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Role of CD59 in experimental glomerulonephritis in rats

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Role of CD59 in experimental glomerulonephritis in rats. CD59 is a molecule which is present on the host cell membranes and inhibits formation of membrane attack complex. A monoclonal antibody, 6D1, recognizes a rat analogue of human CD59. 6D1 inhibits function of rat CD59 and can enhance complement-mediated hemolysis *in vitro*. To assess the role of CD59 in complement-mediated glomerular injury, 6D1 was tested in a model of experimental glomerulonephritis induced by a lectin and its antibodies. The left kidney of a rat was perfused either with 200 μg of Lens culinaris hemoagglutinin (LCH) plus 1 mg of 6D1 (IgG1 fraction) (Group I and III) or with LCH only (Group II) through a cannula placed in the left renal artery. All the perfusate was discarded from a cannula in the renal vein. The holes in the artery and vein were repaired by microsurgery and the blood circulation was re-established. Rats were injected either with 0.125 ml of rabbit anti-LCH serum (Group I and II), or with normal rabbit serum (Group III) via tail vein one minute after the recirculation. Fifteen minutes after injection, significant C9 deposition in the glomeruli was observed only in Group I, whereas C3 deposition in Group I and II were comparable. At Day 4, total glomerular cells, proliferating cells, glomerular expression of intercellular adhesion molecule-1 and fibrin deposition in Group I were all significantly increased when compared with Group II. At Day 7, number of total glomerular cells and leukocytes in the glomeruli of Group I were significantly higher than in Group II. The glomeruli in Group III appeared normal throughout experiments. These data indicate that the functional inhibition of a rat analogue of human CD59 worsens complement-mediated glomerular injury *in vivo*.

The membrane inhibitors of complement are a group of proteins which inhibit complement activation at the C3 convertase level or at the level of formation of the membrane attack complex (MAC) [1, 2]. In humans a number of molecules have been identified and shown to play protective roles against homologous complement attack. Among these molecules, CD59 [3], also known as 20 kDa homologous restriction factor (HRF20 [4]), prevents the formation of MAC on the cell membrane [5]. It has recently been shown that CD59 is essential for the protection of nucleated cells against homologous complement attack *in vitro* using cells in culture [6–8].

The kidney is an organ which is always under threat from homologous complement attack. One can frequently see the localization of immune deposits with complement components in diseased kidneys [9]. It has been suggested that presence of high concentration of ammonium ions can activate the alternative pathway of complement and in certain circumstances leads to tubulointerstitial injuries [10, 11]. Thus, the kidney may be injured if there is no inhibitory system of complement activation. CD59 is expressed in the various structures of the normal human kidney and it is hypothesized that renal CD59 plays a protective role against homologous complement attack. Recently, we reported a rat model of complement-mediated experimental glomerulonephritis which was initiated by the interaction of a lectin planted on the surface of glomerular endothelial cells and its antibodies [12]. Using this model, the *in vivo* role of CD59 in the complement-mediated glomerular injury was assessed using a neutralizing monoclonal antibody, 6D1, against rat CD59 [13]. The results obtained in the present work showed that 6D1 enhanced the deposition of rat C9 in the glomeruli and worsened the subsequent glomerular injuries *in vivo*. This is the first *in vivo* demonstration that CD59 plays protective role in any tissue injury including complement mediated glomerular damage.

Methods

Animals

Female Wistar rats weighing about 275 grams were obtained from Chubu Kagaku Shizai Co. Ltd. (Nagoya, Japan) and were allowed free access to food and water.

Reagents

The characteristics of mouse monoclonal antibody 6D1 were described previously [13]. Ascites made from 6D1 hybridoma were obtained and IgG fraction was purified by protein A affinity column chromatography (Pharmacia Fine Chemicals, Uppsala, Sweden). Chromatographically purified LCH was purchased from Sigma (St. Louis, Missouri, USA). Rabbit anti-LCH serum was obtained as described previously [12]. Fluorescein-conjugated monoclonal anti-leukocyte common antigen antibody (MRC OX-1) was purchased from Dainippon Pharmaceutical Company (Osaka, Japan). Monoclonal antibody (1A29) against rat intercellular adhesion molecule 1 (ICAM-1) was a gift from Dr. M. Miyasaka (Tokyo Metropolitan Institute for Clinical Medicine, Tokyo, Japan) [14]. Rabbit anti-rat C9 was obtained according to the method described before [15]. 1A29 and rabbit anti-rat C9 were labeled with biotin according to a method described by Guesdon, Ternynck and Abrameas [16]. Fluorescein-labeled goat antibodies against

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Table 1. Experimental protocol

	Perfusion		
	LCH	6D1	i.v. injection
Group I	200 μg	1 mg	Rb anti-LCH (0.125 ml)
Group II	200 μg	—	Rb anti-LCH (0.125 ml)
Group III	200 μg	1 mg	NRS (0.125 ml)

Abbreviations are: rabbit anti-LCH, rabbit anti-LCH serum; NRS, normal rabbit serum. Kidney specimens were examined 3 hours, 4 days and 7 days after injection by histological and immunohistological methods. Six rats in each group at the selected time point were examined.

rabbit IgG, rat C3 and rat fibrin/fibrinogen were obtained from Cappel Laboratories (Westchester, Pennsylvania, USA).

Kidney perfusion

To localize LCH and 6D1 to the kidney but not to other organs or blood cells in circulation, the isolated kidney perfusion technique was used. The procedure of left kidney perfusion was described in the previous paper [12, 17]. Under ether anesthesia, the left kidney of a 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 buffer saturated with 95% oxygen and 5% carbon dioxide was used as a vehicle. All the perfusate was discarded through a cannula placed in the renal vein. After kidney perfusion, tubes were removed, and the holes in the artery and vein were repaired by microsurgery. Blood circulation of the left kidney was re-established by releasing the ligature. The average time required for perfusion procedure was about 10 minutes. In order to see the localization of 6D1 after kidney perfusion, rats were perfused with 1 mg of 6D1 in 10 ml of buffer with or without 200 μg of LCH according to the procedure described above. Rats were sacrificed one minute after recirculation of the kidney, and localization of mouse IgG in the kidney was examined by immunofluorescence microscopy.

Experimental protocol

Rats were divided into three groups. Rats of group I were first perfused with 200 μg of LCH and 1 mg of 6D1 in 10 ml of buffer and then perfused with 2 ml of buffer to wash out the unbound LCH and 6D1. One minute after recirculation of the left kidney, 0.125 ml of rabbit anti-LCH serum was injected via the tail vein. Rats of Group II were perfused only with LCH and were injected with the same amount of anti-LCH serum. The amount of anti-LCH serum used in the present study was one-eighth of that used in our previous study [12] to minimize the glomerular injury. Rats of Group III were perfused as described for Group I (LCH and 6D1) and were injected with 0.125 ml of normal rabbit serum. Rats were sacrificed periodically at three hours, four days or seven days after perfusion. The number of rats studied in each group at the selected time point was 6. The protocol is shown in Table 1. Since it is known from our previous study that immune deposits are present on the surface of glomerular endothelial cells 15 minutes after injection of anti-LCH antibodies and they localize in the subendothelial space but not on the endothelial surface three hours later, three rats in each group were sacrificed 15 minutes after injection of anti-LCH antibodies and glomerular deposition of rat C9 was studied by immunofluorescence microscopy.

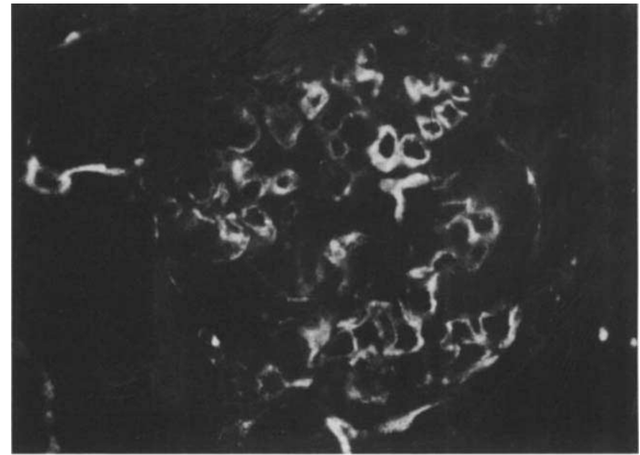

Fig. 1. *Immunofluorescence micrograph showing the binding of 6D1 along glomerular capillary walls one minute after perfusion and recirculation.* The left kidney of a rat was perfused with 6D1. Binding of 6D1 was visualized by FITC-labeled rabbit anti-mouse IgG. (×400)

Histology and immunohistochemistry

At the time of sacrifice, the left kidneys were processed for study by light, electron and immunofluorescence microscopy. For light microscopic study, part of the kidney was fixed in methacaln fixative [18] overnight and was embedded in paraffin. Two μm thick sections were stained with periodic acid-Schiff (PAS).

To detect proliferating cells, paraffin sections were stained with a mouse monoclonal antibody (19A2, Coulter Immunology, Hialeah, Florida, USA) against proliferating cell nuclear antigen (PCNA) [19] according to a method described by Garcia, Coltrera and Gown [20]. Sections were first deparaffinized and rehydrated. They were then incubated with 3% hydrogen peroxide for 15 minutes, and incubated with 19A2 for two hours. The sections were incubated with biotinylated goat anti- mouse IgM for one hour followed by incubation with peroxidase-labeled streptoavidin (VECSTAIN ABC KIT, Vector, Burlingame, California, USA). The reaction was visualized by incubating sections with diaminobenzidine tetrahydrochloride (Nakarai Chemicals, Osaka, Japan) and hydrogen peroxide.

For immunofluorescence microscopic study, small pieces of kidney were snap frozen in liquid nitrogen and were kept at −70°C until use. Two μm thick sections cut by a cryostat were incubated with fluorescein-labeled rabbit antibodies against mouse IgG and rat C3, and goat antibodies against rat fibrin and rabbit IgG (Cappel, Westchester, Pennsylvania, USA). For the detection of ICAM-1, frozen sections were first treated with Avidin D blocking solution (Vector Laboratories) for 15 minutes and then with biotin blocking solution (Vector Laboratories) for 15 minutes at room temperature to minimize background staining. They were then incubated with biotinylated 1A29 (used at the concentration of 10 μg/ml) for 30 minutes at room temperature. For the detection of glomerular C9, sections

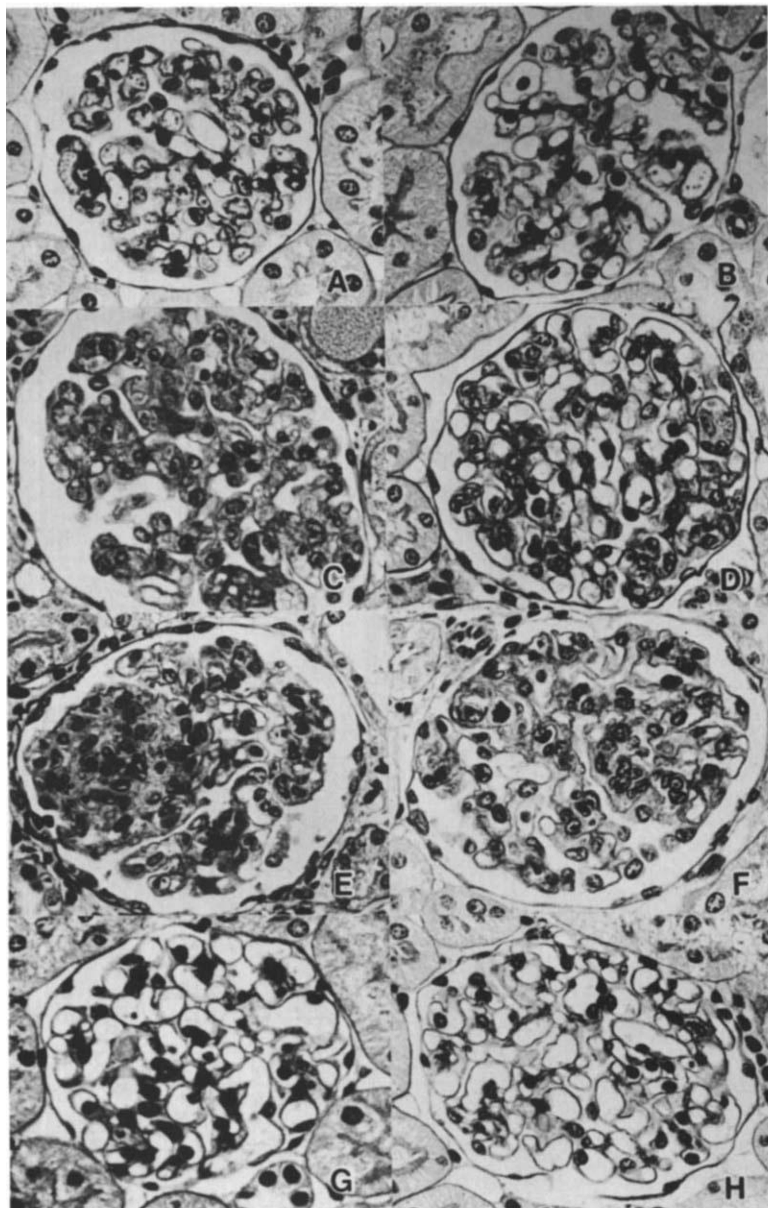

Fig. 2. *Light micrographs (PAS staining).* Each micrograph shows a glomerulus from a rat indicated as follows. (**A**) Group I at 3 hours; (**B**) Group II at 3 hours; (**C**) Group I at 4 days; (**D**) Group II at 4 days; (**E**) Group I at 7 days; (**F**) Group II at 7 days; (**G**) Group III at 3 hours; (**H**) Group III at 7 days. (A through H: ×400)

were incubated with biotinylated anti-rat C9 (at the concentration of 20 μg/ml) for 30 minutes at room temperature. All the sections were then incubated with fluorescein-labeled avidin for another 30 minutes. After final wash in PBS, all the sections were mounted by cover glasses using *p*-phenylenediamine [21] and were observed by Olympus epifluorescence microscopy (Tokyo, Japan).

For electron microscopy, small pieces of kidney tissues were fixed in 2% glutaraldehyde for two hours, and were osmicated. They were then embedded in Epon 812 and ultrathin sections were observed by a JEOL 100CX electron microscopy (Tokyo, Japan).

Quantitation of data

The number of total glomerular cells was assessed by the number of nuclei per equatorially cut glomerular cross section. Similarly, the number of infiltrating cells was assessed by the number of leukocyte common antigen positive cells, and that of proliferating cells by the number of PCNA-positive cells in the glomerular cross section. In each rat, 20 glomeruli were examined and the average number was calculated.

Glomerular ICAM-1 expression was assessed according to distribution and intensity (Distribution Score and Intensity Score). Distribution of ICAM-1 positive area in a glomerulus was graded as 0 to 3+ according to the following criteria: 0, negative; 1, positive in less than one-third of glomerular capillaries; 2, positive in one-third to two-thirds of glomerular capillaries; 3, positive in more than two-thirds of glomerular capillaries. The intensity of glomerular ICAM-1 expression was graded from 0 (negative) to 3 (strongly positive). Glomerular MAC deposition was assessed according to the staining intensity for rat C9. Since part of proximal tubular basement

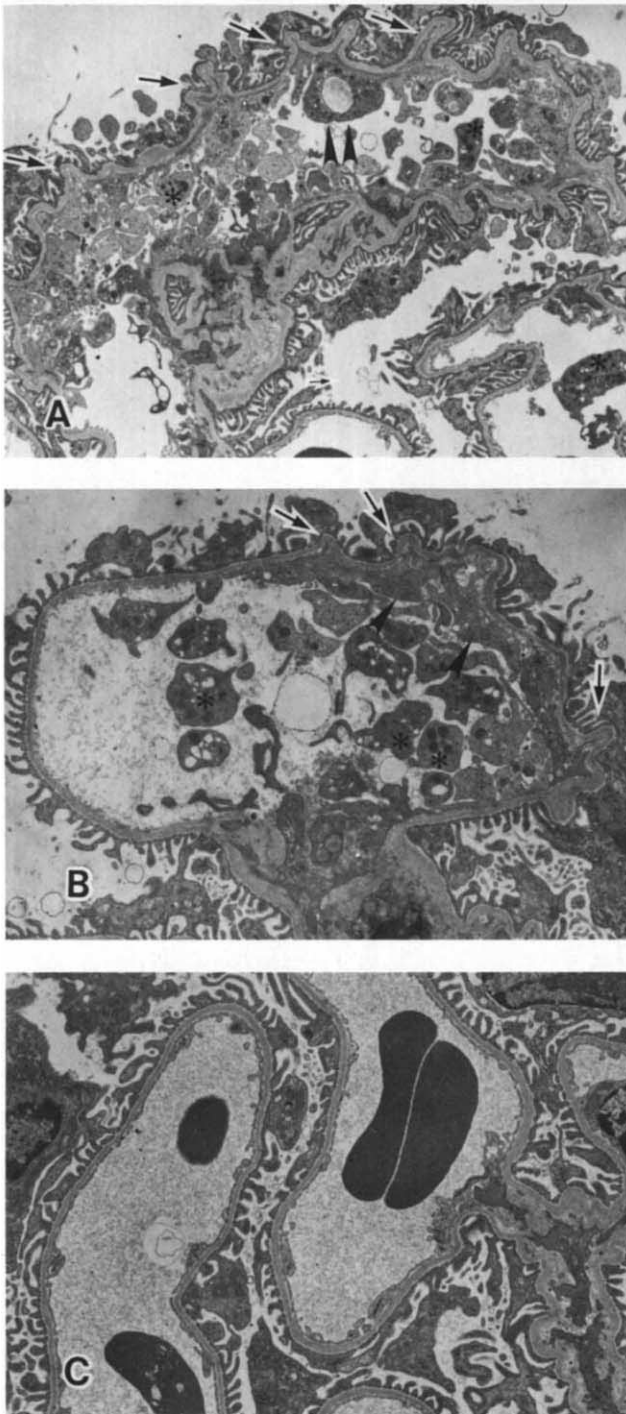

Fig. 3. *Electron micrographs of the kidneys obtained at 3 hours.* (**A**) Group I; (**B**) Group II; (**C**) Group III. Symbols are: arrows, wrinkling of GBM; arrowheads, swollen endothelium; and asterisks, platelets.

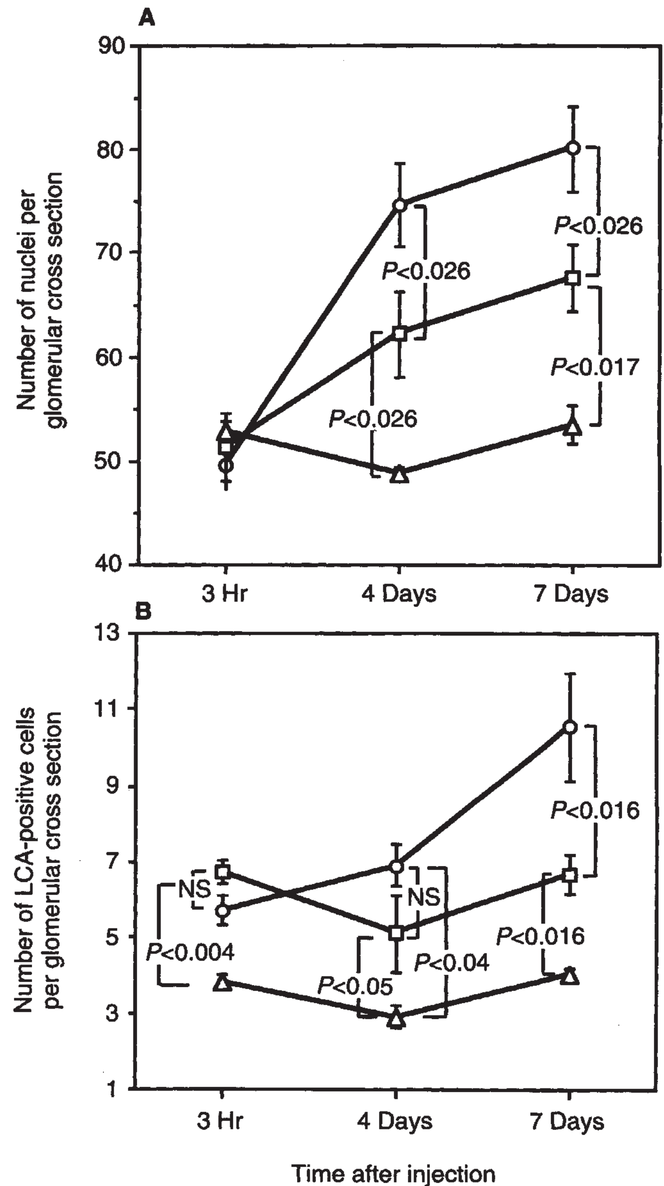

Fig. 4. *Total glomerular cell count* (**A**) *and leukocyte infiltration* (**B**). Glomerular cell count is expressed as the number of nuclei per glomerular cross section. The number of leukocytes in the glomerulus was assessed by the number of leukocyte common antigen positive cells in a glomerular cross section. Symbols are: (○) group I; (□) Group II; (△) Group III.

membranes of the control and nephritic rat kidneys were strongly stained for C9 (Fig. 9), the staining intensity of this was graded as 3. Staining intensity (0 negative to 3 strongly positive) of each glomerulus was observed. Because significant number of glomeruli of Group I and Group II rats showed very faint staining for C9 (that is, staining is not negative but not so strong as 1), we assigned 0.5 to these glomeruli. As described above, 20 glomeruli were observed in each rat and the average grade was calculated.

Glomerular fibrin deposition was observed both in the glomerular capillary lumen and along the glomerular capillary walls. Fibrin deposition in the glomerular capillary lumen was observed as a mass of positive area, while fibrin deposition along glomerular capillary walls was observed as linear staining along GBM. Fluorescein staining intensity was graded from 0 (negative) to 3 (strongly positive). The average grade was similarly calculated in each rat.

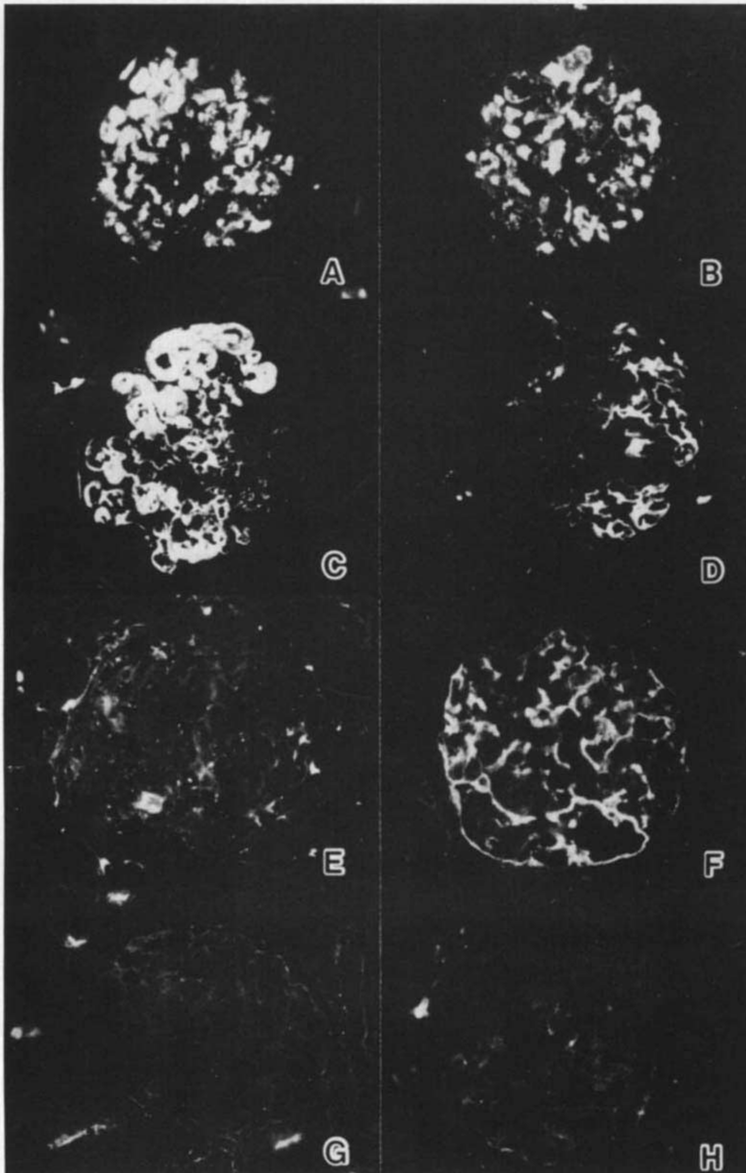

Fig. 5. ***Immunofluorescence micrographs showing glomerular fibrin deposition and ICAM-1 expression.*** Each photograph shows immunostaining described as follows. (**A**) Group I, fibrin, at 3 hours; (**B**) Group II, fibrin, at 3 hours; (**C**) Group I, fibrin, at 4 days; (**D**) Group II, fibrin, 4 days; (**E**) Group III, fibrin, at 4 days; (**F**) Group I, ICAM-1, at 4 days; (**G**) Group II, ICAM-1, 4 days; (**H**) Group III, ICAM-1, 4 days. (A through H: ×400) Note that fibrin deposition is seen as luminal mass in Group I and II rats at 3 hours after induction of glomerular injury.

All the specimens were observed by two blinded persons. Average number or grade was used for statistical analysis.

Urinary protein measurement

Rats were housed in metabolic cages overnight (16 hr) every other day starting at the day of kidney perfusion. Urinary protein was measured by a quantitative sulfosalicylic acid method [22].

Statistical analysis

Statistical analysis was performed by Mann-Whitney U test. Significant difference was set when the *P* value was less than 5% (or z value was more than 1.96) between two groups. All the data are given in mean ± standard error.

Results

Binding of 6D1 following perfusion

6D1 bound mainly to the glomerular capillary walls and peritubular capillaries after perfusion (Fig. 1). Significant binding of 6D1 was not clearly observed in the mesangial area when studied by immunofluorescence microscopy 15 minutes after kidney perfusion and reestablishment of blood flow in Group I and III rats.

Histology and immunohistology

In Group I and II rats there was infiltration of platelets in the glomerular capillary lumen, and the glomerular capillary walls were wrinkled at three hours after initiation of glomerulonephritis (Fig. 2A, B). At the ultrastructural level, vesiculation and

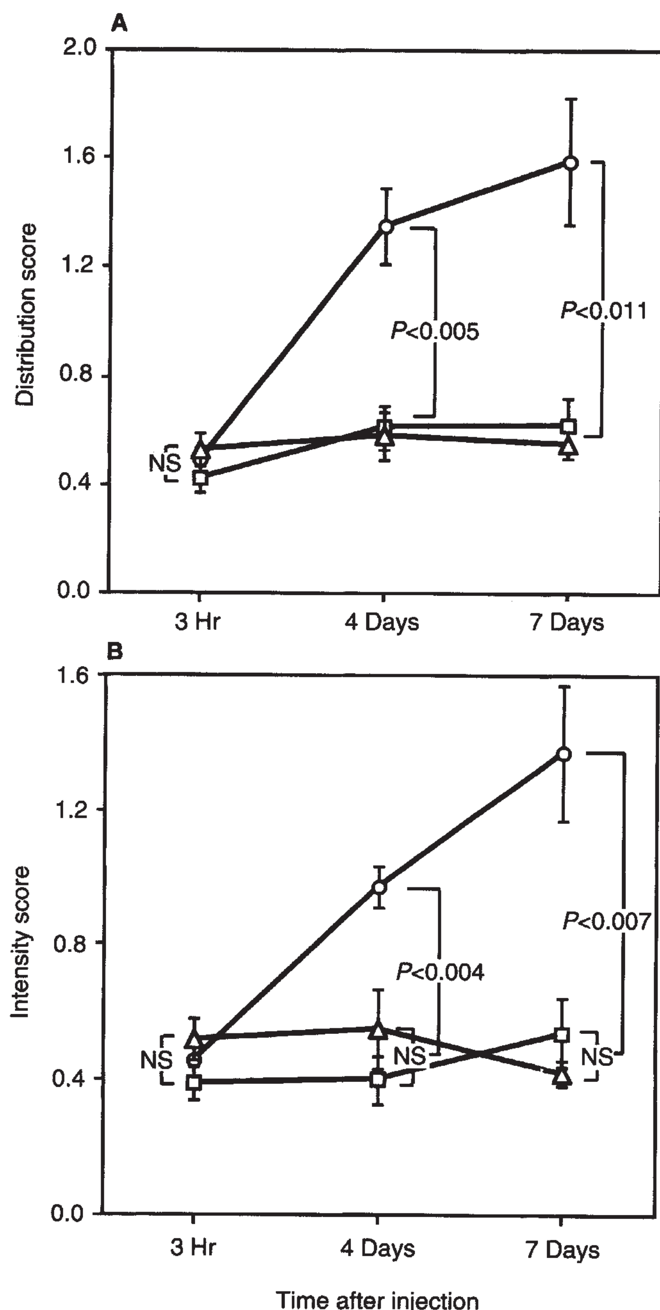

Fig. 6. *Glomerular ICAM-1 expression.* (**A**) shows distribution of glomerular ICAM-1 and (**B**) shows intensity.

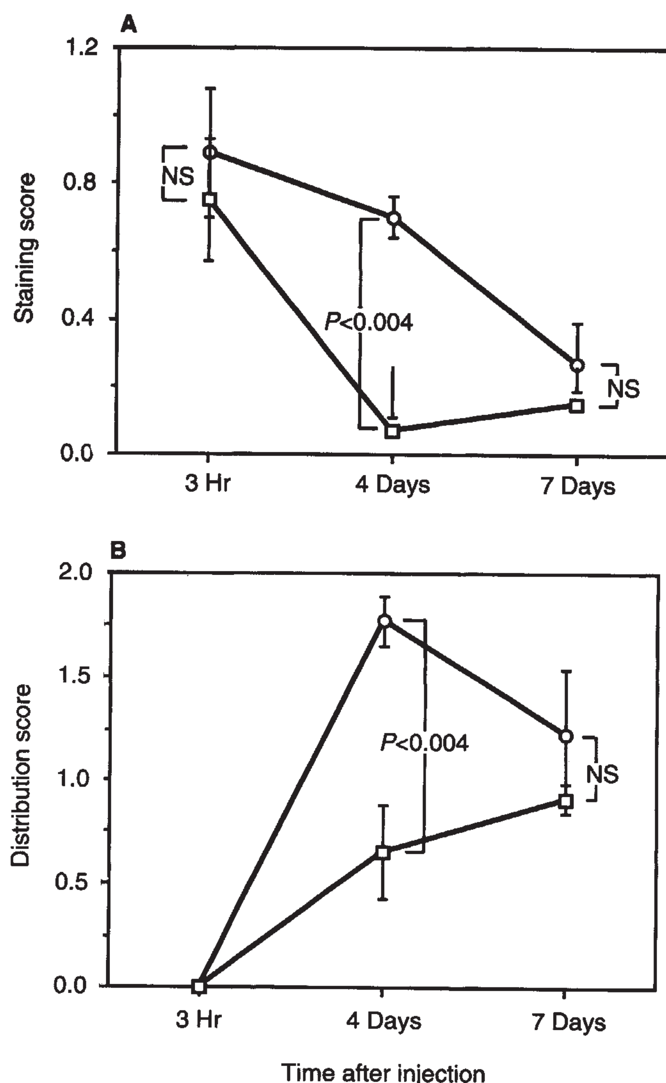

Fig. 7. *Glomerular deposition of fibrin.* Symbols are: (○) Group I; (□) Group II. (**A**) Localization of fibrin in the glomerular capillary lumen. (**B**) Localization of fibrin along glomerular capillary walls. Data of Group III rats were omitted from the graphs because there was no fibrin deposition at all.

swelling of endothelial cells were seen in Group I rats 15 minutes after initiation of nephtitis (not shown). There was endothlial cell swelling, accumulation of platelets, and wrinkling of GBM in Group I and II rats at three hours (Fig. 3A, B). Glomeruli of Group III rats showed normal appearance (Fig. 3C). At Day 4, segmental fibrin thrombi were observed and the capillary lumen was further narrowed in Group I rats. There was moderate hypercellularity seen at this stage in Group I and II rats (Fig. 2C, D). At day 7, hypercellularity was more prominent in Group I rats than in Group II rats (Fig. 2E, F). Rats of Group III showed no abnormality throughout the experiments (Fig. 2G, H).

Immunofluorescence microscopic study showed strong deposition of rabbit IgG and rat C3 along glomerular capillary walls both in Group I and II rats at three hours after initiation of glomerulonephritis. Intensity of the staining for rabbit IgG and rat C3 were comparable in these two groups. Mouse IgG was detected along glomerular capillary walls in Group I and III rats at this stage. Fluorescence intensity for mouse IgG was much weaker than that for rabbit IgG. At Day 4, rabbit IgG and rat C3 were only faintly seen in the glomerulus in Group I and II rats. Mouse IgG was also very faintly seen in Group I and III but not in Group II rats. At Day 7, glomeruli were negative for rabbit IgG, rat C3 and mouse IgG. Immunofluorescence staining for fibrin is described in the section below.

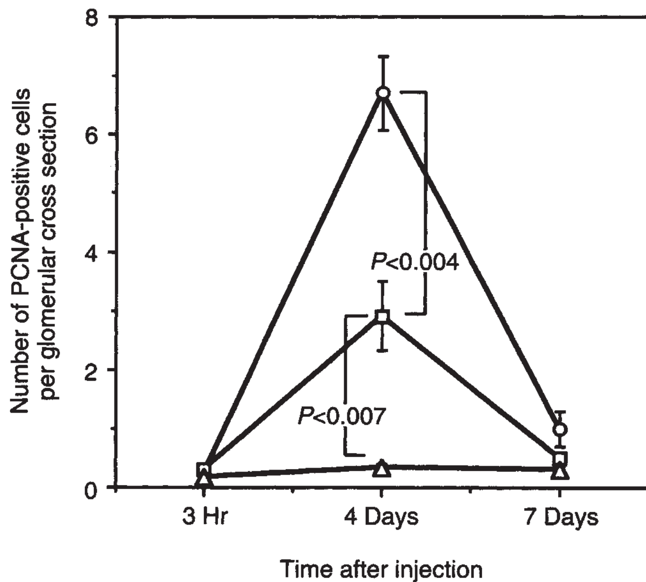

Fig. 8. *Proliferating cells.* Symbols are: (○) Group I; (□) Group II; (△) Group III. Degree of cell proliferation was assessed by the number of PCNA-positive cells in the glomerular cross section. Significant increase of PCNA-positive cells were observed in Group I and II rats only at Day 4.

Hypercellularity and cellular infiltration

The total number of glomerular cells, when assessed by the number of nuclei per glomerular cross section, was significantly increased in Group I and II rats at Day 4 and Day 7. Total glomerular cell count was significantly higher in Group I rats than in Group II at Day 4 and Day 7. Total glomerular cell count remained at the normal level throughout the experiments in Group III rats (Fig. 4A). The number of leukocytes infiltrating into glomeruli was significantly higher in Group I and II rats compared to Group III rats throughout the experiments. At Day 7, there were significantly more leukocytes observed in the glomeruli in Group I rats than in Group II rats (Fig. 4B).

Glomerular ICAM-1 expression

Glomerular ICAM-1 expression was almost identical at three hours after perfusion in all groups, but the extent and intensity of glomerular ICAM-1 expression was significantly increased by later time points in Group I rats. In contrast, glomerular ICAM-1 expression in Group II and III rats did not change during the experiments (Figs. 5, 6).

Glomerular fibrin deposition

Fibrin deposition in the glomerular capillary lumen in Group I and II rats was significantly higher at three hours than that in Group III rats which showed no fibrin deposition. There was still luminal fibrin deposition left in Group I rats at Day 4, while there was very little deposition in Group II rats at this time. Fibrin deposition along Glomerular capillary wall was observed at Day 4 and Day 7 in Group I and II rats. At Day 4, fibrin deposition was significantly stronger in Group I rats than in Group II rats. At Day 7, fibrin deposition became comparable in these two groups. Rats of Group III did not show any significant fibrin deposition throughout the experiments (Figs. 5, 7).

Proliferating cells

PCNA positive cells were hardly detectable at three hours and seven days after perfusion. At Day 4, the number of proliferating cells in Group I and II rats was significantly increased. Among these two groups, proliferation was more prominent in Group I than in Group II (Fig. 8). In Group III rats, there was no detectable proliferation.

Glomerular C9 deposition

Most of the glomeruli showed weak and finely granular deposition of C9 in rats of Group I, while those of Group II and Group III showed faint or negative staining for C9 (Fig. 9). The staining intensity for C9 in the glomerulus was significantly increased in Group I rats compared with the other two groups (Fig. 10).

Proteinuria

There was no significant proteinuria in these three groups throughout the experiments.

Discussion

In humans, CD59 is present on the plasma membrane of cells anchored by glycophosphatidyl inositol (GPI) and inhibits formation of MAC on the cell membranes. CD59 molecule protects host cells from indiscriminate attack by autologous complement at the level of MAC formation, the final step of complement activation. MAC, when formed on the non-nucleated cells like erythrocytes, can induce immediate lysis of the cells. In contrast, MAC formed on the nucleated cells may induce various effects on the function, metabolism and morphology of the cells [23]. For example, MAC formed on the mesangial cell membrane stimulates production of prostanoids/interleukin 1 [24] and reactive oxygen metabolites [25]. In complement-mediated glomerular injuries, formation of MAC on the glomerular cell membrane might induce changes in function and metabolism and affect the subsequent course of injury. Development of 6D1 [13], a mouse monoclonal antibody against the rat analogue of human CD59 which enhances complement-mediated erythrocyte lysis and binds to rat glomeruli [26], enables us to assess the role of CD59 in complement-mediated glomerular injuries *in vivo*.

A model of experimental glomerulonephritis induced by LCH and its antibodies is initiated by antigen-antibody interaction on the surface of glomerular endothelial cells [12]. Based on the observation by Johnson and his colleagues who were using a similar model (concanavalin A and its antibodies), glomerular injuries in this model are likely to be dependent on complement, neutrophils and platelets [27]. Using this model, we investigated the role of CD59 in complement-mediated glomerular injury by neutralizing CD59 molecule *in vivo*. 6D1 was planted along the glomerular capillary walls by kidney perfusion to inhibit the function of CD59 *in situ*. Fifteen minutes after induction of glomerulonephritis, glomerular deposition of C9 was significantly increased in Group I than in other groups while glomerular C3 deposition in Group I and II rats were comparable. This finding suggests that C9 deposition was increased by the functional inhibition of rat CD59 *in vivo*, and that formation of MAC in the glomeruli of Group I rats was presumably enhanced. At three hours after induction of glomerulonephritis, there was no

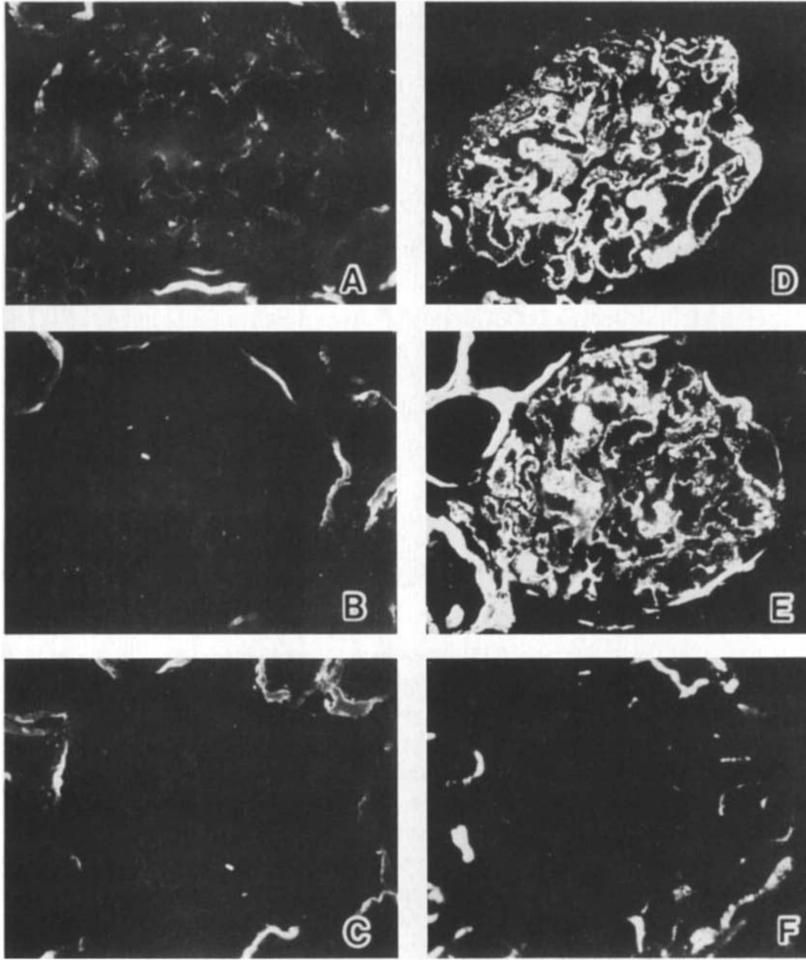

Fig. 9. *Immunofluorescence pictures showing deposition of rat C9 and C3 in the glomerulus 15 minutes after injection of anti-LCH (Group I and II) or non-immune serum (Group III).* (**A**) Group I, rat C9; (**B**) Group II, rat C9; (**C**) Group III, rat C9; (**D**) Group I, rat C3; (**E**) Group II, rat C3; (**F**) Group III, rat C3. (A through F: ×400) Note that C9 is strongly positive in part of tubular basement membranes in all rats, while it is weakly positive in glomeruli only in Group I rats. (A) The fluorescence intensity is grade 1.

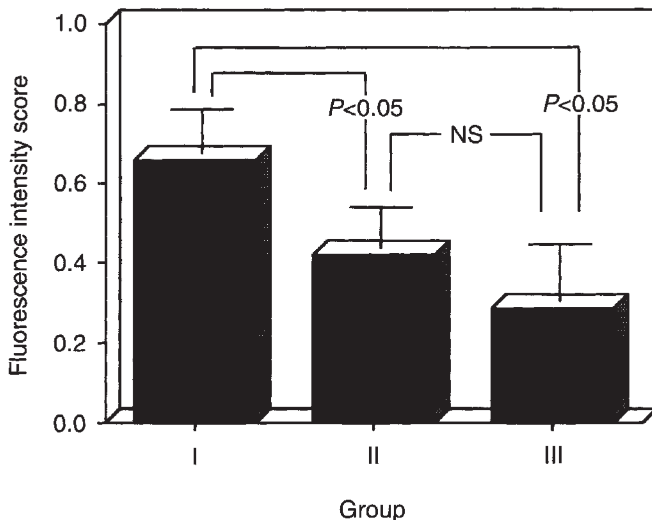

Fig. 10. *Deposition of rat C9 assessed by immunofluorescence staining intensity 15 minutes after injection.*

significant difference with (Group I) or without (Group II) presence of 6D1. However, at four days after induction of glomerular injury there were significant differences in these two groups. Fibrin deposition was prolonged and glomerular ICAM-1 expression was enhanced in Group I rats. The mechanisms of this phenomenon might be explained by non-lethal effects of MAC formed on nucleated cells. Neutralization of CD59 by 6D1 in the glomerular capillaries might have stimulated the glomerular endothelial procoagulant activity through the enhancement of MAC formation on the endothelial cell surface. In favor of this view Hamilton et al, using human umbilical vein endothelial cells in culture, reported that anti-human CD59 antibody augmented the C5b-9-induced cellular responses, including stimulated secretion of von Willebrand factor and expression of the catalytic surface for the prothrombinase enzyme complex [28]. Although there has been no report showing evidence that MAC directly enhanced ICAM-1 expression, expression of glomerular ICAM-1 in Group I rats might be enhanced by the direct mediation of MAC formed on the glomerular endothelial cells. There is a possibility that prolonged glomerular fibrin deposition and enhanced glomerular ICAM-1 expression could be mediated through the interaction between the glomerular endothelial cells and the glomerular infiltrating cells. It is well known that a series of cytokines induce procoagulant activity and ICAM-1 expression of endothelial cells [29]. In the present study, however, fibrin deposition and ICAM-1 expression were significantly higher in Group I rats at four days but not at three hours after initiation of

glomerular injury. In the meanwhile, the degree of leukocyte infiltration in the glomeruli was comparable between Group I and Group II rats at three hours and four days after initiation of glomerulonephritis. Thus, the only difference between Group I and II in the early course of disease exists in the degree of C9 deposition but not in the degree of leukocyte infiltration. Therefore, it was most likely that enhanced glomerular fibrin deposition and ICAM-1 expression was induced by the non-lethal effects of MAC on the glomerular endothelial cells.

There is an interesting report by Okada and coworkers that HRF20 has homology to T cell activating protein and a monoclonal antibody to human HRF20, 1F5, stimulated proliferation of human peripheral T cells [30]. The work of Okada suggests a new role of CD59 other than inhibiting formation of MAC on the cell membranes. It is, therefore, speculated that 6D1 directly stimulated or altered the function of glomerular cells. This effect of 6D1 might remain at the subclinical level in the glomeruli where there is no complement activation. It becomes evident in glomeruli in which activation of complement system has taken place. In the present work, the numbers of total glomerular cells and PCNA-positive cells in Group I rats were larger than those in Group II, while leukocyte infiltration was comparable in these two groups at Day 4. These observations indicate that there was enhanced proliferation of resident glomerular cells in Group I rats. It might be considered that proliferation of the glomerular cells is due either to the effects of enhanced formation of MAC, or to the combined effects of 6D1 and LCH-anti-LCH interaction on the glomerular cells as described above.

In the present work, we have demonstrated *in vivo* that inhibition of glomerular CD59 enhanced complement-mediated glomerular injuries. This is the first direct demonstration of the role of CD59 in complement-mediated tissue injuries *in vivo*. We have previously demonstrated that expression of CD59 in glomerular endothelial cells was increased in the patients with active lupus nephritis but not in those with IgA nephritis or membranous nephropathy [31]. Thus, glomerular endothelial cells may respond to complement activation on the subendothelial deposits by increasing the expression of CD59, presumably to protect endothelial cells from autologous complement attack. Taken together, it is concluded that glomerular CD59, and specifically CD59 on the glomerular endothelial cells, play important roles in glomerular injury induced by autologous complement activation *in vivo*. For a better understanding of the nature of complement-mediated glomerular injury, it is necessary to further study the mechanisms by which glomerular expression of CD59 is regulated.

Acknowledgments

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