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Protective effects of sialyl Lewis X and anti-P-selectin antibody against lipopolysaccharide-induced acute lung injury in rabbits

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Abstract

The prophylactic effects of selectin inhibitors on lipopolysaccharide-induced acute lung injury were studied in rabbits by using sialyl Lewis X-oligosaccharide and PB1.3, an anti-human P-selectin monoclonal antibody. Lipopolysaccharide-induced acute lung injury resembles that of the acute respiratory distress syndrome, in which there is a decrease in arterial blood oxygen tension (PaO_2) and an increase in the difference between alveolar and arterial oxygen tension ($A\text{-}aDO_2$). Prophylactic treatment with the selectin inhibitors, sialyl Lewis X-oligosaccharide (55 mg kg^{-1} i.v. bolus injection immediately before lipopolysaccharide administration + 36 mg kg^{-1} h^{-1} i.v. infusion for 4 h) and PB1.3 (5 mg kg^{-1} i.v. bolus injection immediately before lipopolysaccharide administration), prevented the lipopolysaccharide-induced impairments in pulmonary gas exchange. In contrast, these agents had no significant effects on lipopolysaccharide-induced systemic hypotension, the decrease in the number of circulating white blood cells and platelets, the decline in blood pH, or the increase in arterial CO_2 tension ($PaCO_2$). These results indicate that selectin inhibitors including sialyl Lewis X-oligosaccharide and the anti-P-selectin antibody, PB1.3, attenuate lipopolysaccharide-induced acute lung injury in rabbits. This is the first demonstration that P-selectin is directly involved in the development of lipopolysaccharide-induced impairments in pulmonary gas exchange.

Keywords: Selectin; Anti-P-selectin antibody; Sialyl Lewis X; Acute lung injury; Lipopolysaccharide

1. Introduction

Acute respiratory distress syndrome is an acute, progressive pulmonary disorder characterized by inflammation and increased permeability pulmonary edema, and is associated with hypoxemia, increased lung compliance, and diffuse pulmonary infiltrates on chest radiography (Tate and Repine, 1983). Septic shock caused by Gram-negative bacteria is thought to be one of the most common conditions associated with acute lung injury, including acute respiratory distress syndrome. Acute respiratory distress syndrome develops in approximately 70–80% of patients with Gram-negative sepsis and is associated with a high mortality rate (Sachdeva and Guntupalli, 1997; Artigas et al., 1998). In the last 10 years, a number of novel therapeutic approaches, including the use of anti-cytokine (Natanson et al., 1994), surfactant (Gregory et al., 1997) and anti-oxidant agents (Bernard et al., 1997), have been developed and tested against acute respiratory distress syndrome with sepsis, but few have proved beneficial in reducing mortality. The lipopolysaccharide-induced experimental lung injury model is associated with systemic hypotension, impairment of pulmonary oxygenation, coagulation abnormalities and acidosis in several animal species (Granger and Kubes, 1994; Carvalho et al., 1997). These models have been used to investigate various agents including lysofylline, an inhibitor of phosphatidic acid generation (Hasegawa et al., 1997), methylpredonisolone (Borg et al., 1985), a neutrophil elastase inhibitor (Nishina et al., 1997), nafamostat mesilate (Uchiba et al., 1997) and an anti-interleukin-8 antibody (Carvalho et al., 1997).

The initial step in the acute inflammatory processes, including that of acute respiratory distress syndrome, is the adherence of polymorphonuclear leukocytes to activated endothelial cells (Granger and Kubes, 1994). This first contact and the subsequent rolling step depend largely on the interaction between selectins (E-, L-, and P-selectins) and their ligands including sialyl Lewis X-oligosaccharide

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(Bevilacqua and Nelson, 1993). The polymorphonuclear leukocytes that adhere and migrate to sites of inflammation undergo functional activation and release various mediators, including reactive oxygen radicals, proteolytic enzymes and possibly inflammatory cytokines, which subsequently cause severe damage to the underlying endothelial cells.

Three selectin family members are glycoproteins that share structurally homologous domains, namely, the lectin, epidermal growth factor, complement binding, and cytoplasmic domains (Bevilacqua and Nelson, 1993). L-selectin is constitutively expressed on leukocytes and is rapidly shed following leukocyte activation. The expression of E-selectin is induced by inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β and lipopolysaccharide via de novo protein synthesis. In contrast, P-selectin is stored in α-granules of platelets and in Weibel–Palade bodies of endothelial cells and is rapidly expressed on the cell surface in response to various stimuli such as thrombin and histamine. The sialyl Lewis X-oligosaccharide motif is a carbohydrate ligand for all three selectins (Bevilacqua and Nelson, 1993) and sialyl Lewis X-oligosaccharide has been used as a selectin inhibitor in various studies. Sialyl Lewis X-oligosaccharide inhibits selectin-mediated polymorphonuclear leukocyte adhesion in vitro (Foxall et al., 1992; Kawamura et al., 1995) and attenuates ischemia/reperfusion-induced myocardial necrosis in canine (Lefer et al., 1994), rabbit (Yamada et al., 1998), and rat (Tojo et al., 1996) models as well as ischemia/reperfusion injury in the rabbit ear (Han et al., 1995). We have recently observed that sialyl Lewis X-oligosaccharide attenuates neutrophil-dependent myocardial dysfunction in the isolated rat heart (Ohnishi et al., 1999).

Selectin inhibitors have also been used in various lung injury models, primarily in small animals such as the rat. For example, in rats given lipopolysaccharide intratracheally, the accumulation of polymorphonuclear leukocyte in bronchoalveolar lavage fluid was found to be reduced by the intravenous administration of anti-E-selectin mAb and soluble E-selectin, respectively (Ulich et al., 1994). In the immune-complex-induced lung injury model in rats, the administration of sialyl Lewis X-oligosaccharide (Mulligan et al., 1993) and an anti-P-selectin antibody (Bless et al., 1998) resulted in protection against the injury. In these lung injury models, the effects of drugs are evaluated primarily in terms of inflammatory parameters such as permeability, hemorrhage and myeloperoxidase. The effect of the drugs cannot be evaluated in terms of functional parameters of the lung, such as gas exchange, partly due to the technical difficulties associated with the small animal species used. In contrast, pulmonary function can be analyzed in larger animals, such as pig and rabbit.

Recently, Ridings et al. (Ridings et al., 1997) showed that sialyl Lewis X-oligosaccharide protected against the pulmonary dysfunction induced by the infusion of live *Pseudomonas aeruginosa* in a porcine sepsis model. They observed that the administration of sialyl Lewis X-oligosaccharide improved arterial oxygenation and various inflammatory parameters, thus, demonstrating that the inhibition of selectins protects against lung injury. Since sialyl Lewis X-oligosaccharide inhibits all three selectins (Foxall et al., 1992), their results implied that the inhibition of all three selectins may be protective. However, the possible role of each selectin molecule could not be elucidated from their findings.

We have recently demonstrated that the systemic administration of lipopolysaccharide results in an increase in the plasma P-selectin level in rats (Misugi et al., 1998). Based on this finding and the observations that patients with acute lung injury (Sakamaki et al., 1995) and sepsis (Fijnheer et al., 1997) show an elevated level of soluble P-selectin, we hypothesize that P-selectin is closely involved in the development of pulmonary dysfunction. No study, however, has demonstrated the specific role of P-selectin in the development of pulmonary dysfunction. In the present study, we investigated whether selectin inhibitors can protect against pulmonary dysfunction in a rabbit model by using sialyl Lewis X-oligosaccharide, and then demonstrated the specific contribution of P-selectin by using PB1.3, an anti-P-selectin monoclonal antibody.

2. Materials and methods

2.1. Materials

Male New Zealand White rabbits (SPF, 2.0–2.5 kg) were purchased from Kitayama Labes (Tokyo, Japan). Prior to experiments, they were housed for a minimum of 1 week in a quarantine room with a 12:12-h daily light:dark cycle. Lipopolysaccharide and gallamine triethiodide were purchased from Sigma (St. Louis, MO) and diluted 0.3 mg ml^{-1} and 10 mg ml^{-1} with saline, respectively. PB1.3 is a mouse anti-human P-selectin monoclonal antibody. Sialyl Lewis X-oligosaccharide and PB1.3 were provided by Cytel (San Diego, CA) and diluted appropriately with saline. Sialyl Lewis X-oligosaccharide has been used in various studies with rabbits (Han et al., 1995; Yamada et al., 1998). PB1.3 cross-reacts with rabbit P-selectin, as demonstrated by the PB1.3-induced reduction of rabbit ear swelling caused by reperfusion injury and the immunohistochemical detection of P-selectin with the antibody (Winn et al., 1993). The plasma half-life of PB1.3 is approximately 24 h in the rabbit (Winn et al., 1993). Other reagents used were of the highest grade commercially available.

2.2. Animal preparation

All procedures related to the use of animals in these studies were reviewed and approved by the Institutional

Animal Care and Use Committee at Sumitomo Pharmaceuticals Research Center (Osaka, Japan). Animals were anesthetized by the administration of 60–70 mg per head of sodium pentobarbital through the ear veins. After tracheae were cannulated with silicon tubes, animals were ventilated (concentration of O_2 in inspired gas (FiO_2); 0.52, tidal volume; 30 ml, respiratory rate; 30 rpm) using a volume-controlled respirator (SN-480-5, Sinano, Tokyo, Japan). To arrest spontaneous breathing, animals were injected intramuscularly with 12.5 mg per head of gallamine triethiodide every 2 h. A catheter was placed in the right common carotid artery for monitoring mean arterial blood pressure and heart rate and for sampling blood and a silicon tube was introduced into the right femoral vein for administration of lipopolysaccharide and agents.

Approximately 20–30 min later, when PaO_2 had stabilized, lipopolysaccharide (0.3 mg kg^{-1}) was administered intravenously. Arterial blood collected every hour after lipopolysaccharide injection was subjected to blood gas analysis, using a blood gas analyzer (Type273, Chiron, Boston, MA), and cell numbers were counted using a cell counter (Sysmex F-800, Sysmex, Hyogo, Japan). Arterial blood pressure and heart rate via the right common carotid artery were monitored with an electric recorder (WT-685, Nihon Koden, Tokyo, Japan). Animals were killed with an overdose of sodium pentobarbital 4 h after lipopolysaccharide injection.

A-aDO_2 was calculated according to the formula below (Mellemgarrd, 1966):

$$\begin{aligned} A-aDO_2\,(\mathrm{mmHg}) &= \text{alveolar } O_2 \text{ tension} - PaO_2 \\ &= \left[FiO_2 \times (760-47) - (PaCO_2) \right. \\ &\quad \left. \times \{FiO_2 + (1-FiO_2)/0.8\}\right] \\ &\quad - PaO_2 \\ &= (0.52 \times 713 - 1.12 \times PaCO_2) \\ &\quad - PaO_2 \end{aligned}$$

2.3. Experimental protocols

2.3.1. The treatment with sialyl Lewis X-oligosaccharide

Animals were assigned randomly to five groups. In the saline group ($n = 16$), animals were intravenously injected with saline (1 ml kg^{-1}) and then infused over 4 h with saline (1 ml kg^{-1} h^{-1}). In the lipopolysaccharide group ($n = 16$), animals were intravenously injected with lipopolysaccharide (0.3 mg kg^{-1}, 1 ml kg^{-1}) and then infused over 4 h with saline (1 ml kg^{-1} h^{-1}). In the sialyl Lewis X-oligosaccharide (high-dose) group ($n = 16$), animals were intravenously injected with sialyl Lewis X-oligosaccharide (55 mg kg^{-1}, 1 ml kg^{-1}) immediately followed

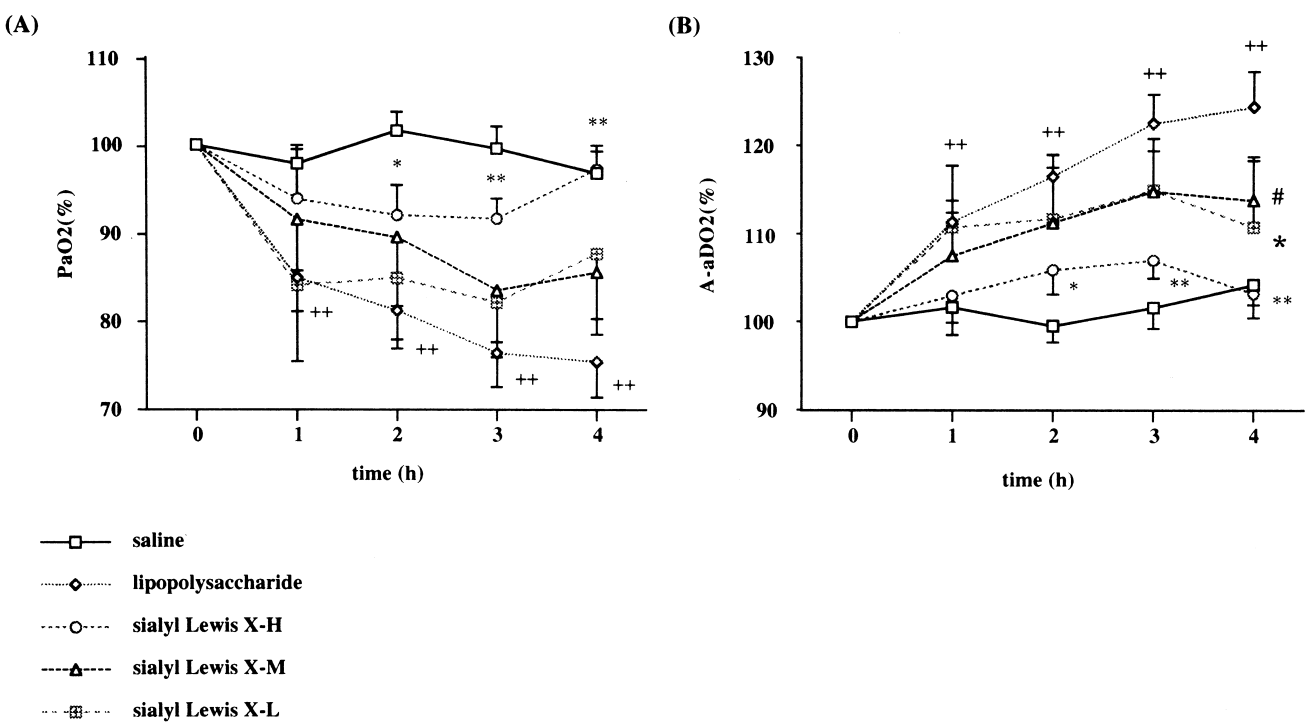

Fig. 1. Effect of sialyl Lewis X-oligosaccharide on PaO_2 and A-aDO_2 in lipopolysaccharide-induced lung injury. Lipopolysaccharide (0.3 mg kg^{-1}) was injected intravenously and PaO_2 (A) was monitored every hour after lipopolysaccharide injection. A-aDO_2 (B) was calculated as described in Section 2. The high-dose group (sialyl Lewis X-H) received sialyl Lewis X-oligosaccharide 55 mg kg^{-1} i.v. bolus + 36 mg kg^{-1} h^{-1} for 4 h, the medium-dose group (sialyl Lewis X-M) received 28 mg kg^{-1} i.v. bolus + 18 mg kg^{-1} h^{-1} for 4 h, and the low-dose group (sialyl Lewis X-L) received 14 mg kg^{-1} i.v. bolus + 9 mg kg^{-1} h^{-1} for 4 h. Each value represents the mean ± SEM of 8–16 determinations. Statistical significance; $^{++}P < 0.01$ for lipopolysaccharide group vs. saline group, $^{**}P < 0.01$ for high-dose group vs. lipopolysaccharide group, $^{*}P < 0.05$ for high-dose group vs. lipopolysaccharide group, $^{\#}P < 0.05$ for medium-dose group vs. lipopolysaccharide group, $^{*}P < 0.05$ for low-dose group vs. lipopolysaccharide group.

by lipopolysaccharide. Then sialyl Lewis X-oligosaccharide (36 mg kg^{-1} h^{-1}, 1 ml kg^{-1} h^{-1}) was infused over 4 h. In the sialyl Lewis X-oligosaccharide (medium-dose) group ($n = 8$), animals were intravenously injected with sialyl Lewis X-oligosaccharide (28 mg kg^{-1}, 1 ml kg^{-1}) immediately followed by lipopolysaccharide. Then sialyl Lewis X-oligosaccharide (18 mg kg^{-1} h^{-1}, 1 ml kg^{-1} h^{-1}) was infused over 4 h. In the sialyl Lewis X-oligosaccharide (low-dose) group ($n = 8$), animals were intravenously injected with sialyl Lewis X-oligosaccharide (14 mg kg^{-1}, 1 ml kg^{-1}) immediately followed by lipopolysaccharide. Then sialyl Lewis X-oligosaccharide (9 mg kg^{-1} h^{-1}, 1 ml kg^{-1} h^{-1}) was infused over 4 h.

2.3.2. The treatment with PB1.3

Animals were assigned randomly to three groups. In the saline group ($n = 8$), animals were intravenously injected with saline (1 ml kg^{-1}) and then infused over 4 h with saline (1 ml kg^{-1} h^{-1}). In the lipopolysaccharide group ($n = 8$), animals were intravenously injected with lipopolysaccharide (0.3 mg kg^{-1}, 1 ml kg^{-1}) and then infused over 4 h with saline (1 ml kg^{-1} h^{-1}). In the PB1.3 group ($n = 8$), animals were intravenously injected with PB1.3 (5 mg kg^{-1}) immediately followed by lipopolysaccharide. Then saline (1 ml kg^{-1} h^{-1}) was infused over 4 h.

2.4. Histopathologic examination

Immediately after the rabbits were killed, all lobes of the left lung were fixed by the instillation of 10% formaldehyde solution through the left bronchus at 50 cm H_2O. The specimens were embedded in paraffin wax and then stained with hematoxylin–eosin.

2.5. Statistical analysis

The significance of differences between two groups, such as saline vs. lipopolysaccharide groups, was analyzed by using Student's *t*-test. The significance of differences between more than two groups was analyzed by using Dunnett's *t*-test with the SAS program (SAS Institute, Cary, NC). Probabilities less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of sialyl Lewis X-oligosaccharide

In the saline group, PaO_2 did not change during the experiment (Fig. 1A). The PaO_2 in the lipopolysaccharide

Table 1
Dose-dependent effect of sialyl Lewis X-oligosaccharide on various parameters

Parameters	Treatment group	Hours after lipopolysaccharide injection				
		0 h	1 h	2 h	3 h	4 h
Mean arterial	saline ($n = 16$)	100	97.1 ± 3.7	89.3 ± 3.1	93.1 ± 5.0	88.2 ± 3.3
blood pressure	lipopolysaccharide ($n = 16$)	100	79.1 ± 3.6[a]	77.6 ± 2.7[a]	76.6 ± 4.7[b]	69.6 ± 4.0[a]
(% of the value at 0 h)	sialyl Lewis X-H ($n = 16$)	100	82.0 ± 2.9	78.3 ± 2.6	74.8 ± 2.9	75.2 ± 2.5[c]
	sialyl Lewis X-M ($n = 8$)	100	90.7 ± 4.2	91.3 ± 3.6	88.1 ± 2.9	83.5 ± 3.7[c]
	sialyl Lewis X-L ($n = 8$)	100	84.2 ± 5.2	86.8 ± 5.8	87.2 ± 6.9	84.9 ± 5.5[c]
Number of peripheral	saline ($n = 16$)	100	68.2 ± 5.0	51.5 ± 5.0	47.0 ± 4.5	53.8 ± 4.0
white blood cells	lipopolysaccharide ($n = 16$)	100	81.4 ± 3.3[a]	45.3 ± 2.9	34.4 ± 2.6[b]	35.8 ± 2.4[a]
(% of the value at 0 h)	sialyl Lewis X-H ($n = 16$)	100	68.3 ± 3.4	39.8 ± 3.5	30.8 ± 2.5	32.9 ± 2.6
	sialyl Lewis X-M ($n = 8$)	100	77.9 ± 6.6	46.3 ± 3.6	33.5 ± 2.2	34.7 ± 1.9
	sialyl Lewis X-L ($n = 8$)	100	68.9 ± 5.6	41.7 ± 2.0	31.8 ± 3.2	33.8 ± 3.6
Number of platelets	saline ($n = 16$)	100	100.3 ± 2.6	94.9 ± 2.9	88.7 ± 3.0	84.8 ± 5.0
(% of the value at 0 h)	lipopolysaccharide ($n = 16$)	100	65.8 ± 3.3[a]	65.1 ± 2.4[a]	60.7 ± 2.9[a]	60.6 ± 3.2[a]
	sialyl Lewis X-H ($n = 16$)	100	69.6 ± 4.5	66.4 ± 5.3	66.3 ± 4.9	62.9 ± 4.5
	sialyl Lewis X-M ($n = 8$)	100	67.7 ± 3.6	68.8 ± 2.7	65.5 ± 1.9	61.8 ± 2.7
	sialyl Lewis X-L ($n = 8$)	100	67.0 ± 2.7	65.9 ± 4.5	53.7 ± 6.0	58.1 ± 3.0
Blood pH	saline ($n = 16$)	7.51 ± 0.02	7.47 ± 0.02	7.47 ± 0.01	7.47 ± 0.01	7.49 ± 0.01
	lipopolysaccharide ($n = 16$)	7.50 ± 0.01	7.38 ± 0.03[a]	7.38 ± 0.02[a]	7.38 ± 0.02[a]	7.36 ± 0.02[a]
	sialyl Lewis X-H ($n = 16$)	7.49 ± 0.02	7.39 ± 0.03	7.40 ± 0.02	7.40 ± 0.01	7.41 ± 0.02
	sialyl Lewis X-M ($n = 8$)	7.42 ± 0.02	7.38 ± 0.01	7.36 ± 0.01	7.36 ± 0.01	7.37 ± 0.01
	sialyl Lewis X-L ($n = 8$)	7.47 ± 0.01	7.39 ± 0.02	7.38 ± 0.02	7.39 ± 0.02	7.39 ± 0.02
Arterial CO_2 tension ($PaCO_2$)	saline ($n = 16$)	100	110.0 ± 4.2	103.1 ± 5.2	103.9 ± 5.5	101.6 ± 6.1
(% of the value at 0 h)	lipopolysaccharide ($n = 16$)	100	123.1 ± 12.8	111.1 ± 8.9	102.8 ± 7.5	99.9 ± 8.9
	sialyl Lewis X-H ($n = 16$)	100	118.1 ± 7.0	111.2 ± 5.2	105.4 ± 3.7	102.9 ± 3.7
	sialyl Lewis X-M ($n = 8$)	100	102.7 ± 4.9	91.5 ± 2.8	95.2 ± 3.3	93.0 ± 4.3
	sialyl Lewis X-L ($n = 8$)	100	119.7 ± 7.0	110.9 ± 4.8	105.4 ± 3.5	110.4 ± 4.4

Sialyl Lewis X-H received sialyl Lewis X-oligosaccharide 55 mg kg^{-1} i.v. bolus + 36 mg kg^{-1} h^{-1} for 4 h, sialyl Lewis X-M received 28 mg kg^{-1} i.v. bolus + 18 mg kg^{-1} h^{-1} for 4 h, and sialyl Lewis X-L received 14 mg kg^{-1} l.v. bolus + 9 mg kg^{-1} h^{-1} for 4 h. Each point represents mean ± S.E. Statistical significance; [a] $P < 0.01$ vs. saline group by Student's *t*-test, [b] $P < 0.05$ vs. saline group by Student's *t*-test, [c] $P < 0.05$ vs. lipopolysaccharide group by Dunnett's *t*-test.

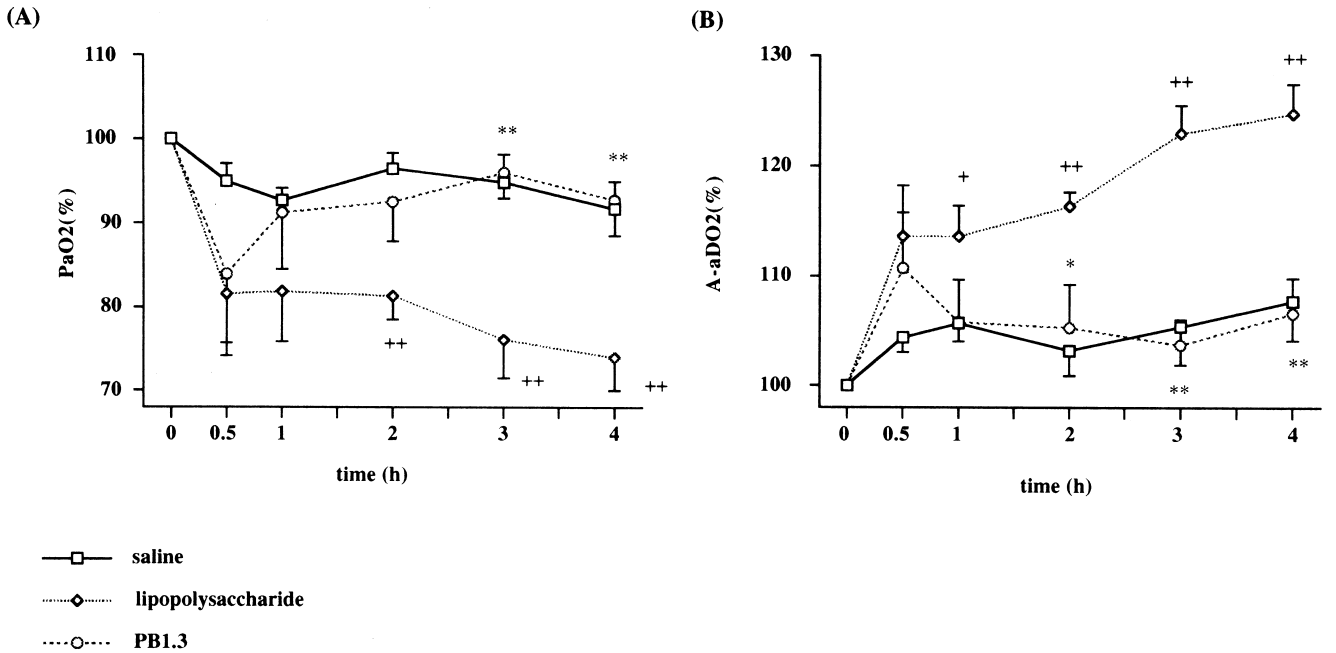

Fig. 2. Effect of PB1.3 on PaO_2 and A-aDO_2 in lipopolysaccharide-induced lung injury. Lipopolysaccharide (0.3 mg kg^{-1}) was injected intravenously and PaO_2 (A) was monitored from 0.5 h after lipopolysaccharide injection. A-aDO_2 (B) was calculated as described in Section 2. Each value represents the mean ± SEM of eight determinations. Statistical significance; $^{++}P < 0.01$ for lipopolysaccharide group vs. saline group, $^{**}P < 0.01$ for PB1.3 group vs. lipopolysaccharide group, $^{*}P < 0.05$ for PB1.3 group vs. lipopolysaccharide group.

group progressively decreased. The levels decreased by approximately 15% at 1 h and by approximately 25% at 4 h as compared with the level at 0 h. The PaO_2 level of the lipopolysaccharide group was significantly different from that in the saline group in the 4 h period examined. In contrast, the PaO_2 level in the sialyl Lewis X-oligosaccharide (high-dose) group was significantly higher 2 h after lipopolysaccharide injection than that in the lipopolysaccharide group. In the two sialyl Lewis X-oligosaccharide (medium-dose and low-dose) groups, the lipopolysaccharide-induced decrease in PaO_2 level appeared to be partly prevented, but was not statistically significant between the lipopolysaccharide group and the two sialyl Lewis X-oligosaccharide groups (Fig. 1A).

Similar results were obtained for the improvement of A-aDO_2 (Fig. 1B). The A-aDO_2 levels in the lipopolysaccharide group increased by approximately 10% at 1 h and by approximately 20% at 4 h after lipopolysaccharide injection. The level became significantly different from that in the saline group from 1 h after lipopolysaccharide

Table 2
Effect of PB1.3 on various parameters

Parameters	Treatment group	Hours after lipopolysaccharide injection					
		0 h	0.5 h	1 h	2 h	3 h	4 h
Mean arterial blood pressure	saline ($n = 8$)	100	97.6 ± 4.4	98.2 ± 6.4	89.5 ± 5.6	97.5 ± 7.7	93.4 ± 4.8
(% of the value at 0 h)	lipopolysaccharide ($n = 8$)	100	87.9 ± 3.6	76.6 ± 5.2[b]	76.2 ± 3.3	73.5 ± 5.8[b]	72.6 ± 5.9[b]
	PB1.3 ($n = 8$)	100	93.2 ± 3.1	84.5 ± 8.0	83.0 ± 6.2	79.7 ± 5.1	80.6 ± 4.7
The number of peripheral	saline ($n = 8$)	100	68.6 ± 5.5	64.4 ± 2.7	46.5 ± 4.4	40.2 ± 5.4	47.8 ± 5.8
white blood cells	lipopolysaccharide ($n = 8$)	100	92.6 ± 5.1[b]	84.4 ± 5.4[b]	47.1 ± 4.1	34.9 ± 4.2	35.7 ± 3.3[b]
(% of the value at 0 h)	PB1.3 ($n = 8$)	100	86.6 ± 6.1	75.3 ± 10.9	44.5 ± 5.2	26.6 ± 3.9	24.7 ± 2.4[c]
The number of platelets	saline ($n = 8$)	100	89.8 ± 7.3	97.1 ± 1.7	89.2 ± 3.6	83.0 ± 5.0	79.6 ± 7.8
(% of the value at 0 h)	lipopolysaccharide ($n = 8$)	100	36.2 ± 4.4[a]	64.4 ± 5.7[a]	63.9 ± 4.3[a]	59.9 ± 5.4[a]	60.9 ± 6.2
	PB1.3 ($n = 8$)	100	50.2 ± 4.2[c]	63.1 ± 3.0	65.0 ± 4.2	67.7 ± 5.2	61.2 ± 3.4
Blood pH	saline ($n = 8$)	7.53 ± 0.03	7.51 ± 0.02	7.49 ± 0.02	7.48 ± 0.02	7.49 ± 0.02	7.50 ± 0.02
	lipopolysaccharide ($n = 8$)	7.52 ± 0.02	7.41 ± 0.05	7.38 ± 0.05	7.39 ± 0.03[b]	7.40 ± 0.03[b]	7.39 ± 0.03[b]
	PB1.3 ($n = 8$)	7.52 ± 0.04	7.45 ± 0.05	7.43 ± 0.04	7.43 ± 0.02	7.43 ± 0.02	7.44 ± 0.02
Arterial CO_2 tension ($PaCO_2$)	saline ($n = 8$)	100	108.4 ± 9.5	112.2 ± 8.2	107.1 ± 9.8	106.1 ± 11.0	106.7 ± 11.9
(% of the value at 0 h)	lipopolysaccharide ($n = 8$)	100	131.7 ± 20.0	131.6 ± 25.7	117.6 ± 17.4	106 ± 15.0	113.2 ± 16.1
	PB1.3 ($n = 8$)	100	119.1 ± 10.5	113.9 ± 10.6	106.6 ± 4.9	102.5 ± 4.6	101.4 ± 7.0

PB1.3 group received 5 mg kg^{-1} PB1.3 immediately after LPS injection. Each point represents mean ± S.E. Statistical significance; $^{a}P < 0.01$ vs. saline group by Student's *t*-test, $^{b}P < 0.05$ vs. saline group by Student's *t*-test, $^{c}P < 0.05$ vs. lipopolysaccharide group by Student's *t*-test.

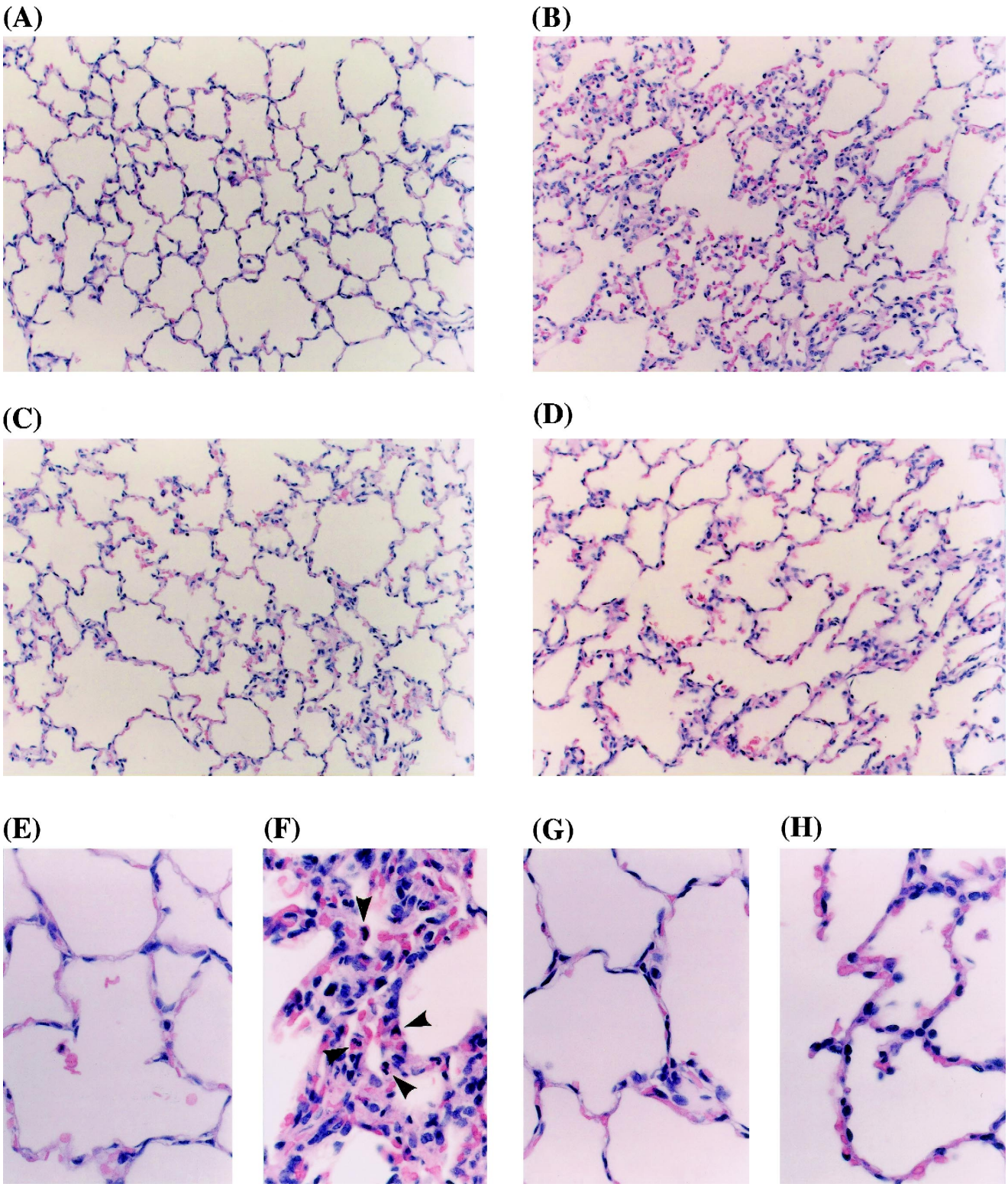

Fig. 3. Histopathologic examination of rabbit lungs of lipopolysaccharide-induced lung injury (hematoxylin and eosin). Lipopolysaccharide (0.3 mg kg^{-1}) was injected intravenously into male New Zealand White rabbits. Lung tissues collected 4 h after lipopolysaccharide injection were fixed with 10% formalin. Tissue slices were stained with hematoxylin and eosin. Typical micrographs of lung tissues are shown. Lower magnification micrographs (×200); (A) saline group, (B) lipopolysaccharide group, (C) sialyl Lewis X-oligosaccharide (high-dose) group, (D) PB1.3 group. Higher magnification micrographs (×600); (E) saline group, (F) lipopolysaccharide group, (G) sialyl Lewis X-oligosaccharide (high-dose) group, (H) PB1.3 group. The infiltrating polymorphonuclear leukocytes are indicated by the arrow heads.

injection onward, and in the level of the sialyl Lewis X-oligosaccharide (high-dose) group, A-aDO_2 was significantly lower from 2 h after lipopolysaccharide injection onward than it was in the lipopolysaccharide group. Treatment with the two other doses of sialyl Lewis X-oligosaccharide (medium-dose and low-dose) caused no significant improvement in the level of A-aDO_2.

The mean arterial pressure, the number of peripheral white blood cells and platelets and the blood pH significantly decreased in the lipopolysaccharide group as compared with the saline group. The $PaCO_2$ level transiently increased in the lipopolysaccharide group as compared with the saline group. Sialyl Lewis X-oligosaccharide did not significantly affect these parameters at all three doses examined (Table 1).

3.2. Effect of PB1.3

In the saline group, PaO_2 did not change during the experiment (Fig. 2A). In the lipopolysaccharide group, the PaO_2 level progressively decreased and was approximately 25% at 4 h, compared to that at 0 h, after lipopolysaccharide injection. The decreased levels became significantly different from 2 h after lipopolysaccharide injection onward from those of the saline group. The PaO_2 level in the PB1.3 group was significantly higher at 3 and 4 h than that in the lipopolysaccharide group (Fig. 2A). Similarly, a significant improvement in the A-aDO_2 level was observed after 2 h in the PB1.3 group when compared with the lipopolysaccharide group (Fig. 2B).

PB1.3 did not significantly affect the lipopolysaccharide-induced decrease in mean arterial pressure, number of white blood cells and platelets, blood pH and the increase in the $PaCO_2$ level relative to the effect of saline (Table 2).

3.3. Histopathologic examination

Histopathologic examination of the lungs was performed. Typical microphotographs of lung tissue in each group are shown in Fig. 3. In the lipopolysaccharide group, signs of inflammation, such as hemorrhage, increased thickness of the alveolar wall, polymorphonuclear leukocyte infiltration of the interstitial space and polymorphonuclear leukocytes adhered to the pulmonary vascular wall, were observed (Fig. 3B). In contrast, the lungs treated with sialyl Lewis X-oligosaccharide (high-dose) (Fig. 3C) and PB1.3 (Fig. 3D) showed no visible signs of inflammation and appeared to be indistinguishable from those in the saline group (Fig. 3A). Using higher magnification micrographs of the lungs, cellular infiltration was examined in more detail. In the lipopolysaccharide group, a significant number of infiltrating cells were observed (Fig. 3F), whereas few cells were detected in the alveoli of the lungs of the saline (Fig. 3E), sialyl Lewis X-oligosaccharide (high-dose) (Fig. 3G) and PB1.3 (Fig. 3H) groups.

4. Discussion

Acute lung injury is associated with various diseases including sepsis, acute pancreatitis, burns and trauma. The destruction of microvascular endothelial cells mediated by polymorphonuclear leukocytes is implicated in the development of acute lung injury, resulting in increased permeability, the formation of interstitial edema in lung, and subsequent organ dysfunction (Tate and Repine, 1983). Bronchoalveolar lavage fluid and lung tissues obtained from patients with acute respiratory distress syndrome contained an increased number of polymorphonuclear leukocytes (Tate and Repine, 1983). In addition, in an animal model of sepsis, the rapid sequestration of polymorphonuclear leukocytes into the lung was demonstrated using radiolabeled polymorphonuclear leukocytes (Hangen et al., 1990). Therefore, inhibition of the interaction between polymorphonuclear leukocytes and endothelial cells might be a useful approach for improving sepsis-induced acute lung injury.

The role of selectins in cell adhesion has been extensively studied and they are implicated in various diseases. For example, the level of plasma E-selectin is known to be elevated in patients with sepsis (Engelberts et al., 1992; Friedman et al., 1996). In addition, the fact that the administration of anti-E-selectin antibody improves gas exchange function in patients with sepsis (Friedman et al., 1996) strongly suggests that the inhibition of E-selectin is therapeutically beneficial. In the case of P-selectin, however, little is known of the effect of P-selectin inhibitor on the lung pulmonary dysfunction associated with acute lung injury, although the lipopolysaccharide-induced expression of P-selectin has previously been demonstrated in various animal models (Sanders et al., 1992; Eppihimer et al., 1996; Gupta et al., 1996). The level of soluble plasma P-selectin is elevated in patients with acute lung injury (Sakamaki et al., 1995) as well as sepsis (Fijnheer et al., 1997). We have recently developed an ELISA system that enables us to measure rat soluble P-selectin and demonstrated for the first time that the level of plasma soluble P-selectin is elevated in response to lipopolysaccharide and peaks at 24 h after lipopolysaccharide administration (Misugi et al., 1998). These results indicate a possible contribution of P-selectin to the development of acute lung injury.

Recently, it was demonstrated in a porcine model that lipopolysaccharide-induced pulmonary dysfunction was ameliorated by sialyl Lewis X-oligosaccharide, an inhibitor of all three selectins (Ridings et al., 1997). Among the selectins, we hypothesized that P-selectin has a predominant role in acute inflammation. We therefore examined the effect of sialyl Lewis X-oligosaccharide as well as PB1.3, a mAb specific to P-selectin, on lipopolysaccharide-induced acute lung injury in rabbits. An advantage of using rabbits is that it has already been demonstrated, by intravital microscopy, that leukocyte rolling in pulmonary microvessels is reduced by fucoidin, an inhibitor of L- and

P-selectins, in this animal species (Kuebler et al., 1997). However, no studies have been carried out to determine the effect of sialyl Lewis X-oligosaccharide and the P-selectin monoclonal antibody against lipopolysaccharide-induced acute lung injury in rabbits.

In the present study, we demonstrated that the administration of sialyl Lewis X-oligosaccharide and PB1.3 attenuated the physiological and histopathologic deterioration of lipopolysaccharide-induced lung injury in the rabbit. Treatment with sialyl Lewis X-oligosaccharide (Fig. 1) improved the dysfunction of oxygenation in a dose-dependent manner. This is the first report of the dose-dependent protective effect of sialyl Lewis X-oligosaccharide against acute lung injury. Our results for rabbits are consistent with those of a previous work that reported the efficacy of sialyl Lewis X-oligosaccharide against sepsis-induced acute lung injury in pigs (Ridings et al., 1997). In addition, a similar protective effect was obtained with PB1.3 (Fig. 2), which clearly demonstrates the role for P-selectin in the development of the pulmonary dysfunction. It should be noted that the protective effect was not a result of the possible neutropenic effect of PB1.3 since we (Yamada et al., 1998) and others (Winn et al., 1993) have previously demonstrated that the antibody does not reduce the number of peripheral polymorphonuclear leukocytes.

On microscopic observation of lung tissue of the lipopolysaccharide group, various inflammatory changes including hemorrhage, increased thickness of the alveolar wall, and polymorphonuclear leukocyte infiltration in the interstitial space were observed. In contrast, the alveolar architecture in the sialyl Lewis X-oligosaccharide (high-dose) and PB1.3-treated groups was well-preserved and appeared to be indistinguishable from that seen in the saline group (Fig. 3).

Neither sialyl Lewis X-oligosaccharide nor PB1.3 had an effect on the lipopolysaccharide-induced decrease in mean arterial blood pressure, blood pH, number of peripheral white blood cells or platelets and increase in the $PaCO_2$ level (Tables 1 and 2). These results are consistent with the results reported for a porcine model, namely, that the administration of sialyl Lewis X-oligosaccharide dose not improve these parameters (Ridings et al., 1997). The derangements in the mean arterial blood pressure are likely mediated by various mediators, such as TNF-α, products of the kallikrein–kinin system, and possibly nitric oxide (Ridings et al., 1997). Decreased production of these mediators is not expected to occur as a result of selectin blockade.

Myeloperoxidase activity has been used as a parameter of lung injury and is known to be increased in animal models of sepsis-induced acute lung injury. The protective effects of drugs including sialyl Lewis X-oligosaccharide (Ridings et al., 1997) and lysofylline (Hasegawa et al., 1997) against the pulmonary dysfunctions are associated with a reduced level of myeloperoxidase activity, indicating that their effects are, at least in part, due to the inhibition of polymorphonuclear leukocyte accumulation. In our model, however, it should be noted that the level of myeloperoxidase activity in the lung was not elevated in the lipopolysaccharide group and was indistinguishable from that in the saline group (data not shown), and therefore myeloperoxidase was not used as a parameter to evaluate these inhibitors. It appears to be in contrast to our histopathologic observations that an increased number of infiltrating polymorphonuclear leukocytes were detected in the lung interstitial regions in the lipopolysaccharide group, whereas few polymorphonuclear leukocytes were observed in the saline group or in the drug-treated groups (Fig. 3). We believe that the lipopolysaccharide-induced decrease in PaO_2 is likely caused by the accumulation of polymorphonuclear leukocytes, and that the difference in the level of polymorphonuclear leukocyte accumulation between the lipopolysaccharide and the control groups cannot be detected by measuring myeloperoxidase activity under our experimental conditions.

Although it is likely that the protective effect of selectin inhibitors observed in the present study resulted from the inhibition of polymorphonuclear leukocyte accumulation in rabbit lung, we cannot rule out the possibility that the effect was also mediated by the inhibition of other types of leukocytes, such as monocytes and macrophages. It is known that selectins are also involved in the accumulation of monocytes in inflammatory sites (Spertini et al., 1992), and that the inhibition of monocyte migration by the blockade of E- and P-selectins improves arthritis and cutaneous inflammation in mice (Walter and Issekutz, 1997a,b). At present, we have no data about the relative contribution of monocytes and macrophages to the lung injury in our model. It is known that resident alveolar macrophages in the lung play a central role in defending the lung against a variety of potentially harmful substances (Tsuchida et al., 1997). Once stimulated by mediators such as complement-derived fragments and lipopolysaccharide, these macrophages release various inflammatory cytokines such as interleukin-6, TNF-α and interleukin-1β (McIntosh et al., 1996), leading to inflammatory responses. Further studies will be required to elucidate the possible role of monocytes and macrophages in acute lung injury. In addition, it has recently been shown that apoptosis is induced by lipopolysaccharide in mouse lung endothelial cells (Haimovitz-Friedman et al., 1997). However, microscopic examination of hematoxylin and eosin-stained lung tissue did not reveal irregularly shaped nuclei or chromosomal condensation characteristic of apoptosis. It remains to be determined whether the apoptotic cascade is involved in the lipopolysaccharide-induced dysfunction of oxygenation in our model. For this purpose, more specific detection methods are needed, such as TUNEL staining.

In conclusion, we demonstrated the protective effects of sialyl Lewis X-oligosaccharide and PB1.3 against pulmonary dysfunction associated with lipopolysaccharide-induced acute lung injury in the rabbit. In addition, we

demonstrated for the first time the specific role of P-selectin in the development of pulmonary dysfunction. The possible role of other selectins in the pathogenesis of lung injury was recently suggested by the demonstration that binding of antibody to E- and L-selectins (EL-246) protected against lung injury in a porcine sepsis model (Ridings et al., 1995). Surprisingly, it was recently reported that the EL-246 antibody does not prevent lung injury or mortality in a septic baboon model (Carraway et al., 1998). In addition, various signs of deterioration, including decreased urine output and survival time, were observed in baboons treated with the EL-246 antibody. It remains to be determined whether the ineffectiveness and the toxicity observed in baboons is associated with the EL-246 antibody. In contrast, no toxicity has been observed with sialyl Lewis X-oligosaccharide and PB1.3, which were used in our study, at their effective doses in vivo (data not shown). In addition, these selectin inhibitors showed protective effects against lipopolysaccharide-induced tissue injury not only in the lung (this study) but also in other tissues including the kidney (Hayashi et al., unpublished observation). The present results suggest that the selectin inhibitors used in our study may provide a potential therapeutic treatment for patients with acute lung injury, including acute respiratory distress syndrome associated with sepsis.

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