Interleukin-11 Attenuates Nephrotoxic Nephritis in Wistar Kyoto Rats

PING-CIN LAI,* H. TERENCE COOK, ‡ JENNIFER SMITH,* JAMES C. KEITH, JR., ‡ CHARLES D. PUSEY,* and FREDERICK W. K. TAM*

*Renal Section and ‡Department of Histopathology, Imperial College School of Medicine, Hammersmith Hospital, London, United Kingdom; and ‡Department of Immunology and Hemostasis, Discovery Research, Wyeth/Genetics Institute Inc., Andover, Massachusetts.

Abstract. Interleukin-11 (IL-11) is a multifunctional cytokine with anti-inflammatory activity. The effect of IL-11 was studied in an experimental model of necrotizing glomerulonephritis induced in Wistar Kyoto rats by an injection of anti–glomerular basement membrane antibody (nephrotoxic serum). Intrapерitoneal injection was chosen as the route of IL-11 administration in all experiments. In experiment 1, recombinant human IL-11 (1360 μg) was given 2 h before nephrotoxic serum, then once daily until day 6. In experiment 2, a lower dose of IL-11 (800 μg/d) was used. Rats were treated either with IL-11 400 μg twice daily intraperitoneally or with 800 μg once daily intraperitoneally for 6 d. In experiment 3, the lower dose of IL-11 was given 2 h before nephrotoxic serum, then twice daily until day 2. In experiment 1, IL-11 significantly reduced proteinuria (13.2 ± 3.3 versus 63.2 ± 4.3 mg/24 h), fibrinoid necrosis (0.58 ± 0.08 versus 1.52 ± 0.06 quadrants/glomerular cross section [gcs]), macrophage infiltration (ED1-positive cells, 24.4 ± 1.8 versus 39.3 ± 1.9 cells/gcs), apoptosis (1.11 ± 0.1 versus 2.39 ± 0.2 apoptotic bodies/gcs), and proliferating cell nuclear antigen–positive cells (24.4 ± 2.0 cells/gcs) vs 37.3 ± 2.3 cells/gcs). Inducible nitric oxide synthase–positive cells were significantly increased (3.1 ± 0.3 versus 2.0 ± 0.2 cells/gcs). In experiment 2, a lower dose of IL-11 significantly reduced proteinuria and fibrinoid necrosis. Macrophage infiltration was similar in treated and control groups, although the number of sialoadhesin-positive macrophages (ED3+) was significantly reduced in the IL-11–treated rats. In experiment 3, quantitative competitive reverse transcriptase–polymerase chain reaction showed that the mRNA ratio of IL-1/β/β-actin in the treated rats was reduced compared with controls. By the use of probes designed from mouse IL-11 receptor α-chain sequence, it was also shown that rat mesangial cells and macrophages expressed IL-11 receptor α-chain, demonstrating that they were capable of responding to IL-11. In this model of necrotizing glomerulonephritis, high-dose IL-11 treatment markedly reduced both proteinuria and fibrinoid necrosis. At the lower dose, there was a reduction in glomerular injury and macrophage sialoadhesin expression, but without an alteration of macrophage numbers, suggesting that IL-11 may be acting in part to reduce macrophage activation.

Immune-mediated glomerulonephritis is characterized by glomerular accumulation of macrophages, and in certain experimental models, glomerular injury is macrophage dependent (1). Macrophages in inflamed glomeruli have an activated phenotype, and their production of proinflammatory cytokines, tumor necrosis factor alpha (TNF-α), and interleukin-1 (IL-1β) appears to play a central role in macrophage-mediated injury (1). There is evidence that the transcription factor NF-κB is a key intermediate in macrophage activation (2–4) and is involved in transcriptional activation of a variety of genes expressed during glomerular inflammation, such as TNF-α, IL-1β, and IL-12 p40 (4,5). Mediators that are able to reduce macrophage activation are therefore potential therapeutic agents in glomerulonephritis.

IL-11 is a multifunctional cytokine involved in hematopoiesis and regulation of inflammation (6,7). Recent studies have demonstrated that IL-11 has significant effects on macrophage activation. In vitro, IL-11 inhibits the lipopolysaccharide (LPS)-induced synthesis of TNF-α, IL-1β, IL-12, and nitric oxide by murine peritoneal macrophages (8). This is accompanied by a reduction in mRNA for TNF-α and IL-1β (9). In human macrophages, IL-11 is a potent inhibitor of IL-12 production and reduces accumulation of the p35 and p40 subunits of IL-12 (10). These effects of IL-11 are independent of other anti-inflammatory molecules such as IL-10, IL-6, and TGF-β. In an in vivo model of endotoxemia, IL-11 downregulated serum levels of the LPS-induced proinflammatory cytokines TNF-α, IL-1β, and interferon gamma (IFN-γ) (8). The effect on macrophages is mediated, at least in part, by inhibition of NF-κB activity (9). Recently, inhibition of NF-κB binding by administration of decoy oligonucleotides complexed with hemagglutinating virus of Japan liposomes has been shown to ameliorate proteinuria and to improve renal histology in glomerulonephritis (11). These results suggest that
IL-11 may have a potential therapeutic role in diseases characterized by macrophage activation and synthesis of proinflammatory cytokines.

IL-11 has been shown to be effective in treating a number of experimental models of inflammation, including colitis (12), collagen-induced arthritis (13), graft-versus-host disease (14), and endotoxin-induced lung injury (15). In humans, IL-11 was effective in reducing expression of proinflammatory cytokines and ameliorating disease in a phase 1 trial of psoriasis (16). IL-11 has not been assessed for the treatment of renal inflammation, and, therefore, we have studied the effect of IL-11 in a florid model of macrophage-dependent, focal and segmental necrotizing glomerulonephritis in the Wistar Kyoto (WKY) rat (17).

Materials and Methods

Reagents

Nephrotoxic serum, a rabbit antiserum to rat glomerular basement membrane, was prepared as described previously (18). Recombinant human IL-11 was supplied by Wyeth/Genetics Institute (Cambridge, MA) and had a specific activity of 1.6 x 10^6 U/mg as determined by T10 proliferation assay (19).

Nephrotoxic Nephritis

Male WKY rats weighing 200 to 230 g were given 0.1 ml of nephrotoxic serum intravenously (17). Urine was collected by housing the rats in metabolic cages for 24-h periods with free access to food and water. Urinary protein was measured by the sulfosalicylic acid method (20). At the end of the experiment, rats were killed while under general anesthesia induced by isoflurane. Samples of kidney, liver, lung, and spleen were placed in 10% formal saline, and samples of kidney were snap-frozen in optimal cutting temperature compound by means of isopentane cooled in liquid nitrogen. All animal experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act.

Experimental Design

Experiment 1. In experiment 1, rats (n = 10) were treated with intraperitoneally administered IL-11 1360 µg in 0.2 ml of 0.1% bovine serum albumin in saline once daily for 6 d. Control rats (n = 8) were given 0.2 ml of vehicle once daily. The first injection was given 2 h before induction of nephritis. Rats were placed in metabolic cages from day 5 to day 6 and killed on day 6.

Experiment 2. Experiment 2 used a lower dose of IL-11 with either once-daily or twice-daily dosing. In experiment 2a, WKY rats (n = 6) were treated with IL-11 400 µg intraperitoneally twice daily for 6 d. In experiment 2b, another set of WKY rats (n = 6) were treated with IL-11 800 µg intraperitoneally once a day for 6 d. Control rats (n = 6) in each experiment received intraperitoneal vehicle injections on the same schedule. The first injection was given 2 h before induction of nephritis. Rats were placed in metabolic cages from day 5 to day 6 and killed on day 6.

Experiment 3. Experiment 3 was designed to study the effect of IL-11 on IL-1β mRNA expression. Rats (n = 3) were treated with IL-11 400 µg intraperitoneally twice daily for 2 d. Control rats (n = 3) were given 0.4 ml of vehicle twice daily. The first injection was given 2 h before induction of nephritis. Rats were killed 48 h after induction of glomerulonephritis. Glomeruli were purified from the kidneys of each rat by differential sieving (21). Total RNA was extracted from glomeruli of each rat by RNAzol B (Biogenesis, Poole, UK) according to the manufacturer’s protocol. Reverse transcription was performed immediately after RNA extraction. The enzyme used to synthesize first strand cDNA was RNNaseH reverse transcriptase from MMLV (SuperScript II, Life Technologies, Paisley, UK). Oligo (dT)12-18 primer (Life Technologies) was used to prime the reaction. The reaction mixture was incubated for 50 min at 42°C, then heat-treated at 70°C for 15 min to inactivate the reverse transcriptase. Samples were stored at −20°C for subsequent competitive polymerase chain reaction (PCR) assay.

Histology and Immunohistochemistry

Glomerular fibrinoid necrosis was assessed in hematoxylin-and eosin–stained sections and was quantified by scoring the number of quadrants of each glomerulus involved. Fifty glomeruli were counted in blinded, randomized sections, and a mean score of quadrants per glomerular cross section (gcs) was calculated (21). Apoptosis was identified by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling method (ApopTag, Intergen, Purchase, NY) in paraffin sections. Paraffin sections were used for immunohistochemistry for macrophages (monoclonal antibody [mAb] ED1; Serotec, Oxford, UK), inducible nitric oxide synthase (iNOS; mouse anti-iNOS mAb; Affiniti, Exeter, UK), proliferating cell nuclear antigen (PCNA; mAb PC10; Dako, Ely, UK), and CD8+ cells (MRC OX-8 mAb; Serotec). For all antibodies except PCNA, sections were pretreated by microwaving for 3 × 5 min. The primary antibodies were used at the following concentrations: ED1, 1:500; iNOS, 1:50; PCNA, 1:50; and MRC OX-8, 1:200. ED1 was detected with an indirect peroxidase technique that used peroxidase-labeled rabbit antibody to mouse immunoglobulin (Ig); Dako), and iNOS, PCNA, and OX-8 were detected by use of biotinylated rabbit anti-mouse antibody (Dako) and streptavidin-biotin-peroxidase complex (Dako). Macrophages expressing sialoadhesin were detected by staining with mAb ED3 (Serotec) (22) in frozen sections by means of the streptavidin-biotin-peroxidase technique. For each antibody, cells were counted in blinded, randomized sections, and 50 glomeruli were counted per section.

Competitive PCR Assay

A multispecific rat competitor DNA fragment was a gift from Dr. H.-D. Volk (Institute for Medical Immunology, Berlin, Germany) (23). To quantify cDNA, competitive PCR was performed with a range of competitor fragment concentrations. In the first step, serial 10-fold dilutions of competitor fragment, ranging from 25 pg/µl to 0.25 fg/µl, were coamplified with a constant amount of glomerular cDNA. On the basis of the results of these reactions, serial twofold dilutions of competitor, spanning from 10- to 0.1-fold of the predicted cDNA amount, were coamplified with constant amounts of glomerular cDNA. For each experiment, cells were counted in blinded, randomized sections, and 50 glomeruli were counted per section.

Direct Immunofluorescence Microscopy

Direct immunofluorescence microscopic studies to assess deposition of rat and rabbit IgG in glomeruli were carried out on frozen sections. Sections were air-dried for 20 min, then washed in phosphate-buffered saline (PBS). Sections were incubated with either...
fluorescein isothiocyanate–labeled goat anti-rabbit IgG (1:80, Sigma, Poole, UK) or fluorescein isothiocyanate–labeled rabbit anti-rat IgG (1:20, Dako). For quantitation of fluorescence staining, images were captured with a color CoolView camera (Phottonic Science, Robertbridge, UK) and analyzed by Image-Pro Plus software (Media Cybernetics). Images were converted to gray scale, and the average pixel intensity of individual glomeruli was recorded. Results are shown as arbitrary units of intensity. Twenty glomeruli were evaluated in each section.

Enzyme-Linked Immunosorbent Assay

Circulating rat anti-rabbit antibodies were measured in sera from WKY rats by a sandwich enzyme-linked immunosorbent assay (24). Rabbit IgG was coated onto 96-well polystyrene microtitrator plates (ImmuNoPlate, NUNC, Na1ge NUNC International, Paisley, UK), at 50 μl/well of 10 μg/ml rabbit IgG in 0.015 M carbonate buffer, pH 9.6 (British Drug House, Poole, UK), by incubation at 4°C overnight. Coated plates were washed three times with 0.1% PBS/Tween. The plates were then blocked with 1% bovine serum albumin (Sigma) at 37°C for 1 h. After further washing in PBS/Tween, 50-μl aliquots of an optimal dilution of control or test rat serum (1/20 as determined previously by a dilution curve) in PBS/Tween were added to rabbit IgG–coated wells in triplicate and incubated at 37°C for 1 h. The solution was then decanted, and the plates were washed three times with PBS/Tween and drained. Bound rat anti-rabbit IgG antibody was detected by incubating wells with 50 μl of alkaline phosphatase conjugated rabbit anti-rat IgG (Sigma) at a dilution of 1 in 1000 for 1 h at 37°C. Excess conjugate was washed off, and 50 μl of the substrate p-nitrophenylphosphate disodium (Sigma 104, Sigma) in 0.015 M carbonate buffer (British Drug House, pH 9.6, was added for 40 min at 37°C. The absorbance for each well was read at 405 nm with an Anthos III enzyme-linked immunosorbent assay plate reader (Lab Tech International, Ringmer, UK).

IL-11 Receptor α-Chain PCR and Sequencing

IL-11 receptor α-chain primers, which were a gift from Wyeth/Genetic Institute Inc., were designed from mouse IL-11 receptor α-chain sequence because the rat IL-11 receptor α-chain sequence was unknown. Primers were designed to span over introns to distinguish amplicons of cDNA from those of genomic DNA. The primer sequences used in this experiment were as follows: sense primer: 5'-TTGGGGTCCTCCAGGGGTCCAGTATG-3'; antisense primer: 5'-GGAAGTAAAGGTACCGGTGGGCAAC-3'. Reverse transcription–PCR (RT-PCR) was carried out by means of mRNA extracted from normal glomeruli, from day 7 nephritic glomeruli, and from primary cultured mesangial cells and thioglycollate-elicited peritoneal macrophages (25). The predicted product size was 350 bp. To verify the sequence of the products, bands of the right size from PCR reactions were then column-purified with AutoSeq96 plates (Amer sham Pharmacia Biotech, Little Chalfont, UK) and dried in a speed vacuum. These samples were resuspended in 10 μl of HiDi (Applied Biosystems). By use of an ABI 3700 DNA sequencer (Applied Biosystems), these solutions were run on a 50 cm capillary array in POP6 polymer (Applied Biosystems). The results were analyzed by Gene-tyx-Mac (version 9, Tokyo, Japan) and compared with the mouse sequence.

Statistical Analyses

Results are presented as mean ± SEM. Comparisons between groups are by the Mann-Whitney U test (two-tailed).

Results

Experiment 1

All of the rats survived until the end of the experiment. As described previously, rats receiving vehicle developed proteinuria and a focal and segmental necrotizing glomerulonephritis with prominent infiltration of glomeruli by macrophages (17). IL-11 treatment significantly reduced glomerular injury, as shown by a 79% decrease in proteinuria and 62% decrease in fibrinoid necrosis (P < 0.01). Glomerular macrophages and proliferating cells (PCNA+) were decreased by 38 and 35%, respectively (P < 0.05). Cells expressing iNOS were significantly increased by 55% in the IL-11–treated animals (P < 0.05). Apoptosis in glomeruli was significantly reduced by 54% in the treated groups (P < 0.05). There was no significant difference in the number of CD8+ cells between the groups (Table 1; Figure 1).

Experiment 2

When we used a lower dose of IL-11, we still found a protective effect with either twice-daily or once-daily dosing. With 400 μg IL-11, twice daily, glomerular fibrinoid necrosis and proteinuria were decreased by 74 and 86%, respectively (P < 0.01). Once-daily treatment with 800 μg of IL-11 achieved a similar therapeutic effect: fibrinoid necrosis decreased by 65% and proteinuria decreased by 64% (P < 0.01). Interestingly, at this dose, there was no reduction in the number of macrophages within glomeruli. However, activated macrophages as assessed by the expression of sialoadhesin (ED3+) were significantly reduced by 71% in the twice-daily experi-

Table 1. Effect of interleukin-11 treatment in experiment 1 on glomerular injury and histology

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-11 Treated (n = 10)</th>
<th>Vehicle Treated (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinoid necrosis (quadrants/gcs)</td>
<td>0.58 ± 0.1b</td>
<td>1.52 ± 0.1</td>
</tr>
<tr>
<td>Proteinuria (mg/24 h)</td>
<td>13 ± 3b</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Macrophages/gcs</td>
<td>24.4 ± 1.8c</td>
<td>39.3 ± 1.9</td>
</tr>
<tr>
<td>PCNA+ cells/gcs</td>
<td>24.4 ± 2.0c</td>
<td>37.3 ± 2.3</td>
</tr>
<tr>
<td>iNOS+ cells/gcs</td>
<td>3.1 ± 0.3c</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>CD8+ cells/gcs</td>
<td>1.6 ± 0.3</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Apoptotic bodies/gcs</td>
<td>1.11 ± 0.19b</td>
<td>2.39 ± 0.27</td>
</tr>
</tbody>
</table>

a IL-11, interleukin-11; gcs, glomerular cross section; PCNA, proliferating cell nuclear antigen; iNOS, inducible nitric oxide synthase.

b P < 0.01.
c P < 0.05.
Figure 1. Histology of untreated (A, C, E, and G) versus interleukin-11 (IL-11)–treated rats (B, D, F, and H) with nephrotoxic nephritis. Hematoxylin-and-eosin–stained sections show fibrinoid necrosis (amorphous eosinophilic material in the glomerulus) in untreated rats (A), which was reduced by IL-11 treatment (B). Immunostaining of ED1-positive cells (brown cytoplasmic staining) showed macrophage infiltration in the glomeruli of untreated rats (C), which was reduced by IL-11 treatment (D). Sialoadhesin (ED3) expressing macrophages (arrows) were significantly reduced in IL-11–treated rats (F) when compared with the control rats (E). IL-11–treated rats had higher glomerular inducible nitric oxide synthase (iNOS) level (brown cytoplasmic staining) (H) than did the control rats (G). Original magnification, ×300.
ment and 35% in the once-daily experiment (P < 0.05) (Figures 1 and 2). The number of iNOS-positive cells was higher in the glomeruli of IL-11–treated rats, and the difference reached statistical significance in the 800 μg once-daily experiment. The number of CD8+ cells in glomeruli was similar in both groups (Table 2).

Figure 2. Glomerular ED1 and ED3 counts in experiment 2. In this experiment, Wistar Kyoto rats were treated either with interleukin-11 (IL-11) 400 μg twice daily intraperitoneally or with 800 μg once daily intraperitoneally. (A and B) Results of twice-daily dosing. (C and D) Once-daily dosing. In each case, ED1+ macrophage numbers in glomeruli were similar in both groups and the number of activated macrophages (ED3+) was significantly reduced by 71 and 35%, respectively. gcs, glomerular cross section.

Enzyme-linked immunosorbent assay for circulating rat anti-rabbit antibodies was carried out on the rats treated once daily and showed that IL-11–treated rats had significantly higher levels (P < 0.05; Figure 3). However, direct immunofluorescence staining showed no difference in glomerular deposition of either rat or rabbit Ig (Figure 4).

Table 2. Effect of interleukin-11 treatment in experiment 2 on glomerular injury and histology

<table>
<thead>
<tr>
<th>Variable</th>
<th>Twice Daily Experiment</th>
<th></th>
<th>Once Daily Experiment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-11 Treated</td>
<td>Vehicle Treated</td>
<td>IL-11 Treated</td>
<td>Vehicle Treated</td>
</tr>
<tr>
<td></td>
<td>(400 μg ip; n = 6)</td>
<td>(n = 6)</td>
<td>(800 μg ip; n = 6)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Fibrinoid necrosis (quadrants/gcs)</td>
<td>0.29 ± 0.11b</td>
<td>1.1 ± 0.21</td>
<td>0.59 ± 0.24b</td>
<td>1.68 ± 0.14</td>
</tr>
<tr>
<td>Proteinuria (mg/24 h)</td>
<td>11 ± 3b</td>
<td>81 ± 19</td>
<td>36 ± 14b</td>
<td>99 ± 5</td>
</tr>
<tr>
<td>iNOS+ cells/gcs</td>
<td>2.7 ± 0.6</td>
<td>1.3 ± 0.3</td>
<td>3.3 ± 0.3b</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>CD8+ cells/gcs</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a IL-11, interleukin-11; gcs, glomerular cross section; iNOS, inducible nitric oxide synthase; ND, not done.
b P < 0.01.
Experiment 3

To explore further the mechanism of this renal protective effect, we examined the effect of IL-11 on the expression of the proinflammatory cytokine, IL-1β, before the onset of tissue injury. To avoid the confounding effect of an alteration in macrophage numbers, we chose the lower dose of IL-11 (used in experiment 2) because we had found that this did not affect glomerular macrophage infiltration. Forty-eight hours after induction of glomerulonephritis, glomerular RNA was extracted. Expression of IL-1β and β-actin mRNA were quantified from this RNA by means of competitive RT-PCR. Figure 5 shows an example of the plot obtained in a representative competitive PCR reaction, comparing the ratio of the intensity of bands amplified from the control fragment and the glomerular sample to the starting concentration of the control fragment. To control for variation in RNA amount, the level of IL-1β mRNA was normalized to the level of β-actin mRNA level. The results showed that the ratio of IL-1β/β-actin mRNA in the IL-11–treated rats was reduced when compared with the control group (Figure 6).

Rat IL-11 Receptor α-Chain

By using PCR primers designed from the mouse IL-11 receptor α-chain sequence, we showed that rat glomeruli, primary cultured mesangial cells, and macrophages contained mRNA that produced a PCR product of the expected size (Figure 7). The PCR product was sequenced and compared with mouse sequence by the Blast test. This result showed that the rat sequence shared 91% homology with mouse IL-11 receptor α-chain (Figure 8).

Discussion

In this study, we have shown a beneficial effect of treatment with IL-11 on proteinuria and glomerular injury in a model of focal and segmental necrotizing glomerulonephritis. The
model we have chosen is induced in the WKY rat by a small dose of rabbit anti-rat glomerular basement membrane antiserum (26). We have previously published a detailed description of the natural history of this model (17). In that study, we found that infiltration of glomeruli by monocytes or macrophages was first detectable by 2.5 h, reaching a maximum at day 4. By day 6, there was fibrinoid necrosis in the majority of glomeruli, which led to florid crescent formation by day 10. Others have shown upregulation of IL-1β and TNF-α in glomeruli as early as 1 h after induction of disease, with levels of mRNA increasing to day 3 (27). Activated NF-κB is detectable...
from day 1 until at least day 14 (28). We studied this model for the following reasons: (1) it is a reproducible model of severe necrotizing glomerulonephritis, histologically resembling human focal and segmental necrotizing glomerulonephritis; (2) there is infiltration of glomeruli by activated macrophages and the glomerular injury is macrophage dependent (29); and (3) there is upregulation of IL-1β and TNF-α and activation of NF-κB, all of which have been reported to be downregulated by IL-11. We chose to study the acute phase of the model, in the first instance, to test the anti-inflammatory effect of IL-11.

IL-11 is a pleiotropic cytokine that was first identified in 1990. Human IL-11 cDNA encodes a protein of 199 amino acids, with a leader sequence of 21 amino acids and a molecular mass of 19 kD (reviewed in Schwertschlag et al. [7]). It is expressed in multiple tissues, including the central nervous system, lung, bone, connective tissue, skin, and kidney (6,7), but the cells responsible for its synthesis in the kidney are unknown. It is a member of a family of cytokines that use the gp130 receptor subunit for signal transduction. The other members of the family are IL-6, ciliary neurotropic factor, leukemia inhibitory factor, oncostatin M, and cardiotropin-1. IL-11 was shown to work on the induction phase of inflammation, where the mechanism of action is unclear. It has been suggested that IL-11 may work through inhibition of NF-κB, thus preventing its nuclear translocation and transcriptional activity (9). We therefore hypothesized that IL-11 would reduce glomerular inflammation in immune-mediated glomerulonephritis.

We found that IL-11 did reduce glomerular damage as assessed by proteinuria and by segmental glomerular fibrinoid necrosis in the model of nephrotoxic nephritis in the WKY rat. At the higher dose, this was associated with a reduction in glomerular macrophage infiltration, but importantly, at the lower dose, there was no reduction in macrophage infiltration, although there was still a reduction in injury. We therefore asked whether we could detect any change in macrophage activation that could account for the reduced injury. We studied macrophage expression of sialoadhesin, which is a well recognized marker of activation in tissue macrophages (22,31), and found that there was a reduction in the number of macrophages expressing sialoadhesin in the IL-11–treated animals. This is similar to the results we have previously published that used the unrelated anti-inflammatory cytokine IL-4, which, if given after the onset of macrophage infiltration, reduced injury and macrophage sialoadhesin expression without an effect on glomerular macrophage numbers (21). Because this model is dependent on CD8+ cells (26), we also quantified glomerular CD8 cells in glomeruli but found that IL-11 had no effect, excluding this as a mechanism in the reduction of injury.

Macrophages are the major effector cells in human and experimental glomerulonephritis (1). Glomerular macrophage accumulation can occur through several mechanisms, including humoral and cellular immunity (32,33). In immune complex disease, ligation of Fcγ receptors of macrophages and possibly mesangial cells appears to play a central role, with subsequent induction of adhesion molecule expression and chemokine secretion. The macrophage response can be divided in two phases: the induction phase of chemotaxis, adhesion, and migration; and the effector phase of cytokine secretion, phagocytosis, tissue modeling, and destruction. Currently, most studies in glomerulonephritis have focused on reagents that work on the induction phase of inflammation, where the reduction of glomerular injury is often accompanied by reduction of infiltrating macrophages. This has made interpretation of the role of macrophage function in glomerulonephritis difficult. To address this question clearly, in our study, we reduced the dose of IL-11 in experiment 2 and found that even with a similar number of glomerular macrophages in treated and control rats, IL-11–treated animals still had significantly reduced glomerular injury.
less proteinuria and fibrinoid necrosis. These results showed that IL-11 exerted its renal protective effect not only by reducing glomerular macrophage infiltration, but also through inactivating macrophage function.

One possible way in which IL-11 could exert its effect is by reducing the glomerular binding of rabbit or rat Ig. We excluded this possibility by performing quantitative immunofluorescence. Interestingly, however, we found that IL-11 treatment led to an increase in circulating rat anti-rabbit Ig. This is consistent with the findings of a previous study that showed that IL-11 enhanced antibody synthesis in a primary immune response in mice (34).

We also examined macrophage expression of iNOS. iNOS is expressed by macrophages in inflamed glomeruli, and isolated glomeruli synthesize NO in vitro (35–38). However, the role of NO in the inflamed glomerulus is not clear. It has been suggested that NO may have a damaging effect either directly or after conversion to peroxynitrite, or alternatively, that it may have beneficial effects by inhibiting glomerular thrombosis, adhesion molecule expression, and inflammatory cytokine production (39). IL-11 in vitro has been shown to reduce NO synthesis by LPS or IFN-γ-treated murine macrophages (8) but did not reduce iNOS mRNA levels, suggesting that the control of NO synthesis was at the translational or post-translational level (9). In experimental murine Lyme disease, iNOS mRNA was higher in the spleens of IL-11–treated mice (40). We therefore assessed the effect of IL-11 on macrophage iNOS expression in our model. By immunohistochemistry, we found that there was a significant increase in macrophages expressing iNOS. Further experiments will be necessary to determine how this is related to levels of iNOS mRNA and the synthesis of NO, which is critically dependent on substrate and cofactor availability. Our results illustrate the complex nature of the relationship between glomerular injury and iNOS expression.

To elucidate further the possible mechanism of action of IL-11 in this model, we examined the expression of the proinflammatory cytokine IL-1β. We chose to study the lower dose of IL-11 because we had established that this did not affect macrophage numbers in the glomerulus, and therefore we hoped to demonstrate an effect on macrophage activation independent of an effect on glomerular infiltration. By using the method of quantitative RT-PCR, we found that at day 2 of the model, there was a marked reduction in the levels of IL-1β mRNA in the glomeruli of the IL-11–treated animals. The importance of IL-1β in mediating glomerular inflammation has been shown in studies that used antagonists such as IL-1 receptor antagonist (41), and we think that the downregulation of IL-1β is likely to be an important mechanism by which IL-11 exerts its anti-inflammatory effect.

In a model of combined radiation therapy and chemotherapy in mice, IL-11 was able to suppress intestinal epithelial cell apoptosis and therefore increased survival (42). A similar protective effect has also been shown after thoracic irradiation (43). In our experiment, we found that glomerular apoptosis in the IL-11–treated animals was significantly reduced. Whether this is a direct effect of IL-11 or whether it reflects a reduction in glomerular inflammation remains unclear. However, in view of the known effect of TNF-α and IL-1β on apoptosis, and in view of the fact that IL-11 can reduce production of these mediators by downregulating macrophage function, one of the possible mechanisms of this effect is that IL-11 reduces glomerular apoptosis indirectly through its effect on glomerular macrophages.

PCNA is an accessory protein required for DNA synthesis and repair of DNA (44). In an in vitro study, Peterson et al. (45) showed that IL-11 had a cytoprotective effect by reducing the proliferation rate of a rat intestinal cell line and this growth inhibition effect correlated with delayed entry into S phase of the cell cycle. In this study, we also found that IL-11–treated rats had significantly lower PCNA in the glomeruli. This result indicates that IL-11 may also have a growth inhibitory effect on intraglomerular cells. Further study is necessary to clarify this effect.

Like other cytokines, to have its biologic effect on target cells, IL-11 has to interact with its receptor complex on the cellular surface. The receptor for IL-11, like other members of the IL-6 family of cytokines, is composed of a gp130 signal transduction unit together with a unique IL-11 receptor α-chain (46). Gp130 is an important signal transduction unit that is shared by many cytokines and expressed in almost every organ, including kidney (47). We therefore investigated whether rat macrophages and mesangial cells expressed IL-11 receptor α-chain. Because the rat IL-11 receptor α-chain sequence is still unknown, we used primer pairs designed from mouse IL-11 receptor α-chain sequence. The PCR results revealed that glomeruli, primary cultured mesangial cells, and peritoneal macrophages of WKY rats all possessed IL-11 receptor α-chain. The PCR product was sequenced and showed 91% homology with mouse IL-11 receptor α-chain. We think that this is the first demonstration of the rat IL-11 receptor α-chain sequence.

Because IL-11 is a member of the IL-6 cytokine family, it is important to compare its effect with other members of the family. Clearly, the effects of the different members are not identical. For example, IL-11 inhibits macrophage synthesis of IL-12, whereas IL-6 does not (10). Our group has previously studied the effect of continuous infusion of IL-6 in a model of accelerated nephrotoxic nephritis in the Lewis rats and has shown a reduction of albuminuria, glomerular macrophage infiltration, and glomerular sialoadhesin expression similar to the results we have found here with IL-11 (48). Further studies will be necessary to elucidate the relative therapeutic potential of IL-11 compared with IL-6 and other potential therapeutic anti-inflammatory cytokines such as IL-4 and IL-10. One of the possible benefits of IL-11, in addition to its role in reducing macrophage activation, is its ability to divert the immune response from a Th1 to a Th2 response (14). The possible relevance of this to glomerular inflammation will require the use of different models such as experimental autoimmune glomerulonephritis in the rat (49) and Th1-dependent crescentic nephritis in the mouse (50), and this is the subject of future experiments.

To our knowledge, this is the first study to examine the effect of IL-11 in glomerulonephritis. We found that in a model
of severe necrotizing glomerular injury, IL-11 reduced glomerular necrosis and proteinuria. At a higher dose, there was a reduction in glomerular macrophage infiltration. At a lower dose, macrophage numbers were unaffected, but there was evidence of a reduction in macrophage activation and in glomerular IL-1β expression. We think IL-11 is worthy of further investigation in the treatment of glomerulonephritis.

Acknowledgments

This work was supported by a grant from the National Kidney Research Fund (NKRF). Dr. Lai was supported by Biomedical Scholarship from Chang-Gung Memorial Hospital, Taiwan. Dr. Tam was an NKRF Senior Research Fellow. We thank Dr. H. D. Volk (Institute for Medical Immunology, Berlin, Germany) for the gift of the multi-specific rat competitor DNA fragment for quantitative PCR.

References

30. Yonemura Y, Kawakita M, Masuda T, Fujimoto K, Takatsuki K: Effect of recombinant human interleukin-11 on rat megakaryo-


