Kidney International, Vol. 44 (1993), pp. 967–973

Modulation of antibody-mediated glomerular injury *in vivo* by interleukin-6

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Modulation of antibody-mediated glomerular injury *in vivo* by interleukin-6. We have shown previously that pretreatment with small doses of bacterial lipopolysaccharide (LPS), human recombinant interleukin-1β (hrIL-1β) and human recombinant tumor necrosis factor-α (hrTNF) increase injury in the heterologous phase of nephrotoxic nephritis (NTN). All three pretreatments induce synthesis of interleukin-6 (IL-6) which in some systems down-regulates synthesis of IL-1 and TNF. We have now investigated the influence of IL-6 on injury in both heterologous and autologous phases of NTN in rats. Injection of hrIL-6 in doses sufficient to induce hepatic synthesis of acute phase proteins (assessed by plasma α2-macroglobulin concentration) had no effect on glomerular injury in the heterologous phase of NTN (albuminuria in NTAb alone 9 ± 6; LPS/NTAb 34 ± 10 and IL-6/NTAb 2 ± 1 mg/24 hr, $P < 0.001$, Wilcoxon test). In contrast, IL-6 pretreatment partially abrogated the effect of LPS on albumin excretion (NTAb 4 ± 2; LPS/NTAb 85 ± 11 and IL-6/LPS/NTAb 32 ± 6 mg/24 hr, $P < 0.002$), percentage of glomerular capillary thrombi (3 ± 1%; 39 ± 8%; and 6 ± 1%, $P < 0.001$) and glomerular neutrophil infiltrate (29 ± 3; 58 ± 5; and 34 ± 2 neutrophils/50 glomeruli in section, $P < 0.001$, respectively) at 24 hours. The effect of IL-6 was also evident four hours after induction of nephritis and was associated with a marked reduction in glomerular concentration of mRNA for IL-1β and TNF, without change in that of tubulin. Serum TNF concentrations were also significantly reduced at four hours in IL-6 treated rats. Glomerular macrophage counts were unaffected by the treatment of IL-6 at four (53 ± 1; 55 ± 3 and 64 ± 7) or 24 hours (201 ± 12; 198 ± 9 and 202 ± 9, respectively). A single injection of IL-6 also decreased albumin excretion by 42% in the autologous phase of NTN, and reduced the prevalence of glomerular capillary thrombosis by 68%. These results show that IL-6 has significant anti-inflammatory properties in this model of antibody mediated injury *in vivo*.

Interleukin-6 (IL-6) is a cytokine with a large number of different functions but whose role *in vivo* is uncertain. It is produced in substantial amounts by monocytes/macrophages [1], especially when stimulated by lipopolysaccharide (LPS), interleukin-1 (IL-1) or tumor necrosis factor-α (TNF) [2, 3]. It is also produced in smaller amounts by many other cells including neutrophils [4], endothelial [5], and mesangial cells [2]. It is a co-mitogen for T cells, and causes terminal differentiation of activated B-cells into plasma cells [6]; it also has hematopoietic effects [7], and may be important in intra- as well as intercellular signaling.

Early studies suggested that IL-6 might be pro-inflammatory like IL-1 and TNF because it shares many of their properties, including induction of fever [8], and hepatic synthesis of acute phase proteins [9]. Increased serum concentrations of IL-6 correlate with mortality rate in a variety of situations including clinical [10] and experimental endotoxemia [8, 11], and passive immunization against IL-6 has been reported to reduce mortality in this situation [12]. However, in contrast to IL-1 and TNF, IL-6 does not cause hemodynamic disturbances [13] or other toxicity even when injected in high doses [14]. It does not induce tolerance to endotoxin, nor it does influence the tolerance induced by IL-1 [15]. Furthermore, again unlike IL-1 and TNF, IL-6 does not activate endothelial cells *in vitro* [16], and does not induce endothelial cell adhesion molecules, synovial collagenase, phospholipase A2, nor nitric oxide synthase [17]. It inhibits the proliferation of bone marrow and peritoneal macrophages *in vitro* [18]. Moreover, IL-6 has been reported to down-regulate IL-1 and TNF synthesis by LPS stimulated macrophages *in vitro* [19] and to abrogate the systemic [20] and local effects [21, 22] of LPS *in vivo*.

The present study was designed to determine the effects of IL-6 pretreatment on an antibody mediated model of acute inflammation *in vivo*, using a passive model of nephrotoxic nephritis (NTN) in rats. The injury in this model is induced by injection of heterologous antibodies to the glomerular basement membrane (GBM), and is neutrophil dependent [23]. The use of this model has two advantages: first, injury can be quantified accurately, both functionally and morphometically, and related to the dose of anti-GBM antibody injected, and second, the injury can be enhanced by pretreatment with small doses of LPS, IL-1 or TNF [24], an effect largely abrogated by passive immunization against TNF, or IL-1β but not IL-1α [25]. We now show that IL-6 pretreatment does not increase injury in the way that LPS, TNF and IL-1β do, but instead inhibits the effect of LPS to the same degree as passive immunization against the cytokines. Such protection is associated with reduced steady state concentrations of mRNA for IL-1β and TNF in inflamed glomeruli, and of circulating TNF concentrations. These data demonstrate the therapeutic effect of IL-6 on glomerular injury.

Received for publication April 7, 1993
and in revised form June 9, 1993
Accepted for publication June 10, 1993

Methods

Animals

Male Sprague-Dawley rats weighing 180 to 200 g were used in all the experiments. They were housed individually in metabolism cages for 24-hour urine collections and had free access to food and water.

Reagents

Rabbit serum containing high titers of antibodies to rat GBM was prepared as previously described [25]. Immunoglobulin G (IgG) was purified from the nephrotoxic serum using a staphylococcus protein-A column [26], with special care to avoid contamination with endotoxin. The resulting IgG (anti-GBM Ab, nephrotoxic antibody, NTAb) was tested for contamination with LPS by Limulus amoebocyte lysate assay, and the final concentration was always less than 50 pg/ml of NTAb. A stock solution of 2.5 mg/ml of LPS (*Salmonella typhimurium*; Sigma Chemical Co., St. Louis, Missouri, USA) was prepared and stored at 4°C until used. Human recombinant IL-6 was prepared at the Glaxo Institute of Molecular Biology (Geneva), and found active at a concentration of 0.1 pM, as determined by the hybridoma growth activity of 7TDI cells, with a specific biological activity of 2.8×10^{10} units/mg [27]. Its endotoxin contents is less than 5 pg/ml (Limulus test). The cDNA probes for rat IL-1β and mouse TNF were provided by Dr. Alan Shaw (Biogen, Geneva). The cDNA probe for human tubulin ka1 is a 1.6 kb insert subcloned into pSP64 plasmid which was used as a control to cross hybridize to rat mRNA [28].

Induction of nephrotoxic nephritis

In the heterologous phase, nephritis was induced by a single intravenous injection of 10 mg of NTAb (nephrotoxic antibody) in 1 ml. From previous experiments this dose was known to cause minimal proteinuria in normal rats and severe nephritis in rats pretreated with an intraperitoneal injection of 0.25 μg of LPS one hour previously. In the autologous phase, nephritis was induced by a single intravenous injection of 10 mg of NTAb one week after immunizing all rats with 1 mg of normal rabbit IgG in Freund's complete adjuvant subcutaneously.

The severity of nephritis was assessed by urinary albumin excretion, the percentage of glomeruli with capillary thrombi with at least one thrombus per glomerulus, the number of neutrophils infiltrating glomeruli, using previously described methods [24], and by the total number of macrophages per 50 glomeruli in each section.

Immunohistochemical staining for glomerular macrophages

Four micron-thick frozen sections were prepared and cut from rat kidneys from different experimental groups as well as from normal rats. These were mounted on poly-L-lysine-coated glass slides, air dried for about one hour, and then fixed for 10 minutes in cold acetone. The slides were next rehydrated in PBS (phosphate-buffered saline) and treated for 30 minutes with hydrogen peroxide in PBS to block endogenous peroxidase activity and washed again with PBS. Next they were treated with a 1:50 dilution of blocking normal sheep serum for 30 minutes and then exposed to 1:50 dilution of (ED1) monoclonal mouse anti-rat macrophage antibody (Serotec, Oxford, UK) and similar dilutions of control sera. After overnight incubation in humid chamber at 4°C, the slides were rinsed and washed three times with PBS, overlaid with peroxidase-conjugated secondary polyclonal sheep anti-mouse antibody (dilution 1:100) (Amersham International, UK), and incubated one hour, followed by three additional rinses and washes with PBS. At this point, sections were overlaid with substrate DAB (3,3'-Diaminobenzedine tetrahydrochloride dihydrate, 97%; Aldrich Chemical Co.) with hydrogen peroxide in PBS and incubated for 15 minutes at room temperature to allow for color development. Harris's hematoxylin was used as a counterstain. Glomerular macrophages were counted per 50 glomeruli in each section.

Northern blot analysis

Kidney tissue was collected for mRNA studies after the lower pole of the left kidney was removed for histology while the rats under ether anesthesia. This is followed by perfusion with 50 ml of phosphate buffered saline (PBS) at room temperature and 10 ml of ice cold PBS. Glomeruli were purified from the rest of the renal tissues by a standard seiving technique (250 mm, 105 mm, 80 mm) with ice cold PBS. Purified glomeruli were immediately homogenized (Ika-ultra-Turbax T25 Janke and Kunkal at 24000 rpm) in 4 M guaninidium thiocyanate solution. Total RNA was purified from the homogenate with the cesium chloride gradient ultracentrifugation method [29]. Equal amounts of total glomerular RNA were electrophoresed on a 3.2% formaldehyde, 1 × MOP, 1% agarose gel and transferred to Genescreen plus™ (New England Nuclear, Boston, Massachusetts, USA), by capillary blot using 10 × SSC solution (1 × SSC = 15 mM trisodium citrate, 150 mM sodium chloride). Total RNA was extracted from a rat spleen four hours following an intraperitoneal injection of 0.25 μg of LPS, and from LPS-stimulated cultured bone marrow macrophages which both were used as positive controls for Northern hybridization. Filters were pre-hybridized in 50% formamide, 1% SDS, 10% dextran sulphate, 1 M NaCl and 200 mg/ml denature salmon testes DNA (Sigma) overnight at 42°C. Complementary DNA probes were radiolabeled with ^{32}P-CTP with Klenow DNA polymerase (Sambrook, Fritsch, Maniatis). Filters were hybridized in the same pre-hybridization buffer at 42°C with radiolabeled probe overnight. They were washed twice in 1% SDS + 1 × SSC solution at 42°C for 30 minutes, and twice in 1% SDS + 0.1 × SSC solution for another 30 minutes at the same temperature. Some filters were further washed in 1% SDS + 0.01 to 0.1 × SSC solution to reduce background radioactivity. Washed filters were exposed to X-romat Kodak film with intensifier screen at room temperature or −70°C for various durations up to 14 days. Multiple durations of exposure were made, so that optimally exposed film was available for densitometry. Filters were stripped of radioactivity by washing in boiling 0.1% SDS and 0.1 × SSC solution before the next hybridization. The degree of hybridization was assessed by densitometric scanning (Chromoscan 3, Joye Loebl) of suitable autoradiographs. Any difference in RNA loading in each lane was corrected by normalization to the tubulin hybridization.

Measurement of serum TNF

Five hundred microliters of blood were drawn off at each time point. Blood samples were transferred to endotoxin free tubes,

Table 1. Effect of a single intravenous injection of hrIL-6, compared with that of LPS and NS, on plasma alpha 2-macroglobulin concentration and creatinine clearance

Treatment	Number of rats	Alpha 2-macroglobulin *μg/ml*				Creatinine clearance *ml/min*
		2 hr	4 hr	8 hr	24 hr	24 hr
HrIL-6	6	698 ± 19	603 ± 145	1074 ± 102	1865 ± 183[a]	0.36 ± 0.04
LPS	3	556 ± 29	463 ± 27	1176 ± 361	2177 ± 409[a]	0.40 ± 0.10
NS	3	278 ± 27	211 ± 39	216 ± 49	298 ± 2	0.38 ± 0.04

Plasma alpha 2-macroglobulin and creatinine clearance at the start of the experiment were 180 ± 15 μg/ml and 0.47 ± 0.10 ml/min, respectively. There is no significant difference in creatinine clearance values between all treatments.
[a] $P < 0.001$ compared with other values at different times

incubated for 20 minutes at 37°C for blood to clot, and centrifuged at 1500 rpm for 15 minutes. The serum was removed and stored at −20°C until TNF was measured by WEHI 164 clone 13/2F2 bioassay [30].

Assessment of the hepatic acute phase response

The hepatic acute phase response was assessed functionally by measurement of plasma alpha 2-macroglobulin (α_2m) concentration as previously described [25].

Creatinine clearance

Both plasma and urine were assayed for creatinine concentrations, using a standard colorimetric assay (Beckman autoanalyzer) which is specific for creatinine and subject to minimal interference from endogenous noncreatinine chromogens, and endogenous creatinine clearance (C_{Cr}) was calculated in all rats.

Statistical analysis

The data are presented as means together with standard errors (SE). The probabilities that differences between the groups were significant were calculated using Wilcoxon rank sum test.

Results

Effect of a single intravenous injection of IL-6

A dose of 4 μg of IL-6 was chosen because of its systemic effect and ability to induce hepatic acute phase response in rats [31]. IL-6 was administered intravenously because it is more potent than other routes [9], and to avoid any possible local effect, especially when given before the intraperitoneal injection of LPS. Six rats were injected with IL-6 in 1 ml of pyrogen-free normal saline (NS), and three with 1 ml of NS alone. Another three rats were injected intraperitoneally with 0.25 μg LPS in 1 ml of NS as a positive control. Neither IL-6 nor LPS in these doses caused any apparent clinical disturbance. Specifically, all the rats remained active and none developed ruffling of fur, diarrhea or bloody nasal discharge. However, both reagents caused comparable hepatic synthesis of acute phase proteins as assessed by plasma concentrations of α_2m which increased from 180 ± 15 to a maximum of 1865 ± 183 μg/ml at 24 hours in the case of IL-6 and to 2177 ± 409 μg/ml with LPS ($P < 0.001$ Wilcoxon; Table 1). Neither treatment caused albuminuria or a significant change in endogenous creatinine clearance. These results demonstrate that the preparation of human recombinant IL-6 is active in rats.

Effect of IL-6 on glomerular injury in the heterologous phase of NTN

Nephritis was induced by a single intravenous injection of 10 mg NTAb in 1 ml of PBS. One hour before the induction of nephritis group 1 ($N = 9$) was injected intravenously with 0.5 ml NS, group 2 ($N = 5$) with 0.25 μg LPS in 0.5 ml of NS, and group 3 ($N = 8$) with 4 μg IL-6 in 0.5 ml of NS. The results are shown in Table 2. As in our previous studies, LPS pretreatment significantly increased the antibody mediated injury [24, 25]. The mean albumin excretion increased from 9 ± 6 to 34 ± 10 mg/24 hr ($P < 0.05$) in LPS treated rats, and the prevalence of glomerular thrombosis from 0 ± 0 to 46 ± 9% ($P < 0.001$). The glomerular neutrophil infiltrate was similarly increased by LPS pretreatment, being 37 ± 11 and 88 ± 15 neutrophils per 50 glomeruli in each section ($P < 0.001$) for group 1 and 2 rats, respectively. By contrast, pretreatment with IL-6 had no detectable effect on glomerular injury when given one hour before NTAb (Table 2). Because of this, we assessed the effect of IL-6 given at the same time with NTAb, and again it did not enhance the injury (albuminuria 1 ± 0.4 mg/24 hr). Neither treatment effected the creatinine clearance, but both increased plasma concentration of α_2m significantly. These results show that IL-6, unlike IL-1 and TNF [24], does not increase injury in this model, and so we examined whether IL-6 influences the severity of the LPS effect.

Pretreatment effect of IL-6 on LPS enhanced glomerular injury in the heterologous phase of NTN

Six rats were intravenously injected with 0.5 ml NS, followed 30 minutes later by 1 ml NS intraperitoneally and one hour later by 10 mg NTAb intravenously. Eleven rats were given 0.5 ml NS, 0.25 μg (1 ml) LPS, and 10 mg NTAb using the same times and routes, and fifteen rats were similarly treated except that 4 μg IL-6 were injected intravenously before LPS and NTAb instead of NS. Pretreatment with LPS again caused a significant increase in glomerular injury and this was partially abrogated by pretreatment with IL-6, which caused a highly significant 60% reduction in albuminuria ($P < 0.002$, compared to nephritic rats preinjected with LPS, Table 2). There was a comparable reduction in the incidence of glomerular capillary thrombi, and the glomerular neutrophil infiltrate (Table 2). However, neither LPS nor IL-6 affected the macrophage infiltration of nephritic glomeruli at either time point. All the rats developed a vigorous acute phase response but α_2m was significantly higher in those injected with either LPS or IL-6; however, there was no

Table 2. Pretreatment effect of hrIL-6 on the heterologous phase of nephrotoxic nephritis in rats

Treatment	Time *hours*	Number of rats	Albuminuria *mg/24 hr and mg/4 hr*	C_{Cr} *ml/min*	Alpha 2-m *μg/ml*	GCT %	Neutrophils/ 50 glomeruli in section	Macrophages/ 50 glomeruli in section
NTAb	24	9	9 ± 6	0.36 ± 0.1	1221 ± 118	0 ± 0	37 ± 11	ND
LPS + NTAb	24	5	34 ± 10 [b]	0.44 ± 0.1	4071 ± 268 [b]	46 ± 9 [b]	88 ± 15 [b]	ND
hrIL-6 + NTAb	24	8	2 ± 1	0.34 ± 0.03	2509 ± 773	0 ± 0	31 ± 4	ND
NTAb	24	6	4 ± 2	0.42 ± 0.1	2019 ± 445 [a]	3 ± 1	29 ± 3	201 ± 12
LPS + NTAb	24	11	85 ± 11 [a]	0.38 ± 0.1	8039 ± 751	39 ± 8 [b]	58 ± 5 [b]	198 ± 9
hrIL-6 + LPS + NTAb	24	15	32 ± 6	0.33 ± 0.03	9991 ± 285	6 ± 1	34 ± 2	202 ± 9
NTAb	4	4	1 ± 0.3	ND	ND	0 ± 0	87 ± 6	53 ± 1
LPS + NTAb	4	4	51 ± 15 [b]	ND	ND	76 ± 7 [b]	177 ± 23 [b]	55 ± 3
hrIL-6 + LPS + NTAb	4	4	3 ± 1	ND	ND	16 ± 6	72 ± 20	64 ± 7

In normal rats ($N = 8$), the total number of macrophages is 35 ± 1 per 50 glomeruli in section. This is unchanged in rats ($N = 8$) treated with hrIL-6 alone (33 ± 2). There is no significant difference in the number of macrophages between all treatments. There is also no significant difference in the C_{Cr} (creatinine clearance) values between all treatments. Rats were killed either at 24 or 4 hours and albumin excretion was measured accordingly. Abbreviations are: HrIL-6, human recombinant interleukin-6; NTAb, nephrotoxic antibody (anti-GBM antibody); LPS, bacterial lipopolysaccharide; and alpha 2m, alpha 2-macroglobulin; ND, not done.

[a] $P < 0.002$ and [b] $P < 0.001$

difference between these two groups (Table 2). Thus IL-6 partially abrogated the enhanced injury provoked by LPS, and so we examined its effect on IL-1 and TNF production.

Effect of IL-6 on IL-1β and TNF synthesis

Separate groups of four rats were studied to assess the effect of IL-6 pretreatment on systemic TNF concentration and on glomerular TNF and IL-1β gene expression in these models. The rats were killed four hours after induction of nephritis when the glomerular neutrophil infiltrate was maximal. Even at this stage albuminuria, glomerular capillary thrombi and neutrophil infiltrate were significantly less in IL-6 pretreated group (Table 2). IL-1β and TNF mRNA were not detected by Northern analysis in glomeruli removed from normal rats, but were easily detected four hours after the induction of nephritis. The degree of hybridization was substantially increased after LPS pretreatment and this effect was markedly attenuated by IL-6 (Fig. 1A) without change in that of tubulin (Fig. 1B). TNF was not detected in serum of any of the rats at the start of the experiment or in rats given NTAb alone (<5 RU/ml). However, rats injected with LPS one hour before induction of nephritis had a substantial increase in serum TNF to 538 ± 182 RU/ml which was significantly less in the IL-6 treated group (148 ± 66 RU/ml, $P < 0.05$). Thus, glomerular expression of IL-1β and TNF genes was reduced by IL-6, as was systemic TNF synthesis.

Effect of IL-6 in the autologous phase of NTN

The results in the heterologous phase of NTN prompted us to examine the effect of IL-6 on glomerular injury in the autologous phase of NTN. Twenty-one rats were immunized subcutaneously with 1 mg of normal rabbit IgG in Freund's complete adjuvent. One week later they were randomized into two groups and nephritis was induced by a single intravenous injection of 10 mg of NTAb. One hour before the induction of nephritis one group ($N = 11$) was pretreated with a single intravenous injection of 4 μg IL-6 diluted in 0.5 ml NS and a second group with 0.5 ml NS. All the rats were killed at 72 hours and urinary albumin excretion (mg/24 hr) was monitored at 24, 48, and 72 hours after the induction of nephritis. A single intravenous injection of IL-6 (4 μg) had 42% less in albumin excretion at day 1 (from 606 ± 108 mg/24 hr to 356 ± 73 mg/24 hr) and 68% fewer in glomerular thrombi at day 3 (from 41 ± 7% to 28 ± 6%). However, there was no significant difference in glomerular cellular influx of either neutrophils or macrophages when the rats were killed at 72 hours (Table 3). TNF was not detected in serum of any of the rats at any of time points over the first 72 hours. The creatinine clearance at the start of the experiment was 0.60 ± 0.12 ml/min which dropped significantly after the induction of nephritis to 0.31 ± 0.04 ml/min ($P < 0.05$), but was not statistically different from the group pretreated with IL-6 (0.36 ± 0.01 ml/min, Table 3). Similarly, there was no significant difference in plasma α_2m concentration between both groups.

Discussion

Relations between TNF, IL-1, and IL-6 are complicated. IL-1 and TNF stimulate many cell types to synthesize large amounts of IL-6; these include neutrophils, monocytes, and glomerular mesangial cells. Conversly, IL-6 down-regulates IL-1 and TNF synthesis by monocytes [19] and macrophages [22] exposed to LPS. A similar phenomenon has been reported *in vivo* in rodents injected with LPS, in whom pretreatment with IL-6 reduces circulating TNF concentrations, and IL-1β gene expression [20–22]. Whether this is a direct effect on macrophages or works indirectly, for example through the pitutary adreno-cortical axis [32, 33], has not been ascertained. The situation *in vivo* is more complex because not all effects of IL-1 and TNF are opposed by IL-6, and some may even be enhanced by it [34, 35]. It appears in some systems that the timing of endogenous IL-6 release or exogenous IL-6 administration is crucial in determining whether IL-6 antagonizes or synergizes with IL-1 [34].

The results reported here show that pretreatment with IL-6, unlike LPS, IL-1β, and TNF, does not exacerbate the glomerular injury but instead largely abrogates the IL-1 and TNF dependent effect of LPS in exacerbating injury in this model. This protection is associated with a significant reduction in serum TNF concentration and of glomerular expression of

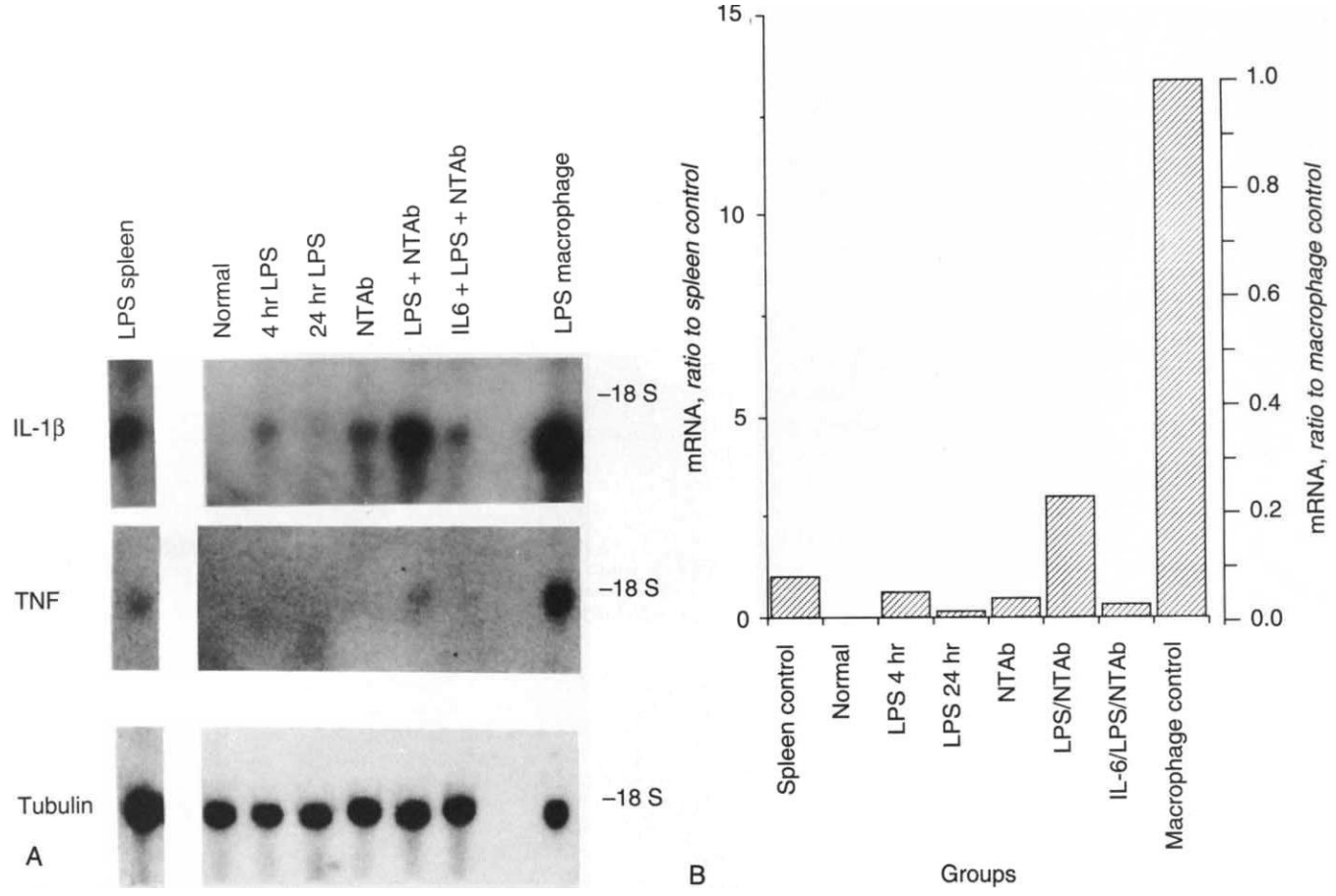

Fig. 1A. *Northern blotting analysis of glomerular mRNA for IL-1β and TNF.* Northern blotting analysis of glomerular RNA showing the same blot sequentially probed for IL-1β, TNF, and tubulin. Samples include glomerular RNA from normal rats killed at 4 hours, rats injected with 0.25 μg LPS one killed at four hours and another at 24 hours, and rats injected with NTAb, LPS/NTAb, and IL-6/LPS/NTAb. Splenic RNA from rats injected with LPS and from bone marrow macrophages stimulated with LPS were the positive control for hybridization. **B.** Densitometric analysis of glomerular IL-1β mRNA concentration from Northern blots. Results were expressed as ratios to positive controls; (1) splenic RNA from an LPS injected rat (left Y axis) and (2) RNA from LPS stimulated bone marrow macrophages (right axis).

Table 3. Pretreatment effect of hrIL-6 on the autologous phase of nephrotoxic nephritis in rats

Treatment	Number of rats	Albuminuria *mg/24 hr* Day 1	Day 2	Day 3	C_{Cr} *ml/min*	α_2m *μg/ml*	GCT %	Neutrophils/ 50 glomeruli	Macrophages/ 50 glomeruli
NTAb	10	606 ± 108	598 ± 115	812 ± 137	0.31 ± 0.04	11298 ± 3711	41 ± 7	61 ± 9	353 ± 11
IL-6 + NTAb	11	356 ± 73	381 ± 65	602 ± 88	0.36 ± 0.01	9135 ± 2327	28 ± 6	64 ± 8	359 ± 16

All rats were killed at 72 hours following induction of nephritis. Pretreatment with IL-6 caused a 42% drop in albumin excretion and 68% in glomerular thrombosis (GCT). However, there was no significant difference in glomerular cellular infiltrate at 72 hours. The creatinine clearance (C_{Cr}) at the start of the experiment was 0.60 ± 0.12 ml/min which significantly dropped following induction of nephritis ($P < 0.05$). However, pretreatment with IL-6 had no effect ($P > 0.05$). Similarly, there was no significant change in the alpha 2-macroglobulin (α_2m) values between both groups. Abbreviation is NTAb, nephrotoxic antibody (anti-glomerular basement membrane antibody).

IL-1β and TNF genes, which confirms the ability of IL-6 to down-regulate these cytokines *in vivo*.

Glomerular inflammation in this model results directly from binding of antibodies to the GBM, which causes transient neutrophil dependent injury. Neutrophil infiltration of glomeruli is maximal between four and six hours, and is accompanied by monocyte infiltration which persists for longer, even after injury has subsided. It is notable that pretreatment with IL-6 significantly reduced glomerular neutrophil infiltration. This effect has striking parallels with results from two models of acute lung inflammation [21, 22] in which injury and neutrophil infiltration were inhibited by IL-6, without effect on the number of glomerular ED1 positive macrophages in this or other model of acute inflammation [22]. They are also in agreement with recent experiments in which neutrophils but not macrophage infiltration was inhibited by IL-6 in an LPS induced model of peritonitis in rats (Karkar, Tam, and Rees, manuscript in preparation). Thus, the differential effect of IL-6 on neutrophil and macrophage migration is a general phenomenon, but whether this is due to differences in expression of adhesion molecules by

leukocytes or endothelial cells or to the synthesis of chemotactic cytokines is not known.

Partial abrogation of glomerular injury caused by IL-6 could be explained by its direct effect on LPS induced TNF and IL-1β synthesis [19]. It is clear from the measurement of circulating TNF concentrations that IL-6 pretreatment caused a systemic reduction in TNF synthesis, and we have shown previously that passive immunization against this cytokine or IL-1β abrogates the glomerular injury [25]. IL-6 pretreatment also decreased glomerular concentrations of specific mRNA for IL-1β and TNF which are synthesized in inflamed glomeruli experimentally [36, 37] and in humans [38], and have been shown to influence injury [24]. Glomerular mesangial cells [39, 40] and infiltrating leukocytes [37, 41] from rats are both capable of releasing IL-1 and TNF, but the source of specific glomerular mRNA in our experiments has not been ascertained. Furthermore, it is not possible to say whether the reduced concentrations of specific mRNA (and by inference reduced concentrations of IL-1β and TNF) are responsible for reduced injury, or are merely a consequence of fewer inflammatory leukocytes within the glomerulus.

Another theoretical possibility is that the effect of IL-6 was due to stimulation of hepatic synthesis of acute phase proteins [9], including proteinase inhibitors with anti-inflammatory effects. It has been shown that α_2m and α1-proteinase inhibitor, purified from inflamed rat serum, suppresses rat neutrophil chemotaxis *in vitro* [42], and local and systemic injections of α_2m suppress edema and neutrophil infiltrate in carrageenin-induced inflammation in rats [43]. Deficiency of α1-proteinase inhibitor is associated with glomerulonephritis and emphysema [44], and synthetic inhibitors of cysteine and serine proteinases decrease proteinuria in experimental models of nephritis [45]. However, the protective effect of IL-6 in the present study is unlikely to be explained by increased α_2m synthesis as there was little difference in α_2m concentrations between the LPS and IL-6 pretreated groups.

The demonstration that IL-6 can reduce acute glomerular inflammation is pertinent because both rat [2, 46] and human mesangial cells [47, 48] in culture produce copious amounts of IL-6 when incubated with IL-1 and TNF, and IL-6 can be detected in glomeruli and urine of patients with glomerulonephritis [47]. Until now these findings have been interpreted as suggesting a proinflammatory role in these circumstances, but it is possible that local synthesis of IL-6 by mesangial cells helps to down-regulate glomerular injury. However, the situation is complicated because administration of IL-6 and other agents such as IL-1ra and soluble IL-1 receptors that reduce glomerular injury in nephrotoxic nephritis also reduce glomerular expression of IL-6 (Karkar, Tam, and Rees, manuscript in preparation). IL-6 has also been reported to induce proliferation of rat mesangial cells *in vitro* [46, 47], but this is controversial [48, 49]. Our findings in the autologous phase injury suggest that IL-6 may be capable of modulating injury in an active model of nephritis, but definitive studies using infusions of IL-6 have yet to be performed.

In conclusion, we have shown that manipulation of the cytokine network by IL-6 can attenuate antibody-mediated glomerular injury, and it seems likely that our results could be extrapolated to other forms of immunologically mediated inflammation.

Acknowledgments

Part of this work has been published in an abstract form in the American Society of Nephrology, November, 1992. This study was supported by grants from the United Kingdom Medical Research Council.

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Appendix. Abbreviations

NTN, nephrotoxic nephritis; GBM, glomerular basement membrane; NTAb, nephrotoxic antibody; hrIL-6, human recombinant interleukin-6; IL-1, interleukin-1; IL-1ra, interleukin-1 receptor antagonist; TNF, tumor necrosis factor; LPS, lipopolysaccharide; α_2m, alpha 2-macroglobulin.

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