R1, but included regulation of TNFR1 expression and function.

**Reconstitution of MIF in MIF<sup>−/−</sup> cells restores p55 TNFR expression**

To confirm the regulation of cytokine receptors by MIF, we next investigated whether reconstitution of MIF in MIF<sup>−/−</sup> cells could restore cytokine receptor expression. We examined the effects of MIF transfection on p55 TNFR expression in studies using MIF<sup>−/−</sup> MEF. Reduced expression of p55 TNFR was confirmed in MIF<sup>−/−</sup> MEF compared with WT MEF by real-time PCR (Fig. 4C). MIF<sup>−/−</sup> MEF were cotransfected with an EGFP reporter plasmid (pEGFP<sup>−</sup>) together with an empty vector (pcDNA3.1<sup>+</sup>) or an MIF-expressing vector (pcDNA3.1<sup>−</sup>MIF) for 48 h, then p55 TNFR expression was determined using flow cytometry as described in Materials and Methods. MIF transfection significantly increased p55 TNFR expression in MIF<sup>−/−</sup> MEF (Fig. 4D). These findings were also confirmed in MDFs (Fig. 4E). Expression of p55 TNFR was increased in both transfected (EGFP<sup>−</sup>) and untransfected (EGFP<sup>+</sup>) cells, indicating autocrine-paracrine effects of MIF.

**p55 TNFR expression is regulated via MAPK/AP-1 activation**

We next investigated the molecular mechanism by which MIF modulates p55 TNFR expression. We hypothesized that down-regulation of cytokine receptor expression in the absence of MIF is dependent on reduced MAPK/AP-1 transcriptional activity. The
FIGURE 3. Down-regulation of IL-1R1 and p55 TNFR expression in MIF \(^{-/-}\) cells. A, Hyporesponsiveness of MIF \(^{-/-}\) cells is cytokine specific. MIF WT and MIF \(^{-/-}\) cells were treated with 5 ng/ml IL-1 or 0.5 M sorbitol for 30 min; phospho-p38 was measured by Western blot, and total p38 was used as a loading control (representative of \(n = 3\)). B, Hyporesponsiveness of MIF \(^{-/-}\) cells to IL-1 occurs upstream of MAPK activation. WT and MIF \(^{-/-}\) cells were treated with IL-1 for 30 min, MKK3/6 phosphorylation was measured by Western blot, the membrane was serially stripped, reprobed for phosphorylated p38 (phospho-p38), and total p38 was used as a loading control (representative of \(n = 3\)). C, IL-1R1 expression was analyzed by flow cytometry in WT and MIF \(^{-/-}\) cells. A representative histogram shows background staining in gray (left panel). The percentage of IL-1R1-positive cells was analyzed (right panel; mean \(\pm\) SEM; \(n = 11\)). \(*, p < 0.05\) compared with MIF WT cells. D, IL-1R1 mRNA expression was also measured in WT and MIF \(^{-/-}\) cells by real-time PCR. The results, based on a ratio of IL-1R1 mRNA/\(\beta\)-actin amplification, are presented as the fold induction in IL-1R1 mRNA expression relative to control samples (mean \(\pm\) SEM; \(n = 3\)). \(*, p < 0.05\) compared with MIF WT cells. E, p55 TNFR expression was analyzed by flow cytometry in MIF WT and MIF \(^{-/-}\) cells. A representative histogram shows background staining in gray (left panel). The percentage of p55 TNFR-positive cells was analyzed (right panel; mean \(\pm\) SEM; \(n = 6\)). \(*, p < 0.05\) compared with MIF WT cells. F, p55 TNFR expression was also measured in WT and MIF \(^{-/-}\) cells by real-time PCR. The results, based on a ratio of p55 TNFR mRNA/\(\beta\)-actin amplification, are presented as the fold induction in p55 TNFR mRNA expression relative to control samples (mean \(\pm\) SEM; \(n = 3\)). \(*, p < 0.05\) compared with MIF WT cells.

upstream kinase MEKK is known to be a strong activator of the MAPK/AP-1 pathway (28–30). We examined whether activation of the MAPK/AP-1 pathway via MEKK could up-regulate TNFR expression in MIF \(^{-/-}\) dermal fibroblasts. pFC-MEKK, or a control plasmid, were cotransfected with pEGFP in MIF \(^{-/-}\) cells and MIF \(^{-/-}\) cells were treated with 5 ng/ml IL-1 or 0.5 M sorbitol for 30 min, phospho-p38 was measured by Western blot, and total p38 was used as a loading control (representative of \(n = 3\)). \(*, p < 0.05\) compared with MIF WT cells. E, p55 TNFR expression was analyzed by flow cytometry in MIF WT and MIF \(^{-/-}\) cells. A representative histogram shows background staining in gray (left panel). The percentage of p55 TNFR-positive cells was analyzed (right panel; mean \(\pm\) SEM; \(n = 6\)). \(*, p < 0.05\) compared with MIF WT cells. F, p55 TNFR expression was also measured in WT and MIF \(^{-/-}\) cells by real-time PCR. The results, based on a ratio of p55 TNFR mRNA/\(\beta\)-actin amplification, are presented as the fold induction in p55 TNFR mRNA expression relative to control samples (mean \(\pm\) SEM; \(n = 3\)). \(*, p < 0.05\) compared with MIF WT cells.

Discussion

Unlike other cytokines, abundant preformed and renewable stores of MIF are present in multiple immune and nonimmune cell types (4, 16, 31–33). Constitutively expressed MIF has immunoregulatory functions in sepsis, inflammatory diseases, and tumorigenesis (4, 12, 13, 34, 35). Thus, MIF is well-placed to potentially mediate cellular responses in the context of reduced MIF is associated with increased p55 TNFR expression (Fig. 4F), suggesting the involvement of the MAPK/AP-1 pathway in the reduced TNFR expression observed in MIF \(^{-/-}\) cells. MEKK transfection was confirmed to induce activation of downstream components of the MAPK cascade Elk 1, CHOP, and c-jun, compared with control transfected cells.

Table I. IL-1R1 and p55 TNFR expression

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<th>MIF WT</th>
<th>MIF (^{-/-})</th>
<th>(p) Value</th>
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<tbody>
<tr>
<td>IL-1R1 MFI</td>
<td>19.45 ± 7.50</td>
<td>2.90 ± 0.53</td>
<td>0.01</td>
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<tr>
<td>p55 TNFR MFI</td>
<td>7.73 ± 0.77</td>
<td>3.98 ± 0.56</td>
<td>0.0006</td>
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</table>

\(^a\) IL-1R1 and p55 TNFR expression was measured by flow cytometry and MFI was calculated after subtraction of MFI in control-Ab-labeled cells. Values are expressed as the mean \(\pm\) SEM for IL-1R1 (\(n = 11\)) or p55 TNFR (\(n = 6\)) positive cells. Values of \(p\) are for MIF WT compared with MIF \(^{-/-}\) cells.

and IL-1 have established roles in the pathogenesis of multiple immunopathologies, however, the mechanism(s) underlying their interactions is unknown. MIF is released as early as or earlier than IL-1 or TNF from cellular stores. In vivo, MIF deficiency or neutralization leads to protection against lethal bacterial sepsis and septic shock induced by Gram-negative endotoxin (17, 36) or Gram-positive exotoxin (37), and amelioration of other chronic inflammatory diseases in the context of reduced MIF is associated with reduced circulating or local TNF and IL-1 production (17, 38, 39). In vitro, MIF-deficient cells exhibit impaired TNF production in response to LPS, an effect mediated via MIF regulation of TLR-4-dependent cellular responses. Herein, we demonstrate that MIF regulates cellular receptor-dependent MAPK/AP-1 activation responses to key proinflammatory cytokines independent of NF-\(\kappa\)B.

We observed an essential role for endogenous MIF in cytokine-induced MAPK activation and basal and cytokine-induced MAPK-dependent proliferation. This is consistent with the action of exogenous MIF on MAPK/AP-1 activation (3, 4, 8, 9, 40, 41), and MAPK-dependent control of cell growth and tumorigenesis (7, 42, 43). In NIH3T3 cells, MIF was required for ERK-induced cell proliferation (3, 4, 6). In these same cells, both exogenous and endogenous MIF modulated adhesion-dependent sustained ERK activation, cyclin D1 expression, and cell cycle progression (5). Similarly, MIF induces ERK-dependent RA FLS proliferation (9, 14), whereas MIF immunoneutralization prevents cytokine-induced RA FLS proliferation (9). MIF immunoneutralization also prevents IL-1-induced ERK-dependent PLA2, and p38-dependent COX-2 expression (9), further supporting a permissive role of MIF in cytokine-induced MAPK-dependent cellular activation.
In contrast, in our study, MIF was not required for cytokine-induced NF-κB activation or NF-κB-dependent IL-6 expression. This finding is in keeping with several previous reports. In RA FLS, for example, MIF does not induce NF-κB at concentrations that activate ERK (21). Moreover, anti-MIF mAb inhibits IL-1-induced cell activation, without effects on NF-κB, and NF-κB inhibition does not inhibit the biological effects of MIF (21). Upregulation of NF-κB DNA binding by MIF has been reported in RA FLS, but only at supraphysiologic concentrations (1 μg/ml) (44), whereas induction of AP-1 DNA binding occurred at a more physiological concentration. MIF has been reported not to directly affect IκB kinase activity (45) TNF-induced NF-κB was identical in control and MIF antisense-transfected cells (46). In contrast, in the context of reduced TLR-4 expression, MIF-deficient macrophages were hyporesponsive to LPS-induced NF-κB activity (13). Both p38 MAPK and NF-κB have been reported to be implicated in the regulation of IL-6 expression (10, 25). However, NF-κB is essential as NF-κB p50/p65-deficient fibroblasts do not express IL-6 in response to IL-1 (47). In our study, IL-1-induced IL-6 remained intact despite a profound reduction in IL-1-induced p38 phosphorylation in MIF−/− cells, and NF-κB antagonism lead to near complete abrogation of IL-1-induced IL-6. This suggests that NF-κB is essential for IL-1-dependent IL-6 expression, and that this is independent of MIF. This is supported by data demonstrating no difference in LPS-induced IL-6 expression in MIF−/− or WT peritoneal macrophages (17), and increased IL-6 expression in MIF−/− macrophages following cutaneous Leishmania major infection (48).
MIF deficiency was associated with a marked reduction in TNF and IL-1R expression. An upstream defect in cytokine receptor-induced MAPK pathway activation was supported by impairment of cytokine-induced MKK3/6 phosphorylation in MIF−/− cells, whereas osmotic stress-induced p38 phosphorylation was intact. Reduced MAPK/AP-1 activation in MIF-deficient cells could be explained by, or alternately be the explanation for, reduced cytokine receptor expression. MIF up-regulates macrophage TLR-4 expression via the transcription factor PU.1 (13). The IL-1R shares homology with TLR-4, and the promoter region contains binding sites for the PU.1 and AP-1 transcription factors (49, 50). PU.1 transfection of MIF−/− cells with the upstream activator of MAPK, MEKK, was associated with activation of Elk 1, CHOP, and ε-jun, and up-regulation of p55 TNF receptor expression. This suggests that MIF deficiency may impair proinflammatory cytokine receptor via the effects observed here on the MAPK/AP-1 pathway.

In addition to the role of endogenous MIF in regulating IL-1R1 and TNFR1 expression, we observed uncoupling of cytokine-induced MAPK-dependent cell proliferation from NF-κB-dependent IL-6 expression. Binding of TNF to the p55 TNFR leads to activation of a multiprotein signaling complex with release of silencer of death domains, recruitment of TNFR-associated death domain protein, and the adaptor proteins TNFR-associated factor 2 (TRAF2) and receptor-interacting protein, which ultimately activate MAPK and NF-κB pathways involved in inflammation, proliferation, and apoptosis (53). Uncoupled receptor-dependent MAPK and NF-κB signaling has been previously described for other TNFR superfamily members and TLR-4, either at the receptor level or through recruitment of other downstream components (54, 55). Recent studies suggest that regulation of p55 TNFR and downstream components such as TRAF2 may also lead to divergent effects on MAPK and NF-κB signaling. For example, down-regulation of p55 TNFR by phosphorylation has been shown to selectively decrease MAPK activation in the presence of preserved NF-κB function (56). In contrast, preferential activation of MAPK but not NF-κB has been reported to be determined by the location of the p55 TNFR in lipid or nonlipid raft components of the plasma membrane (57). Upon activation by TNF, p55 TNFR is segregated to predominantly lipid rafts in the plasma membrane. In parallel, ERK MAPK localizes to the same compartment whereas IkB remains in the nonlipid compartment. In addition, downstream components of the p55 TNFR complex such as TRAF2 recruited by silencer of death domains display similar diversity in regulation of MAPK independent of NF-κB (58, 59).

In conclusion, endogenous MIF has been shown here to exert a permissive regulatory role in cellular responsiveness to key proinflammatory cytokines TNF and IL-1 via up-regulation of cytokine receptor-dependent MAPK signaling independent of NF-κB. Autocrine up-regulation of cytokine-mediated MAPK-dependent events by endogenous MIF may provide a previously unrecognized mechanism for the amplifying role of MIF in immunity, and tumorigenesis, and the observed benefits of MIF deficiency in experimental models of inflammatory disease. Due to the widespread expression of endogenous MIF, IL-1R1, and the p55 TNFR in both immune and nonimmune cells, anti-MIF therapies would be anticipated to have significant anti-inflammatory effects. These data emphasize the role of MIF in regulating the set-point of the cytokine activation response and the potential impact of anti-MIF strategies in inflammatory disease.

Disclosures
The authors have no financial conflict of interest.

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