Role of Intrapulmonary Release of Eicosanoids and Superoxide Anion as Mediators of Pulmonary Dysfunction and Endothelial Injury in Sheep with Intermittent Complement Activation

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SUMMARY. In 30 anesthetized sheep, we show that repeated bolus injections of autologous zymosan-activated plasma produce pulmonary hypertension, hypoxemia, intrapulmonary thromboxane release, pulmonary leukostasis, and sustained increases in lung lymph flow and protein clearance. Studies with platelet-rich plasma demonstrated that addition of zymosan-activated plasma does not induce platelet aggregation or thromboxane release. We studied the role of cyclooxygenase products as mediators of these pathophysiologica responses by pretreating sheep with either meclofenamate (4 mg/kg) or ibuprofen (12.5 mg/kg). Both drugs inhibited thromboxane release and hypoxemia. Ibuprofen, but not meclofenamate, reproducibly attenuated the hypertensive responses and the increases in lymph flow and protein clearance. Neither drug prevented pulmonary leukostasis. These results demonstrate that cyclooxygenase products mediate the development of complement-induced hypoxemia but are not sole mediators of pulmonary hypertension or increases in vascular permeability. Furthermore, ibuprofen has anti-inflammatory actions, not shared by meclofenamate, which enhance the effectiveness of this drug. Since activated leukocytes release reactive oxygen metabolites, we treated sheep with superoxide dismutase (2800 U/kg per hour) to determine the role of superoxide anions in these responses. This treatment significantly attenuated the increases in lung lymph flow and protein clearance. The results suggest that multiple mediators, which may originate from activated leukocytes sequestered in the pulmonary circulation, contribute to the pathophysiologica changes seen with intermittent complement activation. Cyclooxygenase products of arachidonic acid contribute to the hypertension and are solely responsible for the hypoxemia. Reactive oxygen metabolites are important mediators of the complement-induced increases in lung vascular permeability. (Circ Res 53: 574-583, 1983)

THE origin of the lung vascular injury seen after pulmonary embolism (Flick et al., 1981a), phorbol myristate acetate (Loyd et al., 1983), endotoxin (Helfin and Brigham, 1981), and zymosan-activated plasma (Gee et al., 1983) has been attributed to agents generated by activated leukocytes sequestered in the pulmonary circulation. These agents include vasoactive substances, such as cyclooxygenase and lipoxygenase products of arachidonic acid, and cytotoxic substances, such as reactive intermediates of oxygen metabolism.

A common sequence of pathophysiological events is shared by many of these models of lung injury. This begins with a generally transient, but severe, hypoxemia and pulmonary hypertension, as well as pulmonary leukostasis, and is then followed by a sustained increase in lung vascular permeability. Inhibition of the cyclooxygenase pathway of arachidonic acid eliminates the transient hypertension and hypoxemia without affecting the leukocyte sequestration or sustained change in endothelial permeability (Newman et al., 1982; Demling et al., 1981; Fountain et al., 1980; Gee et al., 1983). Snapper and coworkers (1983) recently presented evidence suggesting that the degree of endotoxin-induced leukopenia in sheep is related to the severity of the increase in lung vascular permeability. Other experiments have shown that treatment with superoxide dismutase, a free radical scavenger, prevents the endothelial injury caused by activated leukocytes (Sacks et al., 1978).

To define further the mechanisms responsible for these pathophysiological changes and the role of leukocytes in the acute lung injury, we developed a model using repeated injections of zymosan-activated plasma in anesthetized sheep. This model allows separation of the transient from the sustained events, and the findings suggest that the hypoxemia, acute pulmonary hypertension, and lung vascular injury are mediated by different factors released from sites such as activated leukocytes sequestered in the pulmonary circulation.

Methods

Animal Preparation

Adult sheep (n = 17) weighing 21.8–37.3 kg were anesthetized intravenously with pentobarbital (30 mg/kg)
and secured in the left decubitus position. We made a
tracheotomy and ventilated the sheep with room air at a
tidal volume of 10 ml/kg with 10 cm H2O end expiratory
pressure. Respiratory rate was set to maintain Paco2 at
about 35 mm Hg. One polyethylene catheter was placed
in the aorta to record systemic arterial pressure (AP) and
two were placed in the left jugular vein for drug and
complement infusion. A Swan-Ganz thermodilution cath-
eter was placed in the pulmonary artery via the right
jugular vein to record pulmonary artery (Ppa) and pul-
monary artery wedge (Pw) pressures and to measure
cardiac outputs by thermodilution (KMA, Inc., model
3500). Catheters were connected to transducers (Statham
P23DC) positioned at the level of the left atrium and
pressures were continuously recorded on a direct-writing
oscillograph (Grass model 7). After catheter placement,
heparin (100 U/kg) was administered via the pulmonary
artery catheter.

Through two right thoracotomies, we cannulated an
efferent vessel of the caudal mediastinal lymph node and
ligated the tail of the node to minimize extra-pulmonary
contamination (Staub et al., 1975). Pentobartibal was sup-
plemented as needed throughout the experiment. Lymph
was collected for 30-minute intervals in graduated centri-
fuge tubes treated with heparin and indomethacin. Arte-
rial blood samples and pressure records were taken at the
midpoint of each lymph collection period. Samples were
placed in tubes with heparin and indomethacin, centri-
fuged it for 15 minutes, and drawn off the cell-free
plasma (PRP) was injected as a 5 ml bolus, iv, every 30
minutes for 3 hours. To prepare ZAP, we took 100 ml of blood,
centrifuged it for 15 minutes, and drew off the cell-free
plasma. The plasma was incubated for 30 minutes at 37°C
with 1.0 mg/ml zymosan (Sigma Chemical Co.), centri-
fuged, and the zymosan-activated plasma separated from
the zymosan pellet.

In the first set of experiments, the sheep were separated
into experimental or untreated control (n = 5) groups. The experimental group received sodium meclofenamate (n = 4), or added
ZAP (n = 5) or superoxide dismutase (SOD)-
treated (n = 7) groups. For the SOD-treated group, three
sheep received SOD alone (2800 U/kg per hour) as a
constant infusion, beginning 30 minutes before the injec-
tion period and lasting throughout the period. Four sheep
received, in addition, 10,000 U heparin as a bolus injection
at the start of the SOD infusion. We found that the
presence of heparin made no difference to the results, so
we combined these sheep into the SOD-treated group.

Preparation of Platelet-Rich Plasma
Twenty milliliters of arterial blood were drawn from
seven sheep into a syringe containing 3.8% sodium citrate,
nine parts blood to one part citrate. The blood was centri-
fuged at 300 g for 15 minutes to prepare platelet-rich
plasma (PRP), placed in cuvettes, stirred, and heated to
37°C. Aggregation experiments were carried out using a
Chrono-log Whole-Blood Aggregometer (model 500).
Platelet aggregation was detected in a 1.0-ml sample of
PRP that contained less than 1% contaminating cells by
measuring infrared light transmission through the sample.
To the PRP we either made no addition (n = 4), or added
ADP (for a final concentration of 20 μM) (n = 7), collagen
(for a final concentration of 200 μg/ml) (n = 5), or 20 μl
ZAP (n = 7). After allowing 5 minutes for aggregation to
occur, the PRP was centrifuged to remove the platelet
aggregates and assayed for TxB2 and 6-keto-PGF1α
concentrations using the radioimmunoassays as described
above.

Data Analysis
Numerical data for groups are reported as means ± SEM.
We used a t-test for unpaired data to compare data from
the treatment groups with those from the control
group (Snedecor and Cochran, 1967). A t-test for paired
data was used to analyze data within a group. We accepted
a probability of less than 5% to indicate statistically sig-
nificant changes.
Results

Table 1 summarizes baseline values for all groups of sheep. There were no significant differences among the groups. Repeated bolus injections of zymosan-activated plasma result in highly reproducible transient increases in pulmonary artery pressure (Ppa). The rapid rise in Ppa is associated with a decrease in systemic arterial pressure (AP), apparently caused by the sudden increase in right ventricular afterload. Both pulmonary artery and systemic arterial pressures return to baseline values between injections.

Figure 1 shows the effect of cyclooxygenase inhibitors on peak Ppa values after repeated injections of activated plasma. In untreated control sheep, Ppa increased from baseline values of 28 ± 3 cm H2O to 72 ± 5 cm H2O after the first injection and to an average of 62 ± 4 cm H2O after the last five injections. Meclofenamate pretreatment significantly attenuated the increase in Ppa seen after the first injection to 47 ± 9 cm H2O. After the remaining five injections, however, the peaks in Ppa reached values not significantly different from those seen in untreated control sheep. Ibuprofen pretreatment significantly attenuated the peak in Ppa after the first injection to 54 ± 6 cm H2O and after the subsequent injections to an average of 47 ± 7 cm H2O. Figure 1 also illustrates the peaks in Ppa after treatment with superoxide dismutase (SOD). In this set of experiments, the Ppa values in untreated control sheep increased from baseline values of 24 ± 2 cm H2O to 72 ± 5 cm H2O after the first injection and to an average of 62 ± 4 cm H2O after the last five injections. Treatment with SOD had no effect on these pressure changes.

Figure 2 shows the effect of cyclooxygenase inhibition on the hypoxemia associated with repeated injections of activated plasma. In untreated control sheep, PacO2 fell from baseline values of 75 ± 3 mm Hg to an average of 68 ± 2 mm Hg during the injection period. Sheep pretreated with either meclofenamate or ibuprofen did not show any decrease in PacO2 from baseline values of 85 ± 3 mm Hg and 83 ± 6 mm Hg, respectively. Figure 2 also shows the effect of SOD treatment on the hypoxemia. PacO2 values in this group of untreated control sheep fell from baseline values of 83 ± 4 mm Hg to an average of 55 ± 3 mm Hg during the injection period. SOD treatment had no effect on this response.

The effect of repeated injections of activated
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plasma on TxA2 (as TxB2) and PGI2 (as 6-keto-PGF1α) lymph and plasma concentrations in untreated control sheep is shown in Figure 3. TxB2 concentrations were significantly increased after the first three injections in plasma and all six injections in lymph. In contrast to the remarkable reproducibility of the peaks in pulmonary artery pressure, TxB2 concentrations were highest after the first injection and progressively declined with subsequent injections. PGI2 concentrations in plasma and lymph did not change throughout the injection period. Pretreatment with either meclofenamate or ibuprofen abolished any increase in TxB2 concentrations in both plasma and lymph (Fig. 4). This is in contrast to the differing efficacies of the two cyclooxygenase inhibitors in modifying the increase in pulmonary artery pressure.

Figure 5 shows the effect of SOD treatment on the changes in TxB2 concentration in both plasma and lymph after repeated injections of activated plasma. In this group of untreated control sheep, TxB2 levels in plasma were significantly increased after the first five injections but did not increase after the sixth injection. TxB2 levels in lymph were significantly increased throughout the injection period. PGI2 concentrations were unchanged during the injection period. SOD treatment did not affect the change in TxB2 concentrations from those seen in untreated sheep. In addition, SOD treatment had no effect on PGI2 levels in plasma or lymph.

To investigate further the source of the TxB2, we took simultaneous systemic arterial and mixed venous blood samples (AV samples) immediately before and after each injection. Figure 6 summarizes the changes in pulmonary artery pressure and AV

Figure 3. Effect of 5-ml bolus injections of zymosan-activated plasma (numbers 1-6) on TxB2 and PGI2 (as 6-keto-PGF1α) concentrations in lymph and plasma in untreated control sheep. Vertical bars are ± 1 SEM. Brackets indicate levels significantly different from baseline at P < 0.05.

Figure 4. Effect of 5-ml bolus injections of zymosan-activated plasma on TxB2 concentrations in plasma and lung lymph in untreated control sheep and sheep treated with meclofenamate or ibuprofen.

Figure 5. Effect of 5-ml bolus injections of zymosan-activated plasma (numbers 1-6) on TxB2 concentrations in plasma and lung lymph in untreated control and SOD-treated sheep. Vertical bars are ± 1 SEM. Brackets indicate levels significantly different from baseline at P < 0.05.
plasma TxB₂ concentrations with each injection. The developed pulmonary hypertension was similar to that found in the other two groups of untreated control sheep in that the severity of the response was reproducible after each injection and pressures returned to baseline between injections. TxB₂ concentrations in systemic arterial blood increased at the peak of each pressor response and fell toward baseline levels between injections. TxB₂ concentrations in mixed venous blood increased during the injection period but the increases were significantly less than those in systemic arterial blood. These data indicate that TxA₂ was synthesized and released within the pulmonary circulation.

Tables 2 and 3 show the effect of repeated injections of activated plasma on circulating levels of white blood cells expressed as experimental over baseline values. Baseline leukocyte counts in all groups of sheep are shown in Table 1. In Table 2, untreated sheep from the first set of experiments showed a reproducible decrease in cell numbers of 45 ± 6% after each injection, followed by a rebound increase in numbers to values progressively greater than baseline. Meclofenamate pretreatment did not alter this response. In sheep pretreated with ibuprofen, however, cell numbers did not rebound to levels greater than baseline. Table 3 shows that treatment with SOD had no effect on the leukocyte response. In these untreated control sheep, circulating leukocyte counts decreased by 60 ± 1% after each injection.

In addition to illustrating AV differences in TxB₂ concentration, Figure 6 also summarizes changes in AV leukocyte counts with each injection of activated plasma. After each injection, leukocytes in systemic arterial blood decreased in a similar manner to the other two groups of untreated sheep. Cell counts in mixed venous blood did not decrease, indicating that the decrease seen in arterial blood is due to

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Untreated control sheep</th>
<th>Meclofenamate-treated sheep</th>
<th>Ibuprofen-treated sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection 1</td>
<td>58 ± 4</td>
<td>68 ± 5</td>
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<tr>
<td>Recovery 1</td>
<td>112 ± 9</td>
<td>119 ± 11</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>Injection 2</td>
<td>59 ± 4</td>
<td>59 ± 11</td>
<td>38 ± 12</td>
</tr>
<tr>
<td>Recovery 2</td>
<td>114 ± 8</td>
<td>116 ± 4</td>
<td>101 ± 7</td>
</tr>
<tr>
<td>Injection 3</td>
<td>64 ± 5</td>
<td>83 ± 11</td>
<td>40 ± 11</td>
</tr>
<tr>
<td>Recovery 3</td>
<td>120 ± 11</td>
<td>125 ± 10</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>Injection 4</td>
<td>54 ± 6</td>
<td>54 ± 6</td>
<td>37 ± 13</td>
</tr>
<tr>
<td>Recovery 4</td>
<td>130 ± 13</td>
<td>132 ± 5</td>
<td>97 ± 7</td>
</tr>
<tr>
<td>Injection 5</td>
<td>69 ± 13</td>
<td>65 ± 9</td>
<td>33 ± 10</td>
</tr>
<tr>
<td>Recovery 5</td>
<td>148 ± 15</td>
<td>141 ± 13</td>
<td>101 ± 8</td>
</tr>
<tr>
<td>Injection 6</td>
<td>78 ± 18</td>
<td>64 ± 10</td>
<td>31 ± 9</td>
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<tr>
<td>Recovery 6</td>
<td>180 ± 25</td>
<td>152 ± 12</td>
<td>102 ± 9</td>
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Values shown are mean ± 1 SEM. Injections refer to 5-ml intravenous boluses of zymosan-activated plasma. These samples were taken 5 minutes after each injection. The recovery samples were obtained 25 minutes after the preceding injection.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Untreated control sheep</th>
<th>Superoxide dismutase-treated sheep</th>
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</thead>
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<tr>
<td>Injection 1</td>
<td>46 ± 5</td>
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<td>Recovery 3</td>
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<tr>
<td>Injection 4</td>
<td>51 ± 6</td>
<td>49 ± 8</td>
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<tr>
<td>Recovery 4</td>
<td>120 ± 20</td>
<td>116 ± 8</td>
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<tr>
<td>Injection 5</td>
<td>43 ± 9</td>
<td>50 ± 8</td>
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<tr>
<td>Recovery 5</td>
<td>127 ± 24</td>
<td>123 ± 9</td>
</tr>
<tr>
<td>Injection 6</td>
<td>40 ± 8</td>
<td>50 ± 11</td>
</tr>
<tr>
<td>Recovery 6</td>
<td>136 ± 27</td>
<td>138 ± 15</td>
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</table>

Values shown are means ± 1 SEM. Injections refer to 5-ml intravenous boluses of zymosan-activated plasma. These samples were taken 5 minutes after each injection. The recovery samples were obtained 25 minutes after the preceding injection.
aminate pretreatment delayed, but did not prevent, the increases in lymph flow and lymph protein clearance. In contrast, ibuprofen pretreatment attenuated the increases in both \( Q_l \) and \( Q_l \times L/P \) to 2.0 ± 0.3 times baseline, which is significantly different from the increase seen in untreated control sheep.

Figure 8 shows the effect of SOD treatment on changes in lymph flow and lymph protein clearance. In these untreated control sheep, \( Q_l \) and \( Q_l \times L/P \) increased 5.0 ± 0.3 and 4.6 ± 0.4 times baseline, respectively. SOD treatment significantly attenuated the increases in \( Q_l \) and \( Q_l \times L/P \) to 2.9 ± 0.2 and 2.6 ± 0.2 times baseline, respectively. This occurred even though SOD treatment had no effect on the pulmonary hypertension.

**Discussion**

In these experiments, we describe the pulmonary response of intact sheep to repeated bolus injections of zymosan-activated plasma. Zymosan, a yeast polysaccharide activates complement by the alternate pathway (Gotze and Muller-Eberhard, 1971). The present studies confirm those of Cooper et al. (1980) that intravenous administration of zymosan-activated plasma causes leukocyte sequestration in the pulmonary circulation. In many respects, the responses we observed are similar to those found in other models of acute lung injury including endotoxin (Brigham et al., 1979; Demling et al., 1981), phorbol myristate acetate (Loyd et al., 1983), and \( \alpha \)-naphthylthiourea (ANTU) (Havill et al., 1982). The unique feature of the present model is that by repeated exposure to a short-lived agent, complement fragments, we show that some of the transient effects, notably pulmonary hypertension and leukostasis, are remarkably reproducible. This model, therefore, presents an ideal opportunity to study mediators of each of the pathophysiological changes independently of one another.

**Table 4**

<table>
<thead>
<tr>
<th>Addition to PRP</th>
<th>Aggregation response</th>
<th>Thromboxane release (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>0.47 ± 0.27</td>
</tr>
<tr>
<td>ZAP (20 μl)</td>
<td>None</td>
<td>0.67 ± 0.20</td>
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<tr>
<td>(n = 9)</td>
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<tr>
<td>ADP (20 μm)</td>
<td>Aggregation</td>
<td>2.57 ± 0.95</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
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<tr>
<td>Collagen (200 μg/ml)</td>
<td>Aggregation</td>
<td>20.32 ± 3.05</td>
</tr>
</tbody>
</table>

Thromboxane concentrations are means ± 1 SEM. PRP, platelet-rich plasma; ZAP, zymosan-activated plasma; ADP, adenosine diphosphate.

**Figure 7.** Effect of 5-ml bolus injections of zymosan-activated plasma (numbers 1–6) on lymph flow and lymph protein clearance expressed as experimental over baseline values in untreated control sheep (C) and sheep treated with meclofenamate (M) or ibuprofen (I). Vertical bars are ± 1 SEM. Brackets indicate levels significantly different from respective control values at \( P < 0.05 \).

**Figure 8.** Effect of 5-ml bolus injections of zymosan-activated plasma (numbers 1–6) on lymph flow and lymph protein clearance expressed as experimental over baseline values in untreated control sheep (C) and sheep treated with SOD (S). Vertical bars are ± 1 SEM. Brackets indicate levels significantly different from respective control values at \( P < 0.05 \).

Leukocyte sequestration in the pulmonary circulation.

Table 4 summarizes results obtained with in vitro experiments on platelet-rich plasma (PRP). PRP alone or with 20 μl of autologous activated plasma produced no aggregation or thromboxane release. Further additions of activated plasma to the same sample were also without effect. Addition of ADP produced aggregation and a variable thromboxane release response such that there was no significant increase compared to PRP alone. Collagen aggregated platelets and was a potent stimulus for synthesis and release of thromboxane \( A_2 \). These results suggest that platelets are not the source of the TxA2 release after injections of activated plasma.

In these experiments, we describe the pulmonary response of intact sheep to repeated bolus injections of zymosan-activated plasma. Zymosan, a yeast polysaccharide activates complement by the alternate pathway (Gotze and Muller-Eberhard, 1971). The present studies confirm those of Cooper et al. (1980) that intravenous administration of zymosan-activated plasma causes leukocyte sequestration in the pulmonary circulation. In many respects, the responses we observed are similar to those found in other models of acute lung injury including endotoxin (Brigham et al., 1979; Demling et al., 1981), phorbol myristate acetate (Loyd et al., 1983), and \( \alpha \)-naphthylthiourea (ANTU) (Havill et al., 1982). The unique feature of the present model is that by repeated exposure to a short-lived agent, complement fragments, we show that some of the transient effects, notably pulmonary hypertension and leukostasis, are remarkably reproducible. This model, therefore, presents an ideal opportunity to study mediators of each of the pathophysiological changes independently of one another.
Studies with single exposures to endotoxin (Demling et al., 1981), ANTU (Gee et al., 1981), and activated plasma infusion (Fountain et al., 1980; Gee et al., 1983) clearly demonstrated that treatment with cyclooxygenase inhibitors significantly attenuates the resulting pulmonary hypertension and hypoxemia without affecting the pulmonary sequestration of leukocytes. With the present model, it is possible to separate further the mediation of the pulmonary vasoconstriction from that of the hypoxemia. Meclofenamate, at the dose used, effectively inhibited cyclooxygenase activity. Pretreatment with this drug was not effective, however, in preventing the complement-induced pulmonary hypertension. The hypertension produced after first exposure to activated plasma was attenuated by pretreatment with meclofenamate, confirming published reports using single exposure-activated plasma infusions. By repeating the exposure, however, we found that mediators other than cyclooxygenase products of arachidonic acid are involved in producing this response.

Ibuprofen also effectively inhibited cyclooxygenase activity at the dose used. Both meclofenamate and ibuprofen attenuated the pulmonary hypertension after the first exposure to activated plasma to a similar degree. The difference between the initial pressor response in these two groups compared with that in untreated sheep, then, can be attributed to cyclooxygenase products. However, ibuprofen was more effective than meclofenamate in reproducibly attenuating the induced pulmonary vasoconstriction after subsequent injections. Apparently, ibuprofen has effects other than cyclooxygenase inhibition which are not shared by meclofenamate. Ibuprofen may be either inhibiting release or antagonizing the actions of other vasoconstricting mediators. One possibility is that ibuprofen, like many cyclooxygenase inhibitors, also inhibits the lipoxygenase pathway of arachidonic acid. Activated white blood cells release products of the 5-lipoxygenase pathway, including 5-hydroxyeicosatetraenoic acid (5-HETE) (Borgeat et al., 1976) and leukotrienes B4 and C4 (Borgeat and Samuelsson, 1979). HETEs are chemotactic for neutrophils (Goetzl et al., 1980), while leukotriene B4 is one of the most potent chemotactic and aggregating agents for neutrophils known. Leukotriene C4 is a major component of slow-reacting substance of anaphylaxis (SRS-A) which apparently causes constriction of vascular smooth muscle and increases in vascular permeability (Radmark et al., 1980; Samuelsson, 1983).

Other investigators, using isolated perfused lung systems, showed that oxygen radicals generated by activated granulocytes can cause pulmonary vasoconstriction (Tate et al., 1982). Furthermore, Simchowitz et al. (1979) showed that ibuprofen inhibits superoxide generation in vitro by neutrophils activated with N-formyl-methionyl-leucyl-phenylalanine (FMLP), a potent synthetic chemotactic factor for neutrophils. However, superoxide anions do not appear to be the vasoconstricting agent in the present experiments, since treatment with superoxide dismutase had no effect on the pulmonary pressor responses.

Although more than one mediator appears to be responsible for the pulmonary hypertensive responses, the mechanism for initiation of the hypoxemia seems much clearer. Inhibition of cyclooxygenase activity with either meclofenamate or ibuprofen was an effective means of preventing the hypoxemia. Snapper and coworkers (1981) showed that pretreatment with cyclooxygenase inhibitors prevents acute bronchoconstriction in sheep given endotoxin. We have shown that pretreatment with imidazole, a thromboxane synthetase inhibitor, is effective in preventing hypoxemia in sheep given ANTU (Gee et al., 1981). It is likely that the hypoxemia, in at least these three models, is mediated by cyclooxygenase products of arachidonic acid, the most potent of which is TXA2. Studies with specific thromboxane synthetase inhibitors, however, are required to test this hypothesis.

The source of TXA2 synthesis and release remains to be determined. TXA2 is actively synthesized and released by platelets. Our in vitro experiments with platelet-rich plasma suggest that platelets may not be the source of TXA2 after exposure to zymosan-activated plasma. This confirms in vivo experiments in sheep where platelet depletion did not alter the response to activated plasma infusion (McDonald et al., 1983). However, these studies do not rule out the possibility that platelets may be activated to release thromboxane as a secondary response, perhaps induced by mediators released by complement-activated leukocytes. By comparing differences in arterial and venous TXB2 levels, we determined that the pulmonary circulation is the site of TXA2 synthesis and release. Endothelial cells have been shown to synthesize TXA2 as well as PGI2 (Ingerman-Wojenski et al., 1981). McDonald et al. (1983) found that lung parenchymal cells and pulmonary artery strips are capable of producing both TXA2 and PGI2 after incubation with arachidonic acid. Another possibility is that activated leukocytes sequestering in the lung after each injection may be responsible for the increases in TXB2 concentration. Human polymorphonuclear leukocytes have been shown to synthesize TXA2 in vitro after incubation with zymosan-activated serum (Goldstein et al., 1978). Other white cell populations may also contribute to the TXA2 release. Both human lymphocytes (Parker et al., 1979) and alveolar macrophages (Chang et al., 1982; Laviolette et al., 1983) synthesize and release TXA2 after exposure to appropriate stimuli.

Cooper et al. (1980) showed that leukocytes sequester in the pulmonary circulation during intra-venous infusion of activated plasma. Our results confirm and extend these findings. Leukocytes will repeatedly sequester in the pulmonary circulation to the same degree after the sixth exposure to activated plasma as after the first exposure. Furthermore,
there is some tendency for cell counts to rebound between exposures to levels progressively greater than baseline counts. Upon single exposure to activated plasma or purified C5a, this rebound phenomenon is sustained over many hours and has been attributed to demargination and cell recruitment (Webster et al., 1982; Craddock et al., 1977). Treatment with meclofenamate or superoxide dismutase had no effect on leukocyte responses to activated plasma, while ibuprofen did appear to alter these responses. Sequestration of cells occurred with each exposure, indicating that ibuprofen did not inhibit aggregation in response to activated plasma. Jacob and coworkers (1982) showed that ibuprofen had a dose-dependent inhibitory effect on complement-induced granulocyte aggregation in vitro. We were unable to demonstrate this effect in vivo. Ibuprofen did appear to prevent the rebound in cell numbers. This may indicate that ibuprofen inhibits release of a chemotactic or a cell mobilization factor or interferes with the action of this factor.

The 4- to 5-fold increases in both lung lymph flow and protein clearance in the control groups of sheep suggest that intermittent complement activation with leukocyte sequestration increases lung vascular permeability to fluid and protein. We assume that the increases in filtration are magnified by the transient increases in pulmonary artery pressure, but this relationship is complex. For example, when comparing the two untreated control groups, lymph flow increased to the same extent in both groups despite a 15 cm H2O difference in the average peak values of pulmonary artery pressure (Fig. 9). In contrast, the increase in lymph flow in the SOD-treated group was attenuated 45% from the control values even though the pulmonary pressor response was the same in the SOD-treated and the untreated control sheep. Ibuprofen attenuated the increase in lymph flow 65% from control values, while peak pulmonary artery pressures averaged 30 cm H2O less than control. In view of these results, it seems unlikely that the dramatic decrease in lymph flow with ibuprofen can be attributed solely to the attenuated pulmonary pressor responses. The relationship between lymph flow and pulmonary artery pressure is difficult to interpret in the meclofenamate group because the pattern of lymph flow increase is different from that seen in any of the other groups. The increase in lymph flow was delayed longer than was expected, based on the rapid recovery of the pressor responses and the rates of increase seen in the control and the SOD-treated groups. Furthermore, toward the end of the injection period, a clear steady state in the lymph flow increase had not been established so that flow was apparently continuing to climb. Comparison of the results obtained with the two cyclooxygenase inhibitors suggests that ibuprofen, but not meclofenamate, attenuated the increase in vascular permeability. Ibuprofen did not change the pattern of the lymph response, but the increases were significantly attenuated throughout the experimental period. These results support conclusions made on the basis of other studies of acute lung injury that cyclooxygenase products do not mediate sustained increases in lung vascular permeability (Newman et al., 1982; Ogletree et al., 1979; Gee et al., 1981).

Treatment with superoxide dismutase had no effect on the pulmonary pressor responses, whereas it significantly attenuated increases in lymph flow and protein clearance. This indicates that SOD treatment did, in fact, attenuate the increase in vascular permeability. These data suggest that generation of superoxide anions by activated leukocytes sequestered in the pulmonary circulation may be a primary event initiating vascular endothelial injury. This confirms studies by Till et al. (1981) in rats with activation of the complement system by cobra venom factor. Rats which were neutrophil depleted or pretreated with superoxide dismutase showed a significant attenuation of lung injury, as assessed by leakage of 125I-rat IgG into the extravascular space and by morphological studies. Sacks et al. (1978) showed that complement-activated leukocytes in vitro cause endothelial cell damage which is prevented by superoxide dismutase. Leukocyte activation and subsequent release of superoxide radicals have also been implicated in other models of lung injury such as glass bead embolization (Johnson and Malik, 1982) and

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**Figure 9.** Relationship between lymph protein clearance and peak pulmonary artery pressure (Ppa) seen after the sixth bolus injection of zymosan-activated plasma in untreated control sheep (C) and sheep treated with meclofenamate (M), ibuprofen (I), or SOD (S). Vertical bars are ± 1 SEM. An asterisk indicates lung lymph protein clearance significantly different from control values.

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air embolization (Flick et al., 1981a, 1981b). In addition to superoxide anion, hydrogen peroxide has also been implicated as a mediator of lung injury (Johnson and Ward, 1982). Further studies using catalase, which catalyzes the conversion of hydrogen peroxide to water, must be made to address this issue in this particular model.

Therefore, cyclooxygenase products affect changes in vascular permeability indirectly through alterations in hydrostatic gradients favoring net fluid filtration, and, perhaps, by alterations of perfused surface area. In contrast, generation of toxic oxygen metabolites can induce increases in endothelial permeability without directly affecting hydrostatic gradients.

In this and several other models of acute lung injury, four pathophysiological changes are apparent: hypoxemia, pulmonary hypertension, pulmonary leukostasis, and sustained increases in vascular permeability. Of these, hypoxemia is clearly mediated by synthesis and release of cyclooxygenase products of arachidonic acid. Acute pulmonary hypertension is induced by a more complex series of events, only partially dependent on release of cyclooxygenase products. With intermittent complement activation, as with endotoxin, air emboli, and thromboembolism, pulmonary leukostasis seems to be a key event in the initiation of endothelial permeability increases. These cells, activated and trapped within the pulmonary circulation, are a probable source of vasoactive substances and toxic oxygen metabolites.

Ibuprofen, in doses that inhibit cyclooxygenase activity, exerts other effects not shared by meclofenamate. Ibuprofen seems to have direct effects on the activity, exerts other effects not shared by meclofenamate. Ibuprofen seems to have direct effects on the activity, exerts other effects not shared by meclofenamate.

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Role of intrapulmonary release of eicosanoids and superoxide anion as mediators of pulmonary dysfunction and endothelial injury in sheep with intermittent complement activation.

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