Role of intrinsic renal cells versus infiltrating cells in glomerular crescent formation

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Role of intrinsic renal cells versus infiltrating cells in glomerular crescent formation.

Background. Studies were undertaken to characterize the cellular composition that occurs in glomeruli and the tubulointerstitium of a passive model of complement-independent crescentic nephritis in mice.

Methods. Glomerulonephritis was induced by the injection of antibody to whole rabbit glomeruli, and tissue was examined histologically at 7, 14 and 28 days.

Results. Mice developed proteinuria, glomerular crescents, and progressive glomerulosclerosis and tubulointerstitial fibrosis. The majority of the cells within the crescents appeared to be intrinsic ezrin-positive epithelial cells of visceral or parietal origin. Many of the ezrin positive cells were proliferating and expressing the PDGF receptor. Despite expression of the macrophage adhesive protein, osteopontin, the early crescents were devoid of infiltrating macrophages, T cells or myofibroblasts, which could be explained by the finding that the Bowman's capsule remained intact. Tubulointerstitial damage also occurred, and included tubular dilation and atrophy, periglomerular and patchy interstitial infiltration and interstitial fibrosis with increased interstitial deposition of type IV collagen and laminin. Interstitial infiltrating cells included macrophages, CD4+ T lymphocytes, CD8+ T lymphocytes, and activated myofibroblasts. Tubular osteopontin expression was increased in the areas of tubulointerstitial damage and was associated with interstitial macrophage infiltration.

Conclusions. We describe an experimental model of complement-independent murine crescentic nephritis associated with tubulointerstitial injury. Proliferating glomerular epithelial cells are the main cellular components of the crescents in this model.

Crescentic glomerulonephritis (GN), often called rapidly progressive glomerulonephritis, refers to a pattern of glomerular injury in which there is an extracapillary collection of cells within Bowman's capsule that has the appearance of a 'crescent' on histologic cross-section [1]. The presence of crescents on biopsy is often associated with a deterioration of renal function over days to weeks, independent of whether the glomerular injury is initiated by immune complex, anti-glomerular basement membrane (GBM) antibody or antibody independent mechanisms [1, 2]. Understanding the cellular composition and pathogenesis of crescent formation is therefore of substantial interest.

Experimentally, crescentic nephritis has been most commonly induced by antibodies to whole glomeruli ('nephrotoxic nephritis') or to isolated GBM ('anti-GBM' disease) [3–10]. Disease has been induced by active immunization of glomeruli or GBM, by injecting heterologous anti-GBM antibody into pre-immunized animals ('accelerated autologous' disease), or by the passive administration of anti-GBM or anti-glomerular antibody [3–10]. These studies as well as immunohistochemical studies in humans [11] have suggested that crescents are composed of both parietal and visceral glomerular epithelial cells (GEC) as well as infiltrating monocytes, macrophages, lymphocytes, and later, fibroblast-like cells [12]. The cellular composition of the crescents has varied in these studies and may be influenced by whether or not Bowman's capsule is intact [13, 14].

In this study, we present a model of passive nephrotoxic nephritis in mice induced by heterologous antibody to heterologous glomeruli. These mice develop a relatively uniform crescentic nephritis in the absence of any light microscopic evidence of rupture of Bowman's capsule. Interestingly, the early development of crescents in this model appears to involve a proliferation of parietal and visceral GECs in the absence of detectable macrophage or lymphocyte infiltration. The increased proliferation appears to be linked to increased expression of PDGF receptor (β-subunit) within the crescents. The macrophage adhesive factor, osteopontin, is also expressed in the crescents and may have a role in the later macrophage infiltration. These studies suggest that in this model early crescent formation does not involve a direct macrophage or T-cell infiltration into Bowman's space.

Table 1. Antibodies used to detect specific antigens in renal biopsy tissue

Antigen	Primary antibodies	Second. Antibodies	Detection System
Proliferating cell nuclear antigen (PCNA)	A murine monoclonal IgM against PCNA (Coulter Immunology, Hialeah, FL, USA)	A peroxidase-conjugated monoclonal rat anti-mouse IgM (Zymed Laboratories, San Francisco, CA, USA)	Diaminobenzidine (DAB; (Sigma Chemical CO, St. Louis, MO, USA)
Ezrin	A rabbit polyclonal antibody against ezrin 90/3 (gift from Heinz Furthmayr, Stanford University School of Medicine, Stanford, CA, USA)	A biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA)	Avidin-biotin system and DAB
α-Smooth muscle actin	A monoclonal IgG2a antibody against α-smooth muscle actin (Sigma)	A peroxidase-conjugated monoclonal rat anti-mouse IgG2a (Zymed)	DAB
Mouse macrophages (F4/80)	A rat monoclonal IgG2b antibody against mouse macrophages (F4/80) (Caltag Laboratories, Burlingame, CA, USA)	A biotinylated anti-rat IgG (Vector)	Avidin-biotin system and DAB
Mouse neutrophils	A rat monoclonal IgG2a antibody against mouse neutrophils (clone 7(4)(Caltag)	A biotinylated anti-rat IgG (Vector)	Avidin-biotin system and DAB
CD4+ T lymphocytes	A rat monoclonal IgG2a antibody against mouse CD4 antigen (Caltag)	A biotinylated anti-rat IgG (Vector)	Avidin-biotin system and DAB
CD8+ T lymphocytes	A rat monoclonal IgG2a antibody against mouse CD8a (Ly2) antigen (Caltag)	A biotinylated anti-rat IgG (Vector)	Avidin-biotin system and DAB
Osteopontin (OPN)	A goat polyclonal antibody against OPN (OP199; gift from Cecilia M Giachelli, University of Washington, Seattle, WA, USA)	A peroxidase-conjugated monoclonal mouse anti-goat IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA)	DAB
Platelet-derived growth factor receptor β-subunit (PDGF-Rβ)	A rabbit antibody against human PDGF-Rβ(7649); (gift from Ronald A Seifert, University of Washington)	A biotinylated anti-rabbit IgG (Vector)	Avidin-biotin system and DAB
Type IV collagen	A biotinylated rabbit polyclonal antibody against type IV collagen (Becton Dickinson Labware, Bedford, MA, USA)	Horseradish peroxidase conjugated avidin D (Vector)	DAB
Laminin	A biotinylated rabbit polyclonal antibody against laminin (Chemicon International Inc, Temecula, CA, USA)	Horseradish peroxidase conjugated avidin D (Vector)	DAB

All antigens were immunostained using methylcarnoy's fixed tissue, except that the CD4+ and CD8+ lymphocytes were stained using acetone-fixed (10 min) snap frozen tissue.

METHODS

Induction of murine crescentic nephrotoxic glomerulonephritis

Sheep anti-rabbit glomeruli antibody was produced in a sheep immunized three to four times at two-week intervals with lyophilized whole rabbit glomeruli emulsified in complete Freud's adjuvant at the first immunization and in incomplete Freud's adjuvant at later immunizations. Rabbit glomeruli for immunization were isolated by differential sieving techniques and contained less than one tubular fraction per 100 glomeruli. Antisera were heat inactivated at 56°C for 30 minutes and immune IgG was isolated by using caprylic acid [15].

Experimental crescentic GN was induced in 15 C57/BL6 mice (Simonsen Laboratories, Gilroy, CA, USA) by intraperitoneal injection of sheep anti-rabbit GBM antibody (0.5 ml/20 g body wt/day for 2 consecutive days). Mice were sacrificed on days 7, 14, and 28 ($N = 5$ at each time point), and sera and renal biopsies were obtained from each animal. Controls consisted of normal mice ($N = 6$) or mice injected with equivalent amounts of nonimmune sheep IgG ($N = 3$ at day 7). Biopsies were fixed in either formalin or methyl Carnoy's solution and were embedded in paraffin [16]. For staining of frozen sections, tissue was embedded in O.C.T. Compound (Miles, Elkart, IN, USA) and rapidly snap frozen in isopentane in liquid nitrogen as previously described [16]. Twenty-four-hour urine collections were performed on days 7, 14, and 28 and analyzed for protein content by the micro BCA method (Pierce Chemical, IL, USA).

Immunohistochemistry

To examine renal histology, 4 μm sections were stained with periodic acid and Schiff's reagent (PAS) or with methenamine-silver. An indirect immunoperoxidase method was used as previously described [16] to detect a variety of antigens (Table 1). This included the analysis of proliferating cells (expressing the proliferating cell nuclear antigen or PCNA), α-smooth muscle actin (which marks activated mesangial cells and myofibroblasts [17]), various leukocyte populations (macrophages, CD4 and CD8 positive T lymphocytes and neutrophils), ezrin (an epithelial cell cytoskeletal protein) [18], platelet-derived growth factor receptor

(β-subunit) [19], osteopontin (a macrophage adhesive protein [20], and various extracellular matrix proteins (collagen IV and laminin).

To determine the nature of the proliferating cells in the crescents, double immunostaining for PCNA and ezrin was performed. Tissue sections were first stained for PCNA using the DAB detection system (Table 1) followed by detection of ezrin using the Vectastain ABC-AP Kit with Vector Red (Vector Laboratories).

Immunofluorescence

To examine the glomerular binding of the injected anti-glomerular antibody, and the deposition of complement, 4 μm frozen sections were stained with FITC-conjugated antibodies to sheep IgG (Cappel; Organon Teknika Corp, Durham, NC, USA), mouse IgG (Cappel) and mouse C3 (Cappel). The presence of fibrin in the crescents was also examined by staining the tissue for fibrinogen with FITC-labeled antibody to rat fibrinogen (cross-reactive with mouse fibrinogen) (Cappel).

Histological assessment of glomerular and tubulointerstitial injury

In each biopsy, the following histopathological parameters were evaluated in a blinded fashion on PAS-stained sections: (*1*) the percentage of glomeruli with crescents, (*2*) the percentage of glomeruli with sclerosis, and (*3*) scoring of glomerular sclerosis. Glomeruli were considered to exhibit crescent formation when at least two layers of non-tubular cells were observed in the Bowman's space [21]. Glomerular sclerosis is a histological lesion characterized by segmental or global glomerular capillary obliteration with increased mesangial matrix deposition, intracapillary hyaline deposits, and focal or global adhesions of the capillary tuft into Bowman's capsule [22]. Scoring of glomerular sclerosis utilized five grades: 0 = normal glomerulus; 1+ = sclerosis involving less than 25% of glomerular surface area; 2 = sclerosis involving 25 to 50%; 3+ = 50 to 75%; and 4+ = sclerosis involving 75% to 100% of glomerular surface area. A minimum of 100 glomeruli were assessed to determine the percentage of crescentic and sclerotic glomeruli, and the sclerosis score in each animal.

The number of neutrophils, macrophages, CD4 positive T-cells and CD8 positive T-cells in the glomerular tufts and the crescents were quantified (as identified by immunostaining). At least 100 glomeruli were examined in the methylcarnoy's fixed biopsy (used to identify macrophages and neutrophils) and 30 glomeruli were examined per acetone-fixed frozen tissue (used to identify T lymphocytes).

Tubulointerstitial injury was assessed in PAS stained tissue by the presence of inflammatory cells within the interstitium, by the presence of tubular dilation, atrophy, and cast formation, and by the presence of tubular basement membrane (TBM) thickening and interstitial widen-

ing. Tubulointerstitial inflammation, tubular injury, and interstitial fibrosis were scored semiquantitatively utilizing four grades: 0 = normal interstitium and tubules; 1+ = minimal injury ($< 25\%$ of tissue section affected); 2+ = mild injury (25 to $< 50\%$ of tissue section affected); 3+ = moderate injury (50 to 75% of tissue section affected); and 4+ = severe injury ($> 75\%$ of tissue section involved).

Complement depletion

To examine the role of complement in the development of glomerular crescents in this model, we induced complement depletion in a separate group of four mice by intraperitoneal injections of cobra venom factor (CVF; 1,000 mg/kg body wt) [23] (Diamedix, Miami, FL, USA) given prior to the first dose of anti-GBM antibody (day -1) and at day 3 after the second dose of antibody. A second group of four mice received intraperitoneal saline as controls. At days 3 and 7, sera from CVF-treated and saline-treated mice were collected for measurement of mouse C3. Renal biopsies were taken at day 7 of disease and renal histology was studied as described above.

Mouse C3 levels were measured by radial immunodiffusion [24]. Briefly, goat anti-mouse C3 antibody (CIN, Costa Mesa, CA, USA) was diluted in 1% agarose made in 0.5 M veronal buffer and 2-mm wells were punched in the agarose. Sera from CVF-treated and saline-treated mice were loaded into the wells and the precipitation halos were measured at 24 hours and compared with dilutions of a normal mouse serum standard.

Statistical analysis

The percentage of glomeruli exhibiting crescents, the percentage with sclerosis, and the number of the various leukocyte populations within the glomerular tuft and crescents are expressed as mean $\pm$ SEM. Comparison between groups was evaluated by the Student's *t*-test with the Bonferroni correction.

RESULTS

Immunofluorescence studies

By direct immunofluorescence, a strong linear pattern of sheep IgG (4+) and mouse IgG (3 to 4+) was present along the GBM with weak (1+ to 2+) deposition along the TBM at all time points studied. C3 deposition was present in a granular pattern at similar sites and the intensity of the deposition of C3 increased from day 7 to day 14 but decreased at day 28.

Glomerular changes

After injection of sheep anti-rabbit glomerular antibody, all animals developed severe glomerular lesions with crescent formation at all time points studied (Table 2 and Fig. 1A). At day 7, segmental and circumferential cellular crescents were present in 20% to 34% (average $32 \pm 1.1\%$)

Table 2. Characteristic histologic features in murine crescentic nephrotoxic nephritis

	Day 7	Day 14	Day 28
Glomerular changes			
Crescents (%)	32.4 ± 1.14	27.2 ± 5.63	29.2 ± 3.11
Cellularity (0–4+)	+++	++ − +++	++
Sclerosis			
Sclerotic glomeruli %	14.8 ± 1.8	22.8 ± 4.8	34.6 ± 4.7
Sclerosis score (0–4)	2.4 ± 0.2	2.6 ± 0.3	3.2 ± 0.3
Tubulointerstitial changes (0–4+)			
Inflammation	++++	+++ − ++++	+++ − ++++
Tubular damage	+++	+++	++++
Fibrosis	0 − +	0 − +	++ − +++

of total glomeruli. This was associated with vacuolization of visceral GECs (podocytes) and segmental parietal GEC proliferation, resulting in the formation of synechiae, with adhesion of the glomerular capillary tuft to Bowman's capsule. Bowman's capsules were still intact. Segmental sclerosis was observed in 15 ± 1.8% of total glomeruli (Fig. 1B). Evidence for mesangial cell activation was demonstrated by the *de novo* expression of α-smooth muscle actin by the mesangial cells, and by increased expression of the PDGF-R β-subunit (Fig. 2). Interestingly, only occasional infiltrating neutrophils and macrophages were present in the glomerular tufts (Table 3), but collections of macrophages were observed outside the capsules in a periglomerular distribution (Fig. 3A).

At days 14 and 28, the percentages of crescentic glomeruli were 27.2 ± 5% and 29.2 ± 3%, respectively (Table 2), and were not different from day 7. However, there was a progressive increase over time in the percentage of sclerotic glomeruli (23 ± 5% at day 14, and 35 ± 5% at day 28, $P < 0.01$) and in the severity of glomerular matrix expansion as shown by sclerotic indices (2.6 ± 0.3 at day 14 and 3.2 ± 0.3 at day 28, $P < 0.05$; Table 2). At day 28, many glomeruli were obsolescent, and crescents had become more fibrocellular.

The induction of glomerular disease with anti-glomerular antibody was also associated with the development of proteinuria (Table 4).

Composition of crescents: Proliferating GECs are the primary cell type

The composition of the cells within the crescents was examined by immunostaining for various cell types (Table 3). Infiltrating leukocytes (neutrophils, macrophages, and CD4 positive and CD8 positive T lymphocytes) could not be identified in the early crescents noted on day 7 and 14 (Fig. 3). In contrast, small numbers of macrophages and CD4 positive lymphocytes were identified in the crescents at day 28. No α-smooth muscle actin myofibroblasts could be identified in crescents at all time points studied.

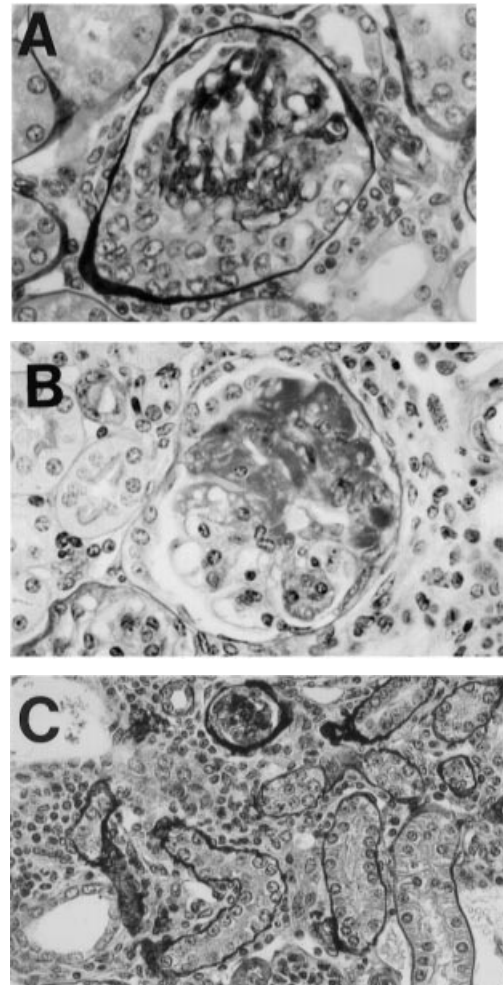The observation that the majority of the cells within the crescents were not leukocytes or α-smooth muscle actin-

Fig. 1. Nephrotoxic nephritis in mice. (*A*) Acute glomerulonephritis with crescent formation (arrow) and an intact Bowman's capsule (day 7, methenamine silver, ×630). (*B*) Segmental glomerulosclerosis (day 28, PAS stain, ×630). (*C*) Tubulointerstitial changes include tubular dilation and atrophy, interstitial mononuclear cell infiltration and interstitial fibrosis (day 7, methenamine silver; ×400).

positive myofibroblasts suggested that the proliferating cells were derived from an endogenous cell population within the glomerulus. Recently, we have identified a cytoskeletal protein, ezrin, that is expressed by parietal and visceral GECs in the rat, but not by mesangial and endothelial cells even in the presence of injury or proliferation [18]. As in the rat, ezrin is expressed by visceral and parietal GECs in the normal mouse glomerulus (Fig. 3C). In crescentic GN, the vast majority of the cells within the crescents were also ezrin-positive (Fig. 3D).

These ezrin-positive cells within the crescents were also proliferating, as noted by double immunostaining with PCNA (Fig. 3E). Many of the cells within the crescents also expressed the β subunit of the PDGF receptor (Fig. 2D), suggesting that PDGF may be one of the mitogens for crescent development. Furthermore, the observation that many of the ezrin-positive cells within the crescents were
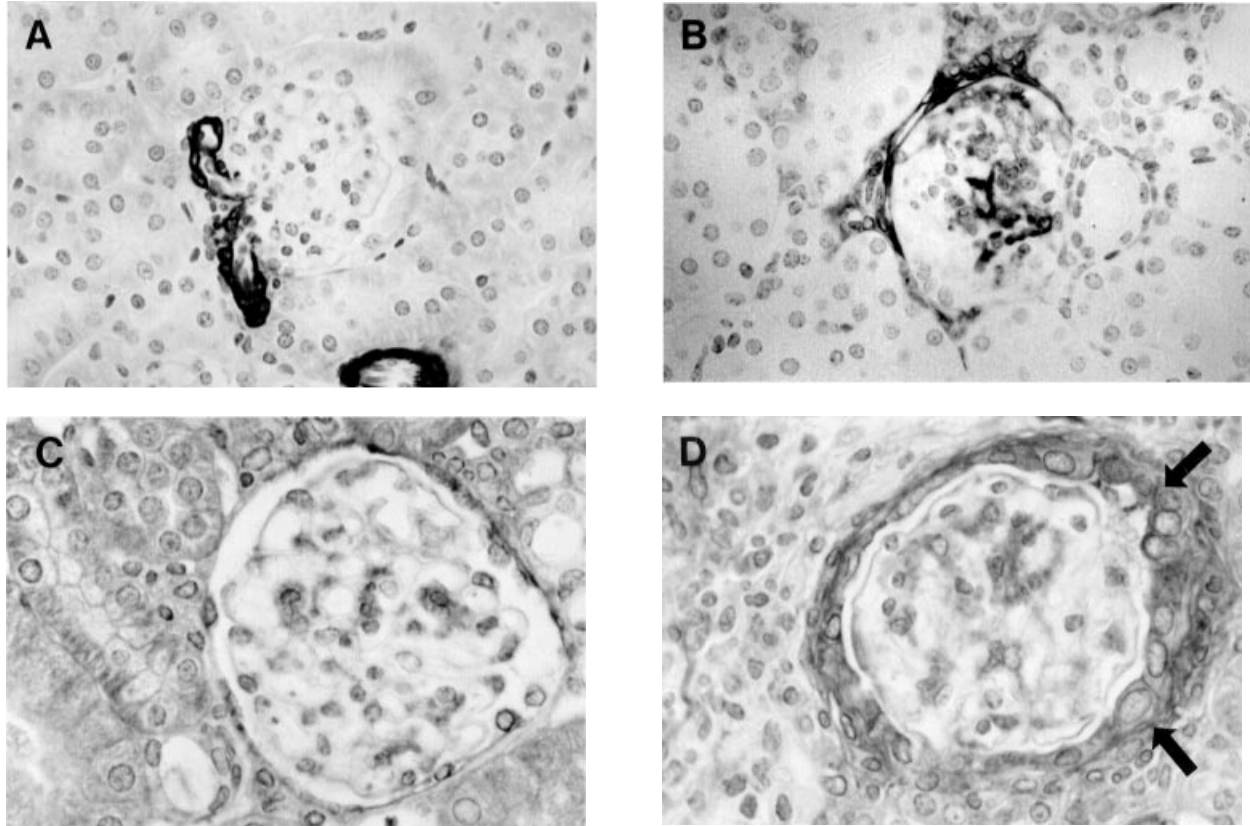
Fig. 2. Expression of α-smooth muscle actin and PDGF β receptor in nephrotoxic nephritis. (*A*) In the normal mouse, α-smooth muscle actin is expressed in arterioles with no expression in the glomerular tuft (×400). (*B*) At day 7 of nephrotoxic nephritis GN there is a marked increase in the expression of α-smooth muscle actin in the glomerular tuft and periglomerular area (×400). (*C*) There is minimal mesangial expression of PDGF-Rβ in the normal mouse glomerulus (×630). (*D*) At day 7 of crescentic GN there is a marked increase in the expression of PDGF-Rβ in the glomerular tuft and in the crescent (×630).

Table 3. Quantitation of PMNs, macrophages, and CD4+ and CD8+ T lymphocytes in the glomeruli and the glomerular crescents

	Normal glomeruli	Day 7		Day 14		Day 28	
		Crescents	Glomeruli	Crescents	Glomeruli	Crescents	Glomeruli
PMNs	0.038 ± 0.015	0 ± 0	0.262 ± 0.093	0 ± 0	0.167 ± 0.073	0 ± 0	0.136 ± 0.057
Macrophages	0.023 ± 0.001	0 ± 0	0.037 ± 0.014	0 ± 0	0.023 ± 0.014	0.052 ± 0.023	0.156 ± 0.075
CD4+ T cells	0.017 ± 0.018	0 ± 0	0.022 ± 0.027	0 ± 0	0.028 ± 0.025	0.027 ± 0.037	0.033 ± 0.024
CD8+ T cells	0.011 ± 0.017	0 ± 0	0.017 ± 0.018	0 ± 0	0.017 ± 0.018	0 ± 0	0.013 ± 0.018

expressing PDGF receptor suggested that these were parietal GECs, for in humans parietal but not visceral GEC express the PDGF-receptor [25].

The tissue sections were also stained for fibrin. In contrast to most crescentic models [12, 26], the crescents in this model were negative for fibrinogen staining except in rare glomeruli.

Tubulointerstitial changes

Marked tubulointerstitial injury occurred in this model (Table 2 and Fig. 1C). Early changes included periglomerular and patchy tubulointerstitial infiltration, tubular dilation, and tubular atrophy. By days 14 and 28, there was an increasing degree of tubular atrophy and interstitial fibrosis. PCNA-positive cells were identified in the areas of periglomerular and interstitial infiltration (Fig. 4A). The nature of the cellular infiltrates were studied by immunostaining for specific cellular markers. A major component of the interstitial infiltrates were macrophages (Fig. 4B), CD4+ T lymphocytes (Fig. 4C), and CD8+ T lymphocytes. There was also an increase in number of interstitial myofibroblasts, as shown by the presence of α-smooth muscle actin-positive cells (Fig. 4D), particularly at day 28. An increased deposition of collagen IV and laminin, as noted by immunostaining, was also present in the areas of tubulointerstitial injury (Fig. 4 E, F).
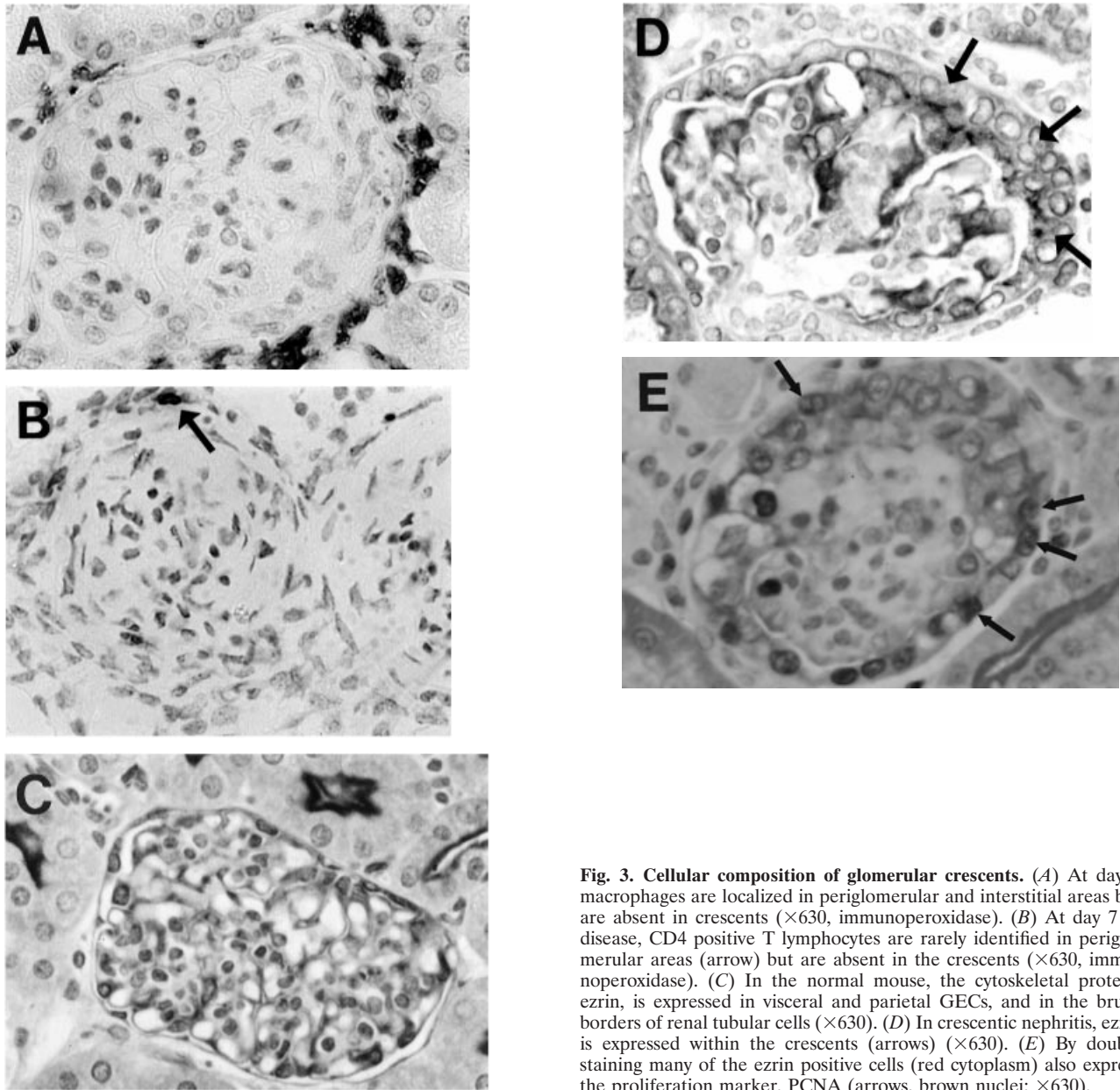
Fig. 3. Cellular composition of glomerular crescents. (*A*) At day 7 macrophages are localized in periglomerular and interstitial areas but are absent in crescents (×630, immunoperoxidase). (*B*) At day 7 of disease, CD4 positive T lymphocytes are rarely identified in periglomerular areas (arrow) but are absent in the crescents (×630, immunoperoxidase). (*C*) In the normal mouse, the cytoskeletal protein, ezrin, is expressed in visceral and parietal GECs, and in the brush borders of renal tubular cells (×630). (*D*) In crescentic nephritis, ezrin is expressed within the crescents (arrows) (×630). (*E*) By double staining many of the ezrin positive cells (red cytoplasm) also express the proliferation marker, PCNA (arrows, brown nuclei; ×630).

Table 4. Urinary protein excretion in murine crescentic nephritis (mg/d $N = 5$ per group)

	Protein excretion mg/day
Normal	2.4 ± 1.5
Day 7	45.4 ± 17.3[a]
Day 14	43.0 ± 29.7[a]
Day 28	72.3 ± 59.3[a]

[a] $P < 0.02$ vs. normal

Increased osteopontin expression in the crescents, and damaged tubules

Osteopontin (OPN), a macrophage adhesive protein [20], has been shown to be expressed in parietal GEC and in proximal and distal tubules in various models of glomerular diseases [27, 28]. In the current study, there was expression of OPN within crescents (Fig. 5). Interestingly, OPN expression was also increased in some visceral GECs of damaged glomeruli (not shown). There was also an increased expression of OPN in renal tubules, particularly in the areas of tubulointerstitial damage (Fig. 5C). Interstitial macrophage infiltration was associated with the areas of increase tubular OPN expression.

Complement depletion does not prevent the development of crescents

After CVF treatment, mice had complete depletion of C3 at days 3 and 7 of disease (0% of standard control,
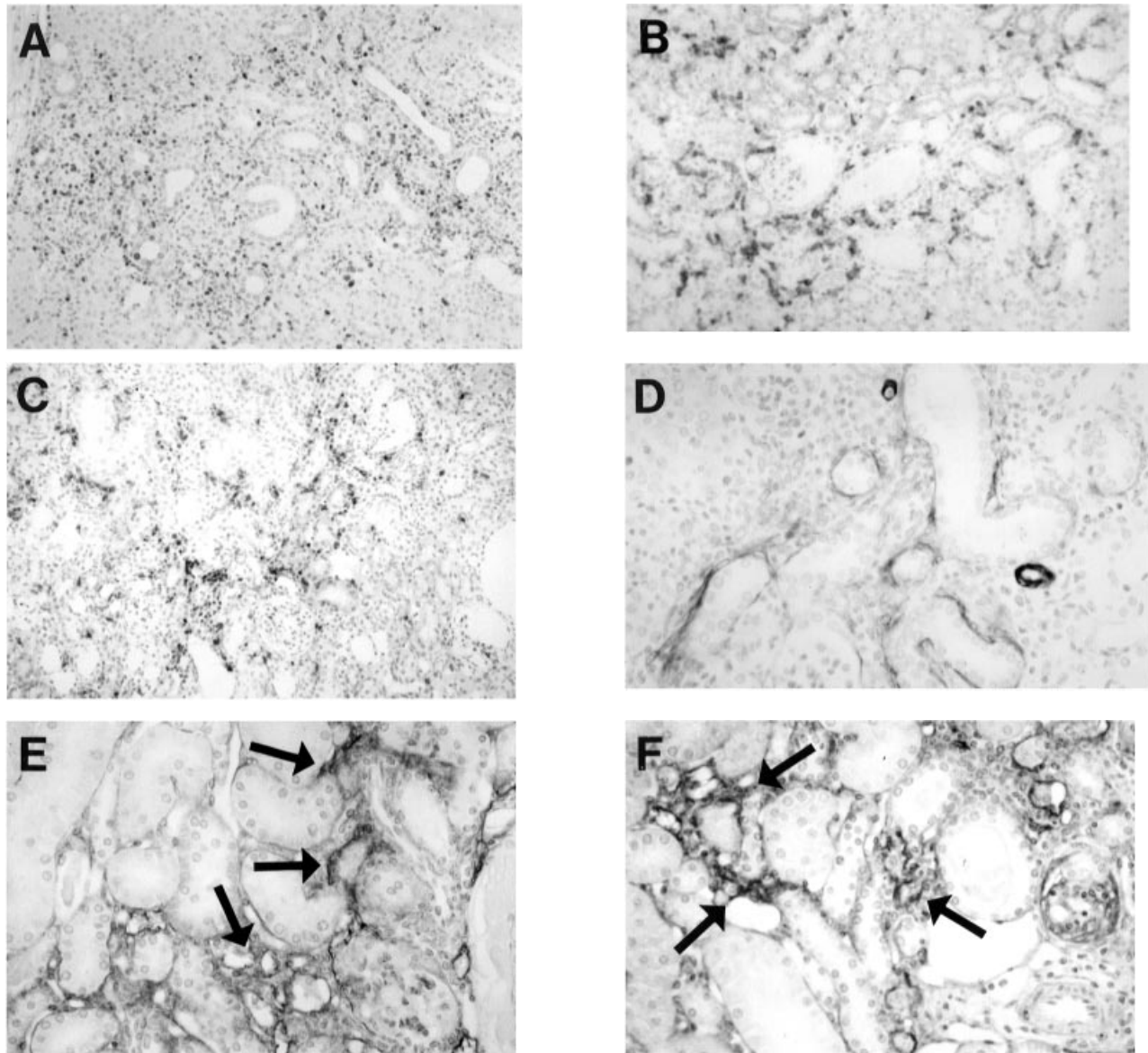
Fig. 4. Crescentic glomerulonephritis is associated with tubulointerstitial injury. (*A*) There was a marked increase in PCNA-positive cells in the tubulointerstitium at day 7 (×200). (*B*) Macrophages (×400) and (*C*) CD4+ T lymphocytes (×200) were the major cellular components of the periglomerular and interstitial infiltrates at day 7. (*D*) Cell expressing of α-smooth muscle actin were also identified in areas of interstitial injury (×400). (*E*) Type IV collagen and (*F*) laminin were also increased in interstitial areas by immunostaining at day 28 of disease (arrows) (×630).

Table 5) while saline-treated mice had a mild decrease of C3 at day 3 (60 ± 22% of standard control) and normal C3 at day 7 (102 ± 44% of standard control). CVF-treated mouse kidneys had no C3 deposition by immunofluorescence at days 3 and 7, whereas control mouse kidneys had mild C3 deposition at day 3 and moderate C3 deposition at day 7 (Table 5). However, there was no difference in the percentage of crescents between CVF-treated and saline-treated mice (Table 5).

DISCUSSION

In this study we describe a passive model of nephrotoxic nephritis induced in mice by the injection of a sheep antibody to rabbit glomeruli. The model results in severe glomerular and tubulointerstitial injury, with proteinuria, crescent formation and the progressive development of glomerulosclerosis and tubulointerstitial fibrosis.

Several interesting findings were observed in this study. The first major finding was that the early crescent formation observed at days 7 and 14 appeared to be primarily due to a proliferation of intrinsic glomerular cells. No macrophages, CD4 positive T cells or CD8 positive T cells could be detected in the lesions (as documented by immunocytochemistry) during this time. Similarly, the crescents were negative for α-smooth muscle actin, a marker of myofibroblasts [17, 29]. Nevertheless, numerous myofibroblasts and
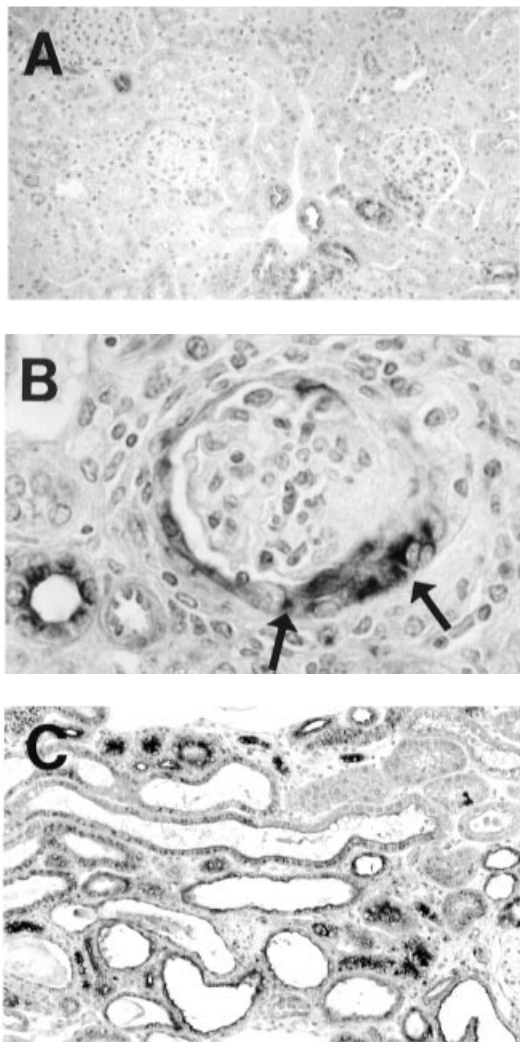
Fig. 5. Osteopontin (OPN) expression in crescentic glomerulonephritis. Normal mice express OPN in some tubules. (*A*; ×200) In nephrotoxic nephritis, OPN is expressed in both glomerular crescents (arrows) (*B*; ×630, day 7) and is increased in tubules at sites of injury (*C*; ×200, day 7).

Table 5. Effects of complement depletion (CVF) in murine crescentic nephrotoxic nephritis

	CVF-treated mice	Saline-treated mice
Serum mouse C3 levels		
% of standard		
day 3	0	60 ± 21.52
day 7	0	101.67 ± 43.19
Mouse C3 deposition in the glomeruli at day 7 (0–4+)	0	+++ − ++++
Crescents at day 7 (%)	27.0 ± 3.54[a]	27.8 ± 3.78[a]

[a] $P > 0.05$

two groups have reported that PDGF B-chain mRNA and protein are expressed in crescents [31, 32]. This suggests that local PDGF could be involved in the initial crescent formation.

Another interesting finding was that osteopontin was expressed within the crescents, an observation that has also been recently reported by Lan et al [28]. Osteopontin is an adhesive protein for a variety of cell types including macrophages [33, 34]. In models of tubulointerstitial injury, osteopontin is expressed by injured tubules, and the degree and site of osteopontin expression correlates with the macrophage infiltration [20]. Mice lacking the osteopontin gene (so-called 'knockout' mice) develop less tubulointerstitial inflammation and macrophage accumulation after ureteral obstruction as compared to control mice [35]. This suggests that osteopontin expression *in vivo* may have a role in macrophage accumulation.

However, in this model osteopontin was expressed in the crescents despite no accumulation of macrophages. One possible explanation is that the macrophages do not have access to Bowman's space in the early period of crescent fomation. Studies by Bonsib suggest that crescentic nephritis can result in areas of GBM rupture which might allow access of some leukocytes into Bowman's space [36], but this could be a late finding. Another mechanism by which macrophages and fibroblasts could enter into Bowman's space would be through breaks in Bowman's capsule [37]. Once the Bowman's capsule is ruptured, large numbers of macrophages have been documented in the crescents [38]. Some of the macrophage accumulation is also due to local macrophage proliferation [39].

The observation that the initial crescent formation was devoid of macrophages and T cells does not imply that this phase of crescent formation was independent of these cells, as these cells could be influencing the behavior of the parietal and visceral GEC via the release of cytokines and other proinflammatory molecules. Indeed, several studies have shown that crescent formation is mediated by leukocyte adhesion molecules [40] and specific T cell populations [41, 42].

In conclusion, we report a model of crescentic nephritis in the mouse. The early formation of crescents in this model appeared to be due to a proliferation of intrinsic

macrophages could be documented surrounding the glomeruli outside Bowman's capsule (Figs. 2B and 3A).

Most of the cells within the crescent were shown to be proliferating (as noted by the PCNA staining) and to be positive for the cytoskeletal protein ezrin. Ezrin is an actin-binding cytoskeletal protein that is expressed by both visceral and parietal GEC but not by mesangial or endothelial cells [18]. The observation that most of these cells were ezrin-positive is consistent with them being of either parietal or visceral origin, and is consistent with reports that parietal GEC constitutes a major component of crescents [11].

Many of these ezrin positive cells within the crescent were expressing the PDGF receptor β-subunit. We have previously reported that human parietal GEC express the PDGF receptor [25], whereas visceral GEC lack PDGF receptors and do not respond to PDGF [30]. Furthermore,

glomerular (most likely parietal or visceral GEC) cells and is associated with local PDGF receptor expression. Despite the local expression of the macrophage adhesive factor, osteopontin, no macrophages and T cells could be identified during the early phase of crescent formation. This may be due to the fact that at this stage of the lesion (before a documentable rupture of Bowman's capsule), the macrophages and T cells have limited access into this sequestered space.

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APPENDIX

Abbreviations used in this article are: CVF, cobra venom factor; GBM, glomerular basement membrane; GEC, glomerular epithelial cells; GN, glomerulonephritis; Ig, immunoglobulin; OPN, osteopontin; PCNA, proliferating cell nuclear antigen; PDGF-R β, platelet-derived growth factor receptor β-subunit; RPGN, rapidly progressive glomerulonephritis; TBM, tubular basement membrane.

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