

In Vivo Effects of Monoclonal Antibodies that Functionally Inhibit Complement Regulatory Proteins in Rats

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Summary

The present work was designed to evaluate the effects of functional suppression of complement regulatory proteins in vivo. Male Wistar rats were anesthetized with Nembutal and were intravenously injected with 1 mg/kg of F(ab')₂ or Fab fraction of either monoclonal antibody 5I2, which inhibits the function of rat counterpart of mouse Crry/p65, or monoclonal antibody 6D1, which inhibits the rat counterpart of CD59. Mean arterial pressure was continuously measured for 30 min. When 5I2 was injected, there was a biphasic change of mean arterial pressure, namely, the rapid increase immediately after the injection (~2 min, phase 1) and the subsequent fall and slow recovery (~4–30 min, phase 2). These effects were completely abrogated by pretreatment of rats with cobra venom factor. Pretreatment with carboxypeptidase inhibitor, which inhibits inactivation of anaphylatoxins C3a and C5a, induced enhanced reduction of blood pressure. Circulating leukocytes and platelets were rapidly decreased 5 min after antibody injection and became normal by 2 h. Hematocrit and erythrocyte count were continuously increased up to 2 h after injection, suggesting that there was hemoconcentration due to increased vascular permeability. Immunofluorescence study revealed binding of antibody fragments and rat C3 along the capillaries of lung, heart, and liver 5 min after injection. In contrast to 5I2, F(ab')₂ fraction of 6D1, though localized to the same areas and in similar amounts, had no significant effect on the parameters measured. These data suggest that the rat counterpart of mouse Crry/p65 plays a vital role in vivo by preventing the activation of autologous complement on vascular endothelium.

The complement system plays important roles in the elimination of foreign organisms. Complement proteins, when activated, become attached not only to the foreign bodies but also nearby host cells. Usually the host cells are protected from the complement-mediated injury by a number of complement regulatory proteins. C3 and C4 play central roles in the complement cascade and at least six regulatory proteins have been reported in human which bind C3b/C4b and regulate the complement activation (1). Among these regulatory proteins, decay accelerating factor (DAF)¹ (2, 3) and

membrane cofactor protein (MCP) (4–6) are cell membrane-associated proteins which are widely distributed not only on blood-borne cells but also on nonhematological cells such as endothelial cells and epithelial cells of various organs (7). Although the fact that deficiency of DAF and 20-kD homologous restriction factor (HRF20, CD59) in the erythrocyte membrane results in the development of paroxysmal nocturnal hemoglobinuria (8–10) suggests the protective role of these proteins against complement-mediated erythrocyte lysis, the role of cell membrane-associated complement regulatory proteins in other diseases is largely unknown. One of the main reasons for this is that functional analogues of DAF, MCP, and HRF20 have not been well characterized in experimental animals until recently, and, therefore, it was impossible to study the function of these regulatory proteins in various disease models.

In mice, a DAF-like molecule (11) and another complement regulatory protein, Crry/p65 (12, 13), have been re-

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¹ Abbreviations used in this paper: CPI, carboxypeptidase inhibitor; CVF, cobra venom factor; DAF, decay accelerating factor; HRF20, 20-kD homologous restriction factor; MCP, membrane cofactor protein.

cently identified as functional counterparts of human MCP and/or DAF. Until now no other molecule has been found that has functional and distributional similarities to human MCP and/or DAF. Crry/p65 transfected in human K562 cells inhibits neuraminidase treatment-induced deposition of mouse C3 on cells, and soluble Crry/p65 inhibits deposition of mouse C3 on the zymozan particles (14). Thus, Crry/p65 is thought to be a major cell membrane-associated complement regulatory protein of C3 convertase in mice. A rat mAb to Crry/p65 has only recently been developed (15) and in vivo roles of this molecule are yet to be clarified.

In rats, Takizawa (16) recently developed a mouse mAb (designated as 5I2, IgG1 subclass) against rat erythrocyte membrane which inhibited function of a rat cell membrane-associated complement regulatory protein. Fab fragment of 5I2 caused deposition of C3b on rat myeloma cells after treatment with rat serum. The antigen recognized by this mAb (5I2Ag) was considered a rat counterpart of mouse Crry/p65 because of the similarities in function, distribution, and amino acid sequences (16, 17). This has been verified by the high homology in base sequence of recently cloned 5I2Ag-cDNA with that of mouse Crry/p65 -cDNA (18). 5I2Ag is present in widespread organs and 5I2, when administered intravenously even in small amounts, clearly bound to these organs. Another mAb, 6D1, was recently developed against rat CD59 by Hughes et al. (19). The rationale for the present study was to examine the in vivo effects of these reagents on the clinical and pathological parameters. Fab or F(ab')₂ fragments of 5I2 and 6D1 bound to the capillary walls in various organs, but whereas 5I2 caused profound changes in blood pressure and other vascular and hematologic parameters in complement-replete animals, 6D1 had little effect. These studies provide novel information on the in vivo roles of these two complement regulators.

Materials and Methods

Animals. Male Wistar rats weighing ~275 g and male BALB/c mice weighing ~20 g were purchased from Chubu Kagaku Shizai Company (Nagoya, Japan). They were allowed free access to food and water.

mAbs. Production and characterization of 5I2 and 6D1 were described previously (16, 19). These mAbs belonged to the same subclass (IgG1) and showed similar binding distribution in rat organs (17). IgG1 fractions of these mAbs were prepared using protein A affinity column chromatography (Pharmacia Japan, Tokyo, Japan). The fractions were pooled, concentrated, and dialyzed against PBS. For the preparation of F(ab')₂ fragments, 15 mg/ml of IgG1 fraction of each mAb was prepared in 1 M citrate buffer, pH 4.5, and 0.5 mg/ml of pepsin was added. The mixture was incubated for 8 h at 30°C, and then the sample was diluted 1:4 in glycine buffer (1.45 M, pH 8.9). Undigested IgG1 and free Fc fragments were removed by protein A affinity column chromatography. The pass through fraction was concentrated and dialyzed against PBS and it was applied to a Sephadex G75 column. The first peak containing the F(ab')₂ fragments was collected, concentrated, and dialyzed against PBS. Fab fragments of 5I2 were prepared by incubating IgG1 fraction of 5I2 with immobilized papain for 5 h at 37°C followed by the absorption of Fc fragments and undigested IgG1 by a protein A affinity column chromatography. Size of IgG1, F(ab')₂,

and Fab fragments was analyzed by a SDS-PAGE. F(ab')₂ fragment was not contaminated by IgG1, and Fab fragment did not contain IgG1 or F(ab')₂ fragment. When reactivity of these fragments with rabbit antibodies against mouse IgG-Fc by an Ouchterlony method, only native IgG1 showed positive result. Binding capacity of each fragment was tested by an indirect immunofluorescence test on the normal rat lung as described below, and it was found that the reactivity was well preserved after enzyme digestion.

Measurement of Bacterial Endotoxin in the Antibody Fragments. Contamination of bacterial endotoxin in the antibody fragments used in the present study was tested by an endotoxin detection reagent (Pregel-M; Sekagaku Kougyou Co. Ltd., Tokyo, Japan) according to the manufacturer's direction. Bacterial LPS was used as a control. Sensitivity of Pregel-M was ~50 pg/ml (or 0.25 EU/ml).

Measurement of Serum Complement Activity. 500 µl of freshly obtained whole blood was mixed with 100 µl of 0.1 M ethylenediamine tetraacetate (EDTA-2Na). EDTA-plasma was obtained by centrifugation of blood and was stored at -70°C until use. For the determination of serum complement activity, CH50 was measured according to a manufacturer's direction using sensitized SRBC (Ishizu Pharmaceutical Co., Osaka, Japan).

Cobra Venom Factor. Cobra venom factor (CVF) was routinely purified on DEAE sephadex from lyophilized cobra venom (Naja Naja; Sigma Chemical Co., St. Louis, MO) and was further purified by electrofocusing to remove phospholipase A (~100-150 kD) (20). When 25 U of purified CVF was intravenously injected into a rat, the complement activity measured by CH50 became undetectable 24 h after the injection.

Measurement of Blood Pressure and Pulse Rate. Animals were anesthetized by intraperitoneal injection with 50 mg/kg of pentobarbital sodium (Abbott Laboratories, North Chicago, IL). They were strapped in a supine position during surgery and experiments. A polystyrene catheter (SP25; Natsume Company, Tokyo, Japan) was placed into the left femoral artery approximately to the level of the aortic bifurcation, and was used for the measurement of arterial pressure and heart rate, which were monitored with transducer for 30 min. Data were shown by percent change of mean arterial pressure and heart rate from the pretreatment value. Percent change of mean arterial pressure at the selected time point was calculated by the formula described below (see *Hematological Study*).

Experimental Protocol. Rats were stratified into seven groups. After stabilizing for a few minutes, rats were treated according to the following protocols. Group I rats were injected with 1.0 mg/kg of F(ab')₂ fraction of 5I2 in 0.6 ml of saline. Group II rats were intravenously injected with 25 U of purified CVF 24 h before the administration of F(ab')₂ fraction. Serum complement activity determined by CH50 was undetectable just before the antibody administration as described in group I rats. Group III rats were intravenously injected with 1.0 mg/kg of Fab fraction of 5I2 instead of F(ab')₂ fraction in order to examine the effects of valence of the antibody fraction. Group IV rats were intravenously injected with 20 mg of carboxypeptidase inhibitor (CPI, DL-2-mercaptomethyl-3-guanidinoethyl thiopropanoic acid; Calbiochem Corp., La Jolla, CA) 2 h before administration of F(ab')₂ fraction of 5I2. CPI was reported to inhibit the inactivation of generated C5a and C3a by competitively inhibiting the carboxypeptidase, an inactivator of C3a and C5a that removes the terminal arginine residues of these vasoactive molecules (21). Group V rats were injected with 0.6 ml of saline from the tail vein for 5 s. These rats were used as controls. Group VI rats were intravenously injected with 25 U of purified CVF in order to see the effects on blood pressure of rapid activa-

tion of complements in fluid phase. In rats of group VII, 1.0 mg/kg of F(ab')₂ fraction of 6D1 was injected instead of 5I2. Mean arterial pressure and heart rate were monitored in all rats for 30 min after administration of antibody fractions (groups I through V) or with CVF (group VI). In groups I, II, VI, and VII, separate sets of rats were killed 5 and 30 min after the antibody injection for the immunofluorescence studies. These protocols are summarized in Table 1.

Immunohistology. Lung, heart, and liver of all groups of rats except for group IV were studied by direct immunofluorescence test according to the previously described procedure (22, 23) 5 and 30 min after injection. Briefly, tissues were snap-frozen in liquid nitrogen. 2- μ m-thick sections were cut by a cryostat and fixed in acetone at room temperature for 10 min. They were then incubated with fluorescein-conjugated goat antibodies against mouse IgG and rat C3 (Cappel Laboratories, West Chester, PA). After washing in PBS, sections were covered with 90% glycerol containing *p*-phenylenediamine (24) and were observed by an epifluorescence microscope (Olympus Optical Co., Tokyo, Japan).

In group I, V, and VII rats, the number of leukocytes present in lung, heart, and liver was quantitated by direct immunofluorescence method. Tissues were obtained at 5 min, 30 min, 2 h, and 24 h after antibody injection and they were processed for direct immunofluorescence study as described above. Frozen sections were stained by fluorescein-conjugated mAb against rat leukocyte common antigen (OX-1; Dainippon Pharmaceutical Company, Osaka, Japan). In the case of lung, heart, and liver, the numbers of leukocyte common antigen-positive cells present in high power field ($\times 400$) were counted under the epifluorescence microscopy. An average number of positive cells from 10 different fields was calculated in each rat.

Hematological Study. In rats of groups I, II, V, and VII, blood samples were obtained from the tail vein before, 5 min, 30 min, 2 h, and 24 h after injection. Leukocytes, platelets, erythrocytes, and hematocrit were calculated by automatic blood cell counter. Data at each time point were expressed by percent change according to the following formula: $100 \times [(the\ value\ at\ the\ selected\ point - preinjection\ value)/preinjection\ value]$.

Statistical Analysis. Statistical analysis was performed in non-parametric manners by the Mann-Whitney U-test for unpaired data,

and by the Wilcoxon signed rank test for paired data. Significant difference between two groups was determined when the *p* value was <0.05 (5%).

Results

Endotoxin in the Antibody Fragments. All the stock solutions of antibody fragments of 5I2 and 6D1 (5 mg/ml each in endotoxin-free saline) were negative for endotoxin test by Pregel-M reagent.

Effects of 5I2 on Mean Arterial Pressure and Pulse Rate in Rats. When rats were injected with F(ab')₂ fraction of 5I2 (group I), there was an immediate increase in mean arterial pressure by $\sim 30\%$ after the injection (~ 2 min, phase 1). The decrease of mean arterial pressure followed at 4 min after the injection and thereafter. Blood pressure recovered slowly by 30 min (phase 2). Administration of Fab (group III) showed quite similar results to those as seen in group I rats. These data are depicted in Fig. 1 A. The change in pulse rate in these rats was within 5% of the preinjection value.

Effects of Pretreatment with CVF and CPI. When complement was depleted by CVF before injection of 5I2 (group II), there was no decrease in mean arterial pressure. Rather it was slightly increased 10 and 15 min after the antibody injection but there was no significant difference between group II and V rats in general. When rats were pretreated with CPI before injection of 5I2 (group IV), rats showed severe respiratory distress 1–2 min after antibody administration and 4 out of 8 rats died within several minutes. In the surviving rats, blood pressure was decreased by $>50\%$ 15 min after the administration of antibody fragment and the recovery of mean arterial pressure during phase 2 was seldom seen (Fig. 1 B). The degree of change in pulse rate was within 10% of the preinjection value in group II rats. Similarly, the change of pulse rate in group IV rats was not significantly different from that of group V rats.

Effects of Rapid Activation of Complement by CVF on Arterial Pressure and Pulse Rate. When rats were intravenously

Table 1. Protocols for Immunofluorescence Studies

Group	Number of rats*	Pretreatment with	Antibody fraction (antibody injected)	Hematological study†	IF study‡
I	5	None	F(ab') ₂ (5I2)	5	4
II	5	CVF§	F(ab') ₂ (5I2)	5	5
III	6	None	Fab (5I2)	Not tested	4
IV	8 (4)¶	CPI¶	F(ab') ₂ (5I2)	Not tested	Not tested
V	7	None	Saline	5	5
VI	6	None	CVF§	Not tested	5
VII	5	None	F(ab') ₂ (6D1)	5	5

Protocol and number of rats for each experimental group are shown.

* The number of rats used for blood pressure measurement is indicated.

† The number of rats used for hematological and immunofluorescence studies is indicated.

§ Purified CVF was injected 24 h before antibody administration.

¶ The number in parentheses indicates number of rats which were dead within several minutes after antibody administration.

¶ CPI was administered 2 h before antibody administration. See Materials and Methods and Results for details.

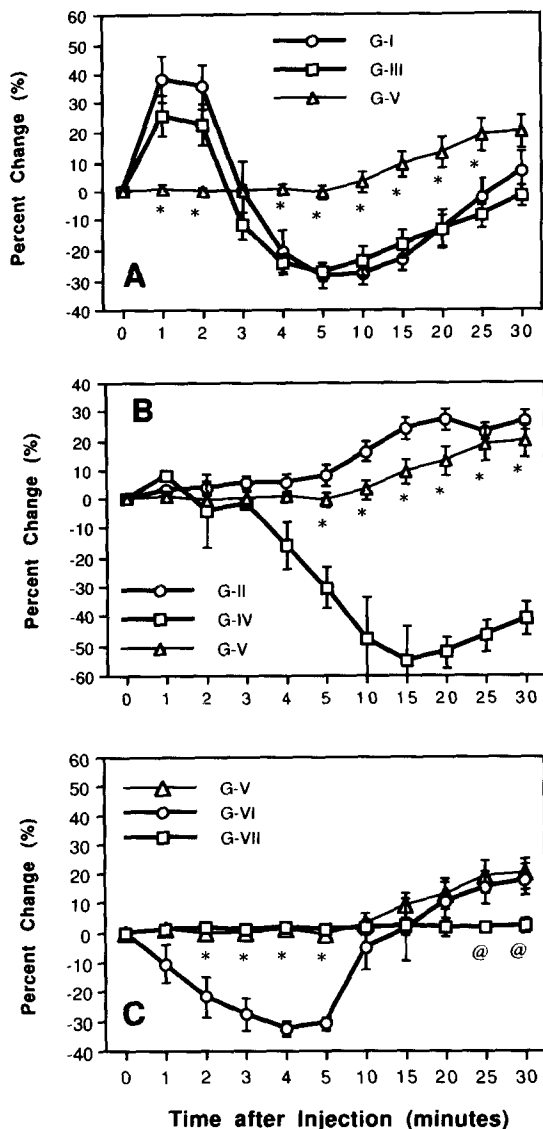


Figure 1. Percent change of mean arterial pressure. In A and C, asterisks show the significant change at the selected time point between control (group V) and each experimental group. (A) (*) Significant difference between experimental groups and group V. (B) (*) Significant difference between groups IV and V. There was no significant difference between groups V and II. (C) (*) Significant difference between groups V and VI and (@) between groups V and VII.

injected with 25 U of purified C5, serum complement activity determined by CH50 became undetectable within 30 min and this condition lasted at least for 48 h. Mean arterial pressure measured during this period (group VI) showed a decrease in the initial phase (2–5 min after injection), and quick recovery by 10 min. These data are shown in Fig. 1 C. Although pulse rate was significantly increased at 25 min after injection of C5 when compared with that of group V rats, overall change of pulse rate in group VI rats was not different from that in group V.

Effects of 6D1 on Blood Pressure and Pulse Rate. Administration of F(ab')₂ fragment of 6D1 did not affect blood pressure (Fig. 1 C) or pulse rate.

Organ Binding of Antibody and Complement. When F(ab')₂ or Fab fraction of 5I2 (1 mg/kg) was injected into rats via the tail vein (group I and III), binding of antibody to the lung, heart, and liver was observed 5 min after injection. In the lung and heart, antibody was localized along the capillaries (Fig. 2, A and B). In the liver, the binding of antibody was observed along the sinusoidal space (Fig. 2 C). Binding of rat C3 was also clearly observed in a similar area of these organs (Fig. 3, A–C). The binding of antibody and C3 became very weak in lung and liver 30 min after antibody administration, whereas it was not significantly changed in heart. In complement-depleted rats (group II), there was binding of antibody as seen in rats of group I and III but deposition of C3 was not observed at all. In rats of group VI, there was faint binding of C3 in the capillaries of heart tissue but there was no significant binding of C3 in lung and liver 5 and 30 min after C5 administration. Rats injected with 6D1 showed binding of antibody to lung, heart, and liver (Fig. 2, D–F) like in group I and III rats, but there was no C3 deposition in these organs (Fig. 3, D–F). These results are summarized in Table 2.

Hematological Study. Peripheral leukocytes and platelets were decreased by 60% compared with the preinjection value in group I rats 5 min after antibody injection. Leukocyte count remained depressed 30 min after injection and returned to normal by 120 min after injection (Fig. 4 A). Platelet count showed a similar change as seen for leukocytes (Fig. 4 B). Concentration of erythrocytes and hematocrit level increased continuously, reaching 30% above preinjection, 120 min after the administration of F(ab')₂ fraction of 5I2 (Fig. 4, C and D). All these hematocrit parameters returned to the preinjection values by 24 h after antibody injection in group I rats. Pretreatment of rats with C5 totally abrogated the effects 5I2 on hematological parameters (group II). In rats injected with F(ab')₂ of 6D1 (group VII), there was a small but significant increase of leukocytes at 5 min and 24 h after injection (Fig. 4 A). There was also significant decrease of red blood cells and hematocrit at 24 h after injection (Fig. 4, C and D). There was no significant change of platelet count throughout the experiment.

Leukocyte Infiltration in Organs. After injection with F(ab')₂ fraction of 5I2 (group I), there was rapid accumulation of leukocytes in the organs studied. The number of leukocytes returned to the normal range 24 h after injection (Fig. 5, A–C). In contrast, rats injected with 6D1 (group VII) showed a mild increase of leukocyte accumulation only in the lung at 24 h after injection (Fig. 5 A). Rats injected with saline (group V) did not show increased leukocyte infiltration in these organs.

Discussion

MCP and DAF have been shown to exist on the surface of vascular endothelium and it was suggested that the pres-

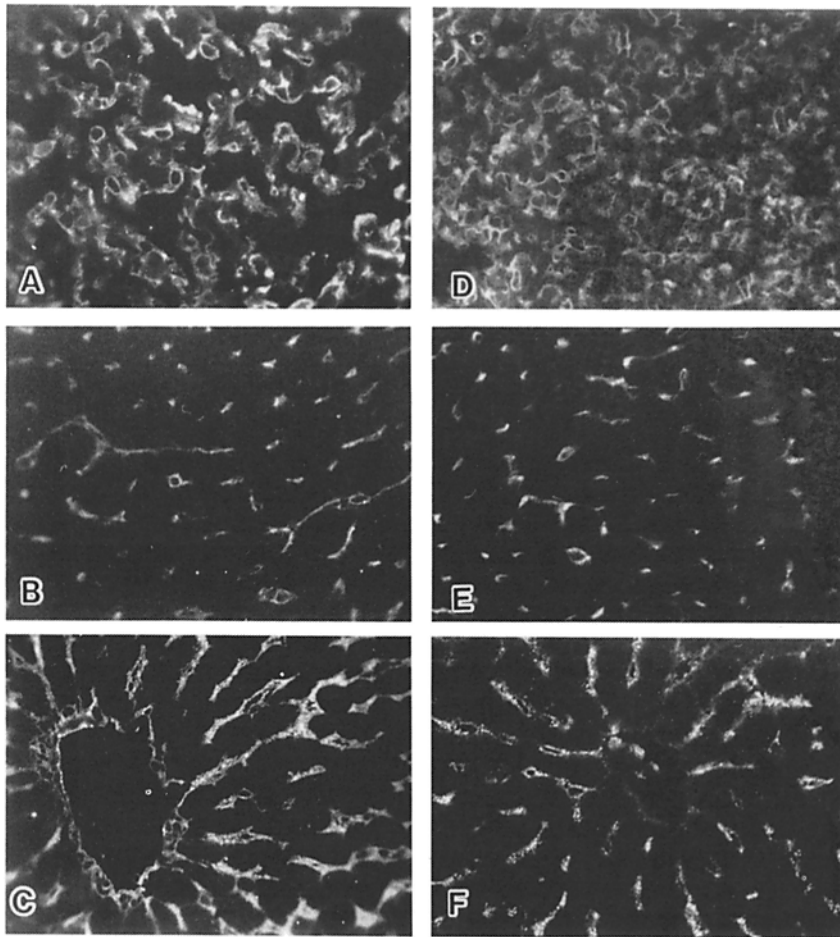


Figure 2. Immunofluorescence pictures depicting the binding of antibody fraction in various organs 5 min after administration of F(ab')₂ fraction of 5I2 (group I) or 6D1 (group VII). (A and D) Lung; (B and E) heart; (C and F) liver; (A-C) group I; (D-F) group VII. ×400.

ence of MCP and/or DAF protected these cells from the serum complement proteins (7). Data obtained in the present work revealed that 5I2 antigen (a rat counterpart of mouse Crry/p65) (16, 18) on the endothelial membrane played an important role at least in maintaining the blood pressure and vascular permeability. When this molecule was functionally suppressed by a mAb, 5I2, complement activation occurred on the surface of vascular endothelium. Since F(ab')₂ and Fab fragments of 5I2 were used in this study, the events observed after antibody injection took place by the functional suppression of 5I2Ag, but not by direct activation of complement through antigen-antibody interaction. It should also be noted that bacterial endotoxin was not present in the 5I2 antibody fragments used in this study. The fact that 6D1, a mAb which inhibits function of rat CD59 and has similar distribution in vivo, had little influence on any parameter studied in this experiment revealed the importance in vivo of a cell membrane molecule (rat Crry/p65) regulating complement activation at C3 level. Vascular endothelial surface, which does not activate complements under normal conditions, may become a slow activator of alternate pathway of complement when complement regulatory protein is inhibited by 5I2. After neutralization of rat CD59, the endothelium remains nonac-

tivating and hence no significant effect is seen in vivo despite neutralization of this important inhibitor. The mechanisms of rapid change in mean arterial pressure and increased vascular permeability after the antibody administration can be explained as follows. First, generation of vasoactive fragments of complement cascade such as C5a and C3a lead to the change in blood pressure (25). This hypothesis is supported by the following findings. Pretreatment of CVF, which completely depleted the serum complement activity, totally abrogated the effects of 5I2. Furthermore, CPI, which inhibits the inactivation of generated C5a and C3a (21), enhanced the shock-promoting effect of 5I2 and markedly inhibited the recovery of blood pressure in phase 2.

Second, activation of complement system on the surface of vascular endothelium alters the endothelial function. Continuous elevation of hematocrit and RBC concentration in group I rats indicated the increase of vascular permeability. This notion was deduced from the experiment studying the direct effects of CVF, which has a complement-activating effect similar to the C3 convertase and causes rapid consumption of complement components in the fluid phase but should not cause C3 deposition on the cell membranes. The fact that C3 deposition was only faintly observed in the capillaries of

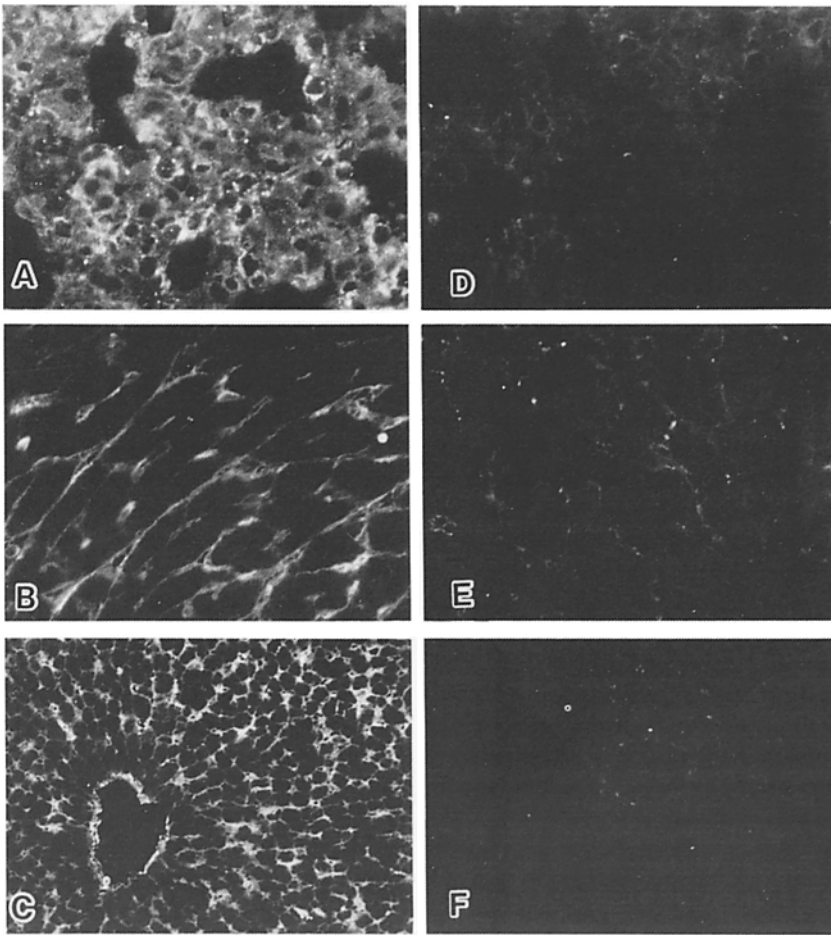


Figure 3. Immunofluorescence pictures showing deposition of rat C3 in various organs of the same animals of Fig. 2. (A and D) Lung; (B and E) heart; (C and F) liver; (A-C) group I; (D-F) group VII. $\times 400$.

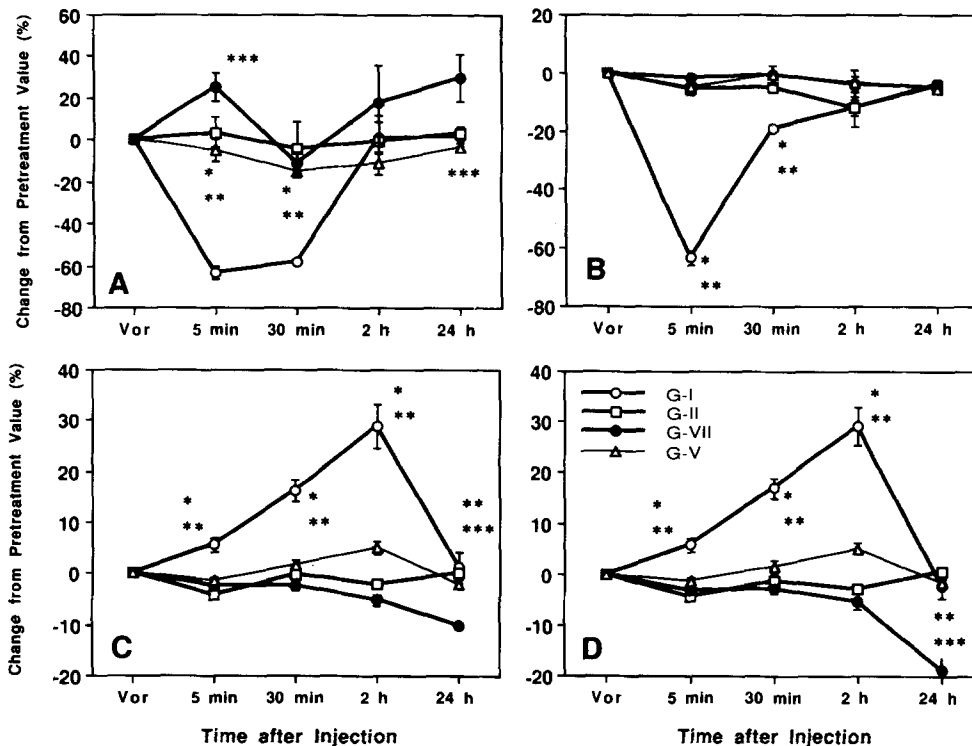


Figure 4. Sequential changes in hematological parameters after the administration of F(ab')₂ fraction of 512 (group I) in comparison with those of group V and group VII. Data are shown by percent change from the pretreatment value (see Materials and Methods for the formula). (A) Peripheral leukocyte count. (B) Peripheral platelet count. (C) Peripheral red blood cell count. (D) Hematocrit. (*) Significant difference between groups I and V; (**) significant difference between groups I and VII; (***) significant difference between groups V and VII. There was no significant difference between groups II and V.

Table 2. Deposition of Antibody Fragments and Rat C3 in Organs 5 min after Injection

Group	Antibody fragments	Rat C3
I	+	+
II	+	-
III	+	+
V	-	-
VI	-	-
		(\pm in capillaries of heart)
VII	+	-

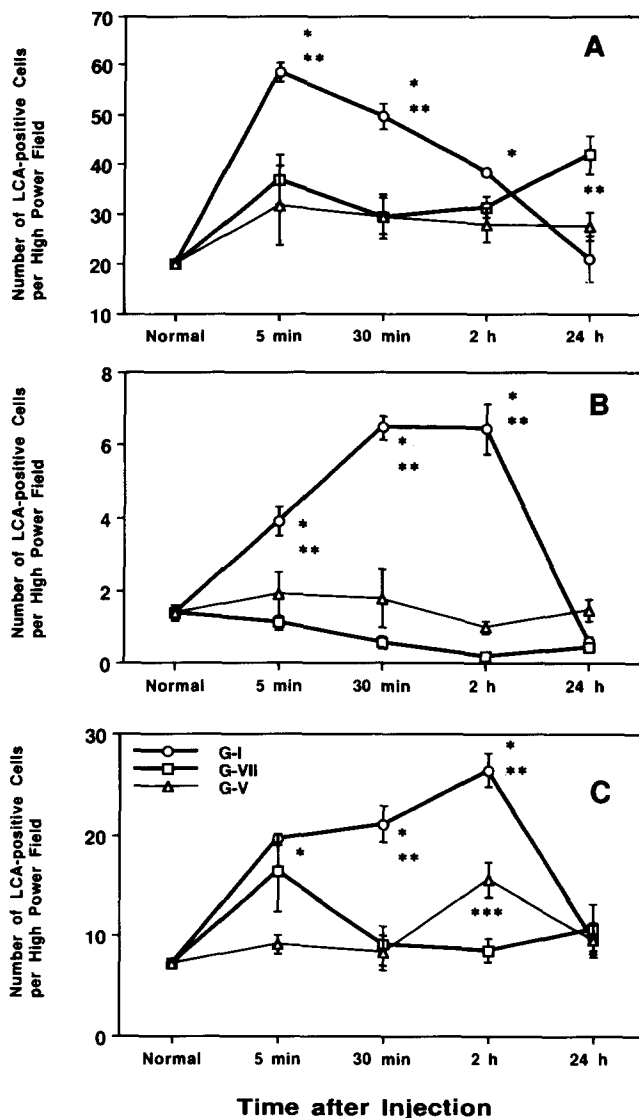


Figure 5. Leukocyte Infiltration in organs. Numbers of leukocytes were counted on the frozen sections at high magnification ($\times 400$) by direct immunofluorescence staining method. (A) Lung; (B) heart; (C) liver. (*) Significant difference between groups I and V; (**) significant difference between groups I and VII; (***) significant difference between groups V and VII.

heart and not at all in the other organs in group VI rats supports this notion. Thus, it could be easily imagined that there was rapid generation of C5a and C3a in the circulation but these could also be quickly inactivated by the carboxypeptidase in the plasma and only transient fall of blood pressure resulted. In contrast, prolonged effects of 5I2 suggested that impairment of vascular endothelial cell function due to the activation of complement on the endothelial cell surface was rather slow but continuous since deposition of C3 was clearly observed in the vasculature of all organs studied.

Third, the rapid decrease and recovery of leukocytes and platelets in the circulating blood suggested that they were transiently captured or stuck to the vascular endothelium of some organs. C3b deposited on the vascular endothelial surface induced by 5I2 injection might be a ligand for C3b receptor present on the surface of leukocytes. On the other hand, most blood cells express 5I2 Ag on their surface (17). Neutralization of 5I2Ag on leukocytes might also render them complement activating, resulting in deposition of complement fragments, release of C3a and C5a, and consequent release of other vasoactive substances from leukocytes attached in the vasculature. The outcome would be a cell-dependent increase in vascular permeability (26). This hypothesis remains to be tested.

Recently it has been shown that the complement system was activated during the endotoxin shock (27-29) and antibodies against C5a abolished the shock-promoting effects of endotoxins (25). In turn, administration of C5a in rabbits has been reported to induce endotoxin shocklike symptoms (30, 31). It is now widely accepted that complement system is deeply involved in the pathogenesis of endotoxin shock (32). Although the relationship between the shock-promoting effects of endotoxins and the cell membrane-associated regulators of complement activation has never been studied, the present work clearly demonstrated that the functional suppression of complement regulatory proteins on the cell membranes leads to the profound hemodynamic changes. Although there has been no report describing the effects of cytokines on the expression of membrane inhibitors of complements, it is interesting from the present results to study the relationship between shock-promoting effects of endotoxins and the expression of these molecules on the endothelial cell membrane.

In humans, vascular endothelial cells express both DAF and MCP presumably to protect them from indiscriminate attack by autologous complement (3, 33). The reason why suppression of only one molecule (a rat analogue of mouse Crry/p65) induced dramatic changes in rats might be explained by a recent study showing that mouse Crry/p65 has both MCP and DAF activities. Takizawa et al. (16) demonstrated that 5I2Ag is a rat counterpart of Crry/p65 from the structural and functional similarities. Thus in rats, it is likely that a single molecule, the 5I2Ag, subserves the functions of both DAF and MCP in humans. Suppression of this molecule thus dramatically reduces control at the C3 convertase stage, inducing severe disease through complement activation on the vascular endothelium.

Finally, it was shown that suppression of rat CD59 by a

mAb 6D1 induced decrease of hematocrit and erythrocyte concentration in vivo. Since direct evidences showing intravascular hemolysis such as increase of plasma LDH or free hemoglobin level (detected by absorbance at OD₄₁₄) were not demonstrable in the present study, the precise mechanism is currently unknown. Further studies are needed to solve the problem.

In conclusion, mAb 5I2, induced the biphasic change in mean arterial pressure. The effects of 5I2 on blood pressure were complement-dependent and due to the functional suppression of a complement regulatory protein (5I2Ag, a rat counter part of mouse Crry/p65) present on vascular endothelial cells and the subsequent activation of complement

on the endothelial surface. In contrast, suppression of CD59, an inhibitor of the terminal complement pathway, did not render the cells susceptible to complement attack and had no effect on blood pressure. Nevertheless, it is likely that simultaneous suppression of both regulators will enhance complement-mediated damage in vivo. These studies are currently in progress. Since there has been no report concerning the effects of functional suppression of cell membrane-associated complement regulatory proteins in vivo, we believe that the findings presented here give new insights into the roles of membrane complement regulatory molecules in suppressing complement activation in vivo and in maintaining homeostasis.

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