

Rat Mesangial Cells Express Macrophage Migration Inhibitory Factor *In Vitro* and *In Vivo*

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Abstract. Mesangial cells are thought to promote glomerular macrophage accumulation in glomerulonephritis. This may occur through the production of macrophage migration inhibitory factor (MIF), a molecule known to regulate macrophage accumulation at sites of inflammation. To study this, glomerular MIF expression and macrophage accumulation were examined in rat anti-Thy-1 disease, a model of mesangioproliferative nephritis. *In situ* hybridization and immunohistochemistry showed that MIF is expressed by some podocytes in normal rat glomeruli. *De novo* MIF expression by glomerular endothelium was seen on day 1 of anti-Thy-1 disease. On day 6, glomerular MIF mRNA and protein expression were prominent in segmental proliferative lesions, which was also the location of most infiltrating macrophages. Double-staining identified *de novo* MIF mRNA and protein expression by proliferating mesangial cells within these lesions.

Cytokine regulation of mesangial cell MIF expression was examined *in vitro*. Northern blotting showed that cultured rat mesangial cells express a single 0.6-kb species of MIF mRNA, and Western blotting detected a single protein band of 12.5 kD. Six-hour stimulation of mesangial cells with interferon- γ or platelet-derived growth factor significantly increased MIF mRNA levels. However, the addition of recombinant MIF to mesangial cells did not affect mesangial cell proliferation or constitutive transforming growth factor- β mRNA expression, nor did MIF induce monocyte chemoattractant protein-1 mRNA expression. In conclusion, this is the first study to demonstrate that mesangial cells can produce MIF *in vivo* and *in vitro*. It is postulated that mesangial cell MIF production in response to injury acts to promote macrophage accumulation within segmental proliferative lesions in rat anti-Thy-1 nephritis. (J Am Soc Nephrol 9: 417–424, 1998)

In human glomerulonephritis, glomerular macrophage infiltration is associated with mesangial hypercellularity and increased mesangial matrix deposition (1–3). Depletion studies in animal disease models have demonstrated that the macrophage plays an important role in the mesangial cell response to injury, leading to increased mesangial cellularity and glomerulosclerosis (4–6). Mesangial cells are thought to contribute to glomerular macrophage accumulation through the production of chemotactic cytokines and adhesion molecules, such as monocyte chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule-1 (ICAM-1) (7–12). A key molecule in macrophage accumulation at sites of inflammatory injury is macrophage migration inhibitory factor (MIF). Recent studies have shown that blocking the action of MIF markedly reduces macrophage accumulation seen in the cutaneous tuberculin delayed-type hypersensitivity response, experimental endotoxemia, and in rat crescentic glomerulonephritis (13–15). An examination of rat crescentic glomerulonephritis has shown that renal MIF expression is upregulated during disease (16); however, it is not known whether mesangial cells express MIF.

Therefore, we have examined: (1) whether glomerular cells, and in particular mesangial cells, make MIF in anti-Thy-1 nephritis a model of mesangioproliferative disease; and (2) whether there is a temporal association between glomerular MIF expression and macrophage accumulation in anti-Thy-1 nephritis.

Materials and Methods

Anti-Thy-1 Disease Model

In-bred male Wistar rats (150 to 180 g) were obtained from Monash Animal Services (Melbourne, Australia). Disease was induced by a single intravenous injection of 5 mg/kg purified OX-7 monoclonal antibody (IgG1). Groups of four animals were killed on days 1, 4, 6, 8, 10, and 14 after OX-7 IgG injection. In addition, a group of four healthy animals were examined.

Antibodies

A mouse IgG1 monoclonal antibody (mAb), termed III.D.9, and a rabbit polyclonal antibody were prepared against recombinant human and mouse MIF, respectively. Both of these antibodies recognize rat and mouse MIF by Western blotting and immunohistochemistry and neutralize MIF bioactivity *in vitro* and *in vivo*, including suppression of CD3-mediated T cell proliferation and the skin delayed-type hypersensitivity response in mice and rats (13–17). Other mAb used were: mouse antihuman proliferating cell nuclear antigen, IgG2a (PC10, Dakopatts, Glostrup, Denmark) (18); mouse anti-rat CD68 (mAb, IgG1 (ED1) (19); and 1A4 (IgG2a), anti- α smooth muscle actin (Sigma Chemical Co., St. Louis, MO). Isotype control antibodies were

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mouse antihuman CD45R, IgG1 (73.5) (20), mouse antihuman CD45, IgG2a (71.5) (20), and normal rabbit IgG. Secondary antibodies included: rabbit anti-mouse IgG2a conjugated with either horseradish peroxidase (HRP) or alkaline phosphatase (AP) (Zymed, San Francisco, CA); goat anti-mouse IgG conjugated with either HRP or AP (Dakopatts); sheep anti-rabbit IgG conjugated with HRP; complexes of mouse anti-HRP IgG conjugated with HRP (mPAP, Dakopatts); complexes of mouse anti-AP IgG conjugated with AP (mAPAAP, Silenus, Melbourne, Australia).

Immunohistochemistry

For double-labeling of tissue sections, fresh tissue was fixed in 4% formalin for 18 to 24 h and then processed into paraffin blocks. Tissue sections (4 μ m) were placed on slides coated with 3-aminopropyltriethoxysilane (Sigma), deparaffinized, and then either washed in phosphate-buffered saline (PBS) or microwave-treated for 10 min in 10 mM sodium citrate buffer, pH 6.0, as described previously (21,22). Microwave treatment was necessary to denature residual OX-7 antibody within tissue sections to prevent its detection with goat anti-mouse (GAM)-HRP. Tissue sections were then preincubated in 10% normal rabbit serum (NRBS)/10% normal sheep serum (NSHS) followed by 10% bovine serum albumin (BSA). These sections were incubated with primary antibody (ED-1 or PC10) overnight at 4°C, blocked for endogenous peroxidase by treatment with 0.3% H₂O₂ in methanol for 20 min, incubated sequentially with GAM-HRP and mPAP for 40 min each, and developed with diaminobenzidine (Sigma). The tissue sections were then microwave-treated for 10 min to block antibody cross reactivity, inactivate endogenous alkaline phosphatase activity, and facilitate detection of the MIF antigen (22). Sections were preincubated with 10% NRBS/10% NSHS followed by 10% BSA and then incubated with mouse antihuman MIF mAb (1:1000) overnight at 4°C. Bound MIF mAb was detected by sequentially incubating with GAM-AP and mAPAAP for 40 min each, and then developing with fast blue BB salt (Ajax Chemicals, Melbourne, Australia). Double-labeled sections were counterstained with periodic acid-Schiff reagent.

For immunostaining of cultured mesangial cells, 1097 cells were grown to near confluence on chamber well tissue culture slides (Labtek, Nunc, Naperville, IL), washed with PBS, and then fixed for 20 min with 2% paraformaldehyde-lysine-periodate (PLP). These slides were preincubated with 10% NRBS/10% NSHS followed by 10% BSA. The slides were then incubated with 1 μ g/ml mouse antihuman MIF IgG1 in 1% BSA for 1 h. After washing in PBS, the slides were blocked for endogenous peroxidase. The slides were then incubated sequentially with GAM-HRP and mPAP for 40 min each and developed using diaminobenzidine (Sigma).

Quantitation of Immunohistochemistry

Antibody-labeled sections were scored for MIF-positive, ED-1-positive, or ED-1-proliferating cell nuclear antigen (PCNA)-positive cells within the glomerular tuft. Fifty glomeruli were scored for each animal, and labeled cells were expressed as mean \pm SD per glomerular cross section. Counting was performed on blinded slides under high magnification (\times 400).

Probes

A 1.26-kb cDNA fragment of rat interleukin-1 β (IL-1 β) and a 980-base cDNA fragment of rat transforming growth factor- β 1 (TGF- β 1) were prepared by reverse transcription-PCR and cloned into the pMOSBlue T-vector (23). The cDNA for rat MCP-1 was kindly provided by Dr. Teizo Yoshimura (National Cancer Institute, Fred-

erick, MD) (24). Antisense digoxigenin (DIG)-labeled cRNA probes were prepared using an RNA labeling kit, according to the manufacturer's instructions (Boehringer Mannheim, Mannheim, Germany). A 440-bp fragment of rat MIF cDNA (25) and a 358-bp fragment of rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (26) were amplified by reverse transcription-PCR and DIG-labeled using the High-Prime random priming kit (Boehringer Mannheim).

In Situ Hybridization

In situ hybridization was performed on 4- μ m paraffin sections of formalin-fixed tissue, using a microwave-based protocol (16). After dewaxing, sections were treated with a microwave oven for 2 \times 5 min at pH 6.0 as described above, incubated with 0.2 M HCl for 15 min, followed by 1% Triton X-100 for 15 min, and then digested for 20 min with 10 μ g/ml proteinase K at 37°C (Boehringer Mannheim). Sections then were washed in 2 \times SSC, prehybridized, and then hybridized with 0.3 ng/ μ l DIG-labeled sense or antisense MIF cRNA probe overnight at 37°C in a hybridization buffer containing 50% deionized formamide, 4 \times SSC, 2 \times Denhardt's solution, 1 mg/ml salmon sperm DNA, and 1 mg/ml yeast tRNA. Sections were washed finally in 0.1 \times SSC at 37°C, and the hybridized probe was detected using sheep anti-DIG antibody (Fab) conjugated with alkaline phosphatase and color development with nitroblue tetrazolium/X-phosphate (Boehringer Mannheim). Some sections were treated with microwave oven heating and stained with the ED-1 or 1A4 mAb, using the three-layer PAP method described above and counterstained with periodic acid-Schiff. Sections were mounted in an aqueous medium. No signal was seen with the MIF sense riboprobe labeled to the same specific activity.

Tissue Culture

A well-characterized cloned mesangial cell line (1097) isolated from Sprague Dawley rats (27,28) was used between passages 20 and 30. A mouse macrophage cell line (P388D1) was used as a positive control. Cells were cultured in RPMI 1640 (Life Technologies, Grand Island, NY) with 1, 2, or 10% fetal calf serum (FCS), 10 mM Hepes buffer, 50 U/ml penicillin, and 30 μ g/ml streptomycin in a humidified 5% CO₂ atmosphere at 37°C. This medium was determined to be endotoxin-free by limulus amoebocyte lysate testing (E-Toxate, Sigma). For Northern and Western blot studies, cells were grown in 175-cm² tissue culture flasks to near confluence and then stimulated for 6, 12, or 24 h with lipopolysaccharide (LPS; 2 μ g/ml), recombinant mouse tumor necrosis factor- α (rmTNF- α ; 10 ng/ml), recombinant mouse interleukin-1 α (rmIL-1 α ; 10 ng/ml), recombinant rat interferon- γ (rIFN- γ ; 1000 U/ml), recombinant human platelet-derived growth factor-AB (rhPDGF-AB; 10 ng/ml), recombinant mouse TGF- β 1 (rmTGF β 1; 10 ng/ml), recombinant mouse interleukin-10 (rmIL-10; 50 ng/ml), or recombinant mouse MIF (rmMIF; 50 ng/ml) (29) or recombinant human MIF (rhMIF; 50 ng/ml) (29), in 2% FCS/RPMI 1640.

For proliferation studies, mesangial cells were added to 96-well flat-bottomed microtiter plates in RPMI 1640/10% heat-inactivated FCS and allowed to adhere overnight. The subconfluent cells were then cultured for 2 to 3 d in 2% heat-inactivated FCS with or without 0.1 to 100 ng/ml recombinant murine or human MIF, rhPDGF-AB (10 ng/ml), the neutralizing III.D.9 anti-MIF mAb (3 to 30 μ g/ml), or an isotype control mAb (3 to 30 μ g/ml). During the last 6 h of culture, 0.5 μ Ci/well of ³H-thymidine (Amersham, Buckinghamshire, United Kingdom) was added. Cells were harvested by removing the medium, washing three times in PBS, and dissolving in 100 μ l of 0.2 M NaOH. This solution was neutralized with HCl, and scintillation counting was

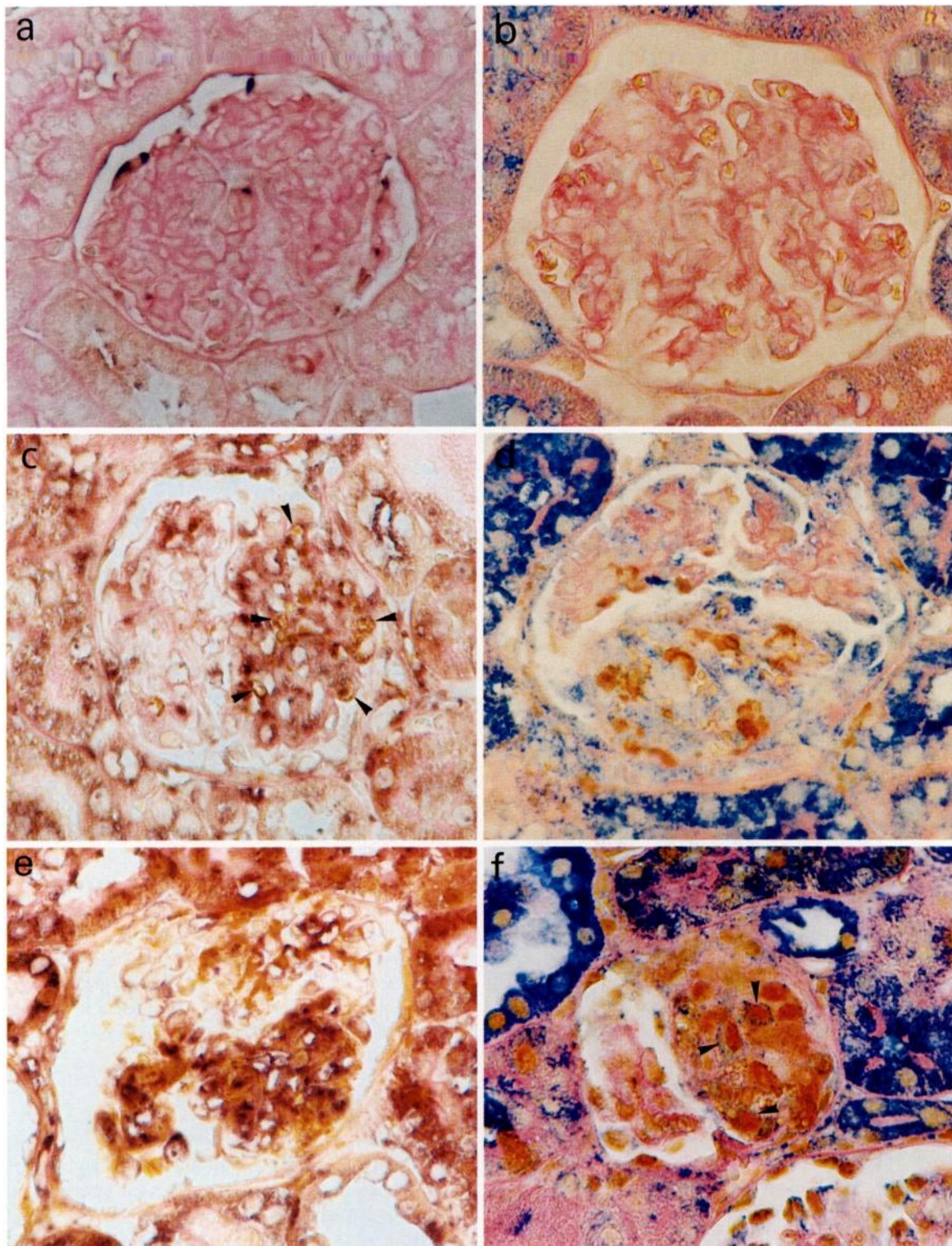


Figure 1. Glomerular macrophage migration inhibitory factor (MIF) expression in rat anti-Thy-1 nephritis assessed by *in situ* hybridization (a, c, and e) and immunohistochemistry (b, d, and f). (a) *In situ* hybridization showing a few MIF mRNA-positive podocytes (purple) in normal rat kidney. (b) Double-immunostaining showing occasional, weak glomerular MIF-positive cells (blue) in normal rat kidney. (c) Combined *in situ* hybridization and immunohistochemistry showing many MIF mRNA-positive cells (purple) in a segmental area of mesangial cell proliferation on day 6 of anti-Thy-1 nephritis. Note the presence of ED-1-positive macrophages (brown cytoplasmic stain, arrowheads) within the segmental lesion. (d) Double-immunostaining showing MIF protein expression (blue) by many cells in a proliferative lesion in association with accumulation of ED-1-positive macrophages (brown, arrowheads) on day 6 of disease. (e) Combined *in situ* hybridization and immunohistochemistry showing colocalization of MIF mRNA expression (purple) and α -smooth muscle actin (brown cytoplasmic stain) in a focal proliferative lesion. (f) Double-immunostaining showing MIF protein expression (blue) by proliferating cell nuclear antigen (PCNA)-positive cells (brown nuclei) within a segmental lesion on day 6 of disease (arrowheads). All sections were counterstained with periodic acid-Schiff minus hematoxylin. Magnification, $\times 400$.

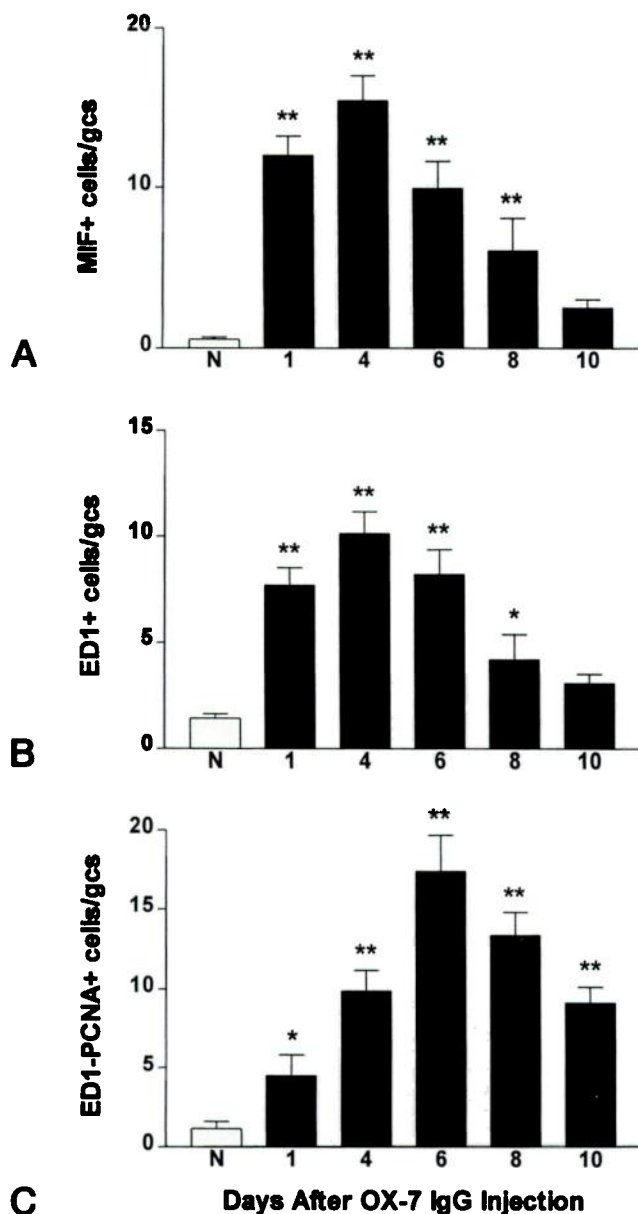


Figure 2. Quantitation of glomerular MIF expression, glomerular macrophage accumulation, and mesangial cell proliferation in normal rats (open bars) and during anti-Thy-1 nephritis (filled bars). (A) Glomerular MIF expression. (B) Accumulation of ED-1-positive glomerular macrophages. (C) Mesangial cell proliferation assessed as ED-1-negative-PCNA-positive cells. Data are shown as mean cells per glomerular cross section \pm SD. Comparisons between healthy animals and diseased groups were performed by ANOVA, using Tukey's test. * $P < 0.05$; ** $P < 0.005$ versus healthy rats.

performed with a Wallac Rackbeta Counter (Turku, Finland). Replicates of six wells were used in all experiments.

Northern Blotting

Cells were removed from tissue culture flasks by trypsinization. These cells were washed in cold PBS and then lysed in Trizol reagent (Life Technologies). Total cellular RNA was prepared by the Trizol method (according to the manufacturer's instructions), and Northern blotting was performed according to a previously described method

(30). Briefly, RNA samples were denatured with glyoxal and DMSO, size-fractionated on 1.0% agarose gels, and capillary-blotted onto Hybond-N membranes (Amersham). Membranes were hybridized overnight at either 42°C with DIG-labeled cDNA probes or 68°C with DIG-labeled cRNA probes in 5× saline-sodium phosphate-ethylenediamine tetra-acetic acid/50% formamide/0.2 mg/ml herring sperm DNA/0.2% sodium dodecyl sulfate (SDS)/0.1% *N*-lauryl sarcosine. After hybridization, membranes were washed in 0.2× SSC/0.1% SDS at either 42 or 68°C. Bound probes were detected using sheep anti-DIG antibody (Fab) conjugated with alkaline phosphatase, which was developed using CPD-star enhanced chemiluminescence (ECL) (Boehringer Mannheim). ECL emissions captured on Kodak XAR film were measured by densitometry, using the Cue 2 Image Analyzer program (Olympus, Lake Success, NY). Northern blot experiments were performed in triplicate, and all results are expressed relative to the GAPDH signal.

Western Blotting

Mesangial cells and P388D1 macrophages (2×10^7 /ml) were lysed on ice for 30 min in 25 mM Tris-HCl lysis buffer, pH 8.0, containing 1% NP-40, 150 mM NaCl, 10 mM ethylenediamine tetra-acetic acid, 1 mM phenylmethylsulfonyl fluoride, and 5 mM iodoacetamide. Cellular debris was removed by centrifugation at $15,000 \times g$ for 10 min, and the lysate was stored at -80°C until use. Cell lysates (1 to 5×10^5 cells/well) were run on 15 to 18% acrylamide SDS-polyacrylamide gel electrophoresis gels under reducing and nonreducing conditions. Proteins were then transferred to nitrocellulose (Hybond-ECL, Amersham) by electroblotting at 0.5 mA/cm^2 in 10 mM 3-(cyclohexylamino)-L-propane sulfonic acid buffer, pH 11.0, 20% methanol for 16 to 18 h at 4°C , using a Bio-Rad Transblot apparatus. These blots were briefly washed in blot-washing buffer (50 mM Tris-HCl, pH 7.5, 350 mM NaCl, and 1% Tween 20) and then preincubated for 2 h in blocking buffer (50 mM Tris-HCl, pH 7.5, 350 mM NaCl, 5% nonfat milk powder, 1% BSA, and 0.2% Tween 20). The blots were incubated for 1 h with rabbit antihuman MIF antiserum (1:1000) in binding buffer (50 mM Tris-HCl, pH 7.5, 350 mM NaCl, 1% BSA, 0.2% Tween 20), washed five times for 3 min in washing buffer, and then incubated for an additional hour with sheep anti-rabbit IgG conjugated with HRP (1:10000) in binding buffer containing 2% normal sheep serum. The blots were then washed five times for 3 min with washing buffer, and labeled MIF protein was detected using an ECL detection kit (Amersham). ECL emissions were captured on Kodak XAR film.

Results

Glomerular MIF Expression in Anti-Thy-1 Nephritis

In situ hybridization and immunohistochemistry showed that MIF mRNA and protein are expressed by a few podocytes in glomeruli of normal rat kidney (Figure 1, a and b). After induction of anti-Thy-1 nephritis, there was a marked increase in glomerular MIF expression (Figure 2A). During the early phase of disease (days 1 to 4), immunostaining showed *de novo* MIF expression in dilated capillaries, together with an increase in the number of MIF-positive podocytes. There was *de novo* MIF expression within segmental proliferative lesions on day 6—the peak of mesangial cell proliferation in this disease model. Combined *in situ* hybridization and immunohistochemistry showed the presence of infiltrating ED-1-positive macrophages within segmental lesions, in which many of the cells expressed MIF mRNA (Figure 1c). *De novo* MIF protein

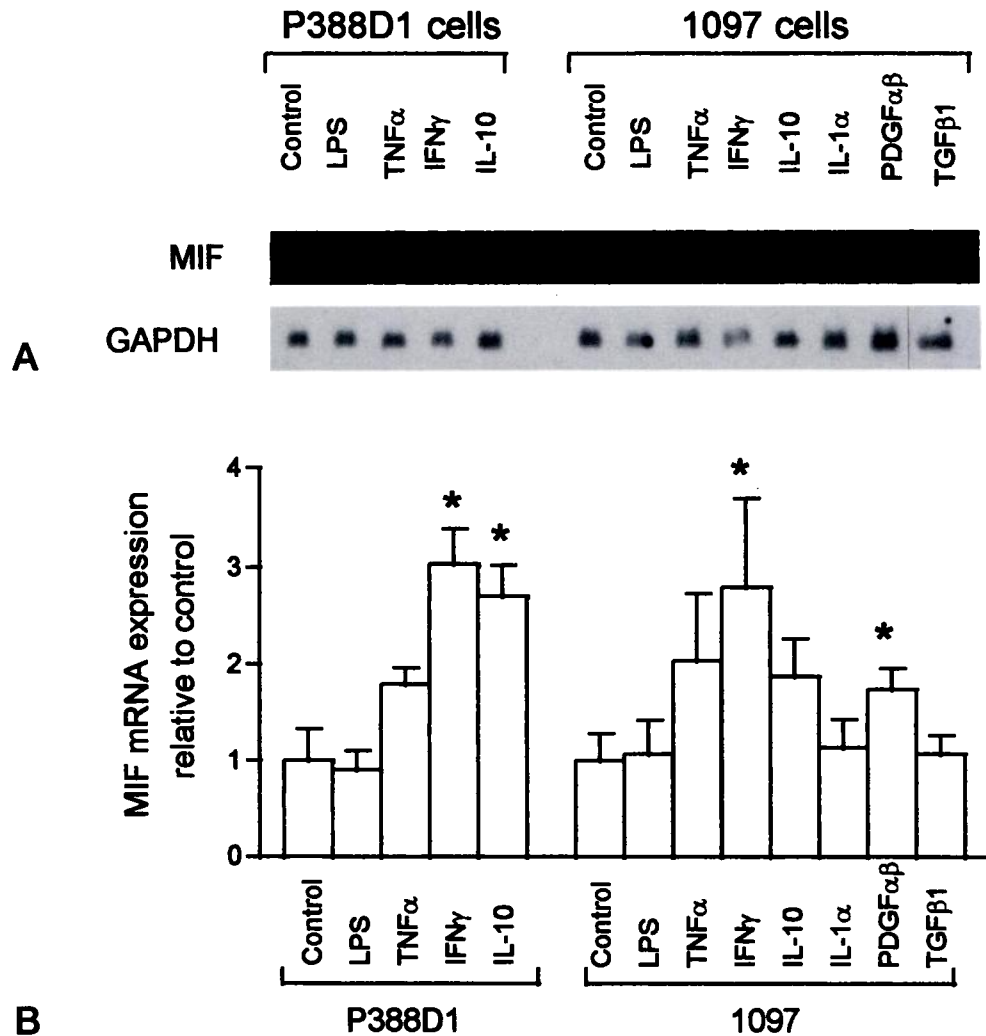


Figure 3. Regulation of MIF mRNA expression by cultured rat 1097 mesangial cells and P388D1 macrophages. (A) Northern blot detection of MIF and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in 1097 and P388D1 cells that were either unstimulated (control) or incubated with lipopolysaccharide or different cytokines for 6 h as indicated. (B) Quantitation of MIF mRNA expression relative to GAPDH, which has been normalized for the unstimulated control cells. Data are shown as mean \pm SD based on three experiments. Comparisons between control and treated groups were performed using the unpaired *t* test with Welch's correction. **P* < 0.05 versus control.

expression was also seen in such segmental lesions in association with accumulation of ED-1-positive macrophages (Figure 1d). To confirm that proliferating mesangial cells were expressing MIF within these lesions, we made use of two well-characterized markers of proliferating mesangial cells in this disease model: PCNA and α -smooth muscle actin (α -SMA) (31,32). Double-immunostaining showed expression of MIF protein by PCNA-positive cells within segmental lesions (Figure 1e), whereas combined *in situ* hybridization and immunohistochemistry clearly showed MIF mRNA expression by α -SMA-positive cells (Figure 1f). Glomerular MIF expression decreased to normal patterns on day 14 of disease, when the transient mesangial proliferative response was finished.

The colocalization of infiltrating macrophages and MIF expression within segmental proliferative lesions was a striking feature of this disease model (Figure 1, c and d). The temporal relationship between glomerular MIF expression, glomerular

macrophage infiltration, and mesangial cell proliferation is shown in Figure 2. There was a highly significant correlation between the number of glomerular cells expressing MIF and glomerular macrophage infiltration over the disease course (Spearman's coefficient $r = 0.94$; $P < 0.0001$). Furthermore, it was found that glomerular macrophage infiltration and MIF expression were maximal on day 4, preceding the peak of mesangial cell proliferation on day 6 (Figure 2).

Regulation of Mesangial Cell MIF Expression

To examine the regulation of MIF production by mesangial cells, we studied a well-characterized mesangial cell line (1097) *in vitro*. Mesangial cells grown *in vitro* were found to constitutively express a single 0.6-kb species of MIF mRNA (Figure 3). The production of MIF protein by cultured mesangial cells was determined by two methods. First, immunostaining with the anti-MIF III.D.9 mAb detected MIF protein ex-

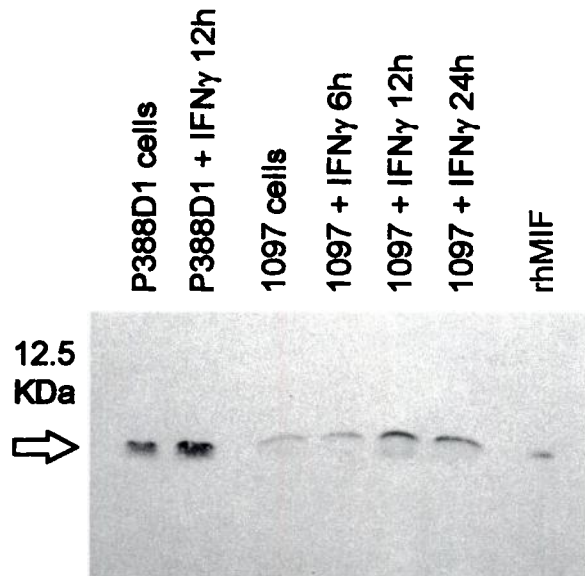


Figure 4. Western blot detection of MIF protein in rat mesangial cells. A 12.5-kD form of MIF protein was detected in normal and IFN- γ -stimulated rat 1097 mesangial cells. This form was also observed in P388D1 macrophages and was identical in size to purified recombinant human MIF protein (rhMIF). These results were reproduced in three separate experiments.

pression in mesangial cells cultured on glass slides (not shown). Second, Western blotting of mesangial cell lysates demonstrated the presence of a single 12.5-kD form of MIF protein under both reducing and nonreducing conditions (Figure 4).

Mesangial cell expression of MIF mRNA was significantly increased by stimulation for 6 h with either IFN- γ (\uparrow 179%, $P < 0.05$) or PDGF-AB (\uparrow 74%, $P < 0.05$), whereas the increases seen with TNF- α and IL-10 did not reach significance; LPS, IL-1 α , and TGF- β had no apparent effect (Figure 3, A and B). Western blot analysis showed that IFN- γ stimulation also produced an increase in MIF protein levels within mesangial cells (Figure 4).

A mouse macrophage cell line, P388D1, was used as a positive control for the detection of MIF expression (24). Stimulation with IFN- γ and other cytokines/growth factors had an effect on P388D1 macrophages similar to that observed with mesangial cells (Figure 3, A and B).

Effects of MIF on Mesangial Cell Proliferation and Cytokine Production

Cell culture studies measuring ^3H -thymidine uptake by mesangial cells in three separate experiments found no effect of murine or rhMIF (0.1 to 100 ng/ml) or neutralizing anti-MIF III.D.9 mAb (0.3 to 30 $\mu\text{g}/\text{ml}$) on the proliferation of rat mesangial cells cultured in 1, 2, or 10% FCS. However, an increase in mesangial cell proliferation was seen in all experiments after addition of PDGF-AB (10 ng/ml) as a positive control (not shown).

Strong *de novo* expression of MCP-1 mRNA expression in rat mesangial cells was induced by a 6-h stimulation with LPS

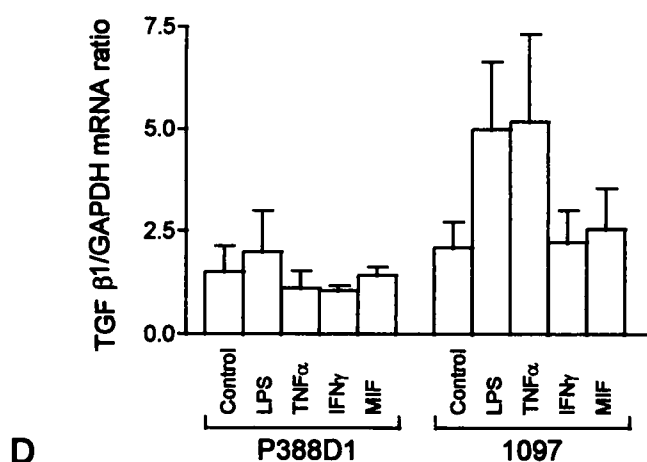
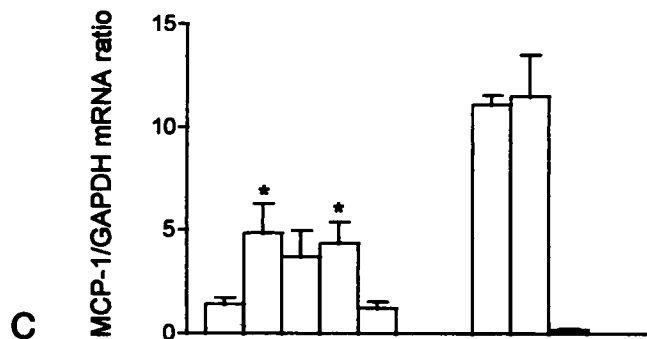
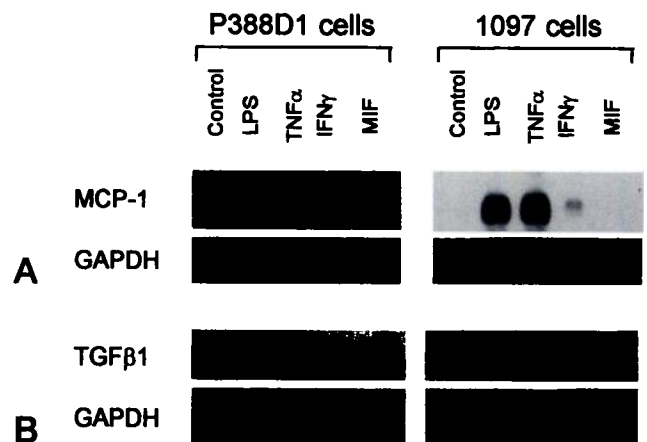


Figure 5. Effect of exogenous recombinant MIF on mesangial cell expression of monocyte chemoattractant protein-1 (MCP-1) and transforming growth factor- β 1 (TGF- β 1) mRNA. Rat 1097 mesangial cells and P388D1 macrophages were cultured for 6 h in the presence of 2% fetal calf serum with and without additional stimuli, as indicated. Northern blot analysis showed that MIF did not induce MCP-1 mRNA expression (A) and did not affect constitutive TGF- β 1 mRNA expression (B) by rat mesangial cells or P388D1 macrophages. This was confirmed by quantitation of the ratio of specific mRNA expression to that of GAPDH mRNA, as shown in C and D. Data in C and D are shown as mean \pm SD based on three experiments. Comparisons between normal and treated groups were performed using the unpaired *t* test with Welch's correction. * $P < 0.05$ versus control.

or TNF- α (Figure 5, A and C). In contrast, IFN- γ stimulation produced only a mild induction of MCP-1 mRNA, and MIF

failed to induce MCP-1 expression (Figure 5, A and C). In addition, stimulation with rmMIF did not affect the constitutive TGF- β 1 mRNA expression by rat mesangial cells or P388D1 mouse macrophages (Figure 5, B and D). A 24-h incubation with MIF also showed no effect on MCP-1 or TGF- β 1 mRNA expression (data not shown). The biological activity of rmMIF in the rat was confirmed by the ability of rmMIF to stimulate nitric oxide production in normal and IFN- γ -primed rat peritoneal macrophages *in vitro* as measured by the Greiss assay (data not shown), consistent with the ability of MIF to stimulate nitric oxide production in mouse macrophages (24).

Discussion

The main findings of this study are that mesangial cells can express MIF both *in vivo* and *in vitro* and that glomerular MIF expression correlates with macrophage accumulation in rat anti-Thy-1 nephritis. Although some kidney cell types, such as podocytes and tubular epithelial cells, constitutively express MIF, mesangial cells in the normal rat glomerulus do not. However, proliferating mesangial cells showed *de novo* MIF expression in rat anti-Thy-1 nephritis. This finding was based on combined *in situ* hybridization and immunohistochemistry, which demonstrated MIF mRNA and protein expression by PCNA-positive and α -SMA-positive cells in proliferative lesions—two well-characterized markers of proliferating mesangial cells in this disease model (31,32).

To examine MIF synthesis by mesangial cells, we used a well-characterized rat mesangial cell line (1097). Rat mesangial cells were shown to express a single 0.6-kb MIF mRNA species and a 12.5-kD MIF protein that was identical in size to rhMIF and MIF derived from mouse P388D1 macrophages. We explored the regulation of MIF expression in mesangial cells by studying the effects of various cytokines and growth factors on MIF mRNA production. Cytokines known to up-regulate MIF expression by macrophages, such as IFN- γ and TNF- α (33), also increased MIF expression by mesangial cells. We also showed that PDGF, a growth factor known to promote mesangial cell proliferation in anti-Thy-1 nephritis (31), up-regulated MIF mRNA expression by cultured rat mesangial cells. This is the first demonstration that PDGF can modulate MIF expression. In addition, stimulation with IL-10 caused an increase in MIF mRNA in macrophages and mesangial cells.

There was a temporal association between glomerular macrophage accumulation and the number of glomerular cells expressing MIF in anti-Thy-1 nephritis. In the early phase of disease (days 1 to 4), MIF was predominantly expressed by glomerular endothelium and this was associated with infiltrating monocytes. However, at the peak of mesangial cell proliferation (day 6), glomerular MIF expression was most frequently observed in proliferating mesangial cells within segmental lesions where the majority of infiltrating macrophages accumulated. This pattern of expression is similar to that observed for MCP-1 expression during the progression of anti-Thy-1 nephritis (9).

MIF production by mesangial cells may have a number of functional roles in mesangioproliferative disease. MIF secreted by mesangial cells may halt macrophage migration through the

mesangium, thus promoting macrophage accumulation in the vicinity of mesangial cells. MIF is also a potent inducer of macrophage activation (29,33). It is recognized that activated macrophages can secrete a number of mesangial cell growth factors, including PDGF, IL-1, and basic fibroblast growth factor (34–36), suggesting a possible mechanism by which MIF may promote mesangial cell proliferation. However, a direct link between MIF activation of macrophages and secretion of mesangial cell growth factors remains to be established, and functional blocking studies in this disease or other, similar models are still needed.

Although we have demonstrated that mesangial cells make MIF, this factor does not appear to act in an autocrine manner. This was evident by the inability of exogenous MIF to affect proliferation, constitutive TGF- β 1 mRNA expression, or induce MCP-1 mRNA expression. In addition, endogenous MIF production did not contribute to mesangial cell proliferation on the basis that incubation with a neutralizing anti-MIF antibody did not modulate basal levels of mesangial cell proliferation, although the same antibody has been shown to inhibit anti-CD3 antibody-driven T cell proliferation (17).

In conclusion, this study has demonstrated that proliferating mesangial cells express MIF and has shown that glomerular MIF expression in anti-Thy-1 disease is associated with glomerular macrophage accumulation. These findings provide new insight into the mechanisms of glomerular macrophage accumulation in mesangioproliferative diseases.

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