Kidney International, Vol. 36 (1989), pp. 582–592

Renal injury in DOCA-salt hypertensive C5-sufficient and C5-deficient mice

LEOPOLDO RAIJ, AGUSTIN P. DALMASSO, NANCY A. STALEY, and ALFRED J. FISH

Veterans Administration Medical Center, and Departments of Medicine, Laboratory Medicine and Pathology and Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota, USA

Renal injury in DOCA-salt hypertensive C5-sufficient and C5-deficient mice. We induced hypertension by uninephrectomy and treatment with desoxycorticosterone (DOCA) and 1% NaCl in the drinking water in congenic mice that differ in the single gene locus responsible for the presence or absence of the complement component C5 and compared them to uninephrectomized normotensive (no DOCA-NaCl) mice. In contrast to C5-sufficient (C5S) mice, C5-deficient (C5D) mice can neither generate C5a nor assemble C5b-9. After four weeks of treatment, DOCA-C5S and -C5D mice developed similar degrees of hypertension; mice receiving no DOCA remained normotensive. Only hypertensive mice developed glomerular injury. Hypertensive DOCA-C5D mice developed more glomerular capillary loop dilatation and larger glomerular capillary tuft volumes than DOCA-C5S mice (1.0 ± 0.1 vs. $0.7 \pm 0.03 \times 10^6$ μm^3, respectively, $P < 0.05$). However, DOCA-C5S mice, compared to DOCA-C5D mice, had significantly more glomerular cell proliferation (64.5 ± 2 vs. 42 ± 3 nuclei/glomerulus), cell necrosis (injury score 22 ± 1 vs. 17 ± 1), extracapillary proliferation (26 ± 4 vs. $2.5 \pm 2\%$ of glomeruli) and proteinuria (5.9 ± 0.8 vs. 3.7 ± 0.5 mg/24 hr; all $P < 0.05$). By immunofluorescence microscopy both DOCA-C5S and -C5D had mesangial C3 deposits but only DOCA-C5S mice had C9 deposits. After 16 weeks of DOCA-NaCl C5S mice, in comparison to C5D mice, had more severe glomerulosclerosis (injury score 50 ± 6 vs. 12 ± 4), proteinuria (16.6 ± 0.1 vs. 9 ± 0.1 mg/24 hr), and renal insufficiency (serum creatinine 0.25 vs. 0.15 mg/dl), all $P < 0.05$. These changes occurred despite levels of hypertension that were similar in DOCA-NaCl C5S and C5D throughout the whole study period. We conclude that C5a and/or C5b-9 may play an important role in hypertensive glomerular injury. Moreover, these studies demonstrate that differences in host responses may determine target organ susceptibility to similar injurious mechanisms.

Studies on the role of the complement system in the pathogenesis of tissue damage have recently focused on the participation of the late-acting components C5 through C9 (C5-9) in the production of injury in several disease states [1–7]. C5a is a chemoattractant and anaphylatoxin of major biological significance [8]. In addition, in the kidney C5a increases glomerular efferent arteriolar resistance which results in elevations in intraglomerular capillary pressure [9]. Membrane-bound C5b-9, also designated membrane attack complex or MAC, causes cell lysis as the result of its interaction with membrane lipid bilayers [4–7]. Recently, it has been demonstrated that the MAC in sublytic amounts can stimulate resident glomerular cells to release inflammatory mediators, reactive oxygen species, and an IL_1-like growth factor [10–12].

Several studies have demonstrated deposition of C5b-9 and C9 neoantigen, a marker of terminal complement activation, not only in tissues damaged by immune mechanisms [1, 2], but also in tissues injured by nonimmunological mechanisms [2]. However, immunohistochemical demonstration of C5b-9 does not necessarily imply that C5b-9 is responsible for producing tissue damage, particularly in lesions where C5b-9 was found to colocalize with the S protein [3]. In these circumstances it is unknown whether the S protein was added to C5b-9 locally after C5b-9 had an opportunity to induce tissue damage or whether SC5b-9 was passively deposited from the circulation [3]. Experimentally, the pathogenetic role of C5-9 in the production of tissue injury either through the action of C5a or C5b-9 has been conclusively demonstrated in several immune-mediated models of renal disease [4, 5, 13].

Recent studies have shown that the hemodynamic stress of hypertension may lead to glomerular and vascular injury in the kidney [14, 15]. Furthermore, we and others have shown experimentally that immune injury and hypertension synergistically interact in the progression of glomerular injury [16–18]. Clinically, hypertension often accelerates the progression of renal disease [19, 20]. However, the basic mechanisms by which hypertension induces vascular injury are still unclear. Recent studies demonstrated the presence of C9 neoantigen in renal blood vessel walls as well as in juxtaglomerular and mesangial regions in hypertension and diabetes mellitus [2], two diseases not mediated by immune mechanisms. These findings suggested to us that in these conditions C5-9 might be involved in the amplification of tissue injury. Therefore, we thought it important to investigate whether C5-9 plays a role in the progression of renal damage during the course of experimentally-induced hypertension.

We induced hypertension by uninephrectomy and treatment with desoxycorticosterone acetate (DOCA) and 1% NaCl in the drinking water in groups of congenic mice which differ in the single gene locus responsible for the presence or absence of C5. This experimental model was chosen because: a) C5-deficient mice can neither generate C5a nor assemble the C5b-9 complex, and b) DOCA-salt hypertension results in high glomerular capillary pressure accompanied by severe glomerular injury [21, 22]. The results showed that, at similar levels of systemic

Received for publication April 6, 1988
and in revised form April 14, 1989
Accepted for publication May 10, 1989

hypertension, C5-deficient (C5D) mice developed significantly less morphologic damage, proteinuria and renal insufficiency than normocomplementemic, C5-sufficient (C5S) mice.

Methods

Five-week-old B.10.D2NSN C5S and B.10.D20SN C5D male mice were obtained from Jackson Laboratory (Bar Harbor, Maine, USA). Mice were fed standard mice chow (Ralston Purina Company, St. Louis, Missouri, USA). Blood pressure (BP) was measured in unanesthetized mice by the tail-cuff method with an IITC apparatus (IITC Life Science, Woodland Hills, California, USA). The BP was measured during the morning in a quiet environment every two weeks [17]. The average of three successive readings was recorded. Mice were anesthetized with 1.5 mg of pentobarbital intraperitoneally and underwent right nephrectomy through a midline incision. Three days after uninephrectomy the baseline BP was determined and then C5S and C5D mice were assigned randomly to experimental groups that received 6 mg of desoxycorticosterone acetate (DOCA; Ciba, Summit, New Jersey, USA) subcutaneously once a week and 1% NaCl as drinking water or to control groups which did not receive DOCA and were given tap water.

Total hemolytic complement assays were performed to confirm the C5S and C5D phenotypes utilizing a microtiter modification of a CH50 assay described previously [23]. Rabbit red blood cells were sensitized with goat hemolysin (Cappel Laboratories, Inc., Cochranville, Pennsylvania, USA) and washed in cold gelatin-Veronal buffered saline. Mouse blood was obtained from the retroorbital plexus and placed immediately on ice. Serum dilutions were then added to the sensitized red blood cells and incubated for one hour at 37°C. At serum dilutions from C5S mice causing lysis at 80 to 100% of the complete lysis control, sera from C5D mice had negligible hemolytic activity.

Serum creatinine, 24-hour urinary protein excretion and renal tissue samples were obtained from mice 4 and 16 weeks after initiation of the experimental protocol. For urine collection mice were kept in metabolic cages. Total urinary protein was determined by the Biuret method [16, 17] and serum creatinine by a modified colorimetric Jaffe reaction [16, 17]. Groups of DOCA-treated C5S mice ($N = 12$) and C5D mice ($N = 12$) as well as controls C5S ($N = 9$) and C5D ($N = 10$) mice were bled and sacrificed four weeks after initiation of the study. In addition, groups of C5S ($N = 11$) and C5D ($N = 10$) mice received DOCA and 1% NaCl as drinking water for 16 weeks, at which time they were bled and sacrificed for examination of renal tissues.

Renal tissue was obtained for light, immunofluorescent and electron microscopy according to techniques previously described [16, 17]. Tissue samples were evaluated by immunofluorescent and light microscopy independently by two investigators (LR, AJF) without prior knowledge of the group to which the mice belonged. Coded samples prepared for electron microscopy were studied by one investigator (NS).

The following light microscopy features were evaluated: a) intracapillary cellular proliferation; b) capillary loop aneurysms characterized by dilatation of capillary loops greater than two to three times normal capillary diameter; c) glomerulosclerosis, defined as the disappearance of cellular elements from the tuft, collapse of the capillary lumen, and folding of the glomerular basement membrane with entrapment of amorphous material; d) cellular necrosis evidenced by loss of recognition of cellular membranes and by karyorrhexis; e) extracapillary cellular proliferation (crescents); and f) tubulointerstitial changes, including tubular atrophy, interstitial fibrosis and cellular infiltrates. Morphologic changes a-d were quantitated on a scale of 1 to 4 [17]. A score of 1 was equivalent to 25% of the glomerulus affected by the particular morphologic change, and 4 represented involvement of 100% of the glomerulus. Intermediate values were assigned a value of 0.5. An injury score was then obtained by multiplying the severity of the change by the percentage of glomeruli with the same severity of change [17]. The extent of injury for each individual tissue specimen was then obtained by the addition of these scores. Morphologic changes e and f were expressed directly as the percentage of glomeruli (e) or tubulointerstitial tissue (f) involved. As the injury score for individual tissue specimens derived by each investigator varied from 15 to 20%, the scores obtained by the two investigators were averaged.

In addition in blinded tissue samples of control and DOCA-C5S and -C5D mice sacrificed at four weeks, we counted glomeruli nuclei and measured glomerular volumes in 30 glomeruli randomly selected from six mice in each group. Glomerular tuft volumes were measured by stereological point counting according to techniques previously described [24, 25]. For this purpose a projection head was fitted to the microscope for the projection of an 81 point grid with 1 cm between interjections (total magnification ×530). The mean glomerular volume is obtained as $V(G) = \times [A(G)]^{3/2}$ where = 1.38 pertains to spheres, k (a distribution coefficient) is taken to be 1.10 [24, 25] and A(G) is the glomerular cross-sectional area. Glomerular nuclei and volumes were not determined in 16-week-old mice because the presence of glomerulosclerosis in DOCA-C5S mice would have interfered with the measurements [26].

For immunohistologic studies renal tissue was treated with monospecific antisera (rabbit or goat) against mouse IgG, IgM, C3, albumin and fibrin (Cappel Laboratories). Deposits of C9 were identified with a rabbit antiserum raised in our laboratory against purified rat C9 that was found to cross-react with mouse C9. The antisera were either fluorescein labeled or unlabeled and then reacted by indirect staining. Monoclonal antibodies (from Dr. Jeffrey Platt, Dept. of Pediatrics, University of Minnesota) of the following specificities were used to search for lymphocyte populations and monocytes in renal tissue: Lyt 1 (CD5) and Lyt 2 (CD8) (Becton Dickinson, Mountain View, California, USA), Thy 1 and MAC 1 (CR3) (Boehringer, Indianapolis, Indiana, USA), and GK 1.5 (CD4) and IL2R (American Tissue Culture Collection, Washington, D.C., USA). Four micron frozen sections were prepared and mounted on glass slides, air dried, and acetone fixed. Sections were reacted with appropriate primary antibody reagents and then with fluorescein-conjugated goat F(ab')2 anti-rat IgG. For immunofluorescent enhancement, a second fluorochrome of F(ab')2 rabbit IgG against the first fluorochrome layer was used [16, 27]. Control sections were incubated with myeloma-derived Ig of the same isotipe as the monoclonal antibody. Sections were also reacted with rhodamine-conjugated rabbit antisera to type IV collagen and laminin [27]. None of the antisera reacted with glomerular cells from normal C5S or C5D mice.

Data were expressed as mean ± SEM. Data from the different

Table 1. Effect of treatment with DOCA-1% NaCl on blood pressure, protein excretion, serum creatinine and body weight of C5-sufficient and C5-deficient mice

		Duration of treatment							
		4 Weeks				16 Weeks			
Group	DOCA-1% NaCl	BP *mm Hg*	U prot *mg/24 hr*	S_{Cr} *mg/dl*	Body wt *g*	BP *mm Hg*	U Prot *mg/24 hr*	S_{Cr} *mg/dl*	Body wt *g*
C5S[a]	No ($N = 9$)	95 ± 4	2.6 ± 0.3	≤0.1	22 ± 0.3	—	—	—	—
C5S[b]	Yes ($N = 9$)	148 ± 10	5.9 ± 0.8	≤0.1	20 ± 0.5	157 ± 3 ($N = 11$)	16.6 ± 0.1	0.25 ± 0.03	31 ± 1
C5D[c]	No ($N = 10$)	96 ± 3	2.0 ± 0.3	≤0.1	21 ± 0.6	—	—	—	—
C5D[d]	Yes ($N = 10$)	143 ± 7	3.7 ± 0.5	≤0.1	19 ± 0.4	157 ± 4 ($N = 10$)	9 ± 0.1	0.15 ± 0.02	33 ± 0.5

Values are mean ± SEM. Abbreviations are: C5S, C5-sufficient mice, C5D, C5-deficient mice; BP, blood pressure; U prot, urine protein: S_{Cr}, serum creatinine; Body wt, body weight; N = number of mice; —, not done.

The following comparisons between groups yielded $P < 0.05$:

At 4 weeks: BP, a vs. b, d
c vs. b, d
U Prot b vs. a, c, d

At 16 weeks: U Prot, b vs. d
S_{Cr}, b vs. d

4 weeks vs. 16 weeks: U prot, b and d
S_{Cr}, b
Body wt, b and d

groups were compared by ANOVA using the Scheffe F-test. $P < 0.05$ was considered significant.

Results

Mice studied at four weeks

Control C5S and C5D mice remained normotensive with blood pressures of 95 ± 4 mm Hg and 96 ± 3 mm Hg, respectively. C5S and C5D mice which received DOCA-NaCl became equally hypertensive, with blood pressures of 148 ± 10 mm Hg and 143 ± 7 mm Hg, respectively. During this period three C5S and two C5D mice died. The surviving mice in all groups appeared healthy and achieved similar body weights (Table 1). Compared with respective control groups, DOCA-C5S but not DOCA-C5D mice had significant increase in urine protein excretion (Table 1). The urinary protein excretion of DOCA-C5S mice was higher than that observed in DOCA-C5D mice (Table 1). All groups had similar serum creatinine (Table 1).

The kidney weight of control C5S and C5D was similar, 0.16 ± 0.01 versus 0.16 ± 0.02 g, respectively. The kidney weight of C5S and C5D mice which received DOCA was significantly increased compared with the respective control mice, 0.31 ± 0.03 and 0.28 ± 0.02 g, respectively. Light microscopy showed normal glomerular and interstitial architecture in normotensive control C5S and C5D mice. Morphologically, glomeruli of normotensive C5S and C5D mice had similar number of glomerular cells (32 ± 2 vs. 27 ± 1 nuclei/glomerulus) and glomerular volumes (0.27 ± 0.01 vs. 0.23 ± 0.02 × 10^6 μm^3, respectively; Table 2). Hypertensive DOCA-C5S and DOCA-C5D mice developed morphologic abnormalities (Tables 2 and 3). Glomeruli of DOCA-C5S mice showed diffuse intraglomerular capillary proliferation (64.5 ± 2 nuclei per glomerulus) which affected primarily the mesangium. Cellular necrosis (score 22 ± 1) was randomly distributed among glomerular lobules; 26% ± 4 of glomeruli had cellular crescent formation (Figs. 1 and 2). The tubules and interstitium were well preserved. These morphologic changes contrasted with those observed in hypertensive DOCA-C5D mice. Although DOCA-C5D mice had intracapillary proliferation and cellular necrosis, these changes were 35% and 23% less severe than in DOCA-C5S mice, respectively (Tables 2 and 3). Crescents were present only in 2.5% of glomeruli of DOCA-C5D mice. However, striking aneurysmal dilatation of capillary loops equivalent to 2 to 3 normal capillary loops were more evident in DOCA-C5D (score 112 ± 10) than in DOCA-C5S (score 53 ± 7) mice (Fig. 2). The glomerular capillary volume of hypertensive DOCA-C5S and DOCA-C5D was significantly increased compared with the respective control mice (Table 2). However, the glomerular capillary volumes of DOCA-C5D mice were signif-

Table 2. Glomerular nuclei number and glomerular volumes in normotensive and hypertensive C5S and C5D mice after 4 weeks of uninephrectomy or uninephrectomy plus DOCA-1% NaCl respectively

Group	DOCA	Nuclei/glomerulus	Glomerular volume × 10^6 μm^3
C5S[a]	No	32 ± 2	0.27 ± 0.01
C5D[b]	No	27 ± 0.8	0.23 ± 0.02
C5S[c]	Yes	64.5 ± 2	0.61 ± 0.02
C5D[d]	Yes	42 ± 3	1.0 ± 0.1

$N = 6$ in each group. Values are mean ± SEM.

The following comparisons between groups yielded $P < 0.05$ for both nuclei/glomerulus and for glomerular volumes:

a vs. c and d
b vs. c and d
c vs. d

Table 3. Renal pathological changes in C5-sufficient and C5-deficient mice given DOCA-1% NaCl for either 4 or 16 weeks

	Duration of treatment			
	4 Weeks		16 Weeks	
Pathological changes	C5S[a] (N = 9)	C5D[b] (N = 10)	C5S[c] (N = 11)	C5D[d] (N = 10)
Glomerular				
Cellularity	137 ± 9	87 ± 6	107 ± 7	78 ± 7
Cell necrosis	22 ± 1	17 ± 1	20 ± 2	10 ± 2
Crescents	26 ± 4	2.5 ± 2	16 ± 2	2.5 ± 2
Capillary dilatation	53 ± 7	112 ± 10	16 ± 5	107 ± 8
Sclerosis	0	0	50 ± 6	12 ± 4
Interstitial	8 ± 2	2.5 ± 2	23 ± 2	5 ± 2

Values are mean ± SEM. Abbreviations are: C5S, C5-sufficient mice; C5D, C5-deficient mice; N, number of mice; cellularity, necrosis, sclerosis and capillary dilation are expressed as injury scores; crescents and interstitial changes are expressed as percent of glomeruli or interstitium involved, respectively. Normotensive C5S and C5D mice had normal glomeruli and interstitium both at 4 and 16 weeks.

The following comparisons between groups yielded $P < 0.05$:
Cellularity: a vs. b, c, d and c vs. d
Cell necrosis: a vs. b, d and c vs. d
Crescents: a vs. b, c, d and c vs. d
Capillary dilatations: a vs. b, c, d and c vs. d
Sclerosis: c vs. a, b, d
Interstitial changes: a vs. b, c, and c vs. d

icantly larger than those in DOCA-C5S (1.0 ± 0.1 vs. 0.61 ± 0.02 × 10^6 μm^3, respectively). Tubulointerstitial changes were minimal in both C5S and C5D mice.

By immunofluorescence microscopy, staining with all antisera was negative in normotensive control mice. In hypertensive mice staining for IgG was negative. Staining for IgM, fibrin and C3 was present primarily in the mesangium and in some peripheral glomerular capillary loops of both DOCA-C5S and DOCA-C5D mice (Fig. 3). Fibrin was present in crescents. The intensity of IgM and fibrin was greater in C5D mice, particularly in the mesangium and peripheral capillary loops. These animals also had C3 deposition in the subendothelium of dilated glomerular capillaries. C9 was present in a focal and segmental distribution in capillary loops and mesangium of DOCA-C5S mice but was absent in DOCA-C5D mice (Fig. 4). The extent of mesangial expansion described above by light microscopy was further assessed by immunofluorescent staining for type IV collagen and laminin which are known to be present in the mesangium. Compared to normotensive mice both groups of hypertensive mice had expanded distribution of type IV collagen and laminin within the mesangium. No significant glomerular infiltration of lymphoid cells was detected in either DOCA-C5S or -C5D mice; however, positive staining for MAC 1 was observed in some crescents.

By electron microscopy normotensive C5S and C5D mice had normal glomeruli. In hypertensive C5S mice glomeruli showed proliferation of mesangial cells as well as widening of mesangial channels. The proliferation of mesangial cells encroached upon the capillary lumens leading to a reduction in the lumen of some capillary loops and in others to a compensatory dilatation of the capillary loop towards the periphery (Fig. 5). In some glomeruli extracapillary proliferation of epithelial cells in the form of crescents could be observed. In occasional glomeruli of C5S mice gaps in the glomerular basement membrane were seen in association with crescent formation (Fig. 5). Focal fusion of epithelial cells foot processes was present in some glomeruli. Fibrillar material with the appearance of fibrin was present in the crescents but was rare within capillary loops. Neither polymorphonuclear leukocytes nor monocytes could be identified within the mesangial areas or in their vicinity. On the other hand some mononuclear cells with the appearance of monocytes could be identified in crescents in proximity to proliferating epithelial cells. As observed by light microscopy, intraglomerular as well as extraglomerular capillary proliferation was less marked in hypertensive C5D mice. In glomeruli of DOCA-C5D mice strikingly dilated capillary loops without evidence of peripheral capillary wall rupture were observed. The dilatation appeared to be due to stretching of the capillary loops themselves which resulted in tears in the anchoring points of the mesangium (Fig. 6). However, contrasting with the glomeruli of C5S mice, the dilated capillary loops were not filled by proliferating mesangial cells. In many capillary loops of both DOCA-C5S and DOCA-C5D the endothelium was focally disrupted leading to subendothelial accumulation of proteinaceus material. The visceral epithelial cells of C5D mice were remarkably well preserved despite being stretched by the dilated glomerular capillaries (Fig. 6).

Mice studied at 16 weeks

The severity of hypertension was similar in C5S and C5D mice throughout the study period. Moreover, the blood pressure of mice which received DOCA-NaCl for 16 weeks was not significantly different from the blood pressure of mice sacrificed after only four weeks of DOCA-NaCl (Table 1). The urinary protein excretion of both DOCA-C5S and DOCA-C5D mice was higher at 16 weeks than at four weeks. However, the urinary protein excretion of DOCA-C5S mice was markedly higher than that of DOCA-C5D mice (Table 1). The serum creatinine, which at four weeks was similar in all groups, at 16 weeks was significantly higher in hypertensive C5S than in C5D mice (Table 1).

By light microscopy morphological differences similar to those observed between hypertensive C5S and C5D mice at four weeks were also seen at 16 weeks. Sixteen week DOCA-C5S mice had intraglomerular capillary proliferation and occasional cellular necrosis (Table 3). However, the glomerular crescents were less cellular and tended to be more fibrotic. Glomerulosclerosis, which was absent at four weeks, could be seen in 16 week DOCA-C5S mice (score 50 ± 6), particularly in corticomedullary glomeruli. In DOCA-C5D mice glomerular capillary proliferation, necrosis and crescents were less severe than in DOCA-C5S mice (Table 3). Capillary aneurysmal ballooning similar to that observed at four weeks was still observed at 16 weeks in DOCA-C5D mice; however, glomerulosclerosis was minimal in these mice (score 12 ± 4, Table 2).

Discussion

These studies were performed to investigate the possible participation of the terminal complement components in hypertensive renal injury. C5-9 can produce renal damage through the chemotactic and anaphylatoxin properties of C5a or through the effects of C5b-9 on membranes [4–7]. Although it is uncertain whether C5b-9 can directly damage extracellular matrices such as glomerular basement membranes, recent work indicates that

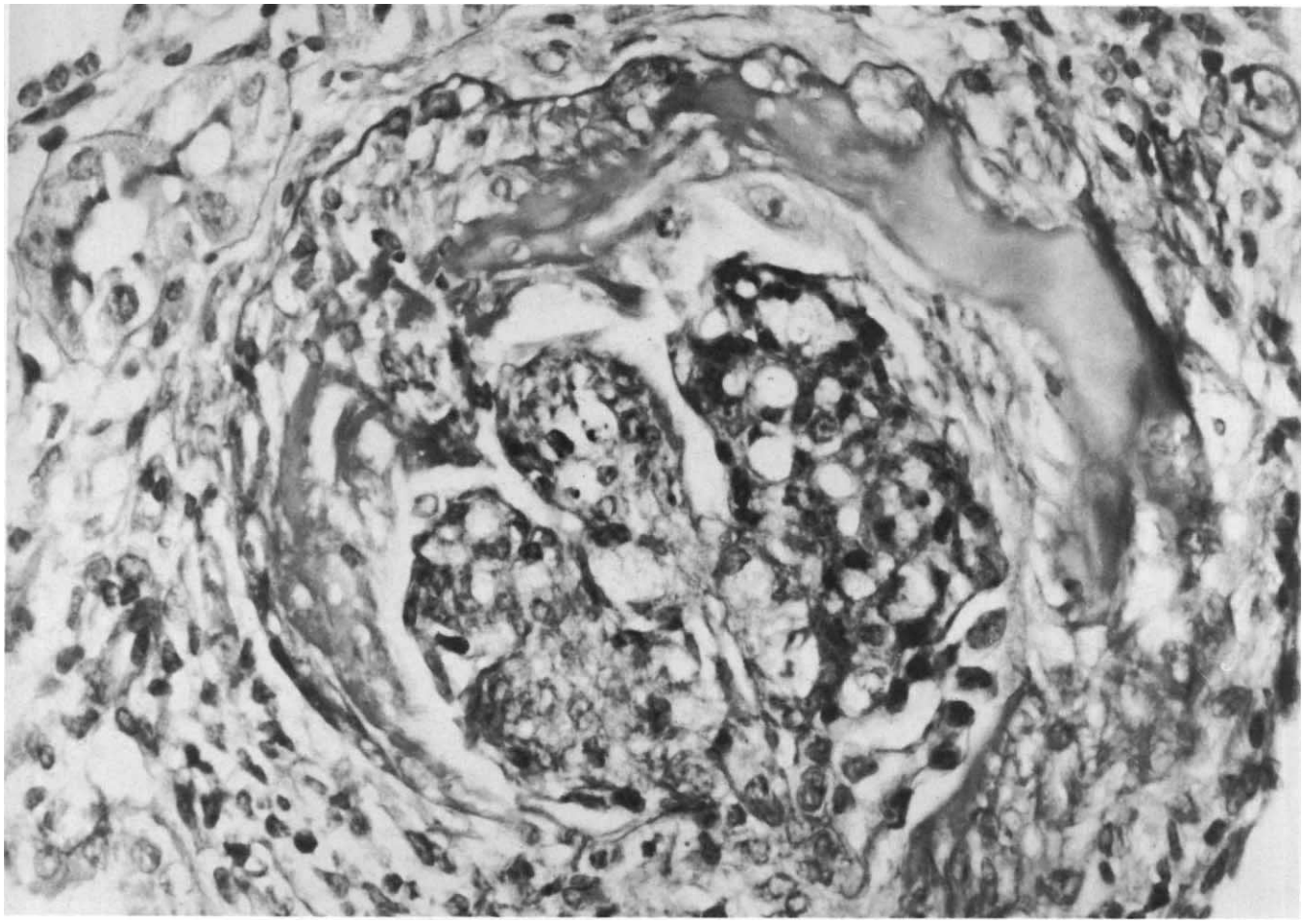

Fig. 1. *Glomerulus from an hypertensive C5-sufficient mouse showing an extracapillary glomerular crescent.* Magnification ×600.

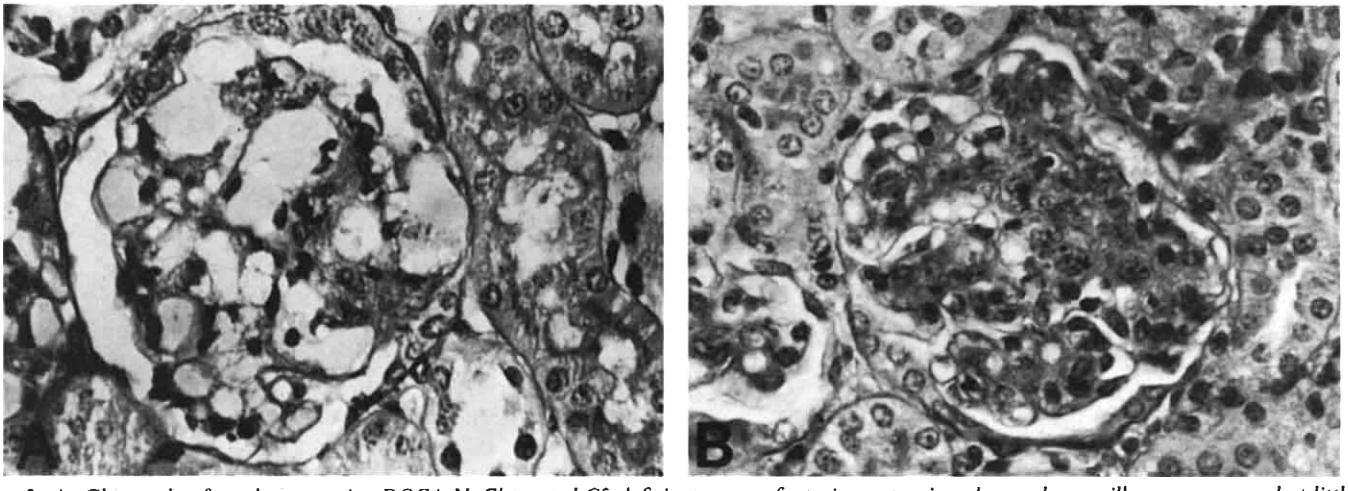

Fig. 2. A. *Glomerulus from hypertensive DOCA-NaCl treated C5-deficient mouse featuring extensive glomerular capillary aneurysms but little cellular proliferation.* Magnification ×400. **B.** Glomerulus from similarly hypertensive C5-sufficient mouse showing glomerular capillary endothelial proliferation, capillary loop narrowing, and mesangial proliferation. Magnification ×470.

C5b-9 in sublytic amounts is able to trigger cellular reactions that are potentially capable of producing renal injury [5, 13]. Sublytic amounts of C5b-9 have been shown to induce release by resident glomerular mesangial cells of inflammatory mediators [10–12], reactive oxygen species [12], and IL_1-like growth factor [11], as well as serotonin and thromboxane B_2 by platelets and macrophages [28, 29]. In addition C5a can raise intraglomerular capillary pressure [9].

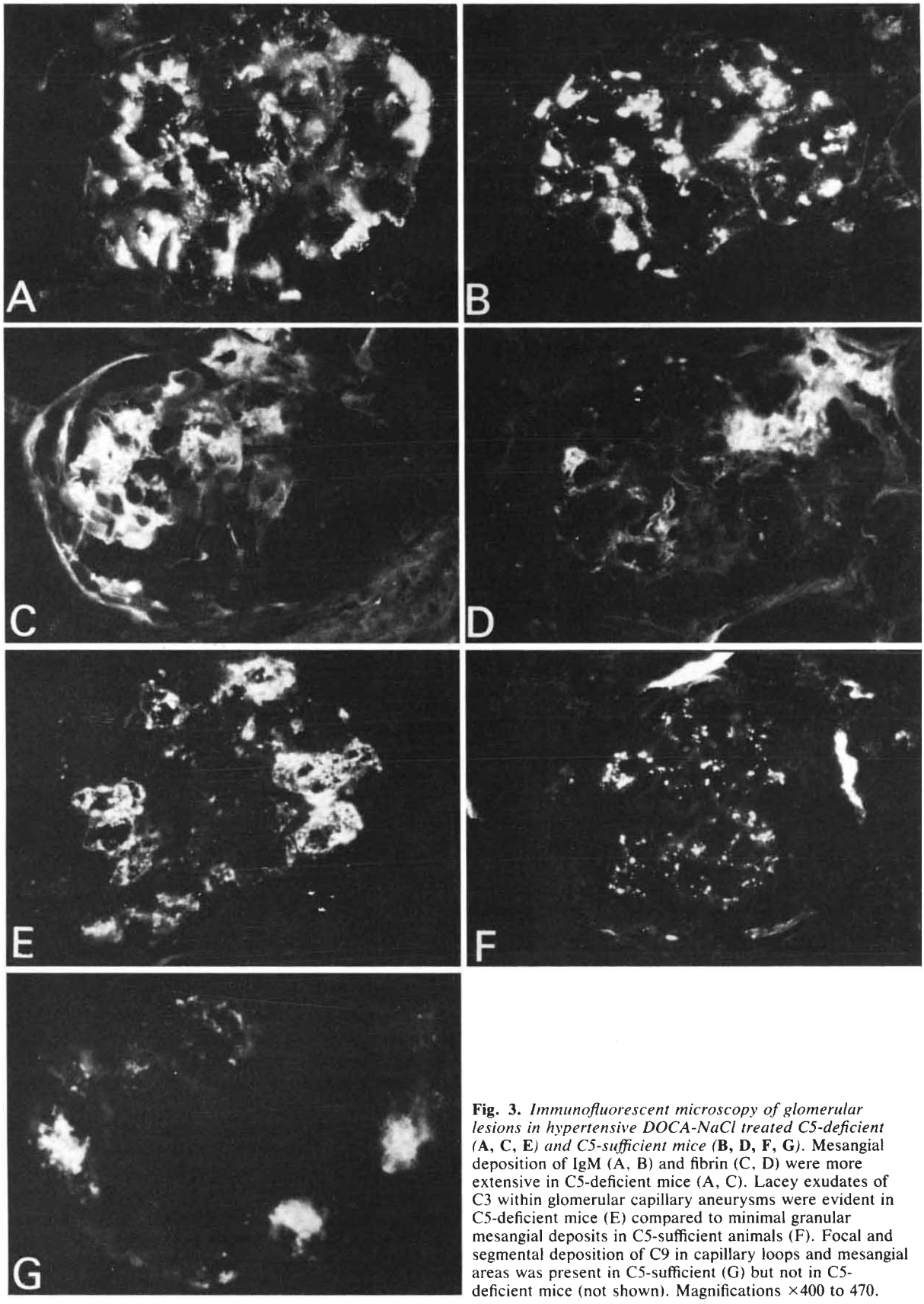

Fig. 3. *Immunofluorescent microscopy of glomerular lesions in hypertensive DOCA-NaCl treated C5-deficient (***A, C, E***) and C5-sufficient mice (***B, D, F, G***).* Mesangial deposition of IgM (A, B) and fibrin (C, D) were more extensive in C5-deficient mice (A, C). Lacey exudates of C3 within glomerular capillary aneurysms were evident in C5-deficient mice (E) compared to minimal granular mesangial deposits in C5-sufficient animals (F). Focal and segmental deposition of C9 in capillary loops and mesangial areas was present in C5-sufficient (G) but not in C5-deficient mice (not shown). Magnifications ×400 to 470.

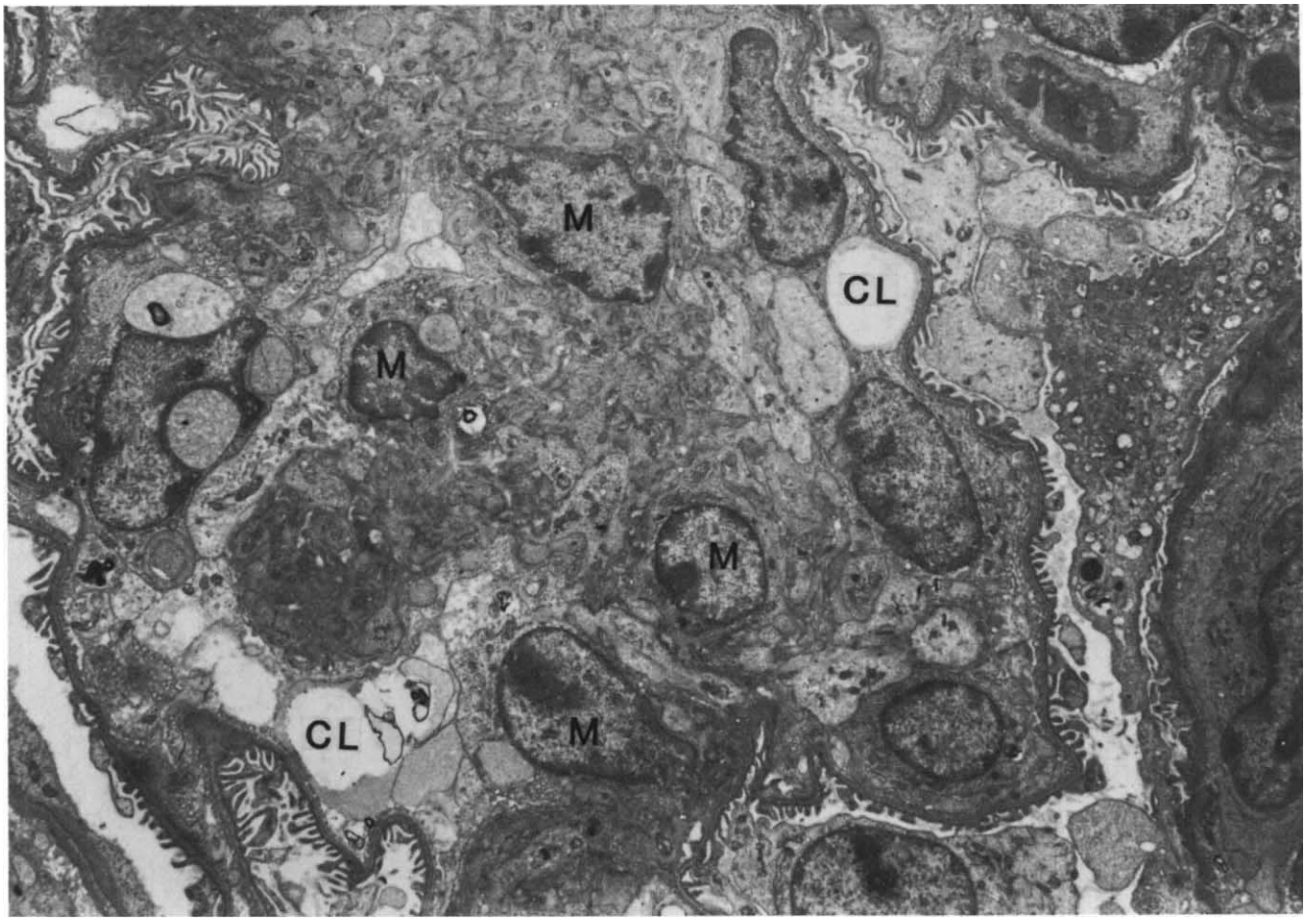

Fig. 4. *Electron micrograph of a glomerulus from a hypertensive DOCA-NaCl C5-sufficient mouse shows proliferation of mesangial cells (M) which impinge on capillary lumens (CL).* Magnification ×3840.

By means of immunohistochemical methods small amounts of C5b-9 were found in normal human tissues, possibly due to binding of C5b-9 to remnant cell membranes during normal senescence [2, 5, 30]. However, studies in disease states have shown large deposits of C5b-9 not only in tissues damaged by immune mechanisms [1, 2], but also in tissues injured by nonimmune mechanisms, such as in diabetes mellitus and hypertension [2]. Although indirect, these observations suggest a possible pathogenic role of complement in these conditions. Therefore we postulated that in hypertension complement might be involved in amplification of renal injury. The basic mechanisms that cause vascular injury in hypertension are still unclear. Studies have suggested that the hemodynamic stress of hypertension can lead to glomerular and vascular injury in the kidney [14–19]. Furthermore, we and others have shown experimentally that immune injury and hypertension interact synergistically in the progression of glomerular injury [16–18]. Clinically, hypertension often accelerates the progression of renal disease [19, 20, 30, 31]. In hypertension, damaged endothelial and subendothelial structures might initiate in situ complement activation. Indeed, previous studies have shown that damaged cells can bind C1q and generate stable C3 convertases [32] and that noncomplement proteolytic enzymes can activate C5 [33].

To explore whether the terminal complement components play a pathogenetic role in hypertensive renal disease we compared the degree of renal injury in a model of hypertension in C5S and C5D mice. We induced hypertension by uninephrectomy-DOCA and 1% NaCl in drinking water in congenic mice, which differ in a single locus responsible for the presence or absence of C5, and compared them with uninephrectomized normotensive (no-DOCA/NaCl) mice. C5D mice can neither generate C5a nor form the C5b-9 complex. In the rat, DOCA-salt hypertension results in elevated glomerular capillary pressures, as demonstrated by micropuncture techniques [22], and severe glomerular injury [21, 22].

In the present studies, similar levels of hypertension developed after four weeks of DOCA in C5S and C5D mice (Table 1). Uninephrectomized C5S and C5D mice receiving no DOCA remained normotensive. Both DOCA-C5S and -C5D mice developed glomerular injury. However, DOCA-C5S mice had significantly more intraglomerular capillary proliferation, cellular necrosis and extracapillary crescents than DOCA-C5D mice.

Increased glomerular capillary volumes and dilatation of capillary loops were particularly striking in DOCA-C5D mice. Since identical capillary changes have been observed in DOCA-

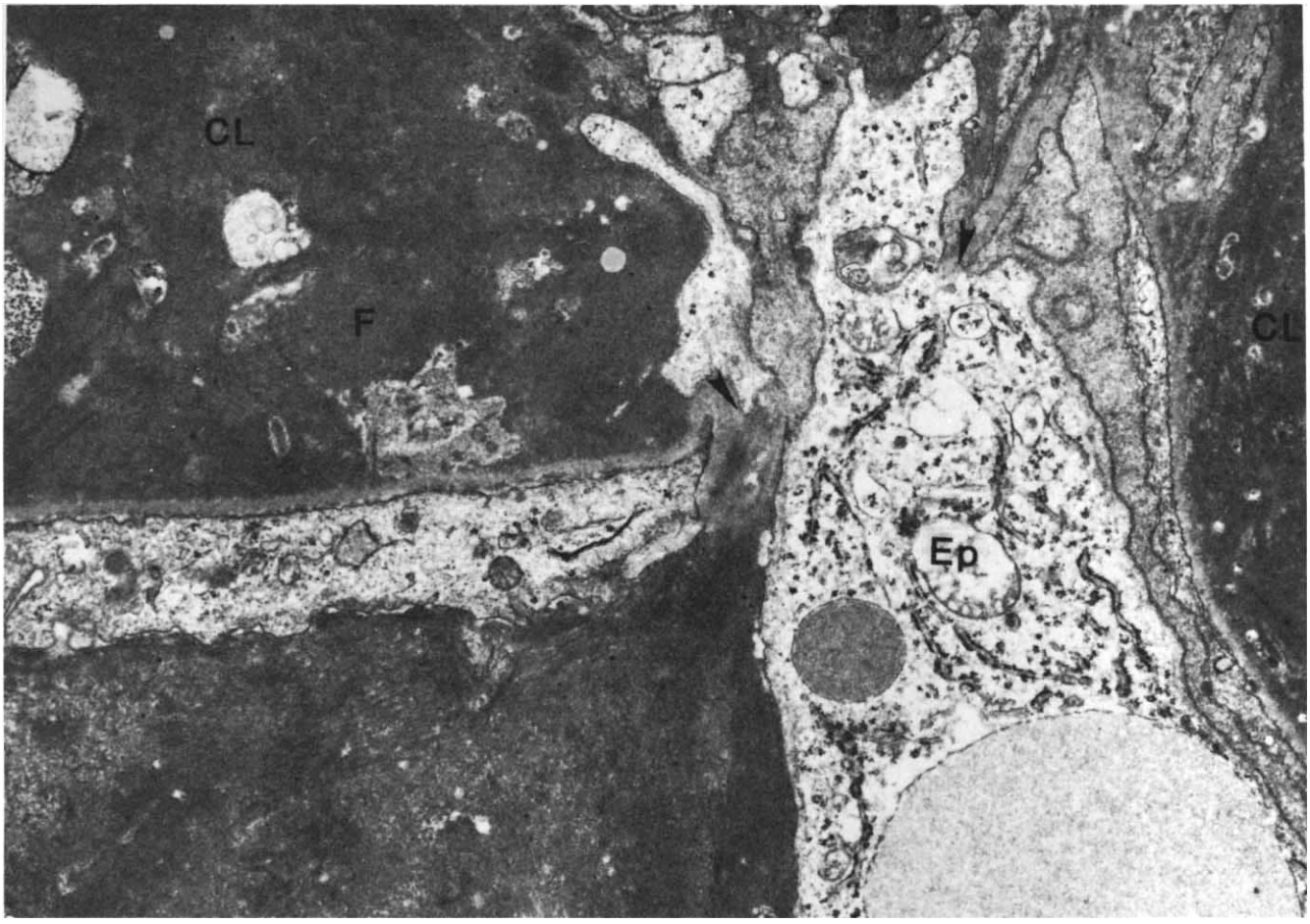

Fig. 5. *Electron micrograph of a glomerulus from a hypertensive DOCA-NaCl C5-sufficient mouse shows rupture (gap between arrows) of the glomerular basement membrane.* Fibrin (F) is present in adjacent capillary lumens (CL) and urinary space. Visceral epithelial cell process (Ep) protrudes through the gap in the glomerular basement membrane. Magnification ×14800.

salt rats with high intraglomerular capillary pressure [22], similar mechanisms might be operative in hypertensive DOCA mice. By electron microscopy glomerular capillary dilatation in DOCA-C5D mice was accompanied by thinning of the mesangium as well as rupture of the mesangial anchoring points of capillary loops, a process similar to the "mesangiolysis" described in rats given Habu snake venom [34]. Moreover, in the rat administration of rabbit anti-thymocyte serum reactive with Thy1-like antigen caused mesangial injury (mesangiolysis) followed by mesangial cell proliferation [35]. In this model, both the mesangial cell injury as well as the subsequent proliferative response appear independent of leukocytes but dependent upon MAC formation [35, 36]. Thus in experimental models in which mesangial disruption occurs [34], the rupture zone is quickly filled by proliferating mesangial cells. This may explain why in hypertensive C5S mice the capillary lumens were filled by proliferating mesangial cells. Indeed glomerular staining with monoclonal antibodies against various lymphocyte types was negative in normotensive as well as in hypertensive C5S and C5D mice. This suggests that the increased glomerular capillary cellularity was not due to blood-borne leukocytes but to proliferation of resident glomerular cells. Furthermore, by electron microscopy polymorphonuclear leukocytes and monocytes were not seen in either DOCA-C5S or -C5D mice at either four or 16 weeks. Proliferating cells, which were particularly increased in DOCA-C5S mice, appeared to be primarily mesangial cells. Increased proliferation of glomerular mesangial cells in C5S mice compared with C5D mice has also been observed in a model of immune complex glomerular disease [37]. Recently, Lovett et al have shown in in vitro experiments that sublytic amounts of C5b-9 stimulate mesangial cells to produce interleukin 1, a powerful growth factor [11].

C3 was not present in glomeruli of normotensive mice. However, we observed C3 deposition in the mesangium of both DOCA-C5S and -C5D mice. Moreover, C9 was present in DOCA-C5S mice but was absent in DOCA-C5D mice. Since DOCA-C5D mice showed much milder mesangial proliferation, we hypothesize that in situ activation of C5-9 may have provided an important stimulus for mesangial cell proliferation in hypertensive DOCA-C5S mice [11]. Whether the increased staining of IgM and C3 in hypertensive C5D mice represents increased deposition and/or trapping or decreased removal of these proteins is at present unclear.

Progressive dilatation of capillary loops in the presence of

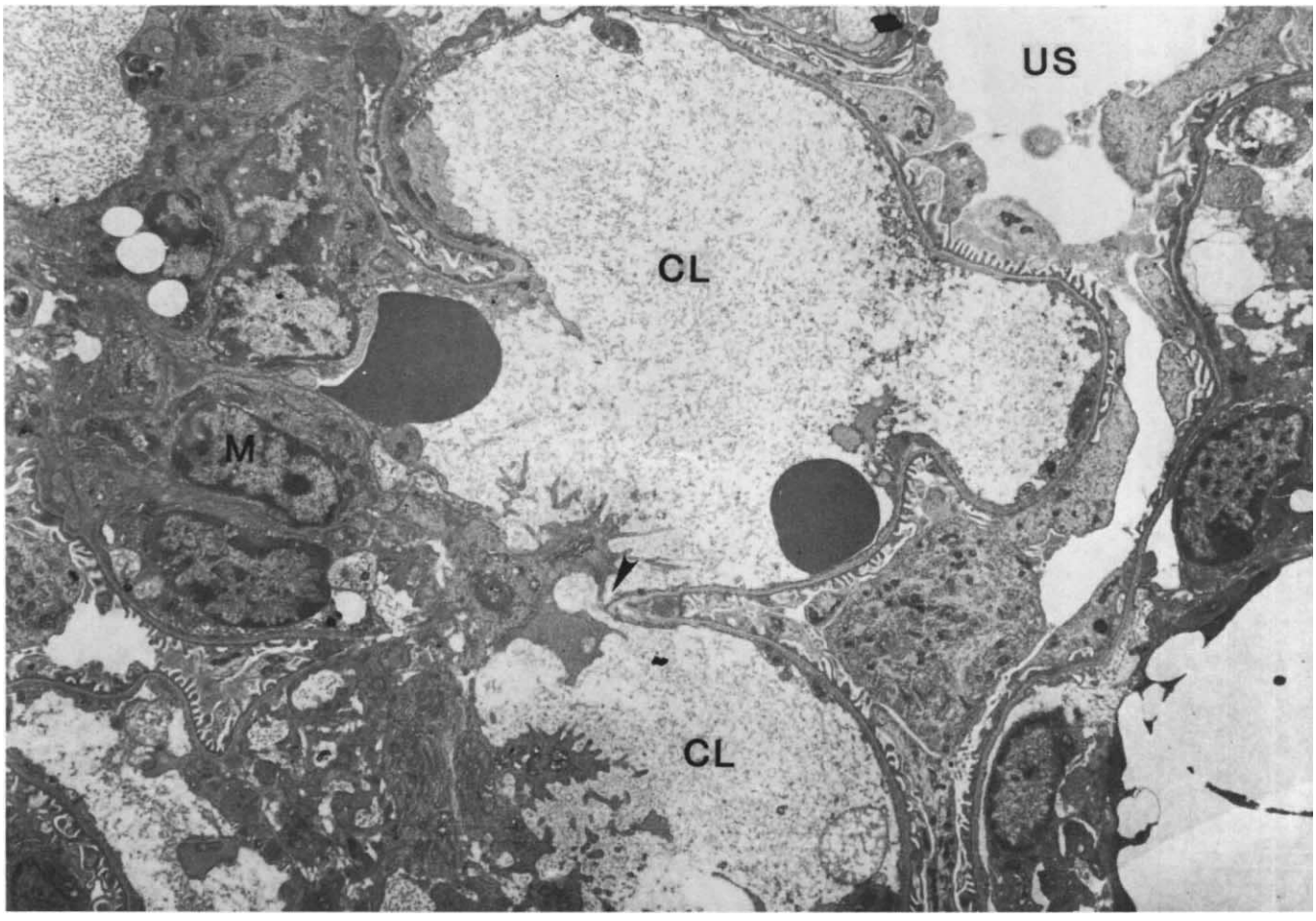

Fig. 6. *Electron micrograph of a glomerulus from a hypertensive DOCA-NaCl C5-deficient mouse shows dilatation of capillary lumens (CL) and focal detachment (arrow) of the glomerular basement membrane from the mesangium (M).* Magnification ×3600.

continuously high intracapillary pressure should increase the tension of the capillary wall according to La Place law. Given the marked dilatation of capillary loops and concomitant increase in glomerular capillary volumes observed in DOCA-C5D mice, it is reasonable to infer that the capillary wall tension would be higher in these mice. In both DOCA-C5S and -C5D mice detachment of endothelial cells was observed. However, gaps in the glomerular basement membrane were noted only in DOCA-C5S mice. Moreover, glomerular crescents, which are associated with capillary rupture, were more common in DOCA-C5S mice than in DOCA-C5D mice. The above described findings would suggest that a) increased capillary wall tension and dilatation may not play a relevant role in this model of hypertensive glomerular injury and/or b) that terminal complement components are pathogenetically important in the mediation or amplification of glomerular damage observed in C5S mice.

Platelets might participate in the production of complement dependent renal damage in hypertension. It is known that the MAC is capable of interacting with platelets, causing their activation [38, 39]. Platelet activation by the MAC may result in release of mitogenic substances in the glomerulus that could contribute to glomerular cell proliferation [40]. Thus the differences in renal lesions between C5D and C5S mice might be mediated, at least in part, by complement-dependent platelet activation. Fibrin and leukocytes, primarily monocytes, were observed in the crescents by both electron microscopy and immunofluorescent staining using monoclonal antibodies. Similar findings have been observed in other types of glomerular injury in which extraglomerular capillary crescents are formed. The reason for the absence of leukocytes in intracapillary locations in DOCA-C5S mice is unclear. This does not appear to be due to abnormal leukocyte function since Falk and Jennette found leukocytes in the glomeruli of C5S mice with immune complex glomerulonephritis [37].

Associated with the more severe glomerular injury at four weeks of treatment, DOCA-C5S mice had higher urinary protein excretion than C5S mice. At this time, however, serum creatinine levels were similar in all groups whether normotensive or hypertensive (Table 1). To determine if the differences at four weeks between C5S and C5D mice were only transient, and if protracted injury would result in progressive glomerular destruction and abnormal renal function, we studied C5S and C5D mice given DOCA-NaCl for 16 weeks. Similar blood pressures were recorded in mice that received DOCA for 16 weeks or for only four weeks. At 16 weeks DOCA-C5D mice

continued to have aneurysmal dilatation of the capillary loops, discrete intraglomerular capillary proliferation and minimal crescents. In these mice the injury score for glomerulosclerosis was low. On the other hand, DOCA-C5S mice developed severe glomerulosclerosis. In addition, at 16 weeks tubulointerstitial changes were minimal in DOCA-C5D mice but moderately severe in DOCA-C5S mice. At 16 weeks both the urinary protein excretion and the serum creatinine of DOCA-C5S mice were significantly higher than in C5D mice, indicating more severe renal damage in C5S mice.

In conclusion, our findings in hypertensive C5S and C5D mice suggest that a) C5-9 may participate in the initiation and/or amplification of the hemodynamically-mediated glomerular injury of hypertension which ultimately results in glomerulosclerosis and renal insufficiency, b) provide insight into the clinically and experimentally observed synergistic interaction between immune mediated and hypertensive glomerular injury [16–19], and c) emphasize the importance of differences in host responses in determining target organ susceptibility to similar injurious mechanisms.

Acknowledgments

This work was supported by grants from the National Institute of Health (HL 29720 and AI 10704), Viking Childrens Fund and funds from the Veterans Administration Medical Center. Monoclonal antibodies used in this study were a gift from Dr. Jeffrey Platt, Department of Pediatrics, University of Minnesota, USA. We thank Ms. Diana Jacobson for assistance in preparation of the manuscript and Ms. Linda Hartich for her technical assistance.

Reprint requests to Dr. Leopoldo Raij, VAMC, Renal Section 111-J, 1 Veterans Drive, Minneapolis, Minnesota 55417, USA.

References

1. Biesecker G, Katz S, Koffler D: Renal localization of the membrane attack complex in systemic lupus erythematosus nephritis. *J Exp Med* 154:1779, 1981
2. Falk RJ, Dalmasso AP, Kim Y, Tsai CH, Scheinman JI, Gewurz H, Michael AF: Neoantigen of the polymerized ninth component of complement. Characterization of a monoclonal antibody and immunohistochemical localization in renal disease. *J Clin Invest* 72:560–573, 1983
3. Falk RJ, Podack E, Dalmasso AP, Jennette JC: Localization of S protein and its relationship to the membrane attack complex of complement in renal tissue. *Am J Pathol* 127:182–190, 1987
4. Biesecker G: Membrane attack complex of complement as a pathologic mediator. *Lab Invest* 49:237–249, 1983
5. Couser WG, Baker PJ, Adler S: Complement and the direct mediation of immune glomerular injury: A new perspective. *Kidney Int* 28:879–890, 1985
6. Müller-Eberhard HJ: The membrane attack complex of complement. *Ann Rev Immunol* 4:503–528, 1986
7. Dalmasso AP: Complement in the pathophysiology and diagnosis of human diseases. *Crit Rev Clin Lab Sci* 24:123–183, 1986
8. Hugli TE: Structure and function of the anaphylatoxins. *Springer Semin Immunopathol* 7:193–219, 1984
9. Pelayo JC, Chenoweth DE, Hugli TE, Wilson CB, Blantz RC: Effects of the anaphylatoxin C5a, on renal and glomerular hemodynamics in the rat. *Kidney Int* 30:62–67, 1986
10. Lovett DH, Sterzel RB, Ryan JL, Atkins E: Production of an endogenous pyrogen by glomerular mesangial cells. *J Immunol* 134:670–672, 1985
11. Lovett DH, Haensch GM, Goppelt M, Resch K, Gemsa D: Activation of glomerular mesangial cells by the terminal membrane attack complex of complement. *J Immunol* 138:2473–2480, 1987
12. Adler S, Baker PJ, Johnson RJ, Ochi RF, Pritzl P, Couser WG: Complement membrane attack complex stimulates production of reactive oxygen metabolites by cultured rat mesangial cells. *J Clin Invest* 77:762–767, 1986
13. Cybulsky AV, Rennke HG, Feintzeig ID, Salant DJ: Complement induced glomerular epithelial cell injury. Role of the membrane attack complex in rat membranous nephropathy. *J Clin Invest* 77:1096–1107, 1986
14. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons: a potential adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
15. Raij L: Role of hypertension in progressive glomerular injury in glomerulonephritis. *Hypertension* 8 (Suppl I):I30–I33, 1986
16. Raij L, Azar S, Keane WF: Role of hypertension in progressive glomerular immune injury. *Hypertension* 7:398–404, 1985
17. Raij L, Azar S, Keane WF: Mesangial immune injury, hypertension, and progressive glomerular damage in Dahl rats. *Kidney Int* 26:137–143, 1984
18. Neugarten J, Feiner HD, Schacht RJ, Gallo GR, Baldwin DS: Aggravation of experimental glomerulonephritis by superimposed clip hypertension. *Kidney Int* 22:257–263, 1982
19. Baldwin DS: Chronic glomerulonephritis: Non-immunologic mechanisms of progressive glomerular damage. *Kidney Int* 21: 109–120, 1982
20. Mogensen CE: Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *Br Med J* 285:685–688, 1982
21. Hill GS, Heptinstall RH: Steroid-induced hypertension in the rat: A microangiographic histologic study on the pathogenesis of hypertensive and vascular glomerular lesions. *Am J Pathol* 52:1–39, 1968
22. Dworkin LD, Hostetter TH, Rennke HG, Brenner BM: Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. *J Clin Invest* 73:1448–1461, 1984
23. Tanaka S, Suzuki T, Nishioka K: Assay of classical and alternative pathway activities of murine complement using antibody-sensitized rabbit erythrocytes. *J Immunol Meth* 86:161–170, 1986
24. Hirose K, Osterby R, Nozawa M, Gundersen HJG: Development of glomerular lesions in experimental long-term diabetes in the rat. *Kidney Int* 21:689–695, 1982
25. Weibel ER: Stereological Methods, in *Practical Methods for Biological Morphometry*, London, Academic Press, 1979, pp. 1–415
26. Yoshida Y, Fogo A, Ichikawa I: Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int* 35:654–660, 1989
27. Platt JL, LeBien TW, Michael AF: Interstitial mononuclear cell populations and renal graft rejection: Identification by monoclonal antibodies in tissue sections. *J Exp Med* 155:17–30, 1982
28. Polley MJ, Nachman RL, Weksler BB: Human complement in the arachidonic acid transformation pathway in platelets. *J Exp Med* 153:257–268, 1981
29. Hansch GM, Seitz M, Martinotti G, Betz M, Rauterberg EW, Cemsa D: Macrophages release arachidonic acid, prostaglandin E2, and thromboxane in response to late complement components. *J Immunol* 133:2145–2150, 1984
30. Hinglais N, Kazatchkine MD, Bhakdi S, Appay MD, Mandet C, Grossetete J, Bariety J: Immunohistochemical study of the C5b-9 complex of complement in human kidneys. *Kidney Int* 30:399–410, 1986
31. Klahr S: The kidney in hypertension-villain and victim. *N Engl J Med* 320:731–733, 1989
32. Baker PJ, Adler S, Yang Y, Couser WG: Complement activation by heat-killed human kidney cells: Formation, activity, and stabilization of cell-bound C3 convertases. *J Immunol* 133:877–881, 1984
33. Wetsel RA, Kolb WP: Complement-independent activation of the fifth component (C5) of human complement: Limited trypsin digestion resulting in the expression of biologic activity. *J Immunol* 128:2209–2216, 1982
34. Morita T, Churg J: Mesangiolysis (Editorial Review). *Kidney Int* 24:1–9, 1983

35. YAMAMOTO T, WILSON CB: Quantitative and qualitative studies of antibody-induced mesangial cell damage in the rat. *Kidney Int* 32:514–525, 1987
36. YAMAMOTO T, WILSON CB: Mesangial cell (MC) injury and proliferation produced by antibody: A role for the membrane attack complex (MAC). (abstract) *Kidney Int* 31:333, 1987
37. FALK RJ, JENNETTE JC: Immune complex induced glomerular lesion in C5 sufficient and deficient mice. *Kidney Int* 30:678–686, 1986
38. ZIMMERMAN TS, KOLB WP: Human platelet-initiated formation and uptake of the C5-9 complex of human complement. *J Clin Invest* 57:203–211, 1976
39. POLLEY MJ, NACHMAN RL: Human complement in thrombin-mediated platelet function. Uptake of the C5b-9 complex. *J Exp Med* 150:633–645, 1979
40. SHULTZ PJ, DICORLETO PE, SILVER BJ, ABBOUD HE: Mesangial cells express PDGF mRNAs and proliferate in response to PDGF. *Am J Physiol* 255:F674–F684, 1988