Stimulation of Pulmonary Intravascular Macrophages Increases Microvascular Permeability in Awake Sheep

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SUZUKI, S., TANITA, T., KUBO, H., ASHINO, Y., CHIDA, M., KOIKE, K. and FUJIMURA, S. *Stimulation of Pulmonary Intravascular Macrophages Increases Microvascular Permeability in Awake Sheep.* Tohoku J. Exp. Med., 1993, **169** (2), 121-130 —— The purpose of this study is to determine if stimulation of pulmonary intravascular macrophages (PIMs) increase microvascular permeability in sheep. We infused latex microbeads, 1 μm in diameter, for 1 hr continuously and analysed lung hemodynamic and lymph-dynamic changes. More than 70% of latex microbeads in the lung were assigned to PIMs, and caught in their phagosomes as determined by morphological examination. This implies that infused latex microbeads predominantly stimulate PIMs. Pulmonary arterial pressure increased during the infusion period, and returned to baseline after the infusion period. Lung lymph flow increased and remained high while the lymph to plasma protein ratio ultimately increased above baseline. This implies that infusion of latex microbeads increases pulmonary microvascular permeability. The increase in lung lymph protein clearance was blocked completely by pretreatment with indomethacin, but not with a thromboxane synthetase inhibitor (OKY-046). These data indicate that the increase in microvascular permeability is mediated by an arachidonic acid cyclooxygenase metabolites but not by thromboxane. We conclude that PIMs can act as an initiator to increase pulmonary microvascular permeability by releasing arachidonic acid cyclooxygenase metabolites through their stimulation with latex beads. —— index terms ; sheep with chronic lung lymph fistulas; latex microbeads; arachidonic acid cyclooxygenase metabolites

Previous reports using sheep with chronic lung lymph fistulas have indicated that infusion of bacteria and endotoxin leads to increase in pulmonary microvascular permeability (Brigham et al. 1974, 1979). This response is interpreted as acute inflammation in the alveolar capillaries based on microscopic examinations

which revealed infiltration of inflammatory cells such as polymorphonuclear leukocytes (PMNs) (Meyrick and Brigham 1983). Although, many studies have focused on PMNs in the lung as the most important cell component for development of increased microvascular permeability, it is still unclear what serves as the initiator of acute inflammation which in turn leads to an increase in permeability of the alveolar capillaries.

In sheep, it has been indicated that almost all bacteria infused intra-arterially are localized rapidly in the lung, and they are seen within phagocytic cells in the alveolar capillaries named pulmonary intravascular macrophages (PIMs) (Warner and Brain 1986). Studies on clearance kinetics of the foreign bodies from blood stream have revealed that sheep lung have drastic clearance activities (Warner et al. 1987). It is now certain that PIMs are one of the more important components of the mononuclear phagocyte system in sheep and are similar to Kupffer cells in the liver of other species such as dogs and rats.

Previous study revealed that infusion of micro particles results in pulmonary hypertension by arachidonic acid metabolites in sheep (Albertine and Staub 1984). Recently, it has been confirmed that infused foreign bodies are assigned to PIMs selectively and PIMs are the most important source of chemical mediators such as arachidonic acid metabolites in acute inflammatory responses (Niehaus et al. 1984 ; Miyamoto et al. 1988). An in vitro study has indicated that PIMs can release a wide array of arachidonic acid metabolites when they are exposed to foreign bodies (Bertram et al. 1988). Some arachidonic acid metabolites constrict pulmonary arterial smooth muscle, increase microvascular permeability and increase adherence of PIMs to endothelial cells, and it is now certain that they could affect inflammatory responses in the lung (Goetzl 1981).

Thus, these previous studies lead us to hypothesize that PIMs serve as initiators of the inflammatory response. If this is true, PIMs stimulation would increase pulmonary microvascular permeability. The purpose of this study is to describe the effects of stimulation of PIMs by latex microbeads infusion on sheep lung hemodynamics and lymph-dynamics.

MATERIALS AND METHODS

Animal preparation

We prepared sheep with chronic lung lymph fistulas as described in other publications (Staub et al. 1985 ; Koike et al. 1986). In brief, male sheep (40-50 kg) were anesthetized with intravenous administration of thiopental sodium (10 mg/kg), intubated and maintained on constant volume (10 ml/kg) positive pressure ventilation (Model 600 ; Harvard Apparatus Co., Millis, MA, USA) with 2% halothane and 50% oxygen. We made a right thoracotomy and resected the tail of the caudal mediastinal lymphnode (CMN) at the level of the posterior margin of the pulmonary ligament, and placed a tube (602-155 ; Dow Corning Co., Midland, MI, USA) into the efferent duct of CMN. We inserted a thermal dilution pulmonary catheter (93A-131-7F ; Edwards Co., Santa Ana, CA, USA) into the main pulmonary artery via the right jugular vein, and cannulated the right carotid artery. Sheep were allowed to recover from the surgery for 5-7 days.

Experimental protocols

Distribution of latex microbeads. In three sheep, we infused 9.12×10^9 beads/kg of fluorescent latex microbeads (Polybeads 04-0171-54; Polyscience Inc., Warrington, PA, USA), 1 μm in diameter, through an arterial catheter over 1 hr. Immediately after the infusion, we sacrificed the sheep and removed the left lung for fixation by instillation of 2.5% glutaraldehyde in potassium phosphate buffer (pH 7.2) with 20 cm of water pressure. For fluorescent microscopic examination, sections 2 μm thick were examined with an excitation wavelength at 420-490 nm. A hundred fluorescent latex microbeads per section were identified as being phagocytized by pulmonary intravascular macrophages (PIMs), polymorphonuclear leukocytes (PMNs), or others. We also removed spleen and liver from the sheep, and examined by fluorescent microscopy as described earlier.

Latex microbeads infusion. In 5 sheep, after a 2 hr baseline period, we continuously infused 5.46×10^{10} beads/kg of latex microbeads, 1 μm in diameter (N-1000; Sekisui Kagaku Co., Tokyo) through an arterial catheter and observed the sheep for an additional 4 hr. The dosage of latex microbeads in 60 ml of saline were 5.46×10^{10} beads/kg in total. In addition we infused one tenth the number of latex microbeads (5.46×10^9 beads/kg in total) in another sheep (46 kg in body weight)

Arachidonic acid cascade inhibition. One hour prior to the period of latex microbeads infusion, we infused 3 mg/kg of indomethacin (Sigma Chemical Co., St Louis, MO, USA) with 5 mg/kg of anhydrous sodium carbonate in 30 ml of saline in 5 sheep, and 10 mg/kg of thromboxane synthetase inhibitor (OKY-046; Kissey Chemical Co., Tokyo) in 30 ml of saline in 4 other sheep, through the pulmonary catheter over a half hour, and followed the sheep responses over a 4 hr period.

Measurements

We measured pulmonary arterial pressure continuously, and pulmonary capillary wedge pressure every 15 min with a pressure transducer (P23ID; Gould Statham, Co., Hato Rey, Puerto Rico), and measured cardiac output by thermal dilution method using a cardiac output computer (9520; Edwards Co., Santa Ana, CA, USA) every 15 min. We measured lung lymph flow by weight every 15 min, and measured total protein concentration by biuret method on an analyser (AU-500; Olympus Co., Tokyo) for the determination of lymph to plasma protein concentration ratio (L/P) every 15 min. We counted peripheral white blood cells using a microcell counter (CC-800; Toa Co., Tokyo).

Data analyses

We calculated pulmonary vascular resistance (PVR) using the formula,

$$PVR = (PAP - PCW)/CO$$

where PAP and PCW are mean pulmonary arterial and pulmonary capillary wedge pressures, respectively, and CO is cardiac output.

We calculated lung lymph protein clearance (Clym) using the formula,

$$Clym = Qlym \times (L/P)$$

where Qlym is lung lymph flow by weight of 15 min, and L/P is lymph to plasma protein concentration ratio.

Data were expressed as a group of mean$\pm$S.D. Analysis of variance was used for statistical analysis. $p < 0.05$ was accepted as indicating statistical significance.

RESULTS

Distribution of latex microbeads. On fluorescent microscopic expansion, we could easily find numerous fluorescent microbeads spots on tissue sections from the

lung. In contrast, we could recognize only 0–2 spots per tissue section from the liver or spleen. Table 1 presents distribution of fluorescent microbeads in the lung. More than 70% of latex microbeads were assigned to PIMs, but only 5% to PMNs. We could not find latex bead aggregates or emboli in the pulmonary arteries or capillaries upon histological examination.

Latex microbeads infusion. Fig. 1 illustrates elapsed time of experiments of latex microbeads infusions. Latex microbeads infusion (5.46×10^{10} beads/kg in total) rapidly elevated pulmonary arterial pressure during the infusion period, and quickly returned to the baseline after cessation of beads infusion. Lung lymph flow began to increase during the infusion period, and remained high. L/P decreased transiently during the infusion period apparently due to high pulmonary microvascular pressure, and then increased to exceed the baseline values (thick lines). In contrast, the one tenth dose of latex microbeads (5.46×10^9 beads/kg in total) moderately elevated pulmonary arterial pressure with no
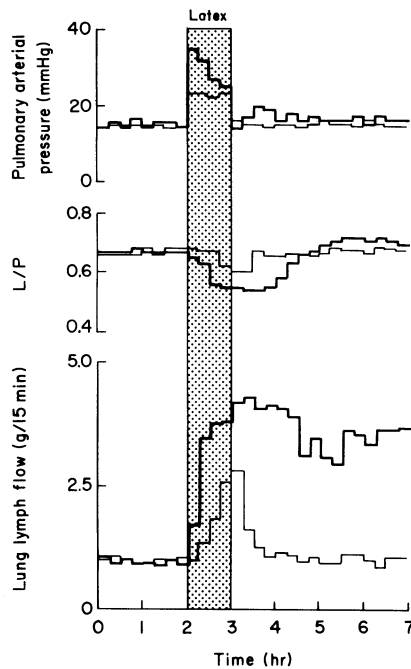

Fig. 1. Elapsed time experiment for latex microbeads infusions. Pulmonary arterial pressure markedly increased during the period of latex microbeads infusion (5.46×10^{10} beads/kg in total), then rapidly returned to baseline. Lung lymph flow began to increase during the infusion period, and remained high. L/P decreased transiently during the infusion period, then increased to exceed the baseline values (thick lines). In contrast, one tenth the amount of latex microbeads infusion (5.46×10^9 beads/kg in total) caused a moderate increase in pulmonary arterial pressure, and no observable changes in lung lymph flow or L/P (thin lines).

TABLE 1. *Distribution of latex microbeads in lungs*

Sheep	PIMs	PMNs	Others	Total
1	71	3	26	100
2	71	8	20	100
3	79	5	16	100

PIMs, pulmonary intravascular macrophages; PMNs, polymorphonuclear leukocytes.

changes in lung lymph flow or L/P (thin lines) (Fig. 1).

Arachidonic acid cascade inhibition. Fig. 2 illustrates one latex microbeads infusion after arachidonic acid cascade inhibition in vivo experiment. Latex microbeads infusion after indomethacin did not change pulmonary arterial pressure, lung lymph flow or L/P throughout the experimental period (thick lines).
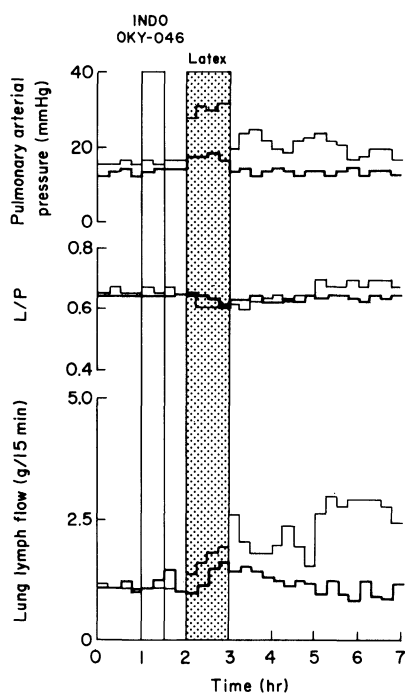

Fig. 2. Elapsed time of experiment for latex microbeads infusion after arachidonic acid cascade inhibition in vivo for 1 experiment. Latex microbeads infusion after indomethacin did not change pulmonary arterial pressure, lung lymph flow, or L/P (thick lines). In contrast, latex microbeads infusion after OKY-046 caused a moderate increase in pulmonary arterial pressure during the infusion period. Lung lymph flow began to increase during the infusion period and remained high. L/P decreased transiently during the infusion period, then increased to exceed baseline (thin lines).

In contrast, latex microbeads infusion after OKY-046 moderately increased pulmonary arterial pressure during the infusion period. Lung lymph flow increased after the infusion period, and remained high. L/P decreased slightly during the infusion period, and increased to exceed the baseline (thin lines) (Fig. 2).

Fig. 3 illustrates the effects of arachidonic acid cascade inhibition on the increase in pulmonary vascular resistance during the latex microbeads infusion period. Indomethacin essentially blocked PVR alterations, whereas, and OKY-046 blocked about 70% of the PVR changes. Fig. 4 illustrates the effects of arachidonic acid cascade inhibition on the increase in lung lymph protein clear-
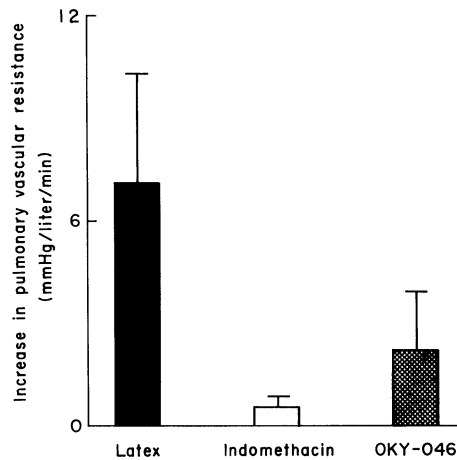
Fig. 3.   Effects of arachidonic acid cascade inhibition on the increase in pulmo-
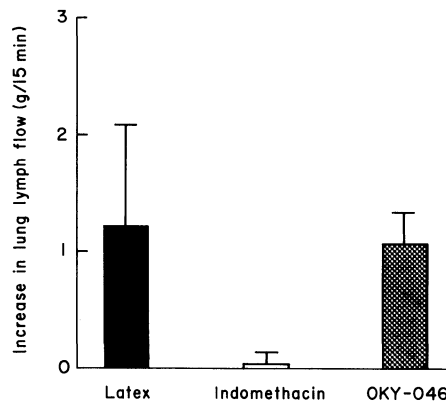nary vascular resistance during the infusion period.

Fig. 4.   Effects of arachidonic acid cascade inhibition on the increase in lung
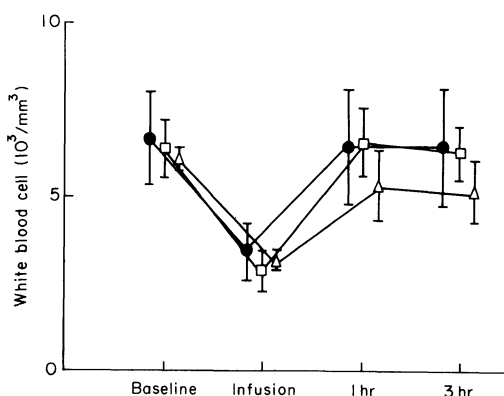lymph protein clearance 3 hr after the infusion period.

Fig. 5. Latex microbeads infusion decreased peripheral white blood cell count transiently (●). The magnitudes and duration of these changes were not influenced by indomethacin (□) or OKY-046 (△) pretreatment.

ance at 3 hr after the infusion period. Indomethacin completely inhibited the latex beads-induced increases in lung lymph protein clearance, yet OKY-046 had no significant effect.

Fig. 5 illustrates the change in peripheral white blood cell count. It decreased transiently during the infusion period, and returned to the baseline. These effects were not influenced by indomethacin or OKY-046.

DISCUSSION

The reasons why we choose latex microbeads are that they are not biochemically active such as endotoxin or phorbol acetate, and they are not fragile beads such as liposomes so that stimulation to PIMs may be continuous. The diameter of latex microbeads we infused in this study was 1 μm, and small enough to pass through all systemic capillaries. We could easily find numerous latex microbeads in the tissue sections from the lungs mainly in PIMs, but not in the spleen or liver on fluorescent microscopic examination. In addition, we could not find any aggregate or emboli of latex microbeads in the pulmonary arteries or alveolar capillaries. The latex microbeads found in PIMs were located in the phagosomes on electron microscopic examination. It has been reported that 85–90% of circulating foreign bodies in sheep rapidly sequester in the lung and that PIMs were the cause of the sequestration (Warner et al. 1986). We believe that intra-arterially infused latex microbeads selectively stimulated PIMs.

Intra-arterial infusion of latex microbeads resulted in lung hemodynamic and lymph-dynamic changes in sheep, and an apparent new steady state was reached in about 3 hr after the infusion period. Although the lung hemodynamic response during the infusion period was observed as dose dependent, the lung lymph effects after the infusion period were entirely different. Small amounts of latex microbeads (5.46×10^9 beads/kg in total) resulted in only slight increase in

lung lymph flow and decrease in L/P during and within 1 hr after the infusion period, and there were no changes at 3 hr after the infusion period. This small change in lung lymph-dynamics can be explained due to increase in pulmonary microvascular pressure (Erdmann et al. 1975). In contrast, a large amount of latex microbeads (5.46×10^{10} beads/kg in total) caused increases in both lung lymph flow and L/P at the new steady state. These data suggest an increase in pulmonary microvascular permeability (Staub et al. 1975; Albertine et al. 1984).

In this study, pretreatment with indomethacin or OKY-046 indicates that the changes in lung hemodynamics and lymph-dynamics were entirely mediated by arachidonic acid metabolites. The increase in pulmonary vascular resistance during the infusion period was blocked completely by indomethacin, and by 70% with OKY-046. This indicated that the pulmonary hypertensive response was mediated by arachidonic acid cyclooxygenase metabolites, especially TXA2. In contrast, there was significant difference between the effects of indomethacin and OKY-046 on the lung lymph-dynamics 3 hr after the infusion period. The increase in lung lymph protein clearance was completely blocked by indomethacin, but not by OKY-046. This strongly suggests that the increase in pulmonary microvascular permeability is mediated by another arachidonic acid cyclooxygenase product(s), but not TXA2.

Infusion of latex microbeads decreased circulating peripheral white blood cell count transiently. Disappearance of white blood cells from peripheral circulation is interpreted as PMN sequestration in alveolar capillaries (Staub et al. 1985). Thus, the recovery of peripheral white blood cell counts after the infusion period indicates that sequestrated PMNs in the lung were released into the peripheral circulation again. Therefore, the increase in microvascular permeability may be independent on PMNs in the lungs.

Indomethacin and OKY-046 failed to influence the changes in peripheral white blood cell count during latex microbeads infusion. This might be simply explained by leukocyte chemotactic effects of lipoxygenase products, for example, leukotrien (LT) B4. This would imply that sequestration of PMNs into the alveolar capillaries during the infusion of latex microbeads had little effect on the increase in microvascular permeability.

How do we combine our results to prove that stimulation of PIMs increased pulmonary microvascular permeability? It has been indicated that both lung clearance of circulating foreign bodies and pulmonary hypertension are entirely host species dependent (Crocker et al. 1981). These phenomena may be due to the predominancy of PIMs in those species more sensitive to the challenge of foreign bodies such as sheep, pigs and calves (Warner et al. 1986, 1987). Moreover, it has been shown that PIMs are the most important source of TXA2 for the pulmonary hypertensive response after the injection of liposomes (Miyamoto et al. 1988). An in vitro study on characterization of arachidonic acid metabolites released from PIMs have shown that there were at least eight identify metabolites including

prostaglandin (PG) E2 and others (Bertram et al. 1988). For example, PGE2 is known to have vasoactive properties, and is thought to contribute to the increase in microvascular permeability (Goetzl 1981). Some arachidonic acid cyclooxygenase products, except for TXA2, may play an important role in the increase in microvascular permeability after latex microbeads infusion. Unfortunately we were not able to identify the precise mediator(s) in this study. It is possible that there is a serial relation between cause and effect for these two results, one being stimulation of PIMs and the other being arachidonic acid metabolite(s), mediated the increase in pulmonary microvascular permeability. Moreover, it is also possible that PMN sequestration in the alveolar capillaries is only an effect of PIMs stimulation mediated by arachidonic acid lipoxygenase metabolites from PIMs, and does not directly cause the increase in microvascular permeability.

Is there another explanation for these results ? Histological examinations have shown that infused latex microbeads were rapidly phagocytized (within 1 hr) by PIMs, which would indicate that the beads were opsonized. Thus, it is possible that latex microbeads activate complement pathways and are opsonized by C3b. This would promote vasoactive complement fragment synthesis, for example C3a and C5a. Thus, we cannot rule out completely the possibility that C3a and C5a could participate in this model. It has been reported that complement mediated increases in microvascular permeability is entirely dependent on PMNs (Till et al. 1982). However, our results in this study suggest that latex microbeads infusion increases permeability independently of PMNs. Thus, we believe that the increase in pulmonary microvascular permeability is a direct effect of PIMs stimulation by latex microbeads. PIMs may serve as the initiator of the inflammatory response in alveolar capillaries.

Acknowledgment

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