IFN-γ Mediates Crescent Formation and Cell-Mediated Immune Injury in Murine Glomerulonephritis

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Abstract. Features of crescentic glomerulonephritis suggest that it results from a T helper 1 (Th1) nephritogenic immune response. Interferon-γ (IFN-γ), produced by Th1 cells, is involved in T cell-directed macrophage activation in effector Th1 responses. The hypothesis that endogenous IFN-γ contributes to the development of crescentic glomerulonephritis was tested by comparing the development of glomerulonephritis (induced by a planted antigen) and immune responses in normal C57BL/6 mice (IFN-γ +/-) and in mice genetically deficient in IFN-γ (IFN-γ −/−). Ten days after the initiation of glomerulonephritis, IFN-γ −/− mice developed fewer glomerular crescents (5 ± 1% versus 26 ± 3%, P < 0.005), less severe glomerular injury, and less renal impairment. Effectors of delayed-type hypersensitivity (CD4+ T cells, macrophages, and fibrin) in glomeruli were reduced in IFN-γ −/− mice. Skin delayed-type hypersensitivity to sheep globulin was reduced. Total antigen-specific Ig and splenocyte interleukin-2 production were unchanged, but antigen-specific serum IgG2a was reduced. Markers of an antigen-specific Th2 response (serum IgG1, splenocyte interleukin-4) were unchanged. Studies 22 d after the initiation of glomerulonephritis showed that IFN-γ −/− mice still had fewer crescents (11 ± 2% versus 22 ± 3%, P = 0.02) and glomerular CD4+ T cells and macrophages than IFN-γ +/- mice. These studies demonstrate that endogenous IFN-γ mediates crescentic glomerulonephritis by promoting cell-mediated immune injury. They support the hypothesis that crescentic glomerulonephritis is a manifestation of a Th1 nephritogenic immune response.

Glomerulonephritis (GN) is characterized by cognate immune responses resulting in glomerular injury. Although some forms of GN are mediated predominantly by humoral effectors of injury (1), others, including proliferative and crescentic forms of GN, are associated with accumulation of T cells, macrophages, tissue factor, and fibrin, components of the delayed-type hypersensitivity (DTH) response (2). Crescent formation complicates proliferative forms of GN, denotes severe immune glomerular injury, and is associated with a poor prognosis. DTH is driven by T helper 1 cell (Th1) responses (3). In two experimental models of crescentic GN, there is evidence that crescent formation is a manifestation of a nephritogenic Th1 response (4,5). One of these models involves the development of autoimmune via immunization with glomerular basement membrane (GBM) (5). The other examines the host’s immune response to an antigen planted in glomeruli (4). Crescent formation in the latter model, used in this study, is CD4+-dependent in the effector phase (4) and occurs in the absence of autologous antibody (6).

Interferon-γ (IFN-γ) is produced by Th1 cells (7). It plays a role in macrophage activation, regulation of Ig isotype switching, and induction of MHC class I and class II molecules (8,9). Mice deficient in IFN-γ develop decreased skin DTH and fail to resist infection with Mycobacterium bovis, suggesting an important role for IFN-γ in effector Th1 responses (8). IFN-γ is necessary for the expression of experimental hypersensitivity pneumonitis, a granulomatous lung disease (10). However, studies in organ-specific autoimmune diseases such as experimental allergic encephalomyelitis, thought to be Th1-mediated, have not confirmed a pathogenic role for IFN-γ (11,12), although the mechanism behind this lack of effect, or even negative effect, is unclear (13). A regulatory role for IFN-γ in allogeneic responses is suggested by the development of increased cytotoxic activity in the mixed lymphocyte reaction using cells from IFN-γ −/− mice (8) and by studies in experimental allogeneic heart transplantation that have shown that deficiency of endogenous IFN-γ did not permit engraftment (14,15).

The current studies use a model of crescentic GN induced by a planted exogenous antigen, sheep globulin, to address the hypothesis that endogenous IFN-γ contributes to crescent formation and cell-mediated immune glomerular injury. The use of this model addresses the role of IFN-γ in renal immunopathology without needing to consider its role in the context of the development of autoimmunity or allogeneic responses. This hypothesis was tested by comparing the development of GN in C57BL/6 mice deficient in the gene for IFN-γ (IFN-γ −/−) with GN in genetically normal C57BL/6 mice (IFN-γ +/-), by examining glomerular crescent formation and effector pathways of glomerular injury, and by assessing the nature of the systemic immune response to the nephritogenic antigen.
Materials and Methods

Induction of Accelerated Nephrototoxic Serum Nephritis

A sheep was repeatedly immunized against homogenized murine renal cortex in Freund’s complete adjuvant (Sigma Chemical Co., St. Louis, MO) and later Freund’s incomplete adjuvant (Sigma). To produce a globulin fraction, this serum was adsorbed twice with mouse red blood cells (10% by volume) and then precipitated twice with ammonium sulfate at a final concentration of 50%. Mice with a genetic deletion of IFN-γ on the C57BL/6 background (8) were obtained from Jackson Laboratories (Bar Harbor, ME) and bred at Monash University, (Melbourne, Australia). Male IFN-γ −/− (n = 15) and genetically normal C57BL/6 mice (n = 22), 8 to 10 wk of age, were sensitized by subcutaneous injection of a total of 100 μg of sheep globulin in 100 μl of Freund’s complete adjuvant in divided doses in each flank. Ten days later, GN was initiated by intravenous administration of 3.3 mg of nephrotoxic serum (raised in sheep, as above), a dose that does induce proteinuria in naïve mice (data not shown). Glomerular injury and immune responses were assessed after an additional 10 d (IFN-γ −/− n = 7, IFN-γ +/+ n = 12). To assess the development of more chronic injury in mice, GN was studied in two other groups at day 22 (IFN-γ −/− n = 8, IFN-γ +/+ n = 10).

Assessment of Glomerular Crescent Formation

Kidney tissue was fixed in Bouin’s fixative and embedded in paraffin, and 3-μm tissue sections were cut and stained with periodic acid-Schiff. A glomerulus was considered to exhibit crescent formation when more than two layers of cells were observed in Bowman’s space. A minimum of 50 glomeruli was assessed to determine the crescent score for each animal.

Glomerular T Cell, Macrophage, and Neutrophil Accumulation

Kidney tissue was fixed in periodate lysine paraformaldehyde for 4 h, washed in 7% sucrose solution, then frozen in liquid nitrogen. Tissue sections (6 μm) were stained to demonstrate CD4+ cells, CD8+ cells, and macrophages using a three-layer immunoperoxidase technique, as described previously (16,17). The primary monoclonal antibodies were GK1.5 (18), anti-mouse CD4 (American Type Culture Collection, [ATCC], Rockville, MD), 53-6.7 (anti-mouse CD8, ATCC), and M1/70 (anti-mouse Mac-1, ATCC), which recognizes subcutaneous injection of a total of 100 μg of sheep globulin in 100 μl of Freund’s complete adjuvant in divided doses in each flank. Ten days later, GN was initiated by intravenous administration of 3.3 mg of nephrotoxic serum (raised in sheep, as above), a dose that does induce proteinuria in naïve mice (data not shown). Glomerular injury and immune responses were assessed after an additional 10 d (IFN-γ −/− n = 7, IFN-γ +/+ n = 12). To assess the development of more chronic injury in mice, GN was studied in two other groups at day 22 (IFN-γ −/− n = 8, IFN-γ +/+ n = 10).

Glomerular Deposition of Fibrin, Sheep Globulin, Mouse Ig, and C3

Tissue was embedded in OCT compound, frozen in liquid nitrogen, and stored at −70°C. Immunofluorescence was performed on 4-μm cryostat cut tissue. Glomerular fibrin deposition was detected on a minimum of 30 glomeruli per mouse using FITC-goat anti-mouse fibrin/fibrinogen serum (Nordic Immunological Laboratories, Berks, United Kingdom) at a dilution of 1:20 and scored semiquantitatively (0 to 3+) as follows: 0, no fibrin deposition; 1, fibrin occupying up to one-third of the glomerular cross-sectional area; 2, fibrin occupying one-third to two-thirds of the glomerulus; 3, greater than two-thirds of the glomerular cross section covered by fibrin. Deposition of sheep globulin was evaluated using FITC-rabbit anti-sheep Ig (Nordic) at a dilution of 1:500, and sections were scored 0 to 3+ on the basis of fluorescence intensity. Two methods were used for assessment of mouse Ig (FITC-sheep anti-mouse Ig [Silenus, Hawthorn, Victoria, Australia]) and C3 (FITC-goat anti-mouse C3 [Cappel, Durham, NC]) in glomeruli. First, fluorescence intensity was assessed semiquantitatively (0 to 3+) using a dilution of 1:200 for mouse Ig and 1:100 for C3. Sections in which only some glomeruli were positive were graded as 0.5 (i.e., +/-). Second, serial dilutions of these antibodies were made to determine the end point positive titer in each animal, and results were expressed as the log2 of this end point titer.

Serum Creatinine and Proteinuria

Serum creatinine concentrations at the end of each experiment were measured by the alkaline picric acid method using an autoanalyzer. Urinary protein concentrations were determined by a modified Bradford method on 24-h urine collections. For mice studied at day 10, urine collections were made from day 1 to 2 and from day 9 to 10 of disease. For mice studied at day 22, a urine collection was made from day 21 to 22.

Measurement of Interleukin-2 and Interleukin-4 Production by Splenocytes

Spleens from mice with GN were removed aseptically and placed in Dulbecco’s modified Eagle’s medium (DMEM) 5% fetal calf serum (FCS). Single cell suspensions were prepared, and erythrocytes were lysed by incubation in 0.15 M NH4Cl, 0.01 M NaHCO3, 0.1 mM EDTA, pH 7.3, for 1 min. Splenocytes were washed in DMEM 5% FCS and incubated (4 × 106 cells/ml in DMEM 10% FCS, 50 μM 2-mercaptoethanol) for 72 h at 37°C, 5% CO2 in 48-well plates with protein G-purified normal sheep IgG (10 μg/ml). Interleukin-4 (IL-4) in culture supernatants were measured by enzyme-linked immunosorbent assay (ELISA) as described previously (19), using rat anti-mouse IL-4 (11B11; ATCC) and biotinylated rat anti-mouse IL-4 (BDV6; DNAX, Palo Alto, CA) monoclonal antibodies and 0.1 M 2,2’-azino-di-3-ethylbenzthiazoline sulfonate (Boehringer Mannheim, Mannheim, Germany) in 0.02% H2O2 as a substrate. IL-2 was measured using a commercial ELISA (Intertest-2X, mouse IL-2 ELISA kit, Genzyme, Cambridge, MA).

Skin DTH to Sheep Globulin

In the experimental groups where GN was induced and studied at day 22, mice were challenged 24 h before the end of the experiment by intradermal injection of sheep globulin (500 μg in 50 μl of phosphate-buffered saline [PBS]) into the plantar surface of a hind foot. An irrelevant antigen (horse globulin) was injected in the opposite foot pad as a control. DTH was assessed 24 h later by measuring the difference between the sheep globulin- and horse globulin-injected foot pads in each mouse using a micrometer (Mitutoyo Corp., Japan).

Titers of Serum Anti-Sheep Globulin Ig and Ig Isotypes

Titers of total mouse anti-sheep globulin Ig and anti-sheep globulin IgM, IgG1, IgG2a, IgG2b, and IgG3 were measured by ELISA on serum collected at the end of each experiment as described previously (19). Plates were coated with 10 μg/ml normal sheep globulin, washed, then blocked with 1% BSA in PBS for total Ig, IgM, IgG1, and IgG2b assays (2% casein in PBS for IgG2a and IgG3 assays). Plates were washed, then incubated with mouse serum at the dilution indicated, and incubated for 1 h at 37°C (overnight at 4°C for IgG2a and IgG3 assays). Bound mouse Ig was detected with horseradish peroxidase-conjugated antibodies: sheep anti-mouse Ig (1:2000; Amersham, Little Chalfont, United Kingdom) for total Ig and goat anti-mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 (1:4000; Southern Biotechnology, Birmingham, AL) for Ig isotypes. 2,2’-Azino-di-3-ethylbenzthiazoline sulfonate substrate solution was
added and the absorbance was read at 405 nm. Sera from five nonimmunized mice were tested to provide normal controls.

Statistical Analyses

Results are expressed as the mean ± SEM. The significance of differences between IFN-γ +/+ mice and IFN-γ −/− mice was determined by the Mann–Whitney U test.

Results

Development of Crescentic GN in Normal C57BL/6 Mice

Proliferative and crescentic GN had developed in IFN-γ +/+ mice 10 d after initiation of GN (Figure 1A). These mice developed periodic acid-Schiff-positive material in glomeruli and moderate tubulointerstitial damage. An average of 25 ± 3% of glomeruli were affected by crescent formation (Figure 2). Effectors of DTH were present in glomeruli: CD4+ T cells, macrophages, and fibrin (Figure 2). Occasional CD8+ cells and neutrophils were present in glomeruli. Sheep globulin was deposited in a linear pattern along the GBM.

IFN-γ Deficiency Reduces Crescent Formation and Cell-Mediated Immune Renal Injury at Day 10 of GN

Renal injury was less severe in IFN-γ −/− mice 10 d after the initiation of GN (Figure 1B). There was equivalent deposition of the nephritogenic antigen, sheep globulin, in glomeruli of IFN-γ +/+ and IFN-γ −/− mice (IFN-γ +/+ : 2.6 ± 0.1; IFN-γ −/− : 2.6 ± 0.2 [score 0 to 3+]). Glomerular crescent formation was attenuated, with a corresponding reduction in CD4+ cells, macrophages, and glomerular fibrin deposition (Figure 2). Consistent alterations in glomerular deposition of humoral mediators of injury were not observed in IFN-γ −/− mice (Table 1), although there was a nonstatistically significant increase in end point positive titer of autologous antibody deposition, and decreased C3 deposition when assessed semiquantitatively. Glomerular CD8+ cells were increased compared with normal mice with GN, but were still present only in relatively low numbers (Figure 2). Glomerular neutrophil numbers were not affected (IFN-γ +/+ : 0.25 ± 0.04 c/gcs; IFN-γ −/− : 0.20 ± 0.02 c/gcs). The impairment of renal function found in IFN-γ −/− mice with GN was attenuated, compared with IFN-γ +/+ mice (IFN-γ +/+ : 39 ± 4; IFN-γ −/− : 28 ± 2 μmol/L) (Figure 3A). Proteinuria at day 1 to 2 was reduced compared with IFN-γ +/+ mice with GN (Figure 3B). This decrease in proteinuria was not statistically significant at the end of the experiment (day 9 to 10, P = 0.13).

Systemic Immune Responses to Sheep Globulin in IFN-γ +/+ and IFN-γ −/− Mice with GN

Skin DTH to sheep globulin (measured as the difference in swelling between the footpad challenged with sheep globulin and the footpad challenged with horse globulin) was reduced, but not abrogated, in IFN-γ −/− mice (IFN-γ +/+ : 0.28 ± 0.03 mm; IFN-γ −/− : 0.16 ± 0.03 mm, P = 0.02). IL-2 and IL-4 were measured in sheep IgG-stimulated supernatants of splenocytes isolated from mice with GN. Levels of IL-2 were unaltered by IFN-γ deficiency (IFN-γ +/+ : 128 ± 28; IFN-γ −/− : 105 ± 25 pg/ml). Deficiency of endogenous IFN-γ did not result in an increased Th2 response to sheep globulin. Levels of the prototypic Th2 cytokine IL-4 were not increased in splenocyte supernatants from IFN-γ −/− mice. Due to considerable overlap between the experimental groups, the apparent reduction in IL-4 was not statistically significant (IFN-γ +/+ : 181 ± 54 pg/ml; IFN-γ −/− : 70 ± 21 pg/ml, P = 0.23).

Sheep globulin-specific antibody responses were measured by ELISA on serum collected from mice at the end of the experiment (Table 2). Genetic deficiency of IFN-γ did not alter titers of total antigen-specific Ig. However, there was a significant decrease in IgG2a titers. There was a trend toward a reduction in IgG3 titers, whereas IgG1 titers were nonsignificantly increased in IFN-γ −/− mice compared with IFN-γ +/+ mice. Levels of sheep globulin-specific IgG2b and IgM were unchanged. These results show that IFN-γ deficiency has
little effect on the total antibody or IgM response to the nephritogenic antigen, but changes in IgG subclasses were consistent with IFN-\(\gamma\) deficiency, given its role in the induction of IgG subclass switching (20).

**IFN-\(\gamma\)-Deficient Mice Have Fewer Glomerular Crescents at Day 22 of Disease**

Renal injury in normal mice with GN and IFN-\(\gamma\) \(-/-\) mice was studied after 22 d of disease, to assess the effects of IFN-\(\gamma\) deficiency on more chronic immune renal injury. Genetically normal mice showed features of more chronic renal injury, with proliferative GN with focal and segmental areas of necrosis. Glomerular crescent formation was present, which was mainly cellular, although some crescents were fibrocellular. There were focal areas of interstitial inflammation and tubular atrophy. Mice deficient in IFN-\(\gamma\) showed a lesser degree of glomerular crescent formation (IFN-\(\gamma\) \(+/+\): 22 \pm 3\%; IFN-\(\gamma\) \(-/-\): 11 \pm 2\%; \(P = 0.02\)) (Figure 4), with fewer glomerular T cells and macrophages. Heavy proteinuria continued at day 21 to 22 in control mice with GN, and remained lower in IFN-\(\gamma\) \(-/-\) mice (Figure 5B) that had levels comparable to mice studied at earlier time points (day 1 to 2 and day 9 to 10). There was no difference in serum creatinine values between IFN-\(\gamma\) \(-/-\) and IFN-\(\gamma\) \(+/+\) mice at day 22 (IFN-\(\gamma\) \(+/+\): 32 \pm 2; IFN-\(\gamma\) \(-/-\): 33 \pm 1 \(\mu\)mol/L) (Figure 5A).

**Discussion**

In this model of crescentic GN, mice lacking endogenous IFN-\(\gamma\) developed less severe GN than genetically normal

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**Table 1. Humoral effectors of glomerular injury: deposition of autologous antibody and C3 in glomeruli of mice 10 d after initiation of GN**

<table>
<thead>
<tr>
<th>Effector</th>
<th>IFN-(\gamma) (+/+)</th>
<th>IFN-(\gamma) (-/-)</th>
</tr>
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<tbody>
<tr>
<td>Autologous antibody</td>
<td></td>
<td></td>
</tr>
<tr>
<td>end point positive titer</td>
<td>10.8 (\pm) 0.2</td>
<td>11.5 (\pm) 0.4(^b)</td>
</tr>
<tr>
<td>score (0 to 3+)</td>
<td>1.6 (\pm) 0.1</td>
<td>1.7 (\pm) 0.2</td>
</tr>
<tr>
<td>C3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>end point positive titer</td>
<td>11.0 (\pm) 0.2</td>
<td>10.7 (\pm) 0.4</td>
</tr>
<tr>
<td>score (0 to 3+)</td>
<td>1.6 (\pm) 0.2</td>
<td>0.9 (\pm) 0.2(^c)</td>
</tr>
</tbody>
</table>

\(^a\) Autologous antibody and C3 deposition in glomeruli were assessed by immunofluorescence. Results are expressed as the mean \(\pm\) SEM of the log 2 of the end point-positive titer and as the mean \(\pm\) SEM of a semiquantitative score for each animal. GN, glomerulonephritis; IFN-\(\gamma\), interferon-\(\gamma\).

\(^b\) \(P = 0.06\).

\(^c\) \(P = 0.02\).
C57BL/6 mice. Crescent formation and elements of DTH were reduced in glomeruli of IFN-γ −/− mice, and functional injury, measured by serum creatinine and urinary protein excretion, was diminished in IFN-γ-deficient mice. Skin DTH to the nephritogenic antigen was reduced, consistent with the observed reductions in glomerular injury. Serum titers of antigen-specific IgG2a and IgG3, associated with IFN-γ and Th1 responses, were reduced in IFN-γ2/2 mice, but splenocyte IL-2 levels were unaffected. As IL-2 is produced by Th1 cells (7), this result suggests that the effects of endogenous IFN-γ on Th1 responses were mediated directly by IFN-γ itself, and not by a generalized downregulation of all components of the Th1 response. Th2 elements of the immune response to sheep globulin were unaffected. There was a nonsignificant increase in the Th2-associated subclass IgG1, and total antibody titers and splenocyte IL-4 levels were not increased.

Although renal injury was substantially reduced in IFN-γ −/− mice compared with genetically normal mice at day 10 of disease, renal injury was not abrogated. A minority of glomeruli were affected by crescent formation, and abnormal numbers of glomerular CD4+ cells and macrophages were present. Functionally, IFN-γ −/− mice still developed proteinuria and some renal impairment at day 10. The effects of IFN-γ deficiency lasted into a more chronic phase of this model (22 d after the initiation of GN). Crescent formation was still significantly less than in genetically normal mice with GN at day 22, although the percentage of glomeruli affected by crescent formation in IFN-γ −/− mice was increased to 11% at this time point. Numbers of CD4+ T cells and macrophages in glomeruli were still reduced in IFN-γ −/− mice, as was proteinuria. However, the serum creatinine in IFN-γ −/− mice was not different from that of IFN-γ +/+ mice.

The fact that injury, although reduced, was not absent in IFN-γ −/− mice is not surprising, and could be due to alternative pathways of macrophage activation, and/or to injury induced by humoral mediators. It is unlikely that IFN-γ is the

Table 2. Sheep globulin-specific total immunoglobulin and immunoglobulin isotypes 10 d after initiation of GN in sera of IFN-γ +/+ and IFN-γ −/− mice with GN, with normal unimmunized mice as controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal Mouse</th>
<th>IFN-γ +/+</th>
<th>IFN-γ −/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ig</td>
<td>0.026 ± 0.003</td>
<td>0.427 ± 0.075</td>
<td>0.428 ± 0.072</td>
</tr>
<tr>
<td>IgG1</td>
<td>0.016 ± 0.006</td>
<td>0.667 ± 0.042</td>
<td>0.844 ± 0.090b</td>
</tr>
<tr>
<td>IgG2a</td>
<td>0.046 ± 0.020</td>
<td>0.109 ± 0.022</td>
<td>0.038 ± 0.009c</td>
</tr>
<tr>
<td>IgG2b</td>
<td>0.004 ± 0.003</td>
<td>0.286 ± 0.076</td>
<td>0.340 ± 0.069</td>
</tr>
<tr>
<td>IgG3</td>
<td>0.021 ± 0.009</td>
<td>0.153 ± 0.038</td>
<td>0.072 ± 0.012d</td>
</tr>
<tr>
<td>IgM</td>
<td>0.053 ± 0.008</td>
<td>0.174 ± 0.028</td>
<td>0.130 ± 0.020</td>
</tr>
</tbody>
</table>

* Sera were assessed by enzyme-linked immunosorbent assay at a dilution of 1:500 for total sheep globulin-specific immunoglobulin, 1:50 for IgG2a and IgG3 levels, 1:100 for IgM, and 1:200 for IgG1 and IgG2b isotypes. Results are expressed as the mean OD405 ± SEM. Abbreviations as in Table 1.
  b P = 0.08.
  c P = 0.015.
  d P = 0.10.

Figure 4. Glomerular crescent formation and effectors of cell-mediated immune injury in glomeruli in IFN-γ +/+ and IFN-γ −/− mice 22 d after the initiation of renal injury. c/gcs, cells per glomerular cross section. * P = 0.02; ** P < 0.005.

Figure 5. Renal function and urinary protein excretion, measured at day 22 in IFN-γ +/+ mice (□) and IFN-γ −/− mice (◼) with GN. Dotted lines represent values for normal mice without GN. (A) There was no difference in serum creatinine between the two groups at day 22. (B) However, there was a significant reduction in proteinuria at day 22 in IFN-γ mice compared with IFN-γ −/− mice. * P = 0.01.
sole determinant of macrophage activation, or of the generation of Th1 responses. Skin DTH to sheep globulin, while attenuated, was not absent in IFN-γ-deficient mice, consistent with previous reports on the role of IFN-γ in DTH responses (21) and the phenotype of IFN-γ and IFN-γ-receptor-deficient mice (8,9). Deficiency of the IFN-γ receptor did not affect the ability of mice to mount a Th1 response in Leishmania major infection (22). Although IFN-γ clearly has an important role in promoting macrophage effector functions, it is not the sole known activator of macrophages (23–25).

An additional likely contributing factor to the renal injury present in IFN-γ−/− mice with GN is the persistence of humoral factors. Glomerular deposition of autologous antibody was unchanged in these mice. Although autologous antibody is not required for crescent formation, humoral mediators do result in glomerular injury in this model (4,26). Therefore, deposition of antibody and complement in glomeruli is likely to have contributed to the proteinuria and the renal impairment that was present in IFN-γ−/− mice. It is possible that a reduction in the complement-fixing IgG2a isotype in IFN-γ-deficient mice may have contributed to the reduction in renal injury in these mice. However, several lines of evidence from other studies in this model suggest that alterations in humoral immune responses are in themselves not sufficient to alter the degree of crescentic injury. Crescent formation is independent of the presence of autologous antibody (6). CD4+ cell depletion in the effector phase of the immune response prevented crescent formation but did not alter serum antigen-specific antibody levels (4). Studies in IL-4-deficient BALB/c mice have shown that they do not develop glomerular crescent formation despite alterations in IgG subclasses (increased IgG2a and IgG3) that would, at least in theory, favor increases in glomerular injury (27).

Endogenous IFN-γ upregulates MHC class II in kidneys in the context of inflammatory stimuli (28). The unchanged levels of serum antigen-specific Ig and of splenocyte IL-2 argue against a prominent role for IFN-γ in antigen presentation in this study. However, the decreased glomerular CD4+ cell infiltration in IFN-γ−/− mice suggests effects of endogenous IFN-γ beyond its effector functions of macrophage activation and IgG3 subclass switching. Given the recent demonstration that MHC class II expression by intrinsic renal cells is required for antigen recognition in the development of crescentic GN (29), a lack of IFN-γ-induced MHC II expression could explain the reduced glomerular CD4+ cells found in this study.

Although present in relatively low numbers, CD8+ cells in glomeruli were increased in IFN-γ−/− mice with GN at day 10 (although there was no change at day 22). These findings are consistent with the regulatory effects of IFN-γ on the CD8+ subset observed in initial studies using IFN-γ−/− mice, reporting increased cytotoxic function and proliferation in IFN-γ-deficient mice attributed to CD8+ cells (8). An important pathogenic role for cytotoxic CD8+ cells in crescentic GN has not yet been demonstrated. Crescentic GN is not attenuated in CD8+−/− mice (30), and although depletion of CD8+ cells reduces glomerular injury in rats, cytotoxic T cell activity is not prominent, suggesting that CD8+ cells are functioning predominantly as T helper cells in rats (31).

In addition to expression of MHC class II, intrinsic glomerular cells have the capacity to respond directly to IFN-γ (32,33). The magnitude of this response in vivo is not known. However, in addition to glomerular injury induced by IFN-γ-activated macrophages (as predicted by knowledge of the effects of IFN-γ on the immune system and the effector function of macrophages in cell-mediated immune responses), it is possible that some of the effects of endogenous IFN-γ in crescentic GN could relate to T cell-derived IFN-γ acting directly on intrinsic glomerular cells.

Two previous studies have examined IFN-γ in experimental crescentic GN. In the first, neutralizing IFN-γ using a neutralizing monoclonal antibody reduced crescent formation and glomerular macrophage infiltration without affecting glomerular CD4+ cell numbers (4). The reduction in CD4+ cells observed in the current study may relate to the effects of complete absence of IFN-γ via the use of genetically deficient mice. In the second, genetically normal 129Sv mice developed moderate disease, with 11% of glomeruli affected by crescent formation (34). 129Sv mice genetically deficient in the IFN-γ receptor developed less disease on assessment of several parameters, but the reduction in crescent formation did not reach statistical significance.

Although in many forms of human GN the antigens involved are unknown, GN may result from immune responses to exogenous antigens, whereas in other forms of GN the immune response is directed against self antigens. Glomerulonephritis may develop as an organ-specific autoimmune disease, e.g., anti-GBM GN, or as part of systemic autoimmunity, e.g., lupus nephritis. IFN-γ has been detected within the inflammatory cell infiltrate in autoimmune murine anti-GBM GN (5). The role of IFN-γ has been studied in murine lupus, where, although not all of the results of studies have been concordant, studies in mice rendered deficient in IFN-γ or the IFN-γ receptor (35,36) by gene targeting have demonstrated a role for endogenous IFN-γ in the development of murine lupus. The effects of IFN-γ gene deletion have been attributed largely to reductions in the IgG2a and IgG3 autoantibodies, in contrast to the findings of the current study, which emphasize the role of cell-mediated immunity in crescentic GN. IFN-γ has been administered to humans as treatment for rheumatoid arthritis/systemic lupus erythematosus, and was reported to induce proliferative/crescentic GN (37,38).

These studies use a model of proliferative, crescentic GN induced by a planted exogenous antigen, which is CD4+/− dependent in the effector phase (4) and independent of the presence of autologous antibody (6). They establish a role for endogenous IFN-γ in the pathogenesis of severe crescentic GN through selective modulation of effector Th1 responses and reductions in cell-mediated immune renal injury. The presence of residual cell-mediated injury in IFN-γ−/− mice, particularly at day 22, suggests that although IFN-γ is important in cell-mediated immune responses, there may be other mediators relevant to the activation of macrophages. They provide evi-
dence that glomerular crescent formation is a manifestation of a Th1 nephritogenic immune response.

Acknowledgments

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