Kidney International, Vol. 58 (2000), pp. 1569–1579

CD59 protects rat kidney from complement mediated injury in collaboration with Crry

Midoriko Watanabe, Yoshiki Morita, Masashi Mizuno, Kazuhiro Nishikawa, Yukio Yuzawa, Nigishi Hotta, B. Paul Morgan, Noriko Okada, Hidechika Okada, and Seiichi Matsuo

Internal Medicine III, Nagoya University School of Medicine, Nagoya, Japan; Department of Medical Biochemistry, University of Wales College of Medicine, Cardiff, Wales, United Kingdom; and Department of Molecular Biology, Nagoya City University School of Medicine, Nagoya, Japan

CD59 protects rat kidney from complement mediated injury in collaboration with Crry.

Background. As previously reported, the membrane-bound complement regulator at the C3 level (Crry/p65) is important in maintaining normal integrity of the kidney in rats. However, the role of a complement regulator at the C8/9 level (CD59) is not clear, especially when activation of complement occurs at the C3 level. The aim of this work was to elucidate the in vivo role of CD59 under C3 activating conditions.

Methods. Two monoclonal antibodies, 5I2 and 6D1, were used to suppress the function of Crry and CD59, respectively. In order to activate alternative the pathway of complement, the left kidney was perfused with 5I2 and/or 6D1 and was recirculated.

Results. In the kidneys perfused with 5I2 alone, deposition of C3 and membrane attack complex (MAC) was observed in the peritubular capillaries, vasa recta, and tubular basement membranes. Cast formation, tubular dilation and degeneration, and cellular infiltration were observed at days 1 and 4, and they recovered by day 7. Further suppression of CD59 by 6D1 significantly enhanced the deposition of MAC and worsened the already exacerbated tubulointerstitial injury. These effects of 6D1 were dose dependent. Perfusion with 6D1 alone did not induce histologic damage or MAC deposition in the tubulointerstitium.

Conclusions. In rats, CD59 maintains normal integrity of the kidney in collaboration with Crry in rats against complement-mediated damage in vivo.

The complement system functions as a protective machinery against foreign microorganisms. One of the key mechanisms by which the complement system distinguishes self from non-self is via expression of complement-regulatory proteins on the plasma membrane of host cells. In humans, decay accelerating factor (DAF; CD55) [1], membrane cofactor protein (MCP, CD46) [2], and CD59 [3–7] are widely distributed in many organs and vessels and play central roles to protect host cells from autologous complement attack. MCP and DAF regulate complement activation at the C3 level, and CD59 acts at the level of formation of membrane attack complex (MAC). The protective role of C3 level membrane inhibitors has been demonstrated in vivo in our previous studies [8, 9] using monoclonal antibody (mAb) 5I2, which blocks the function of rat Crry, a complement regulator acting at the C3 level [10]. Rat Crry, the target antigen of mAb 5I2, is the rat homologue of mouse Crry [11], which inhibits mouse C3 convertase [12–14]. Broad tissue distribution of rat Crry has been demonstrated [15]. The protective role of CD59 has been elucidated in many disease conditions, such as paroxysmal nocturnal hematuria [4, 6], acute myocardial infarction [16], and xenotransplantation [17]. Inhibition of CD59 in the synovial tissues induced transient inflammation [18]. Intravenous administration of mAb 5I2 induced a transient decrease in blood pressure, while mAb 6D1 (a neutralizing mAb against rat CD59) [19] showed no effect [8]. In the normal human kidneys, complement-regulatory proteins at both C3 and MAC levels are abundantly expressed in most compartments, including peritubular capillaries [20]. In rats, Crry and CD59 are also expressed in the peritubular capillaries [21]. Blocking of Crry by perfusion of the kidney with mAb 5I2 caused increased permeability of peritubular capillaries [22] and the subsequent interstitial inflammation with deposition of C3 and MAC in the renal interstitium [21]. These data confirm the importance of rat Crry in the protection against autologous complement attack. In contrast, the contribution of CD59 has not been fully investigated in the kidney. Suppression of the function of CD59 alone does not induce

Key words: C3, membrane attack complex, peritubular capillaries, tubulointerstitial injury, renal protective agents.

Received for publication November 18, 1999
and in revised form March 31, 2000
Accepted for publication May 1, 2000

any inflammatory changes in the kidney when complement activation is not initiated [21]. It remains to be elucidated whether CD59 plays protective roles under C3-activated condition. The present work was designed to study the role of CD59 in the rat kidney when complement was activated at the C3 level. The results obtained in the present work show clearly that CD59 protects kidneys from complement-mediated injury in collaboration with Crry in rat kidneys.

METHODS

Animals

Male Wistar rats weighing 260 to 280 g were purchased from Chubu Kagaku Shizai Co. Ltd. (Nagoya, Japan) and were allowed free access to food and water. The experiments were performed according to the Animal Experimentation Guide of Nagoya University School of Medicine (Nagoya, Japan).

Antibodies

Characteristics of rat Crry and mouse mAb 5I2 were described previously [10]. A mouse mAb 6D1 that inhibits its function of rat CD59 [19] was also described previously. F(ab′)2 fragments of mAb 5I2 and 6D1 were prepared as reported earlier and were used in all in vivo studies [8, 21]. Mouse mAb 2A1 [23] directed against rat MAC was kindly provided by Dr. W.G. Couser (University of Washington, Seattle, WA, USA). The 2A1 was labeled with biotin according to the method described by Guesdon, Ternynck, and Abrameas [24]. FITC-labeled mouse mAb against rat leukocyte common antigen (LCA; clone OX-1) was purchased from Dainippon Pharmaceutical Co. (Osaka, Japan).

Urinary protein excretion

Rats were housed in metabolic cages overnight (16 h) every other day from day 1 to day 7. Urinary protein was measured by the pyrogallol red method [25].

Kidney perfusion

To localize 5I2 and/or 6D1 solely in the left kidney and to avoid their systemic effects, isolated left kidney perfusion was performed according to the method described previously [26, 27]. Under nembutal anesthesia, the left kidney of rat was exposed. Polyethylene tubes were placed in the left renal artery and vein, and the proximal portions of the vessels were temporarily ligated. The left kidney was perfused at a rate of 2 mL/min using a peristaltic pump. Modified Tyrode's buffer [26] saturated with 95% O_2 and 5% CO_2 was used as a vehicle. All of the perfusate was discarded through the cannula placed in the renal vein. After kidney perfusion, tubes were removed, and the holes in the vessels were repaired by microsurgery. Then blood circulation was re-established by releasing the clamps. The average time required for the whole procedure was less than 15 minutes.

Table 1. Experimental protocol

Group	Antibody[a] *mg/body*	15 min[b]	Day 1[b]	Day 4[b]	Day 7[b]
I	5I2 (0.3)	3	6	6	6
II	5I2 (0.3) + 6D1 (3.0)	3	6	6	6
III	5I2 (0.3) + 6D1 (0.3)	3	6	6	6
IV	6D1 (3.0)	3	6	6	6
V	Vehicle[c]	3	6	6	6

[a] The left kidney was perfused with each dose of antibody
[b] The number in each column indicates the number of rats examined
[c] Modified Tyrode's buffer

Experimental protocol

Rats were divided into five groups. Rats of group I were perfused with 0.3 mg of F(ab′)2 fragment of 5I2 in 10 mL of Tyrode's buffer. Rats of group II were perfused with 0.3 mg of 5I2 F(ab′)2 and 3.0 mg of F(ab′)2 fragment of 6D1. Rats of group III were perfused with 0.3 mg of 5I2 F(ab′)2 and 0.3 mg of 6D1 F(ab′)2. Group IV rats were perfused with 3.0 mg of F(ab′)2 fragment of 6D1. Rats in group V were perfused with Tyrode's buffer (vehicle) only and were used as controls. Rats were killed at one, four, and seven days after perfusion. To study the binding of mAbs and rat complement, rats were also killed 15 minutes after perfusion. The protocol and the number of rats examined are summarized in Table 1.

Histology and immunohistology

For light microscopic examination, methacarn-fixed and paraffin-embedded kidney tissues were cut at 2 μm and stained with periodic acid-Schiff (PAS). To assess tubulointerstitial injuries, kidney sections were arbitrarily divided into three regions, that is, cortex, outer medulla, and inner medulla. Using semiquantitative indices, sections were analyzed for the evaluation of acute tubulointerstitial damage. In each region, extents of tubular cast formation and tubular dilation were scored by two blinded observers according to the following criteria: 0 = normal; 1 = <30%; 2 = 30 to 70%; 3 = >70% of the pertinent area [21].

For immunofluorescence microscopy, kidney tissues were snap frozen, cut at 2 μm, and fixed in acetone. Sections were stained by FITC-labeled rabbit antibodies against mouse IgG and rat IgG, and FITC-labeled goat antibodies against human C4 (Biogenesis Ltd., Poole, UK) and rat C3 (Cappel Laboratories, West Chester, PA, USA). The antibody against human C4 cross-reacts with rat C4. For the detection of MAC, sections were first treated with avidin D and biotin-blocking solution (Vector Laboratories, Burlingame, CA, USA). They were then incubated with biotinylated 2A1 followed by the incubation with FITC-labeled streptoavidin (Cappel Laboratories).

Deposition of rat C3 and MAC in the kidney was semiquantitatively scored according to the extent of deposition in each region using the following criteria: 0 = normal; 1 = <30%; 2 = 30 to 70%; and 3 = >70% of the pertinent area [21]. Since normal rat tubular basement membrane of cortex was positively stained for rat C3 and MAC in a focal and interrupted pattern, only circumferential staining was counted as positive. In renal medulla, C3 and MAC were negative in normal rats. For the analysis of interstitial leukocyte infiltration, sections were stained by FITC-OX1. The number of positive cells was counted in randomly selected 10 microscopic fields under higher magnification (×400). After washing with phosphate-buffered saline, all the sections were covered with 90% glycerol containing p-phenylenediamine [28] and were examined by two blinded observers using an epifluorescence microscope (Olympus Optical Co., Tokyo, Japan).

Statistical analysis

All values are provided as mean ± SD. Statistical analysis was performed by one-factor analysis of variance (ANOVA). When significant difference was present, statistical analysis was further performed using Scheffe's *F*-test between two groups. Significant difference was set when the *P* value was less than 0.05 (5%).

RESULTS

Light microscopic findings

A significant tubulointerstitial change was observed by light microscopy in rats of groups I, II, and III. Cast formation, dilation of tubules, vacuolar degeneration, numerous mitotic figures, loss of brush border, and detachment of tubular epithelial cells were also prominent in these rats (Fig. 1). Cellular infiltration was also significantly present (quantitative data are shown in the next section). The tubulointerstitial changes observed by light microscopy tended to heal by day 7 in rats of groups I and III, whereas those changes in group II rats remained until day 7 (Fig. 2). In rats of groups IV and V, there was no histologic alteration in the left kidneys throughout the experiments. Further semiquantitative analysis revealed that the extent of tubular cast formation, and dilation of tubules was significantly larger in group II rats than in group I and III rats (Fig. 3). In contrast to tubulointerstitial tissue, there was no significant alteration in the glomerulus throughout the experiments in any group.

Immunohistological findings

Distribution of antibody in vivo. Fifteen minutes after kidney perfusion, binding of mouse IgG was seen along glomerular, peritubular, and medullary capillaries, including the vasa recta of outer medulla in rats of groups I, II, and III. Mouse IgG also bound to the basolateral membrane of the proximal tubules. At day 1 (24 hours after perfusion), mouse IgG was still present in the tubules and vascular bundles, but had become almost undetectable in glomeruli and peritubular capillaries. After four days, mouse IgG disappeared from the kidney except for weak staining in the vasa recta. In rats of group IV, 6D1 bound to glomerulus, peritubular capillaries, vasa recta, and distal tubules 15 minutes after perfusion. The fate of 6D1 in the kidney was almost identical to that of 5I2 in rats of group I. Staining for rat IgG was not detectable in any rat throughout the experiment.

Deposition of C3. Fifteen minutes after perfusion/recirculation, strong deposition of C3 was detected in group I, II, and III rats in peritubular capillaries of cortex, vasa recta, basement membranes of proximal tubules, and inner medulla. C3 deposition was also present in glomerulus at this time, but was not detectable at day 1 and later, whereas at other sites, C3 was present at all time points. The extent of deposition of C3 was almost the same among groups I, II, and III. In rats of groups IV and V, there was no abnormal deposition of C3. These data were analyzed semiquantitatively and are presented in Figure 4. C4 was not detected throughout the experiments.

Deposition of MAC. After perfusion/recirculation, MAC was detected in rats of groups I, II, and III in almost the same distribution as C3, although glomerular deposition was absent. MAC deposition was still strong in these groups, particularly along the cortical tubular basement membrane where circumferential binding of MAC was observed. The deposition of MAC in peritubular capillaries and vasa recta had disappeared by day 7, whereas the deposition in tubular basement membrane (TBM) still remained at day 7. The degree of MAC deposition in group II was significantly higher than that in groups I and III. In rats of groups IV and V, there was no abnormal deposition of MAC in the kidney. MAC deposition was not detected in the glomerulus in any of the groups at any time point (Figs. 4 and 5).

Cellular infiltration. In rats of groups I, II, and III, cellular infiltration was not so prominent at day 1 in cortex and medulla. There was, however, significant leukocyte infiltration at days 4 and 7. In addition, the extent of leukocyte infiltration in group II was significantly greater than groups I and III. Cellular infiltration was most prominent in the outer medulla. In other groups of rats, cellular infiltration was minimal and did not change throughout the experiments. Leukocyte infiltration was not seen in the glomeruli of any of the rats at any time point. These results are shown in Figure 6.

Urinary protein excretion

Urinary protein loss significantly increased in rats of groups I, II, and III compared with groups IV and V at days 0 to 1. The maximum amount of urinary protein loss in group II rats was significantly larger than in groups

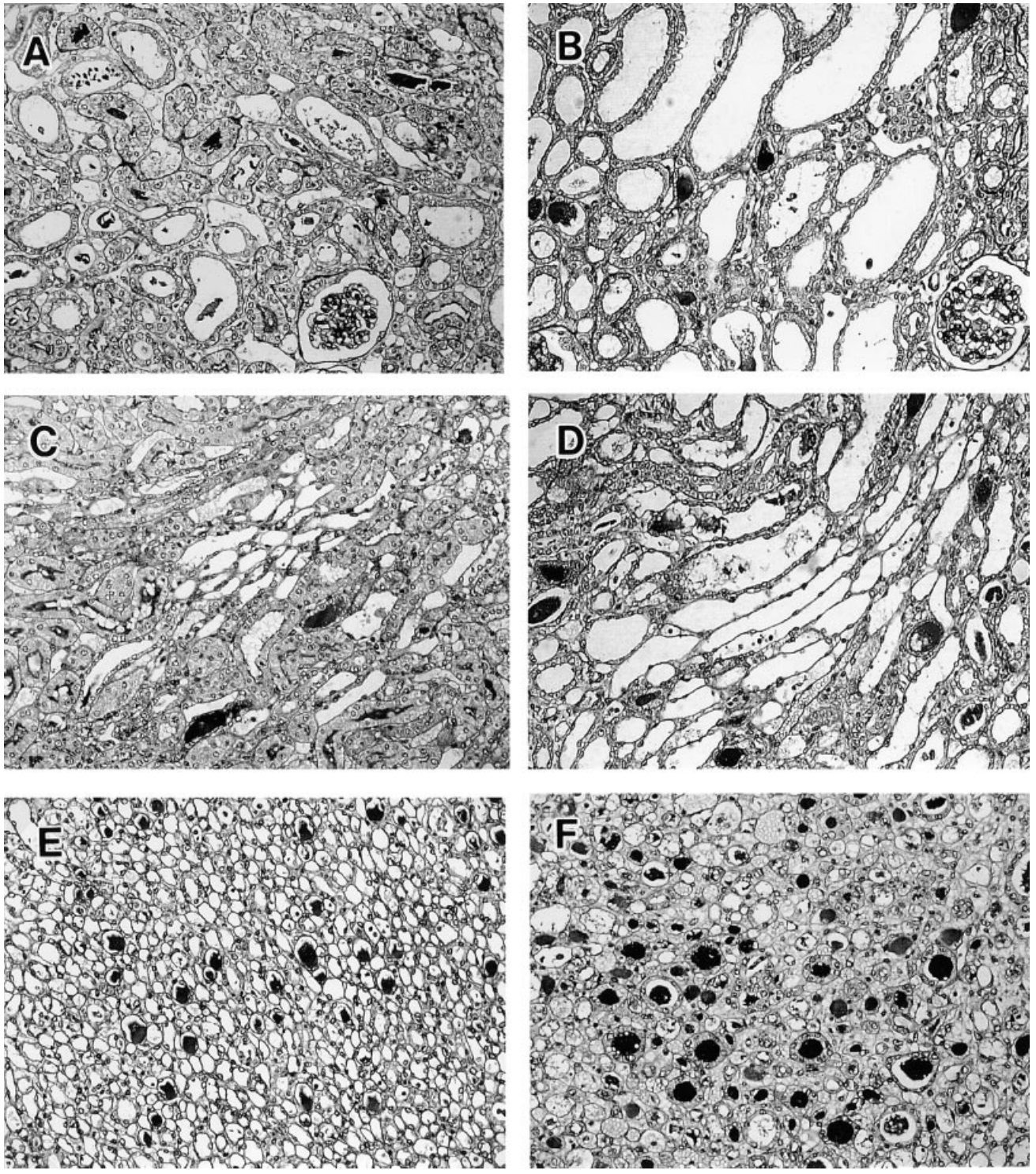

Fig. 1. Light microscopy at day 4 in groups I and II (*A* and *B*) cortex, (*C* and *D*) outer medulla, and (*E* and *F*) inner medulla. Tubular dilation and cast formation are seen in rat of both groups, but these changes are more prominent in group II. In the outer medulla, the structure of vasa recta is not clear in group II (Group I, PAS, ×200, A, C, and E); Group II, B, D, and F).

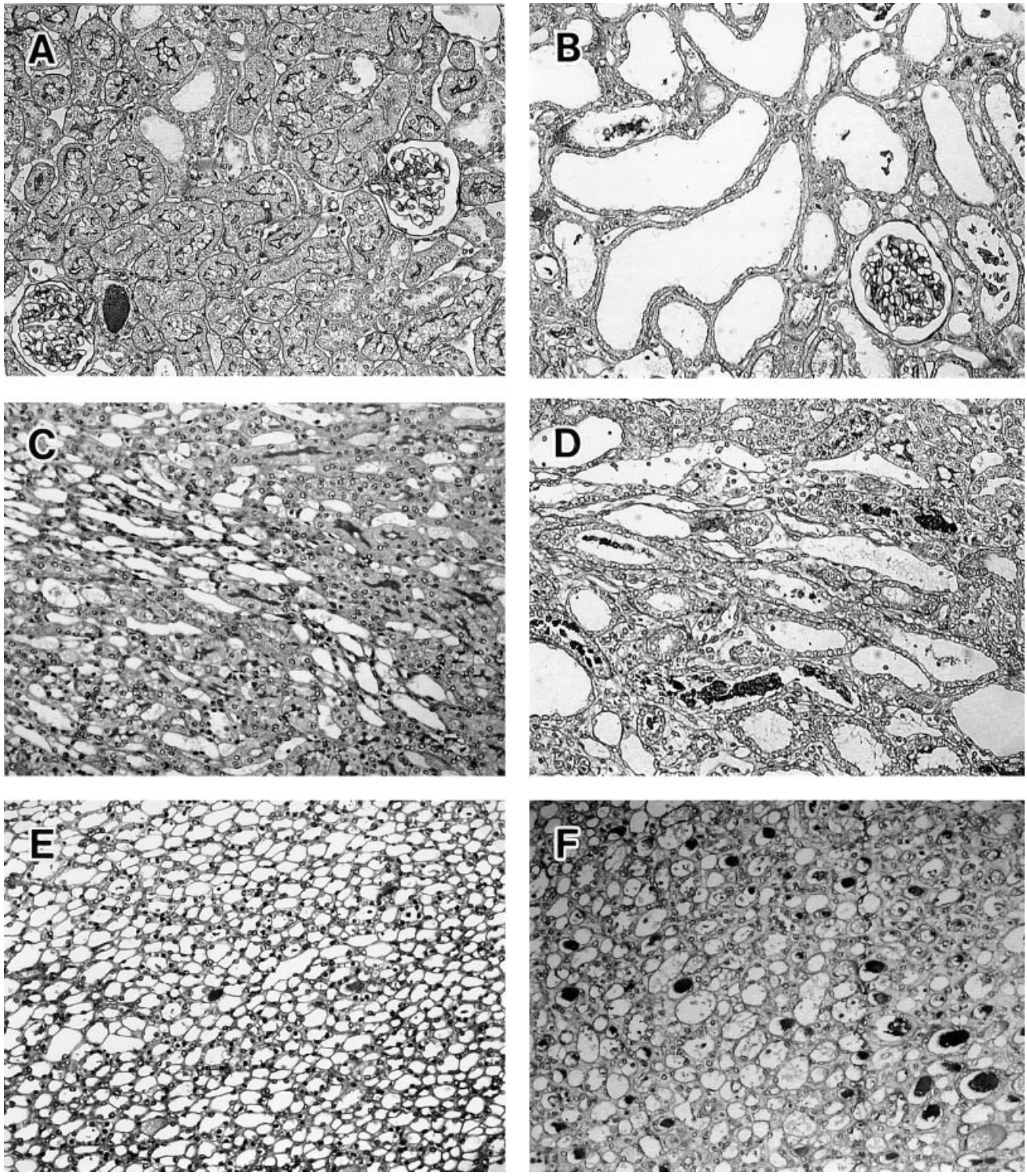

Fig. 2. Light microscopy at day 7 in groups I and II (*A* and *B*) cortex, (*C* and *D*) outer medulla, and (*E* and *F*) inner medulla. Tubular dilation and cast formation are still present in group II, while these changes are almost healed in group I (Group I, PAS, ×200, A, C, and E; Group II, B, D and F).

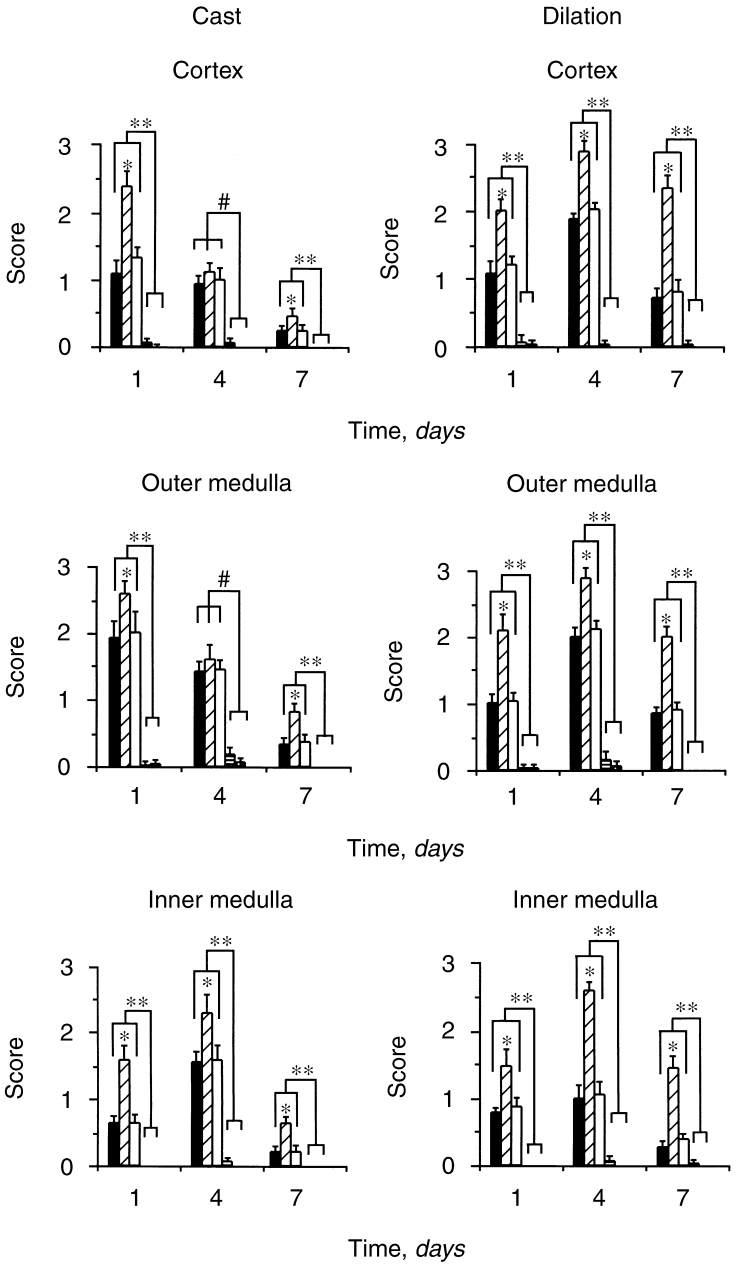

Fig. 3. Semiquantitative analysis of tubular changes by light microscopy. Note that the changes are most prominent in group II and that the tubular changes are still prominent at day 7. Symbols are: (■) group I; (▨) group II, (□) group III; (▤) group IV; (▩) group V; $*P < 0.01$ group II vs. other groups; $**P < 0.01$ groups I or III vs. groups IV or V; $\#P < 0.01$ groups I or II or III vs. group IV or V; no significant difference among groups I, II, and III.

I and III. Urinary protein excretion in group II recovered slowly, reaching the normal range at day 5, whereas groups I and III had recovered to the normal range by day 3 and onward (Fig. 7).

DISCUSSION

In a previous study, we showed that suppression of the function of renal CD59 alone did not induce complement-mediated tubulointerstitial injury. Our previous data have demonstrated that regulation at the C3 level was most important for the protection of renal tubulointerstitium from autologous complement attack. In the present work, this notion was confirmed by examining the effects of kidney perfusion with a large amount (3.0 mg in the present study vs. 0.3 mg in the previous work) of anti-rat CD59 antibody (6D1; group IV). There was no significant tubulointerstitial injury in this group. However, once complement activation was initiated at the C3 level by blocking the function of rat Crry, CD59 was revealed to be playing an essential role to protect kidneys against autologous complement attack. This notion is supported by the following facts. First, additional suppression of CD59 by relatively large amounts (3.0 mg) of 6D1 [F(ab′)2 fragment] significantly worsened tubulointerstitial injury. Since a relatively low dose of

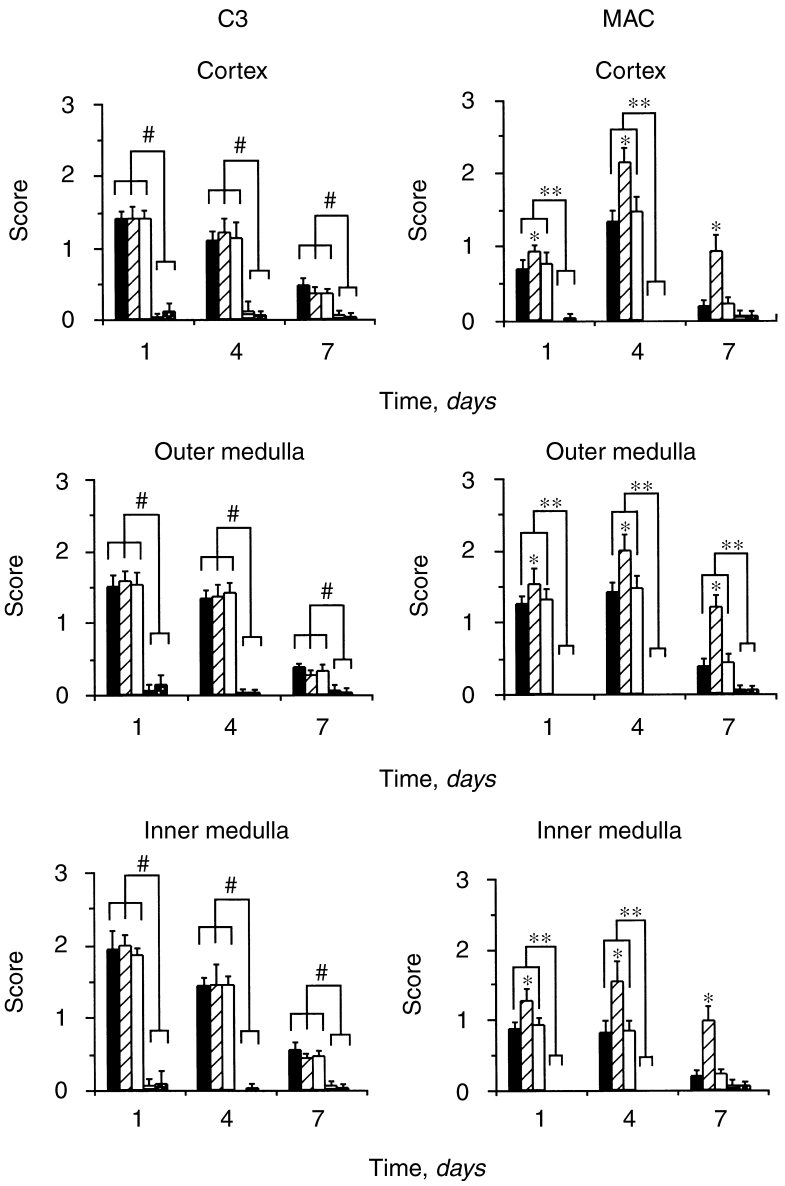

Fig. 4. Semiquantitative analysis of deposition of C3 and MAC in the kidney by immunofluorescence miscroscopy. Note that deposition of C3 is comparable among groups I, II, and III, while MAC deposition is most prominent in group II. Symbols are: (■) group I; (▨) group II, (□) group III; (▤) group IV; (▩) group V; #$P < 0.01$, there is no significant difference among groups I, II, and III. *$P <$ 0.01 group II vs. other groups; **$P < 0.01$ group I or III vs. group IV or V.

6D1 (0.3 mg) was not enough to enhance the 5I2-induced renal injury, it is likely that there is a dose dependency of 6D1 in the suppression of CD59. Second, the degree of C3 deposition was not different among animals in groups I, II, and III, while the extent of MAC deposition was much more prominent in group II. Since histological and immunohistological parameters closely correlated with the extent of MAC deposition, we concluded that the worsening of tubulointerstitial injury was due to the increased MAC deposition.

Complement-mediated injury in the renal vasculature, especially in the peritubular capillaries and vasa recta, is likely to be the initial events in the present model of tubulointerstitial injury. The primary binding site of monoclonal antibodies 15 minutes after perfusion and recirculation was seen in these areas [21]. Hatanaka et al have shown that the vascular permeability for plasma proteins was increased after suppression of Crry [22]. Thus, the primary event in the present model is likely to be the complement-mediated microvascular injury in the interstitium. Subsequent leakage of plasma proteins, including complement components into the interstitial compartment, will permit complement activation and deposition of MAC in tubular basement membranes and basolateral membranes of tubular cells. Interstitial inflammation and MAC deposition in the tubules will then induce tubular injury. In contrast to the tubulointerstitium, there was no significant morphological alteration in the glomeruli. This finding was also reported by Nangaku et al [29]. The different responses following functional suppression

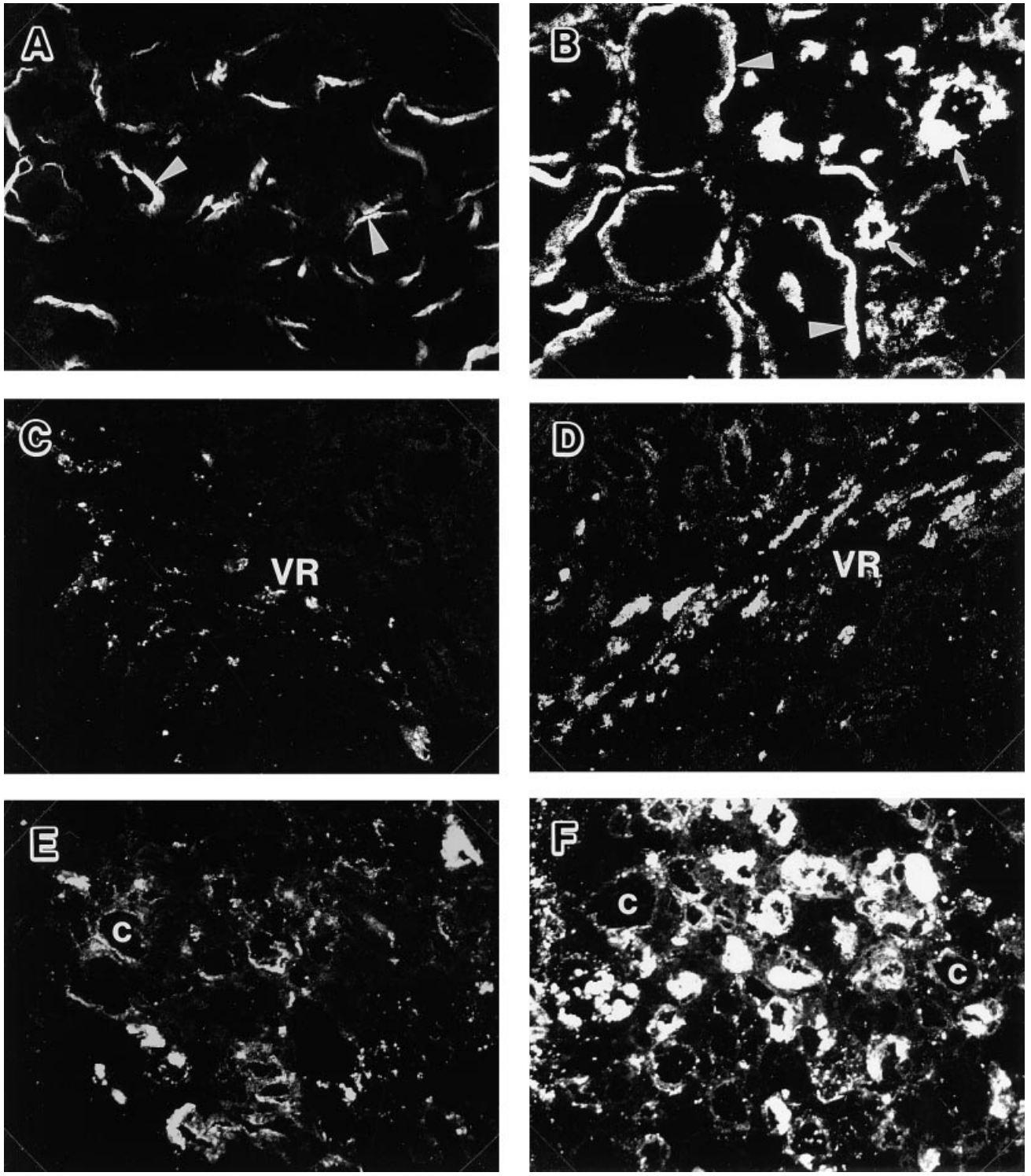

Fig. 5. Immunofluorescence showing deposition of MAC at day 4 in (*A* and *B*) cortex, (*C* and *D*) outer medulla, and (*E* and *F*) inner medulla. Circumferential binding of MAC was observed along cortical tubular basement membranes (arrow heads) in both groups, whereas deposition is also detected in peritubular capillaries (arrows), vasa recta (VR), and the medullary capillaries in group II. Staining in these area of group I rats is faint. C, collecting duct (Group I, ×200, A, C, and E; Group II, B, D, and F).

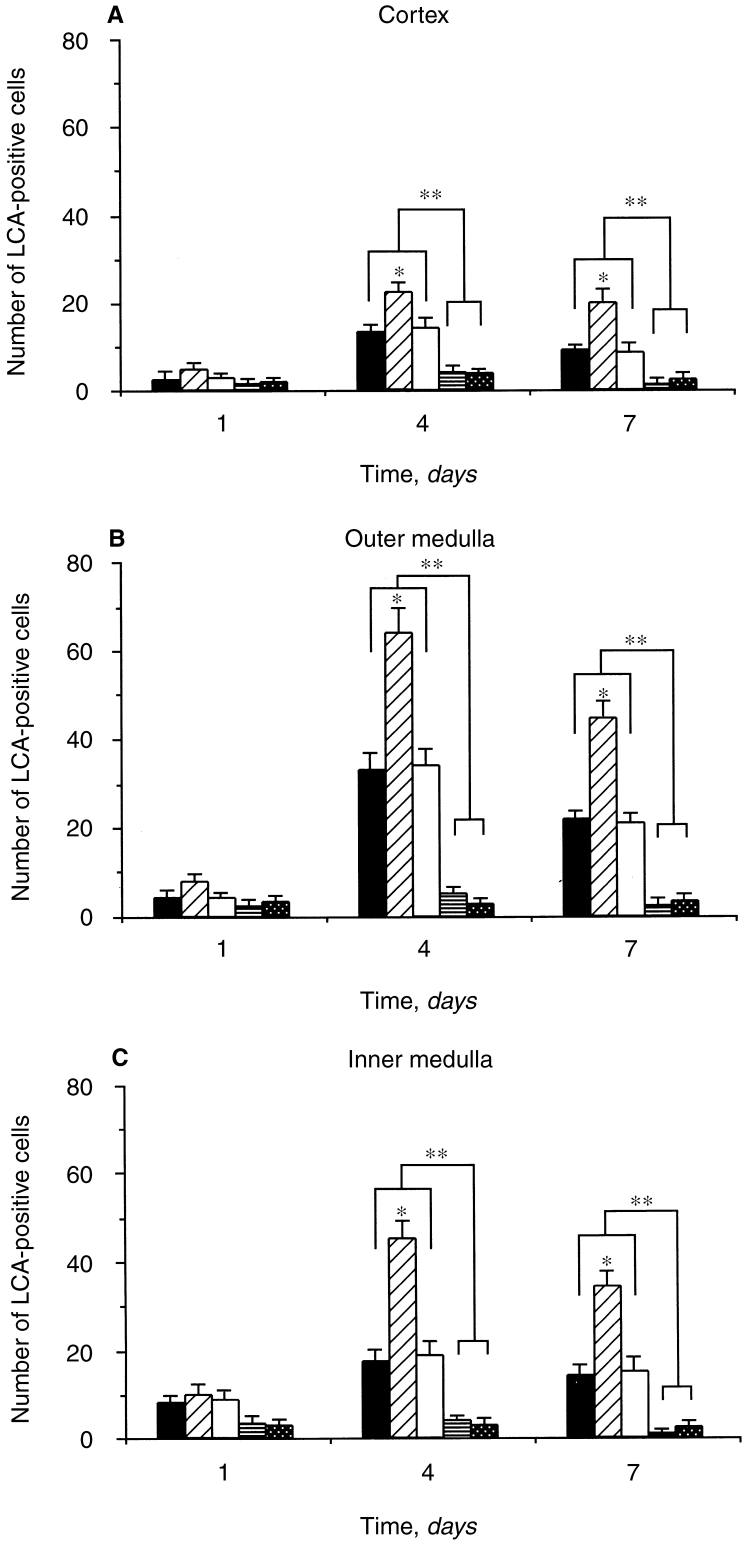

Fig. 6. Quantitative analysis of leukocyte infiltration in the tubulointerstitium. Note that infiltration of LCA-positive leukocytes is most prominent in group II at day 4 and day 7. Symbols are: (■) group I; (▨) group II, (□) group III; (▤) group IV; (▩) group V; $*P <$ 0.01 group II vs. other groups; $**P < 0.01$ groups I or III vs. groups IV or V.

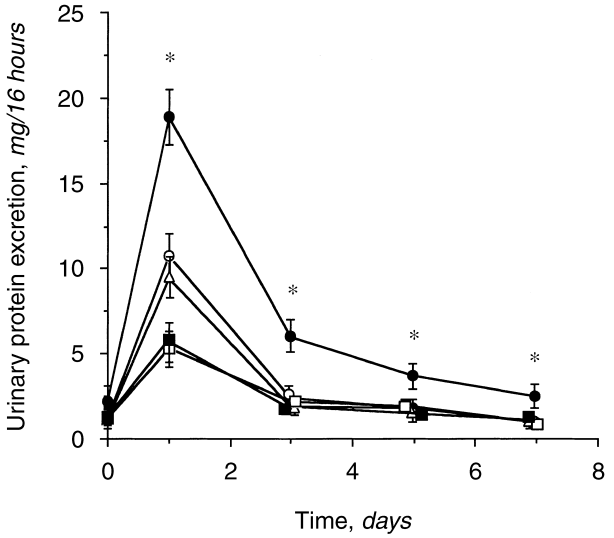

Fig. 7. Urinary protein excretion. Urinary protein excretion was transiently increased at day 1 in all groups. Note that rats of group II (●) showed significantly higher protein excretion than other groups throughout the time course. In groups I (○), III (△), IV (□), and V (■), urinary protein excretion returns to the normal level by day 3. *$P < 0.01$ group II vs. other groups.

of a complement regulator(s) between these two compartments are surprising but might be explained as follows: First, ammonium is known as a C3 activator through amidation of the C3 molecule [30]. Ammonium is generated in the proximal tubular cells and can be transported into interstitium and peritubular capillaries. Therefore, the tubulointerstitial compartment offers the environment in which complement is easily activated via the alternative pathway [31]. In the glomeruli, there is no such stimulus to activation. Second, there are several candidates of complement regulators other than Crry in the rat glomeruli. For example, rat DAF has been recently cloned by Hinchliffe et al [32]. Rat DAF is abundantly expressed in the glomerulus but is absent from the tubulointerstitial tissue [33]. This fact may explain why mAb 5I2 can selectively induce tubulointerstitial injury. Third, proteoglycans have been reported to suppress complement activation [34, 35] and are the candidates for inhibitors of complement activation in the glomerulus. Unfortunately, it is not clear whether these proteoglycans are also present in the tubulointerstitial area. The absence of significant histopathological changes in the glomeruli indicate that the mild proteinuria observed in this model is of tubular origin; that is, the injury affects reabsorption of low molecular weight proteins. In support of this notion, analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of urinary protein in rats from this study revealed that the protein was composed of albumin and lower molecular weight proteins only (data not shown).

Complement-mediated renal injury takes place when complement activation overwhelms its regulation mechanisms. The kidney is an organ that is always exposed to complement-activating stimuli. The present data demonstrated that Crry and CD59 work together as the important regulators of complement in the living kidney.

ACKNOWLEDGMENTS

Part of this work was supported by the 1999 Grant-in-Aid for Scientific Research from Ministry of Education and Culture of the Japanese government (No. 11671033). The authors appreciate the excellent technical assistance of N. Suzuki, N. Asano, T. Katahara, and Y. Fujitani.

Reprint requests to Seiichi Matsuo, M.D., Division of Nephrology, Internal Medicine III, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.
E-mail: smatsuo@med.nagoya-u.ac.jp

REFERENCES

1. Nicholson-Weller A, Burge J, Fearon DT, Weller PF, Austen KF: Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertases of the complement system. *J Immunol* 129:184–189, 1982
2. Seya T, Turner J, Atkinson JP: Purification and characterization of membrane cofactor protein (gp45-70) which is a cofactor for cleavage of C3b and C4b. *J Exp Med* 163:837–855, 1986
3. Okada N, Harada R, Fujita T, Okada H: A novel membrane glycoprotein capable of inhibiting membrane attack by homologous complement. *Int Immunol* 1:205–208, 1989
4. Davies A, Simmons DL, Hale G, Harrison RA, Tighe H, Lachmann PJ, Waldmann H: CD59, an Ly-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. *J Exp Med* 170: 637–654, 1989
5. Zalman LS, Wood LM, Moller-Eberhard HJ: Isolation of a human erythrocyte membrane protein capable of inhibiting expression of homologous complement transmembrane channels. *Proc Natl Acad Sci USA* 83:6975–6979, 1986
6. Holguin MH, Fredrick LR, Bernshaw NJ, Wilcox LA, Parker CJ: Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. *J Clin Invest* 84:7–17, 1989
7. Sugita Y, Nakano Y, Tomita M: Isolation from human erythrocytes of a new membrane protein which inhibits the formation of complement transmembrane channels. *J Biochem* 104:633–637, 1988
8. Matsuo S, Ichida S, Takizawa H, Okada N, Baranyi L, Iguchi A, Morgan BP, Okada H: In vivo effects of monoclonal antibodies which functionally inhibit complement regulatory proteins in rats. *J Exp Med* 180:1619–1627, 1994
9. Nishikawa K, Matsuo S, Okada N, Morgan BP, Okada H: Local inflammation caused by a monoclonal antibody that blocks the function of the function of the rat membrane inhibitor of C3 convertase. *J Immunol* 156:1182–1188, 1996
10. Takizawa H, Okada N, Okada H: Complement inhibitor of rat cell membrane resembling mouse Crry/p65. *J Immunol* 152:3032–3038, 1994
11. Sakurada C, Seno H, Dohi N, Takizawa H, Nonaka M, Okada N, Okada H: Molecular Cloning of the rat complement regulatory protein, 5I2 antigen. *Biochem Biophys Res Commun* 198:819–826, 1994
12. Molina H, Wong W, Kinoshita T, Brenner C, Foley S, Holers VM: Distinct receptor and regulatory properties of recombinant mouse complement receptor 1 (CR1) and Cry, the two genetic homologues of human CR1. *J Exp Med* 175:121–129, 1992

13. Martin BK, Weis JH: Functional identification of transcription control sequences of the mouse Cry gene. *J Immunol* 151:857–869, 1993
14. Foley S, Li B, Dehoff M, Molina H, Holers VM: Mouse Crry/p65 is a regulator of the alternative pathway of complement activation. *Eur J Immunol* 23:1381–1384, 1993
15. Funabashi K, Okada N, Matsuo S, Yamamoto T, Morgan BP, Okada H: Tissue distribution of complement regulatory membrane proteins in rats. *Immunology* 81:444–451, 1994
16. Tada T, Okada H, Okada N, Tateyama H, Suzuki H, Takahashi Y, Eimoto T: Membrane attack complex of complement and 20 kDa homologous restriction factor (CD59) in myocardial infarction. *Virchows Arch* 430:327–332, 1997
17. Peter JC, Christine AS, Trixie AS, Marina K, Atousa A, Margarita R, Margaret JT, Martin JP, Anthony JF: High-level endothelial expression of human CD59 prolongs heart function in an ex vivo model of xenograft rejection. *Transplantation* 65:826–831, 1998
18. Mizuno M, Nishikawa K, Goodfellow RM, Piddlesden SJ, Morgan BP: The effects of functional suppression of a membrane-bound complement regulatory protein, CD59, in the synovial tissue in rats. *Arthritis Rheum* 40:527–533, 1997
19. Hughes TR, Piddlesden SJ, Williams JD, Harrison RA, Morgan BP: Isolation and characterization of a membrane protein from rat erythrocytes which inhibits lysis by the membrane attack complex of rat complement. *Biochem J* 284:169–176, 1992
20. Ichida S, Yuzawa Y, Okada H, Yoshioka K, Matsuo S: Localization of the complement regulatory proteins in the normal human kidney. *Kidney Int* 46:89–96, 1994
21. Nomura A, Nishikawa K, Yuzawa Y, Okada H, Okada N, Morgan BP, Sara JP, Nadai M, Hasegawa T, Matsuo S: Tubulointerstitial injury induced in rats by a monoclonal antibody which inhibits function of a membrane inhibitor of complement. *J Clin Invest* 96:2348–2356, 1995
22. Hatanaka Y, Yuzawa Y, Nishikawa K, Fukatsu A, Okada N, Okada H, Mizuno M, Matsuo S: Role of a rat membrane inhibitor of complement in anti-basement membrane antibody-induced renal injury. *Kidney Int* 48:1728–1737, 1995
23. Schulze M, Baker PJ, Perkinson DT, Johnson RJ, Ochi RF, Stahl RAK, Couser WG: Increased urinary excretion of C5b-9 distinguishes passive Heymann nephritis in the rat. *Kidney Int* 35:60–68, 1989
24. Guesdon J, Ternynck T, Abrameas S: The use of avidin-biotin interaction in immunoenzymatic techniques. *J Histochem Cytochem* 27:1131–1139, 1979
25. Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K, Tokuda K: Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. *Clin Chem* 32:1551–1554, 1986
26. Matsuo S, Yoshida F, Yuzawa Y, Hara S, Fukatsu A, Watanabe Y, Sakamoto N: Experimental glomerulonephritis induced in rats by a lectin and its antibodies. *Kidney Int* 36:1011–1021, 1989
27. Matsuo S, Nishikage H, Yoshida F, Nomura A, Piddlesden SJ, Morgan BP: Role of CD59 in experimental glomerulonephritis in rats. *Kidney Int* 46:191–200, 1994
28. Platt J, Michael AF: Retardation of fading and enhancement of intensity of immunofluorescence by p-phenylene-diamine. *J Histochem Cytochem* 31:840–842, 1983
29. Nangaku M, Charles EA, Jeffrey P, Stuart JS, Kurokawa K, Stephen A, Morgan BP, Richard JJ, William GC: CD59 protects glomerular endothelial cells from immune-mediated thrombotic microangiopathy in rats. *J Am Soc Nephrol* 9:590–597, 1998
30. Hostetter MK, Gordon DL: Biochemistry of C3 and related thioester proteins in infection and inflammation. *Rev Infect Dis* 9:97–109, 1987
31. Nath KA, Hostetter MK, Hostetter TH: Pathophysiology of chronic tubulointerstitial disease in rats: Interactions of dietary acid load, ammonia, and complement component C3. *J Clin Invest* 76:667–675, 1985
32. Hinchliffe SJ, Spiller OB, Rushmere NK, Morgan BP: Molecular cloning and functional characterization of the rat analogue of human decay-accelerating factor (CD55). *J Immunol* 161:5695–5703, 1998
33. Spiller OB, Hanna SM, Morgan BP: Tissue distribution of the rat analogue of decay-accelerating factor. *Immunology* 97:374–384, 1999
34. Quigg RJ: Inhibition of the alternative pathway of complement by glomerular chondroitin sulphate proteoglycan. *Immunology* 76:373–377, 1992
35. Raats CJI, Mariken EL, Marinka AHB, Annemieke VDW, Peter H: Reduction in glomerular heparan sulfate correlates with complement deposition and albuminuria in active Heymann nephritis. *J Am Soc Nephrol* 10:1689–1699, 1999