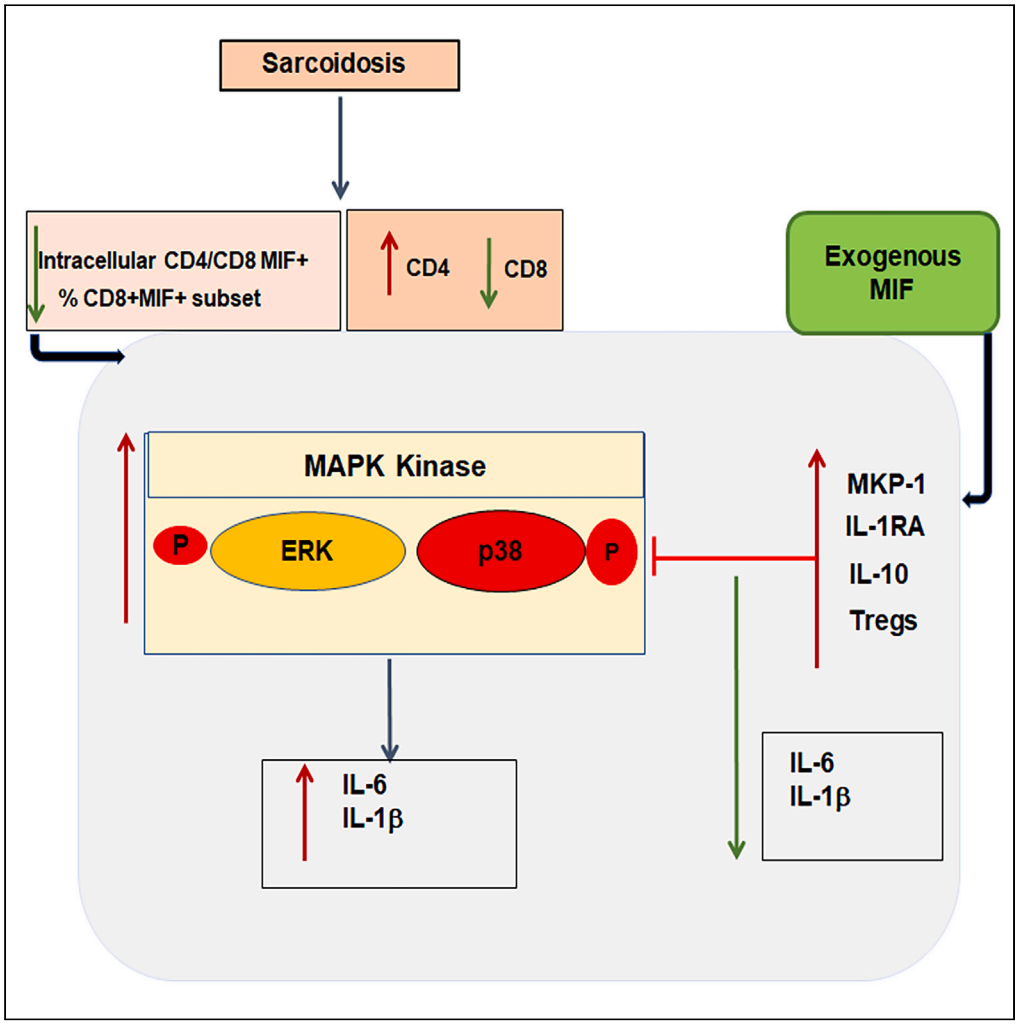
iScience

CellPress
OPEN ACCESS

Article

MIF modulates p38/ERK phosphorylation via MKP-1 induction in sarcoidosis



Jaya Talreja,
Changya Peng,
Lobelia Samavati

ay6003@wayne.edu

Highlights

Sarcoidosis CD4 and CD8 T-cells exhibit lower intracellular MIF

Sarcoidosis is associated with dysregulation of MAP-kinase (p38) activation MKP-1

Exogenous MIF upregulated MKP-1 and modulates p38 and ERK1/2 phosphorylation

Exogenous MIF decreased IL-6 and IL-1® production and induced IL-1RA and Tregs

Talreja et al., iScience 27, 108746
January 19, 2024
https://doi.org/10.1016/j.isci.2023.108746

iScience

CellPress
OPEN ACCESS

Article

MIF modulates p38/ERK phosphorylation via MKP-1 induction in sarcoidosis

Jaya Talreja,[1,3] Changya Peng,[1] and Lobelia Samavati[1,2,*]

SUMMARY

Macrophage migration inhibitory factor (MIF) is a versatile cytokine that influences a variety of cellular processes important for immune regulation and tissue homeostasis. Sarcoidosis is a granulomatous disease characterized by extensive local inflammation and increased T helper cell mediated cytokines. We have shown that MIF has a modulatory role in cytokine networks in sarcoidosis. We investigated the effect of exogenous MIF on sarcoidosis alveolar macrophages (AMs), CD14$^+$ monocytes and peripheral blood mononuclear cells (PBMCs). Our results showed that MIF negatively regulates the increased MAPKs (pp38 and pERK1/2) activation by inducing Mitogen-activated protein kinase phosphatase (MKP)-1. We found that MIF decreased IL-6 and IL-1β production, increased the percentage of regulatory T-cells (Tregs), and induced IL-1R antagonist (IL-1RA) and IL-10 production. Thus, the results of our study suggest that exogenous MIF modulates MAPK activation by inducing MKP-1and Tregs as well as IL-10 and IL-1RA, and hence plays a modulatory role in immune activation in sarcoidosis.

INTRODUCTION

Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine with multiple roles in pathology, tissue homeostasis, and in regulation of cellular activities.[1–3] It is produced by variety of cells including immune cells (macrophages, T cells, dendritic cells, eosinophils, and neutrophils), non-immune cells (epithelial and endothelial cells) and is a pituitary-derived hormone.[1,4,5] Preformed MIF protein is stored in the intracellular vesicles and secreted by a non-conventional pathway upon stimulation. Stimuli such as inflammatory cytokines, microbial products, oxidized lipids, mitogens, hypoxia, necrotic cells and corticosteroids have been demonstrated to trigger the MIF secretion.[6–9] MIF has pro-inflammatory, protective and antiapoptotic properties depending on the cellular context.[3,10–12] MIF has been implicated in the pathogenesis of various autoimmune and chronic inflammatory diseases.[13–15] Recent studies have shown that MIF plays a key role in the maintenance of hemostasis by promoting cell survival, antioxidant signaling, angiogenesis and tissue repair.[16] The protective role of MIF has been reported in the immunity against various infections and oxidative stress-mediated DNA damage.[3,11,12,17] MIF exerts its effect either through its cognate receptor (CD74) or through a non-classical endocytic pathway.[18] Upon MIF engagement, CD74 recruits CD44 and subsequently mediates downstream signal transduction through PI3K/Akt and MAPK pathways among others.[19–21] The chemokine receptors CXCR2, CXCR4, and CXCR7 are also able to form complexes with CD74/- MIF heterodimer.[22] Depending on the cellular context and stimulation status, MIF can bind to these receptors and trigger several signaling pathways[23] and modulate innate and adaptive immune responses.

Sarcoidosis is a systemic granulomatous disease characterized by extensive local or systemic inflammation associated with an increase in activated macrophages and CD4 T cells. A paradoxically decreased CD8 T cells in sarcoidosis is associated with T cell exhaustion and elevated PD-1 expression.[24,25] The T cell exhaustions occur in cancers, chronic infection and various autoimmune conditions and attributed to antigen persistence and loss of T cell effector function.[26] Various mechanisms contribute to the T cell exhaustion including, elevated PD-1, MAP kinase activation and p38 phosphorylation.[26,27]

Previously, we have shown that sarcoidosis alveolar macrophages (AMs) exhibit higher levels of activated p38 MAPK and a decreased expression of dual specificity phosphatase (DUSP1 or MKP-1).[28–30] Recently, we have shown that MIF has a modulatory role in cytokine networks in sarcoidosis and not necessarily has a detrimental effect.[31] Our study showed that the serum MIF levels in sarcoidosis patients positively correlate with IFNγ and IL-10 and improved pulmonary function testing. In current study, first, we assessed which cells produced MIF in sarcoidosis. Additionally, we evaluated the effects of exogenous MIF and TCR activation of peripheral blood mononuclear cells (PBMCs) isolated from sarcoidosis and healthy controls. In addition, we elucidated the potential pathways associated with MIF regulatory functions in sarcoidosis AMs. We found that at baseline despite increased number of CD4 cells in sarcoidosis, the intracellular MIF was lower compared to healthy controls and that TCR activation did not lead to further increase of intracellular MIF, neither in CD4 nor CD8 cells in sarcoidosis.

[1]Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, Wayne State University, School of Medicine and Detroit Medical Center, Detroit, MI 48201, USA
[2]Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI 48201, USA
[3]Lead contact
*Correspondence: ay6003@wayne.edu
https://doi.org/10.1016/j.isci.2023.108746

Table 1. Subject demographics

Characteristics	Patient Subjects, n = 60	Healthy subjects = 12
Age, years (mean ± SD)	45 ± 9	42 ± 5
Sex (female)	24 (40)	5(41)
BMI, kg.m-2 (mean ± SD)	31 ± 5	28 ± 6
Race (n, %)		
African American	58 (96)	6 (60)
White	1 (1)	2 (20)
Asian	1 (1)	2 (20)
Organ involvements (n, %)		
Pulmonary involvement	59 (98)	NA
Extra pulmonary involvement	48 (80)	NA
Skin	20 (33)	NA
Eye	15 (25)	NA
Heart	6 (10)	NA
CNS[a]	3 (5)	NA
Other	4 (6)	NA
Initial chest radiograph stages (n, %)		
Stage 0	2 (3)	NA
Stage 1	9 (16)	NA
Stage 2	31 (56)	NA
Stage 3	10 (10)	NA

[a]CNS, central nervous system

Furthermore, we found that exogenous MIF modulates cytokine production in sarcoidosis AMs and PBMCs by dampening the activation of p38 and ERK MAPK through MKP-1 and Tregs induction.

RESULTS

Activation of PBMCs increased the percentage of CD8+MIF+ cells

Sarcoidosis is characterized by an increased number of $CD4^+$ T lymphocytes.[24,29,30] The ratio CD4/CD8 in PBMCs samples was confirmed by flow cytometry. Sarcoidosis PBMCs exhibit an increased CD4 cells (mean = 53%) as compared to CD8 cells (mean = 18%). Healthy controls (HC) PBMCs showed an average of 46% of $CD4^+$ cells and 32% of $CD8^+$ cells (Figure S1A). The CD4/CD8 ratio in sarcoidosis PBMCs was about 2.9 and in healthy controls was 1.5. Subjects' characteristics (n = 70) are demonstrated in Table 1.

MIF is produced and secreted by the activated lymphocytes.[1] It is not known which lymphocytes population in sarcoidosis predominantly produce MIF. We assessed and compared the percentage of MIF+ $CD3^+$ T-cells and MIF+$CD19^+$ B-cells in PBMCs of healthy controls (HC) and sarcoid by flow cytometry. The percentage of $CD19^+$ MIF+ B-cells (5%) was comparable in both groups (Figures S1B and S1C). Figures 1A and 1B show that the percentage of $CD3^+$ MIF+ T-cells in HC is 69% and higher mean fluorescent intensity (MFI) for intracellular MIF as compared to sarcoid CD3+MIF+ cells. Due to an increase in CD4/CD8 ratio in sarcoidosis, we evaluated the percentage of double-positive $CD4^+$ MIF+ and $CD8^+$ MIF+ cells and how anti-CD3 challenge impacts on MIF+ cells. PBMCs were challenged with anti-CD3 for 96 h and then surface stained for CD4 and CD8 followed by intracellular staining to detect the intracellular MIF. As shown in Figures 1C and 1D, at the unstimulated condition, HC had a lower percentage of CD4+MIF+cells (about 50%) as compared to sarcoidosis PBMCs (75%). HC PBMCs exhibited a higher percentage of CD8+MIF+ cells (about 23%), while less than 1% CD8 cells were MIF+ in sarcoidosis PBMCs (Figures 1E and 1F). Anti-CD3 activation of HC PBMCs led to 30% increase in the MFI for intracellular MIF in $CD4^+$ cells without further augmentation of the percentage of CD4+MIF+ cells (compare Figures 1C and 1G). In contrast, anti-CD3 activation of sarcoidosis PBMC did not significantly increase the MFI for intracellular MIF or the percentage of CD4+MIF+ cells (compare Figures 1D–1H). The overlay of CD4+MIF+ subset for HC and sarcoid is shown in Figure 1I. In contrast, anti-CD3 activation of HC PBMCs led to a significant increase in the percentage of double-positive $CD8^+$ MIF+ (bright) cells from 24% to 32%, and additionally, enhanced the MFI by 40% for the intracellular MIF staining of CD8+MIF+ (bright) (compare Figures 1E and 1J). In sarcoidosis PBMCs, at baseline $CD8^+$ MIF+ were only 0.7%, which increased upon anti-CD3 activation to 6% (compare Figures 1F and 1K), but this activation led to only a marginal increase (11%) in the MFI for intracellular MIF. The overlay of CD8+MIF+ subset for HC and sarcoid is shown in Figure 1L. We determined the secreted MIF levels in the culture supernatants and results showed that anti-CD3 challenge significantly increased the release of MIF in both sarcoid and HC PBMCs (Figure S1D). This result shows that

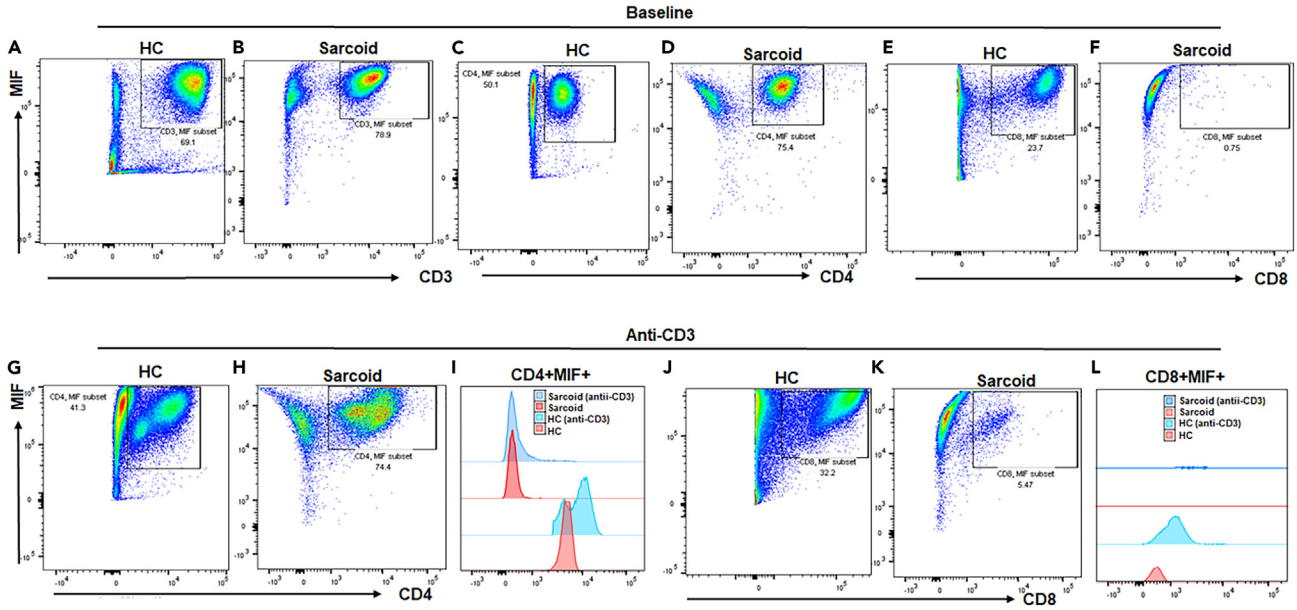


Figure 1. Activation of PBMCs increased the percentage of CD8+MIF+ cells

PBMCs of HC and sarcoid subjects were cultured with or without stimulation with anti-CD3 (1 μg/mL) for 96 h. For intracellular MIF staining, cells were cultured with 3 μm monensin for 2 h before staining. Cells were harvested after 96 h of culture and immunostained with fluorescein conjugated antibodies CD3, CD19, CD4, and CD8 and then permeabilized with 0.5% saponin and stained for MIF and analyzed by flow cytometry using Flow-jo software. Representative scatterplots show FACS analysis of both HC and sarcoidosis PBMCs. The percentage of $CD3^+$ MIF+ T-cells (70%) at baseline in HC.

(A) The percentage of $CD3^+$ MIF+ T-cells (78%) at baseline in sarcoidosis.

(B) The percentage of CD4+MIF+ cells at baseline in HC was 50%.

(C) The percentage of CD4+MIF+ cells in sarcoid was 76% at baseline.

(D) The percentage of CD8+MIF+ cells was 24% at baseline in HC.

(E) The percentage of CD8+MIF+ cells was 0.5% at baseline.

(F) Stimulation of PBMCs with anti-CD3 did not increase the percentage of CD4+MIF+ cells in both HC (G) and sarcoidosis.

(H) The overlay of baseline and anti-CD3 activated, CD4+MIF+ subsets in HC and sarcoid. The histogram depicts HC baseline CD4+MIF+ subset (pink), anti-CD3 activated CD4+MIF+ subset (turquoise blue), sarcoid baseline CD4+MIF+ subset (dark pink), anti-CD3 activated CD4+MIF+ cells (blue).

(I) Stimulation of PBMCs with anti-CD3 increased the percentage of CD8+MIF+ cells in HC from 24% to 32% (J) and 0.5%–6.24% in sarcoidosis.

(K) The overlay of baseline and anti-CD3 activated, CD8+MIF+ subsets in HC and sarcoid. The histogram depicts HC baseline CD8+MIF+ subset (pink), anti-CD3 activated CD8+MIF+ subset (turquoise blue), sarcoid baseline CD8+MIF+ subset (dark pink), anti-CD3 activated CD8+MIF+ subset (blue).

(L) The presented data is the representative of 5 independent experiments.

in contrast to sarcoidosis, CD4 and CD8 cells in HC exhibit higher levels of intracellular MIF and the TCR activation led to an even higher MFI as well as higher level of released MIF compared to baseline.

Exogenous MIF increases the percentage of regulatory T-cells

MIF is produced by activated T-cells and regulates T cell mediated immune responses and neutralization of MIF inhibits T cell proliferation and IL-2 production *in vitro*, suppresses antigen-driven T cell activation and antibody production *in vivo*.[1,12] To elucidate the effect of exogenous MIF on the T cells markers (CD25, CD69 and Tregs) and antigen-presenting cells markers (CD80, CD86), sarcoidosis PBMCs were cultured in the presence and absence of anti-CD3 (1 ug/mL) alone or the combination of anti-CD3 and rMIF (60 ng/mL). After 96 h cells were surface stained for CD80, CD86, CD69, CD4, CD25 followed by intracellular staining for the forkhead/winged helix transcription factor (FoxP3) and analyzed by flow cytometry. The gating strategies and controls are shown in Figure S2. The rMIF had no significant effects on the expression of APC activation markers CD80, CD86 and CD69 (Figure S3). The baseline expression of $CD4^+CD25^+$ was 3% in sarcoidosis PBMCs (Figure 2A). The rMIF treatment did not change the percentage of $CD4^+CD25^+$ cells (Figure 2B). In contrast, the challenge of sarcoidosis PBMCs with anti-CD3 increased the percentage of $CD4^+CD25^+$ cells to 25% (Figure 2C), but when the exogeneous MIF was added no further increase was observed (Figure 2D). Similarly, we evaluated the effect of rMIF on markers of T regulatory cells (Tregs, $CD4^+$CD25+FoxP3+) in presence and absence of anti-CD3 challenge. Exogenous MIF alone did not change the percentage of Tregs as compared to the vehicle treated cells (Figures 2E and 2F). Activation of sarcoidosis PBMCs with anti-CD3 increased the percentage of Tregs from 0.3% to 7% (Figure 2G) and the combination of anti-CD3 and rMIF led to a further increase in the percentage of anti-CD3 induced Tregs from 7 to 10% (Figure 2H). We found a higher percentage of Tregs (1.4%) in HC PBMCs as compared to sarcoid patients (Tregs,0.68%). The

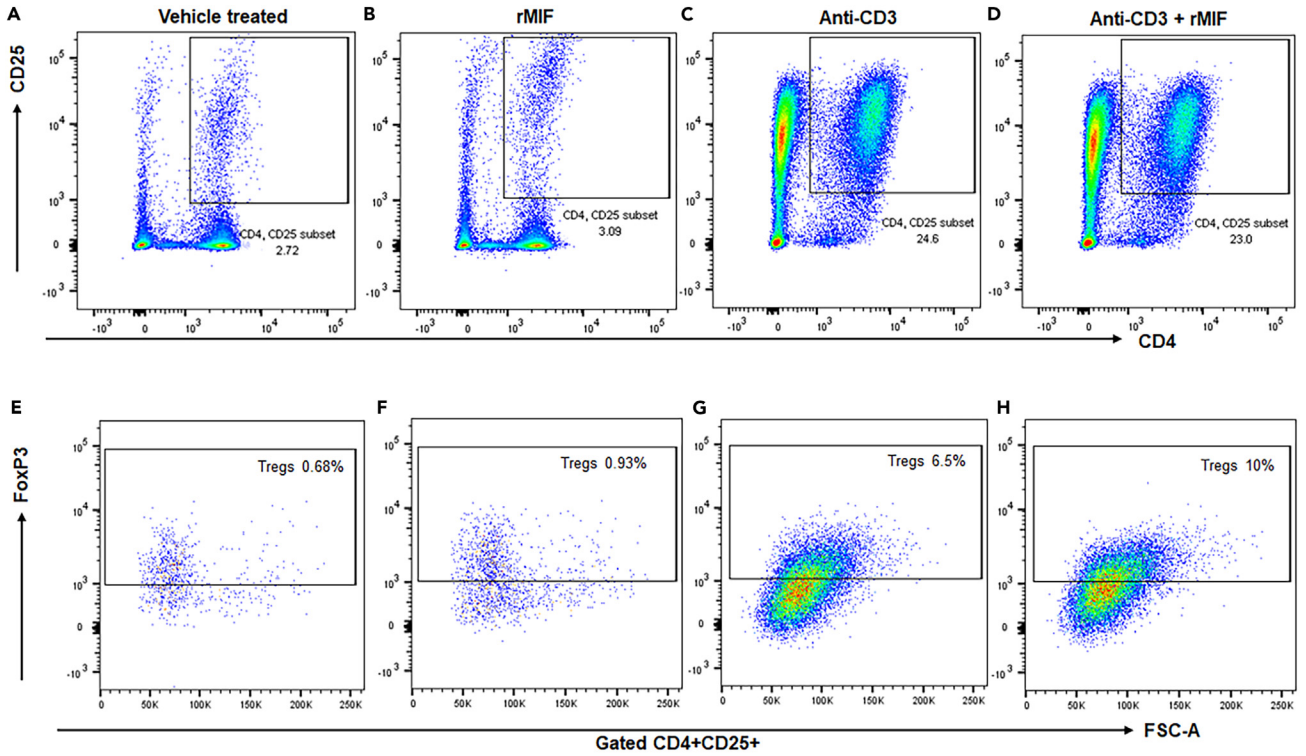


Figure 2. MIF increases the percentage of Tregs

PBMCs of sarcoid subjects were treated with rMIF (60 ng/mL) alone or were stimulated with anti-CD3 (1 μg/mL) in the presence or absence of rMIF for 96 h. Cells were harvested after 96 h of culture and immunostained with fluorescein conjugated antibodies CD4 and CD25 and then permeabilized with 0.5% saponin and stained for FoXP3 and analyzed by flow cytometry using Flow-jo software. The gating strategy is shown in Figure S2. Representative scatterplots show FACS analysis of CD4 and CD25 expression of sarcoidosis PBMCs.

(A–D). The percentage of CD4 and CD25 double-positive, representing activated T-cells, were 3% in untreated PBMCs (A). Treatment with rMIF did not increase the percentage of $CD4^+CD25^+$ cells (3%) (B). PBMCs stimulated with anti-CD3 the percentage of $CD4^+CD25^+$ double-positive T-cells increased to 25% (C). Sarcoidosis PBMCs were stimulated with anti-CD3 in the presence of rMIF. The percentage of $CD4^+CD25^+$ double-positive cells did not increase in the presence of rMIF (D). $CD4^+CD25^+$ double-positive cells were gated and analyzed for FoxP3 representing Tregs. The percentage of Tregs in untreated PBMCs was 0.38 (E), rMIF increased the percentage of Tregs from 0.38 to 0.52.

(F). Activation of PBMCs with anti-CD3 increased the percentage of Tregs from 0.38 to 6.5 (G), rMIF increased the percentage of anti-CD3 induced Tregs from 6.5 to 10%.

(H) Data presented is a representative of 3 independent experiments.

activation of HC PBMCs with anti-CD3 increased the percentage of Tregs from 1.4% to 4% and the combination of anti-CD3 and rMIF led to an increase in the percentage of Tregs from 4 to 7% (Figures S4E–S4H). We concluded that rMIF potentiates the Treg development.

Exogenous MIF modulates the cytokine production in sarcoidosis

Because our recent study showed that in sarcoidosis MIF has a potential modulatory role in cytokines networks,[23] we assessed the effect of exogenous MIF on cytokines relevant to sarcoidosis. Previously, we have shown that sarcoidosis patients PBMCs exhibit significantly higher production of inflammatory cytokines IL-6 and IL-1β.[29,30] To determine the effect of exogenous MIF on cytokine production, sarcoidosis or HC PBMCs were challenged with anti-CD3 in presence or absence of rMIF for 96 h. The conditioned media were assessed for IL-6, IL-1β, IFN-γ and IL-10 production via ELISA. The challenge of PBMCs with anti-CD3 led to a significant increased IL-6 production in sarcoid. Sarcoid PBMCs exhibited significantly higher production of IL-6 as compared to HC. The exogenous MIF significantly decreased the anti-CD3 induced IL-6 in sarcoid PBMCs. Anti-CD3 challenge of HC PBMCs led to a moderate increase in IL-6 production and the addition of rMIF had no significant effect on IL-6 production in HC (Figure 3A). Similarly, the challenge of PBMCs with anti-CD3 led to significant increased production of IL-1β in both sarcoid and HC. However, sarcoid PBMCs exhibited significantly higher production of IL-1β as compared to HC. The rMIF significantly decreased the anti-CD3 induced IL-1β in sarcoid PBMCs (Figure 3B), whereas it did not impact anti-CD3 induced IL-1β in HC PBMCs. Next, we assessed the IFN-γ production in response to anti-CD3 and rMIF. IFN-γ production was significantly induced in both HC and sarcoid PBMCs in response to anti-CD3 challenge. However, in contrast to IL-6 and IL-1β, rMIF potentiated the production of anti-CD3 induced IFN-γ in sarcoid PBMCs. The exogenous rMIF had no significant effect on the upregulation of anti-CD3 induced IFN-γ in HC (Figure 3C). Anti-CD3 challenge significantly induced IL-10 in both HC and sarcoid

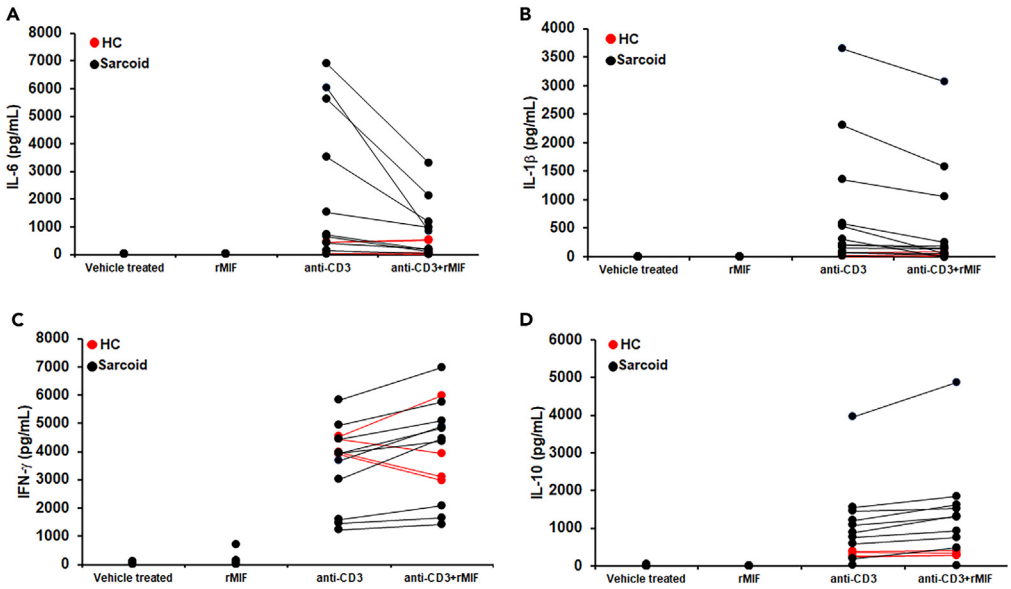


Figure 3. MIF modulates the cytokine production in PBMCs

PBMCs from healthy (n = 4) or sarcoidosis subjects (n = 10) were cultured in absence (vehicle treated) or presence of rMIF (60 ng/mL) and activated with anti-CD3 (1 μg/mL). Conditioned media were collected after 96 h and were assessed for IL-6, IL-1β, IFNγ and IL-10 via ELISA. Anti-CD3 induced IL-6 in both HC and sarcoid PBMCs. The range of IL-6 production in HC was 43–447 pg/mL and in sarcoid was 125–8500 pg/mL rMIF significantly ($p = 0.02$) dampened the anti-CD3 induced IL-6 in sarcoid (range:24–1089 pg/mL) but had no effect on IL-6 production in HC.

(A) Anti-CD3 induced IL-1β in both HC (range: 2–73 pg/mL) and sarcoid PBMCs (range: 70–3641 pg/mL). rMIF significantly ($p = 0.007$) reduced anti-CD3 induced IL-1β in sarcoid ranging from 2 to 3065 pg/mL but had no effect in HC.

(B) Anti-CD3 induced IFNγ in both HC (range: 3973-4446 pg/mL) and sarcoid PBMCs (range:1238–5800 pg/mL). rMIF significantly ($p = 0.0004$) increased the anti-CD3 induced IFNγ in sarcoid (range:1800–6995 pg/mL) but did not potentiate the anti-CD3 induced IFNγ in HC (range: 3940–5990 pg/mL).

(C) Anti-CD3 induced IL-10 in both HC (range:230-373 pg/mL) and sarcoid PBMCs (range: 5–3804 pg/mL). rMIF significantly ($p = 0.004$) increased the anti-CD3 induced IL-10 production in sarcoid (range:15–4507 pg/mL) but did not potentiate the anti-CD3 induced IL-10 in HC (range: 281–330 pg/mL) (D).

PBMCs. IL-10 production in sarcoid was significantly higher than HC. The exogenous MIF significantly potentiated the production of anti-CD3 induced IL-10 in sarcoid PBMCs (Figure 3D). rMIF alone did not induce the production of any cytokine in PBMCs, but it modulated the anti-CD3 induced cytokine production in sarcoid PBMCs.

MIF decreased the p38 and ERK1/2 phosphorylation and induced MKP-1 expression in sarcoidosis AMs

Previously, we have identified that sarcoidosis AMs exhibit heightened p38 activation, in part due to the lack of MKP-1expression and the inhibition of p38 phosphorylation or MKP-1 induction decrease the cytokine production in sarcoidosis AMs and PBMCs.[28–30] Because MIF regulates the macrophage responses, and we observed that exogeneous MIF decreased the IL-1 β and IL-6 (Figure 3), we hypothesized that rMIF might modulate MAPK activation. To test this, AMs were cultured in the presence or absence of rMIF (60 ng/mL) for 30 min. Western blot was performed using the cell lysates to evaluate the levels of phosphorylated form of p38 MAPK (pp38). Figures 4A and 4B show that sarcoid AMs exhibit increased expression of pp38 at the baseline and additional rMIF treatment significantly reduced the expression of pp38. We also determined the effect of rMIF on the phosphorylation of ERK1/2. Similarly, rMIF significantly reduced the phosphorylation of ERK1/2 (Figure C and D). To assess the effect of rMIF on p38 phosphorylation in sarcoidosis AMs, AMs were cultured in chamber slides and treated with rMIF alone, LPS (100 ng/mL) in presence or absence of rMIF for 30 min, and then stained with antibody against pp38. As shown, sarcoid AMs exhibit enhanced pp38 levels at the baseline confirming our previous results[28,29] (Figure 4E). Challenge with rMIF decreased baseline levels of pp38 fluorescence staining in sarcoidosis AMs (Figure 4F). As expected LPS challenge led to an increase in levels of the pp38 staining (Figure 4G). rMIF treatment in the presence of LPS led to a decrease in the LPS-mediated pp38 staining (Figure 4H). The secondary antibody control is shown in Figure 4I. The quantification of fluorescence intensity and indicator for p38 activation showed that rMIF significantly decreased the activation of p38 (Figure 4J).

MKP-1 is a dual specificity MAPK phosphatase that is considered as an important feedback control of MAPK activation.[32] MKP-1 regulates the immune response by dephosphorylating the Thr-Xaa-Tyr motifs on the activated MAPKs.[28,32–34] MKP-1is highly expressed by innate immune cells and belongs to the immediate-early gene family.[35] Many immunomodulatory compounds, like corticosteroid and rapamycin are positive regulators of MKP-1 expression.[34,36] Because exogenous MIF decreased the phosphorylation of p38 and ERK1/2 MAPKs, we determined the effect of rMIF on MKP-1 expression in sarcoid AMs. AMs were cultured in the presence or absence of rMIF (60 ng/mL) for 30 min. Western blot was performed using the AMs cell lysates to evaluate the levels of MKP-1 protein. rMIF treatment

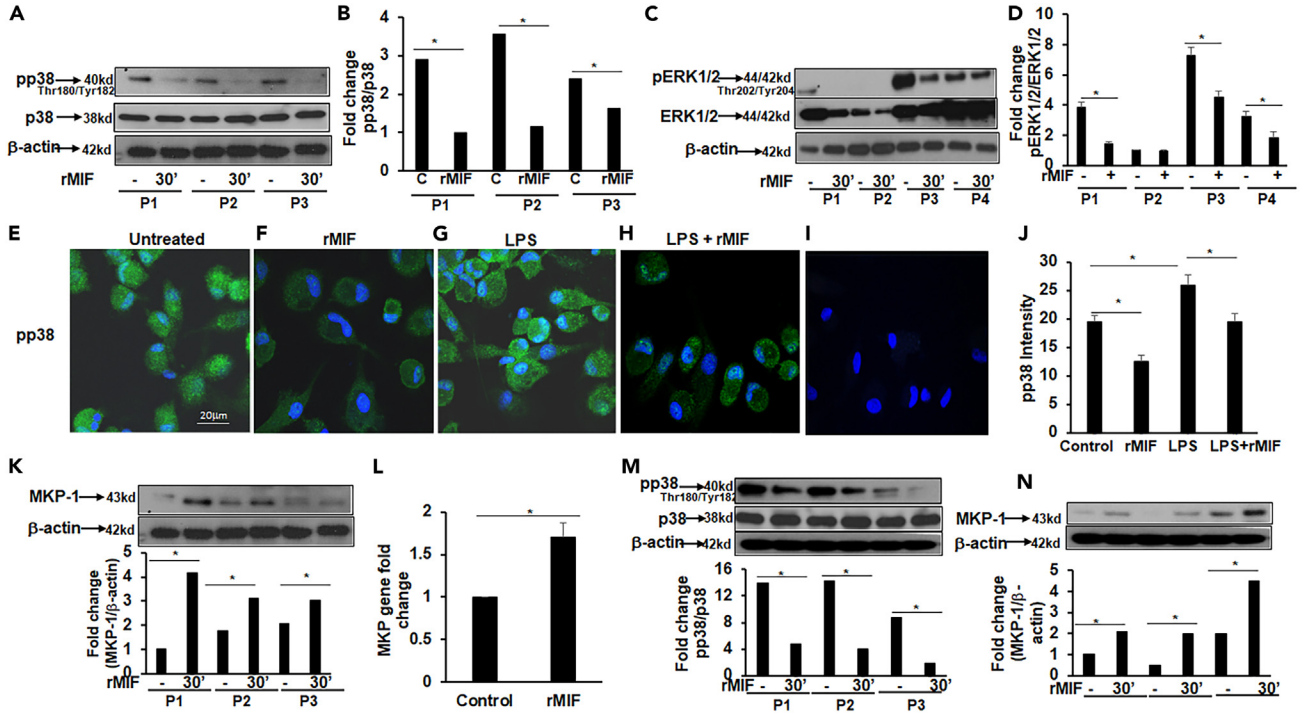


Figure 4. Exogenous MIF decreases the activation of p38 and ERK1/2 MAPKs and induces the MKP-1 expression

Sarcoidosis AMs were treated with rMIF (60 ng/mL) for 30 min. Whole cell lysates were subjected to immunoblotting to assess the p38 and ERK1/2 activation. Values were normalized to total p38 or total ERK1/2 and are shown as relative expression to untreated cells. Densitometry analysis is expressed as fold increase of the ratio of specific phosphorylated protein/total protein. Sarcoid AMs (n = 3 patients) showed the enhanced p38 activation at baseline and treatment with rMIF significantly decreased the activation of p38 (A) Densitometry analysis of blot.

(B) Western blot of pERK1/2 (C) and densitometry analysis of immunoblot (D) shows that treatment of sarcoid AMs with rMIF significantly decreased the activation of ERK1/2. Sarcoidosis AMs (1×10^5) were allowed to adhere overnight on chamber slides. Next day cells were treated with rMIF or LPS (100 ng/mL) in the presence or absence of rMIF (60 ng/mL) for 30 min and then were washed briefly with PBST and fixed with 4% paraformaldehyde. Cells were permeabilized with 0.1% saponin, blocked (10% FCS), and then incubated with anti-pp38 antibody overnight at 4°C. The secondary antibody was Alexa Fluor 488 - conjugated goat anti-rabbit antibody (green). Nuclei were stained with DAPI (blue). Images were analyzed by Confocal laser scanning microscopy (CLSM-310, Zeiss). Images show enhanced baseline activation of p38 (E), rMIF decreased the p38 activation.

(F) LPS treatment increased the activation of p38 (G) and rMIF decreased the LPS-induced p38 activation.

(H) Secondary antibody control.

(I) The quantification of fluorescence intensity showed that rMIF significantly decreased the activation of p38.

(J) Treatment of AMs (n = 3 patients) with rMIF increased the expression of MKP-1 (upper panel, K), densitometry analysis of blot (lower panel, K). Sarcoidosis AMs (n = 5) were treated with rMIF (60 ng/mL) for 1 h and cells were harvested to isolate the RNA. Total RNA was extracted using TRIzol reagent and reverse transcribed. The MKP-1 and a reference gene, GAPDH primers were used to amplify the corresponding cDNA by using iQ SYBR Green Supermix. PCR amplification was performed in a total volume of 20 μL containing 2 μL each cDNA preparation and 20 pg primers. Relative mRNA levels were calculated after normalizing to GAPDH. rMIF significantly induced the expression of MKP-1 mRNA.

(L) Sarcoid $CD14^+$ monocytes isolated from PBMCs were treated with rMIF (60 ng/mL) for 30 min. Whole cell lysates were subjected to immunoblotting to assess the p38 activation and MKP-1expression. Values were normalized to total p38 or ®-actin and are shown as relative expression to untreated cells. Sarcoid $CD14^+$ monocytes (n = 3 patients) showed the enhanced p38 activation at baseline and treatment with rMIF significantly decreased the activation of p38 (upper panel, M), and densitometry analysis of blot (lower panel, M). Treatment of sarcoid $CD14^+$ monocytes (n = 3 patients) with rMIF increased the expression of MKP-1 (upper panel, N), densitometry analysis of blot (lower panel.

(N) The Western blot and densitometric results are representative from three patients out of total of 6 patients. *, $p < 0.05$ and was considered significant.

of sarcoidosis AMs led to a significant MKP-1 induction at the protein level (Figure 4K). MKP-1 expression is tightly regulated both at transcriptional and post transcriptional levels.[37] As shown, the treatment of AMs with rMIF significantly upregulated the MKP-1 transcript (Figure 4L). Previously, we have shown that sarcoid patients exhibit higher percentage of double-positive $CD14^+$ phospho p38 positive monocytes,[29] therefore, we determined the effect of rMIF on the circulating $CD14^+$ monocytes isolated from sarcoid PBMCs. Purified $CD14^+$ monocytes were cultured in the presence or absence of rMIF (60 ng/mL) for 30 min. Western blot was performed using the monocytes cell lysates to evaluate the levels of pp38 and MKP-1 protein. The results showed that sarcoid monocytes at baseline exhibit enhanced pp38 levels and treatment with rMIF decreased the phosphorylation of p38 MAPK (Figure 4M). The rMIF treatment of $CD14^+$ monocytes significantly enhanced the MKP-1 expression (Figure 4N).

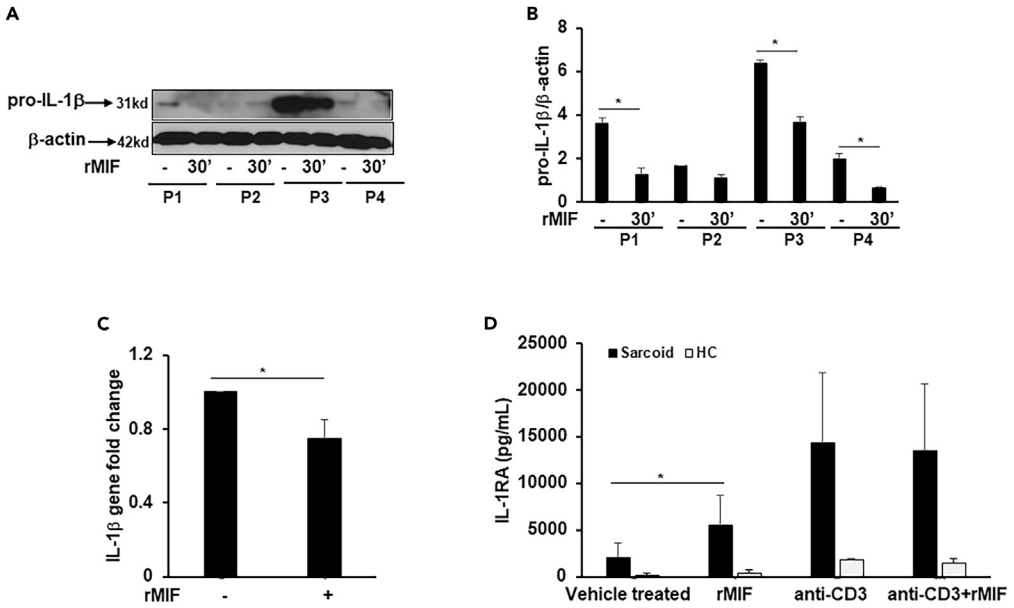


Figure 5. Exogenous MIF decreases the pro-IL-1β protein and gene expression and induces IL-1RA

Sarcoidosis AMs were treated with rMIF (60 ng/mL) for 30 min. Whole cell lysates obtained after 30 min of treatment were subjected to immunoblotting to assess the pro-IL-1β expression. Values were normalized to β-actin and are shown as relative expression to untreated cells. Densitometry analysis is expressed as fold increase of the ratio of specific protein/β-actin. Sarcoid AMs showed the enhanced pro-IL-1® expression at baseline and treatment with rMIF significantly decreased the pro-IL-1β expression.

(A) Densitometry analysis of blot.

(B) The Western blot and densitometric results are representative from three patients out of total of 6 patients. *, $p < 0.05$ and was considered significant. The IL-1β and a reference gene, GAPDH primers were used to amplify the corresponding cDNA by using iQ SYBR Green Supermix. Relative mRNA levels were calculated after normalizing to GAPDH. rMIF significantly reduced the expression of IL-1β mRNA.

(C) Sarcoid or HC PBMCs were activated with anti-CD3 (1 μg/mL) in the presence or absence of rMIF (60 ng/mL). Conditioned media were collected after 96 h and were assessed for IL-1RA via ELISA. The range of baseline IL-1RA in HC was 73–316 pg/mL and in sarcoid 25–10000 pg/mL rMIF significantly increased the IL-1RA production in sarcoid PBMCs (range: 545–18526 pg/mL) but did not significantly increase the IL-1RA levels in HC (range:79–642 pg/mL.

(D) ELISA results represent mean ± SEM from 10 independent experiments. *, $p < 0.05$ and was considered significant.

MIF decreased the pro-IL-1β and induced the production of IL-1 receptor antagonist (IL-1RA)

Previously, we have shown that *ex vivo* sarcoidosis AMs exhibit elevated levels of pro-IL-1β and IL-1 receptor antagonist (IL-1RA).[28–30] IL-1 RA is considered as a decoy receptor for IL-1β and thought that IL-1RA competitively binds to IL-1β and forms a nonsignaling complex that acts as a molecular trap by inhibiting IL-1beta activity on cells.[38] Thus, we explored the effect of exogeneous MIF on pro-IL1β and IL1β in cultured sarcoidosis AMs. As previously reported, sarcoidosis AMs exhibit increased levels of pro-IL-1β at baseline, which significantly decreased in response to the rMIF treatment (Figures 5A and 5B). We also determined the effect of rMIF on the IL-1β mRNA expression in sarcoidosis AMs in response to rMIF treatment. The exogenous MIF led to a significant downregulation of IL-1β transcripts (Figure 5C). These data indicates that exogenous MIF decreases IL-1β both at transcript and protein levels. Next, we asked if exogenous MIF has any effect on the production of IL-1RA. Sarcoidosis or HC PBMCs were cultured in the presence of rMIF alone or challenged with anti-CD3 in presence or absence of rMIF (60 ng/mL) for 96 h and IL-1RA levels were measured in the conditioned media via ELISA. The baseline IL-1RA mean value (194 pg/mL) in HC was significantly lower than the sarcoid patients (2061 pg/mL). Exogenous MIF led to increased IL-1RA in HC (from 194 to 360 pg/mL) as well as in sarcoidosis PBMCs (from 2061 to 5500 pg/mL). The challenge of PBMCs with anti-CD3 significantly induced IL-1RA in both HC and sarcoid PBMCs but rMIF had no significant additive effects on anti-CD3 mediated IL-1RA induction (Figure 5D).

DISCUSSION

MIF is a highly conserved ubiquitously present protein that exhibits multiple properties of lymphokine, hormone, enzyme, chaperone, oxidoreductase, and a growth factor.[39–41] It is constitutively expressed in most tissues and is stored in the intracellular vesicles.[42] MIF secretion occurs through a noncanonical pathway as it lacks an N-terminal signal sequence for translocation into the endoplasmic reticulum (ER)/Golgi.[43] Released MIF has both autocrine and paracrine effects, hence plays an essential role in the maintenance of cellular homeostasis, tissue repair and is involved in the regulation of both innate and adaptive immune responses. MIF is a multifaceted protein interacting with multiple binding partners and regulating fundamental cellular functions. Exogenous MIF exerts its biological effects either through the cell surface receptors/co-receptors CD74/CD44 axis or by non-receptor mediated endocytosis.[5] It has been shown that the intracellular MIF or endocytosed exogenous MIF can interact with the intracellular proteins/transcriptional coactivator and modulate signaling pathways.[16,44] Intracellular MIF activities are linked

to the thiol protein oxidoreductase activity of MIF, the tumor suppressor protein p53, and c-Jun activation domain binding protein-1 (JAB1).[16,45] Recent experimental evidence indicates that MIF plays a beneficial role by protecting from tissue senescence and DNA damage.[37,46,47] For instance, MIF binds to superoxide dismutase 1 (SOD1) and inhibits the accumulation of misfolded SOD1 in neurodegenerative disease.[48]

Sarcoidosis is characterized as a systemic and local disease affecting barrier organs like lung, skin, and eyes with aberrant immune response to undefined environmental or infectious triggers.[49] In sarcoidosis, CD4 cells are highly represented in the lungs but there is a relative lower representation of CD8 cells and Tregs.[28,49] The excessive amount of activation signals often leads to a decreased $CD8^+$ T cells, gradual deterioration of T cell function and inefficient control of persisting antigens.[26,27] Several mechanisms are associated with T cell exhaustion, including elevated PD-1 levels and sustained p38 activation.[27,50] Inhibition of p38 has been shown to enhance the anti-tumor efficacy of T-cells and T cell cytotoxicity through the increased production of IFN-γ.[27] Another study showed that PD-1 or p38 blockade can reverse the proliferation and effector function of memory $CD8^+$ T cells.[50] Our results show that exogenous MIF decreased the p38 phosphorylation and potentiated the IFN-γ production. Interestingly, TCR activation of sarcoidosis PBMCs did not lead to a significant augmentation of intracellular MIF in CD4 and CD8 cells. In contrast, TCR activation of healthy control PBMCs enhanced the MFI for intracellular MIF in CD4 as well as CD8 cells. Whether the lack of accumulation of intracellular MIF in sarcoidosis in response to TCR activation is associated with T cell exhaustion needs further investigation.

Recently, we have shown that based on serum MIF levels there were two distinct classes of sarcoidosis patients: (1) a group with high levels of MIF, and (2) a group with low MIF levels.[31] The high MIF group was associated with elevated IL-10 and IFN-γ, whereas the low MIF group exhibited a lower IL-10 and IFN-γ level. Our current experimental data support our clinical observation, as exogenous MIF dampened the IL-6 and IL-1β production but upregulated IL-10 and IFN-γ production. IFN-γ has been shown to have both inflammatory and regulatory activities.[51] IL-10 is an anti-inflammatory cytokine that negatively regulates the activity of Th1, NK cells and macrophages.[52] One study showed that when MIF produced at the higher physiological levels acts as an inhibitor of the T cell immune responses.[53] Our data indicate that the MIF regulates the cytokine production in activated sarcoidosis PBMCs by modulating the balance between pro and anti-inflammatory cytokines. Evaluating the MIF effects on the activation markers in sarcoidosis PBMCs, we found that exogenous MIF had no significant effect on the activation markers of T-cells and antigen-presenting cells. In contrast, the *ex vivo* rMIF in anti-CD3 stimulated PBMCs increased the percentage of Tregs from 6 to 10%. $CD4^+$CD25+FoxP3+ Tregs are a subpopulation of T cells that modulates the enhanced immune responses through immunosuppressive cytokines (e.g., IL-10, TGF-β).[54] Our study shows that exogenous MIF influences the immune cell activation in sarcoidosis by promoting IL-10 production and Tregs induction.

Persistent granulomatous inflammation in sarcoidosis is associated with increased p38, RIP2 activation, and an increased in HIF-1α expression in sarcoidosis granuloma.[28–30] Activated AMs play a central role in granuloma formation and their activation is associated with distinct signaling, including sustained p38 activation, lack of MKP-1 expression, abundance of HIF1-α in association with metabolic derangement, elevated proinflammatory cytokine production and activated T cells.[28–30,55] Activation of MAPKs is required for production of various cytokines, including the pro-IL-1β. Our current results show that exogenous MIF inhibits phosphorylation of both p38 and ERK1/2 in sarcoidosis AMs, $CD14^+$ monocytes and PBMCs. Similarly, rMIF decreased the baseline levels of pro-IL-1β in sarcoidosis AMs and IL1-β production in PBMCs. MAPKs are primarily deactivated through dephosphorylation of the regulatory residues (threonine and tyrosine) of their targeted MAPKs by dual specific phosphatases (DUSPs or MKPs). MKP-1 is the key molecule responsible for immune modulatory effects of various drugs, including corticosteroid (GC) and rapamycin.[34,35,56,57] Many chronic inflammatory diseases including asthma, and sarcoidosis have been associated with dysregulation of MKP-1 expression.[28,56] In fact, the immune suppressive effect of GC attributed, at least in part, to MKP-1induction.[58,59] One of the mechanisms in GC resistant asthma is attributed to the lack of MKP-1 induction in response to GC.[60,61] Interestingly, our results demonstrated that exogenous MIF induces MKP-1 in sarcoidosis AMs both at the transcript and protein levels. This explains mechanistically that exogenous MIF induces MKP-1, which in turn decreased the p38/ERK phosphorylation in sarcoidosis AMs and reduced the production of proinflammatory cytokines. $CD14^+$ monocytes are elevated in sarcoidosis patients and have been shown to be involved in the sarcoidosis pathology. Our results showed that $CD14^+$ monocytes expressed higher levels of pp38 and exogenous MIF induced MKP-1 and decreased the phosphorylation of p38 in the isolated monocytes. Interestingly, we found that p38 inhibitor (SB203580) while suppressing several pro-inflammatory cytokines, induced the secretion of MIF in sarcoid PBMCs (data not shown), suggesting positive MIF regulation through p38 inhibition. Numerous studies have shown that GCs in dose dependent manner increases MIF expression and secretion, in contrast to other pro-inflammatory cytokines that are generally suppressed by GC.[4,15,62] In several experimental animal models of sepsis or transformed macrophage cell lines (RAW 264.7), several groups have shown that exogenous MIF abrogates the GC immune suppressive effect on LPS induced proinflammatory cytokines.[62,63] In similar experimental sepsis, it has been shown that MIF inhibit the GCs mediated MKP-1 induction through inhibitory effects on GC-induced leucine zipper (GILZ).[63,64] This evidence is the basis for the hypothesis that MIF functions as an endogenous counter regulator of GCs effects.[62] However, it has been shown that exogenous MIF does not act as a mitogen and MIF is required for T cell activation.[1,62] Important to note that our experimental design completely differs from the experimental endotoxin models, as we did not study the concomitant effect of MIF and GCs.

One of our novel findings is the upregulation of IL-1RA in response to exogenous MIF both in sarcoidosis and healthy control PBMCs. Elevated IL-1RA has been reported in human autoimmune/inflammatory diseases, including rheumatoid arthritis and sarcoidosis.[30,65] Although it is shown that IL-1RA acts as a decoy receptor and binds *in vitro* to IL-1β,[38,66] the mechanism by which it is upregulated is not fully elucidated. Further studies are required to delineate the cellular mechanisms involved in the modulation of IL-1RA production by MIF.

Thus, the results of our study suggest the immunomodulatory role of MIF in sarcoidosis that suppresses the p38 activation, induces the MKP-1, decreases the proinflammatory cytokines (IL-6 and IL-1β) but induces Tregs, IFN-γ, IL-10 and IL-1RA. In conclusion, MIF has apparently

paradoxical functions, responding in different ways depending on physiological concentration, cell types, underlying stimulation context and the timing of and the stage at which it is produced.

Limitations of the study

Our data indicates that MIF does not have mitogenic capacity or potentiate the T or macrophages activation. However, it is still unclear if the modulatory role of MIF is entirely due to upregulation of MKP-1 or though IL-1RA and Tregs. Further experiments are required to address the mechanisms by which MIF induces MKP-1.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Study participant details
- METHOD DETAILS
  - BAL and the isolation of alveolar macrophages (AMs)
  - Isolation of PBMCs and purification of $CD14^+$ monocytes
  - Enzyme- linked immunosorbent assay (ELISA)
  - Flow cytometry and cell surface immunostaining
  - Protein extraction and immunoblotting
  - Immunofluorescent staining
  - RNA extraction and quantitative real-time RT-PCR
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108746.

ACKNOWLEDGMENTS

This work was supported by NHLBI: grant R01HL113508 (LS).

AUTHOR CONTRIBUTIONS

J.T. conducted the experiments and contributed to data analysis and writing the original draft and review of the manuscript. C.P. contributed to doing RT-PCR experiments. L.S. conceived and designed the study, participated in all areas of the research, data analysis and writing of the manuscript.

DECLARATION OF INTERESTS

None of authors has any competing interests to declare.

Received: March 16, 2023
Revised: September 21, 2023
Accepted: December 12, 2023
Published: December 14, 2023

REFERENCES

1. Bacher, M., Metz, C.N., Calandra, T., Mayer, K., Chesney, J., Lohoff, M., Gemsa, D., Donnelly, T., and Bucala, R. (1996). An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proc. Natl. Acad. Sci. USA *93*, 7849–7854.
2. Hofmann, E., Soppert, J., Ruhl, T., Gousopoulos, E., Gerra, S., Storti, G., Tian, Y., Brandhofer, M., Schweizer, R., Song, S.-Y., et al. (2021). The Role of Macrophage Migration Inhibitory Factor in Adipose-Derived Stem Cells Under Hypoxia. Front. Physiol. *12*, 638448.
3. Farr, L., Ghosh, S., and Moonah, S. (2020). Role of MIF Cytokine/CD74 receptor pathway in protecting against injury and promoting repair. Front. Immunol. *11*, 1273.
4. Calandra, T., Froidevaux, C., Martin, C., and Roger, T. (2003). Macrophage migration inhibitory factor and host innate immune defenses against bacterial sepsis. J. Infect. Dis. *187*, S385–S390.
5. Calandra, T., and Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. Nat. Rev. Immunol. *3*, 791–800.

6. Fingerle-Rowson, G., Koch, P., Bikoff, R., Lin, X., Metz, C.N., Dhabhar, F.S., Meinhardt, A., and Bucala, R. (2003). Regulation of macrophage migration inhibitory factor expression by glucocorticoids in vivo. Am. J. Pathol. *162*, 47–56.
7. Grieb, G., Merk, M., Bernhagen, J., and Bucala, R. (2010). Macrophage migration inhibitory factor (MIF): a promising biomarker. Drug News Perspect. *23*, 257–264.
8. Dankers, W., Hasnat, M.A., Swann, V., Alharbi, A., Lee, J.P., Cristofaro, M.A., Gantier, M.P., Jones, S.A., Morand, E.F., Flynn, J.K., and Harris, J. (2020). Necrotic cell death increases the release of macrophage migration inhibitory factor by monocytes/macrophages. Immunol. Cell Biol. *98*, 782–790.
9. Nishihira, J. (2000). Macrophage migration inhibitory factor (MIF): its essential role in the immune system and cell growth. J. Interferon Cytokine Res. *20*, 751–762.
10. Ningyan, G., Xu, Y., Hongfei, S., Jingjing, C., and Min, C. (2015). The role of macrophage migration inhibitory factor in mast cell-stimulated fibroblast proliferation and collagen production. PLoS One *10*, e0122482.
11. Stoppe, C., Averdunk, L., Goetzenich, A., Soppert, J., Marlier, A., Kraemer, S., Vieten, J., Coburn, M., Kowark, A., Kim, B.-S., et al. (2018). The protective role of macrophage migration inhibitory factor in acute kidney injury after cardiac surgery. Sci. Transl. Med. *10*, eaan4886.
12. Koebernick, H., Grode, L., David, J.R., Rohde, W., Rolph, M.S., Mittrücker, H.W., and Kaufmann, S.H.E. (2002). Macrophage migration inhibitory factor (MIF) plays a pivotal role in immunity against Salmonella typhimurium. Proc. Natl. Acad. Sci. USA *99*, 13681–13686.
13. Donnelly, S.C., Haslett, C., Reid, P.T., Grant, I.S., Wallace, W.A., Metz, C.N., Bruce, L.J., and Bucala, R. (1997). Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. Nat. Med. *3*, 320–323.
14. Yamaguchi, E., Nishihira, J., Shimizu, T., Takahashi, T., Kitashiro, N., Hizawa, N., Kamishima, K., and Kawakami, Y. (2000). Macrophage migration inhibitory factor (MIF) in bronchial asthma. Clin. Exp. Allergy *30*, 1244–1249.
15. Calandra, T., Echtenacher, B., Roy, D.L., Pugin, J., Metz, C.N., Hültner, L., Heumann, D., Männel, D., Bucala, R., and Glauser, M.P. (2000). Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat. Med. *6*, 164–170.
16. Nguyen, M.T., Lue, H., Kleemann, R., Thiele, M., Tolle, G., Finkelmeier, D., Wagner, E., Braun, A., and Bernhagen, J. (2003). The cytokine macrophage migration inhibitory factor reduces pro-oxidative stress-induced apoptosis. J. Immunol. *170*, 3337–3347.
17. Roger, T., Schneider, A., Weier, M., Sweep, F.C.G.J., Le Roy, D., Bernhagen, J., Calandra, T., and Giannoni, E. (2016). High expression levels of macrophage migration inhibitory factor sustain the innate immune responses of neonates. Proc. Natl. Acad. Sci. USA *113*, E997–E1005.
18. Harris, J., VanPatten, S., Deen, N.S., Al-Abed, Y., and Morand, E.F. (2019). Rediscovering MIF: new tricks for an old cytokine. Trends Immunol. *40*, 447–462.
19. Shi, X., Leng, L., Wang, T., Wang, W., Du, X., Li, J., McDonald, C., Chen, Z., Murphy, J.W., Lolis, E., et al. (2006). CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. Immunity *25*, 595–606.
20. Leng, L., Metz, C.N., Fang, Y., Xu, J., Donnelly, S., Baugh, J., Delohery, T., Chen, Y., Mitchell, R.A., and Bucala, R. (2003). MIF signal transduction initiated by binding to CD74. J. Exp. Med. *197*, 1467–1476.
21. Noe, J.T., and Mitchell, R.A. (2020). MIF-dependent control of tumor immunity. Front. Immunol. *11*, 609948.
22. Schwartz, V., Lue, H., Kraemer, S., Korbiel, J., Krohn, R., Ohl, K., Bucala, R., Weber, C., and Bernhagen, J. (2009). A functional heteromeric MIF receptor formed by CD74 and CXCR4. FEBS Lett. *583*, 2749–2757.
23. Lue, H., Dewor, M., Leng, L., Bucala, R., and Bernhagen, J. (2011). Activation of the JNK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on CXCR4 and CD74. Cell. Signal. *23*, 135–144.
24. Miyara, M., Amoura, Z., Parizot, C., Badoual, C., Dorgham, K., Trad, S., Kambouchner, M., Valeyre, D., Chapelon-Abric, C., Debré, P., et al. (2006). The immune paradox of sarcoidosis and regulatory T cells. J. Exp. Med. *203*, 359–370.
25. Hawkins, C., Shaginurova, G., Shelton, D.A., Herazo-Maya, J.D., Oswald-Richter, K.A., Rotsinger, J.E., Young, A., Celada, L.J., Kaminski, N., Sevin, C., and Drake, W.P. (2017). Local and systemic CD4+ T cell exhaustion reverses with clinical resolution of pulmonary sarcoidosis. J. Immunol. Res. *2017*, 3642832.
26. Yi, J.S., Cox, M.A., and Zajac, A.J. (2010). T-cell exhaustion: characteristics, causes and conversion. Immunology *129*, 474–481.
27. Chan, J.D., Beavis, P.A., and Darcy, P.K. (2020). p38 kinase: a key target for driving potent T cells for adoptive immunotherapy. Cancer Cell *37*, 756–758.
28. Rastogi, R., Du, W., Ju, D., Pirockinaite, G., Liu, Y., Nunez, G., and Samavati, L. (2011). Dysregulation of p38 and MKP-1 in response to NOD1/TLR4 stimulation in sarcoid bronchoalveolar cells. Am. J. Respir. Crit. Care Med. *183*, 500–510.
29. Talreja, J., Talwar, H., Ahmad, N., Rastogi, R., and Samavati, L. (2016). Dual Inhibition of Rip2 and IRAK1/4 Regulates IL-1beta and IL-6 in Sarcoidosis Alveolar Macrophages and Peripheral Blood Mononuclear Cells. J. Immunol. *197*, 1368–1378.
30. Talreja, J., Talwar, H., Bauerfeld, C., Grossman, L.I., Zhang, K., Tranchida, P., and Samavati, L. (2019). HIF-1alpha regulates IL-1beta and IL-17 in sarcoidosis. Elife *8*, e44519.
31. Elahi, M., Talreja, J., Steinbauer, B., Koth, L.L., and Samavati, L. (2022). Modulatory role of macrophage migration inhibitory factor on cytokines and clinical features of sarcoidosis. Sci. Rep. *12*, 16906–16911.
32. Liu, Y., Shepherd, E.G., and Nelin, L.D. (2007). MAPK phosphatases–regulating the immune response. Nat. Rev. Immunol. *7*, 202–212.
33. Talwar, H., Bauerfeld, C., Bouhamdan, M., Farshi, P., Liu, Y., and Samavati, L. (2017). MKP-1 negatively regulates LPS-mediated IL-1beta production through p38 activation and HIF-1alpha expression. Cell. Signal. *34*, 1–10.
34. Rastogi, R., Jiang, Z., Ahmad, N., Rosati, R., Liu, Y., Beuret, L., Monks, R., Charron, J., Birnbaum, M.J., and Samavati, L. (2013). Rapamycin Induces Mitogen-activated Protein (MAP) Kinase Phosphatase-1 (MKP-1) Expression through Activation of Protein Kinase B and Mitogen-activated Protein Kinase Kinase Pathways. J. Biol. Chem. *288*, 33966–33977.
35. Li, L., Chen, S.-F., and Liu, Y. (2009). MAP kinase phosphatase-1, a critical negative regulator of the innate immune response. Int. J. Clin. Exp. Med. *2*, 48–67.
36. Chi, H., Barry, S.P., Roth, R.J., Wu, J.J., Jones, E.A., Bennett, A.M., and Flavell, R.A. (2006). Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. Proc. Natl. Acad. Sci. USA *103*, 2274–2279.
37. Florez-Sampedro, L., Soto-Gamez, A., Poelarends, G.J., and Melgert, B.N. (2020). The role of MIF in chronic lung diseases: looking beyond inflammation. Am. J. Physiol. Lung Cell Mol. Physiol. *318*, L1183–L1197.
38. Arend, W.P., and Guthridge, C.J. (2000). Biological role of interleukin 1 receptor antagonist isoforms. Ann. Rheum. Dis. *59*, i60–i64.
39. Kang, I., and Bucala, R. (2019). The immunobiology of MIF: function, genetics and prospects for precision medicine. Nat. Rev. Rheumatol. *15*, 427–437.
40. Israelson, A., Ditsworth, D., Sun, S., Song, S., Liang, J., Hruska-Plochan, M., McAlonis-Downes, M., Abu-Hamad, S., Zoltsman, G., Shani, T., et al. (2015). Macrophage migration inhibitory factor as a chaperone inhibiting accumulation of misfolded SOD1. Neuron *86*, 218–232.
41. Thiele, M., and Bernhagen, J. (2005). Link between macrophage migration inhibitory factor and cellular redox regulation. Antioxidants Redox Signal. *7*, 1234–1248.
42. Sumaiya, K., Langford, D., Natarajaseenivasan, K., and Shanmughapriya, S. (2022). Macrophage migration inhibitory factor (MIF): a multifaceted cytokine regulated by genetic and physiological strategies. Pharmacol. Ther. *233*, 108024.
43. Flieger, O., Engling, A., Bucala, R., Lue, H., Nickel, W., and Bernhagen, J. (2003). Regulated secretion of macrophage migration inhibitory factor is mediated by a non-classical pathway involving an ABC transporter. FEBS Lett. *551*, 78–86.
44. Song, S., Xiao, Z., Dekker, F.J., Poelarends, G.J., and Melgert, B.N. (2022). Macrophage migration inhibitory factor family proteins are multitasking cytokines in tissue injury. Cell. Mol. Life Sci. *79*, 105.
45. Mitchell, R.A., Liao, H., Chesney, J., Fingerle-Rowson, G., Baugh, J., David, J., and Bucala, R. (2002). Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. Proc. Natl. Acad. Sci. USA *99*, 345–350.
46. Marsh, L.M., Cakarova, L., Kwapiszewska, G., von Wulffen, W., Herold, S., Seeger, W., and Lohmeyer, J. (2009). Surface expression of CD74 by type II alveolar epithelial cells: a potential mechanism for macrophage migration inhibitory factor-induced epithelial repair. Am. J. Physiol. Lung Cell Mol. Physiol. *296*, L442–L452.
47. Nemajerova, A., Mena, P., Fingerle-Rowson, G., Moll, U.M., and Petrenko, O. (2007). Impaired DNA damage checkpoint response in MIF-deficient mice. EMBO J. *26*, 987–997.
48. Shvil, N., Banerjee, V., Zoltsman, G., Shani, T., Kahn, J., Abu-Hamad, S., Papo, N., Engel, S., Bernhagen, J., and Israelson, A. (2018). MIF inhibits the formation and toxicity of

misfolded SOD1 amyloid aggregates: implications for familial ALS. Cell Death Dis. *9*, 107.
49. Gerke, A.K., and Hunninghake, G. (2008). The immunology of sarcoidosis. Clin. Chest Med. *29*, 379–390. vii.
50. Henson, S.M., Macaulay, R., Riddell, N.E., Nunn, C.J., and Akbar, A.N. (2015). Blockade of PD-1 or p38 MAP kinase signaling enhances senescent human CD8+ T-cell proliferation by distinct pathways. Eur. J. Immunol. *45*, 1441–1451.
51. Lees, J.R. (2015). Interferon gamma in autoimmunity: A complicated player on a complex stage. Cytokine *74*, 18–26.
52. Couper, K.N., Blount, D.G., and Riley, E.M. (2008). IL-10: the master regulator of immunity to infection. J. Immunol. *180*, 5771–5777.
53. Yan, X., Orentas, R.J., and Johnson, B.D. (2006). Tumor-derived macrophage migration inhibitory factor (MIF) inhibits T lymphocyte activation. Cytokine *33*, 188–198.
54. Vignali, D.A.A., Collison, L.W., and Workman, C.J. (2008). How regulatory T cells work. Nat. Rev. Immunol. *8*, 523–532.
55. Geamanu, A., Gupta, S.V., Bauerfeld, C., and Samavati, L. (2016). Metabolomics connects aberrant bioenergetic, transmethylation, and gut microbiota in sarcoidosis. Metabolomics *12*, 35.
56. Quante, T., Ng, Y.C., Ramsay, E.E., Henness, S., Allen, J.C., Parmentier, J., Ge, Q., and Ammit, A.J. (2008). Corticosteroids reduce IL-6 in ASM cells via up-regulation of MKP-1. Am. J. Respir. Cell Mol. Biol. *39*, 208–217.
57. Hoppstädter, J., and Ammit, A.J. (2019). Role of dual-specificity phosphatase 1 in glucocorticoid-driven anti-inflammatory responses. Front. Immunol. *10*, 1446.
58. Wang, X., Meng, X., Kuhlman, J.R., Nelin, L.D., Nicol, K.K., English, B.K., and Liu, Y. (2007). Knockout of Mkp-1 enhances the host inflammatory responses to gram-positive bacteria. J. Immunol. *178*, 5312–5320.
59. Wang, X., Nelin, L.D., Kuhlman, J.R., Meng, X., Welty, S.E., and Liu, Y. (2008). The role of MAP kinase phosphatase-1 in the protective mechanism of dexamethasone against endotoxemia. Life Sci. *83*, 671–680.
60. Manley, G.C.A., Parker, L.C., and Zhang, Y. (2019). Emerging regulatory roles of dual-specificity phosphatases in inflammatory airway disease. Int. J. Mol. Sci. *20*, 678.
61. Zhang, Y., Leung, D.Y.M., and Goleva, E. (2014). Anti-inflammatory and corticosteroid-enhancing actions of vitamin D in monocytes of patients with steroid-resistant and those with steroid-sensitive asthma. J. Allergy Clin. Immunol. *133*, 1744–1752.e1.
62. Calandra, T., Bernhagen, J., Metz, C.N., Spiegel, L.A., Bacher, M., Donnelly, T., Cerami, A., and Bucala, R. (1995). MIF as a glucocorticoid-induced modulator of cytokine production. Nature *377*, 68–71.
63. Aeberli, D., Yang, Y., Mansell, A., Santos, L., Leech, M., and Morand, E.F. (2006). Endogenous macrophage migration inhibitory factor modulates glucocorticoid sensitivity in macrophages via effects on MAP kinase phosphatase-1 and p38 MAP kinase. FEBS Lett. *580*, 974–981.
64. Fan, H., Kao, W., Yang, Y.H., Gu, R., Harris, J., Fingerle-Rowson, G., Bucala, R., Ngo, D., Beaulieu, E., and Morand, E.F. (2014). Macrophage migration inhibitory factor inhibits the antiinflammatory effects of glucocorticoids via glucocorticoid-induced leucine zipper. Arthritis Rheumatol. *66*, 2059–2070.
65. Arend, W.P., Malyak, M., Guthridge, C.J., and Gabay, C. (1998). Interleukin-1 receptor antagonist: role in biology. Annu. Rev. Immunol. *16*, 27–55.
66. Arend, W.P., Joslin, F.G., and Massoni, R.J. (1985). Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor. J. Immunol. *134*, 3868–3875.
67. Crouser, E.D., Maier, L.A., Wilson, K.C., Bonham, C.A., Morgenthau, A.S., Patterson, K.C., Abston, E., Bernstein, R.C., Blankstein, R., Chen, E.S., et al. (2020). Diagnosis and Detection of Sarcoidosis. An Official American Thoracic Society Clinical Practice Guideline. Am. J. Respir. Crit. Care Med. *201*, e26–e51.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Human MIF Antibody	R&D	Cat# MAB289, RRID:AB_2281975
Anti-p38 MAPK Antibody	Cell Signaling	Cat# 9212, RRID:AB_330713
Phospho-p38 MAPK (Thr180/Tyr182) Antibody	Cell Signaling	Cat# 9211, RRID:AB_331641
ERK 1/2	Cell Signaling	Cat# 9102, RRID:AB_330744
pERK1/2	Cell Signaling	Cat# 9101, RRID:AB_331646
Anti-rabbit IgG HRP-linked Antibody	Cell Signaling	Cat# 7074, RRID:AB_2099233
Anti-mouse IgG HRP-linked Antibody	Cell Signaling	Cat# 7076, RRID:AB_330924
MKP-1	Santa Cruz	Cat# sc-1102, RRID:AB_2094224
Beta Actin	Abcam	Cat# ab8227, RRID:AB_2305186
FITC Mouse Anti-Human CD4	BD Biosciences	Cat# 340133, RRID: AB_400007
PE-Cy™7 Mouse Anti-Human CD8	BD Biosciences	Cat# 566858, RRID:AB_2869912
FITC Mouse Anti-Human CD19	BD Biosciences	Cat# 555412, RRID:AB_395812
PE Mouse Anti-Human CD25	BD Biosciences	Cat# 341009, RRID: AB_400203
Alexa Fluor® 647 Mouse anti-Human FoxP3	BD Biosciences	Cat# 561184, RRID:AB_10584328
Purified Mouse Anti-Human CD3	BD Biosciences	Cat# 550367, RRID:AB_393638
FITC Mouse Anti-Human CD86	BD Biosciences	Cat# 557343, RRID: AB_396651
PE Mouse Anti-Human CD80	BD Biosciences	Cat# 557227, RRID: AB_396606
APC anti-human CD74 Antibody	Biolegend	Cat# 326812, RRID:AB_2564389
Goat anti-Rabbit IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Thermofisher	Cat # A-11008
Biological samples		
Human Blood samples	WSU, Detroit	
Human bronchoalveolar lavage (BAL) samples	WSU, Detroit	
Chemicals, peptides, and recombinant proteins		
Recombinant Human MIF Protein	R&D	Cat# 289-MF
UltraComp eBeads™ Compensation Beads	Thermofisher	Cat# 01-2222-42
Lipopolysaccharide (LPS)	Invivogen	Cat# vac-3pelps
Cytiva Amersham™ ECL™ Western Blotting Detection Reagents	Fisherscientific	Cat# 45-000-878
ProLong™ Gold Antifade Mountant with DNA Stain DAPI	Thermofisher	Cat# P36931
TRIzol reagent	Thermofisher	Cat# 15596026
Ficoll-Histopaque 1077	Sigma-Aldrich	Cat# 1077
Critical commercial assays		
Human IL-1 beta/IL-1F2 DuoSet ELISA	R&D	Cat# DY201
Human IL-6 DuoSet ELISA	R&D	Cat# DY206
Human IL-10 DuoSet ELISA	R&D	Cat# DY217B
Human IFN-gamma DuoSet ELISA	R&D	Cat# DY285B
Oligonucleotides		
GAPDH forward:GTCTCCTCTGACTTCAACAGCG	IDT	
GAPDH reverse:ACCACCCTGTTGCTGTAGCCAA	IDT	
MKP-1 forward: CAACCACAAGGCAGACATCAGC	IDT	
MKP-1 Reverse: GTAAGCAAGGCAGATGGTGGCT	IDT	

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
IL-1beta forward: CTTCGACACATGGGATAACG	IDT	
IL-1beta reverse: ATGGACCAGACATCACCAAG	IDT	
Software and algorithms		
ImageJ	NIH	
Flow Jo	BD Biosciences	
Other		
LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit	Thermofisher	Cat# L34976
iScript™ Reverse Transcription Supermix for RT-qPCR	Bio-rad	Cat# 1708841
Fast SYBR Green Master Mix	Thermofisher	Cat# A46109

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact: Jaya Talreja (jtalreja@.med.wayne.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data presented in this manuscript is available.
- Code availability: Code will be shared by the lead contact upon request.
- Other item availability: Microscopy data, flow cytometry and any additional information reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study participant details

The Committee for Investigations Involving Human Subjects at Wayne State University approved the protocol for obtaining bronchoalveolar lavage (BAL) and blood samples from patients with sarcoidosis (n = 60). The IRB number for this study is 055208MP4E. All methods were performed in accordance with the relevant guidelines and regulations. Informed consent was obtained from all subjects enrolled in the study. Sarcoidosis diagnosis was based on the ATS/ERS/WASOG statement.[67] The enrollment criteria for sarcoidosis patients were as previously described.[28–30]

METHOD DETAILS

BAL and the isolation of alveolar macrophages (AMs)

BAL was collected during bronchoscopy after administration of local anesthesia and before tissue biopsies.[28–30] Briefly, a total of 150–200 mL of sterile saline solution was injected via fiberoptic bronchoscopy; the BAL fluid was retrieved, measured, and centrifuged. Cells recovered from the BAL fluid were filtered through a sterile gauze pad and washed three times with phosphate-buffered saline (PBS), resuspended in endotoxin-free RPMI 1640 medium (HyClone) supplemented with L-glutamine (Life Technologies), penicillin/streptomycin (Life Technologies), and 1% fetal calf serum (HyClone), and then counted. BAL cells were cultured on adherent plates for 1 h at 37°C in air containing 5% CO2. Non-adherent cells were removed by aspiration; adherent cells were washed three times and used as AMs. Viability of these populations was routinely about 97% and by morphologic criteria 99% of cells were AMs.[20,28–31]

Isolation of PBMCs and purification of $CD14^+$ monocytes

PBMCs were isolated from heparinized blood using Ficoll-Histopaque (Sigma, St. Louis, MO) density gradient separation followed by washing twice with PBS. Cell suspension was made in endotoxin-free RPMI 1640 medium (HyClone) supplemented with L-glutamine (Life Technologies), penicillin/streptomycin (Life Technologies), and 10% fetal calf serum (HyClone). Cells were cultured in 12-well plates for further experiments.[28–30] CD14 + monocytes were purified from PBMCs by using the MACS monocyte isolation kit (Miltenyi Biotech, San Diego, CA) according to the manufacturer's instructions. The purity of enriched monocytes was evaluated by flow cytometry using fluorochrome-conjugated CD14 antibody; the purity of monocytes was about 95%.

Enzyme- linked immunosorbent assay (ELISA)

The levels of, IL-6, IFN-©, IL-10 and IL-1® and MIF in the conditioned media were measured by ELISA according to the manufacturer's instructions (ELISA DuoKits; R and D Systems, Minneapolis, MN).

Flow cytometry and cell surface immunostaining

PBMCs from subjects with sarcoidosis were isolated, cultured, and after appropriate treatment for 96 h, cells were stained for cell surface markers using fluorescent labeled antibodies for CD4-FITC, CD8-PE-cy7, CD25-PE CD80-PE, CD86-FITC, CD69-PerCP, CD3-PE-cy7, CD19-FITC. Intracellular staining of PBMCs was done for MIF and FoxP3. Briefly, PBMCs were first surface stained for CD4, CD8, CD3, CD19 (for MIF) or stained for CD4, CD25 (for FoxP3+ Tregs) and then fixed using 100 μL of 1% paraformaldehyde for 30 min and then permeabilized with permeabilization buffer (0.5% saponin) for 15 min at room temperature. Cells were centrifuged and resuspended in 100 μL of permeabilization buffer and stained with FoxP3-AF647 antibody for 30 min. Cells were washed and resuspended in staining buffer. For MIF staining, PBMCs were cultured for 96 h and 3 μM monensin for 2 h. PBMCs were first surface stained for CD4, CD8 and then fixed and permeabilized with permeabilization buffer (0.5% saponin) and stained with MIF antibody followed by the Alexa 488 secondary antibody (#A11070, Molecular Probes). After 30 min cells were washed twice, resuspended in staining buffer, and analyzed for CD3+MIF+, CD19+-MIF+, CD4+MIF+ and CD8+MIF+ cells or $CD4^{+}$CD25+FoxP3+ (Tregs) cells by flow cytometry. Flow cytometry was performed on a BD LSR II SORP and data analysis was performed using FlowJo software (FlowJo, LLC, Ashland, OR).[29,30] Samples were gated on cells using FSC/SSC and doublet discrimination was performed to identify singlets using SSC-W vs. SSC-A. Exclusion of dead cells was done using Live/Dead Near IR dye. Ultracomp beads were stained with each single-color fluorophore conjugated antibodies used in the immunostaining to set voltage and gating parameters. The flow cytometry work was done at the Microscopy, Imaging and Cytometry Resources (MICR) Core at the Karmanos Cancer Institute, Wayne State University.

Protein extraction and immunoblotting

Total cellular proteins were extracted by addition of RIPA buffer containing a protease inhibitor cocktail and antiphosphatase I and II (Sigma Chemicals). Protein concentration was measured with the BCA assay (Thermo Scientific, CA). Equal amounts of proteins (10 μg) were mixed 1/1 (v/v) with 2x sample buffer (20% glycerol, 4% sodium dodecyl sulfate, 10% 2-βME, 0.05% bromophenol blue, and 1.25 M Tris-HCl, pH 6.8), and fractionated on a 10% sodium dodecyl sulfate–polyacrylamide gel. Proteins were transferred onto a polyvinylidene difluoride membrane (Bio-Rad) for 60 min at 18 V using a SemiDry Transfer Cell (Bio-Rad). The polyvinylidene difluoride membrane was blocked with 5% nonfat dry milk in TBST (Tris-buffered saline with 0.1% Tween 20) for 1 h, washed, and incubated with primary Abs (1/1000) overnight at 4°C. The blots were washed with TBST and then incubated for 1 h with horseradish peroxidase–conjugated secondary anti-IgG Ab using a dilution of 1/10,000 in 5% nonfat dry milk in TBST. Membranes were washed four times in TBST. Immuno-reactive bands were visualized with a chemiluminescent reagent (Amersham GE, PA). Images were captured on Hyblot CL film (Denville; Scientific, Inc; Metuchen, NJ) using a JPI automatic X-ray film processor model JP-33. Optical density analysis of signals was performed using ImageJ software.[28,30,34]

Immunofluorescent staining

The expression of pp38 in sarcoidosis AMs was visualized by immunofluorescence staining. AMs (1×10^{5}) were allowed to adhere overnight on chamber slides. Next day cells were activated with rMIF for 30 min and then were washed briefly with PBST and fixed with 4% paraformaldehyde. Cells were washed and permeabilized with 0.1% saponin, blocked (10% FCS), and then incubated with anti-pp38 overnight at 4°C. The next day cells were washed three times with PBS for 5 min and stained with Alexa-fluor 488- conjugated goat anti-rabbit secondary antibody for 45 min. The secondary antibody control well was not incubated with the primary antibody (pp38) but stained with secondary antibody only. Then the cells were washed with PBS and the slide was mounted with a drop of ProLong Gold antifade reagent with DAPI (Invitrogen). The slide was examined using an Axiovert 40 CFL fluorescence microscope (Carl Zeiss MicroImaging, Inc).[30]

RNA extraction and quantitative real-time RT-PCR

Total RNA was extracted using TRIzol reagent (Life Technologies) and reverse transcribed using the ABI Reverse Transcription System (Life Technologies). The primers (targeting MKP-1 and a reference gene, GAPDH) were used to amplify the corresponding cDNA by using iQ SYBR Green Supermix (Life Technologies). Quantitative analysis of mRNA expression was performed using the ABI instrument (Life Technologies). PCR amplification was performed in a total volume of 20 μL containing 2 μL each cDNA preparation and 20 pg primers (Invitrogen). The PCR amplification protocol was performed as described previously.[29,34] Relative mRNA levels were calculated after normalizing to GAPDH. Data were analyzed using the unpaired, two-tailed Student's *t* test, and the results were expressed as relative fold of change. The following primers were used in the PCR reactions: GAPDH forward, 5′- GTCTCCTCTGACTTCAACAGCG -3′ and reverse, 5′- ACCACCCTGTTG CTGTAGCCAA -3′; MKP-1, forward, 5′-CAACCACAAGGCAGACATCAGC-3′and reverse, 5′-GTAAGCAAGGCAGATGGTGGCT-3′, IL-1® forward, 5′-CTTCGACACATGGGATAACG-3′ and reverse, 5′-ATGGACCAGACATCACCAAG-3′.

QUANTIFICATION AND STATISTICAL ANALYSIS

ELISA results were expressed as mean ± SEM. For all analyses, a two-way ANOVA was used to compare independent variables among groups and treatments. A p values of less than 0.05 were considered to be significant.