

## COMPLEMENT SYSTEM IS INVOLVED IN ANAPHYLACTOID REACTION INDUCED BY LIPOPOLYSACCHARIDES IN MURAMYLDIPEPTIDE-TREATED MICE

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**ABSTRACT**—We previously reported that an intravenous injection of specified bacterial lipopolysaccharides (LPS) induced anaphylactoid shock in muramyldipeptide (MDP)-primed mice of various strains, including LPS-resistant C3H/HeJ, accompanied with occasional mortality of mice within 1 h. Prior to shock, rapid accumulation of blood platelets into the lungs and liver followed by degradation of the platelets and tissue destruction were observed. In this report we present the following evidence suggesting that complement activation by LPS is responsible for the anaphylactoid reaction. In C5-deficient DBA/2 mice, the platelet degradation and anaphylactoid reactions did not occur following injection of *Prevotella intermedia* LPS, although transient platelet accumulation into the lungs and liver was observed. Anti-complement agents K-76 COOH (C5 inhibitor) and cobra venom factor (C5 consumer) protected MDP-primed C3H/HeJ mice from mortality in the anaphylactoid reaction induced by *P. intermedia* and *Salmonella typhimurium* LPS, respectively. K-76 COOH also inhibited platelet degradation, but not accumulation, induced by *P. intermedia* LPS in C3H/HeN mice. LPS specimens carrying mannose-homopolymer (MHP) prepared from wild-type *Klebsiella* O3 and *Escherichia coli* O8 and O9 and recombinant *E. coli* O8 and O9 strains, which have been reported to markedly activate the human complement system probably through the lectin pathway, induced anaphylactoid reactions in MDP-primed C3H/HeJ mice. In contrast, LPS from R-mutant of *Klebsiella* O3 and the parental strain of the recombinant *E. coli* strains, which lacked MHP, did not induce anaphylactoid reaction. Based on these findings together with those of our previous studies, we postulated the following mechanism for the anaphylactoid reaction: strong complement activation by specified LPS preparations induced degradation of platelets which have accumulated in the lungs and liver, resulting in acute inflammation accompanied with severe tissue destruction, especially in the lungs, which in turn leads to anaphylactoid reaction. However, the mechanism of platelet accumulation induced by LPS is not yet clear.

**KEYWORDS**—Anaphylactoid shock, C5, DBA/2 mice, C3H/HeJ mice, *Klebsiella*, lectin pathway, mannose-homopolymer, platelets, serotonin

### INTRODUCTION

Some preparations of lipopolysaccharide (LPS) induced acute shock in muramyldipeptide (MDP)-treated mice (1). The reaction occurred 5 to 15 min after intravenous injection of LPS, and the mice exhibited loss of consciousness, severe convulsions, and rolling, and some mice died within 1 h. We designated the reaction as the anaphylactoid reaction. This reaction was distinguishable from usual endotoxin shock with regard to the following points: i) Endotoxin-non-responsive C3H/HeJ mice were sensitive to the reaction, while some endotoxin-responsive strains such as AKR and DBA/2 were resistant (2); ii) low-endotoxic LPS prepared from oral

black-pigmented bacteria (BPB) such as *Porphyromonas gingivalis* and *Prevotella intermedia* induced the reaction, while some LPS and lipid A with high endotoxicity did not (1, 2); and iii) usual endotoxic mediators such as tumor necrosis factor (TNF)- $\alpha$  seem not to be involved in the reaction because TNF- $\alpha$  was not detected in the sera in C3H/HeJ mice injected with LPS and the reaction occurred prior to TNF- $\alpha$  induction in endotoxin-sensitive mice (2). Endo and colleagues (3, 4) found that LPS induced rapid accumulation of platelets in the lungs and liver followed by degradation of the platelets prior to the anaphylactoid reaction without MDP-priming. They also found that MDP-priming enhanced the platelet reactions in responsive strains of mice. We speculated that the platelet and anaphylactoid reactions might be partially responsible for the clinical features of endotoxic shock.

Recently, we noticed that DBA/2 and AKR mice, which are resistant to anaphylactoid reaction as noted above, are deficient in complement C5 (5–7). Therefore, we assumed that some LPS specimens markedly activated the complement system and

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induced the degradation of platelets, resulting in the induction of anaphylactoid reaction accompanied by acute mortality within 1 h. In this study we examined the possible involvement of the complement system in the anaphylactoid and platelet reactions induced by the specified LPS preparations.

## MATERIALS AND METHODS

### Mice

Male and female C3H/HeJ mice (8–15-weeks-old) were obtained originally from Clea Japan (Osaka) and were bred in the animal facility of the Kagoshima University Dental School. Male C3H/HeN and DBA/2 mice (6–7-weeks-old) were supplied by the facility of Tohoku University and SLC Japan (Shizuoka, Japan), respectively. All protocols used in this study were approved by the Animal Research Committee of Kagoshima University Dental School and complied with the Guidelines for Care and Use of Laboratory Animals of Tohoku University. The experiments were conducted in line with the National Institutes of Health guidelines on the Care and Use of Laboratory Animals.

### LPS and other reagents

LPS prepared from *Salmonella typhimurium* and *Escherichia coli* O111:B4 and O55:B5 by the hot phenol-water extraction method were purchased from Difco Laboratories (Detroit, MI) and Sigma Chemical Co. (St. Louis, MO), respectively. LPS from *P. intermedia* ATCC 25611 was prepared by the phenol-chloroform-petroleum ether (PCP) extraction method as described previously (8). LPS extracted from *Klebsiella* O3 strain LEN-1 (smooth form wild type) by hot-phenol water carries mannose homopolymer (MHP) as the O-specific polysaccharide. In contrast, LPS extracted from the rough form mutant strain LEN-113 by PCP extraction is devoid of O-specific polysaccharide. Several LPS specimens carrying MHP were also extracted by hot-phenol water from the wild-type and recombinant strains of *E. coli* O8 and O9. Wild-type O8 and O9 LPS were prepared from strains CO8 and B993, respectively (9). The recombinant strains were prepared in rough-type *E. coli* K-12 JA221 as described previously (10). The structures of the repeating units in the O-specific polysaccharides of the LPS used in this study are shown in Table 1.

*N*-Acetylmuramyl-L-alanyl-D-isoglutamine, muramyl dipeptide (MDP) was provided by Daiichi Pharmaceutical Co. (Tokyo). The anti-complement agent K-76 monocarboxylic acid (6,7-dihydroxy-2,5,5,8a-tetramethyl-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene-1-spiro-2'-(7'-carboxyl)-6'-formyl-4'-hydroxy-2',3'-dihydrobenzofuran, K-76 COOH), which inhibits complement activity, especially at the C5 step (11), was generously supplied by Dr. K. Miyazaki (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). Cobra venom factor (CoVF) purchased from Sigma was kept in our laboratories.

### Assay for anaphylactoid reaction

In most experiments, groups of 3 to 6 mice were injected intravenously with 100  $\mu$ g of MDP dissolved in 0.2 mL of pyrogen-free physiological saline. Four hours later, the mice were challenged by intravenous injection of 200  $\mu$ g of test LPS specimens in 0.2 mL of pyrogen-free physiological saline. The mice were

observed for 1 h, and the incidences of anaphylactoid reaction and death were recorded. In the anaphylactoid reaction, mice exhibited severe convulsions, spreading, and spells of unconsciousness 5 to 20 min after LPS injection, with occasional deaths within 15 to 60 min (usually around 30 min) after LPS injection. In some experiments mice were treated by intraperitoneal injection of K-76 COOH or CoVF simultaneously with or at various intervals prior to LPS injection, and inhibition of anaphylactoid reactions and curing of mice were recorded.

### Assay for platelet reaction

Serotonin (5-hydroxy-tryptamine, 5HT) is specifically present in the granules in platelets, and free 5HT in blood is rapidly cleared from the circulation (12). Therefore, as described in our previous papers (4, 13), translocation of platelets was monitored by measuring 5HT levels in blood and various tissues. C3H/HeN or DBA/2 mice received an intravenous injection of *P. intermedia* LPS. In the inhibitor assay C3H/HeN mice were given an intraperitoneal injection of K-76 COOH (2 mg) 1 h prior to LPS injection. 5HT levels in blood, liver, lung, and spleen from mice were measured at 5 to 60 min after LPS injection as described previously (13). Experimental values for 5HT are given as the mean  $\pm$  SD.

### Assay for anti-complement activity of LPS

To test the anti-human complement activity of LPS, 100  $\mu$ L of LPS (100  $\mu$ g/mL) in 10 mM veronal buffer containing 0.148 M NaCl, 0.1% gelatin, 0.15 mM CaCl<sub>2</sub>, and 0.5 mM MgCl<sub>2</sub> (GVB<sup>2+</sup>, pH 7.4) were incubated with 100  $\mu$ L of human serum at 37°C for 30 min. As a control, human serum was incubated with GVB<sup>2+</sup>. After incubation, complement activities (CH50) were determined by the method of Mayer (14) except that the total volume of reaction mixture was 3 mL. Briefly, human serum treated with LPS or GVB<sup>2+</sup> was diluted to a total volume of 5 mL with GVB<sup>2+</sup>. GVB<sup>2+</sup> was mixed with various amounts of diluted serum (0.5, 0.6, 0.7, 0.8, 1.0, and 1.5 mL) to make a total volume of 2.6 mL, and then 0.4 mL of antibody-sensitized sheep erythrocytes was added. The reaction mixture was incubated at 37°C for 1 h. After centrifugation, the optical density of the supernatant was determined at 541 nm. CH50 was determined from the percentage hemolysis and the anti-complement activity of LPS was calculated as % CH50 consumption = [(CH50<sub>control</sub> - CH50<sub>LPS</sub>) / CH50<sub>control</sub>]  $\times$  100.

### Statistical analyses

In the experiments on anaphylactoid reactions, differences among groups were evaluated by Fisher's Exact Test. In the platelet reaction, significance of differences was assessed using Dunnett's comparison test after analysis of variance. In both analyses, *P* values of less than 0.05 were considered significant.

## RESULTS

### Inhibitory effects of anti-complement agents on anaphylactoid reaction

First, we examined whether the anti-complement agent K-76 COOH inhibited the lethality of the anaphylactoid reaction in

TABLE 1. Structures of repeating units of LPSs used in the present study

O antigen	Structure of the repeating unit
<i>E. coli</i> O8	$\alpha$ -Man3Me-(1 $\rightarrow$ 3)- $\beta$ -Man-(1 $\rightarrow$ 2)- $\alpha$ -Man-(1 $\rightarrow$ 2)- $\alpha$ -Man-(1 $\rightarrow$ ) <sub>n</sub>
<i>E. coli</i> O9	$\beta$ 3)- $\alpha$ -Man-(1 $\rightarrow$ 3)- $\alpha$ -Man-(1-2)- $\alpha$ -Man-(1 $\rightarrow$ 2)- $\alpha$ -Man-(1 $\rightarrow$ 2)- $\alpha$ -Man-(1 $\rightarrow$ ) <sub>n</sub>
<i>Klebsiella</i> O3	$\beta$ 3)- $\alpha$ -Man-(1 $\rightarrow$ 3)- $\alpha$ -Man-(1-2)- $\alpha$ -Man-(1 $\rightarrow$ 2)- $\alpha$ -Man-(1 $\rightarrow$ 2)- $\alpha$ -Man-(1 $\rightarrow$ ) <sub>n</sub>
<i>E. coli</i> O111	$\beta$ 4)- $\alpha$ -Glc-(1 $\rightarrow$ 4)- $\alpha$ -Gal(1 $\rightarrow$ 3)- $\beta$ -GlcNAc-(1 $\rightarrow$ ) <sub>n</sub> $\alpha$ 1.6 Col
<i>E. coli</i> O55	Col-(1 $\rightarrow$ 3)- $\alpha$ -Gal $\beta$ 1.3
<i>S. typhimurium</i>	$\beta$ 6)- $\beta$ -GlcNAc-(1 $\rightarrow$ 3)- $\beta$ -Gal-(1 $\rightarrow$ 3)- $\alpha$ -GalNAc-(1 $\rightarrow$ ) <sub>n</sub> 2-Ac-Abe $\alpha$ 1.3 $\alpha$ 1.4 $\beta$ 2)- $\alpha$ -Man-(1 $\rightarrow$ 4)- $\alpha$ -Rha-(1 $\rightarrow$ 3)- $\alpha$ -Gal-(1 $\rightarrow$ ) <sub>n</sub>

*P. intermedia* LPS is a so-called lipooligosaccharide, and lacks repeating sugar units including MHP (2, 8).

mice elicited by LPS. Groups of 4 to 15 C3H/HeJ mice received an intraperitoneal injection of 1, 4, 5, or 10 mg of K-76 COOH, followed by intravenous injections of 100  $\mu$ g of MDP and 200  $\mu$ g of *P. intermedia* LPS 2 and 6 h later, respectively. Four and 5 mg of the agent protected 1 of 4 and 12 of 15 mice, respectively, from lethality in the anaphylactoid reaction (Table 2). All of 8 mice receiving 10 mg of K-76 COOH survived the reaction. Second, the kinetics of the inhibitory effect of K-76 COOH were investigated. As shown in Table 3, when administered from 2 to 10 h before LPS injection, K-76 COOH (5 mg) completely protected the mice. The inhibitory effect was observed from 30 min to 1 day before LPS injection, but simultaneous injection of K-76 COOH with LPS had no effect.

CoVF is a powerful activator of the alternative pathway of complement, and it consumed complement components (15–18). As shown in Table 4, C3H/HeJ mice given an intraperitoneal injection of CoVF (50  $\mu$ g) did not show anaphylactoid reactions following combination injections of MDP and *S. typhimurium* LPS, and survived during the 1-h observation period, although some mice died 1 day after LPS injection.

#### Complement system is involved in the acute platelet reaction induced by LPS

As reported previously (4), intravenous injection of 100  $\mu$ g of *P. intermedia* LPS induced rapid platelet reactions in C3H/HeN mice: rapid (within 5 min) translocation of platelets from blood to the lungs and liver, especially the lungs (approximately 80%), followed by severe destruction of platelets within 30 min. In this system we investigated whether K-76 COOH inhibited the platelet reaction induced by *P. intermedia* LPS. Groups of 4 C3H/HeN mice received an intraperitoneal injection of K-76 COOH (2.5 mg) or physiological saline. One hour later, the mice were challenged with an intravenous injection of *P. intermedia* LPS (100  $\mu$ g), and the 5HT levels in the blood and tissues of each mouse were measured at 5 to 60 min after LPS injection. In both groups of mice, the blood 5HT level dropped rapidly and tissue 5HT, especially that in the lungs, rapidly increased in a similar pattern (Fig. 1). Within 10 min, the non-treated mice exhibited severe anaphylactoid reaction. K-76 COOH-treated mice showed no anaphylactoid reaction and 5HT levels in blood and tissues returned to

TABLE 2. Inhibitory effect of K-76 COOH on acute lethal toxicity induced by *P. intermedia* LPS in MDP-primed C3H/HeJ mice (dose-response)

K-76 COOH (mg/head)	Incidence	
	Anaphylactoid reaction	Death within 1 h
Without treatment	4/4	4/4
1.0	4/4	4/4
4.0	4/4	3/4
5.0	3/15***	3/15***
10.0	0/8***	0/8***

C3H/HeJ mice received an intraperitoneal injection of K-76 COOH. Two and 6 h later, the mice were injected intravenously with 100  $\mu$ g of MDP and 200  $\mu$ g of *P. intermedia* LPS, respectively. The incidence of death within 1 h was recorded. \*\*\*, Significantly different from control (without treatment) group by Fisher's Exact Test ( $P < 0.001$ ).

TABLE 3. Inhibitory effect of K-76 COOH on acute lethal toxicity induced by *P. intermedia* LPS in MDP-primed C3H/HeJ mice (kinetic study)

Interval (h) between K-76 COOH and LPS injections	Incidence	
	Anaphylactoid reaction	Death within 1 h
Without treatment	4/4	4/4
0	4/4	4/4
0.5	2/4	2/4
1	2/4	2/4
2	0/4*	0/4*
4	0/4*	0/4*
6	0/4*	0/4*
10	1/4	0/4*
16	2/4	1/4
28	2/4	2/4
76	4/4	4/4

C3H/HeJ mice were pretreated by intraperitoneal injection of K-76 COOH (5 mg) at various intervals before intravenous injection of *P. intermedia* LPS (200  $\mu$ g). Two mice were also primed with MDP (100  $\mu$ g) 4 h prior to the LPS injection. The incidence of death within 1 h was recorded. \*, Significantly different from control (without treatment) group by Fisher's Exact Test ( $P < 0.05$ ).

TABLE 4. Inhibitory effect of cobra venom factor (CoVF) on anaphylactoid reaction induced by *S. typhimurium* LPS in MDP-primed C3H/HeJ mice

Interval (h) between CoVF and LPS injections	Incidence	
	Anaphylactoid reaction	Death within 1 h (1 day)
Without treatment	10/10	10/10
1	0/3**	0/3** (2/3)
4	0/3**	0/3**
6	0/3**	0/3** (1/3*)

C3H/HeJ mice were pretreated by intraperitoneal injection of CoVF (50  $\mu$ g) at various intervals before intravenous injection of *S. typhimurium* LPS (200  $\mu$ g). The mice were also primed with MDP (100  $\mu$ g) 4 h prior to the LPS injection. The incidence of death within 1 h and 1 day was recorded. \* and \*\*, Significantly different from control (without treatment) group by Fisher's Exact Test (\* $P < 0.05$ , \*\* $P < 0.01$ ).

almost normal levels within 60 min, probably without platelet destruction.

We also examined blood and tissue 5HT levels after intravenous injection of *P. intermedia* LPS (100  $\mu$ g) in DBA/2 mice. In the C5-deficient mice, a transient decrease in blood 5HT level and comparable increase in the lungs were observed within 15 min, although spleen 5HT level increased continuously during the experimental period (Fig. 2). These mice did not show anaphylactoid reactions. These findings suggested that platelet destruction in the tissues was not induced by LPS in these mice.

#### MHP-containing LPS markedly activated the human complement system and induced severe anaphylactoid reactions

In our previous studies LPS carrying MHP as the *O*-specific polysaccharide exhibited strong complement activation probably via the lectin pathway initiated by the interaction between MHP and mannose-binding lectin (MBL) (10, 19). Therefore, we examined the possible relationship between complement activation and anaphylactoid reaction using the MHP-carrying

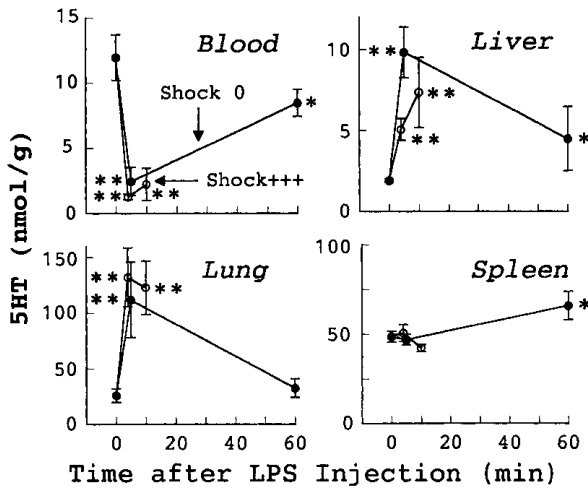


FIG. 1. Effect of K-76 COOH on platelet reaction induced by *P. intermedia* LPS in C3H/HeN mice. C3H/HeN mice pretreated by intraperitoneal injection of K-76 COOH (2.5 mg) (closed circles) or non-treated controls (open circles) were challenged with an intravenous injection of *P. intermedia* LPS (100 µg). Blood and tissues were taken at the indicated times after LPS injection, and 5HT levels were measured. Values are the means ± SD from 4 mice. \* and \*\*, Significantly different from normal level (0 time) by Dunnett's multiple comparison test (\**P* < 0.05, \*\**P* < 0.01). Shock +++ indicates that all mice died within 10 min in the severe anaphylactoid reaction.

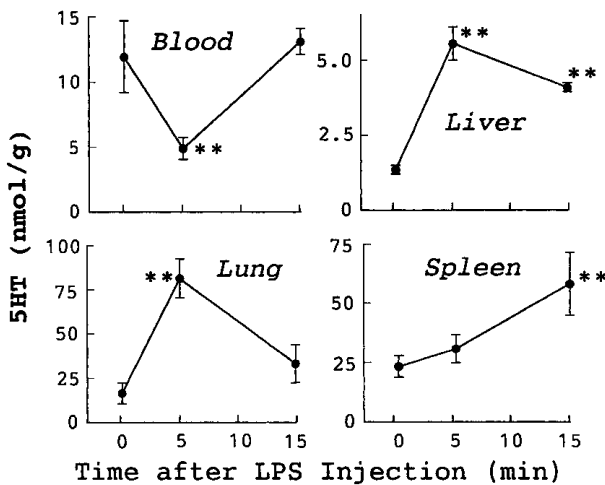


FIG. 2. Platelet reaction induced by *P. intermedia* LPS in C5-deficient DBA/2 mice. DBA/2 mice received an intravenous injection of *P. intermedia* LPS (100 µg). Blood and tissues were taken at the indicated times after LPS injection, and 5HT levels were measured. Values are the means ± SD from 4 mice. \*\*, Significantly different from normal level (0 time) by Dunnett's multiple comparison test (*P* < 0.01).

LPS (LPS prepared from *Klebsiella* O3, wild-type, and recombinant strains of *E. coli* O8 and O9) and the respective reference LPS-lacking MHP (R-type LPS specimens from the mutant strain LEN-113 of *Klebsiella* O3 and the parental strain, K-12, of the recombinant *E. coli* O8 and O9). Five LPS specimens carrying the MHP exhibited definite complement activation (Table 5). Most notably, *Klebsiella* O3 and *E. coli* O9 (both wild type and recombinant) LPS exhibited marked complement activation, and induced anaphylactoid reactions in MDP-primed C3H/HeJ mice and death within 1 h at an incidence of 100%. The levels of complement activation by *E. coli*

TABLE 5. MHP-carrying LPS specimens induced severe anaphylactoid reaction in MDP-primed C3H/HeJ mice

LPS origin	MHP number	Complement activation (% CH50 consumption) <sup>†</sup>	Incidence	
			Anaphylactoid reaction	Death within 1 h
<i>Klebsiella</i> O3	+	95	3/3**	3/3**
<i>Klebsiella</i> O3-R	-	15	0/3	0/3
<i>E. coli</i> O8 (wild type)	+	52	3/3**	2/3
<i>E. coli</i> O8 (recombinant)	+	53	2/3	2/3
<i>E. coli</i> O9 (wild type)	+	96	3/3**	3/3**
<i>E. coli</i> O9 (recombinant)	+	93	3/3**	3/3**
<i>E. coli</i> K-12	-	22	0/3	0/3
<i>E. coli</i> O111:B4	-	9	0/3	0/3
<i>E. coli</i> O55:B5	-	7	0/3	0/3
<i>S. typhimurium</i>	-	34	3/3**	3/3**
<i>P. intermedia</i>	-	11	3/3**	3/3**
Saline	-	-	0/10	0/10

C3H/HeJ mice were injected intravenously with MDP (100 µg). Four hours later, the mice received an intravenous injection of various LPS specimens (200 µg). The incidences of anaphylactoid reaction and rapid mortality were recorded for 1 h after LPS injection. #, Presence or absence of MHP as an O-specific polysaccharide. †, Anti-human complement activity of LPS was determined in terms of the percent of CH50 consumption as described in text. \*\*, Significantly different from control (saline) group by Fisher's Exact Test (\*\**P* < 0.01).

O8 (both wild type and recombinant) LPS were less than those of O3 and O9 LPS, and 1 of 3 mice challenged with O8 LPS survived. R-type LPS specimens from the mutant strain LEN-113 of *Klebsiella* O3 and the *E. coli* K-12, the parent strain of the recombinant *E. coli* O8 and O9, both of which lacked O-specific polysaccharide and consequently lacked MHP, exhibited only weak complement activation, and induced neither anaphylactoid reaction nor rapid lethality. Smooth form LPS specimens from *E. coli* O111:B4 and O55:B5, which have different O-specific polysaccharides from MHP and are practically devoid of complement activation, were also inactive in induction of anaphylactoid reaction and lethality within 1 h in 14 and 13 of 15 mice, respectively, whereas the control LPS without MHP did not induce either anaphylactoid reaction or lethality in any of 12 mice. The differences in the incidences of anaphylactoid reaction and lethality within 1 h between MHP(+) and MHP(-) groups were significant (*P* < 0.001) by Fisher's Exact Test. It should be noted here, however, that LPS from *S. typhimurium* and *P. intermedia*, neither of which carry MHP and exhibited at most moderate complement activation, induced anaphylactoid reaction.

DISCUSSION

Here we presented evidence that the complement system is involved in the anaphylactoid reactions induced by specific LPS preparations for example, C5-deficient strains such as DBA/2 and AKR mice were resistant to the reaction (2); the anti-complement agents K76 COOH and CoVF inhibited the

reaction (Tables 2–4); and all of the MHP-containing LPS specimens from 3 wild-type and 2 recombinant bacterial strains, which markedly activated the complement system probably via the lectin pathway (9, 10, 19), induced the reaction (Table 5). In contrast, the LPS specimens from respective mutant and parent strains, lacking MHP, did not induce the reaction.

The LPS specimens including those from *P. intermedia* induced rapid accumulation of platelets in the lungs and liver followed by degradation of the platelets and tissue destruction prior to the anaphylactoid reaction in C3H/HeN mice (4). The platelet reactions were also inhibited by the anti-complement agent K-76 COOH, and did not occur in C5-deficient DBA/2 mice (Figs. 1 and 2). These findings suggested that complement activation is responsible for the degradation of platelets in the tissues, especially in the lungs, whereas platelet accumulation may occur complement-independently because transient platelet accumulation occurred even in K-76 COOH-treated C3H/HeN mice and C5-deficient DBA/2 mice. In this context *Klebsiella* O3 LPS markedly induced the platelet reaction, whereas the respective R-mutant LEN-113 LPS induced only transient platelet accumulation (20). Another possibility is that early component(s) of the complement system such as C4 and/or C2 may be involved in platelet accumulation as these early complement components may be intact in C5-deficient DBA/2 (6) and K-76 COOH-(11) or CoVF-(17, 18) treated mice.

MHP-carrying LPS preparations, which exhibited strong complement activation in a human serum system (9, 10; Table 5), induced severe anaphylactoid reaction accompanied by rapid lethality at a high incidence (Table 5). In contrast, MHP-lacking LPS preparations, irrespective of whether they were S-form (*E. coli* O111:B4 and O55:B5) or R-form (*Klebsiella* strain LEN-113 and *E. coli* K-12), which exhibit at most weak complement activation, did not induce anaphylactoid reaction. Jiang et al. (19) demonstrated that MHP-carrying LPS specimens such as *Klebsiella* O3, *E. coli* O8, and O9 bound to MBL, whereas LPS from *E. coli* O55 and O111 devoid of MHP scarcely bound to MBL. The binding between MHP and MBL may initiate activation of complement through the lectin pathway (21–23). On the other hand, the LPS from *P. intermedia* and *S. typhimurium*, which lacked MHP and exhibited only weak and moderate complement activation, respectively, induced severe anaphylactoid reaction. These findings suggested that complement activation through pathways other than the usual lectin pathway that is initiated from MBL binding may also be involved in the induction of anaphylactoid reaction. We also observed anaphylactoid reactions induced by various Gram-positive bacterial cells, mycobacterial lipoarabinomannan, and *Candida albicans* cells in MDP-primed mice (24, 25; H. Takada, Y. Kawabata, et al.). A common mechanism, complement-dependent platelet degradation, might be involved in these reactions. If this is the case, the platelet and anaphylactoid reactions might be partially responsible for the clinical features of septic shock, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome, which occur subsequent to microbial infection.

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