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Mechanisms of Clearance of Immune Complexes from Peritubular Capillaries in the Rat

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These experiments evaluated extraglomerular sites of renal immune complex (IC) deposition and specific features of host capability to remove these IC. Ex vivo ***perfusion of rat kidneys with the endothelium binding lectin concanavalin A (con A) followed by rabbit anti con A IgG results in a subendothelial IC nephritis in glomerular capillaries (GC) and diffuse IC formation with complement (C_3) deposition in peritubular capillaries (PC). Histologic, immunofluorescence, and ultrastructural studies were performed at 10 minutes and 1, 4, and 24 hours after perfusion. At 10 minutes, strong linear binding of con A, rabbit IgG, and rat C_3 to the endothelium was detected by immunofluorescence in both GC and PC. In GC this was followed by endothelial cell swelling and denudation (1 hour) with platelet and neutrophil infiltration and formation of subendothelial IC deposits which persisted at 4 and 24 hours. In contrast, some PC endothelial swelling was also present at 10 minutes and 1 hour, but ICs (IgG, con A, C_3) were capped and shed into capillary lumina at 1 to 2 hours with complete clearance of IC by 4 hours. Selective neutrophil depletion, by antisera and irradiation, and complement depletion with cobra venom factor, delayed clearance of PC IC by several hours but complete clearance of IC with restored structural integrity of PC was still achieved by 24 hours. Platelet depletion had no effect on PC IC clearance. These studies demonstrate a model for study of PC IC. Such a model may aid our understanding of lupus nephritis in which extensive GC IC deposits associated with severe inflammatory injury may coexist with PC deposits. Efficient clearance of IC in PC compared with GC may be due to differences in hemodynamic forces, amounts of IC formed in each of these sites, differences in binding of IC to subendothelial basement membrane, or phenotypic specialization of the endothelium lining these two different capillary beds. (Am J Pathol 1991, 139:855–867)***

Extraglomerular renal capillary wall immune deposits in humans are commonly encountered in biopsies obtained from patients with proliferative lupus nephritis.[1–6] They have also uncommonly been reported in allograft kidneys undergoing rejection.[1,7] The significance and natural history of these deposits remains poorly understood, and our potential to achieve a better understanding remains hampered by the paucity of relevant experimental models of this type of immune renal injury. This is particularly disappointing in the context of the considerable recent advances made in understanding of the role of the endothelial cell as both a target and modulator of immune and inflammatory tissue injury.[8,9]

We have approached this problem utilizing the concanavalin A (con A)/rabbit anti-con A antibody *ex vivo* renal perfusion model in the rat, which results in endothelial binding of the lectin con A, followed by subsequent formation of con A/rabbit anti-con A IgG immune complexes (IC) *in situ* on the endothelial and subendothelial surfaces of the renal microvasculature.[10,11] Within the glomerulus, we have shown that subsequent glomerular injury and proteinuria is dependent on the influx of neutrophils and platelets as well as activation of complement.[11,12]

We have extended these studies to the renal peritubular capillaries, where the renal artery perfusion of con A followed by antibody to con A also results in rapid formation of IC *in situ* on the endothelial surface. We have found that there are considerable differences in the susceptibility of the glomerular and peritubular capillary beds to persistence of the IC deposits and induction of inflammatory injury. Furthermore, mediators that contribute to capillary wall injury in glomerular capillaries, specifically white

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cells, and complement, are shown to instead have a beneficial role in peritubular capillaries in that they appear to facilitate clearance of IC.

Methods

Description of Immune Complex Nephritis

Immune complex nephritis was induced in rats by the selective renal artery perfusion of the lectin concanavalin A (con A) followed by anti-concanavalin A antibody, as described previously.[11,13] The infusion of con A results in binding to glucose and mannose residues on glomerular endothelial cells and basement membrane (GBM) glycoprotein, and hence allows it to function as a "planted" antigen.[12] The subsequent perfusion of anti-con A antibody results in formation of immune complexes *in situ* on the endothelial cell surface.

Renal Artery Perfusion

The technique for inducing GN with con A and antibody to con A has been described in detail previously.[11] Briefly, male Sprague-Dawley rats (Tyler Labs, Bellevue, Washington, USA) weighing between 200 and 300 g were anesthetized with equithesin (pentobarbital and chloral hydrate) IP (0.3 cc per 100 g body wt), and a left nephrectomy was performed. The aorta was isolated above and below the right renal and superior mesenteric arteries, and blood flow was interrupted. The superior mesenteric artery was cannulated in a retrograde manner with a 30-gauge needle and the right kidney perfused using a constant infusion pump (Sage Instrument Div., Orion Research Inc., Cambridge, MA) at a rate of 0.75 ml/min on a heated operating table. The kidney was initially perfused with 0.5 ml phosphate-buffered saline (PBS), pH 7.2 followed by 1.0 ml of PBS containing 125 μg con A (ICN Biomedicals, Costa Mesa, CA). After a flush of the cannula with 0.2 ml PBS, an additional infusion of polyclonal rabbit anti-con A IgG (19 mg in 0.5 ml PBS) followed by PBS (0.3 ml) was performed. The preparation of the anti-con A IgG has been described previously.[13] Blood flow was then restored to the kidney, the needle removed, and bleeding from the puncture site was stopped by the application of gelfoam (Upjohn Co., Kalamazoo, Michigan) and gentle pressure. Total ischemia of the kidney was always less than eight minutes. The abdominal wound was then closed and the animal was placed under a heat lamp for 1 to 2 hours until he had recovered from anesthesia.

Experimental Protocol

The rats used in this study were initially studied and reported in a separate investigation of the kinetics and leukocyte and platelet influx within the glomerulus in the con A/anti-con A model.[12] Immune complex nephritis was induced in one group of rats by renal artery perfusion of con A and anti-con A antibody as described earlier (n = 18). Control rats underwent renal artery perfusion with PBS alone (n = 16). Other groups studied included rats with immune complex GN that had been previously depleted of complement (n = 16), leukocytes (n = 16), or platelets (n = 16).

Experimental Animals

Each group (n = 4) was killed at 10 minutes, 1 hour, 4 hours, and 24 hours. At the time of sacrifice, the blood was sampled for white cell and platelet counts and C3 levels, and renal tissue was obtained for histologic studies. Two additional rats in the unmodified con A/anti-con A treated group were killed at 2 hours, for total n of 4, 4, 2, 4, and 4 animals at 10 minutes, 1 hour, 2 hours, 4 hours, and 24 hours, respectively in this group.

Complement (C) Depletion

Rats were depleted of complement with cobra venom factor (CVF; Naja naja kaounthia, Diamedix Corporation, Miami, FL).[14] Rats were injected with CVF 300 μ/kg IP in three divided doses beginning 24 hours before renal artery perfusion and received an additional dose of 100 μ/kg IP on the day of surgery and induction of GN. Serial serum C3 concentrations were measured by radial immunodiffusion[15] to monitor the complement status of the animals. Mean serum C3 concentrations on the day of renal artery perfusion and at the time of sacrifice were 4% of the baseline serum C3 level obtained before the first injection of CVF. CVF-treated rats exhibited a leukocytosis (peripheral white blood count 25,500 ± 3088/mm^3 vs. 14,132 ± 1059/mm^2 in normal controls) but had normal peripheral platelet counts (756,000 ± 46,000 vs. 764,000 ± 44,000 in normal controls) at the time of disease induction.[11]

Leukocyte (WBC) Depletion

Rats were depleted of leukocytes (WBC) by irradiation with shielding of the right kidney in a method modified from Tucker et al.[16] After anesthesia with equithesin, rats were placed on a rotating platform and the right kidney

was shielded with a 3-cm thick lead block. Rats received 800 total rads at a rate of 20 rads/min using a unidirectional ^{137}Cs source. Dosimetry studies have confirmed that the shielded kidney receives less than 10% of the total dose (that is, 60–70 rads). Three days after irradiation rats also were given 1.0 ml anti-PMN serum IV, that has been described previously.[13] On day 4 the renal artery perfusion of con A and anti-con A was performed. At the time of surgery all 16 rats had severe leukopenia (mean 80/mm^3; normal controls 14,132 ± 1059/mm^3) and an absence of PMNs (0/mm^3). The peripheral platelet count was slightly depressed (556,000 ± 52,000 vs 764,000 ± 44,000 in normal controls), but complement levels were normal.[11] In contrast, rats studied 24 hours after induction of GN (that is, day 5 postirradiation) were leukopenic (mean WBC < 75/mm^3) and thrombocytopenic (plt ct 50,000 ± 8,000/mm^3), at the time of sacrifice.

Platelet Depletion

Rats were depleted of platelets with polyclonal goat antiplatelet IgG.[11] A dose of 40 to 50 mg/100 g body weight IP results in a selective thrombocytopenia (mean platelet count < 10,000/mm^3) within 8 hours and is sustained for over 40 hours. Platelet depleted rats had a leukocytosis (white blood count 20,800 ± 2420/mm^3 vs. 14,132 ± 1059/mm^3 in control animals) and a modest depression in C3 (mean 61% ± 2% of normal) at the time of surgery.

Renal Morphology

Tissue for light microscopy was fixed in Sorenson's buffered formalin (10% formalin in 0.1 M Na phosphate buffer, pH 7.2) for at least 4 hours, and was then dehydrated in graded ethanols and embedded in glycomethylmethacrylate (Polyscience, Warrington, PA). Sections (2–3 μm) were stained with periodic acid Schiff (PAS) reagent.

Tissue for immunofluorescence (IF) was embedded in OCT (Lab-Tech products, Miles, Naperville, IL), snap-frozen in isopentane, and sectioned with a Tissue-Tek II Microtome/Cryostat (Miles).[10] Con A was detected by indirect immunofluorescence using a biotinylated goat anti-con A antibody (Vector Labs, Burlingame, CA) followed by fluorescein isothiocyanate (FITC)-conjugated strepavidin (Amersham). Rabbit anti-con A IgG was detected using FITC-conjugated goat anti-rabbit IgG, and rat C3 with FITC-conjugated goat anti-rat C_3 (Organon Teknika Corp., BCA Cappel, West Chester, PA). Sections were examined with a Leitz Ortholux II microscope with a Ploempak 2.2 vertical fluorescence illuminator (E. Leitz Inc., Rockleigh, NJ) and the intensity was graded semiquantitatively from 0 to 4+ as described previously.[11]

Tissue for electron microscopy (EM) was fixed in half-strength Karnovsky's Solution (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M Na cacodylate buffer, pH 7.0), postfixed in osmium tetroxide, dehydrated in graded ethanols, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 410 (Philips Export BV, Eindhoven, The Netherlands) electron microscope.

Immune Complex Localization

To confirm the ingestion and removal of con A immune complexes by circulating blood cells, con A conjugated to the electron dense label ferritin (Sigma, St. Louis) was utilized for perfusion studies in unmodified rats sacrificed at 10 minutes, and 1 and 2 hours (n = 2 each group). Subsequent steps after con A-ferritin perfusion were identical to those described earlier. At sacrifice, tissue was divided for renal morphologic studies as mentioned. Ferritin particles were localized by examination of micrographs obtained by transmission electron microscopy.

Results

Unmodified con A/anti-con A Antibody-mediated Injury

Glomerular Capillaries

The glomerular injury in this model has been previously described,[11,12] and is illustrated in Figure 1. The deposition of con A, rabbit anti-con A IgG and complement on the glomerular endothelium, are each readily detectable at 10 minutes after perfusion by immunofluorescence (Figure 2). There is at the same time rapid influx of platelets, which peaks within 10 minutes and subsequent influx of neutrophils and mononuclear cells, with the influx of neutrophils peaking at 1 to 4 hours after renal artery perfusion. The capillary wall localization of con A, rabbit IgG, and rat C_3 noted at 10 minutes remains within glomerular capillaries at similar intensity for the remainder of the 24-hour study period. Electron microscopy showed the presence of an electron-dense "fuzzy" material (presumed antigen-antibody deposits) that coated the endothelium at 10 minutes. By 1 hour, immune deposits remain on the glomerular endothelial cell surface, but also are present as confluent subendothelial electron-dense deposits. The endothelium at this time is swollen and partially denuded.[12] At 24 hours, the glomerular capillary loops still contain numerous neutrophils, and in many

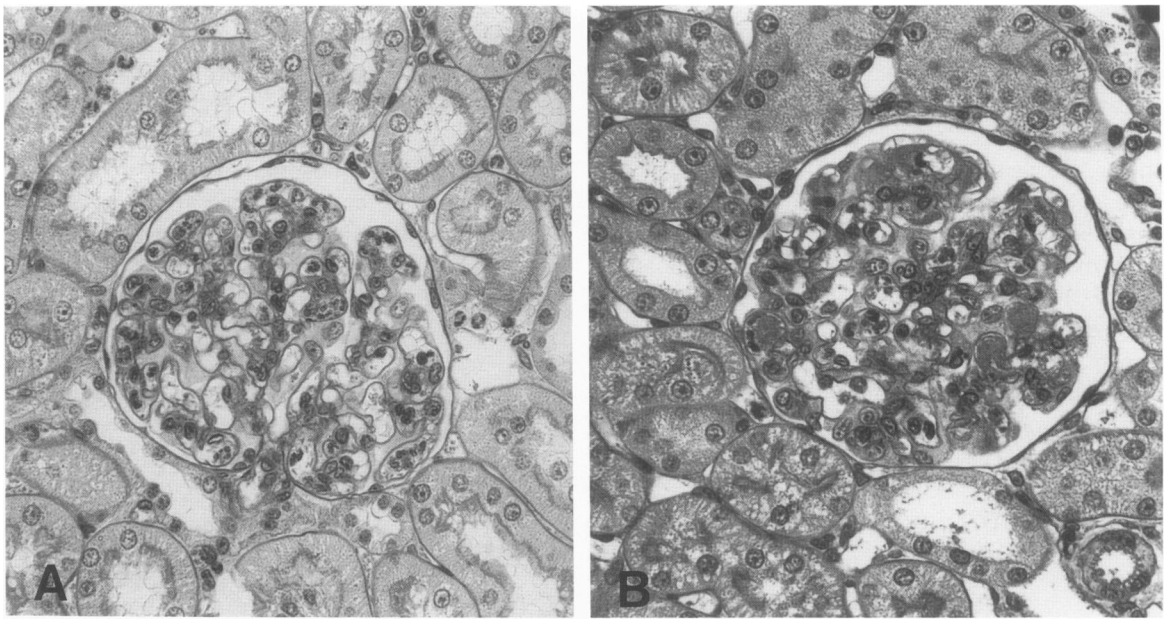

Figure 1. *Histologic appearance of renal parenchyma in unmodified animals at 1 hour* (A) *and 24 hours* (B) *after perfusion of concanavalin A (con A) and rabbit-anti con A antisera. In* (A), *there is a prominent influx of neutrophils in glomerular and peritubular capillaries, but little evidence of alteration of intrinsic renal structures. In* (B), *there is extensive glomerular injury with intracapillary thrombosis and persistence of neutrophils; tubules and peritubular capillaries are morphologically normal.* A, B, ×520.

capillary loops, there are intracapillary thrombi consisting of a mesh of degranulated platelets and fibrin. Endothelium frequently is denuded and there are diffuse, confluent electron-dense deposits on the subendothelial aspect of capillary basement membrane. Occasional small, discrete subepithelial deposits are also encountered in glomerular basement membranes at this time.

Peritubular Capillaries

In the peritubular capillaries, as in glomerular capillaries, at 10 minutes after perfusion of con A and anti-con A, there is intense (4+) linear immunofluorescence staining showing the presence of con A, rabbit anti-con A IgG and rat C_3 (Figure 2). Although there is no evidence of peritubular capillary injury by light microscopic study, electron microscopy shows that there is a diffuse coat of electron-dense "fuzzy" material on the luminal surface of the peritubular capillary endothelium similar to that seen in glomerular capillaries associated with focal endothelial cell swelling. As in the glomeruli, there is also a prominent influx of platelets into these peritubular capillaries; this influx is maximal between 10 minutes and 1 hour after perfusion (Figure 3). By 1 hour, the intense immunofluorescence staining for con A, rabbit IgG, and C_3 persist, but already there is evidence of focal capping and aggregation of immune complexes as indicated by a transformation from a "linear" to an increasingly granular pattern of staining along the peritubular capillary endothelial surface (Figure 2B, 2C). Ultrastructural examination at this time shows there is an influx of neutrophils, which appear to be phagocytosing all residual immune deposits as well as any associated endothelial cytoplasmic debris (Figure 4).

By 2 hours, the transition to a granular and less intense (2+) pattern of immunofluorescence staining in peritubular capillaries for each of con A, rabbit IgG, and C_3 is well established, and it appears numerous immune complexes are being shed into the capillary lumina (Figure 2). Ultrastructural examination shows persistence of the intraluminal neutrophils. At this time much of the electron-dense material coating endothelial cells has disappeared, a process associated with restoration of endothelial cell integrity. By 4 hours, the immune injury in the glomerulus persists, as indicated earlier, while peritubular capillary involvement has completely resolved with complete disappearance of con A, rabbit IgG, and complement from the endothelial cell surface as detected by both immunofluorescence and electron microscopy.

Studies Utilizing a Ferritin Label to con A

Studies in unmodified animals in which con A was conjugated to the electron-dense label ferritin but otherwise following exactly the experimental protocol described earlier showed similar patterns of injury as identified by histologic and immunofluorescence examination. Ultrastructural study localized the ferritin-labeled

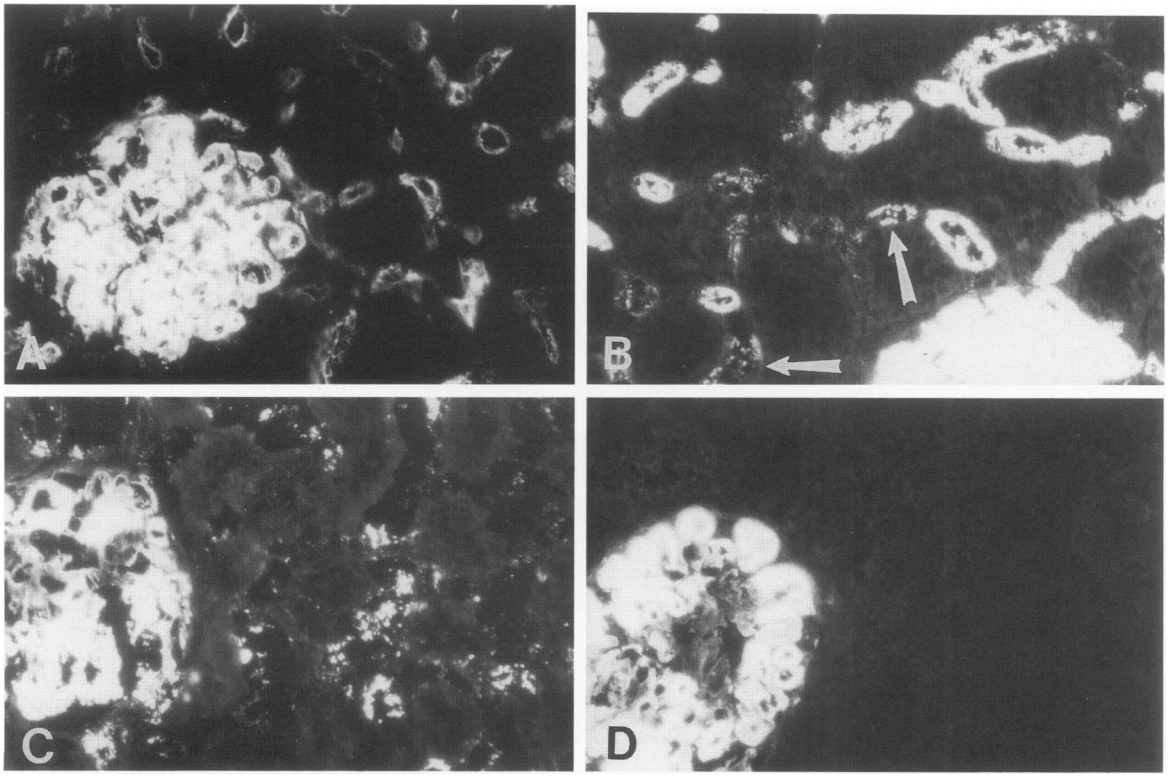

Figure 2. *Composite of typical immunofluorescence findings for localization of rabbit IgG (anti-con A antibody) in unmodified animals at 10 minutes* (A), *1 hour* (B), *2 hours* (C) *and 4 hours* (D) *after perfusion of con A and anti-con A. The intense staining of glomerular and peritubular capillaries seen in* (A) *persists at 1 hour, but there is evidence of focal capping and aggregation of immune complexes as indicated by transformation from a "linear" to an increasingly granular pattern of staining along the peritubular capillaries endothelial surface (arrows). At 2 hours, this granular pattern in peritubular capillaries is well established, and it appears complexes are being shed into the capillary lumina. At 4 hours the immune injury in the glomerulus persists, while peritubular capillary involvement has resolved. Localization of con A and rat C_3 shows a similar pattern. All, ×365.*

material to the electron-dense "fuzzy" material coating the endothelium in both glomerular and peritubular capillaries. Influxing platelets in both capillary beds showed features of activation including centralization of granules and/or degranulation, and filopodia formation. In addition, ferritin-labeled material identical to that coating the endothelium could be readily identified within vacuoles and/or cisternae in a minority of the platelets present, suggestive of a process of either endocytosis or phagocytosis (Figure 3). Clear evidence of ingestion and phagocytosis of ferritin-labeled material by neutrophils was seen at 1 and 2 hours after perfusion (Figure 4). In unmodified animals the aggregation and phagocytic activities of the neutrophils coincided with the almost complete disposal of deposits as determined by ultrastructural visualization.

Effect of Complement Depletion on Peritubular Capillary Injury

At 10 minutes, immunofluorescence studies for con A and rabbit IgG in this group of rats showed similar extensive, predominantly linear, immune complex formation in peritubular capillaries similar to that seen in the unmodified animals (Figure 5). At 1 hour, this group continued to show extensive immune complex deposition in peritubular capillary walls with linear staining (Figure 6). Morphologic studies showed that the influx of platelets and neutrophils into capillary lumina was largely abolished by complement depletion. At 4 hours, there was delayed resolution of peritubular capillary immune complexes as compared with unmodified rats with IC nephritis (Figure 7). At this time point, there was evidence of a process of capping with numerous immune complexes still present on the endothelial cell surface of peritubular capillaries as detected by immunofluorescence microscopy. However, by 24 hours, there was complete clearance of the peritubular capillary immune complexes as detected by immunofluorescence microscopy, similar to what was seen at 4 hours in control rats with nephritis (Figure 8). Immunofluorescence stains for C_3 were appropriately negative at all times.

At each time point throughout the 24-hour period, extensive, predominately linear, immune-complex deposi-

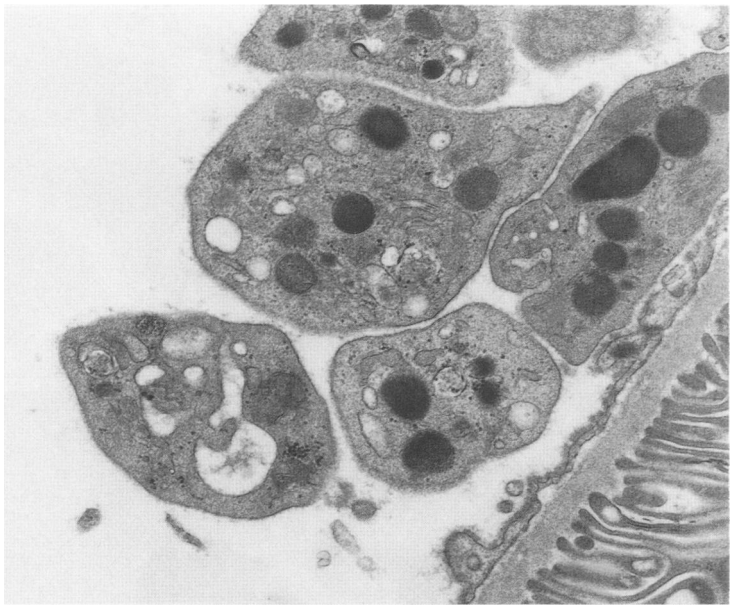

Figure 3. *Electron micrograph showing influx of platelets in peritubular capillary 10 minutes after perfusion of con A and anti-con A IgG. The endothelium is coated by ill-defined "fuzzy" material, some of which also appears to be present in platelet vacuoles and/or cisternae. ×26,250.*

tion, which was indistinguishable from the glomerular deposits seen in unmodified animals (Figures 5–8), persisted in glomerular capillaries.

Effect of White Blood Cell Depletion on Peritubular Capillary Immune-complex Injury

Severe leukocyte depletion (mean peripheral WBC count 80/mm^3) was accomplished in 16 rats with a combination of irradiation and anti-PMN serum before induction of nephritis. As in complement-depleted rats, there was strong staining by immunofluorescence microscopy for con A antigen, rabbit IgG, and rat C_3 at 10 minutes in both glomerular and peritubular capillaries; this staining was indistinguishable from unmodified rats with immune-complex nephritis (Figure 5). At 1 hour, immunofluorescence studies in this group were similar to that in complement-depleted group in that there was extensive immune-complex deposition in capillary walls (Figure 6). Leukocyte depletion had no effect of the initial platelet

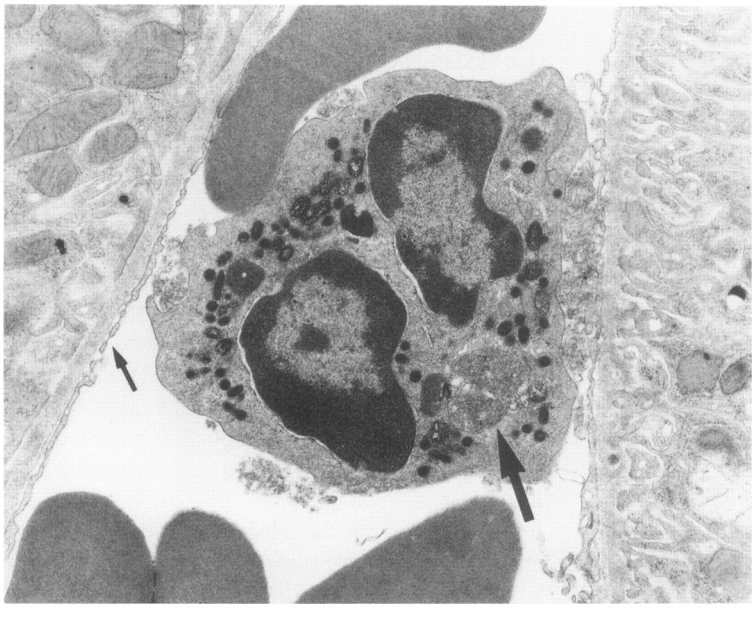

Figure 4. *Electron micrograph showing a neutrophil phagocytosing residual con A/anti-con A immune complexes from the endothelial surface at 1 hour. The con A has been conjugated to the electron dense label ferritin. Some immune complexes are already present intracellularly in a phagolysozome (arrow). Large portions of the capillary endothelium visualized in this micrograph already show complete shedding of complexes and a normal morphologic appearance including the presence of fenestrae (small arrow). ×13,100.*

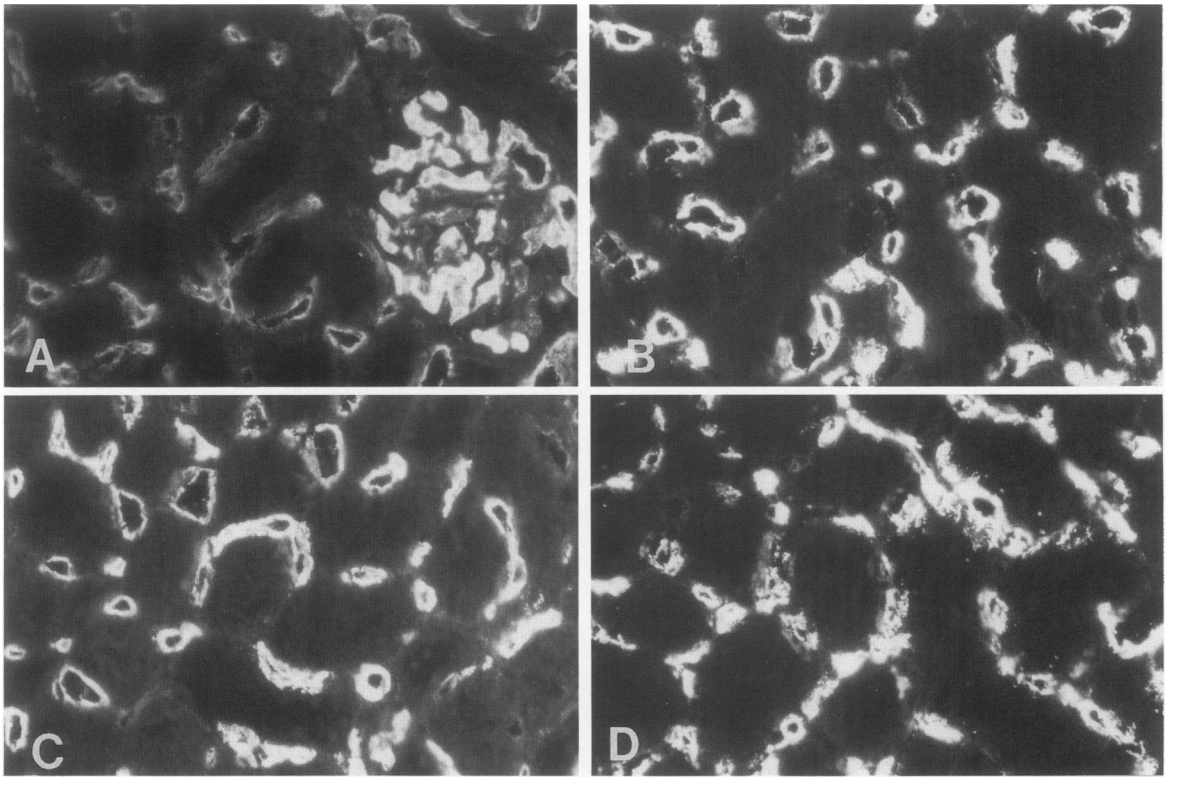

Figure 5. *Composite of typical immunofluorescence localization of rabbit IgG (anti-con A antibody) at 10 minutes in* **(A)** *unmodified,* **(B)** *platelet depleted,* **(C)** *neutrophil depleted, and* **(D)** *complement depleted groups. All groups show extensive immune complex deposition in glomerular (shown in* **A** *only) and peritubular capillary walls. Localization of con A and rat C3 showed a similar pattern in groups* **(A)(B)** *and* **(C)** C_3 *was not detectable in* **(D)**. *All,* ×365.

accumulation seen at 10 minutes and 1 hour. At 4 hours, there was again evidence of marked delay in clearance of peritubular immune deposits as detected by immunofluorescence when compared with unmodified rats with immune-complex nephritis (Figure 7). As in complement-depleted rats, there was evidence of extensive capping of immune-complex aggregates on the endothelial cell surface; these aggregates showed complete clearance at 24 hours (Figure 8).

Effect of Platelet Depletion on Peritubular Capillary Immune Complex Nephritis

Severe thrombocytopenia (mean peripheral platelet count < 10,000/mm^3) had no effect on the extent of deposition of con A antigen, rabbit IgG, or rat C_3 in either glomeruli or peritubular capillaries as detected by immunofluorescence microscopy when compared with normal unmodified rats with immune-complex nephritis at any of the time points examined (Figures 5–8). Platelet depletion had no effect on recruitment of neutrophils into peritubular capillaries at 1 hour. No discernible changes in either endothelial cell morphology or extent of electron-dense "fuzzy" material on the endothelial cell surface was detectable by ultrastructural studies in this group.

Discussion

These studies define a process of antibody-mediated renal microvascular injury whereby antibodies directed to an antigen planted on the surface of peritubular capillary endothelium form immune complexes *in situ,* which under normal conditions are then efficiently removed within 4 hours. Within this time frame, the IC remain confined to the endothelial cell surface and are not detected in peritubular capillary or tubular basement membranes. Our morphologic studies show this process of immune-complex formation is associated with activation and deposition of complement, early intravascular recruitment, and activation of platelets, and subsequent recruitment of neutrophils. Although it is known that each of these mediators may play a role in clearance of immune complexes, the clearance of the con A/anti-con A immune complexes from the peritubular capillary endothelial surface seems to involve primarily a process of clustering and shedding analogous to that previously described in

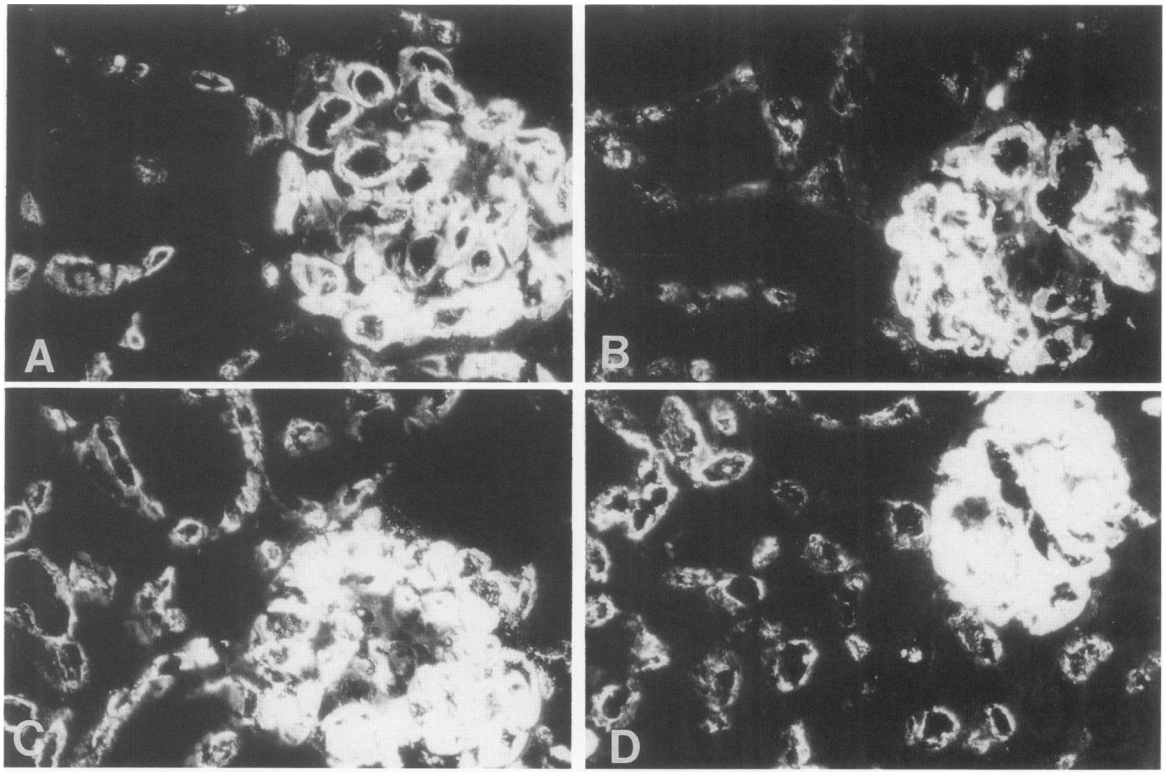

Figure 6. *Composite of typical immunofluorescence localization of rabbit IgG (anti-con A antibody) at 1 hour in* **(A)** *unmodified,* **(B)** *platelet depleted,* **(C)** *neutrophil depleted, and* **(D)** *complement depleted groups. All groups continue to show extensive immune complex deposition in glomerular and peritubular capillary walls. Localization of con A and rat C_3 showed a similar pattern in groups* **(A)(B)** *and* **(C)** *C_3 was not detectable in* **(D)**. *All, ×365.*

studies of ligand binding to lymphocyte surface antigens,[6,17,18] and in studies of anti- angiotensin–converting enzyme antibody-mediated injury to lung and glomerular endothelium.[19,20] These studies have shown that because of the fluid mosaic structure of cell membranes, a ligand, in the present case anti-con A antibody, possibly can bind to structures normally present or planted in the plasma membrane with subsequent redistribution of these bound complexes into discrete aggregates on the cell surface ("capping"). These aggregates are cleared from the cell surface either by endocytosis, or as in this model, by shedding from the cell surface into the vascular space. This process of capping and shedding of IC may be a special feature of microvascular endothelium or the antigen/antibody system used; others have had difficulty demonstrating this process in glomerular epithelial cells *in vitro* using different antigen/antibody systems with alternate, principally electrostatic, mechanisms of ligand binding.[21]

The effect of the depletion experiments involving complement, neutrophils, and platelets showed only limited modification of IC clearance and resolution in peritubular capillary injury, in contrast to our previous studies in which identical manipulations dramatically altered the natural evolution of glomerular capillary injury.[11,12] In the glomerulus, it has been shown that each of these components plays an essential role in augmenting inflammatory injury and glomerular dysfunction. The specific mechanisms by which neutrophils, complement, and platelets interact in the process of resolution of immune-complex deposition in the peritubular capillaries is not well understood. The early influx of platelets, with additional features of platelet activation and extensive contact with capillary endothelium as well as the presence of electron-dense material within platelet vacuolar structures and/or cisternae, have suggested that in the rat, platelets are able to endocytose, and possibly phagocytize, immune complexes. It follows that platelets may thus have a role in antigen processing and/or presentation, or recruitment of other inflammatory cells. To further establish this possibility, several rats were perfused with con A conjugated to the electron-dense label ferritin, which was followed by anti-con A antibody following the protocol described earlier. These rats showed accumulation of ferritin-bound electron-dense material, presumably representing con A/anti-con A immune complexes, within platelets at 10 minutes. Others have also observed apparent platelet phagocytosis in experimental and *in vitro*

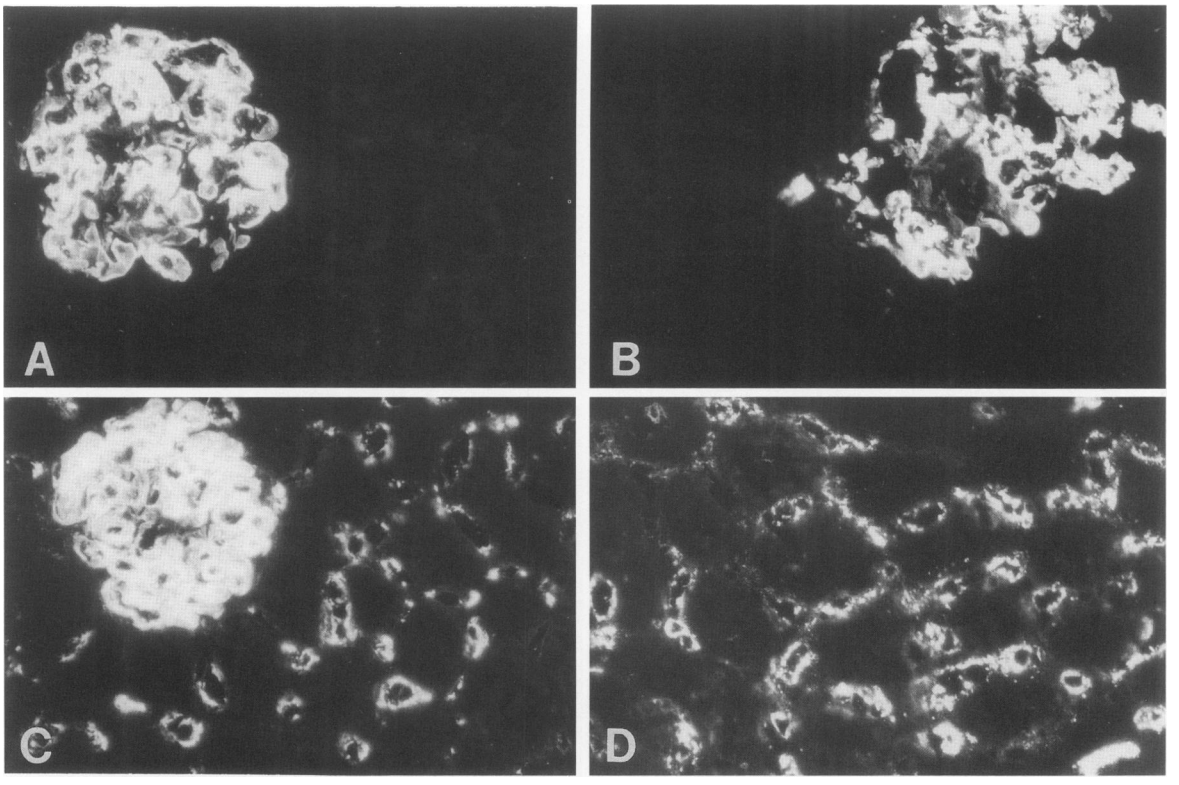

Figure 7. *Composite of typical immunofluorescence localization of rabbit IgG at 4 hours in* (A) *unmodified,* (B) *platelet depleted,* (C) *neutrophil depleted, and* (D) *complement depleted groups. Control and platelet depleted animals have near complete resolution of peritubular capillary, but not glomerular deposits, as seen in* (A) *and* (B); *neutrophil and complement depleted rats show delayed resolution of the peritubular capillary immune complexes. Localization of con A and rat C_3 showed a similar pattern in groups* (A)(B) *and* (C) *C_3 was not detectable in* (D). *All, ×365.*

settings.[22–24] Nonetheless, it is our impression based on observations of transmission electron micrographs and in the absence of any quantifiable data, that the ingestion and possibly phagocytic activities of the platelet exhibited in our studies is likely to involve a minor portion of the immune-complex disposal in unmodified animals compared with the readily identified, extensive phagocytosis of deposits by neutrophils. It is clear from our ablation studies that the platelets have no essential role in the clearance of immune complexes from peritubular capillary endothelium in this model.

Complement might participate in the removal of immune complexes via a number of mechanisms. In addition to its chemoattractant effect for inflammatory and phagocytic cells, complement has a well-known role in immune-complex removal through the process of opsonization.[25,26] An additional role for complement in solubilizing immune complexes to aid in their removal through the circulation has been recently reviewed.[26,27] The delayed clearance of peritubular capillary deposits in complement-depleted animals may be due to one or all of these mechanisms. However, the finding that the immune complexes can be removed within 24 hours in complement-depleted animals is indicative that none of these processes are essential for immune-complex clearance. Similarly, although neutrophil phagocytosis is readily identified in control animals, the fact that clearance of deposits is delayed but not prevented by neutrophil depletion indicates that the neutrophil is helpful but not required for the endothelial cell to respond effectively to this form of immune-complex injury.

A striking finding of this study is the divergence in response to an identical form of immune-complex–mediated injury by the two principal capillary beds of the kidney. The glomerular injury in this model has been previously described and illustrated (Figure 1; [11,12]). After deposition of con A, rabbit anti-con A IgG and complement on the glomerular and peritubular capillary endothelium, there is a rapid influx of platelets within 10 minutes and subsequent influx of neutrophils and mononuclear cells. However, within glomeruli the immune complexes are not cleared by capping and shedding, and there is an evolution to a severe proliferative glomerulonephritis with prominent intracapillary thrombus formation and occasional segmental necrosis of capillary loops, as seen in Figure 1. Ultrastructural examination of

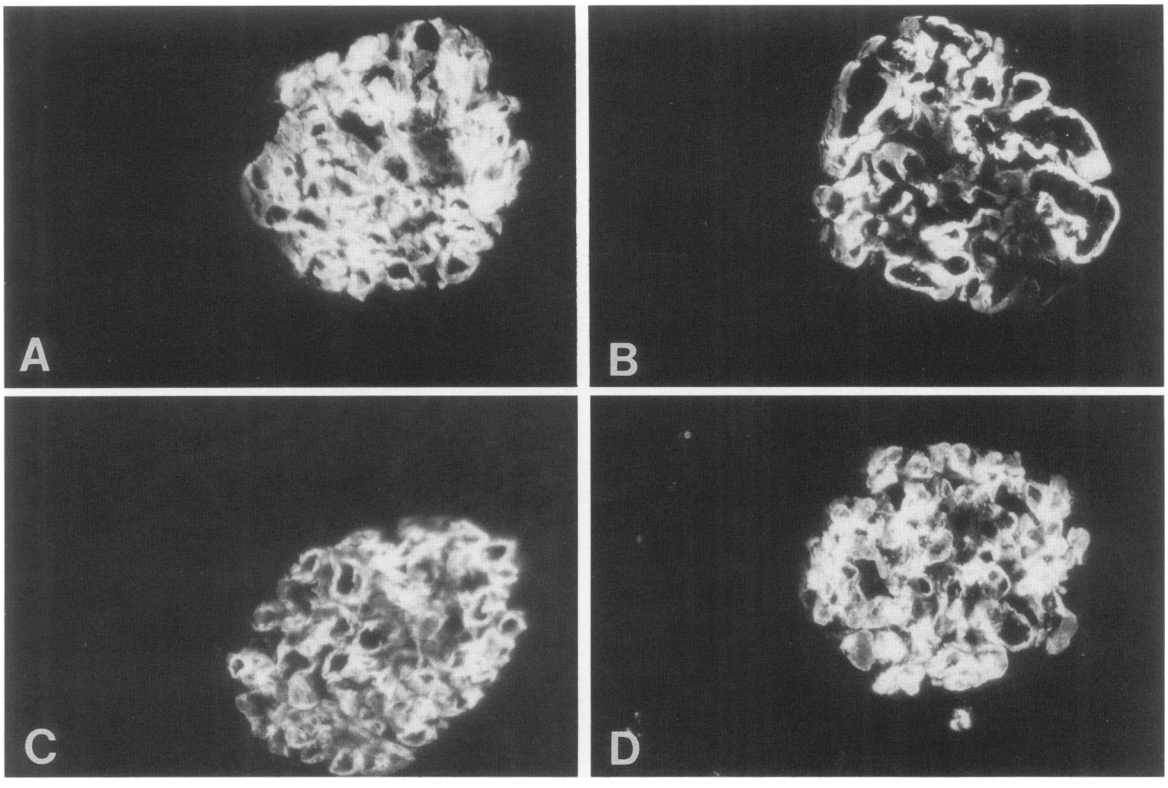

Figure 8. *Composite of typical immunofluorescence localization of rabbit IgG at 24 hours in* (A) *unmodified,* (B) *platelet depleted,* (C) *neutrophil depleted, and* (D) *complement depleted groups. All groups are similar in showing persistent glomerular deposits with virtually complete clearance of immune complexes from peritubular capillaries. Localization of con A and rat C_3 showed a similar pattern in groups* (A)(B) *and* (C) *C_3 was not detectable in* (D). *All, ×365.*

glomeruli at 24 hours after initiation of injury shows extensive denudation of capillary endothelium, extensive confluent subendothelial electron-dense deposits, and infrequent, small subepithelial-dense deposits. This glomerular process develops while adjacent peritubular capillaries show efficient clearance of immune complexes and complete restoration of endothelial cell integrity.

How can we explain these dramatic differences of complete resolution versus severe persistent injury in adjacent capillary beds? By both immunofluorescence and ultrastructural studies, despite a first passage through the glomerular capillary beds, enough con A and anti-con A antibody remain in the renal circulation to allow binding over the surface of virtually the entire peritubular capillary bed. There they form immune complexes, activate complement, and attract platelets and neutrophils, much as in the glomerular capillaries. However, we have no method at present that would allow us to quantitate the amount of con A and IgG deposits in peritubular capillaries. Therefore we cannot directly address the critical question of whether the observed differences in the morphologic injury might be attributable to lesser quantities of either antigen or antibody in the peritubular deposits. It therefore remains a plausible hypothesis that lesser amounts of IC formation within the peritubular capillary bed result in less injury to the endothelial lining compared with the glomerular capillaries. With lesser degrees of injury, the endothelial cell may be either better able to handle IC utilizing the capping and shedding responses described in this study or may present a stronger barrier to IC access to the underlying basement membranes where such complexes might bind in a manner that precludes rapid disposal.

An alternate basis for the difference in morphologic injury may be the result of differences in hemodynamic forces and hydraulic conductivity between the glomerular and peritubular capillary beds, with a higher glomerular intracapillary pressure serving to "drive" the immune deposits into the capillary wall and/or somehow preclude the endothelial cell from mounting an effective clearance response. The studies of Matsuo, et al., which utilized an alternate endothelial-binding lectin (Helix pomatia) and anti-lectin antibody to induce glomerulonephritis also bear on this possibility.[28] In that model, the glomerular endothelium was preserved, but complexes were not

capped and shed. Rather, IC were found to migrate into the glomerular capillary wall. The authors believed this result could be attributable to either hemodynamic forces within the glomerular capillary, or alternatively to compromise of the endothelial barrier to IC access to the glomerular basement membrane because of local inflammation or to initial formation of IC in the subendothelial space with subsequent binding to the glomerular basement membrane. No evidence of peritubular capillary IC formation can be deduced from the immunofluorescence micrographs in that article, and so direct comparisons to the present study are limited. Nonetheless, in the absence of a methodology that would allow us to measure hemodynamic forces or hydraulic conductivity *in situ* in the peritubular capillary bed, the hypothesis that differences in these parameters in the two capillary beds accounts for the differences in handling IC cannot yet be evaluated.

A third explanation for the differences in disease expression may be derived from differences in the biology of the endothelial cells lining these two capillary beds. Although intriguing, little is known about any such differences. Both the glomerular and peritubular capillaries are lined by fenestrated endothelium, as seen in Figure 4, although the fenestrae of the peritubular microvasculature may not be as evenly distributed and hence as readily identifiable as that of the glomeruli.[29–31] Immunohistochemical and enzyme histochemical studies have shown similar expression of MHC class I and II antigens, and similar capacities to bind such endothelial markers as Ulex europaeus lectin[32] by these two capillary beds. Some differences have been identified, including the presence of the enzymes alkaline phosphatase and ATPase in normal peritubular capillary but not glomerular endothelium.[33] The significance of each of these findings is unknown, but would support a premise that heterogeneity of renal capillary endothelium could contribute to the observed differences in capacity to respond to specific, defined injury.

Although our studies clearly document a dissociation between glomerular and peritubular capillary injury within the same animal, our findings in peritubular capillaries bear noteworthy resemblance to findings by Fries et al., in their studies of subendothelial immune complexes in rat glomeruli.[34] In that model of con A/anti-con A mediated injury, in which there are several important differences in the experimental protocol compared with the model described herein, the investigators described the initiation and evolution of glomerular injury over 24 hours that parallels to a great degree the course of unmodified peritubular capillary injury. That study showed immune-complex formation *in situ* was followed by complement deposition, influx of platelets followed by neutrophils, apparent shedding of complexes into the vascular space, and complete resolution of the injury over a longer time course but which was complete by 24 hours. For reasons not entirely clear, the differences in experimental protocol between our study and that of Fries et al., result in dramatically attenuated glomerular endothelial injury in their model, in which the processes of glomerular endothelial denudation, persistence of immune complexes, and development of functional impairment of the glomerular capillary wall as manifest by proteinuria are not achieved. Injury to peritubular capillaries in their study was not detailed. Nevertheless, their studies do document that under some circumstances glomerular endothelium is capable of responding to immune complex deposition in a manner similar to peritubular capillary endothelium, and so lend support to those hypotheses that implicate hemodynamic forces or local concentrations of antigen and antibody as the principal explanation for the differences observed in our study.

These studies may enhance our understanding of human immune-mediated tubulointerstitial disease. Endothelial cell clearance may be an effective mechanism in protecting the kidney parenchyma from some forms of antibody-mediated injury, accounting for the infrequent occurrence of such forms of injury within the tubulointerstitial compartments with the important exception of diffuse proliferative lupus nephritis noted earlier.[1–5] The peritubular capillary bed may be much less successful in defending against cell-mediated forms of immune injury, as suggested by studies of experimental and human renal transplant rejection and other forms of interstitial nephritis such as drug-allergy–associated hypersensitivity reactions.[35–37] Finally, the development of models of peritubular capillary injury may be of particular utility in view of the observations by Bohle et al., whose studies of human glomerulonephritis have led to the premise that injury and obliteration of the peritubular capillary bed is a key element in the development of interstitial fibrosis, which in turn has been recognized as the critical parameter in establishing permanent renal impairment even in patients with a primary glomerular injury.[38]

In summary, this study defines the initiation and evolution of a specific antibody-mediated form of endothelial injury in the renal peritubular vascular bed, and has further characterized how the response to injury may be modified when each of three important arms of the inflammatory response are depleted. These studies document a remarkable capacity for some endothelial cells to defend themselves against immune injury. The basis for the dissociation between extent and resolution of glomerular and peritubular capillary injury observed in this study remains to be established. It does seem likely that the integrity of the endothelial cell, both as a barrier govern-

ing accessibility of antigen and antibody to underlying basement membranes and as an active participant in the handling of immune complexes, is an important determinant of the extent of microvascular injury engendered by the con A/anti-con A model.

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