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Macrophage Migration Inhibitory Factor Deficiency Attenuates Macrophage Recruitment, Glomerulonephritis, and Lethality in MRL/lpr Mice

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Systemic lupus erythematosus (SLE) is a serious systemic autoimmune disease of unknown etiology. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is operative in innate and adaptive immunity and important in immune-mediated diseases such as rheumatoid arthritis and atherosclerosis. The functional relevance of MIF in systemic autoimmune diseases such as SLE is unknown. Using the lupus-prone MRL/lpr mice, we aim to examine the expression and function of MIF in this murine model of systemic autoimmune disease. These experiments revealed that renal MIF expression was significantly higher in MRL/lpr mice compared with nondiseased control mice (MRL/MpJ), and MIF was also markedly up-regulated in skin lesions of MRL/lpr mice. To examine the effect of MIF on development of systemic autoimmune disease, we generated MRL/lpr mice with a targeted disruption of the MIF gene (MIF−/−/MRL/lpr), and compared their disease manifestations to MIF+/+/MRL/lpr littermates. MIF−/−/MRL/lpr mice exhibited significantly prolonged survival, and reduced renal and skin manifestations of SLE. These effects occurred in the absence of major changes in T and B cell markers or alterations in autoantibody production. In contrast, renal macrophage recruitment and glomerular injury were significantly reduced in MIF−/−/−MRL/lpr mice, and this was associated with reduction in the monocyte chemokine MCP-1. Taken together, these data suggest MIF as a critical effector of organ injury in SLE. The Journal of Immunology, 2006, 177: 5687–5696.

Materials and Methods

Mice

MRL/MpJ-Faslpr (MRL/lpr) and MRL-Mpj (MRL+/+) mice were obtained from The Jackson Laboratory. C129S4 (B6) MIF−/− mice were generated via homologous recombination in J1 embryonic stem cells as previously described (1), and were provided by Dr. J. David (Harvard Medical School, Boston, MA). To study the effect of MIF on disease expression in MRL/lpr mice, MIF−/− mice were backcrossed to MRL/lpr mice for a minimum of seven generations, before intercrossing the MIF−/− offspring to generate the homozygous MIF−/− MRL/lpr and MIF−/− MRL/lpr littermates. All study mice were homozygous for lpr/lpr. Mice were bred under specific pathogen-free conditions. Male and female mice were included and in equivalent numbers in all groups. All animal protocols were approved by the Institutional Animal Care and Use Committee at Monash University.

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1 This work was supported by Program Grant 334067 from the National Health and Medical Research Council, Australia, and National Institutes of Health R01 Grant AR51807-01. A.Y.H. was supported by a postgraduate scholarship from the National Health and Medical Research Council. Address correspondence and reprint requests to Assoc. Prof. Eric F. Morand, Centre for Inflammatory Diseases, Monash Institute of Medical Research, Monash University, Locked Bag Number 29, Clayton, Melbourne, Victoria, 3168, Australia. E-mail address: eric.morand@med.monash.edu.au

2 Abbreviations used in this paper: SLE, systemic lupus erythematosus; MIF, macrophage migration inhibitory factor; DNTC, double-negative T cell.
MIF immunohistochemistry

Kidneys and skin were collected at time of death, and fixed in 10% buffered formalin and embedded in paraffin. Ag retrieval was performed as previously described (12), by heating in a microwave for 10 min at 800 W in 0.01 M sodium citrate (pH 6.0). Sections were preincubated with 20% normal rabbit serum in 5% BSA/PBS for 30 min, then labeled with goat anti-human MIF Ab (R&D Systems) at 1 μg/ml overnight at 4°C. After quenching, sections were incubated with rabbit anti-sheep HRP (DakoCy- tomation) at room temperature for 30 min. Staining was developed by diamobenzidine brown (DakoCytomation) and counterstained with he- matoxylin. Sections from MIF−/− MRL/lpr mice were used as negative controls.

MIF ELISA

Kidneys from 12-, 16-, and 20-wk-old MRL/lpr and MRL−/− littermates were harvested, and protein was extracted by homogenization in detergent buffer consisting of 1% Triton X-100, 0.1 M TrisBase, 0.1 M NaCl, complete protease inhibitor (Roche), and adjusted to pH 7.4. MIF concen- trations from supernatant were measured by ELISA as previously de- scribed (13) and adjusted for total protein concentrations as measured by bicinchoninic acid protein assay kit (Pierce).

MIF and lpr genotyping

Genomic DNA was extracted from tail snips (3- to 4-wk-old mice). Primers for PCR for MIF were as follows: wild type, forward: 5′-AGA CGA CCT GCT GCT TAG CTG AG-3′; reverse: 5′-GCA TCG CTA CCT GGT GAT AA-3′; knockout, forward: 5′-AAT GAA CAA GAT GGA TTG CAC-3′; reverse: 5′-CGT CCA GAT CAG CCT GAT C-3′. PCR conditions were as follows: 94°C for 10 min, 40 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and 72°C for 10 min. Primers for PCR for lpr were as follows: Fas A, 5′-AGG TTA CAA AAG GTC ACC C-3′; Fas B, 5′-GAT ACG AAC ATC TTC TCT GGT-3′; and Fas C, 5′-CAAG CAC GAG TCA AAT CGT CTC-3′. PCR conditions were as follows: 94°C for 5 min; 35 cycles of 94°C for 45 s, 59°C for 45 s, and 72°C for 45 s; and 72°C for 7 min. Samples were electrophoresed in a 2% and 2.5% agarose gel for MIF and lpr, respectively, and visualized by ethidium bromide staining.

Survival and incidence of skin disease

MIF+/+ and MIF−/− MRL/lpr littermates were observed weekly from 14 wk of age for signs of disease and the presence of skin lesions. Time of death was defined as the point at which mice were moribund or fulfilled at least two of the following criteria: weight loss > 20% from baseline, ab- normal respiration, or severely reduced activities. The onset of macro- scopic skin disease was recorded. Mice were considered affected when skin over the upper back showed evidence of ulceration, induration, scab formation, or alopecia.

Proteinuria

Urinary protein excretion was assessed by urinary collection over 24 h in metabolic cages. Urinary protein concentration was determined by Bradford assay (14), and total protein excretion was calculated by multiplying urinary protein concentration by total urine volume produced in 24 h. Urinary creatinine was measured by an enzymatic assay (CREA Plus; Boehr- inger Mannheim Systems), and read photometrically by Roche Cobas Bio system. Severe proteinuria was defined as urinary protein excretion > 5 mg/day.

Measurement of serum IgG and anti-dsDNA Abs

Serum was collected from 22-wk-old MIF+/+ and MIF−/− MRL/lpr litter- mates by cardiac puncture. For serum total IgG, plates were coated with hamster anti-mouse Ig (γ-chain specific; Southern Biotechnology Associates) in PBS and incubated overnight. For anti-dsDNA Abs, plates were pre- treated with 0.01% proteamine sulfate for 90 min at room temperature, and then coated with S1 nuclease treated double-stranded murine DNA (The Jackson Laboratory) in 0.1 M NaHCO3 coating buffer overnight at 4°C. After blocking, diluted sera were added for 1 h at 37°C. Bound IgG was detected by incubation with HRP-conjugated goat anti-mouse IgG (γ-chain specific; Southern Biotechnology Associates) or HRP-goat anti-mouse IgG1 or IgG2a (Southern Biotechnology Associates) for 1 h at 37°C. Color development was achieved by the addition of tetramethylbenzidine and OD was read at 450 nm.

Renal histopathology

Kidneys from MIF+/+ and MIF−/− MRL/lpr littermates were examined at 16 wk, 22 wk, and at the time of death. Kidney sections were fixed with 10% buffered formalin, then embedded in paraffin, and stained by periodic acid-Schiff. Slides were independently scored by two individuals. Renal pathol- ogy was scored according to the criteria of Fricke et al. (6), with thickening of Bowman’s capsule wall to two or more cellular layers) and/or sclerosed glomeruli under high-power field (×400). A mini- mum of 60 glomeruli was counted per section.

Glomerular IgG deposition

Snap-frozen kidney sections from 22-wk-old MIF+/+ and MIF−/− MRL/lpr littermates were stained with FITC-conjugated sheep anti-mouse IgG (Chemicon International) after preincubation with 15% normal sheep se- rum in 5% BSA/PBS. Fluorescence was examined under fluorescence mi-croscopy and images were analyzed by Scion Image Software (NIH Im- age). The mean relative glomerular staining intensity was calculated from the average of 10 randomly selected glomeruli, after subtraction of back- ground staining.

Leukocyte immunohistochemistry

Three-layer immunoperoxidase staining was performed as previously described (15). To identify CD91+ T cells, periodic lysine paraformal- dehyde-fixed frozen kidney sections were collected from MIF+/+ and MIF−/− MRL/lpr littermates at 22 wk of age. Rat anti-mouse CD8 Ab (clone 53.6.7, rat IgG2a; American Type Culture Collection) was used to stain for intrarenal CD8+ T cells. To identify of effector monocytes/macrophages, formalin-fixed paraffin-embedded kidney sections, collected at 16 wk, 22 wk, and at the time of death, first underwent Ag retrieval by micro- wave irradiation as described above. Sections were then stained with rat anti-mouse F4/80 (CalTag Laboratories). Sections subsequently under- went color development with diaminobenzidine black (Sigma-Aldrich), and counterstained with Nuclear Fast Red (Sigma-Aldrich). Intra- renal macrophages were enumerated in the glomeruli and interstitium and an average of 20 high-power microscopic fields (×400) was recorded for each mouse.

Flow cytometry

The following Abs were used for double, triple, or quadruple color staining: anti-CD3-FITC, anti-CD3-alkaline phosphatase Cy5, anti-CD4-PE, anti-CD4-FITC, anti-CD8a-PE, anti-CD8a-FITC, anti-CD69-PE, anti-CD54-PE, anti-CD62L-FITC, anti-CD44-PE Cy5 (BD Pharmingen), and anti-CD40L-PE (eBioscience). Single-cell suspensions of splenocytes were washed and incubated with the relevant mAbs for 30 min at 4°C. Mitogen-activated expression of CD69, CD54, and CD40L was measured after splenocytes were treated with 10 μg/ml Con A for 48 h at 37°C. For detection of apoptosis, cells were first labeled for surface markers CD4, CD8, and CD3, and B220 for 30 min on ice, then stained with anti-annexin-V (Roche) according to manufacturer’s protocol. Cells were analyzed on a MoFlo flow cytometer (DakoCytomation).

Real-time PCR analysis

Kidneys were collected from 22-wk-old MIF+/+ and MIF−/− MRL/lpr littermates. Total RNA was extracted using TRizol reagent (Invitrogen Life Technologies) according to manufacturer’s protocol. cDNA was synthe- sized from total RNA (1 μg) using Superscript III reverse transcriptase (Invitrogen Life Technologies) and random primers. PCR amplification was performed on a LightCycler using SYBR Green I (Roche). Primers for murine IL-1, IL-6, TNF (16), and IFN-γ (17) were used as previously described. β-actin expression was used to normalize expression of respec- tive mRNA species.

Urinary MCP-1 excretion

MCP-1 was measured in the urine collected from 22-wk-old MIF+/+ MRL/lpr and MIF−/− MRL/lpr littermates by sandwich ELISA.

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described above, and data were expressed as a ratio of MCP-1 concentration to creatinine concentration.

Statistics

Unpaired Student’s two-tailed $t$ tests and Mann-Whitney two-tailed $U$ tests were used to compare parametric and nonparametric data, respectively. Survival curve data was analyzed using the log-rank test. Differences in skin disease prevalence were analyzed using $\chi^2$ analysis. Statistical analyses were performed using GraphPad Prism. Results were expressed as mean $\pm$ SEM, where appropriate. A value of $p < 0.05$ was considered to be statistically significant.

Results

**MIF expression in MRL/lpr mice**

Previous studies have reported the up-regulation of MIF in inflammatory conditions involving organs affected by the disease process of SLE (6, 7). We first examined MIF expression by immunohistochemistry in the kidneys of lupus-prone MRL/lpr mice, and found that MIF was abundantly expressed in the renal cortex. The expression of MIF was most evident at the basal aspect of proximal tubular epithelial cells (Fig. 1, A–D), which are immunologically active resident cells that can interact with other immune effector cells (18–20). MIF was also detected in some glomerular epithelial cells, as has been previously described in other models of inflammatory renal disease (12). In MRL/lpr mice with advanced renal disease characterized by crescent formation, glomerulosclerosis, and tubular damage, MIF was still detectable around intact tubules.

We quantitated renal expression of MIF in MRL/lpr mice by ELISA, and compared this to healthy nondiseased control (MRL/+/+) mice. Analysis of 12-, 16-, and 20-wk-old MRL/lpr mice revealed an age-dependent increase in renal MIF expression (Fig. 1E). Renal MIF expression was significantly higher in MRL/lpr than in MRL/+/+ mice at 12 (Fig. 1E) and 16 (data not shown) wk of age, preceding the onset of overt proteinuria (Fig. 1F).
time course of renal MIF expression parallels the reported up-regulation of other key inflammatory cytokines and chemokines (21–26). For example, TNF mRNA expression was markedly up-regulated in 24-wk-old MRL/lpr mice compared with 8-wk-old mice, and not detectable in similarly aged MRL/+ mice (22). Similarly, MCP-1 mRNA expression was increased up to 8-fold in 24-wk-old MRL/lpr mice with advanced renal injury, relative to 8-wk-old mice or C57BL/6 mice (21).

The typical skin lesions of MRL/lpr mice are similar to human cutaneous SLE, characterized by dermal hyperplasia and marked inflammatory cell infiltrate. We examined MIF expression in lesional and nonlesional skin of MRL/lpr mice, and found that MIF was present in suprabasal keratinocytes and the outer root sheath of hair follicles in nonlesional skin, and was markedly increased in the hyperplastic basal layer of epidermis of lesional skin, relative to the adjacent nonlesional skin (Fig. 1G).

**MIF deficiency attenuates the disease phenotype of MRL/lpr mice**

To study the effect of MIF deficiency on the disease phenotype of MRL/lpr mice, we compared the survival and development of renal and skin disease in MIF+/+ and MIF−/− litters from heterozygote sibling mating. MIF deficiency conferred a significant protection from lethality, with median survival of MIF−/− mice extended beyond 44 wk, compared with 23.5 wk in MIF+/+ mice (p < 0.01, Fig. 2A).

The most common cause of death in MRL/lpr mice is accelerated immune complex glomerulonephritis, leading to severe proteinuria and end-stage renal failure. Therefore, we examined the effects of MIF deficiency on renal disease in MRL/lpr mice by measuring proteinuria on a weekly basis from 14 wk of age. We found that MIF−/− MRL/lpr mice had significantly less proteinuria than MIF+/+ MRL/lpr mice (Fig. 2B). Comparison of the ratio of urinary protein to creatinine confirmed the difference observed between the two groups (Fig. 2C). The reduction in proteinuria in MIF−/− MRL/lpr mice was also manifested in a significantly lower incidence of severe proteinuria, defined as urinary protein excretion >5 mg/day (Fig. 2D).

MIF−/− MRL/lpr mice also exhibited a lower prevalence of skin lesions (Fig. 2E). The prevalence of skin lesions at 30 wk of age was 67% for MIF+/+ compared with 29% for MIF−/− MRL/lpr mice (p < 0.05).

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** MIF deficiency confers significant protection from lethality and attenuates renal and skin disease. A, MIF−/− MRL/lpr mice had significantly prolonged survival (N7 and N8 littersmates, n = 20) compared to MIF+/+ (N7 and N8 littersmates, n = 26); ***, p < 0.01. Dotted line median survival. B, Urinary protein excretion (mean ± SEM) was significantly lower in MIF−/− MRL/lpr mice. *, p < 0.05; **, p < 0.01 compared to MIF+/+ MRL/lpr. Dotted line threshold for severe proteinuria, defined as urinary protein excretion >5 mg/day. C, Urinary protein to creatinine ratio was significantly lower in MIF−/− MRL/lpr mice. Data shown are from 22-wk-old MIF−/− and MIF+/+ MRL/lpr mice. *, p < 0.05. D, The incidence of severe proteinuria was significantly lower in MIF−/− MRL/lpr mice (N8 littersmates, n = 15) compared to MIF+/+ MRL/lpr mice (N8 littersmates, n = 17) ***, p < 0.01. E, Prevalence of skin lesions was significantly lower in MIF−/− MRL/lpr mice (N7 and N8 littersmates, n = 20) compared to MIF+/+ (N7 and N8 littersmates, n = 26); *, p < 0.05.

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** MIF deficiency has no effect on hypergammaglobulinemia or autoantibody production. Total IgG and autoantibody levels were detected by ELISA. Total IgG (A) and anti-dsDNA IgG (B) were similar in MIF−/− MRL/lpr compared with MIF+/+ MRL/lpr mice. Isotype subclasses of anti-dsDNA were measured, and anti-dsDNA IgG1 (C) and anti-dsDNA IgG2a (D) were similar in MIF−/− and MIF+/+ MRL/lpr mice. Sera were collected from 22-wk-old MIF−/− (n = 6) and MIF+/+ (n = 11) MRL/lpr mice. Data are expressed as mean ± SEM.
MIF deficiency in MRL/lpr mice was also not associated with changes in T cell activation, as measured by constitutive and mitogen-activated expression of CD54, CD40L, or CD69 (Table II). As MIF has antiapoptotic effects via its inhibition of p53 (29, 30), and may therefore play a role in promoting the survival of autoreactive cells, we also examined the effect of MIF on lymphocyte apoptosis. We observed no difference in the percentage of apoptotic CD4, CD8, B, and DNTC lymphocyte subpopulations between MIF+/− and MIF−/− MRL/lpr mice (Table III). The degree of lymphoproliferation, as demonstrated by spleen weights and numbers of splenic DNTCs, was also similar in MIF+/− MRL/lpr mice. These data suggest an overriding effect of the lpr mutation on the lymphoproliferative phenotype, which is not affected by MIF deficiency.

### MIF-deficient MRL/lpr mice are protected from crescent formation and glomerulosclerosis

The most marked difference in the renal histopathology of MIF+/− MRL/lpr mice was protection from crescent formation. MRL/lpr mice develop a proliferative glomerulonephritis, which progresses at a variable rate to the more severe crescentic form later in life. In addition, glomerulosclerosis is a prominent finding in advanced renal lesions, irrespective of the trigger mechanism (31). At 16 wk of age, there were no crescentic glomeruli present in either

<table>
<thead>
<tr>
<th>Splenocyte phenotypes</th>
<th>MIF+/− MRL/lpr</th>
<th>MIF−/− MRL/lpr</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ (% total)</td>
<td>19.1 ± 1.9</td>
<td>18.3 ± 0.9</td>
</tr>
<tr>
<td>CD8+ (% total)</td>
<td>11.0 ± 1.8</td>
<td>14.0 ± 1.6</td>
</tr>
<tr>
<td>CD4+ CD62LlowCD44hi (% CD4+)</td>
<td>81.7 ± 2.0</td>
<td>75.1 ± 4.3</td>
</tr>
<tr>
<td>CD4+ CD62LloCD44lo (% CD4+)</td>
<td>0.2 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>CD8+ CD62LloCD44hi (% CD8+)</td>
<td>35.6 ± 2.2</td>
<td>20.7 ± 4.0*</td>
</tr>
<tr>
<td>CD8+ CD62LloCD44lo (% CD8+)</td>
<td>8.6 ± 1.5</td>
<td>12.1 ± 1.6</td>
</tr>
<tr>
<td>CD3− B220− (% total)</td>
<td>15.7 ± 2.1</td>
<td>13.0 ± 0.4</td>
</tr>
<tr>
<td>CD3− B220+ (% total)</td>
<td>49.9 ± 4.4</td>
<td>49.4 ± 3.6</td>
</tr>
<tr>
<td>CD11b− CD11c+ (% total)</td>
<td>1.3 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

"p < 0.01 compared to MIF+/− MRL/lpr mice (n = 6).

### Table II. Effects of MIF deficiency on splenomegaly and splenocyte phenotypes

<table>
<thead>
<tr>
<th>Splenomegaly and Splenocyte Phenotypes</th>
<th>MIF+/− MRL/lpr</th>
<th>MIF−/− MRL/lpr</th>
</tr>
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<tr>
<td>Spleen size</td>
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<tr>
<td>Weight (g)</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
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### Table III. Effects of MIF deficiency on splenocyte activation markers

<table>
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<tr>
<th>Basal activation markers</th>
<th>MIF+/− MRL/lpr</th>
<th>MIF−/− MRL/lpr</th>
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<tbody>
<tr>
<td>CD4+ CD69+ (% CD4+)</td>
<td>20.8 ± 6.2</td>
<td>15.6 ± 6.3</td>
</tr>
<tr>
<td>CD8+ CD69+ (% CD8+)</td>
<td>9.7 ± 2.7</td>
<td>8.1 ± 1.5</td>
</tr>
<tr>
<td>CD3− B220+ CD69− (% CD3− B220+)</td>
<td>27.4 ± 14.3</td>
<td>28.4 ± 8.1</td>
</tr>
<tr>
<td>CD4+ CD54+ (% CD4+)</td>
<td>31.3 ± 7.8</td>
<td>32.3 ± 8.3</td>
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<tr>
<td>CD8+ CD54+ (% CD8+)</td>
<td>25.7 ± 5.4</td>
<td>17.4 ± 4.2</td>
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<tr>
<td>CD3− B220− CD54− (% CD3− B220+)</td>
<td>35.8 ± 13.8</td>
<td>24.1 ± 8.4</td>
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<tr>
<td>CD4− CD40L− (% CD4−)</td>
<td>2.0 ± 0.4</td>
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<td>CD8− CD40L− (% CD8−)</td>
<td>0.9 ± 0.3</td>
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<table>
<thead>
<tr>
<th>Mitogen-activated</th>
<th>MIF+/− MRL/lpr</th>
<th>MIF−/− MRL/lpr</th>
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<tbody>
<tr>
<td>CD4+ CD69+ (% CD4+)</td>
<td>49.9 ± 0.6</td>
<td>53.6 ± 6.5</td>
</tr>
<tr>
<td>CD8+ CD69+ (% CD8+)</td>
<td>68.6 ± 7.2</td>
<td>77.3 ± 6.2</td>
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<td>CD3− B220+ CD69− (% CD3− B220+)</td>
<td>46.3 ± 5.1</td>
<td>47.9 ± 4.1</td>
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<td>CD4+ CD54+ (% CD4+)</td>
<td>38.9 ± 8.7</td>
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<tr>
<td>CD8+ CD54+ (% CD8+)</td>
<td>47.1 ± 9.1</td>
<td>42.5 ± 3.8</td>
</tr>
<tr>
<td>CD3− B220− CD54− (% CD3− B220+)</td>
<td>48.1 ± 14.6</td>
<td>32.1 ± 5.2</td>
</tr>
<tr>
<td>CD4− CD40L− (% CD4−)</td>
<td>8.1 ± 0.6</td>
<td>9.3 ± 2.1</td>
</tr>
<tr>
<td>CD8− CD40L− (% CD8−)</td>
<td>4.0 ± 0.8</td>
<td>4.0 ± 1.0</td>
</tr>
</tbody>
</table>

a Splenocytes were stimulated with 10 μg/ml Con A for 48 h, and expression of activation markers CD69, CD54, and CD40L was determined. Data are expressed as mean ± SEM (n ≥ 3).
MIF$^{+/+}$ or MIF$^{-/-}$ MRL/lpr mice. By 22 wk of age, crescentic or sclerosed glomeruli were increased in MIF$^{+/+}$ (22 ± 12%) and to a lesser extent in MIF$^{-/-}$ (12 ± 8%) MRL/lpr mice. There was a further increase in crescent score in MIF$^{+/+}$/MRL/lpr mice at time of death, and the difference between MIF$^{+/+}$ and MIF$^{-/-}$/MRL/lpr mice was maximal and significant at this time ($p < 0.01$) (Fig. 4, A and B). This finding suggests that protection from crescent formation and glomerulosclerosis is the main reason for the improved survival of these mice. There was no difference in the degree of mesangial proliferation between MIF$^{+/+}$/MRL/lpr and MIF$^{-/-}$/MRL/lpr mice at earlier time points (data not shown), suggesting the major protective role of MIF deficiency is operative in severe disease.

We next examined immune complex deposition in the kidney by immunohistochemical staining of mouse IgG. There was a significant reduction in glomerular immune complex deposition in MIF$^{-/-}$/MRL/lpr mice, compared with MIF$^{+/+}$ (Fig. 4, C and D).

### Table III. Effects of MIF deficiency on splenocyte apoptosis

<table>
<thead>
<tr>
<th>Splenocyte apoptosis</th>
<th>MIF$^{+/+}$/MRL/lpr</th>
<th>MIF$^{-/-}$/MRL/lpr</th>
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<tr>
<td>CD4$^+$ annexin V$^+$ (% CD4$^+$)</td>
<td>9.9 ± 1.1</td>
<td>11.1 ± 2.5</td>
</tr>
<tr>
<td>CD8$^+$ annexin V$^+$ (% CD8$^+$)</td>
<td>14.3 ± 2.6</td>
<td>12.1 ± 1.8</td>
</tr>
<tr>
<td>CD3$^+$ B220$^+$ annexin V$^+$ (% CD3$^+$ B220$^+$)</td>
<td>32.1 ± 7.5</td>
<td>33.5 ± 15.3</td>
</tr>
<tr>
<td>CD3$^+$ B220$^+$ annexin V$^+$ (% CD3$^+$ B220$^+$)</td>
<td>18.7 ± 1.7</td>
<td>15.7 ± 5.8</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM (n ≥ 3).
MIF deficiency reduces renal macrophage recruitment and MCP-1 expression

Macrophage infiltration is a hallmark of severe lupus nephritis, and macrophages play an important role in amplification of the inflammatory process in the kidney (32–34). Therefore, we examined the extent of macrophage infiltration in MIF\(^{+/+}\) and MIF\(^{-/-}\) MRL/lpr mice. The extent of F4/80\(^{+}\) macrophage infiltration in the renal interstitium followed the same trend as the crescent score in MIF\(^{+/+}\) and MIF\(^{-/-}\) MRL/lpr mice (Figs. 4B and 5B). In MIF\(^{+/+}\) MRL/lpr mice, F4/80\(^{+}\) macrophages were present at 16 wk of age, and their number progressively increased until time of death. In MIF\(^{-/-}\) mice, macrophage infiltration was present at slightly lower levels at 16 and 22 wk of age, and was significantly lower at time of death (\(p < 0.01\)) (Fig. 5, A and B).

Tissue macrophages can be recruited as a consequence of the actions of a range of inflammatory mediators such as cytokines. The expression of the proinflammatory cytokines TNF, IL-1, and IL-6 was assessed by real-time PCR, but no statistically significant reduction was evident in MIF\(^{-/-}\) MRL/lpr mice (Fig. 5C). We also observed no significant reduction in IFN-\(\gamma\) expression in MIF\(^{-/-}\) MRL/lpr mice, and indeed a trend toward higher IFN-\(\gamma\) expression was noted. This is in keeping with the observation that T cell activation markers were not affected by MIF deficiency in this model, and suggests that the well-described pathological effects of IFN-\(\gamma\) on nephritis (35, 36) in MRL/lpr mice are reduced in the absence of MIF.

The infiltration of macrophages to inflammatory lesions is also regulated by chemokines released by injured resident cells. The monocyte-selective chemokine, MCP-1, is expressed by tubular epithelial cells, and is a critical chemokine in the pathogenesis of lupus (21). We hypothesized that MIF may influence macrophage migration by regulation of MCP-1, and given a report of increased urine MCP-1 and correlation of this with disease activity in human SLE patients (37), we compared urine MCP-1 in MIF\(^{+/+}\) and MIF\(^{-/-}\) MRL/lpr mice. We found that urine MCP-1 was significantly reduced in MIF\(^{-/-}\) MRL/lpr mice (Fig. 5D). These data suggest that the effects of MIF deficiency on kidney inflammation were mediated by reduced MCP-1 expression and macrophage recruitment.

Discussion

The current results indicate a critical role for MIF in the development of end organ disease in MRL/lpr mice. We found that not only is MIF up-regulated in disease-affected organs of these lupus prone mice, but MIF deficiency also confers significant protection from the development of severe renal disease and skin lesions and most importantly prolongs survival. This is the first evidence that MIF is a potential therapeutic target in systemic autoimmune diseases such as SLE.

The most prominent effect of MIF deficiency was the improvement in end organ inflammation and protection from lethality, with minimal changes in markers of lymphocyte phenotype and activation, demonstrating that MIF primarily regulates effector pathways of the immune-mediated injury. Therefore, the current results highlight the importance of injury mediated by effector pathways in SLE. As the mechanisms of loss of self-tolerance in human SLE have not been elucidated, modulation of key effector pathway molecules may be an effective therapeutic strategy.
Leukocyte recruitment is a critical step in mediating tissue inflammation, and others have shown that deficiency of molecules important in this pathway attenuates disease in murine lupus (21, 38–40). Macrophages are key effector cells responsible for renal injury in other models of crescentic glomerulonephritis (41, 42). We observed that macrophage infiltration in the kidneys of MIF+/− MRL/lpr mice was significantly reduced, and this was matched by reductions in the percentage of crescents seen in the kidneys at different time points.

MCP-1 can induce transendothelial migration of monocytes (43), and infiltration of monocytes/macrophages can in turn facilitate tissue destruction. Abundant urine MCP-1 expression in MIF+/+ MRL/lpr mice was associated with macrophage recruitment, both of which were significantly reduced in the absence of MIF. MIF has been shown to regulate leukocyte recruitment to the inflamed microvasculature in other tissues (44), and we have recently found that rMIF up-regulates endothelial cell MCP-1 expression and macrophage recruitment (62). It has been previously shown that the up-regulation of MCP-1 in the inflamed kidney interstitium in MRL/lpr mice is most striking in the tubular epithelial cells (26), in a similar distribution to that of MIF. MIF can also have effects on other molecules that promote leukocyte recruitment. VCAM-1 expression, for example, has been shown to be modulated by anti-MIF Abs in a murine autoimmune encephalomyelitis model (45).

Macrophages are both an important source and target of MIF (46). Examination of macrophages from MIF-deficient mice has revealed an array of effects of MIF on macrophages, including modulation of TLR4 and p53 expression (47, 48). MIF can also enhance macrophage functions such as phagocytosis, intracellular killing, and H2O2 generation (49–51). In addition, MIF released by macrophages can act in an autocrine and paracrine fashion to amplify the inflammatory response, by inducing the expression of a variety of proinflammatory cytokines such as TNF and IL-6 (46, 52). Infiltrating mononuclear cells are the major source of TNF and IL-6 in diseased kidneys affected by lupus nephritis (22, 24). The current data, indicating a trend toward reductions in renal TNF and IL-6 in MIF+/− MRL/lpr mice, suggest that in addition to a role for MIF in promoting renal macrophage accumulation, MIF may also act to regulate cytokine expression in infiltrating macrophages.

MIF deficiency significantly protects MRL/lpr mice from lethality and attenuates injury in multiple organs but these tissues were not totally protected. In the kidneys, MIF+/− MRL/lpr mice still exhibit proliferative changes in the mesangium and inflammatory infiltrates in the interstitium, even though progression to severe crescentic glomerulonephritis was reduced. MRL/lpr mice have multiple genetic lupus-susceptibility loci, with the primary defect in the Fas gene resulting in the persistence of DNTCs. Accumulation of DNTCs in lymphoid organs and around blood vessels was observed in MIF+/+ and MIF+/− MRL/lpr mice to a similar extent.

Dissociation between autoantibody production and disease development is not unusual. In humans, autoreactivity as manifested by presence of autoantibodies or autoreactive cells does not necessarily translate into overt autoimmune disease. The effects of MIF deficiency on disease pathology in MRL/lpr mice are not associated with major changes in autoantibody production or lymphocyte phenotypes. A decrease in splenic memory CD8+ T cells (CD62LlowCD44high) was noted but it was not accompanied by changes in CD8+ T cell numbers in renal lesions. Although CD8+ T cells are important in inducing injury in some animal models of nephritis (53), the role of memory CD8+ cells in lupus is less well-defined than that of activated memory CD4+ T cells (54). In MIF−/− MRL/lpr mice, there was no difference in the number of splenic memory CD4+ T cells.

We observed a disparity between the effect of MIF deficiency on autoantibody production and immune complex deposition. In MIF−/− MRL/lpr mice, we observed no major changes in autoantibody production but a reduction in immune complex deposition in the glomeruli, accompanying the reduction in renal pathology. This apparent paradox can be explained by consideration of factors that can influence IgG deposition in the kidney other than the levels of circulating autoantibodies. Indeed, human subjects with autoimmune disease regularly demonstrate elevated circulating autoantibodies without any significant deposition in the kidneys. The current finding may be explained by increased trapping of immune complexes in the diseased glomeruli of MIF+/− MRL/lpr mice. Pathogenic anti-dsDNA Abs and circulating nucleosomes form immune complexes, and become trapped as a result of increased permeability (55). Podocyte integrity is a major determinant of glomerular permeability, and in a MIF-transgenic model, it was shown that podocytes are susceptible to injury as a result of MIF overexpression, resulting in spontaneous heavy proteinuria and progressive renal failure (56). As a result of progressive glomerular injury, circulating Abs may also be more prone to bind to various antigenic structures in the process of in situ immune complex formation (57). Immune complex deposition is therefore as much a reflection of renal injury as of the level of autoantibodies in the circulation.

IFN-γ is a critical nephritogenic cytokine in MRL/lpr mice, important in regulating leukocyte recruitment and subsequent inflammatory injury in the kidney (34–36). Interestingly, IFN-γ was not reduced in the MIF−/− MRL/lpr kidneys, further supporting the contention that the protective effects of MIF deficiency are largely independent of reductions in T cell responses. In fact, IFN-γ is a potent inducer of MIF expression (58), and there is considerable overlap in the functions of these cytokines in effector pathways (59). The current data suggest the possibility that the effects of IFN-γ on end organ injury in MRL/lpr mice require the presence of MIF.

In conclusion, we have shown an important role of MIF in effector pathways of immune-mediated inflammatory injury and death in a model of SLE. MIF is required for increased MCP-1 expression and macrophage recruitment, and subsequent development of severe end organ inflammation in MRL/lpr mice. MIF also has an emerging role in the pathogenesis of atherosclerosis (60), a common cause of death in patients with SLE (61). Taken with the current data, this suggests that therapeutic antagonism of MIF should be investigated as an opportunity for targeted therapy in human SLE.

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Disclosures
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References


