Migration inhibition factor in acute serum sickness nephritis

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Migration inhibition factor in acute serum sickness nephritis. Monocytes have been demonstrated to play an important role in acute serum sickness (AcSS) nephritis. Because accumulation of monocytes within the glomeruli could be the result of local lymphokine production, we studied migration inhibition factor (MIF) activity in supernatants from glomerular cultures, analyzed its temporal relationship with monocyte and lymphocyte accumulation, and tested the effect of anti-T lymphocyte monoclonal antibody on local MIF production. AcSS was induced in 12 rabbits, and one additional rabbit had antigen elimination without proteinuria. Single nephrectomy was performed at the time of antigen elimination in all animals; the remaining kidney was removed four days (4 rabbits) or 14 days afterwards (5 rabbits). In glomerular cross sections (gcs), lymphocytes were identified using monoclonal antibody M108, and monocytes by nonspecific esterase stain (ES). MIF activity was determined in supernatants of cultures of isolated glomeruli by the agarose microdroplet method. Peak of MIF activity (84.3 ± 2.6%, SEM) was observed the first day of proteinuria in association with peak of lymphocyte infiltration (1.15 ± 0.1 lymphocytes/gcs) and monocyte infiltration (2.4 ± 0.3 mean ES score/gcs). MIF activity diminished by day 4 (66.0 ± 6.3%) and reached control levels by day 14 (12.8 ± 3.2%). There was a significant correlation between lymphocyte infiltration and MIF activity (r = 0.776, P < 0.0001) as well as between MIF activity and monocyte accumulation (r = 0.858, P < 0.0001). In five additional rabbits with AcSS, glomeruli were isolated, treated successively with M108 and normal rabbit serum, and supernatants harvested from 24-hour cultures were tested for MIF activity. Negative controls were supernatants treated with T1-lytic monoclonal antibody, as well as untreated glomerular cultures. MIF activity from untreated and T1-lytic treated glomerular cultures was similar. Addition of M108 monoclonal antibody to glomerular cultures produced 70 ± 12.9% inhibition of MIF activity. Our data suggest that lymphocytes infiltrating the glomeruli in AcSS produce lymphokines (MIF) which are related to the monocyte accumulation.

Monocytes may play a significant role in the pathogenesis of acute glomerulonephritis since they are capable of secreting a variety of phlogenic products [reviewed in 1] that may produce damage in several structures within the glomeruli. A series of recent experiments have shown that monocytes infiltrate this structure in experimental acute serum sickness (AcSS) [2, 3] and in human proliferative glomerulonephritis [4–6]. Three possible mechanisms have been suggested to account for the intraglomerular infiltration of monocytes: first, immune adherence of monocytes to the Fc portion of immuno-

globulins deposited in the glomeruli [7], second, monocytes could be attracted by chemotactic factors released during the complement activation [8] and third, by a lymphokine-dependent mechanism similar to that in delayed-type of hypersensitivity [9, 1]. The latter mechanism requires the participation of activated lymphocytes within the glomeruli, and this possibility appears reasonable in view of studies showing that lymphocyte infiltration precedes monocyte accumulation [10]. In fact, lymphokines are known to be produced in other models of glomerulonephritis such as experimental anti-GBM disease [10].

In acute serum sickness, intraglomerular lymphocyte infiltration has been demonstrated during the acute phase of the disease [11], but evidence of lymphokine release has not been obtained. The present experiments were done to investigate the production of migration inhibition factor (MIF) by lymphocytes infiltrating the glomeruli during the acute and recovery phases of acute serum sickness nephritis, and to evaluate temporal correlations with monocyte accumulation in the course of renal disease.

Methods

Two groups of experiments were carried out.

Intraglomerular lymphocytes and monocytes, and migration inhibition factor activity

These experiments were done in 15 rabbits weighing 2.0 to 2.5 kg and housed in metabolic cages. Twenty-four-hour urine collections were obtained twice before immunization for baseline determinations of proteinuria. Following the work of Hunsicker et al [12], the rabbits were pre-immunized in the rear foot pads with 6 mg of BSA (Fraction V, Sigma Chemical Co., St. Louis, Missouri, USA) in 0.25 ml suspension of Bordetella Pertussis (Lederle Laboratories Division, Pearl River, New York, USA). Four days later, the rabbits were immunized with a single intravenous dose of 125 mg/kg of 10% BSA diluted in saline solution and adjusted to pH 7.4. Serum samples were obtained one hour after injection for baseline (100%) concentration of bovine serum albumin (BSA). Starting six days after immunization, urine and serum samples were taken daily for determination of 24-hour urinary protein excretion and serum BSA levels, respectively.

Circulating levels of BSA were measured by radial immunodiffusion [13] of neat serum into 1% agarose containing rabbit anti-BSA antibody (Cappel Laboratories, Organon Teknika Corporation, Westchester, Pennsylvania, USA). The values

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were calculated from a BSA standard curve and the results were expressed as a percent of the baseline serum concentration (obtained 1 hr after immunization). Immunoclearance was defined as BSA level of 5% of baseline, and it occurred the day before or the day of the beginning of proteinuria.

Proteinuria was determined in 24-hour urine collections by the sulfosalicylic acid method [14]. As suggested by Neild et al [15], the results were expressed as mg/dl in urine excreted in 24 hours.

Acute serum sickness nephritis developed in 12 rabbits, one animal had antigen elimination without proteinuria and two rabbits did not have either antigen elimination or proteinuria. In the 12 animals which had antigen elimination and proteinuria single nephrectomy was performed on the first day of proteinuria and the remaining kidney was removed four to five days afterwards (4 rabbits) or 14 days afterwards (5 rabbits). In the rabbit that had antigen elimination but no proteinuria, nephrectomy was done only on the day of antigen elimination. In two animals which did not have either antigen elimination or proteinuria, no biopsies were carried out.

**Histological methods.** Tissue fragments were taken from each nephrectomy. One fragment was embedded in Tissue-Tek II O.C.T. compound (Lab-tek products, Miles Laboratories, Inc., Naperville, Illinois, USA), frozen with a dry ice-acetone mixture and stored at −70°C. Three μm thick sections were cut in a cryostat and stained for C3, IgG and BSA using direct immunofluorescence technique with specific polyclonal antibodies (Cappel Laboratories) [16]. The other fragment was immersed in Dubosq-Brazil fixative solution for two hours, washed and stored in 15% formalin and embedded in Paraplast (Monocollect Scientific division of Sherwood Medical, St. Louis, Missouri, USA); 2 μm sections were stained with hemotoxilin and eosin and periodic acid-Schiff staining.

**Intraglomerular monocytes and lymphocytes.** Glomerular monocytes were identified in frozen sections using non-specific esterase staining [17]. Positivity was graded on an arbitrary scale 0 to 4+ based on the number of esterase positive cells per glomerulus, and results were expressed as the mean score per glomerular cross section (gcs). Lymphocytes were identified in frozen sections by indirect immunofluorescence as well as by indirect immunoperoxidase technique [18], utilizing mouse monoclonal antibody to rabbit lymphocyte M108 (Accurate Chemical and Scientific Corp., Westbury, New York, USA). This antibody is specific for T cells and binds to less than 20% of B cells. For immunoperoxidase staining, 4 μm sections were fixed in acetone for 10 minutes, air dried and incubated for three minutes in saline Tris buffer 0.05 M, pH 7.6 containing 0.3% of hydrogen peroxide. Then, sections were washed with saline-Tris buffer containing 4% rabbit serum and incubated for two hours with 50 μl of the M108 monoclonal antibody at 37°C.

Following incubation with the monoclonal antibody, sections were washed with saline-Tris buffer containing 1% goat serum and then incubated with 50 μl of peroxidase conjugated goat anti-mouse Ig antibody (Cappel Laboratories). After a final wash with saline-Tris, sections were incubated with diamobenzidine and H2O2 in saline-Tris and counterstained with methyl green. Negative controls and positive controls were included with each section. The negative controls were obtained processing the tissue in a similar manner but excluding the addition of the monoclonal antibody. Positive controls were obtained in lymph node and spleen sections; in addition, the interstitial lymphocyte infiltrate present during the acute phase of the disease also served as internal positive control. Methyl green stained nuclei surrounded by a brown plasma membrane were considered reactive cells.

Seven kidney biopsies were also stained with the same M108 antibody by indirect immunofluorescence, using fluorescein conjugated rat anti-mouse IgG and fluorescein conjugated rabbit anti-rat IgG as secondary antibodies.

For each section, positive cells in 30 to 50 glomeruli were counted and results were expressed as the mean number of positive cells per glomerular cross section (gcs). The correlation coefficient between the cell counts done by immunoperoxidase and immunofluorescence was 0.80. Results given correspond to the readings with the immunoperoxidase method.

**Isolation and culture of glomeruli.** Nephrectomies were done in sterile conditions under kethalar-chlorpromazine anesthesia. The renal capsule was removed and the cortices were dissected out from the kidney. Cortical slices were minced with a surgical blade and pressed with a spatula through a stainless steel screen of No 150 mesh (103 μm pore; USA standard sieve series, Newark Wire Cloth Co., Newark, New Jersey, USA) and then rinsed with Hanks' balanced solution (HBS) through successive screens of No 100 mesh (150 μm pore) and No 200 mesh (75 μm pore), placed in series. Tissue consisting 85 to 95% of glomeruli on the No 200 mesh screen was transferred to plastic tubes and washed two times. After a final wash, the glomeruli were suspended in Minimum Essential Medium (MEM, Gibco, Grand Island, New York, USA) containing 50 U/ml of penicillin, 50 μg/ml of streptomycin and 0.01 M HEPES (Flow Laboratories Inc., McLean, Virginia, USA). Suspensions of 10,000 glomeruli/ml were cultured on 24-well tissue culture plates (Costar, Cambridge, Massachusetts, USA) at 37°C in 5% CO2 atmosphere for 24 hours. After this time, cultures were centrifuged at 1000 rpm for five minutes and supernatants were harvested and stored at −70°C until they were used.

**Migration inhibition factor (MIF).** Migration inhibition factor activity was assayed by the agarose microdroplet method [19, 20]. Briefly, guinea pig peritoneal macrophages were obtained by injection of 20 ml sterile liquid parafin in the guinea pig peritoneal cavity. After three days, peritoneal exudate was obtained by washing the peritoneal cavity with 150 ml HBS and centrifuged at 250 g for 10 minutes. Cells were washed with HBS and resuspended in MEM. For determination of MIF activity, 50 × 106 cells were pelleted by centrifugation and mixed with 100 μl of 0.2% seaplate agarose (FMC Bioproducts, Rockland, Maine, USA) in medium 199 containing 1% of newborn calf serum and, 1 μl of the mixture was dispensed in wells of the 96 flat-bottom wells of a microtiter culture plate (Limbro, Hamden, Connecticut, USA). The microdroplets were allowed to solidify by placing the microtiter plate in an ice bath for three minutes. Then, 100 μl of control or experimental glomerular supernatants were added and the horizontal and vertical diameters of the microdroplets were measured by an Orholux light microscope (Leitz, FRG) provided with a micrometer eyepiece (gradation 10 mm = 100 intervals) and using magnification 12.5 × 3.5. A new measurement was done after 24 hours of incubation at 37°C in 5% CO2. The area of migration was calculated by subtracting the area of the original droplet from the area after 24 hour incubation and MIF activity
was expressed as the percent of the inhibited migration, as follows:

\[
\frac{Area\ of\ migration\ with\ experimental\ supernatant}{Area\ of\ migration\ with\ medium\ alone} \times 100
\]

Each sample was tested in triplicate. The intrassay variation coefficient was 11.6 ± 1.9% (mean ± SEM), N = 23 and the interassay variation coefficient was 10.9 ± 1.9% (N = 10).

The positive controls were obtained by stimulation of spleen rabbit cells with Phytohemaglutinin A (PHA, Gibco) as suggested by Borish et al [19].

The negative controls were obtained from supernatants of normal rabbit glomeruli harvested and cultured under the same conditions described earlier for the glomeruli of the rabbits with acute serum sickness.

Statistical analysis was done by conventional methods using a software statistical package (Statgraphics, Statistical Graphics Corporation, Rockville, Maryland, USA). Results are expressed as mean ± SEM.

**Abrogation of MIF activity with monoclonal anti-T lymphocyte antibody**

In this set of experiments, M108 monoclonal antibody was used to inhibit MIF production in glomerular cultures presumed to be due to infiltrating lymphocytes. Eight additional rabbits were used for induction of AcSS. Five of these rabbits developed proteinuria and antigen elimination, one rabbit developed antigen elimination without nephritis and two rabbits did not develop antigen elimination.

Animals which developed AcSS were sacrificed the first day of proteinuria, and the animal which developed antigen elimination without proteinuria was sacrificed the day after antigen elimination. Glomeruli were isolated as described earlier and 1 ml of glomerular suspension containing 10⁶ glomeruli per ml in MEM was centrifuged at 1200 rpm. After removing the supernatant, 100 μl of monoclonal antibody to pan T rabbit lymphocytes (M108) diluted 1:10 were added, incubated one hour at room temperature and washed with 5 ml of MEM. Afterwards, 200 μl of normal rabbit serum (NRS) diluted 1:3 in MEM, as a source of complement, were added to the pellet and incubated one hour at 37°C in 5% CO₂. Glomeruli were then washed, resuspended in 1 ml of MEM and incubated 24 hours at 37°C in 5% CO₂. Finally, the supernatant was harvested and stored at -70°C. T1-lytic Monoclonal antibody (T1) (Coulter Immunology, Hialeah, Florida, USA; cat. 6602454) diluted 1:10 was used as negative control. T1 is directed to human mature T lymphocytes, it belongs to IgG 2a class and it is lytic for T human lymphocytes. Supernatants from 24 hour untreated glomerular cultures were harvested from each rabbit and run in parallel with antibody-treated glomerular cultures.

In vitro binding of M108 to lymphocytes infiltrating into the whole glomerulus was demonstrated in glomerular suspensions from four nephritic rabbits by indirect immunofluorescence technique [21] using fluorescein-conjugated rat anti-mouse IgG and fluorescein-conjugated rabbit anti-rat IgG as secondary antibodies. M108 monoclonal antibody bound to 4.5 ± 1.8 lymphocytes per whole glomerulus, whereas no positive intraglomerular cells were observed when T1 monoclonal antibody was used.

The lytic effect of M108 was demonstrated in rabbit peripheral blood lymphocytes isolated by Histopaque 1.077 (Sigma Chemical Co) as follows: 20 μl of M108 or T1 diluted (1:2, 1:4, 1:8, 1:10, 1:15 and 1:20) were added to 20 μl of the mononuclear cells suspension containing 10 × 10⁶ cells per ml and incubated one hour at 37°C; then, 50 μl of NRS as source of complement was added, and after a one hour incubation the cell suspension was stained with eosin, and percentage of lysis was determined. Simultaneously, the percentage of M108 positive cells in the cell suspension was determined by indirect immunofluorescence. M108 monoclonal antibody diluted 1:10 produced lysis of 60% of M108 immunofluorescent positive cells.

**Results**

**Correlation between migration inhibition factor activity, with lymphocytes and monocytes**

Acute serum sickness was induced in 86% of the immunized rabbits within 9 days of immunization. Pertinent data of the rabbits that showed antigen elimination is shown in Table 1. Proteinuria was present in 12 of 13 animals with antigen elimination and began the day of 95% clearance of the injected BSA or the day immediately after. Proteinuria lasted 3.00 ± 0.42 days. As shown in Table 2, at the moment of first biopsy (first day of proteinuria), the proteinuria was 348 ± 50 mg/dl, while at the time of the second biopsy taken 4 or 14 days afterwards, proteinuria had decreased to levels of 27 ± 8.9 mg/dl (N = 4) and 30 ± 8.2 mg/dl (N = 5), respectively.

**Histological findings**

Histological findings were similar to those described by other authors [16, 22]. In biopsies taken at the time of antigen elimination, widespread hypercellularity and infiltration of mononuclear cells were the most conspicuous findings. Polymorphonuclear leucocytes were rarely observed. Histological findings were resolved by the time of the late biopsy, 14 days after antigen elimination. Monocyte infiltration was prominent in the acute phase of the disease (Tables 1 and 2) and the esterase stain score decreased from a mean value of 2.4 ± 0.3 to mean values of 0.53 ± 0.2 and 0.04 ± 0.02, after 4 and 14 days, respectively. There was a significant linear correlation between monocyte infiltration and proteinuria (Fig. 1; r = 0.748, P < 0.001).

Giemsa infiltration of lymphocytes (Fig. 2) was present in the acute phase and decreased in a fashion similar to that of the esterase stain score (Tables 1 and 2). A significant correlation was found between intraglomerular lymphocyte counts and monocyte stain scores (Fig. 3; r = 0.876, P < 0.0001).

In six of the biopsies (from rabbits 13, 12, 6, 7, 8, 5) obtained at antigen elimination, different degrees of interstitial infiltrate were observed: two biopsies did not show interstitial infiltration, two had mild infiltrate and two had moderate lymphocyte infiltration (Table 3).

**Migration inhibition factor activity**

Supernatants of PHA-stimulated rabbit spleen cells used for positive control had a MIF activity of 95 ± 8.87%. Supernatants from normal glomerular cultures had a MIF activity of 15.3 ± 4.5% (N = 6), as shown in Table 2. MIF activity from
Table 1. Migration inhibition factor and cellular infiltration in acute serum sickness

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Day of BSA elimination</th>
<th>Day of biopsy</th>
<th>Day of biopsy</th>
<th>MIF</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day of biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ES score</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td></td>
<td></td>
<td>per gcs</td>
<td>per gcs</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>8</td>
<td>205</td>
<td>89</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>8</td>
<td>680</td>
<td>91</td>
<td>NA</td>
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<tr>
<td>3</td>
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<td>8</td>
<td>NA</td>
<td>46</td>
<td>0.14</td>
<td>0.9</td>
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<tr>
<td>4</td>
<td>9</td>
<td>9</td>
<td>45</td>
<td>1.04</td>
<td>2.6</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>8</td>
<td>185</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>7</td>
<td>334</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>8</td>
<td>198</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>8</td>
<td>382</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>12</td>
<td>117</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>22</td>
<td>365</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>22</td>
<td>369</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>23</td>
<td>455</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>22</td>
<td>536</td>
<td>1.7</td>
<td>2.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Day after immunization with bovine serum albumin (BSA)

b Concentration of protein in 24-hour urine collection

c MIF = migration inhibition factor activity (%) in supernatant from cultured isolated glomeruli

d Lymphocyte infiltration is expressed as positive cells per glomerular cross section (gcs)

e Monocyte infiltration is expressed as the mean of esterase stain (ES) scores per gcs

Table 2. Migration inhibition factor, cellular infiltration and proteinuria in acute serum sickness

<table>
<thead>
<tr>
<th>Control</th>
<th>Acute serum sickness</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIF (% activity)</td>
<td>15.3 ± 4.5</td>
<td>84.3 ± 2.6f</td>
<td>66.0 ± 6.3f</td>
<td>12.8 ± 3.2f</td>
</tr>
<tr>
<td>Lymphocytes/gcs</td>
<td>0.01 ± 0.01</td>
<td>1.15 ± 0.1f</td>
<td>0.47 ± 0.2e</td>
<td>0.11 ± 0.1f</td>
</tr>
<tr>
<td>Monocytes (ES score/gcs)</td>
<td>0.00</td>
<td>2.4 ± 0.3f</td>
<td>0.53 ± 0.2f</td>
<td>0.04 ± 0.02f</td>
</tr>
<tr>
<td>U protein mg/dl</td>
<td>14.7 ± 2.53</td>
<td>348 ± 30.6f</td>
<td>27.0 ± 8.9</td>
<td>30.0 ± 8.2</td>
</tr>
</tbody>
</table>

Results are mean ± SEM (number of animals).

a Day(s) after appearance of proteinuria

b MIF = migration inhibition factor activity (%)

c Number of lymphocytes per glomerular cross section (gcs)

d Score of nonspecific esterase stain (ES) per gcs

e Urine protein concentration in 24-hour urine collection

f P < 0.001

e P < 0.01

Supernatants of glomeruli harvested in the acute phase of the disease, 4 and 14 days after immune clearance, are shown in Tables 1 and 2. There is a significant correlation between glomerular lymphocyte infiltration and supernatant MIF activity (Fig. 4; r = 0.776, P < 0.0001) as well as between MIF activity and esterase stain score. The latter is an exponential relationship since the increase of esterase scores is observed at levels of MIF activity higher than 46% (Fig. 5; r = 0.858, P < 0.0001).

No correlation between MIF activity and interstitial lymphocyte infiltration was detected.

**Abrogation of migration inhibition factor activity with monoclonal antibody M108**

Untreated cultures and T1 treated cultures had a similar MIF activity (mean ± SEM): 76.80 ± 5.4% and 82.40 ± 5.3%, respectively. Addition of M108 monoclonal antibody to glomer-
Table 3. Lymphocyte interstitial infiltration and MIF activity

<table>
<thead>
<tr>
<th>Animal</th>
<th>Lymphocyte infiltration</th>
<th>MIF activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 AE</td>
<td>5</td>
<td>73</td>
</tr>
<tr>
<td>6 AE</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>7 AE</td>
<td>1</td>
<td>89</td>
</tr>
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<td>8 AE</td>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td>12 AE</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>13 AE</td>
<td>0</td>
<td>88</td>
</tr>
</tbody>
</table>

Lymphocyte infiltrate in interstitium was identified with M108 monoclonal antibody by immunoperoxidase technique. The lymphocyte infiltrate was arbitrarily graded in a scale of 1 to 5.

Discussion

Evidence accumulated during the last 10 years suggests the participation of cell mediated mechanisms in the pathogenesis of acute glomerulonephritis [23, 24]. Macrophages may be pathogenic in the glomerulus since they release lysosomal hydrolases capable of denaturating the glomerular basement membrane. In addition, macrophages produce a wide variety of cytokines that may influence the glomerular injury; these include interleukin 1, platelet activating factor, interferon, arachidonic acid metabolites, tissue necrosis factor and comple-

Fig. 1. Relationship between monocyte accumulation and proteinuria in biopsies taken 1 (■), 4 (□), and 14 (▲) days after the beginning of proteinuria. \( y = 35 + 105.20 x, r = 0.747, P < 0.001 \). Interrupted lines correspond to 95% confidence limits.

Fig. 2. Biopsy taken the first day of proteinuria in rabbit No. 5 showing intraglomerular lymphocyte identified with the immunoperoxidase technique using M108 monoclonal antibody. (Magnification × 320)

Fig. 3. Correlation between intraglomerular lymphocyte and monocyte infiltration per glomerular cross section (gcs). Monocyte infiltration determined by the mean esterase stain score. Data from biopsies taken 1 (■), 4 (□), and 14 (▲) days after the beginning of proteinuria. Interrupted lines are the 95% confidence limits. \( y = 0.097 + 2.01 x; r = 0.876; P < 0.0001 \).

Fig. 4. Correlation between lymphocyte infiltration and migration inhibition factor (MIF) activity in supernatants of glomerular cultures. Data from biopsies taken 1 (■), 4 (□), and 14 (▲) days after the beginning of proteinuria. Interrupted lines are the 95% confidence limits \( y = 26.43 + 44.77 x; r = 0.776; P < 0.0001 \).
of antigen elimination. Lymphocyte infiltration diminished rapidly by day 4 and continued to decrease by day 14 after antigen elimination, in parallel with the decreasing of monocytes. A significant correlation is present between both lymphocyte infiltration and esterase stain score per gcs (Fig. 3).

We have shown an increase of MIF activity in supernatants from glomerular cultures obtained from kidneys removed at the antigen elimination day (Tables 1 and 2). This activity decreased by day 4 after antigen elimination and was comparable to the control normal kidneys by day 14. The linear correlation observed between MIF activity and the lymphocyte infiltration (Fig. 4) suggests that MIF is produced by lymphocytes infiltrating the glomerulus of animals with AcSS. Lymphocyte accumulation and increased MIF activity have been found to coexist in anti-glomerular basement membrane disease [10].

It is difficult to rule out the possibility that passenger or trapped lymphocytes could be a source of MIF activity. Nevertheless, data from two rabbits (# 4, shown in Table 1; and # 21 shown in Table 3) argue against a major participation by trapped lymphocytes. Those animals developed antigen elimination without proteinuria and had a low MIF activity (46 and 27%, respectively). We could assume that those animals had systemic lymphocyte activation because they produced enough antibody to clear BSA and they had IgG and C3 deposits in the glomeruli; nevertheless, MIF activity and monocyte infiltration in these animals were much lower than in the rabbits with proteinuria. Our results are probably not due to contaminating interstitial lymphocytes, because no difference in MIF activity was found in six rabbits which had different degrees of interstitial infiltrate (Table 3). Taken together, these findings suggest that trapped, passenger or interstitial lymphocytes, may contribute to MIF activity but they are not the main source of lymphokine production in this model.

In vitro experiments suggest that macrophages may also be retained as a result of mechanisms dependent of antigen-antibody complexes or polymorphonuclear leukocytes [27]. It is unlikely that polymorphonuclear leukocytes could be responsible for the macrophage accumulation in the glomeruli, in view of the studies of Boyce, Tipping and Holdsworth [10], who showed, in a culture system similar to ours, that nonlymphokine products of inflammation do not inhibit macrophage migration.

Immune complexes may facilitate macrophage accumulation, but it seems unlikely that they could play a determinant role since the highest numbers of intraglomerular macrophages are found early, at the time of antigen elimination when the renal deposition of immune complexes is known to be less prominent [16]. In fact, at day 4, as determined by immunofluorescent studies, the immune complexes were more intense and, at this time, monocyte accumulation had already decreased.

In MIF inhibition experiments we have used two antibodies: M108 and T1, both of which belong to IgG2 class that can activate the classic complement pathway. Both antibodies are directed to T lymphocytes, but M108 is specific for rabbit T lymphocytes whereas T1 reacts and is lytic to human T lymphocytes. Our experiments demonstrated that M108 monoclonal antibody is lytic for rabbit lymphocytes, and binds to intraglomerular lymphocytes. When M108, but not T1, is added to glomeruli from AcSS rabbits, it inhibits MIF activity in the glomerular supernatants (Table 4). The specific inhibition of MIF activity in M108 treated glomeruli suggests that MIF is released by lymphocytes during acute phase of AcSS. Because lymphocyte
infiltration and monocyte accumulation have a temporal relationship with glomerular lymphokine activity, it is possible that MIF activity plays a role in macrophage recruitment in AcSS glomerulonephritis. Nevertheless, our findings do not exclude the participation of other potential mechanisms such as adherence to immune globulins by the monocyte Fc receptor [7].

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References