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Antibody independent crescentic glomerulonephritis in μ chain deficient mice

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Antibody independent crescentic glomerulonephritis in μ chain deficient mice. The hypothesis that crescent formation in glomerulonephritis (GN) is a delayed type hypersensitivity (DTH)-like lesion, not dependent on a humoral immune response, was addressed using mice with deletion of the μ immunoglobulin heavy chain gene (μ chain deficient mice). Homozygous μ chain deficient mice do not develop mature B cells or produce immunoglobulin, but have intact cell mediated immunity. GN was induced in sensitized mice by a subnephritogenic dose of sheep anti-mouse GBM globulin. Heterozygous mice (μ chain +/−) demonstrated normal antibody and DTH responses to sheep globulin and developed a proliferative GN with proteinuria (6.4 ± 1.4 mg/24 hr), renal impairment (serum creatinine 32.6 ± 3.3 μmol/liter) and crescents in 33 ± 2.4% of glomeruli, when this antigen was planted in their glomeruli. This lesion was demonstrated to be T cell dependent by *in vivo* T cell depletion. Homozygous μ chain deficient mice (−/−) also developed proliferative GN, histologically indistinguishable from +/− mice. Proteinuria (3.8 ± 1.0 mg/24 hr), renal impairment (serum creatinine 24.5 ± 3.4 μmol/liter) and crescent formation (29 ± 2% of glomeruli) were no different from =/− mice. Mouse immunoglobulin was absent in their serum and glomeruli, however, cutaneous DTH to sheep globulin was identical to heterozygous mice. These results demonstrate that glomerular crescent formation and injury can occur independent of a humoral immune response to planted glomerular antigen and without glomerular deposition of autologous antibody. This strongly supports the hypothesis that crescent formation is a manifestation of DTH.

Many patients develop crescentic glomerulonephritis (GN) in the absence of detectable glomerular deposition of immunoglobulin in their renal biopsies [1], suggesting that glomerular crescent formation is not dependent on the humoral arm of the immune response. The prominent participation of T cells, macrophages and fibrin in human [2] and experimental [3] crescentic GN suggests a pivotal role for cellular immunity in the development of crescents. Depletion of T helper cells with specific monoclonal antibodies during the effector phase of experimental anti-glomerular basement membrane (anti-GBM) induced GN in rats [3] significantly attenuates development of crescents, further suggesting a key role for T cells in crescentic GN. In these T cell depleted rats, inhibition of crescentic GN is observed without detectable attenuation of the humoral immune response to the disease initiating antigen.

The development of technology to delete genes in the germ line of mice has allowed development of animals with a variety of selective genetic deficiencies, including selective and specific immunological lesions. The μ chain knock-out mouse was created by a gene-targeted knock out of the trans-membrane portion of the IgM molecule, leading to a failure of B cell precursors to progress beyond the early pre-B stage [4]. These mice fail to develop mature B cells and cannot produce immunoglobulin. However, their T cell function is normal [5] and they develop normal cellular immune responses including cutaneous DTH. In heterozygous (μ chain +/−) mice, B cell precursors develop normally into B cells and plasma cells and their immunoglobulin production is normal, consistent with the view that in normal B cell development, productive rearrangement of a single single heavy chain gene results in allelic exclusion at this locus.

A planted antigen model of GN (anti-GBM GN), which results in crescentic disease in normal mice, was used to determine the contribution of humoral immunity to the development of glomerular crescents. This question was addressed by comparing the development of this disease in antibody deficient mice (μ chain −/−) with its development in their heterozygous littermates (μ chain +/−), which have normal humoral immunity and develop normal antibody response to the disease initiating antigen.

Methods

Mice

μ chain deficient mice were originally generated by Prof. Rajewsky [4] from 129/Sv derived embryonic stem cells injected into C57BL/6 blastocysts. These mice were back crossed into the C57BL/6 strain for 8 generations by Dr. D. Tarlinton, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia. Their phenotypes were confirmed by flow cytometric analysis of B220 (CD45R) and surface immunoglobulin on circulating B cells.

Induction of anti-GBM GN

Anti-GBM globulin was prepared from serum of a sheep immunized against a particulate fraction of mouse GBM by absorption with mouse red blood cells and ammonium sulphate precipitation, as previously described [6, 7]. Mice, between 8 and 14 weeks of age, were sensitized to sheep globulin by s.c. injection of a total of 2 mg in 100 μl of CFA (Sigma Chemical Co., St. Louis, MO, USA) in divided doses in each flank. Fourteen days later, GN was initiated by i.v. administration of 7.5 mg of sheep

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anti-mouse GBM globulin. This dose did not induce acute (heterologous phase) proteinuria or histological injury in nonimmunized mice. Histological indices of glomerular injury were assessed 14 days after administration of anti-GBM globulin and proteinuria and DTH over the preceeding 24 hours.

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 (PAS) reagent. Glomerular crescent formation was assessed by an experienced histopathologist (PGT) in a blinded protocol. Glomeruli were considered to exhibit crescent formation when more than three layers of cells were observed in Bowman's space. A minimum of 20 glomeruli were assessed to determine the crescent score for each animal.

Functional assessment of glomerular injury

Proteinuria. Mice were housed individually in cages to collect urine over the final 24 hours of each experiment. Urinary protein concentrations were determined by a modified Bradford method, adapted to a microtiter plate assay as previously described [8]. The 24-hour urinary protein excretion was calculated from the 24-hour urine volume and the urinary protein concentration.

Serum creatinine and 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 creatinine concentrations and the urine volume.

Assessment of immunological effectors in glomeruli

Glomerular T cell and macrophage accumulation. Spleen and kidney tissue was fixed in periodate lysine paraformaldehyde (PLP) for four hours, washed in 7% sucrose solution then frozen in liquid nitrogen cooled isopentane. Tissue sections (3 μm) were stained to demonstrate macrophages and T cells using a three layer immunoperoxidase technique, as previously described [3, 7]. The primary antibodies were GK1.5 (monoclonal anti-mouse CD4; ATCC, Rockville, MD, USA) and M1/70 (monoclonal anti-mouse Mac-1, ATCC). Sections of spleen provided a positive control for each animal and irrelevant rat monoclonal antibody was substituted for primary monoclonal antibody to provide a negative control. A minimum of 20 equatorially sectioned glomeruli per animal were assessed per animal and the results were expressed as cells per glomerular cross section (c/gcs).

Immunoglobulin and fibrin. Tissue sections (3 μm) were cut from snap frozen kidney and stained by direct immunofluorescence with FITC-conjugated sheep anti-mouse immunoglobulin (Silenus, Hawthorn, Victoria, Australia) and FITC conjugated goat anti-mouse fibrinogen (Nordic, Drawer, CA, USA). Semiquantitative assessment of the glomerular deposition of these inflammatory mediators was performed by determining the end point positive titer for detection of staining of these antigens using serial dilutions of each antibody.

Assessment of the systemic immune response

Circulating mouse anti-sheep globulin antibody. Titers of mouse anti-sheep globulin antibody were measured by ELISA on serum collected at the end of each experiment. Polystyrene microtiter plates (Greiner Labortechnik, Germany) were coated with 5 μg/ml normal sheep globulin in carbonate/bicarbonate buffer pH 9.5 by incubation overnight at 4°C, and then blocked with 1% BSA. Plates were washed with PBS containing 0.05% Tween 20, then incubated with serial dilutions of mouse serum. After further washing, bound mouse immunoglobulin was detected with horseradish peroxidase conjugated rabbit anti-mouse immunoglobulin antibody (Sigma) at a dilution of 1 in 1000 using 0.1 M 2,2′-azinodi-3-ethylbenzthiazoline sulphonate (ABTS, Boehringer Mannheim, Germany) in 0.02% H_2O_2 as a substrate. The absorbance at 405 nm was read on a microtiter plate reader (Dynatech Laboratories, Chantilly, VA, USA). Serum from each mouse was tested at serial 1 in 4 dilutions, starting from a dilution of 1 in 50 in each group except for serum from wild-type C57BL/6 mice, which was titered from a starting dilution of 1 in 100.

Delayed type hypersensitivity to sheep globulin. Mice were challenged 24 hours prior to the end of each experiment by intradermal injection of sheep globulin (20 μg in 20 μl of PBS) into the plantar surface of a hind foot. An irrelevant antigen (horse globulin) was injected in the opposite foot pad as a control. DTH was assessed 24 hours later by determining the presence or absence of footpad swelling in a blinded protocol.

T helper cell depletion protocol

T helper cell depletion was induced by i.p. injection of 0.5 mg per mouse of protein G purified rat anti-mouse CD4 monoclonal antibody (GK 1.5), administered daily for four days, starting 24 hours before injection of anti-GBM globulin. A further dose of 0.5 mg was given on the seventh day after anti-GBM globulin. This antibody has been previously established to produce prolonged T helper cell depletion *in vivo* [9, 10]. The extent of T cell depletion in the blood and spleen (using isolated spleen cells) on day 14 after administration of anti-GBM globulin was assessed by flow cytometry by dual labeling with anti-CD3 (29B, Sigma) and anti-CD4 (KT6; Serotec, Oxford, UK) or anti-CD8 (53-6.7; Sigma) antibodies.

Experimental design and statistical analysis

The following groups were studied:

(1.) Normal C57BL/6 mice ($N = 6$);
(2.) Normal heterozygous μ chain deficient mice (μ chain +/−) ($N = 4$);
(3.) Normal homozygous μ chain deficient mice (μ chain −/−) ($N = 4$);
(4.) Heterozygous μ chain deficient mice (μ chain +/−) with anti-GBM GN ($N = 5$);
(6.) T cell depleted, heterozygous μ chain deficient mice (μ chain +/−) with anti-GBM GN ($N = 6$);
(7.) Homozygous μ chain deficient mice (μ chain −/−) with anti-GBM GN ($N = 8$).

The statistical significance of differences between groups was determined by the Mann Whitney *U*-test.

Results

Renal function and immune response to sheep globulin in wild-type and μ chain deficient mice

Normal wild-type, heterozygous and homozygous μ chain deficient mice showed no differences in their 24-hour urinary protein

Table 1. Normal renal function in C57BL/6 (wild type), heterozygous (μ chain +/−) and homozygous (μ chain −/−) deficient mice

	Serum creatinine *μM/liter*	Proteinuria *mg/24 h*
Wild-type	10 ± 1.5	1.6 ± 0.2
μ Chain +/−	8.5 ± 1.3	1.4 ± 0.4
μ Chain −/−	10 ± 1.8	1.9 ± 0.2

excretion and serum creatinines (Table 1) and their kidneys were all histologically normal.

Heterozygous and wild-type mice showed identical cell surface expression of B220 (CD45R) on circulating lymphocytes by flow cytometry indicating the presence of similar numbers of mature B cells (Fig. 1). Their serum titers of anti-sheep globulin antibody after immunization according to the protocol for inducing anti-GBM GN were also identical (Fig. 2). In homozygous (μ chain −/−) μ chain deficient mice, B220 expression on circulating lymphoctyes was not detectable above background levels, indicating an absence of mature B cells (Fig. 1), and anti-sheep globulin was undetectable in their serum after immunization (Fig. 2).

Immunized wild-type, heterozygous and homozygous μ chain deficient mice developed cutaneous delayed type hypersensitivity following challenge with sheep globulin. Pronounced swelling occurred at the site of challenge and T cells and macrophages accumulation was prominent histologically. Challenge with horse globulin in the opposite footpad did not induce skin swelling.

Development of anti-GBM GN in heterozygous μ chain deficient mice

Heterozygous (μ chain +/−) μ chain deficient mice developed a proliferative crescentic GN (Fig. 3A) the same as that previously reported in wild-type C57BL/6 mice using this protocol [11]. Crescents were observed 33.1 ± 2.4% of glomeruli in μ chain +/− mice with anti-GBM GN (Fig. 4), but were not observed in normal mice. This crescentic GN was associated with a significant glomerular accumulation of CD4 positive T cells (0.92 ± 0.08 c/gcs, normal 0.01 ± 0.01 c/gcs, $P < 0.001$) and macrophages (1.47 ± 0.19 c/gcs, normal 0.01 ± 0.001 c/gcs, $P < 0.001$; Fig. 4) and prominent glomerular fibrin deposition. Deposition of mouse immunoglobulin in a linear pattern was detectable in glomeruli (Fig. 3D) at a mean end point titer of 1 in 4000 of sheep anti-mouse immunoglobulin FITC.

Heterozygous mice with anti-GBM GN developed significant proteinuria (6.4 ± 1.4 mg/24 hr, $P < 0.05$) compared with normal μ chain +/− (1.4 ± 0.3 mg/24 hr; Fig. 5) and wild-type C57BL/6 mice of a similar age (0.9 ± 0.2 mg/24 hr). The serum creatinine was also significantly increased (32.6 ± 3.3 μM/liter, $P < 0.05$) compared to normal μ chain +/− (8.5.± 1.8 μM/liter) and wild-type C57Bl/6 (10.0 ± 1.8 μM/liter) mice and the creatinine clearance (90.0 ± 13.4 μl/min) was also significantly reduced compared with normal C57Bl/6 mice (193 ± 10.4 μl/min, $P = 0.02$; Fig. 5).

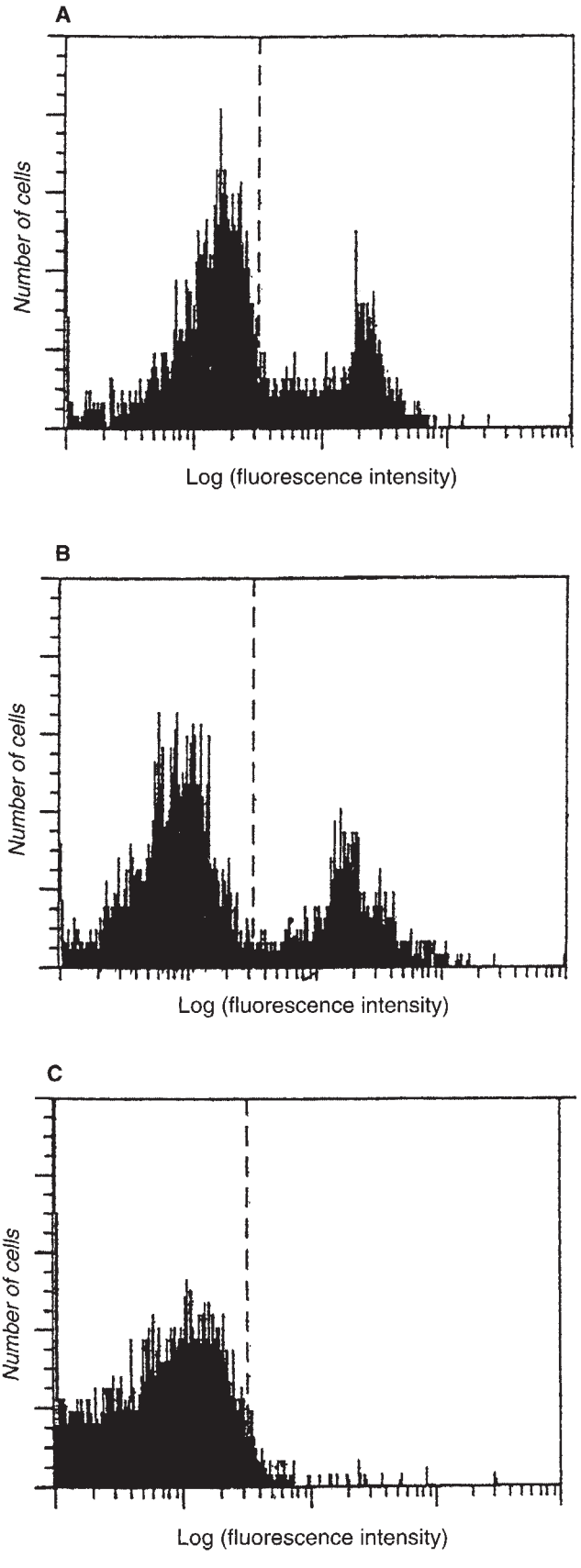

Fig. 1. *Flow cytometric analysis of B220 (CD45R) expression on blood leukocytes from* (**A**) *a normal C57BL/6 (wild-type) mouse,* (**B**) *heterozygous μ chain deficient (μ +/−) mouse, and* (**C**) *a homozygous μ chain deficient (μ −/−) mouse demonstrating equivalent B220 antigen expression in the wild-type and heterozygotes, but no significant expression above background (to the left of the broken line) in the homozygous μ chain deficient mouse.*

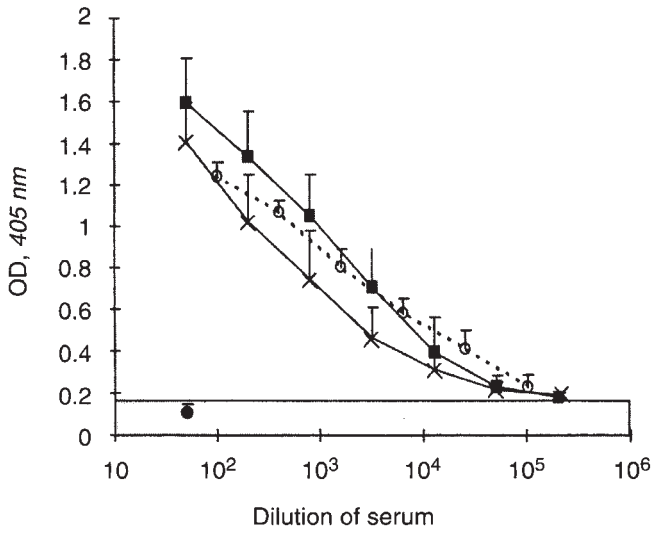

Fig. 2. *Serum titers of mouse anti-sheep globulin antibody in wild-type (○), heterozygote (μ +/−) μ chain deficient (■), T cell depleted heterozygote μ chain deficient (X) and homozygous μ chain deficient (μ−/−) mice on day 14 of anti-GBM GN.* Optical density (OD) of wells with serum from homozygotes μ chain deficient (μ +/−) mice (●) was below background (1% BSA/PBS control) indicated by the shaded area.

In vivo treatment with anti-CD4 antibody produced a significant depletion of CD4 bearing cells in the blood and spleen. In treated mice on day 14, CD3/CD4 positive cells were reduced to 1.45 ± 0.22% of total circulating leukocytes (normal 17.7%), whereas CD3/CD8 positive cells (14.3 ± 1.4%) were unaffected (normal 16.5%). A similar effect was observed in the spleen, with CD3/CD4 positive cells reduced to 0.6 ± 0.05% of total splenocytes (normal 18.1%) and CD3/CD8 positive cells (12.0 ± 1.5%) unaffected (normal 12.1%).

T cell depletion significantly attenuated the development of disease in μ chain +/− mice. Crescent formation was significantly attenuated (1.6 ± 0.8% of glomeruli, $P = 0.006$) in T cell depleted heterozygous mice, as was glomerular accumulation of CD 4 positive T cells (0.30 ± 0.10 c/gcs, $P = 0.011$) and macrophages (0.64 ± 0.13 c/gcs, $P = 0.019$), compared to untreated disease in heterozygotes. Proteinuria (2.7 ± 0.2 mg/24 hr, $P = 0.075$) and serum creatinine (19.5 ± 2.6 μM/liter, $P = 0.028$) were also reduced compared to untreated heterozygous mice with anti-GBM GN (Fig. 5). Creatinine clearance in T cell depleted mice (193 ± 10.4 μl/min, $P = 0.02$) was significantly higher than in untreated mice. The glomerular deposition of mouse immunoglobulin as assessed by immunofluorescence appeared slightly reduced in T cell depleted mice, the mean end point titer of sheep anti-mouse immunoglobulin antibody-FITC for detection was 1 in 2000 compared with 1 in 4000 in untreated heterozygous mice. Circulating titers of mouse anti-sheep globulin were unaffected by T cell depletion (Fig. 2).

Development of anti-GBM GN in homozygous μ chain deficient mice

Homozygous (μ chain −/−) μ chain deficient mice developed a proliferative crescentic GN of similar severity to heterozygous mice (Fig. 3A). Crescent formation (28.9 ± 2.2% of glomeruli), glomerular accumulation of CD4 positive T cells (1.01 ± 0.17 c/gcs) and macrophages (1.60 ± 0.23 c/gcs) was not significantly different from heterozygous mice (Fig. 4). Fibrin deposition in glomeruli was also present to a similar extent, however, mouse immunoglobulin was totally undetectable in glomeruli of μ chain −/− mice (Fig. 3C). The extent of proteinuria (3.8 ± 1.0 mg/24 hr), the elevation of serum creatinine (24.5 ± 3.4 μM/liter) and reduced creatinine clearance (135 ± 29.0 μl/min) in homozygous μ chain deficient mice were not significantly different from heterozygous mice with anti-GBM GN (Fig. 5).

Discussion

Early studies of the immunological events in GN emphasized the role of antibody and complement as effectors of injury [12–16] and resulted in the potential involvement of T cells being largely discounted [17, 18]. However, the observation that many patients develop crescentic GN in the absence of detectable immunoglobulin deposition in their glomeruli [1] renewed interest in the possibility that cell mediated immunity may play an important role in crescentic forms of GN.

Several studies have addressed this hypothesis in the past. Kreisburg, Wayne and Karnovsky demonstrated glomerular localization of lymphocytes during the onset of anti-GBM GN in rats [19]. Bhan produced histological evidence of glomerular injury after passive transfer of sensitized T cells to rats with planted antigens and immune-complexes in their glomeruli [20, 21]. Athymic (nude) mice [22] do not develop humoral or cellular immune responses and fail to develop autologous injury following anti-GBM serum. Athymic rats are also protected from autologous phase anti-GBM GN and develop proteinuria but not crescents following passive transfer of autologous antibody [23]. Effectors of delayed type hypersensitivity have been demonstrated in association of with crescentic GN in humans [2] and strategies have been devised to selectively block either the humoral [24] or cellular effector arm [3] of the immune system in experimental models of GN.

Other evidence supporting the potential of T cells to cause glomerular injury comes from studies in which haptens have been selectively planted in the kidney to induce a predominant T cell dependent immune response. [25, 26]. Oite et al perfused sensitized rats with trinitrobenzene sulphonic acid (TNP) coupled to BSA via the renal artery and induced a marked proliferative GN and transient proteinuria, associated with accumulation of Ia positive cells in glomeruli, in the absence of detectable immunoglobulin and complement [25]. Sensitized rats developed low circulating antibody titers to TNP demonstrating the difficulty in completely preventing a humoral immune response. Rennke et al [26] adopted a similar approach by infusing azobenzenearsonate into the kidneys of sensitized rats. This resulted in a granulomatous cortical inflammatory response with glomerular hypercellularity and crescents, but no abnormal proteinuria. Systemic transfer of sensitized T cells resulted in a similar histological lesion, albeit somewhat attenuated in severity. A diffuse inflammatory infiltrate of macrophages and T cells was present throughout the kidney and despite the presence of circulating antibody to azobenzenearsonate, minimal rat immunoglobulin and complement was detected in glomeruli.

The development of mice with specific genetic deletion of selective parts of the immune effector system now allows the role of the antibody and T cells in crescentic GN to be addressed more definitively. Mice with disruption of one of the membrane exons

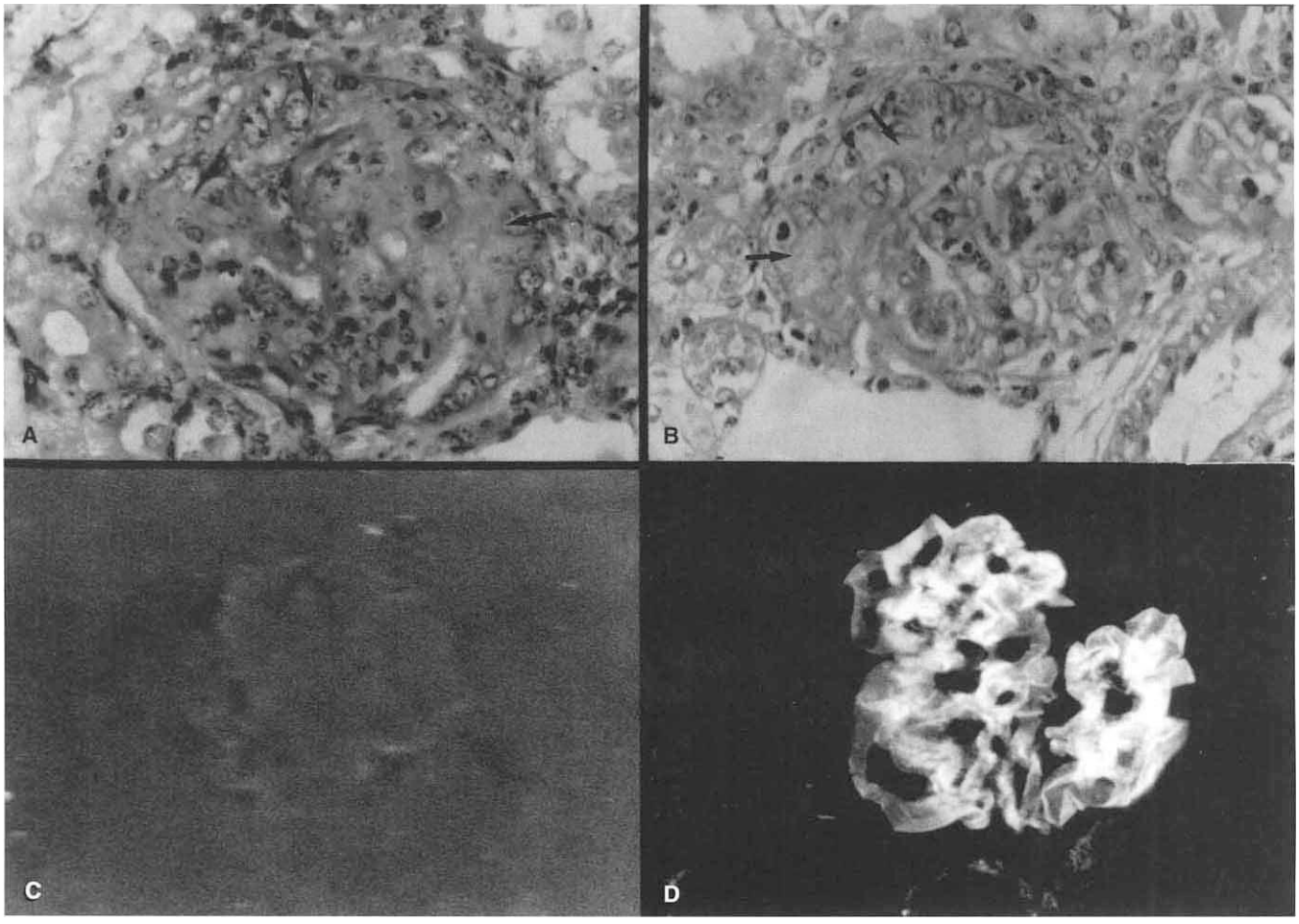

Fig. 3. *Photomicrographs of glomeruli from homozygous (***A** *and* **C***) and heterozygous (***B** *and* **D***) μ chain deficient mice with anti-GBM GN demonstrating an identical proliferative and crescentic GN in both mice (A and B, PAS stain, magnification ×400), with undetectable mouse immunoglobulin in the homozygous mouse (C) and strong linear deposition in the heterozygous mouse (both immunofluorescence, magnification ×400).*

of the gene encoding for the μ-chain constant region of the IgM heavy chain were produced by gene targeting in mouse embryonic stem cells [4]. Heterozygous expression of this defect did not result in any phenotypic abnormalities, however, homozygous mice demonstrated arrest of B cell development at an immature pre-B stage and an absence of mature B cells (identified by surface expression of B220 antigen [27]). These mice have no circulating or surface immunoglobulin [4] and fail to develop antibody responses following antigen challenge. However, they demonstrate normal T cell priming and effector responses to antigens both *in vitro* and *in vivo* [5], and thus provided an ideal system in which to address the requirement for antibody in the development of crescentic GN.

GN was induced by planting an antigen (sheep globulin) on the glomerular basement membrane of mice previously sensitized to this antigen. GN results from an active immune response to this exogenous planted antigen in a similar manner to many of forms of human GN, in which antigens exogenous to the kidney (often bacterial or parasitic) become lodged in the glomerulus and an antigen specific immune response results in local glomerular injury. In this model, there is no loss of tolerance to GBM antigens as occurs in human anti-GBM disease and in experimental GN induced rats [28] and rabbits [29] by immunization with the α3 chain of the noncollagenous domain of type IV collagen. However, the pathological features of GN (severe renal impairment, proteiniuria, prominent crescent formation, glomerular fibrin deposition and accumulation of CD4 positive T cells, macrophages) closely parallel the features of human anti-GBM GN and other types of rapidly progressive crescentic GN.

In heterozygous μ-chain deficient mice, this induced a crescentic GN, characterized by glomerular deposition of autologous (mouse anti-sheep globulin) antibody and accumulation of CD4 positive T cells and macrophages in glomeruli and results in proteinuria and renal impairment. Glomerular T cell and macrophage recruitment, crescent formation and injury were significantly attenuated by *in vivo* depletion of CD4 positive T cells, demonstrating that this form of injury is T cell dependent in the effector phase. In the T cell depletion protocol employed in this study, no effect was seen on circulating autologous antibody measured by ELISA, but a minor reduction of glomerular deposition of autologous antibody was suggested by immunofluorescence. As complete absence of glomerular deposition of autologous antibody in homozygous μ-chain deficient mice did not significantly alter the development of disease, it is unlikely that

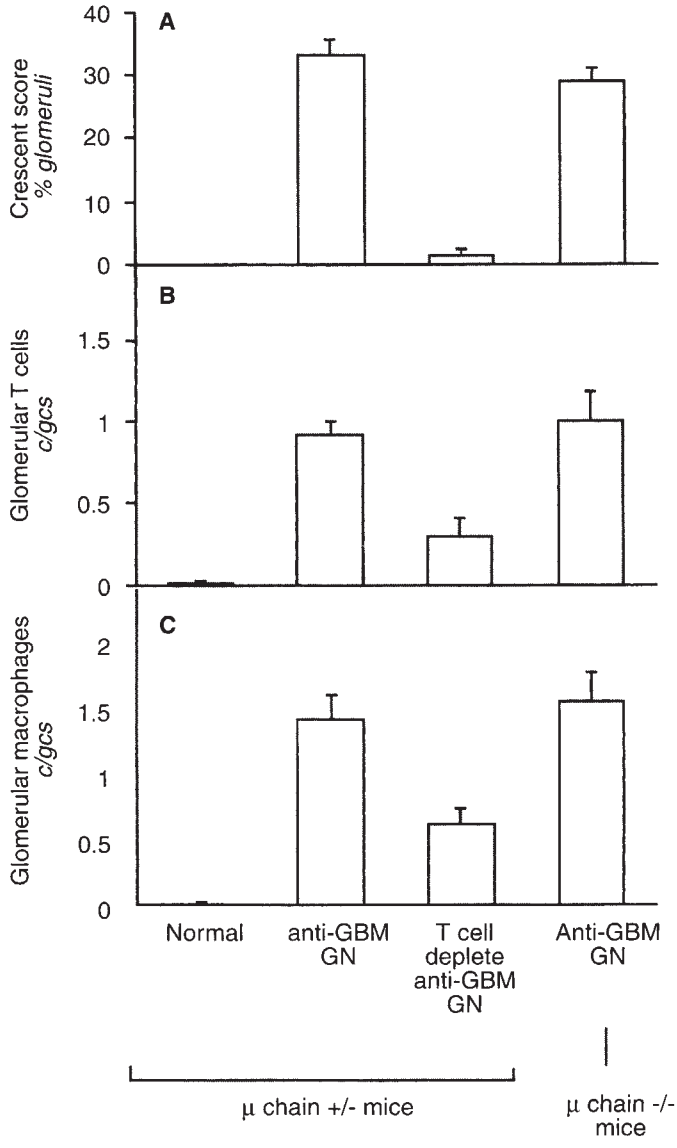

Fig. 4. *(A) Glomerular crescent scores (% of glomeruli demonstrating crescents) and (B) glomerular T cell and (C) macrophage accumulation (cells per glomerular cross-section) in homozygous (μ chain −/−) deficient mice with anti-GBM GN, non-diseased (normal) heterozygous (μ chain +/−) deficient mice and intact and T cell depleted heterozygous mice with anti-GBM GN.*

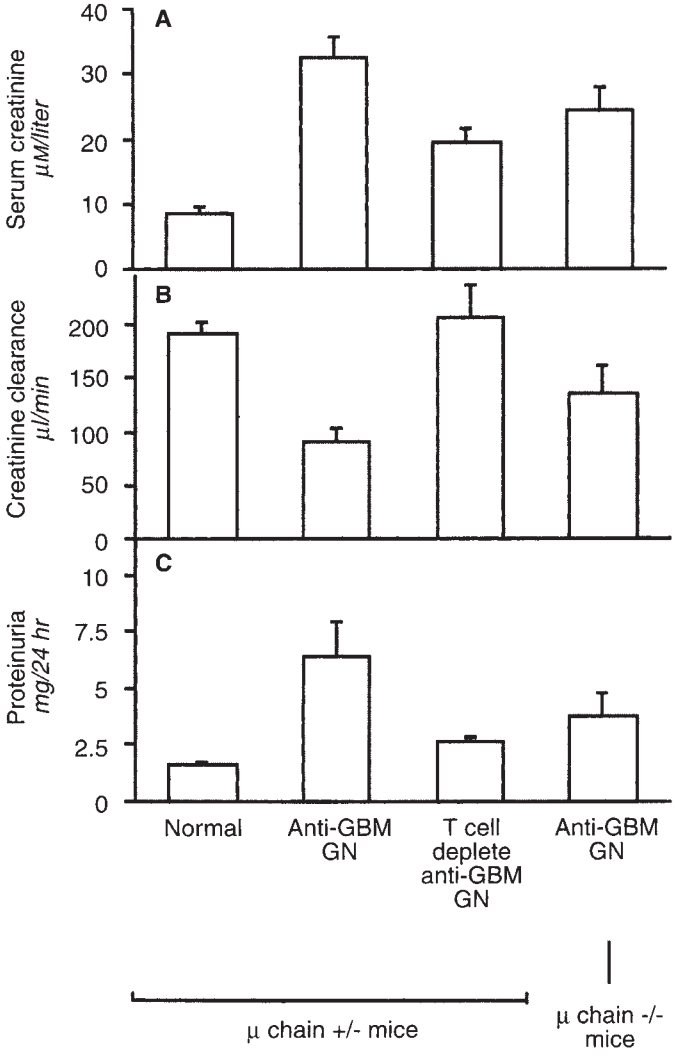

Fig. 5. *(A) Serum creatinine (μM/liter),* (**B**) creatinine clearance (μl/min) and (**C**) proteinuria (mg/24 hr) in homozygous (μ chain −/−) deficient mice with anti-GBM GN, non-diseased (normal) heterozygous (μ chain +/−) deficient mice and intact and T cell depleted heterozygous mice with anti-GBM GN.

this minor effect of T depletion contributed to its profound effects on development of crescentic GN.

In homozygous μ-chain deficient mice, despite complete absence of circulating and glomerular mouse immunoglobulin, crescent formation, proteinuria and renal impairment was essentially the same as in B cell intact mice. These mice also demonstrated cutaneous DTH following challenge with the GN initiating antigen and glomerular accumulation of T cells and macrophages was unaffected. The immunopathological features of disease in these mice are thus similar to those seen in pauci-immune crescentic human GN, although there is no evidence of systemic vasculitis that often accompanies ANCA associated GN.

These studies clearly demonstrate that glomerular antibody deposition is not required for crescent formation in response to a planted glomerular antigen. This is consistent with the previous study of Bolton, Tucker and Sturgill, who induced a model of proliferative GN by immunization of chickens with bovine glomerular basement membrane [24]. These birds developed primitive crescents in association with glomerular accumulation of esterase positive macrophages and subsequently these authors demonstrated that disease could be transferred passively by administration of mononuclear cells from the spleen and kidneys from nephritic birds. Bursectomy using cyclophosphamide resulted in very low or undetectable anti-GBM antibody titers, and appeared to exacerbate the development of proliferative GN and crescents. While bursectomy in birds allowed dissection of T and B cell effector mechanisms, substantial difference between avian and mammalian renal morphology, physiology and pathology diminish the relevance of these studies to human disease. The

mouse model employed in the current studies has similar immunopathological and functional outcomes to those seen in human crescentic GN. Antibody depletion was absolute and the involvement of T cells in the glomerular lesion was demonstrated both histologically and functionally.

A second approach to demonstrating the contribution of the cellular and humoral effector arms of the immune system to development of crescentic GN has been by attempting to selectively block the T cells *in vivo* [3]. The results of these studies also support a pivotal effector role for T helper cells by demonstrating that depletion of either CD4 or CD5 positive T cells in rats with an established antibody response prevented development of crescents and injury in response to a planted glomerular antigen. This attenuation of disease was not associated with detectable reduction of the humoral immune response [3]. The development of crescents in association with T cell and macrophage recruitment and fibrin deposition in glomeruli suggests that crescent formation is an manifestation of DTH-like glomerular injury.

In summary, the current studies demonstrate that glomerular crescents can develop as a result of a T cell dependent immune response to a planted glomerular antigen. They provide a clear demonstration of the pivotal role for cell mediated immunity in the development of crescentic GN in the total absence of any participation of antibody.

Acknowledgments

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