

Interleukin-4 ameliorates crescentic glomerulonephritis in Wistar Kyoto rats

H. TERENCE COOK, SHARON J. SINGH, DAVID E. WEMBRIDGE, JENNIFER SMITH, FREDERICK W.K. TAM, and CHARLES D. PUSEY

Departments of Histopathology and Medicine, Imperial College School of Medicine, Hammersmith Hospital, London, England, United Kingdom

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Background. Activated macrophages play a central role in crescentic glomerulonephritis. Interleukin-4 (IL-4) down-regulates many macrophage proinflammatory activities. We therefore studied the effect of IL-4 on glomerular injury in a model of crescentic glomerulonephritis in the Wistar Kyoto rat.

Methods. Glomerulonephritis was induced by i.v. administration of rabbit antirat glomerular basement membrane antiserum (nephrotoxic serum, NTS). In experiment 1, IL-4 was given from two hours before NTS until day 6. In experiment 2, rats were treated from day 0 to 7 and were then monitored until killed on day 28. In experiment 3, IL-4 was given from day 4 to 7.

Results. Continuous IL-4 treatment (experiment 1) significantly ($P = 0.001$) reduced proteinuria (3 ± 1 mg per 24 hr vs. 56 ± 7), fibrinoid necrosis (0.06 ± 0.04 quadrants/glomulus vs. 1.2 ± 0.1), macrophage infiltration (6.7 ± 2.6 cells/glom vs. 33 ± 2.5), CD8+ cells (1.5 ± 0.6 cells/glom vs. 6.2 ± 1.1), inducible nitric oxide synthase positive cells (0.04 ± 0.04 cells/glom vs. 3.7 ± 0.6), proliferating cell nuclear antigen positive cells (3.2 ± 1 cells/glom vs. 15 ± 2.3), and glomerular intercellular adhesion molecule-1 expression. Follow-up after seven days of treatment (experiment 2) showed that at four weeks, creatinine clearance was higher in treated rats (1.1 ± 0.1 ml/min vs. 0.4 ± 0.1 , $P = 0.011$), and both glomerular scarring ($P = 0.006$) and tubular atrophy ($P = 0.006$) were less. Delayed treatment (experiment 3) reduced proteinuria (41 ± 5 mg per 24 hr vs. 97 ± 9 , $P = 0.004$) and fibrinoid necrosis (0.39 ± 0.05 quadrants/glom vs. 1.6 ± 0.1 , $P = 0.004$). There was no difference in macrophage infiltration, but inducible nitric oxide synthase positive cells were reduced (0.6 ± 0.1 cells/glom vs. 1.8 ± 0.4 , $P = 0.01$) as were ED3+ cells (0.18 ± 0.06 cells/glom vs. 1.86 ± 0.21 , $P = 0.004$).

Conclusion. In this model of crescentic glomerulonephritis, early IL-4 treatment abolished proteinuria and markedly reduced glomerular inflammation. If treatment was stopped after seven days, there was continuing benefit on glomerular and

tubulointerstitial scarring and creatinine clearance at four weeks. If treatment was delayed until inflammation was established, there was still a reduction of injury, but without an alteration of macrophage numbers, suggesting that IL-4 may be acting, in part, to reduce macrophage activation.

Crescentic glomerulonephritis is an important cause of end-stage renal failure. An understanding of its pathogenesis is still incomplete, and current treatment is non-specific and has a high risk of adverse effects. Experimental models of crescentic nephritis in rats and rabbits have provided considerable insight into the mechanisms by which crescents form [1]. Crescent formation results from severe damage to the glomerular capillary wall with disruption of the basement membrane. Macrophages play a critical role in causing this damage [2], in part by the release of proteolytic enzymes. Once the integrity of the basement membrane is disrupted, large plasma proteins (including fibrinogen) and leukocytes pass into Bowman's space, and the clotting system becomes activated, allowing the deposition of fibrin. Activation of the coagulation pathway is predominantly due to tissue factor [3], and activated macrophages are a major source of this activity [4, 5]. There is then accumulation of cells within Bowman's space because of further recruitment of monocytes/macrophages and proliferation of macrophages and glomerular epithelial cells.

When monocytes arrive in the inflamed glomerulus, they rapidly become activated and develop a proinflammatory phenotype. This has been shown by both functional studies, which demonstrate increased secretion of reactive oxygen species [6, 7], tissue factor [5], an altered pattern of eicosanoid synthesis [7], and by immunohistochemical studies showing increased expression of class II histocompatibility antigens [7], inducible nitric oxide synthase (iNOS) [8], and sialoadhesin [9]. A major stimulus to macrophage activation is likely to be the intraglomerular release of proinflammatory cytokines such as interleukin (IL)-1 β and tumor necrosis factor (TNF) [10].

Key words: macrophage activation, inflammation, glomerular injury, proteinuria, tubulointerstitial scarring.

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Thus, cytokines that reduce macrophage activation would be predicted to reduce glomerular damage in the acute phase of crescentic glomerulonephritis, and therefore, we have examined the effect of IL-4.

Interleukin-4 is a 20 kDa immunoregulatory cytokine that is secreted by T cells, mast cells, basophils, and a subset of natural killer cells [11]. It is produced by T helper 2 (Th2) CD4⁺ T cells and facilitates the development of a Th2 phenotype. *In vitro*, it inhibits interferon (IFN)- γ production by activated T cells [12]. IL-4 inhibits many functions of activated macrophages, including the secretion of reactive oxygen intermediates [13] and nitric oxide [14], and the expression of tissue factor [15] and macrophage colony stimulating factor [16]. It suppresses macrophage TNF- α and IL-1 β [17] production and up-regulates expression of IL-1 receptor antagonist [18]. It stimulates macrophage 15-lipoxygenase activity, which may reduce synthesis of the proinflammatory leukotriene B4 [19]. IL-4 also decreases monocyte expression of all three classes of Fc receptor for IgG [20].

We have recently shown that IL-4 ameliorated glomerular injury in a model of accelerated nephrotoxic nephritis in Sprague-Dawley rats when given for four days starting before the injection of nephrotoxic serum [21]. We have now examined the effect of IL-4 on a model of more severe crescentic glomerulonephritis in the Wistar Kyoto (WKY) rat. We have studied the effect of (a) continuous treatment given for six days, beginning just before induction of nephritis; (b) the long-term effect of a short course of IL-4; and (c) the effect of delaying IL-4 treatment until the fourth day, by which time there is a marked glomerular macrophage infiltrate.

METHODS

Reagents

Nephrotoxic serum (NTS), a rabbit antiserum to rat glomerular basement membrane, was prepared as described previously [22]. Recombinant rat IL-4 was produced as cell culture supernatant from a Chinese hamster ovary (CHO)-K1 cell line transfected with rat IL-4 cDNA (a gift from Dr. D. Fowell, MRC Immunology Unit, Oxford, UK). The concentration of IL-4 was measured by enzyme-linked immunosorbent assay using the Biosource rat IL-4 Immunoassay Kit (Lifescreeen, Watford, UK). Two batches of supernatant were used in these experiments. The first batch, used in experiment 1, contained 5.1 $\mu\text{g/ml}$ IL-4, and the second batch, used in experiments 2 and 3, contained 9.6 $\mu\text{g/ml}$ IL-4. Control rats received cell culture supernatant from the parent CHO-K1 cell line.

Nephrotoxic nephritis

Male WKY rats weighing 200 to 220 g were given 0.1 ml of NTS intravenously. Urine was collected by housing the rats in metabolic cages for 24-hour periods, with free

access to food and water. Rats were killed under isoflurane anesthesia, and blood was collected from the abdominal aorta. Samples of kidney, liver, lung, and spleen were placed in 10% formal saline, and samples of kidney were snap frozen in optimal cutting temperature (OCT) compound using isopentane cooled in liquid nitrogen. Urinary protein was measured by the sulfosalicylic acid method [23]. Urinary and serum creatinine concentrations were measured by the Jaffe method on an autoanalyzer.

Experimental design

Three separate experiments were performed.

Experiment 1. Rats were treated with intraperitoneal (i.p.) IL-4 0.25 ml ($N = 8$) or 0.5 ml ($N = 8$) twice daily for six days. Control rats were given i.p. control supernatant 0.5 ml twice daily ($N = 8$). The first injections were given two hours before induction of nephritis. Rats were placed in metabolic cages from day 5 to day 6 and were killed on day 6.

Experiment 2. Rats were treated with 0.5 ml IL-4 i.p. twice daily ($N = 6$) or 0.5 ml control supernatant ($N = 6$). The first injections were given two hours before induction of nephritis, and treatment was continued for seven days. The rats were then followed with weekly 24-hour urinary collections until four weeks, when they were killed.

Experiment 3. Rats were given 0.5 ml IL-4 i.p. twice daily ($N = 6$) or control supernatant ($N = 6$) starting on the fourth day after the induction of nephritis. Rats were placed in metabolic cages from day 6 to day 7 and were killed on day 7.

Histology and immunohistochemistry

In experiments 1 and 3, glomerular fibrinoid necrosis was assessed in hematoxylin and eosin (HE)-stained sections and was quantitated by scoring the number of quadrants of each glomerulus involved. At least 25 glomeruli were counted, and a mean score of quadrants/glomerulus was calculated. In experiment 2, glomerular scarring and tubular damage were assessed in HE-stained sections. Glomerular scarring was assessed by counting the percentage of glomeruli containing fibrous or fibrocellular crescents. Tubular damage was assessed semiquantitatively by estimating the percentage of tubules showing atrophy or dedifferentiation and converting this to a score from 0 to 4. Apoptotic cells were counted in HE-stained sections. A cell was considered apoptotic when it showed chromatin condensation along the nuclear membrane with intensely basophilic staining and/or nuclear fragmentation into spherical structures containing condensed chromatin [24]. All analyses were carried out on randomized sections.

Frozen sections were stained by direct immunofluorescence for rabbit immunoglobulin using fluorescein isothiocyanate (FITC)-conjugated swine antirabbit immunoglobulin (Nordic Immunology, Tilburg, The Neth-

erlands), for rat immunoglobulin using FITC-conjugated rabbit antirat immunoglobulin (Dako, Cambridge, UK), and for fibrinogen using FITC-conjugated goat antirat fibrinogen (ICN, Thame, UK). Intercellular adhesion molecule-1 (ICAM-1) expression was detected in frozen sections by staining with mouse antirat ICAM-1 (R&D Systems, Abingdon, UK) followed by FITC-conjugated rabbit antimouse immunoglobulin (Dako). Paraffin sections were used for immunohistochemistry for macrophages (mAb ED1; Serotec, Oxford, UK), macrophage sialoadhesin (mAb ED3; Serotec), inducible nitric oxide synthase (iNOS; mouse anti-iNOS mAb; Affiniti, Exeter, UK), and proliferating cell nuclear antigen (PCNA, mAb PC10; Dako). The primary antibodies were used at the following concentrations: ED1, 1:500; ED3, 1:200; iNOS, 1:100; and PCNA, 1:50. ED1 was detected with an indirect peroxidase technique using peroxidase-labeled rabbit antimouse immunoglobulin (Dako), and ED3 and iNOS were detected using biotinylated rabbit antimouse antibody (Dako) and streptavidin-biotin-peroxidase complexes (Dako). CD8-positive cells were identified in frozen sections by staining with mAb MRC OX8 (Serotec) using the streptavidin-biotin-peroxidase technique. Cells were counted in randomized sections. Where possible, at least 25 glomeruli were counted per section.

For quantitation of fluorescent staining, images were captured using a color CoolView camera (Photonic Science, Robertsbridge, UK) and were analyzed using Image-Pro plus software (Media Cybernetics, Silver Spring, MA, USA). Images were converted to gray scale, and the average pixel intensity of individual glomeruli was recorded. Results are shown as arbitrary units of intensity.

Statistics

Results are presented as mean \pm SE. Comparisons between treatments were by Mann-Whitney *U*-test.

RESULTS

Experiment 1

All rats survived until the end of the experiment. The rats treated with control supernatant developed proteinuria with prominent glomerular fibrinoid necrosis and glomerular macrophage infiltration (Figs. 1 and 2). Treatment with either dose of IL-4 (0.25 ml or 0.5 ml) completely abolished proteinuria (Table 1). Macrophage infiltration, fibrinoid necrosis, CD8+ cells, iNOS-positive glomerular cells, and glomerular PCNA staining were all significantly reduced by IL-4 in a dose-dependent manner (Figs. 1 and 2; Table 1). Glomerular ICAM-1 staining was significantly reduced by the higher dose of IL-4. After seven days of treatment, the rats treated with IL-4 showed small amounts of ascites. Histology of the liver showed drop out of hepatocytes in acinar zone 3 with hepatocyte apoptosis (Fig. 3A).

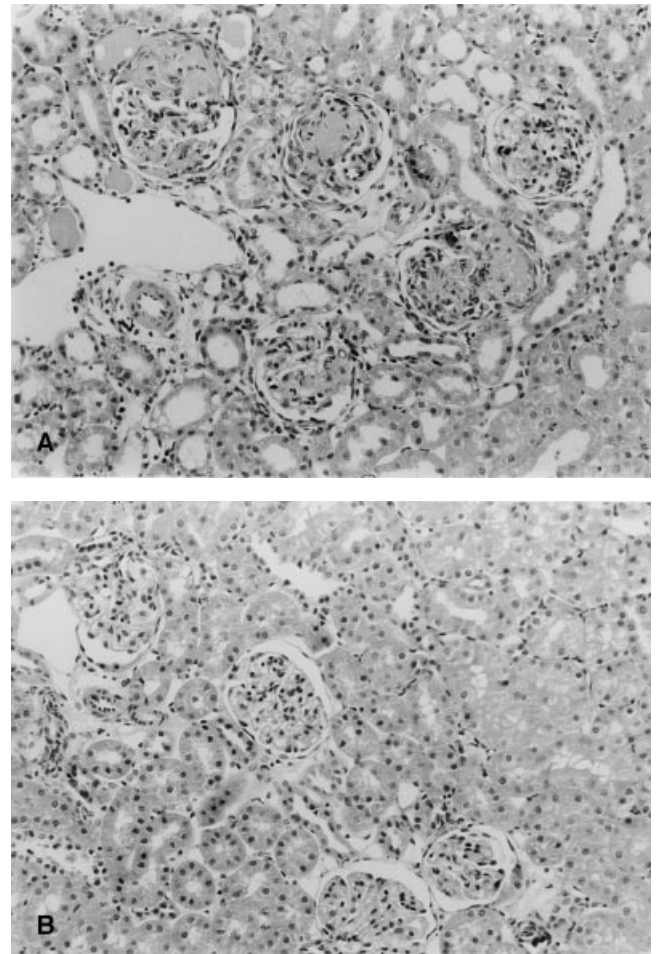


Fig. 1. Renal histology six days after the induction of nephritis in experiment 1. (A) Control. Most of the glomeruli show prominent segmental fibrinoid necrosis. (B) Interleukin-4 (IL-4) treated (0.5 ml b.i.d.). The glomeruli show slight hypercellularity but no fibrinoid necrosis [hematoxylin and eosin (HE) stain, $\times 150$].

Experiment 2

One rat in the IL-4-treated group died at 12 days. As in experiment 1, there was abolition of proteinuria at one week in the IL-4-treated animals (Fig. 4). However, by week 4, the level of proteinuria was the same in the two groups. In the IL-4-treated group, the creatinine clearance at four weeks (1.1 ± 0.1 ml/min) did not differ from that of normal rats, whereas in the control group, it was significantly reduced (0.4 ± 0.1 /min; $U = 1$, $P = 0.011$). Macroscopically, the kidneys of the IL-4-treated rats appeared normal, whereas those of the control rats showed prominent fine granularity of the surface. There was a significant reduction in the percentage of glomeruli showing fibrous or fibrocellular crescents (control, $93 \pm 2\%$; IL-4 treated, $41 \pm 14\%$; $U = 0$, $P = 0.006$) and in the score for tubular damage (control, 3.7 ± 0.2 ; IL-4 treated, 1.0 ± 0.3 ; $U = 0$, $P = 0.006$; Fig. 5). The numbers of glomerular macrophages were increased in the IL-4-

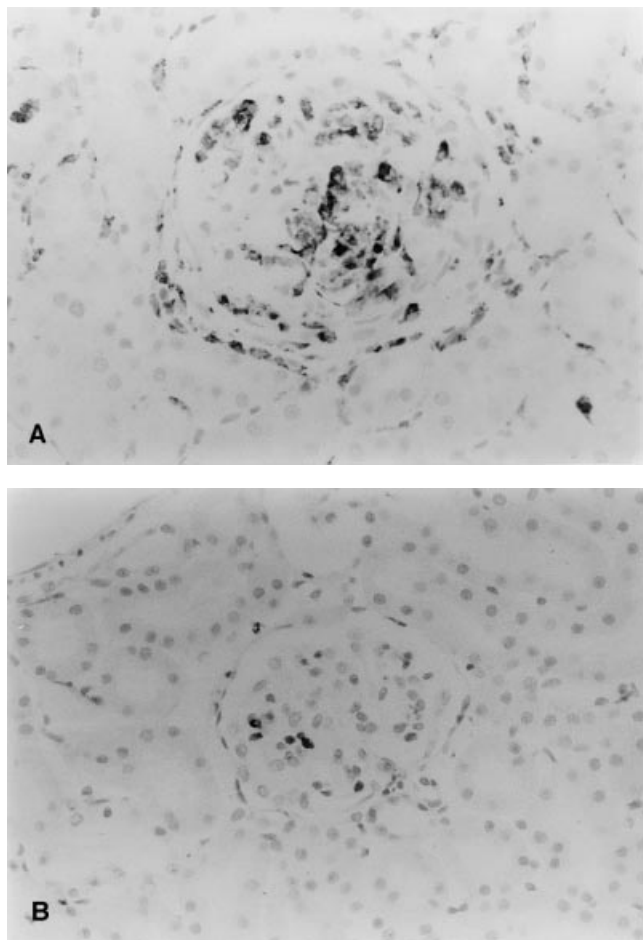


Fig. 2. Macrophage infiltration demonstrated by immunohistochemistry with monoclonal antibody ED1 in experiment 1, six days after induction of nephritis. (A) Control. Many ED1+ macrophages are present in the glomerular tuft. (B) IL-4 treated (0.5 ml b.i.d.). Glomerular macrophage infiltration is markedly reduced compared with control (immunoperoxidase with hematoxylin counterstain $\times 300$).

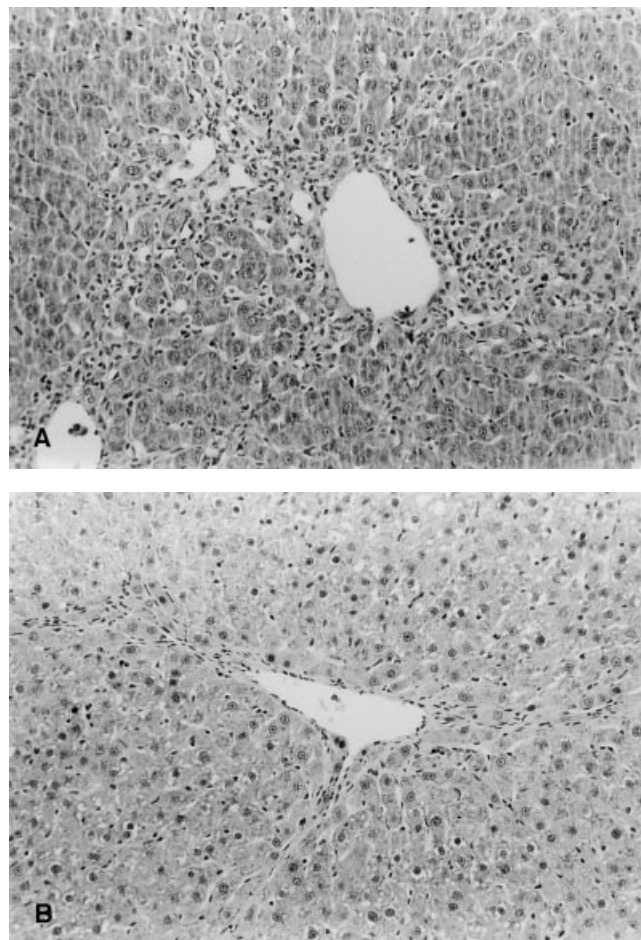


Fig. 3. (A) Liver histology from a rat from experiment 1 treated with IL-4 (0.5 ml b.i.d.). There is prominent hepatocyte loss around a central vein with crowding of the remaining Kupffer cells. (HE $\times 300$). (B) Liver histology from a rat from experiment 2 treated with IL-4 for one week and then killed at four weeks. There is mild bridging fibrosis extending from this central vein (HE $\times 300$).

Table 1. Effect of interleukin-4 (IL-4) given twice daily from the time of induction of glomerulonephritis until day 6

	N	Proteinuria mg/24 hr	Fibrinoid necrosis quadrants/glom	Macrophages/ glom	CD8+ cells/glom	iNOS+ cells/glom	PCNA+ cells/glom	ICAM-1 staining
Control	8	69 \pm 6	1.3 \pm 0.1	32 \pm 1.2	6.2 \pm 1.1	3.0 \pm 0.4	15 \pm 2.3	100 \pm 6.2
IL-4 0.25 ml bid	8	4 \pm 2 ^a	0.21 \pm 0.1 ^a	12.6 \pm 2.3 ^a	3.1 \pm 1.0 ^b	0.9 \pm 0.3 ^a	5.4 \pm 0.5 ^a	84 \pm 5.9
IL-4 0.5 ml bid	8	1 \pm 1 ^a	0.04 \pm 0.02 ^a	4.8 \pm 1.3 ^a	1.5 \pm 0.6 ^a	0.05 \pm 0.03 ^a	3.2 \pm 1.0 ^a	75 \pm 7.9 ^b

Abbreviations are: glom, glomerulus; iNOS, inducible nitric oxide synthase; PCNA, proliferating cell nuclear antigen; ICAM, intercellular adhesion molecule.

^a*P* = 0.001 compared with control

^b*P* = 0.025 compared with control

treated rats (36 \pm 5 macrophages/glom) compared with controls (23 \pm 2 macrophages), but this did not reach statistical significance (*U* = 5, *P* = 0.068). The numbers of CD8+ cells were not significantly different (control, 0.32 \pm 0.09 cells/glom; IL-4 treated, 0.66 \pm 0.38). There was no difference in glomerular deposition of rat immunoglobulin between the two groups (control, 21.3 \pm 3.0 intensity units/glom; IL-4 treated, 20.5 \pm 4.0). No ascites

was seen. The livers of the IL-4-treated rats showed brisk mitotic activity in hepatocytes, with some mild focal bridging fibrosis between portal veins (Fig. 3B).

Experiment 3

When IL-4 treatment was delayed until the fourth day, there was still a significant reduction in proteinuria by day 7, although not as marked as that seen with continu-

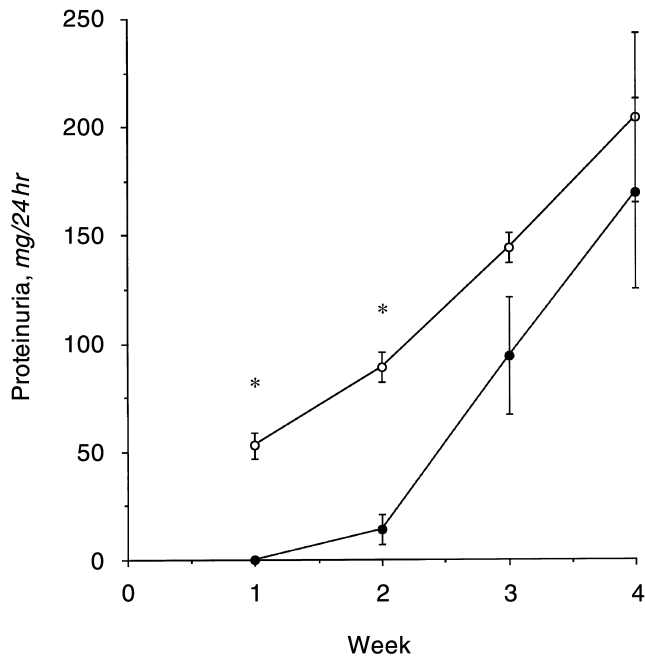


Fig. 4. Graph of proteinuria in experiment 2. Closed symbols denote IL-4 treated rats and open symbols are control nephritic rats. * $P = 0.006$.

ous treatment (Table 2). Glomerular injury, as assessed by fibrinoid necrosis, was significantly reduced in the treated group (Table 2). There was no difference in numbers of glomerular macrophages, but the numbers of iNOS-positive cells and ED3-positive cells were significantly reduced. CD8+ cells were reduced, but this did not reach statistical significance. There was no difference in cells staining for PCNA or in numbers of apoptotic cells (Table 2). Glomerular deposition of rabbit immunoglobulin did not differ between the groups.

DISCUSSION

In order to examine the effect of IL-4 in crescentic nephritis, we have chosen the model of nephrotoxic nephritis in the WKY rat first described by Kawasaki et al [25]. This is a reproducible model with rapid development of glomerulonephritis following a single injection of nephrotoxic serum. Our previous characterization of this model (Tam et al, manuscript submitted for publication) showed that there is a rapid influx of macrophages that plateaus at day four. Proteinuria is first seen at day 3 to 4, and histology on day 4 shows segmental fibrinoid necrosis in glomeruli that becomes more severe by day 6, leading to the development of cellular crescents in a mean of 63% of glomeruli by day 11. By 6 weeks, rats developed renal failure (creatinine of more than 300 $\mu\text{mol/liter}$), with a great majority of the glomeruli showing sclerosis. The very rapid onset of this model, with

established glomerular fibrinoid necrosis by six days, makes it unlikely that an acquired immune response to the planted rabbit immunoglobulin plays an important part in pathogenesis. This is supported by our previous observations: First, deposition of rat immunoglobulin was not seen in the glomeruli until day 6. Second, fluorescence-activated cell sorter analysis of single-cell preparations from glomeruli did not show any evidence of glomerular T-cell infiltration, as assessed by expression of CD3, T-cell receptor $\alpha\beta$, or T-cell receptor $\gamma\delta$, until day 11. We therefore believe that this model allows us to study the effect of IL-4 on the effector phase of glomerular inflammation rather than on the generation of the immune response.

Our first experiment, in which IL-4 was started immediately before the induction of nephritis, showed a dramatic reduction in injury. By six days, a time at which fibrinoid necrosis is maximal in the model, there was a complete abolition of proteinuria with either dose of IL-4 tested. With the lower dose, there was an 84% reduction in fibrinoid necrosis, and with the higher dose there was a 97% reduction. This was accompanied by a reduction in glomerular macrophages of 41% with the lower dose and 85% with the higher dose. This suggests that, although the major reason for the amelioration of injury is probably the reduction of macrophage influx, there may also be another effect on injury once the macrophages have entered the glomerulus. Previous studies have shown that the mechanism of macrophage infiltration involves the interaction of ICAM-1 with lymphocyte function associated antigen-1 (CD11a/CD18). Thus, there is up-regulated expression of ICAM-1 mRNA by one hour [26] and of immunohistochemically detectable ICAM-1 by one day [27], and injection of antibodies against ICAM-1 and CD11a reduced proteinuria, macrophage influx, and crescent formation [27]. Monocyte chemoattractant protein-1 has also been shown to play a role in the early macrophage influx in this model [28]. Our results show that IL-4 treatment reduces glomerular ICAM-1 expression. Potential mediators of the increase in ICAM-1 expression are IL-1 β and TNF- α , which have been shown to increase ICAM-1 expression rapidly in endothelial cells [29] and which are up-regulated early in the course of this model [26]. IL-4 is known to suppress macrophage TNF- α and IL-1 β [17], and we therefore hypothesize that the action of IL-4 in reducing ICAM-1 is via reduced TNF- α and IL-1 β synthesis. Our current studies are addressing these mechanisms.

We have recently studied the effect of IL-4 in accelerated nephrotoxic nephritis in the Sprague-Dawley rat [21]. This differs from the WKY model used in the current study in that it depends on preimmunization with rabbit immunoglobulin to induce an autologous immune response before the injection of nephrotoxic serum. In accelerated nephrotoxic nephritis (NTN), IL-4 treatment

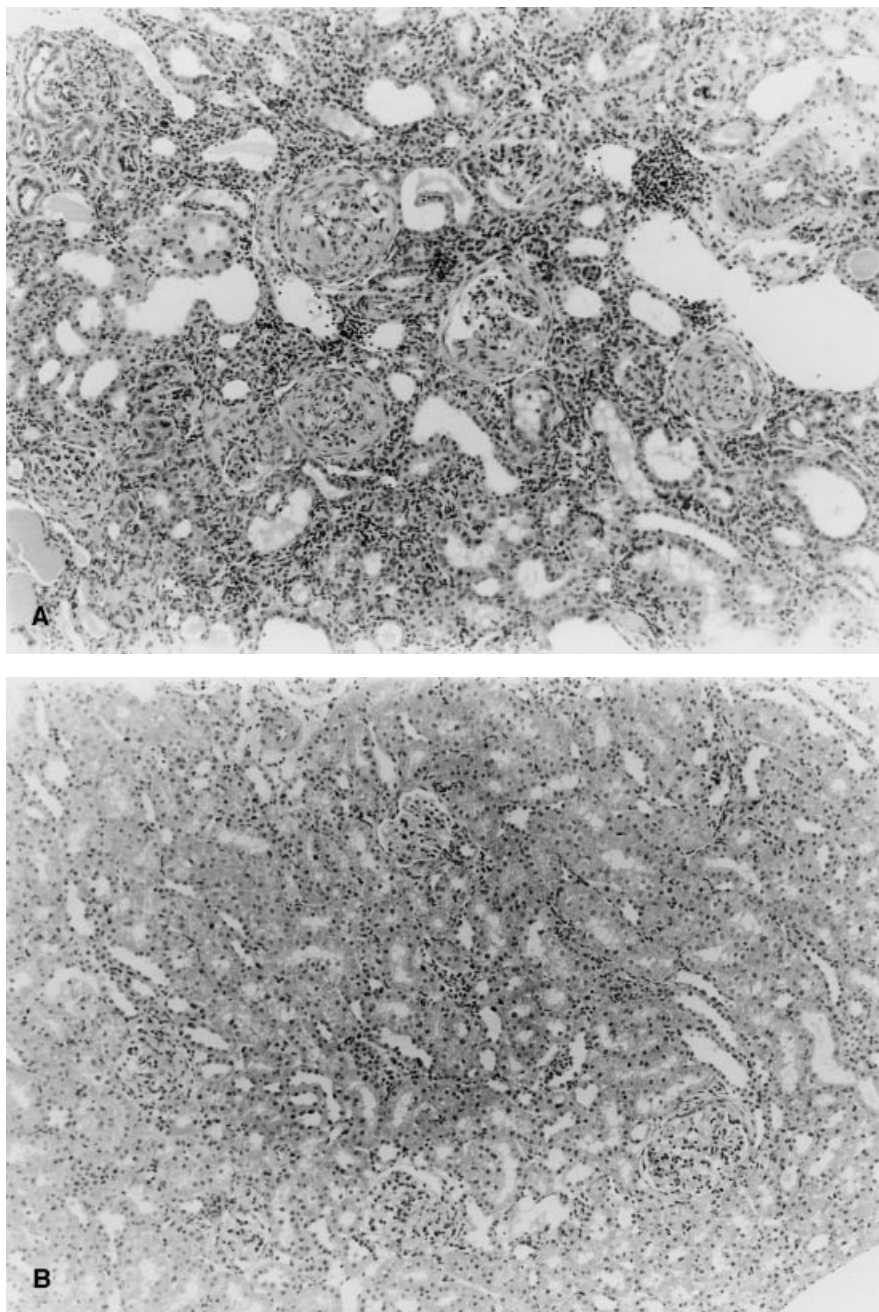


Fig. 5. Renal histology four weeks after the induction of nephritis in experiment 2. (A) Control rat. The glomeruli show prominent segmental scarring and fibrous crescents. The tubules show dedifferentiation, and there is an interstitial chronic inflammatory infiltrate. (B) IL-4 treated rat. There is much better preservation of tubular architecture with no significant inflammation (HE $\times 150$).

started just before the injection of nephrotoxic serum (NTS) produced a 73% reduction in albuminuria and 79% reduction in thrombosis. The model showed only modest glomerular macrophage infiltration (7.0 ± 0.5 macrophages/glom) compared with the WKY model, but there was a small but significant reduction in macrophage infiltration in the IL-4-treated group (5.3 ± 0.4 macrophages/glomerulus). In that study, no difference in expression of IL-1 β mRNA was found at four days; however, there was expression of IL-1 receptor type II (IL-1

decoy receptor) in the glomeruli of treated rats but not control rats.

We wished to study whether the early reduction in injury that we found with a short course of IL-4 would be maintained once the IL-4 treatment was stopped. In experiment 2, we therefore followed the rats for a further three weeks after a seven-day course of IL-4. In the untreated rats, there was a reduction in creatinine clearance with marked glomerular scarring and tubular atrophy. All of these parameters were improved in the IL-4-

Table 2. Effect of IL-4 given twice daily from day 4 to day 7 after induction of glomerulonephritis

	N	Proteinuria mg/24 hr	Fibrinoid quadrants/glom	Macrophages/ glom	iNOS+ cells/glom	PCNA+ cells/glom	ED3+ cells/glom	CD8+ cells/glom	Apoptosis cells/glom
Control	6	97 ± 9	1.59 ± 0.13	61 ± 6	1.83 ± 0.40	17.7 ± 1.6	1.86 ± 0.21	4.55 ± 0.71	0.42 ± 0.04
IL-4	6	41 ± 5 ^b	0.39 ± 0.05 ^b	60 ± 3	0.56 ± 0.10 ^a	17.0 ± 1.1	0.18 ± 0.06 ^b	2.95 ± 0.27	0.40 ± 0.05

Abbreviations are in Table 1.

^a*P* = 0.01 compared with control

^b*P* = 0.004 compared with control

treated animals, showing that reduction of initial injury led to a reduction in subsequent scarring. However, the IL-4-treated animals had developed prominent glomerular macrophage infiltration and proteinuria by this time, and it will be necessary to carry out further long-term follow-up to see whether this resolves without scarring. We considered the possibility that the rapid increase in proteinuria after stopping IL-4 could be due to an enhanced humoral immune response but found no difference in glomerular deposition of rat immunoglobulin.

The only side-effect seen with IL-4 treatment was on the liver. After six days of treatment, there was loss of hepatocytes around central veins, which appeared to be due to hepatocyte apoptosis without significant inflammation. The cause of this unusual change is not clear. It was not seen in the control rats that were given supernatant from nontransfected CHO-K1 cells. Transient elevations of liver enzymes has been reported in a trial of IL-4 in patients with Kaposi's sarcoma [30] and in a phase I dose toxicity trial [31], but no histological changes were described. Importantly, the liver damage we saw appeared to have almost completely resolved three weeks after stopping IL-4, with increased hepatocyte mitosis consistent with regeneration.

The results of the first experiment suggested that the reduction in injury was relatively greater than the reduction in macrophage numbers, and this was similar to what we had found in our previous study on accelerated NTN [21]. This indicates that IL-4 may also have the effect of reducing injury mediated by macrophages that are already in the glomerulus. Therefore, in order to test this hypothesis and to mimic more closely the treatment of clinical renal disease (which presents with established glomerulonephritis), we carried out a third experiment in which treatment was delayed until the fourth day. By this time, proteinuria was apparent, and there was marked glomerular hypercellularity. We found that IL-4 treatment was still effective in reducing proteinuria and fibrinoid necrosis but that there was no effect on macrophage numbers, suggesting that treatment may alter macrophage activation to reduce macrophage-mediated injury. In keeping with this hypothesis, we found that there was a reduction in the number of cells expressing iNOS and also in the expression of sialoadhesin, a marker

of macrophage activation. There was no effect on the number of proliferating cells or on apoptosis.

Interleukin-4 treatment has been previously tested in a mouse model of accelerated NTN. In one study [32], C57BL/6 mice were preimmunized with sheep immunoglobulin, given sheep nephrotoxic serum at 10 days, and then killed 10 days later. IL-4 treatment was given throughout the experiment. This is a strain of mice that mounts a strong Th1 response, and the authors have previously provided evidence that glomerulonephritis in this strain is dependent on CD4+ T cells [33]. IL-4 treatment abolished crescent formation and significantly attenuated glomerular fibrin deposition and both T cell and macrophage accumulation. The authors attributed the improvement to an attenuation of the Th1 response by IL-4. They have also studied the same model but with IL-4 treatment started 72 hours after the initiation of disease and continued until day 10 [34]. In that study, IL-4 did not reduce crescent formation or protect renal function; however, the combination of IL-4 with IL-10 significantly reduced crescent formation and preserved renal function. The failure of delayed treatment with IL-4 to influence crescentic glomerulonephritis in the mouse, compared with the success in our rat model, may be related to differences in dosage, in pathogenesis, or in species.

In the same mouse model, there is also evidence that endogenous IL-4 has an effect on reducing glomerular inflammation. Thus, mice genetically deficient in IL-4 showed more severe glomerulonephritis with increased renal impairment and crescent formation [35]. This was associated with an increased antigen-specific Th1 response shown by increased skin delayed-type hypersensitivity. It is not clear how far the influence of endogenous IL-4 in this model is related to modulation of the immune response and how far to a direct anti-inflammatory effect in the glomerulus.

In summary, we have shown in a model of crescentic glomerulonephritis in the WKY rat that IL-4 given from the time of induction of glomerulonephritis is able to abolish proteinuria and markedly reduce glomerular fibrinoid necrosis. This is associated with a marked reduction in glomerular macrophage accumulation. The beneficial effect of seven days of treatment is still apparent at four weeks, with preservation of renal function and

reduced glomerular and tubulointerstitial scarring. If treatment is started once glomerular inflammation is established, there is no longer an effect on macrophage infiltration; however, there is still reduction in injury, and we have shown that this is associated with a reduction in markers of macrophage activation.

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Reprint requests to Dr. H. Terence Cook, Department of Histopathology, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London, W12 0NN, England, United Kingdom.
E-mail: t.cook@rpms.ac.uk

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