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Regulatory interactions of αβ and γδ T cells in glomerulonephritis

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Regulatory interactions of αβ and γδ T cells in glomerulonephritis.

Background. Several lines of evidence suggest that cellular immune mechanisms contribute to glomerulonephritis.

Methods. The roles of αβ and γδ T cells in the pathogenesis of glomerulonephritis were investigated in a model of nephrotoxic nephritis in mice deficient in either T-cell population [T-cell receptor (TCR)β and TCRδ knockout mice]. The model, induced by the injection of rabbit anti-mouse glomerular basement membrane antibody, is characterized by the development of proteinuria and glomerular damage over a 21-day observation period in wild-type mice.

Results. Mice deficient in either αβ or γδ T cells developed minimal proteinuria and glomerular lesions and had a significant reduction in macrophage accumulation compared with wild-type mice. In γδ T-cell–deficient mice, circulating levels and glomerular deposition of autologous IgG were comparable to wild-type levels, while αβ T-cell–deficient mice had no autologous IgG production. Autologous antibody production was not required for the development of glomerulonephritis since mice that lack IgG and B cells (μ-chain−/−) developed similar proteinuria to that observed in wild-type mice.

Conclusions. These studies suggest a proinflammatory role for both αβ and γδ T cells in glomerular injury, independent of the humoral response. This is the first demonstration, to our knowledge, that both T-cell subsets contribute to the progression of a disease, and it suggests that complex regulatory interactions between αβ and γδ T cells play a role in glomerular injury.

Glomerular accumulation of T cells and other manifestations of cell-mediated immune injury have been observed in patients with proliferative and crescentic forms of glomerulonephritis (GN) [1]. In animals, there is compelling evidence that sensitized nephritogenic T cells, as a result of cell-mediated immune reactions, can initiate and amplify inflammatory renal lesions independently of antibody deposition [2–7]. A recent study in mice suggests that antibody facilitates the development of glomerular disease only in major histocompatibility complex (MHC) haplotypes capable of generating a subset of nephritogenic T cells [8]. Together, these studies indicate that cell-mediated immunity participates in the initiation and progression of glomerular injury in GN.

After priming T cells in secondary lymphoid tissue, the first effector step of cellular immunity in GN is the presentation of the relevant antigen in the renal parenchyma to appropriate T cells for recognition. Intrinsic kidney cells can act as effective antigen-presenting cells in the intact animal [6]. Importantly, cellular immunity to a glomerular antigen in human GN has been described [9], and the transfer of glomerular antigen-sensitized T cells can induce GN [6, 8, 10]. Despite this evidence, the functional role of the two T-cell populations expressing αβ and γδ T-cell receptors in the development of glomerular injury has not been directly assessed. αβ T cells are $CD4^+$ and $CD8^+$ positive cells that recognize MHC-associated peptides. $CD4^+$ αβ T cells provide help, in the form of cytokines, to stimulate B cells, activate macrophages, and promote inflammation and cytotoxic lymphocyte differentiation. $CD8^+$ αβ T cells express cytotoxic functions [11]. γδ T cells are predominantly $CD4^+$ and CD8β negative, but occasionally express CD4 and CD8α [12, 13]. γδ T cells represent only 5% of the circulating T-cell population in humans and mice [11]. The physiological role of γδ T cells is unclear, in part because the ligands recognized by γδ T-cell receptors are largely unknown [14]. In vivo, γδ T-cell–deficient mice show no obvious defects in antigen-specific immunities to a number of foreign antigen challenges [15, 16]. In vitro, γδ T cells can respond to stressed autologous cells, unprocessed stress associated antigens expressed by damaged cells such as heat shock proteins, nonclassic MHC class 1b

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molecules that may be expressed by cell stress, and nonprotein antigens containing critical phosphate moieties [14, 15]. They are known to elaborate chemokines that may recruit macrophages, secrete effector cytokines, and mediate natural killer-like cytotoxicity [14, 17, 18].

Both αβ and γδ T cells are present in normal human kidney, although the majority of the cells are $CD4^+$ and $CD8^+$ αβ T cells. An increase in αβ and γδ T cells in human GN suggests that both T-cell subsets may play a role in the pathogenesis of GN [19]. Infiltration of the kidney by both αβ and γδ T cells has been noted in IgA nephropathy. Interestingly, the presence of γδ T cells was essentially restricted to patients with progressive disease [20].

We examined the role of the αβ and γδ T cells in the pathogenesis of GN by evaluating a complement-dependent Th1 predominant model of accelerated nephrotoxic nephritis [21] in αβ or γδ T-cell–deficient mice generated by targeting the disruption of TCRβ or TCRδ chains, respectively [22–24]. GN in wild-type mice was characterized by significant proteinuria, histologic indices of glomerular damage, and T-cell and macrophage accumulation. In contrast, αβ T-cell–deficient mice developed no proteinuria or histologic evidence of glomerular injury. γδ T-cell–deficient mice developed minimal proteinuria and glomerular injury compared with wild-type mice, in spite of normal renal accumulation of $CD4^+$ T cells, suggesting that $CD4^+$ αβ T cells are not pathogenic in the absence of γδ T cells. Both sets of T-cell–deficient mice had a significant reduction in interstitial macrophage accumulation, whereas glomerular complement deposition was not significantly impaired. Autologous antibody production was absent in αβ T-cell–deficient mice. However, μ-chain–deficient mice that lack B cells and an IgG response developed injury that was comparable to that of wild-type mice, suggesting that the absence of autologous antibody production was not the protective factor in αβ T-cell–deficient mice. Our studies point to an important proinflammatory role for both αβ and γδ T cells in glomerular injury, independent of IgG production, and involving the recruitment of macrophages. The requirement for both T-cell populations for expression of glomerular disease suggests that functional interactions between the αβ and γδ T cells may play a role in the disease process.

METHODS

Study design

Mouse strains on a C57BL/6J background with targeted mutations described later in this article section and their wild-type C57BL/6J controls were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained in the virus/antibody free facility at Longwood Medical Research Center (Boston, MA, USA). The following knockout mice were used: mice with a targeted mutation of the β chain of the T-cell receptor ($TCR\beta^{-/-}$) [25], the δ chain of the T-cell receptor ($TCR\delta^{-/-}$) [24], and the membrane exon of the immunoglobulin μ chain gene ($\mu^{-/-}$) [26]. Male mice between seven and nine weeks were used throughout the studies.

Accelerated nephrotoxic nephritis was induced essentially as previously described [21]. Briefly, mice were preimmunized subcutaneously with 0.1 mg of rabbit IgG (Pel-Freeze Biologicals, Rogers, AZ, USA) dissolved in incomplete Freund's adjuvant and nonviable desiccated Mycobacterium tuberculosis H37Ra (Difco Laboratories, Detroit, MI, USA). Three days later, a tail vein injection of heat-inactivated rabbit anti-mouse glomerular basement membrane (GBM) antiserum at a concentration of 5 mg/20 g body weight was given. Twenty-four–hour urine samples were collected in metabolic cages at days 1, 7, 14, and 21 after induction of disease. At days 14 and 21, mice were anesthetized with methoxyflurane, and peripheral blood was collected by retroorbital bleeding into glass tubes to obtain serum. At these time points, a set of animals was euthanized with CO_2 inhalation, and both kidneys were harvested. The kidneys were cut in half, and the corresponding poles of the contralateral kidneys were either fixed in formalin or snap frozen in Optimum Cooling Temperature (OCT) embedding medium (Miles, Elkhart, IN, USA). In select cases, part of the kidney was homogenized in TRIzol for use in the ribonuclease protection assays (RPAs).

Assessment of glomerular injury

Formalin-fixed renal tissue were embedded in paraffin, cut in 4 μm sections, and stained with periodic acid Schiff's stain (PAS) for histologic analysis. The presence of PAS-positive material within the glomeruli was analyzed semiquantitatively in at least 50 glomeruli per mouse as previously described [21]: 0 = no deposition of PAS-positive material, 1 = up to one third, 2 = one third to two thirds, and 3 = more than two thirds of the glomerular cross-section stain positive for PAS. Only glomeruli cut in equatorial cross section were scored.

Sections cut from frozen tissue (4 μm) were used for immunofluorescence or immunoperoxidase staining using standard techniques. For the detection of heterologous IgG deposition, sections were stained by direct immunofluorescence with FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA). For the detection of autologous IgG, FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc.) was used. Semiquantitative assessment of the glomerular deposition of IgG was performed by determining the end point positive titer for detection of staining using serial dilutions of each antibody.

The three-layer immunoperoxidase technique was used

for the detection of macrophages and T cells in the kidney sections as previously described [21]. Macrophages were stained using a rat anti-mouse mature macrophage antibody (Clone F4/80; Caltag Laboratories Inc., Burlingame, CA, USA), and semiquantitative scoring was performed as follows: 0 = no cells stained positive, 1+ = 5 to 10 cells, 2+ = 10 to 50 cells, 3+ = 50 to 200 cells, and 4+ = over 200 cells stained positive per low-power field. For the detection of $CD4^+$ T cells a rat anti-mouse CD4 monoclonal antibody (mAb) was used (clone L3T3; Pharmingen, San Diego, CA, USA), for $CD8^+$ T cells a rat anti-mouse CD8a mAb (Clone Ly-2; Pharmingen) was used. In all cases, an IgG2a isotype antibody (Clone G155-178; Pharmingen) served as a negative control. Quantitation of T cells was done by counting the number of cells in 10 adjacent high-power fields of cortex and medulla.

Measurement of circulating mouse anti-rabbit globulin antibody

Mouse anti-rabbit globulin titers were assessed by enzyme-linked immunosorbent assay (ELISA) on serum collected at days 14 or 21 as previously described [21]. Serum from each mouse was tested in serial dilutions from 1:100 to 1:800. Results are shown at a dilution of 1:200 and are given in arbitrary units.

Functional assessment of glomerular injury: Albuminuria

Mice were housed individually in metabolic cages to collect urine over 24 hours at the indicated time points. Urinary albumin concentrations were determined by a double-sandwich ELISA as described extensively elsewhere [21]. Additionally, urine creatinine levels were measured by the alkaline picric acid method using a commercially available kit (Sigma Chemicals Co., St. Louis, MO, USA). To standardize urine albumin excretion in animals for glomerular filtration rates, albuminuria was expressed as μg albumin per mg of urinary creatinine.

Analysis of mRNA transcripts by ribonuclease protection assay (RPA)

Kidneys were frozen on dry ice at necropsy. The tissue was homogenized in TRIzol reagent (Life Technologies, Gaithersburg, MD, USA) with a Dounce-type homogenizer. Total RNA was then subjected to a multiprobe RPA system (Riboquant; Pharmingen) using probes for multiple T-cell–related and leukocyte-related RNA molecules as described in the manufacturer's protocols. Dried gels were developed using a phosphorimager (Molecular Dynamics, Sunnyvale, CA, USA), and bands were quantitated using instrument-resident densitometry software (Image-Quant; Molecular Dynamics). Within each sample, the density of each specific mRNA transcript was divided by that of the L32 ribosomal RNA (L32) or glyceraldehyde phosphate dehydrogenase (GAPDH) band. The resulting ratio was then multiplied by 1000. For each specific mRNA species quantitated, differences between control and nephritic wild-type groups were statistically assessed by two-tailed Student's *t*-test.

Statistics

Results were expressed as the mean ± SEM. Significances of differences were determined by the unpaired *t*-test.

RESULTS

Mice deficient in αβ or γδ T cells have minimal proteinuria and glomerular injury

Mice deficient in TCRβ lack mature αβ T cells but maintain a full complement of γδ T cells [23, 25]. TCRδ-deficient mice are deficient in γδ T cell expressing cells but have normal αβ T-cell development, numbers, and T-cell effector functions [24]. To examine the functional role of αβ and γδ T cells in GN, αβ and γδ T-cell–deficient mice of the C57Bl/6 strain, and C57Bl/6 wild-type mice were subjected to a complement dependent Th1 predominant model of GN [21], as described in the **Methods** section. Proteinuria was determined, and a histologic evaluation of glomeruli was performed at intervals up to 21 days after induction of nephritis. Wild-type mice exhibited significant proteinuria at days 14 and 21. Proteinuria in αβ T-cell–deficient mice was absent (Fig. 1A), and γδ T-cell–deficient mice had mild proteinuria, although it was still significantly reduced compared with nephritic wild-type mice (Fig. 1A). Histologically, mild to moderate hypercellularity, focal deposition of PAS material, and occasional small crescents were observed in wild-type mice. These lesions were absent in αβ and γδ T-cell–deficient mice (Fig. 2). To obtain a quantitative score of the extent of glomerular injury the degree of glomerular PAS deposition was evaluated as previously described [21]. Glomerular PAS deposition was present in wild-type mice but was nearly absent in αβ and γδ T-cell–deficient mice (Fig. 1B).

Macrophage accumulation was evaluated in nephritic wild-type mice and mice deficient in αβ or γδ T cells. Although macrophages were occasionally observed in glomeruli, particularly in crescents, the numbers were small and thus not easily quantitated. In contrast, abundant macrophages were evident in the interstitium of wild-type nephritic mice. Quantitation of macrophage infiltration revealed that the numbers of macrophages in nephritic αβ and γδ T-cell–deficient mice were significantly reduced compared with nephritic wild-type mice (Table 1) and was similar to that seen in untreated mice. Our previous studies in C5-deficient mice have demonstrated that proteinuria was complement dependent [21]. In the present study, despite the significant attenuation

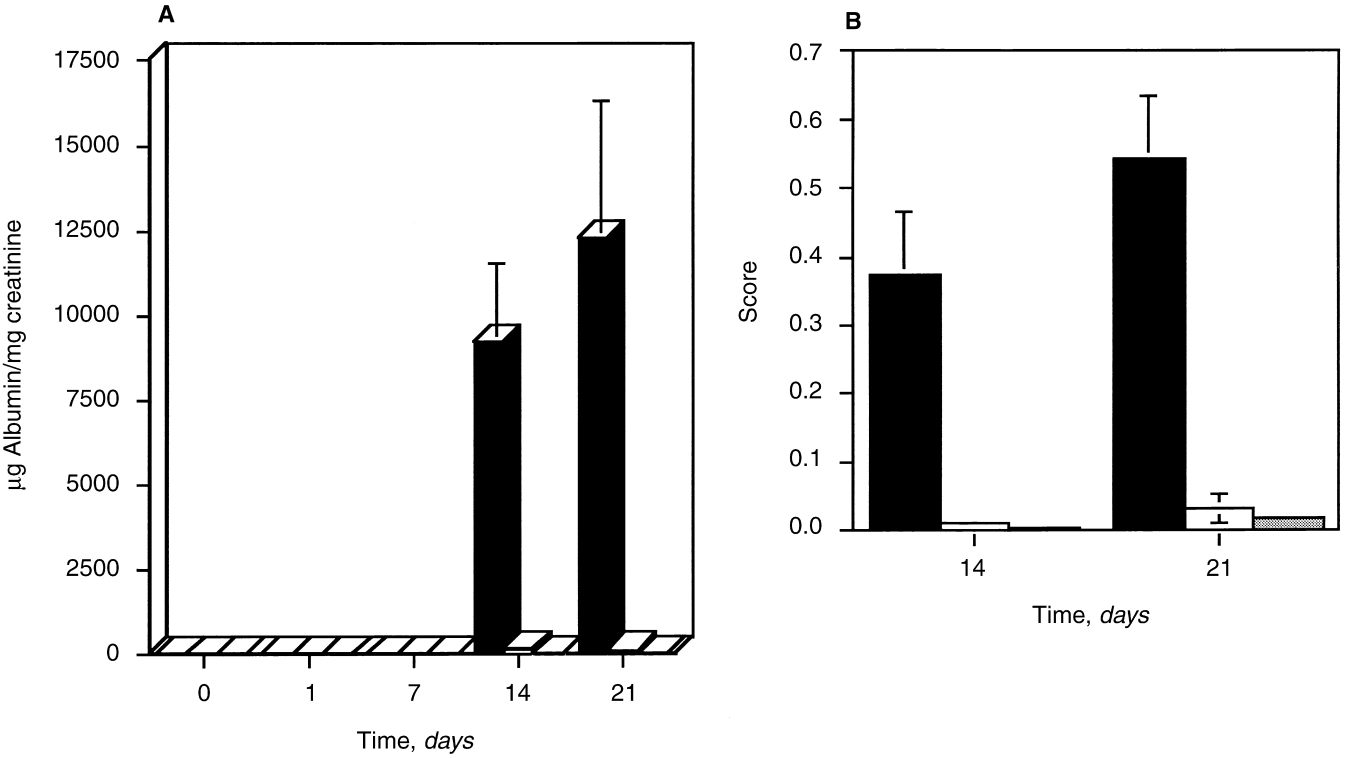

Fig. 1. Mice deficient in αβ and γδ T cells have significantly reduced proteinuria and PAS-positive deposits compared with wild-type mice. Nephritis was induced in wild-type mice (■), αβ (▩), and γδ T-cell–deficient mice (□). Proteinuria (*A*) and PAS+ deposits (*B*) were evaluated at days 0, 7, 14 ($N = 12$ per genotype), and 21 ($N = 8$ per genotype) after anti-GBM injection. (A) Urine albumin excretion (in micrograms) was determined and expressed per mg of urinary creatinine to standardize for the glomerular filtration rate. Wild-type mice had significant albuminuria, while αβ and γδ T-cell–deficient mice had minimal proteinuria. (B) PAS+ deposits were quantitated as a measure of glomerular injury. Scores are: 0 = no deposition of PAS+ material; 1 = up to one third; 2 = one third to two thirds; and 3 = more than two thirds of the glomerular cross-section stain positive for PAS. $P < 0.0005$ in αβ and γδ T-cell–deficient mice compared to wild-type mice.

of proteinuria in αβ- and γδ-deficient mice, intense linear deposition of complement C3 was observed in glomerular capillary loops of the majority of αβ and γδ T-cell–deficient mice and was not significantly reduced compared with that observed in wild-type mice (Fig. 3 and Table 1).

Humoral response to nephritogenic antibody deposition in the glomerulus

The deposition of nephritogenic rabbit anti-mouse GBM in glomerular capillary loops was equivalent in wild-type, αβ- and γδ T-cell–deficient mice following injection of anti-GBM antibody, as assessed by the mean immunofluorescence end point titer required for its detection. However, the deposition of autologous mouse antibody was absent in αβ T-cell–deficient mice and slightly attenuated in the γδ T-cell–deficient mice compared with wild-type mice (Fig. 3 and Table 1). Circulating mouse anti-rabbit IgG titers in the αβ T-cell–deficient mice were undetectable, suggesting a profound deficiency in autologous anti-rabbit IgG production. On the other hand, it was increased in γδ T-cell–deficient mice compared with wild-type mice (Table 1). Thus, αβ T cells, and not γδ T cells, provide B-cell help for mouse anti-rabbit antibody production in experimental GN, and γδ T cells may play an ancillary role in regulating the magnitude of the αβ T cell response.

T-cell infiltrates in nephritic kidneys

The presence of αβ and γδ T cells in kidneys was evaluated by subjecting RNA isolated from the kidneys of day 21 nephritic and untreated wild-type mice to a multiprobe RPA. This allowed simultaneous characterization of multiple T-cell–related RNA molecules, including TCRδ (for γδ T cells) and TCRα (for αβ T cells). TCRδ and TCRα were observed in normal kidney, but only TCRγ was significantly increased in nephritic kidneys (Fig. 4). Immunohistochemical evidence for these two T-cell populations in renal tissue was sought using TCR-specific antibodies. However, the available TCR antibodies were unable to visualize αβ and γδ T cells in the kidney, despite the consistent staining for both T-cell subsets in murine small intestines using the same reagents (data not shown). Although the lack of αβ and γδ T-cell–

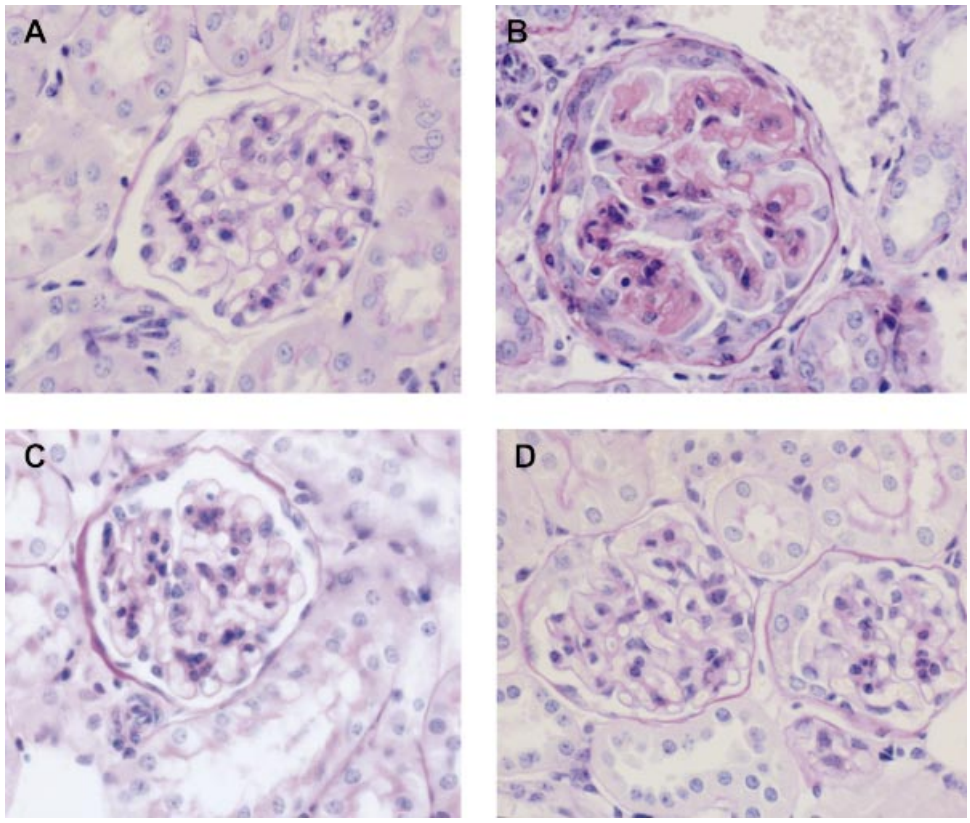

Fig. 2. Representative PAS-stained sections from control mice (*A*), 21-day nephritic wild-type mice (*B*), αβ (*C*), and γδ (*D*) T-cell–deficient mice. Glomeruli from nephritic wild-type mice show focal hypercellularity and PAS-positive deposition along the mesangium and capillary wall. Glomeruli from αβ and γδ T-cell–deficient mice show minimal changes.

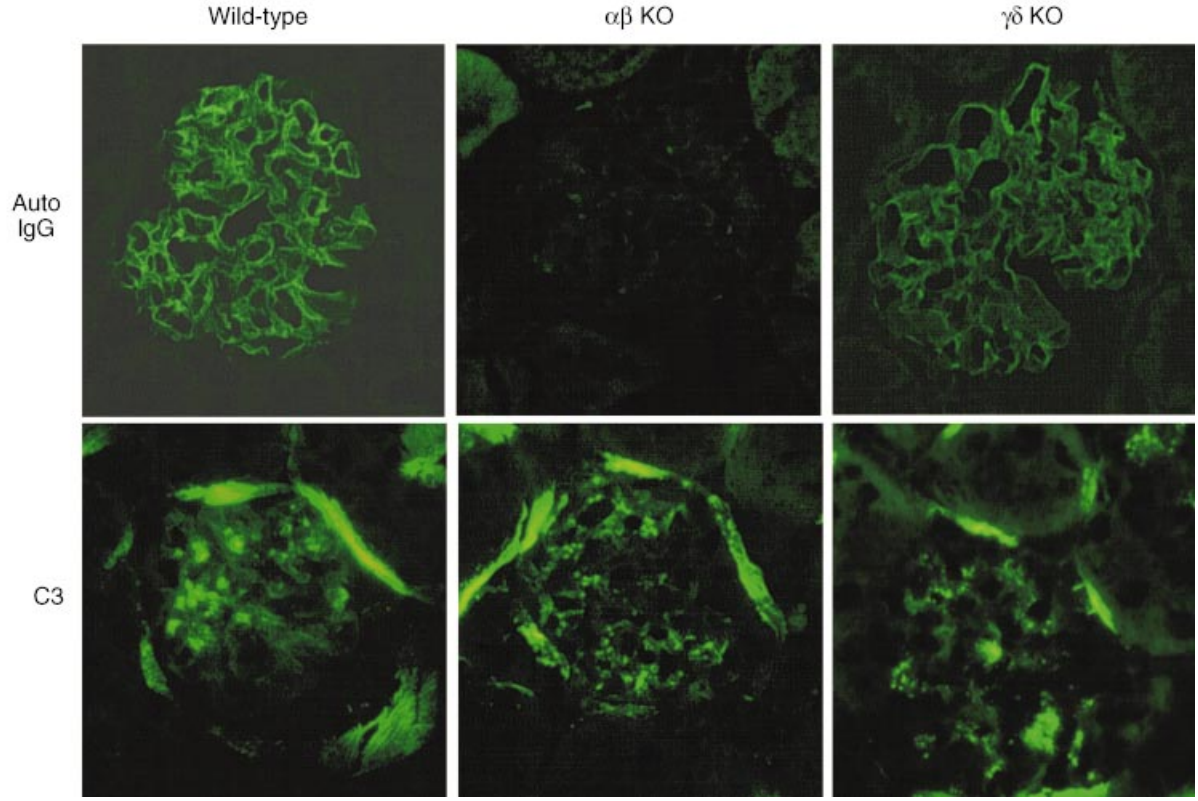

Fig. 3. Glomerular deposition of complement and mouse antirabbit antibody. Autologous anti-rabbit antibody (Auto. IgG) and complement C3 deposition was evaluated in renal tissue harvested from wild-type, αβ T-cell–deficient (αβ KO), and γδ T-cell–deficient (γδ KO) mice 21 days following anti-GBM antibody injection. A representative glomerulus from each experimental group is shown. The glomerulus in wild-type mice had significant antibody deposition, as did that of the γδ KO mice. There was no detectable antibody deposition in the glomerulus of αβ KO mice. Punctate staining for C3 was evident in the glomeruli of mice in all three experimental groups.

Table 1. Comparison of immune effectors in glomeruli of αβ and γδ T-cell–deficient mice subjected to anti-GBM glomerulonephritis

	Wild-type	αβ KO	γδ KO
Complement (glomerulus)			
d14	1.88 ± 0.13	1.25 ± 0.25	1.25 ± 0.14
d21	2.25 ± 0.16	2.25 ± 0.16	1.50 ± 0.27
Macrophages (interstitium)			
d14	2.25 ± 0.27	1.19 ± 0.19[a]	1.44 ± 0.12[a]
d21	2.72 ± 0.18	1.72 ± 0.09[b]	1.21 ± 0.21[b]
Heterologous IgG (glomerulus) end point titer	1:1600	1:1600	1:1600
Autologous IgG (glomerulus) end point titer	1:3200	none detected	1:1600–1:3200
Autologous IgG (serum)			
d14	1620.6 ± 258.1	0	1740.8 ± 268.0
d21	726.3 ± 202.8	0	1118.8 ± 138.1

Analysis for glomerular autologous and heterologous IgG is shown for day 21. Similar trends between the genotypes were seen for samples from d14 (data not shown). Autologous IgG is given in arbitrary units. Complement and macrophage scores are assigned as previously described [21].

Abbreviations are: αβ KO, αβ T-cell–deficient; γδ KO, γδ T-cell–deficient.

[a] $P < 0.05$, [b] $P < 0.0005$ compared with wild-type mice

specific staining in the kidney may be due to technical factors, it is also possible that the TCRs in glomerular localized T cells are down-regulated because of T-cell activation and are thus not detected. Rapid and long-lasting down-regulation of TCR cell surface expression has been shown to occur in response to T-cell–activating stimuli [27–29]. RNA for CD4 T cells but not CD8 T cells was significantly increased in nephritic kidneys compared with control kidneys (Fig. 4), a finding that was confirmed by immunohistochemistry, as described later in this article. F4/80 RNA, reflecting macrophage infiltration, was increased six- to tenfold in nephritic versus control kidneys (Fig. 4).

The time course of $CD4^+$ and $CD8^+$ T-cell infiltration in the nephritic kidneys of wild-type mice studied by immunohistochemistry revealed that 24 hours after injection of anti-GBM antibody, $CD4^+$ and $CD8^+$ numbers significantly decreased but then increased by days 14 and 21, exceeding the numbers in untreated kidney (data not shown). The extent of interstitial infiltration of $CD4^+$ and $CD8^+$ T cells at 14 and 21 days in nephritic αβ and γδ T-cell–deficient mice was then evaluated (Fig. 5). Such analysis in γδ T-cell–deficient mice should reveal whether the renal localization of αβ T cells, which are $CD4^+$ or $CD8^+$, is affected in these mice following nephritis. As shown in Figure 5, the accumulation of $CD4^+$ αβ T cells was similar in γδ T-cell–deficient mice and wild-type mice, suggesting that localization of $CD4^+$ αβ T cells does not require γδ T cells. However, the accumulation of $CD8^+$ T cells was compromised in the absence of γδ T cells. A similar analysis in αβ T-cell–deficient mice was undertaken. In this case, the analysis revealed only the small population of γδ T cells that were CD4 or CD8 positive; the majority of the γδ T cells, which were $CD4^-$ $CD8^-$, were not detected by this approach. In αβ T-cell–deficient mice, $CD4^+$ T cells and a few $CD8^+$ T cells were detected, suggesting that $CD4^+$ and $CD8^+$ γδ T cells accumulate in the kidneys of nephritic αβ T-cell–deficient mice.

B-cell–dependent IgG production is not required for GN

IgG deposition in the glomerulus was absent in αβ T-cell–deficient mice but only slightly altered in γδ T-cell–deficient mice (Table 1). However, despite the normal levels of IgG production and deposition in γδ T-cell–deficient mice, the IgG subtype distribution may have been altered since cytokines produced by γδ T cells may be needed to induce class switching to pathologic Ig isotypes or to alter the affinity of the autologous antibodies [17, 30]. We therefore addressed the importance of B-cell–dependent autologous antibody production in the pathogenesis of GN. We examined the model in wild-type mice and mice that have a targeted mutation in the μ-chain; these μ-chain mutants have no B cells and lack IgG production [26]. Proteinuria in μ-chain knockout mice was similar to that seen in wild-type mice at day 14 but was significantly reduced at day 21 (Fig. 6). The histologic changes, as judged by quantitation of PAS-positive deposits in the glomeruli of knockout mice, were similar to that in wild-type mice (data not shown). As expected, there was no circulating autologous mouse anti-rabbit IgG [WT (in arbitrary units): 1198 ± 123.1; μ-chain−/−; 0) nor was there any deposited in the kidney [WT (end point titer): 1:3200; μ-chain−/−: none detected] at day 21 after injection of anti-GBM antibody. In addition, complement deposition in the glomerulus of μ-chain–deficient mice was similar if not higher than that seen in wild-type mice (WT, 2.00 ± 0.41; μ-chain−/−, 2.80 ± 0.20), and macrophage accumulation in the interstitium was only slightly reduced compared with wild-type mice (WT, 2.5 ± 0.27; μ-chain−/−, 1.9 ± 0.13). Thus, production of autologous antibodies to the heterologous anti-GBM antibody is not required for the devel-

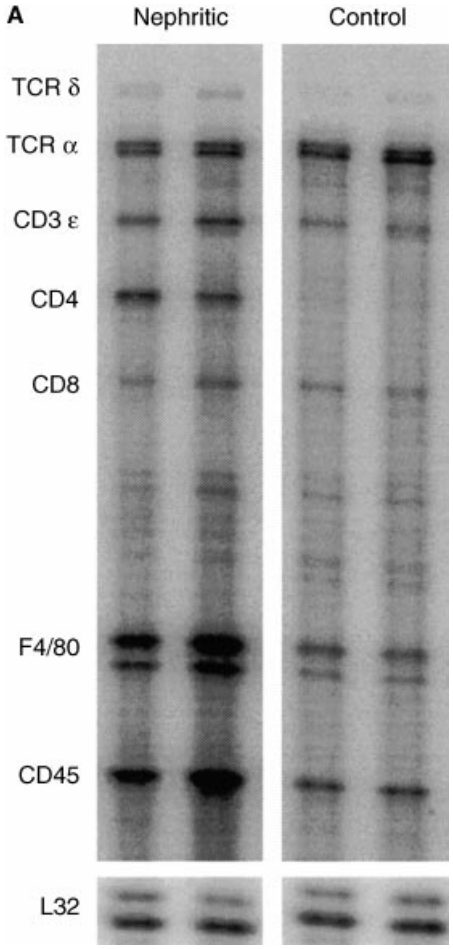

Fig. 4. Analysis of T cell and macrophage mRNA in renal tissue by ribonuclease protection assay. Kidneys were harvested from control wild-type mice (control) or 21 days following the induction of nephritis in wild-type mice (nephritic). Total RNA was extracted, and mRNA for T-cell markers and macrophages, and house keeping genes, L32 and GAPDH were detected in a multiprobe ribonuclease protection assay. T-cell–related markers are as follows: TCRδ (for γδ T cells), TCRα (for αβ T cells), CD3ε (for total T cells), CD4, and CD8α, and CD8β. F4/80 is a marker for macrophages, and CD45 is a marker for all leukocyte subsets. (*A*) A representative autoradiogram of samples from two nephritic and control mice is shown. (*B*) Densitometric quantitation of the data was undertaken. Within each sample, the density of each specific mRNA transcript was divided by the L32 ribosomal RNA (L32). Similar results were obtained when samples were normalized to GAPDH (data not shown). The resulting ratio was then multiplied by 1000. Results from two independent experiments are shown to demonstrate the consistency of the findings. The asterisk indicates a significant increase in nephritic WT mice compared with control WT ($P < 0.05$).

B

	TCR δ	TCR α	CD3 ε	CD4	CD8	F4/80	CD45
Control ($N = 3$)	0.3 ± 0.1	10.7 ± 1.4	5.4 ± 0.8	3.1 ± 0.6	5.7 ± 1.4	23.5 ± 6.1	28.0 ± 6.0
Nephritic ($N = 2$)	0.7 ± 0.1	13.5 ± 1.8	14.2 ± 2.9	10.4 ± 1.7	15.2 ± 4.3	223.9 ± 74.9	151.8 ± 28.1
Fold increase	2.3*	1.3	2.6*	3.4*	2.7	9.5*	5.4*

	TCR δ	TCR α	CD3 ε	CD4	CD8	F4/80	CD45
Control ($N = 4$)	0.5 ± 0.1	33.8 ± 14.2	12.1 ± 1.1	5.8 ± 1.2	13.1 ± 2.6	20.0 ± 1.4	36.7 ± 5.2
Nephritic ($N = 2$)	1.9 ± 0.5	39.3 ± 9.7	27.4 ± 9.3	33.8 ± 2.4	19.0 ± 6.5	124.3 ± 43.3	172.5 ± 89
Fold increase	3.7*	1.2	2.3*	5.8*	1.4	6.2*	4.7*

opment of glomerular lesions following nephritis, but is required for sustaining proteinuria.

DISCUSSION

As in humans, γδ and $CD4^+$ and $CD8^+$ αβ T cells were present in normal murine kidney, and γδ T cells were significantly increased in nephritic kidneys 21 days following the induction of experimental GN. This study has demonstrated a role for both αβ and γδ T cells in glomerular injury. A deficiency in either population of T cells led to a significant attenuation of glomerular injury and proteinuria in both strains of T-cell–deficient mice. αβ T cells alone were responsible for the minor proteinuria in γδ T-cell–deficient mice. To our knowledge, this is the first demonstration that both populations of T cells are required for the pathogenesis of a disease, and this suggests that interactions between these subsets

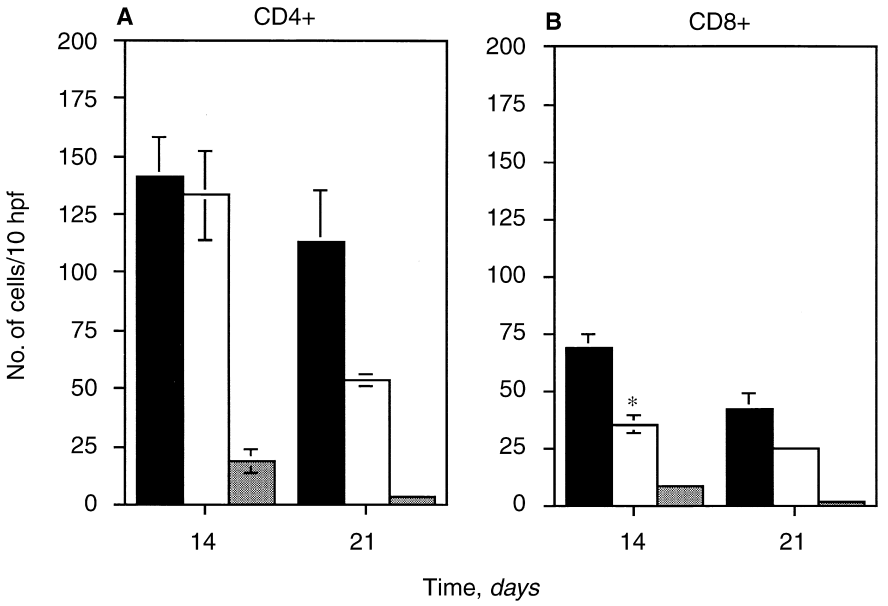

Fig. 5. Immunohistochemical analyses of CD4$^+$ and CD8$^+$ T-cell accumulation in wild-type (■), αβ (▩) and γδ (□) T-cell–deficient mice. An analysis of CD4$^+$ and CD8$^+$ T cells was undertaken in day 14 and day 21 nephritic wild-type mice, αβ T cell, and γδ T-cell–deficient mice. In γδ T-cell–deficient mice, the numbers of interstitial CD4$^+$ T cells were similar to wild-type mice at day 14 but were reduced at day 21, although this was not statistically significant ($P = 0.079$). On the other hand, the number of interstitial CD8$^+$ T cells was reduced compared with wild-type mice at day 14 ($^*P < 0.01$) but did not differ significantly from wild-type mice at day 21. In αβ T-cell–deficient mice, CD4$^+$ and CD8$^+$ T cells were present at day 14 than at day 21 following injection of anti-GBM antibody, suggesting that CD4$^+$ and CD8$^+$ γδ T cells accumulate in the nephritic kidney.

may be required for glomerular inflammation. Further support for this conclusion is that CD4$^+$ αβ T cells accumulated normally in the renal tissue of γδ T-cell–deficient mice, but were not sufficient to produce glomerular injury, suggesting that they were not pathogenic in the absence of γδ T cells. The significant attenuation of GN in γδ T-cell–deficient mice is particularly notable, since γδ T cells have been largely attributed to protecting the host against infection and resolving inflammation [31, 32]. For example, mice deficient in γδ T cells have an exaggerated inflammatory response in response to Listeria monocytogenes infection [31] and have increased mortality in response to *M. tuberculosis* infection [32] compared with T-cell–intact mice.

The role of γδ and αβ T cells has been previously investigated in renal disease associated with systemic autoimmunity, and it was in these studies that γδ T cells were shown to regulate autoimmunity. Studies were undertaken in murine lupus-prone MRL/Mp-*lpr*/*lpr* mice congenitally deficient in αβ (TCRβ−/−) and γδ (TCRδ−/−) T cells. MRL/Mp-*lpr*/*lpr* mice develop autoantibodies and immune complex end-organ disease, including renal disease, as a result of a defect in the Fas apoptosis gene. MRL/Mp-*lpr*/*lpr* mice deficient in γδ T cells developed more severe renal disease and mortality, which was associated with increased hypergammaglobulinemia and autoantibody production, and a polyclonal expansion of CD4$^+$ αβ T cells [33]. αβ T-cell–deficient animals developed a partially penetrant lupus syndrome characterized by mild renal disease, which was associated with immune deposits in kidneys, hypergammaglobulinemia, and anti-DNA autoantibodies. This suggested that γδ T cells played a role in propagating and regulating autoimmunity [34]. In the murine lupus model, autoimmune pathogenesis is attributed to pathogenic autoantibody production driven by autoreactive CD4$^+$ αβ T cells and an intrinsic B cell defect [35–37]. However, in the experimental nephritis model in this study and in murine crescentic GN [38], B cells and IgG production are not essential for the expression of disease and may thus be the basis for the differential role of γδ T cells in experimental GN versus lupus nephritis.

Questions remain as to the mechanism(s) contributing to αβ and γδ T-cell–mediated GN. The delayed-type hypersensitivity response in γδ T-cell–deficient mice is intact [39, 40], suggesting that the αβ T-cell recognition/activation phase in response to antigen is functioning in these mice. Since IgG production and subsequent glomerular deposition are not required for expression of glomerular disease, the humoral response appears to not be a significant determinant of αβ or γδ T-cell–mediated glomerular injury. Finally, our studies suggest that the αβ and γδ T-cell–mediated injury is not dependent on complement deposition, and others have shown that CD8$^+$ T cells do not play a role in the progression of glomerular injury in mice [41].

Clues for the mechanisms of αβ and γδ T-cell–dependent injury come from the following two observations. First, CD4$^+$ and CD8$^+$ αβ T-cell accumulation can occur in the absence of γδ T cells, yet γδ T-cell–deficient mice had no significant inflammatory response, in the glomerulus or the interstitium. Thus, αβ T cells alone are

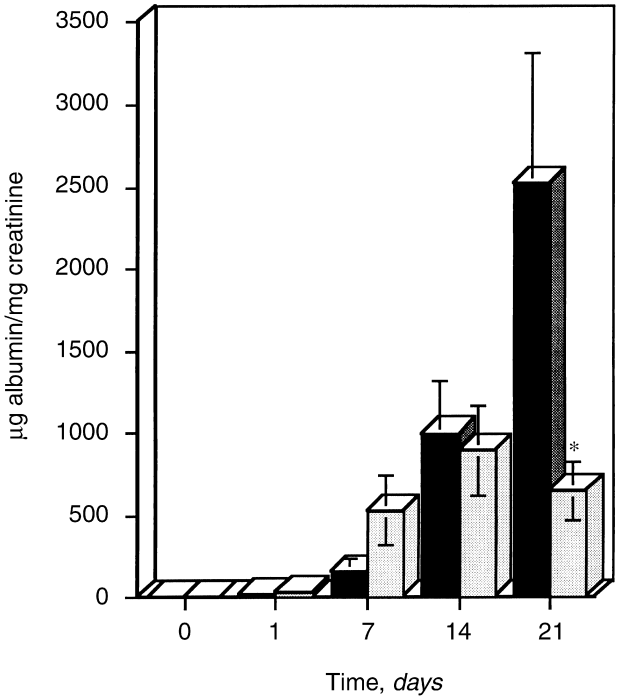

Fig. 6. Analysis of μ-chain–deficient mice subjected to experimental nephritis. Albuminuria was evaluated in wild-type (■; $N = 8$) and μ-chain–deficient (▩; $N = 8$) nephritic mice. Albuminuria was equivalent in both genotypes at day 14, but was reduced in the μ-chain–deficient mice compared with the wild-type mice at day 21 following injection of anti-GBM antibody. $^*P < 0.05$.

insufficient to promote inflammation and may require γδ T cell for their activation. Second, macrophage accumulation is significantly reduced in αβ and γδ T-cell–deficient mice compared with wild-type mice. In GN, macrophages follow an initial T-cell infiltrate in the glomeruli, and T cells are thought to participate in macrophage recruitment in some models of macrophage-mediated injury [42, 43]. Thus, we propose that the role of αβ and γδ T cells in GN is to elaborate cytokines that regulate each others' reactivity and recruitment. This results in the generation of an effector T-cell population that produces Th1-type cytokines [for example, interferon-γ (IFN-γ)], which are responsible for the recruitment and reactivity of effector cells such as macrophages. Several lines of evidence suggest that αβ and γδ T cells functionally interact in vitro. Antigen-activated αβ T cells are known to stimulate γδ T-cell responses [14]. Activated γδ T cells, in turn, are known to secrete cytokines such as IFN-γ and interleukin-4 (IL-4) that promote development of Th1 responses in $CD4^+$ αβ T cells [44]; Th1 cells have been shown to direct delayed-type hypersensitivity (DTH) responses associated with severe proliferative and crescentic GN [45]. The cross-talk between αβ and γδ T cells may also involve the apoptosis of Fas-positive αβ T cells by Fas ligand expressing γδ T cells [46]. This could potentially reduce the excess production of proinflammatory cytokines such as αβ T-cell–derived IFN-γ and tumor necrosis factor-α (TNF-α) at the glomerular lesion. αβ T-cell–derived cytokines are well known to activate macrophages, and recent studies suggest that IFN-γ provided by γδ T cells is required to prime macrophages for production of TNF-α and nitric oxide [14]. Thus, it is possible that IFN-γ is the effector cytokine in αβ and γδ T-cell–dependent glomerular injury. IFN-γ has been previously shown to contribute to renal accumulation of macrophages and the development of glomerular injury [47]. Finally, growth factors such as IL-2, IL-3, and granulocyte macrophage-colony stimulating factors (GM-CSFs) produced by γδ T cells may be capable of promoting the expansion of recruited and activated macrophages and αβ T cells [14]. Although the humoral response was not absolutely required for the expression of glomerular injury, it is required for sustaining proteinuria, as suggested by our experiments in μ-chain–deficient mice. Our study provides data on the role of αβ and γδ T cells in the humoral response. γδ T cells can provide help to B cells to produce IgG, to induce isotype switching, and to form germinal centers, the anatomical hallmark of T-B cell collaboration [17]. This help may be provided in the form of cytokines such as IL-4, IL-5, and IFN-γ secreted by γδ T cells and/or signaling through CD40L on T cells and CD40 on B cells [30]. However, since mouse anti-rabbit IgG antibodies were not detected in the serum of αβ T-cell–deficient mice, it appears that γδ T cells do not provide B-cell help in generating anti-GBM antibodies in GN. γδ T-cell–deficient mice had exaggerated circulating levels of mouse anti-rabbit IgG antibodies, suggesting a prominent role for αβ T cells in antibody production that may be down-regulated by γδ T cells.

Taken together, our results suggest the following model for the role of αβ and γδ T cells in glomerular injury (Fig. 7). Preimmunization of mice with rabbit IgG and the subsequent intravenous injection of rabbit anti-mouse GBM antibody leads to the deposition of anti-GBM antibody in the kidney, and the sensitization of αβ T cells in secondary lymphoid organs to rabbit IgG. Antigen-stimulated αβ T cells localize in the renal tissue and promote the recruitment of γδ T cells. On the other hand, antibody deposition in the glomerulus may also lead to the expression of stress-associated antigens such as heat shock protein, which stimulates γδ T cells and their subsequent localization into renal tissue independently of αβ T cells. Functional interactions between αβ and γδ T cells leads to the generation of an effector T-cell population. The effector T cells, in turn, secrete cytokines such as IFN-γ, which promote the recruitment of macrophages. Macrophages release soluble proinflammatory mediators (oxidants, proteases, and cytokines), bind com-

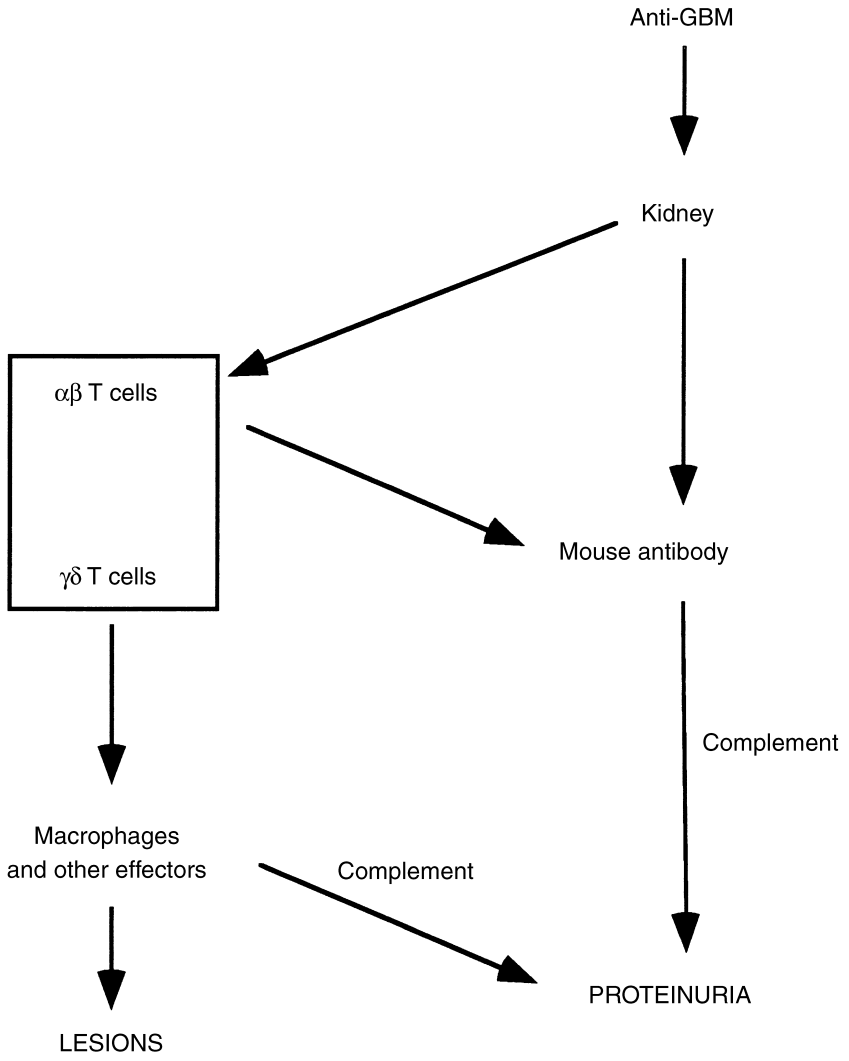

Fig. 7. Model of putative role of αβ and γδ T cells in antiglomerular basement membrane accelerated nephrotoxic nephritis. Anti-GBM deposition in the kidney leads to the recruitment of sensitized αβ and γδ T cells and the production and glomerular deposition of autologous mouse anti-rabbit IgG (mouse antibody). Mouse antibody production is dependent on αβ T-cell help. Cross-talk between αβ and γδ T cells leads to the differentiation of a T-cell subset into effector cells, which promote macrophage recruitment. Complement interaction with mouse antibody or complement receptors on macrophages promotes proteinuria. Macrophage-derived proinflammatory mediators also lead to the formation of glomerular lesions.

plement through complement receptors, and promote fibrin deposition, which leads to the development of glomerular lesions and proteinuria. Injection of anti-GBM antibody also leads to the production of mouse antibody to the injected heterologous antibody through αβ T-cell help. Renal deposition of mouse antibody leads to generation of C5b-C9, the lytic component of complement that promotes GBM degradation and proteinuria.

In conclusion, our findings suggest a pro-inflammatory role for both αβ and γδ T cells in an accelerated model of nephrotoxic nephritis at steps downstream of the humoral response to antigen. Our studies showing a significant reduction in glomerular injury in the absence of γδ T cells alone suggest that targeted therapy against γδ T cells, which represents less than 5% of the circulating T-cell population, may be beneficial in patients with GN.

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