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IL-4 Therapy Prevents the Development of Proteinuria in Active Heymann Nephritis by Inhibition of Tc1 Cells

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The role of IL-4, a key Th2 cytokine, in promoting or inhibiting active Heymann nephritis (HN) was examined. HN is induced by immunization with Fx1A in CFA, and proteinuria in HN is associated with subepithelial IgG and C3 deposition and infiltration of CD8+ T-cytotoxic 1 (Tc1) cells and macrophages into glomeruli, as well as induction of Abs to Crry. Treatment with rIL-4 from the time of Fx1A/CFA immunization stimulated an earlier IgG1 response to Fx1A, induced anti-Crry Abs, and up-regulated IL-4 mRNA in lymphoid tissue, but did not alter proteinuria. Treatment with MRCoX-81, an IL-4-blocking mAb, resulted in greater proteinuria, which suggests endogenous IL-4 regulated the autoimmune response. Delay of rIL-4 treatment until 4 wk post-Fx1A/CFA immunization and just before the onset of proteinuria prevented the development of proteinuria and reduced Tc1 cell infiltration in glomeruli. Delayed treatment with IL-4 had no effect on titer or isotype of Abs to Fx1A or on Ig, C3, and C9 accumulation in glomeruli. Treatment with rIL-13, a cytokine that alters macrophage function such as rIL-4, but has no direct effect on T or B cell function, reduced glomerular macrophage infiltrate, but did not prevent proteinuria or CD8+ T cell infiltrate. Anti-Crry Abs were paradoxically only induced with rIL-4 therapy, not in HN controls with proteinuria. It was concluded that the rIL-4 effect was probably by inhibition of Tc1 cells, which normally mediate the glomerular injury that results in proteinuria. The Journal of Immunology, 2001, 167: 3725–3733.

Immune injury in glomerulonephritis is categorized as either Th1 with infiltration of Th1 cells and macrophages (1–4) or Th2 with Ab deposition (5, 6). Active Heymann nephritis (HN) is a rat model of human membranous glomerulonephritis that has Ab/complement deposition in glomeruli and would thus be expected to be due to a Th2 response. HN is induced in susceptible rat strains by immunization with a rat renal tubular Ag (Fx1A) in CFA (7, 8). Anti-Fx1A Ab titers appear 2–4 wk later, and autoantibody binds to antigenic determinants on Gp330 (Megalin) at the base of glomerular epithelial cells (GEC) (9). Activation of C3 by anti-Fx1A Abs leads to formation of the membrane attack complex (C5b-9/MAC), which is thought to mediate sublytic injury to GEC, synthesis of abnormal glomerular basement membrane (GBM) matrix, and proteinuria (10, 11). In passive HN (PHN), C3 depletion with cobra venom factor abrogates proteinuria, suggesting complement is the principal mediator of glomerular injury (12, 13). Abs responses that block the function of the complement regulatory molecule Crry, the rodent analogue of human decay-accelerating factor and monocyte chemoattractant protein, are also necessary for the development of proteinuria in HN (14). Against an essential role for MAC in HN is the finding that it can be induced in C6-deficient PVG rats that are unable to assemble C5b-9/MAC (15). Our group also observed that the onset of proteinuria in HN is associated with glomerular infiltration of T-cytotoxic 1 (Tc1) cells and macrophages (16). Furthermore, permanent depletion of CD8+ T cells prevents the onset of proteinuria (17). These findings suggest that Th1 responses such as Tc1 cytotoxic cells, not MAC, may mediate glomerular injury. It has been proposed that the T-cytotoxic Tc1 response only develops 4–6 wk after immunization in response to the inflammation caused by Ab deposition and C activation in the glomerulus (17).

IL-4 is the key Th2 cytokine in that it both promotes the development of Th2 responses and induces IgE production and Ig isotype switching to IgG1 (18), as well as inhibiting Th1 responses. IL-4 blocks Tc1 cell development by making them noncytolytic (19). IL-4 also inhibits macrophage activation and their production of cytotoxic molecules, including TNF-α and NO (20–22).

This study examined the effects on HN of both IL-4 therapy and inhibition of endogenous IL-4 with anti-IL-4 mAb. The aim was to see whether Ab/complement responses or Th1 and cytotoxic Tc1 responses were more important. Therapies were given at three phases in the disease; first, at the time of immunization when the initial anti-Fx1A Ig response is induced and there is glomerular deposition of Ig with complement activation; second, between 4 and 6 wk after Fx1A immunization, at a time just before the onset of proteinuria, when Tc1 cells and macrophages infiltrate into glomeruli; and third, from 8–10 wk post-Fx1A immunization, after proteinuria has developed and all inflammatory mediators are in the glomerulus. These studies showed that rIL-4 administration just before onset of proteinuria prevented proteinuria, inhibited CD8+ T cell and macrophage infiltrate, induced anti-Crry Abs, but had no effect on glomerular Ig and C3 deposition. Treatment with anti-IL-4 mAb resulted in greater proteinuria. As the antiinflammatory effect of rIL-4 could be due to inhibition of activation of either CD8+ T cells or macrophages, we also examined the effects of treatment with rIL-13. IL-13 has a similar effect as IL-4 on
Materials and Methods

Experimental animals and induction of HN

Lewis rats (LEW/Ssn) were bred in the animal facility at Liverpool Hospital, and Sprague Dawley rats and BALB/c mice were purchased from the Animal Breeding and Holding Unit (New South Wales, Australia). All animals had water and standard chow available ad libitum. All procedures have been previously described, including preparation and immunization with renal tubular Ag (Fx1A) in CFA (IFA; Sigma, St. Louis, MO) and Mycobacterium bovis BCG (H37Rv, Dietz, MI) (25), monitoring with kidney biopsies, 24-hr urine protein estimations, and sera for anti-Fx1A Abs (16). The Animal Care and Ethics Committee of University of New South Wales approved animal experimental protocols.

Production and administration of rat rIL-4 and rIL-13

Rat rIL-4 was produced as cell culture supernatant from a Chinese hamster ovary (CHO-K1) cell line transfected with rat IL-4 cDNA (a kind gift of D. Mason, Medical Research Council, Cellular Immunology Unit, Oxford, U.K.) (26), as described (27). One unit of rIL-4 was defined as the amount of rIL-4 required to promote 50% of the maximal MHC class II induction on 5 × 10^6 B cells (28). A daily dose of 32,000 U rIL-4 was administered as twice-daily i.p. injections for 10 days. This protocol is based on the published use of rat IL-4 in anti-GBM Ab-mediated glomerulonephritis (3) and in prolongation of neonatal heart allograft survival (29). Rat rIL-13 was produced as cell culture supernatant from a CHO-K1 cell line transfected with rat IL-13 cDNA (30). One unit of rIL-13 was defined as the concentration inducing half-maximal proliferation of a dependent human erythroleukemia cell line (TF1), as described (31). A daily dose of 5000 U rIL-13 was administered as a twice-daily i.p. injection for 10 days, based on the dose of rIL-13 used to prolong allograft survival.4

As a control for administration of cytokines, supernatant from nontransfected CHO-K1 cells was concentrated and administered. All preparations of rIL-4, rIL-13, CHO-K1 supernatant and mAb preparations were assayed for endotoxin levels and had <0.006 U/ml in a Limulus amebocyte lysate assay (Coa test Gel-LAL; Chromogenix, Molndal, Sweden).

Production and administration of mAbs

Clones were MRCOx-81, IgG1 anti-rat IL-4 (a kind gift of D. Mason); MRCOx-33, IgG1 anti-rat IL-13 (27), and MRCOx-8 (30). A combination of FITC- and PE-conjugated mouse anti-rat mAb, including anti-rat CD4, anti-rat CD3, anti-rat CD8, anti-rat CD45R (a kind gift of D. Mason), and anti-rat TNF-α, were produced as cell culture supernatants from a CHO-K1 cell line transfected with rat IL-13 cDNA and were assayed using biotin-conjugated anti-rat IgE Abs and a streptavidin enzyme-linked immunosorbent assay (CoA test Gel-LAL; Chromogenix, Molndal, Sweden).

ELISA assay for anti-Fx1A and Crry Abs

Anti-Fx1A Ab titers (total Ig) were determined by ELISA, as described (33). Fx1A for ELISA was solubilized and purified by fractional salt precipitation, and plates were prepared (25). Triplicate sample ODs were read at 405 nm, corrected for a control sample of known strongly positive serum, and expressed as a percentage of a control positive serum OD (i.e., sample OD/control positive serum OD × 100). The anti-Fx1A Ab titer of the control positive serum was 1:250. IgG subclasses were assayed by the same method using alkaline phosphatase-conjugated mouse mAb to rat IgG1, IgG2a, and IgG2b (BD Pharmingen, San Diego, CA). IgE anti-Fx1A titers were assayed using biotin-conjugated anti-rat IgE Abs and a streptavidin/alkaline phosphatase conjugate (BD Pharmingen).

Anti-Crry Abs were assessed by ELISA, as described (14). Briefly, plates were coated with purified Crry (a gift of R. Quigg, University of Chicago, Chicago, IL), and titers of anti-Crry Ab (total Ig) in experimental sera were compared with control sera and anti-rat Crry/p65 Ab (IgG1) (BD Pharmingen).

Immunoperoxidase cytochemistry of renal cortex and isolated whole glomeruli

An indirect peroxidase-antiperoxidase complex technique was used on renal cortical wedge biopsies or isolated glomeruli, as described (16). Glomeruli were stained with MRCOx-12, which recognizes rat Ig κ-L chains (34), C9 polyclonal rabbit anti-rat Ab (35) (a kind gift of S. Fiddlesden,

Results

Effect of early treatment with rIL-4 and MRCOx-81 at the time of immunization

The development of HN was followed in Lewis rats treated with either rIL-4 or IL-4-blocking Ab (MRCOx-81) for 10 days from the day of immunization with Fx1A. At 2 wk post-Fx1A immunization, significant titers of anti-Fx1A Abs developed in the rats treated with rIL-4 (168.4 ± 17.9%, p < 0.001), which declined subsequently. In control HN and MRCOx-81-treated rats, anti-Fx1A Abs only developed by 4 wk, peaking at 6 wk (Fig. 1a). The IgG1 subclass predominated in the rIL-4 treatment group at all time points, but was the minor subclass in control HN and MRCOx-81-treated groups (Fig. 1b). IgG2a was the main subclass in HN and MRCOx-81 groups, and was higher in MRCOx-81-treated animals (Fig. 1c, p < 0.03). Treatment control groups, given either A6 the IgG1 isotype match for MRCOx-81 or CHO-K1 supernatant as a control for rIL-4, both developed total and IgG subclass Ab titers not statistically different from the HN control group (data not shown). No detectable titers of IgE anti-Fx1A Abs were present in any groups of rats. Anti-Crry Abs were only detected in low titers in the 2-wk sera of rats treated with rIL-4, and were not found in HN control rats or in other treatment groups (Fig. 1e).

At wk 8, there were no differences between treatment groups in deposition of Ig, C3, or C9 in glomeruli between treatment groups (Fig. 1f).

To examine the effect of these therapies on the site of immunization, peripheral lymph nodes draining the site were taken at 2 wk postimmunization. The mean weight of the popliteal lymph nodes from early rIL-4-treated animals (30 ± 4 mg/100 g body weight) was similar to CFA controls (32.2 ± 12.2 mg/100 g, p = NS), but was less than both HN controls (44.7 ± 11.8 mg/100 g, p = 0.03) and MRCOx-81-treated rats (55.7 ± 5.1 mg/100 g, p = 0.003).

FACS analysis of lymphocytes in these 2-wk popliteal lymph nodes showed a reduced B cell percentage in rats immunized with either CFA alone (27.6%), HN (27.6%), rIL-4-treated HN (16.8%), or MRCOx-81-treated HN (22.4%) compared with untreated male Lewis rats (41%). There were significant increases in CD4+ and CD8+ T cell subset percentages in CFA controls and all rats immunized with Fx1A/CFA regardless of treatment, compared with untreated controls (Fig. 2a). The CD3+ count did not rise consistently, which suggests the majority of the CD4+ count rise was due to macrophages, not T cells. RT-PCR of cytokine mRNA in the popliteal nodes showed up-regulation of mRNA for IL-4 in both early rIL-4 and early MRCOx-81-treated animals compared with HN and CFA controls (Fig. 2, b and c). IFN-γ mRNA was up-regulated in all rats immunized with Fx1A/CFA as compared with CFA controls. There was no difference between groups for IL-2 or IL-10 cytokine mRNA (Fig. 2, b and c). These studies showed that rIL-4 had the effect of reducing the accumulation and/or proliferation of lymphocytes in the regional node and enhancing mRNA for IL-4.

Proteinuria developed by 8 wk in all rats immunized with Fx1A in CFA, and control rats immunized with CFA never developed proteinuria (Table I). Proteinuria in the rIL-4-treated group was not different from HN controls at any time. Rats treated early with MRCOx-81 had significantly more proteinuria at 10 wk (217 ± 63 mg/100 g body weight/24 h) than either the early rIL-4-treated group (140.3 ± 73 mg/100 g/24 h) or the HN control group (107.4 ± 19.4 mg/100 g/24 h) (p < 0.05, n = 10). At 12 wk, these differences persisted (MRCOx-81, 268 ± 43.7 mg/100 g/24 h vs HN, 194.6 ± 62.7 mg/100 g/24 h, and early rIL-4, 181.5 ± 40.7 mg/100 g/24 h, p < 0.01). Rats treated with control therapies CHO-K1 and A6 developed proteinuria that was not different from HN controls (Table I). Similar results were obtained when the experiments were repeated on two other occasions.

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**FIGURE 1.** Ab responses. Comparison of Ig responses in early treatment with rIL-4 (●) and anti-IL-4 mAb (○) vs HN no treatment (■) and CFA alone (no Fx1A) controls (□), n = 6 per group. Anti-Fx1A Ab levels expressed as percentage binding of known strongly positive serum (Fx1A titer 1:250), measured by ELISA and expressed as mean ± SEM. a. Rats treated early with rIL-4 show high titer of total Ig by 2 wk as compared with HN- and MRCOx-81-treated groups (**, p < 0.001), but not at later points. b. At 2 wk, the rIL-4-treated group has a significantly stronger IgG1 response than other groups (*, p < 0.001), which persists until 8 wk. MRCOx-81-treated animals developed a stronger IgG2a response than HN- and rIL-4-treated animals (*, p < 0.03). c. IgG2b response was not significantly different. d. Anti-Crry Ab titers from 8-wk sera. Only rIL-4-treated rats had significant titers of anti-Crry Abs. The early (0–2 wk) rIL-4 treatment group (rIL-4§) showed the highest binding (**, p < 0.001 compared with CFA), while rats treated with rIL-4 from 4 wk (rIL-4§) also developed Abs (*, p < 0.01 compared with CFA). e. Glomerular deposition of Ig, C3, and C9 from wk 8 biopsies of CFA (■), HN (■– ■), rIL-4 (■– ■), and MRCOx-81 (■– ■)-treated rats. There was no difference between treatment groups and HN rats.
The mononuclear cell infiltrate in renal cortex was examined at 10 wk. As previously reported, HN was associated with an infiltrate of TCR-αβ+, CD4+ and CD8+ T cells, but not NK cells (3.2.3) compared with CFA controls. Both rIL-4- and MRCOx-81-treated rats had marked TCR-αβ+, CD4+, and macrophage infiltrates compared with CFA controls. There was increased macrophage infiltrate in the early MRCOx-81-treated rats when compared with HN disease controls and rIL-4-treated rats (p < 0.001). The CD8+ infiltrate in HN- and MRCOx-81-treated rats was not statistically different. In contrast, early rIL-4 treatment did not alter the number of infiltrating macrophages stained with ED1, but reduced the infiltrate of CD8+ cells as compared with HN controls and MRCOx-81-treated rats (p < 0.01).

Taken together, these studies show that rIL-4 treatment caused acceleration of the Th2 response, as manifested by high IgG1 anti-Fx1A titers, but this did not accelerate the onset of proteinuria. Anti-IL-4 mAb therapy increased the titer of complement-fixing IgG2a anti-Fx1A Ab, and was associated with increased proteinuria and increased macrophage infiltrate in renal cortex. Development of proteinuria was not dependent upon the presence of anti-Cry Abs, which were only detected with rIL-4 treatment and not in control HN or anti-IL-4 mAb-treated groups (p < 0.05, Fig. 1e).

Effect of treatment with rIL-4 and MRCOx-81 from 4–6 wk after Fx1A/CFA immunization

Therapy with rIL-4 commencing 4 wk after CFA/Fx1A immunization inhibited the development of proteinuria at 8, 10, and 12 wk compared with HN controls and controls sham treated with supernatant from CHO-K1 cells not transfected with cytokine (Table II and Fig. 3a). Only 2 rats of 11 (from two experiments) treated with rIL-4 at 4 wk went on to develop proteinuria. MRCOx-81 therapy again increased proteinuria by 12 wk compared with HN controls and isotype control mAb-treated rats (Table II).

Table II. Proteinuria after treatment with rIL-4 and MRCOx-81 for 10 days commencing at the time of Fx1A immunization (milligrams/100 g/24 h, mean ± SD) 

<table>
<thead>
<tr>
<th>Immunized Treatment</th>
<th>CFA</th>
<th>CFA/Fx1A</th>
<th>CFA/Fx1A</th>
<th>CFA/Fx1A</th>
<th>CFA/Fx1A</th>
<th>CFA/Fx1A</th>
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<tbody>
<tr>
<td>4</td>
<td>10.5 ± 2.5</td>
<td>10.5 ± 2.5</td>
<td>7.7 ± 0.9</td>
<td>7.4 ± 1.8</td>
<td>8.4 ± 1.0</td>
<td>13.1 ± 2.7</td>
</tr>
<tr>
<td>8</td>
<td>51.3 ± 14.2</td>
<td>65.3 ± 9.3</td>
<td>48.4 ± 27</td>
<td>64.6 ± 10.4</td>
<td>75 ± 15</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>107 ± 19.4</td>
<td>140.3 ± 74</td>
<td>113 ± 47</td>
<td>217 ± 63*</td>
<td>108 ± 38</td>
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</tr>
<tr>
<td>12</td>
<td>195 ± 63</td>
<td>181.5 ± 41</td>
<td>201 ± 35</td>
<td>268 ± 44**</td>
<td>195 ± 58</td>
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</table>

* All treatments given >10 days from the time of immunization with Fx1A/CFA. Treatment with MRCOx-81 increased proteinuria to 10 wk (*, p < 0.05) and 12 wk (**, p < 0.02) compared with HN controls. Treatment with rIL-4 at the time of Fx1A/CFA immunization had no effect on proteinuria (minimum of five rats per group; experiment repeated twice).

† Weeks postimmunization.

‡ Cytokine control.

§ IgG1 isotype control Ab.
Effect of treatment with rIL-4 or MRCOx-81 commencing 4 wk after Fx1A/CFA immunization on various parameters. All values are mean ± SD, n = minimum 5 per group. Groups were CFA (círculo), HN (círculo triangular), rIL-4 (círculo redondo), and MRCOx-81 (círculo). a, Proteinuria. Proteinuria in wk 4 rIL-4-treated rats was less than HN rats by 8 and 10 wk (**, p < 0.001), and still significantly less at 12 wk (+, p < 0.01). MRCOx-81-treated rats had more proteinuria at 12 wk (**, p < 0.001). b, Total anti-Fx1A titers. All groups immunized with Fx1A/CFA developed raised titers of anti-Fx1A Ab, but there were no significant differences. c, Glomerular deposition of rat Ig, C3, and C9 in glomeruli of HN and MRCOx-81 groups (p < 0.001). (Figs. 4 and 5). There was a trend to less TCR-αβ+ cells with rIL-4 therapy, but no difference in macrophage infiltrate. The B cell infiltrate was increased with rIL-4 therapy.

Semiquantitative RT-PCR of isolated glomeruli at 12 wk post-Fx1A/CFA immunization in rIL-4-treated rats demonstrated less mRNA for IFN-γ (p < 0.01), perforin (p < 0.01), and TNF-β (p < 0.05) when compared with HN controls (Fig. 4, b and c), with levels not different from CFA controls. Other cytokines were present at levels comparable with HN controls and greater than CFA controls (Fig. 4, b and c).

The onset of proteinuria in HN has been associated with increased glomerular mRNA for Th1 (IL-2, IFN-γ, and TNF-β) and macrophage cytokines (IL-10, TNF-α, and inducible NO synthase (iNOS)). This work has shown that while Th2 and macrophage cytokine levels of rIL-4-treated rats were similar to HN controls and greater than CFA controls, Th1 and Tc1 glomerular cytokine levels in rIL-4-treated rats were reduced to the level of CFA controls.

Effect of treatment with rIL-4 starting 8 wk after immunization with Fx1A

rIL-4 treatment given after the onset of proteinuria in HN was unable to inhibit or reverse proteinuria (Table II).

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**FIGURE 3.** Effect of treatment with rIL-4 or MRCOx-81 commencing 4 wk after Fx1A/CFA immunization on various parameters. All values are expressed as mean ± SEM, n = minimum 5 per group. Groups were CFA (círculo), HN (círculo triangular), rIL-4 (círculo redondo), and MRCOx-81 (círculo). a, Proteinuria. Proteinuria in wk 4 rIL-4-treated rats was less than HN rats by 8 and 10 wk (**, p < 0.001), and still significantly less at 12 wk (+, p < 0.01). MRCOx-81-treated rats had more proteinuria at 12 wk (***, p < 0.001). b, Total anti-Fx1A titers. All groups immunized with Fx1A/CFA developed raised titers of anti-Fx1A Ab, but there were no significant differences. c, Glomerular deposition of rat Ig, C3, and C9 in glomeruli at 12 wk. There were no differences between HN (círculo) and either rIL-4-treated (círculo redondo) or MRCOx-81 (círculo triangular) treatment groups, but all were higher than CFA rats (círculo). d-f, Anti-Fx1A isotype titers. There were no significant differences between groups.
Effect of treatment with rIL-13 given 4–6 wk post-Fx1A immunization

IL-13 is a cytokine that has a similar effect as IL-4 in inhibiting macrophage activation, but has no effect on T cells or Ab responses in rats (23). We therefore used rIL-13 therapy in HN as a means of assessing the importance of activated macrophages in the development of HN. rIL-13 was given in a dose that has a similar effect as rIL-4 on neonatal allograft survival, and has the capacity to reduce macrophage activation (2). In these experiments, rIL-13-treated rats compared with HN controls had less glomerular macrophage infiltration at 12 wk, as well as less glomerular mRNA for the activated macrophage cytokines TNF-α and iNOS (Fig. 6, b and c). Despite this, rIL-13-treated rats had similar amounts of proteinuria compared with HN controls (Fig. 6a).

Discussion

More than 40 years after Heymann’s original description of HN, there is ongoing controversy as to the underlying immune mechanisms that cause injury in HN and human membranous GN. Most studies suggest Ab deposition and complement activation is the main effector mechanism, while our studies demonstrate that Th1 effector cells and macrophages mediate injury. In this study, treatment with IL-4 or anti-IL-4 mAb altered immune responses in HN. IL-4 inhibited the Tc1 cells and enhanced the autoantibody or anti-Crry responses.

When rIL-4 treatment was commenced 4 wk after Fx1A immunization, it prevented development of proteinuria. This was after the anti-Fx1A autoantibody response had developed and Ig and C3 had deposited in glomeruli. Treatment with rIL-4 at this time had no effect on anti-Fx1A titers or the IgG isotypes of these Abs, but did increase the anti-Crry response. This response was paradoxical, as development of these Abs has been reported to be necessary for the development of proteinuria in HN (14).

Complement fixing IgG to Fx1A is thought to bind to Ag in glomeruli, then activate complement to form the C5b-9/MAC complex. Glomerular deposition of IgG, C3, and C9 was not affected by rIL-4 treatment. Rats treated with rIL-4 had reduced infiltrate of CD8+ cells in glomeruli, but glomerular macrophage and other mononuclear cell accumulation was similar to HN controls. mRNA levels for IFN-γ, TNF-β, and perforin were significantly reduced in the glomeruli of rIL-4-treated rats compared with HN controls, consistent with reduced infiltrate of Tc1 cells. Taken together, these results suggested that rIL-4 inhibited the Tc1 response, which we have previously demonstrated is necessary for induction of proteinuria in this model (17). We have proposed that a cytotoxic CD8+ T cell response only develops several weeks post-Fx1A immunization and occurs secondary to the deposition of anti-Fx1A Ab and complement activation in glomeruli (17). The findings in this study are consistent with this hypothesis, since administration of rIL-4 at 4 wk post-Fx1A immunization markedly reduced proteinuria, while rIL-4 treatment at the time of immunization with Fx1A or after the onset of proteinuria had no effect on proteinuria.
Therapy with rIL-4 at the time of Fx1A immunization caused an accelerated autoantibody response with isotype switching to enhance IgG1 and reduce complement-fixing IgG2a, as well as increased IL-4 mRNA expression in the draining lymph nodes. These findings demonstrated rIL-4 induced Th2 cells. Even so, these rats developed proteinuria. The failure of early rIL-4 treatment to stop proteinuria also supports our proposal that a delayed CD8$^+$ T cell response is the mediator of injury in HN, as the short-acting rIL-4 would not be available at the later time when cytotoxic T cells are activated.

Anti-IL-4 mAb (MRCOX-81) therapy when given at the time of immunization or 4–6 wk after immunization with Fx1A resulted

FIGURE 5. Immunoperoxidase staining of isolated glomeruli. Isolated glomeruli from different treatment groups showing deposition of rat Ig (OX-12), rat C3, and rat C9. Infiltration of CD8$^+$ cells (OX8$^+$) was reduced in rIL-4-treated rats to approximately the same level as that seen in CFA controls.

FIGURE 6. a, The effect of rIL-13 on proteinuria in HN CFA ( ), HN ( ), rIL-13 ( ), and CHO-K1 supernatant (cytokine control) ( ). rIL-13 administered for 10 days starting 4 wk after immunization with Fx1A/CFA had no effect on proteinuria. b, Glomerular infiltrate at 12 wk in rats with HN treated with IL-13. The infiltrate of cells staining for ED1 (macrophages/Ma/dendritic cells) and W3/25 (CD4$^+$ cells and some macrophages) was reduced in the rIL-13-treated rats, $p < 0.01$. Results expressed as mean ± SEM of number of cells per glomerulus, $n = 4$ rats per group. c, Expression of cytokine mRNA in isolated glomeruli at 12 wk in rats treated with rIL-13. When compared with HN controls, rats treated with rIL-13 had less glomerular mRNA for TNF-α and iNOS, $p < 0.01$. Results expressed as mean cycle number of first appearance ± SD, $n = 4$ rats per group. Mo, mononuclear cells.
in greater proteinuria than untreated HN controls. Early therapy with MRCOx-81 caused increased IgG2a and reduced IgG1 anti-Fx1A titers consistent with it blocking IL-4 and allowing Th1 cells to facilitate isotype switching to complement-fixing Ig. MRCOx-81-treated rats had increased macrophage infiltrate into renal cortex, which may have been due to an enhanced Th1 response or the greater proteinuria. The higher IL-4 mRNA in popliteal lymph node is consistent with MRCOx-81 blocking released IL-4, but not production of IL-4 at a cellular level. Similar increased IL-4 mRNA has been observed in MRCOx-81-treated transplant recipients (29). The loss of negative feedback from IL-4 may enhance its production by Th2 cells, resulting in higher mRNA for IL-4. The worsening of proteinuria seen with blocking IL-4 is consistent with IL-4 having a natural role in regulation of severity of disease.

Therapy with rIL-13 reduced both glomerular macrophage accumulation and expression of mRNA for activated macrophage cytokines TNF-α and iNOS in glomeruli, but rIL-13 had no effect on proteinuria in HN or on Th1 cell accumulation in glomeruli. This suggests that the glomerular macrophages seen in HN are not the primary cause of proteinuria, and supports the finding that rIL-4 therapy reduced proteinuria by inhibition of Th1 cell, but not macrophage infiltrate into glomeruli. This finding is in distinction to the disease-suppressive effect of IL-13 therapy in experimental autoimmune encephalomyelitis, a disease in which macrophage injury is important and Th1 cells play little or no role (23).

Taken together, the results of this study support the role of cellular immune mechanisms in the glomerular injury of HN. Early work by Heymann and others showed that splenic or lymph node cells from proteinuric rats could transfer nephrosis to naive rats, suggesting the importance of cellular immune mechanisms in the pathogenesis of this model (44, 45). However, subsequent study of active and PHN has focused on the effects of IgG deposition and complement activation, concluding that sublytic MAC injury of the GEC leads to the typical GBM thickening and proteinuria of HN (11). The presence of subepithelial IgG and C3 in the GBM is one of the hallmarks of HN and human membranous nephropathy, and the importance of the early complement components in PHN is well established. Rats depleted of complement with cobra venom factor do not develop proteinuria in PHN (13, 46, 47), and Baker et al. (48) showed that depletion of C6 with a mAb prevented proteinuria in PHN. However, the pathogenic role of the MAC complex has been questioned with the induction of both active HN (15) and PHN (49, 50) in a C6-deficient PVG rat strain. Furthermore, a Th1/Th2 lymphocyte and macrophage infiltrate into the glomeruli of HN rats parallels the course of proteinuria (16), and rats depleted of CD8+ T cells do not develop proteinuria (17).

Since it has been assumed that the pathogenic role of complement is entirely due to MAC, little attention has been paid to other potential immunostimulatory or chemotactic effects of early complement components deposited in the GBM. The finding that early complement components are required for development of PHN is still consistent with our proposal that secondary cellular immune mechanisms are operative in HN, as cellular immune injury is stimulated at sites of immune complex formation or deposition. C3b and C4b are important in facilitating Ag presentation and activation of T cell responses (51–53).

Recent support for complement-mediated injury in HN came from the finding that proteinuria in HN required development of Abs to Crry that blocked the function of this complement-deactivating molecule and allowed complement-mediated injury to proceed (14). It is important to note that Crry (the rodent equivalent of decay-accelerating factor and monocyte chemoattractant protein) inhibits complement activation at the C3 level, while another molecule, CD59, is required for inhibition of the MAC. In the present study, anti-Crry Abs were only detected in rats treated with rIL-4. They were not detected in HN controls or our anti-IL-4 mAb (MRCOx-81)-treated groups, all of which developed massive proteinuria. An alternative explanation for the anti-Crry relationship to proteinuria is that less pure Ag preparations elicit autoantibodies to many glomerular Ags other than to gp330, including Crry. It is these Abs that may be required to trigger the secondary CD8+ T cell response. CD8+ T cell-mediated injury has been found to be the principal mechanism of injury in experimental interstitial nephritis (54–56). Furthermore, antitubular basement membrane-specific CD8+ T cell clones with low IFN-γ and perforin expression do not induce interstitial nephritis (57). Perforin has been shown to effect sublytic cellular injury in nucleated cells (58) analogous to the effects of MAC, and we propose that sublytic injury of the GEC is conducted by nephritogenic Th1 clones.

IL-4 is the prototypic Th2 cytokine (18) that has multiple effects on activated T lymphocytes, including directing the development of Th2 cells and blocking the development of Th1 cells (59, 60). IL-4 has been used successfully as an antinflammatory agent in Th1-type animal models of autoimmune disease, such as rodent models of crescentic (61) and anti-GBM glomerulonephritis (3), experimental autoimmune encephalomyelitis (62), arthritis (63), and diabetes (64). It is generally thought that the antinflammatory effect of IL-4 in these models is due to induction of a Th2 phenotype combined with direct inhibition of the Th1 response. IL-4 is known to cause specific phenotypic as well as functional inhibition of CD8+ Th1 cells (Th1 cells); CD8+ cells activated in the presence of IL-4 stop expressing CD8, IFN-γ, and perforin (19), and are thus rendered noncytotoxic. The reduction in glomerular CD8+ T cell infiltration, but not macrophage infiltration seen with rIL-4 therapy suggests that the rIL-4 effect in HN is mediated through specific inhibition of nephritogenic Th1 lymphocytes.

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References


