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Interleukin-4 deficiency enhances Th1 responses and crescentic glomerulonephritis in mice

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Interleukin-4 deficiency enhances Th1 responses and crescentic glomerulonephritis in mice. Evidence suggests that crescentic glomerulonephritis (GN) is due to T helper cell 1 (Th1) directed delayed-type hypersensitivity (DTH)-like injury. As endogenous interleukin (IL)-4, (the pivotal cytokine in Th2 responses) may attenuate Th1 responses in this disease, we compared the development of crescentic GN, induced by a planted antigen, in mice genetically deficient in IL-4 (*IL-4−/−*) with disease in normal mice (*IL-4+/+*). *IL-4−/−* mice developed more severe GN with increased renal impairment (C_{Cr} 35 ± 7 μl/min vs. 133 ± 14 μl/min, $P < 0.002$) and crescent formation (55.7 ± 8.4% vs. 4.9 ± 1.2%, $P < 0.002$). This was associated with increased glomerular fibrin deposition, glomerular CD4+ T cell infiltration and macrophage recruitment. Systemically, *IL-4−/−* mice showed an increased antigen specific Th1 response indicated by increased skin DTH, and increased IgG3 and IgG2b. Decreased IgG1 levels indicated a reduced Th2 response. These results demonstrate a protective role for endogenous IL-4 in crescentic GN. They show that IL-4 deficiency promotes crescentic glomerular injury and amplifies local and systemic Th1 responses. They support the hypothesis that crescent formation results from Th1 immune responses to antigens in the glomerulus.

The formation of glomerular crescents, which occurs in the setting of proliferative forms of glomerulonephritis (GN), indicates severe disease and is associated with a poor clinical outcome. Important immunopathological events in crescentic GN include recruitment of CD4+ T cells and macrophages and prominent glomerular deposition of fibrin, akin to a T helper cell 1 (Th1) directed, delayed-type hypersensitivity (DTH)-like response. These features have been demonstrated in both human crescentic GN [1, 2] and animal models of crescentic GN [3, 4].

Delayed-type hypersensitivity is mediated by the Th1 subset of CD4+ T cells, which characteristically produce IFN-γ and IL-2 [5, 6]. T helper cell 2 (Th2) CD4+ T cells produce IL-4 and IL-10, which tend to inhibit macrophage functions and stimulate production of IgE and IgG1 [7]. The cytokine profile of antigen stimulated T cells depends on the cytokine milieu at the time of antigen presentation [8], with IL-12 being important in the differentiation of T cells into Th1 cells [9] and IL-4 being crucial to Th2 responses [10]. The concept of Th1 and Th2 responses has been modified to suggest that, while an immune response to a specific antigen may be characterized as predominantly Th1 or Th2 mediated, antigen-specific T cells produce a spectrum of cytokines with pure Th1 and Th2 cytokine secretors being at the two extremes of a spectrum [11].

The hypothesis that endogenous IL-4 serves to direct immunological responses toward Th2, while limiting Th1 responses and thus attenuating crescentic GN, was addressed using mice with a targeted disruption of the IL-4 gene [12]. These mice have been characterized to be deficient in IL-4, and to possess a relative deficiency in the acquisition of antigen-specific Th2 responses [12]. Glomerulonephritis was initiated in mice previously sensitized to sheep globulin (SG) by planting this antigen on the glomerular basement membrane (GBM) using a sheep anti-mouse GBM antibody. The development of the systemic and local immune response to this antigen and the subsequent glomerular injury were compared in the presence and absence of endogenous IL-4.

Key words: interleukin-4 deficiency, crescentic glomerulonephrits, glomerulonephritis, T helper cell.

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METHODS

Animals and induction of anti-glomerular basement membrane glomerulonephritis

Interleukin-4−/− and *IL-4+/+* mice, previously characterized [12], on a mixed background of C57BL/6 and 129Sv were a generous gift of Dr. Manfred Kopf. Mice were bred at Monash University Animal Services (Melbourne, Australia). Anti-glomerular basement membrane globulin was prepared as previously described [13]. Eight- to ten-week-old male mice were sensitized by s.c. injection of a total of 2 mg of sheep globulin in 200 μl of CFA (Sigma Chemical Co., St. Louis, MO, USA) in divided doses in each flank. After 10 days, GN was initiated by i.v. administration of 9.5 mg of sheep anti-mouse GBM globulin.

Histological assessment of glomerular injury

Glomerular crescent formation. Kidney tissue was fixed in Bouin's fixative, embedded in paraffin and 3 μm tissue sections were cut and stained with periodic acid-Schiff reagent. Sections from some animals' kidneys were stained with silver methenamine/acid fuchsin. Glomerular crescent formation was assessed in a blinded protocol. Glomeruli were considered to exhibit crescent formation when three or more layers of cells were observed in

Bowman's space. A minimum of 50 glomeruli were assessed to determine the percentage of glomeruli affected in each animal.

Assessment of glomerular deposition of sheep globulin, mouse immunoglobulin, immunoglobulin isotypes and fibrin. Tissue was embedded in Optimal Cutting Temperature Compound (OCT; Miles Scientific, IN, USA), immediately frozen in liquid nitrogen and stored at −70°C. Immunofluorescence was performed on 4 μm cryostat cut tissue sections and results assessed in a blinded protocol. Glomerular deposition of sheep globulin was evaluated using FITC conjugated donkey anti-sheep immunoglobulin (Silenus, Hawthorn, Victoria, Australia). Serial dilutions of this antibody were made to determine the end point positive titer for glomerular sheep globulin. Deposition of total mouse immunoglobulin was detected using FITC conjugated sheep anti-mouse immunoglobulin (dilution 1 in 500; Silenus). Glomerular deposition of immunoglobulin isotopes was assessed using either monoclonal rat anti-mouse IgG1, IgG2a, IgG2b or IgG3 (dilution 1 in 100; Pharmingen, San Diego, CA, USA) as the primary antibody followed by FITC conjugated sheep anti-rat immunoglobulin (1 in 100; Silenus) absorbed against normal mouse serum. Omission of the primary antibody served as a negative control. Fluorescence intensity was scored semiquantitatively (0 to 3+). Glomerular fibrin deposition was detected on a minimum of 30 glomeruli per mouse using FITC conjugated goat anti-mouse fibrin/fibrinogen serum (Nordic Immunological Laboratories, Berks, UK) at a dilution of 1 in 25, and scored semiquantitatively (0 to 3+) as follows: 0 = no fibrin deposition, 1 = fibrin occupying up to one third of the glomerular cross-sectional area, 2 = fibrin occupying one third to two thirds of the glomerulus, 3 = greater than two thirds of the glomerular cross-section covered by fibrin.

Glomerular T cell and macrophage accumulation. Spleen and kidney tissue was fixed in periodate/lysine/paraformaldehyde for four hours, washed in 7% sucrose solution, then frozen in liquid nitrogen. Tissue sections (6 μm thick) were stained to demonstrate macrophages and CD4+ T cells using a three-layer immunoperoxidase technique, as previously described [3, 14]. The primary antibodies were GK1.5 [monoclonal anti-mouse CD4; American Type Culture Collection (ATCC), Rockville, MD, USA] and M1/70 (monoclonal anti-mouse Mac-1; ATCC). Sections of spleen provided positive controls and protein G purified rat immunoglobulin was substituted for the primary monoclonal antibody to provide a negative control. Glomeruli from non-diseased *IL-4−/−* ($N = 5$) and *IL-4+/+* mice ($N = 5$) were assessed to provide baseline values for glomerular T cell and macrophage numbers. A minimum of 20 equatorially sectioned glomeruli were assessed per animal. Results were expressed as cells per glomerular cross section (c/gcs).

Functional assessment of glomerular injury

Creatinine clearance. Serum and urine creatinine concentrations were measured by the alkaline picric acid method using an autoanalyzer (Cobas Bio; Roche Diagnostic, Basel, Switzerland). Creatinine clearance was calculated from the serum and urine creatinine values and the urine volume.

Proteinuria. Twenty-four hour urine collections from each mouse were made at three time points: prior to disease, from days 1 to 2 of disease, and over the final 24 hours of each experiment. Urinary protein concentrations were determined by a modified Bradford method [15], adapted to a microtiter plate assay as previously described [3]. The 24-hour urinary protein excretion was calculated from the 24-hour urine volume and the urinary protein concentration.

Assessment of the systemic immune response to sheep globulin

Delayed-type hypersensitivity to sheep globulin. Mice were challenged 24 hours prior to the end of each experiment by intradermal injection of SG (50 μg in 30 μl of PBS) into the plantar surface of a hindfoot. An irrelevant antigen (horse globulin) was injected in the opposite foot pad as a control. Delayed-type hypersensitivity was quantified 24 hours later in a blinded protocol by inspecting the footpads and measuring the difference in thickness between SG and horse globulin injected footpads in each mouse using a micrometer (Mitutoyo Corporation, Japan). Results are expressed as the change in footpad thickness (mm) between the horse globulin and the sheep globulin-injected sides.

Measurement of interferon (IFN)-γ production by splenic T cells. Spleens from sensitized mice were removed aseptically and placed in RPMI 1640 5% FCS medium. Single cell suspensions were prepared by gently teasing tissue apart. Erythrocytes were lysed by incubation in Boyle's solution (0.17 M Tris, 0.16 M ammonium chloride) for one minute at 37°C. Cell suspensions were washed in RPMI 1640 5% FCS, then enriched for T cells by passage through nylon wool columns. Samples of enriched T cells (4×10^6 cells/ml in RPMI 1640 10% FCS) were incubated for 72 hours at 37°C, 5% CO_2 in 48-well tissue culture plates with sheep IgG at a concentration of 10 μg/ml. Interferon-γ in culture supernatants was measured by ELISA using flat bottom polystyrene microtiter plates (Greiner Labortechnik, Kremsmünster, Austria) coated with rat anti-mouse IFN-γ monoclonal antibody (RA-6A2; Pharmingen,) at 5 μg/ml and blocked with 1% BSA. Supernatant (100 μl) or recombinant murine IFN-γ (Genzyme, Cambridge, MA, USA) were incubated in wells overnight at 4°C. Biotinylated rat anti-mouse IFN-γ mAb (XMG1.2; Pharmingen) was used as the detecting antibody at a concentration of 1 μg/ml. Plates were washed and incubated with streptavidin horseradish peroxidase complex (Silenus) at a dilution of 1 in 2000, washed then incubated with ABTS (0.1 M 2,2′-azino-di-3-ethylbenzthiazoline sulfonate; Boehringer Mannheim, Germany) in 0.02% H_2O_2. The absorbance at 405 nm was read on a microtiter plate reader (Dynatech Laboratories, Chantilly, VA, USA). The lower limit for detection of IFN-γ in this assay was 6 pg/ml.

Measurement of circulating anti-sheep globulin IgG isotypes. Isotypes of mouse anti-SG antibodies were measured by ELISA. Plates were coated with 10 μg/ml SG in carbonate/bicarbonate buffer (pH 9.6) by incubation overnight at 4°C and blocked with 1% BSA. Plates were washed, then incubated with mouse sera at a dilution of 1 in 200. After further washing, binding of specific mouse immunoglobulin isotypes was detected using horseradish peroxidase conjugated goat anti-mouse IgG1, IgG2a, IgG2b and IgG3 antibodies (Southern Biotechnology Assoc., Birmingham, AL, USA) at a dilution of 1 in 4000. ABTS substrate solution was added, the absorbance was read at 405 nm and the concentration of each isotype between treatment groups was compared. Serum from seven non-immunized mice (*IL-4+/+*, $N = 3$, *IL-4−/−*, $N = 4$) was tested as normal controls.

Experimental design and statistical analysis

Anti-glomerular basement membrane GN was induced as described above and glomerular injury was assessed 10 days after administration of anti-GBM globulin in *IL-4−/−* mice ($N = 6$)

and *IL-4+/+* mice ($N = 9$). Measurements of proteinuria, renal function, crescent formation and serum IgG isotypes were made on six *IL-4−/−* mice and nine *IL-4+/+* mice, while assessments of glomerular fibrin deposition, glomerular T cell and macrophage numbers, glomerular immunofluorescence, footpad DTH and splenic T cell IFN-γ levels were performed on four *IL-4−/−* mice and five *IL-4+/+* mice. Age matched, non-diseased, male *IL-4−/−* ($N = 3$ to 5) and *IL-4+/+* ($N = 4$ to 5) mice were used for baseline measurements. Results are expressed as the mean ± SEM. The statistical significance of differences between groups was determined by the Mann Whitney *U* test.

RESULTS

Interleukin-4 deficient mice develop more severe crescentic glomerulonephritis, with increased glomerular effectors of delayed-type hypersensitivity

Kidneys of non-diseased *IL-4−/−* and *IL-4+/+* mice were histologically normal. Glomerular CD4+ T cells (*IL-4−/−* 0.006 ± 0.006 c/gcs, *IL-4+/+* 0.04 ± 0.02 c/gcs) and macrophages (*IL-4−/−* 0.04 ± 0.02 c/gcs, *IL-4+/+* 0.04 ± 0.02 c/gcs) were detected only rarely. Ten days after initiation of GN, *IL-4+/+* mice developed a diffuse proliferative GN (Fig. 1 A, C), with glomerular T cell and macrophage infiltration (Fig. 1 E, G), glomerular fibrin deposition and occasional crescent formation. *IL-4−/−* mice developed considerably more severe GN than *IL-4+/+* mice (Fig. 1 B, D). There was increased glomerular crescent formation (55.7 ± 8.4% vs. 4.9 ± 1.2% $P < 0.002$; Figs. 1B and 2) and fibrin deposition ($P < 0.02$; Fig. 2). Glomerular CD4+ T cell and macrophage recruitment was significantly enhanced in nephritic *IL-4−/−* mice (Fig. 1 F, H, and Fig. 2) compared to *IL-4+/+* mice with GN. There was more than a doubling of glomerular T cells ($P < 0.02$; Fig. 1F), and a fourfold increase in glomerular macrophage numbers ($P < 0.02$; Fig. 1H). Macrophages were observed in both the glomerular tuft and crescent, as well as in the periglomerular region (Fig. 1H).

Glomerular deposition of sheep globulin and autologous antibody

Glomeruli of mice with GN were stained by immunofluorescence for the nephritogenic antigen (sheep globulin), for total mouse immunoglobulin and for IgG isotypes. Linear deposition of both sheep and mouse globulin was observed in glomeruli. There was no difference in the glomerular deposition of sheep globulin or total mouse immunoglobulin between *IL-4+/+* and *IL-4−/−* mice. However, changes in fluorescence intensity of immunoglobulin isotypes were observed, the results of which are presented in Table 1. Deposition of IgG1 was decreased in IL-4 deficient mice with GN, while deposition of IgG2b and IgG3 were increased compared to *IL-4+/+* animals. Glomerular IgG2a was unchanged. These semiquantitative assessments paralleled the quantitation of serum sheep globulin specific immunoglobulin isotypes measured by ELISA and presented in Table 2.

Interleukin-4 deficient mice develop more severe functional injury in crescentic glomerulonephritis

The *IL-4+/+* mice developed functional evidence of renal injury with proteinuria (Fig. 3 and reduced creatinine clearance (normal mice C_{Cr} 193 ± 10 μL/min, *IL-4+/+* with GN 133 ± 14 μl/min, $P < 0.02$, Fig. 4). However, *IL-4−/−* mice developed more marked renal impairment than *IL-4+/+* mice (35 ± 7 μl/min, $P < 0.002$), with severe renal failure (C_{Cr} less than 20% of normal). Both *IL-4−/−* and *IL-4+/+* mice developed proteinuria, with more severe proteinuria in *IL-4−/−* mice in the early stages of disease (days 1 to 2, $P = 0.05$; Fig. 3). This increase became less statistically significant as renal impairment in *IL-4−/−* mice became more severe (days 9 to 10, $P = 0.157$).

Systemic immune responses of interleukin-4 deficient mice to sheep globulin are more polarized towards a Th1 response

Both *IL-4−/−* and *IL-4+/+* mice developed cutaneous DTH when challenged intradermally with SG injected 24 hours prior to the end of the experiment. However, antigen-specific DTH responses were significantly greater in IL-4 deficient mice ($P < 0.03$; Fig. 5). To further examine the nature of the T cell response to SG, IFN-γ production from splenic T cells of mice with GN was measured. Interferon-γ production was increased in *IL-4−/−* mice compared to *IL-4+/+* mice, but the increase did not reach statistical significance ($P = 0.096$; Fig. 5).

There were significant differences in antigen-specific immunoglobulin isotypes between *IL-4+/+* and *IL-4−/−*, indicating the immune response to SG was further polarized towards a Th1 response in *IL-4−/−* mice (Table 2). There was a significant reduction in antigen specific IgG1 ($P < 0.002$) in *IL-4−/−* mice, and an increase in IgG3 ($P < 0.002$) and IgG2b ($P < 0.002$) levels. Levels of IgG2a were unchanged.

DISCUSSION

Crescentic glomerulonephritis has immunopathological features of a Th1 dependent, DTH response. Evidence to support this view includes the presence of the effectors of DTH (T cells, macrophages, tissue factor and fibrin) in glomeruli of humans with crescentic GN [1] and the fact that many cases of human crescentic GN occur in the absence of significant glomerular deposition of antibody [16]. Studies in experimental crescentic GN have allowed this hypothesis to be specifically tested. Crescent formation occurs despite the absence of autologous antibody [17, 18] and can be prevented by T cell depletion [3, 4].

It has been recently shown in a planted antigen model of crescentic GN similar to the one used in this study that the type of renal injury depends on the balance between Th1 and Th2 cytokine production [4]. Th1 prone mice, C57BL/6, develop crescent formation, glomerular T cells, macrophages and fibrin, skin DTH to the nephritogenic antigen and higher levels of splenic T cell IFN-γ production relative to IL-4 production. Antibody blockade of IFN-γ [4] or administration of IL-4 and IL-10 [19] attenuates crescentic disease. BALB/c mice, which are Th2 prone, also develop severe renal injury [4]. However, their disease is characterized not by crescent formation but by glomerular deposition of autologous antibody and complement, absence of skin DTH to SG, and higher levels of splenic T cell IL-4 production relative to IFN-γ.

Interleukin-4 is a critical cytokine in determining the pattern of cytokine secretion of primed CD4+ T cells. Interleukin-4 facilitates development of a Th2 phenotype and inhibits IFN-γ production by activated T cells *in vitro* [10]. However, IL-12 has the opposite effect [20]. These observations are supported by analyses of the immune responses of mice genetically deficient in IL-4 to

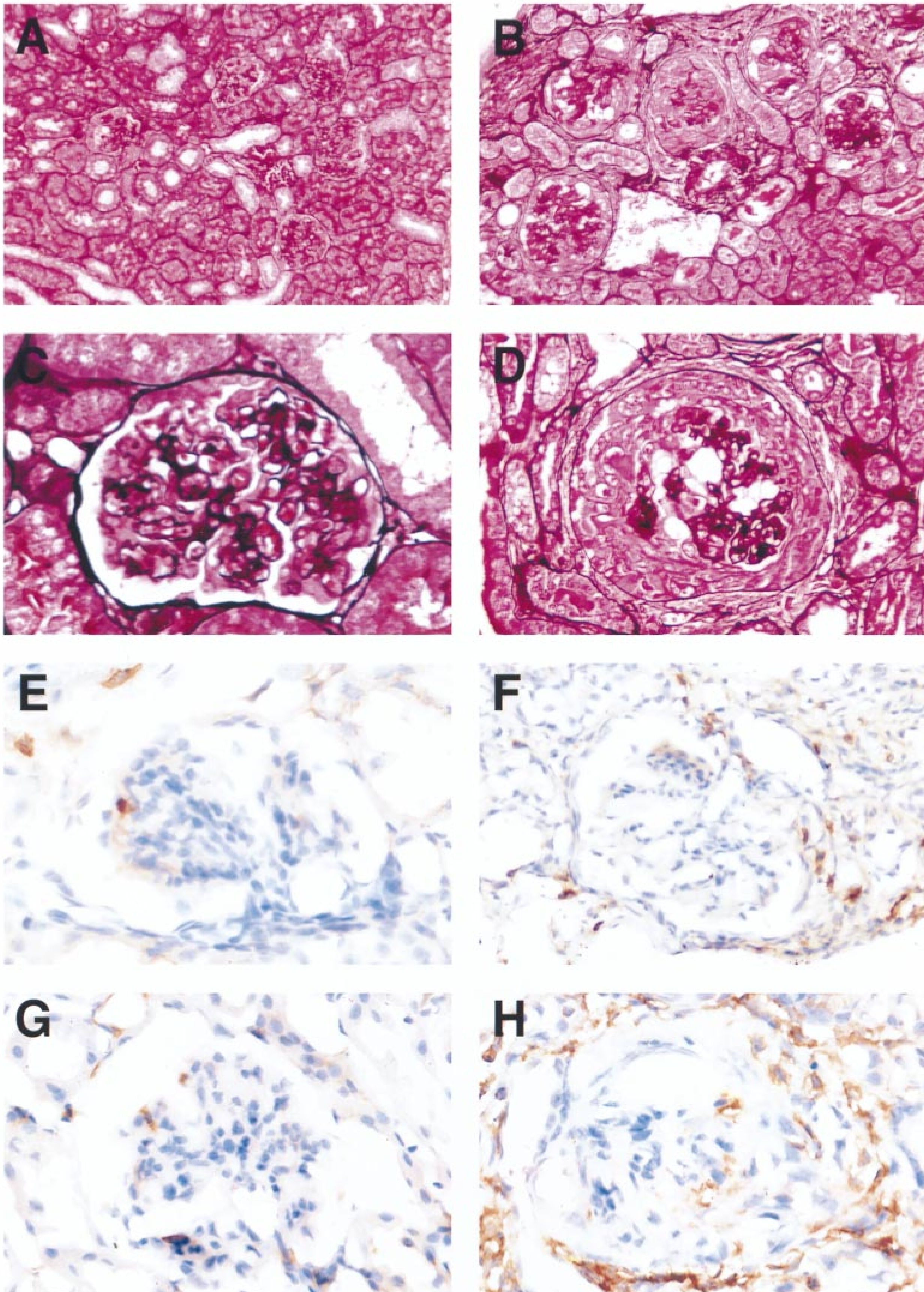

Fig. 1. Histological injury in *IL-4*+/+ and *IL-4*−/− mice with glomerulonephritis. Photomicrographs of glomeruli from *IL-4*+/+ mice with GN show proliferative GN at low power (*A*, silver methenamine/acid fuchsin stain, ×100) and high power (*C*, ×400), with CD4+ T cell infiltration (*E*, immunoperoxidase with hematoxylin counterstain using GK1.5, ×400) and macrophage accumulation (*G*, immunoperoxidase using M1/70, ×400). Mice deficient in IL-4 develop markedly more severe disease with frequent glomerular crescent formation (*B*, ×100 and *D*, ×250), and increased T cells (*F*, ×250) and macrophages (*H*, ×400).

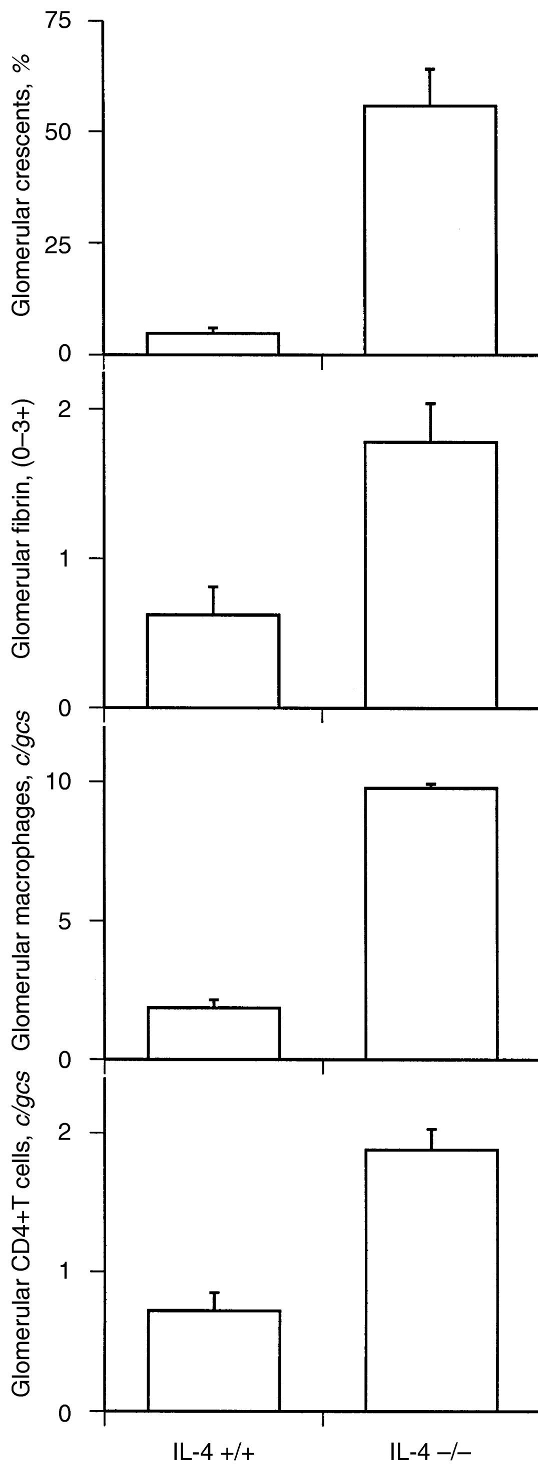

Fig. 2. Features of glomerular disease in *IL-4+/+* and *IL-4−/−* mice with glomerulonephris. Interleukin-4 deficiency markedly exacerbated GN with more frequent glomerular crescents, increased glomerular fibrin deposition and increased numbers of macrophages and CD4+ T cells in glomeruli of diseased *IL-4−/−* mice indicating enhanced features of DTH in glomeruli. Abbreviation c/gcs is cells per glomerular cross section.

Table 1. Deposition of murine IgG isotypes in glomeruli of *IL-4+/+* and *IL-4−/−* mice 10 days after initiation of glomerulonephritis

	IL-4+/+	*IL-4−/−*
IgG1	2+	+
IgG2a	+	+
IgG2b	+	3+
IgG3	+	2+

Changes in the glomerular deposition of immunoglobulin isotypes were evaluated by semiquantitative scoring of fluorescence intensity (0 to 3+), at an antibody dilution of 1 in 100.

Table 2. Sheep globulin specific IgG isotypes in sera of *IL-4+/+* and *IL-4−/−* mice 10 days after initiation of glomerulonephritis

	IgG1	IgG2a	IgG2b	IgG3
IL-4+/+	1.137 ± 0.046	0.352 ± 0.100	0.176 ± 0.027	0.057 ± 0.010
IL-4−/−	0.641 ± 0.042[a]	0.256 ± 0.034	1.414 ± 0.053[a]	0.326 ± 0.073[a]
Normal mouse serum	0.001 ± 0.001	0.012 ± 0.004	0.022 ± 0.004	0.014 ± 0.007

Sera at a dilution of 1 in 200 were assessed by ELISA. Results are expressed as the mean OD_{405} ± SEM.
[a] $P < 0.002$ vs. *IL-4+/+* mice.

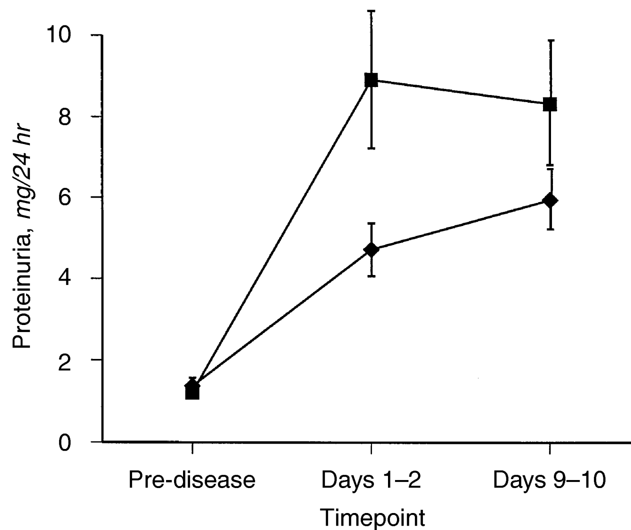

Fig. 3. Proteinuria in *IL-4+/+* (◆) and *IL-4−/−* (■) mice with glomerulonephritis. All nephritic mice developed proteinuria. *IL-4−/−* mice developed more severe proteinuria compared to *IL-4+/+* mice early in the disease.

parasitic infection, [12, 21] showing decreased production of other Th2 cytokines, including IL-10 and IL-13, and increased IFN-γ levels. Serum IgG1 is reduced and IgG2a, IgG2b and IgG3 are increased [21]. Therefore, IL-4 is an important determinant of the nature of the effector immune response. Furthermore, in genetically normal mice, IL-12 profoundly up-regulates antigen specific IgG2a, IgG2b and IgG3 production [22].

The current studies were performed to address the hypothesis that endogenously produced IL-4 is protective in the context of a Th1 directed, non-infective inflammatory immune response. It was hypothesized that in crescentic GN, IL-4 (and thus the Th2 element of the immune response) prevents extreme polarization of the immune response to a Th1 profile. Therefore, deficiency of IL-4 would enhance Th1 responses to a nephritogenic antigen. This would lead to increased crescent formation and renal injury.

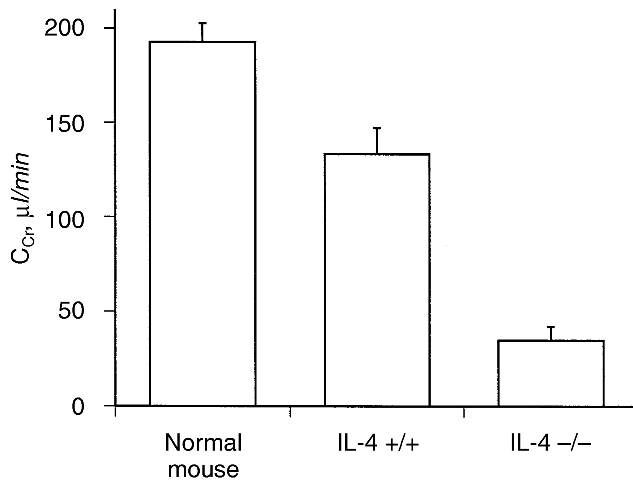

Fig. 4. Renal function in diseased *IL-4*+/+ and *IL-4*−/− mice (day 10 of disease), with normal mice for comparison. Interleukin-4 deficient mice developed severe renal failure compared to *IL-4*+/+ animals.

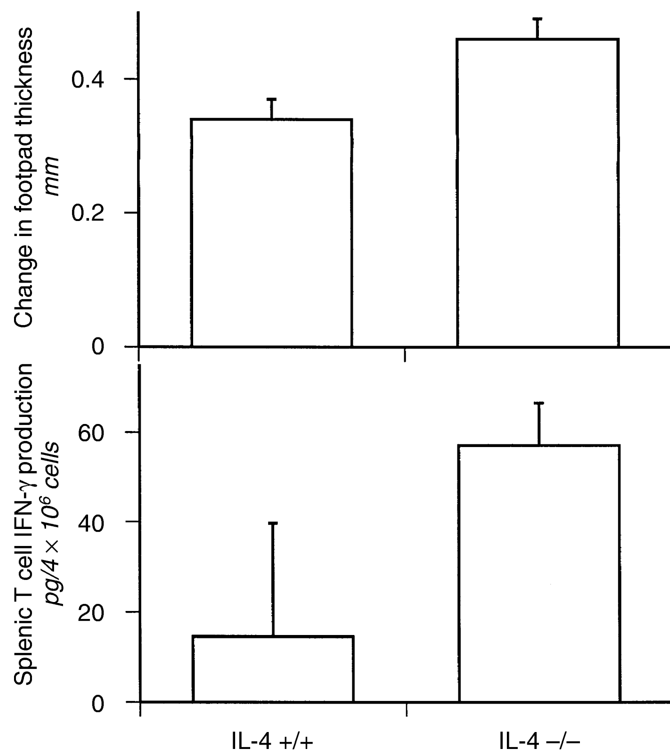

Fig. 5. The effect of IL-4 gene deletion on the DTH response to cutaneous antigen challenge and antigen stimulated production of IFN-γ by splenic T lymphocytes from mice with glomerulonephritis. Cutaneous DTH was increased in *IL-4*−/− mice compared to genetically intact mice. There was a trend to increased production of IFN-γ by splenic T cells isolated from nephritic IL-4 deficient mice that did not reach statistical significance (P = 0.096).

To address this question, immune responses and renal injury were studied in mice genetically deficient in IL-4 using a model of crescentic GN. Glomerulonephritis was induced by planting an antigen, sheep globulin, into kidneys of sensitized mice. Genetically normal mice developed proliferative GN with occasional crescent formation, associated with glomerular T cell and macrophage infiltration and glomerular fibrin deposition. These histological changes were accompanied by proteinuria and renal impairment. Mice developed skin DTH in response to cutaneous antigen challenge. *IL-4*−/− mice developed increased Th1 responses to SG, as evidenced by increased skin DTH, a trend to increased splenic T cell IFN-γ production, and increased serum IgG2b and IgG3. The decrease in antigen specific serum IgG1 levels confirms the decreased Th2 responsiveness of IL-4 deficient mice previously demonstrated [12, 23]. Glomerulonephritis in *IL-4*−/− mice was exacerbated, with markedly increased crescent formation, and increases in glomerular T cells, macrophages and fibrin deposition, features that occur in the setting of DTH. Changes in the deposition of glomerular immunoglobulin isotypes reflected the alterations observed in serum antigen specific immunoglobulin levels.

The lower level of crescent formation found in *IL-4*+/+ mice in this study compared to that seen previously in pure C57BL/6 mice [19] may reflect a strain difference between pure C57BL/6 mice and the C57BL/6 × 129Sv mice used in these studies. However, an alternative explanation is that the dose of anti-GBM globulin used was intentionally lower than that used in a previous study (in C57BL/6 mice) that employed the same batch of antibody [19].

The lack of an increase in antigen-specific IgG2a response in *IL-4*−/− mice compared to *IL-4*+/+ mice does not fit this pattern, but has been observed in previous studies of immune responses in IL-4 deficient mice [21, 24]. Autologous antibody production is not required for crescent formation in this model [17]. Therefore, the changes in immunoglobulin isotype levels in this study are unlikely to be of pathogenetic significance in themselves and can be considered as markers of a shift in the T helper cell response to the nephritogenic antigen. The results of this study, when taken in the context of previous work in both human [16] and experimental [17, 18] crescentic GN, suggest that it is the Th1 derived, *cell mediated* component of the nephritogenic immune response that is critical in the pathogenesis of glomerular crescent formation.

In addition to the shift in the Th1/Th2 balance, other mechanisms may have contributed to the exacerbation of GN in *IL-4*−/− mice. Interleukin-4 has been shown to directly deactivate macrophages *in vitro* [25–27] and to prevent induction of tissue factor mRNA in stimulated human monocytes [28]. There is evidence that IL-4 promotes synthesis of intrarenal 15-lipoxygenase [29], and it is possible that reduced glomeruloprotective prostanoid synthesis may have had deleterious hemodynamic consequences contributing to the increased renal impairment observed in *IL-4*−/− mice with GN.

In summary, genetic deficiency of IL-4, and therefore of endogenous Th2 responses, increases Th1-mediated renal injury, with increased glomerular cell mediated immunity (that is, features of DTH) and evidence of a shift in the systemic immune response to the nephritogenic antigen further towards a Th1 response. These studies demonstrate that endogenous IL-4 is protective in the context of crescentic GN, an injurious Th1 response to antigens in glomeruli.

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APPENDIX

Abbreviations used in this article are: IL, interleukin; GN, glomerulonephritis; Th, T helper cell; DTH, delayed-type hypersensitivity; SG, sheep globulin; GBM, glomerular basement membrane; OCT, optimal cutting temperature compound; IFN-γ, interferon-γ; c/gcs, cells per glomerular cross section.

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