Neutrophil Depletion Does Not Prevent Oxygen-Induced Lung Injury in Rabbits*

J. Usha Raj, M.D.; and Richard D. Bland, M.D.

Recent reports suggest that polymorphonuclear leukocytes play an important role in many types of lung injury, including endothelial damage and edema from prolonged oxygen breathing. Other studies indicate that pulmonary endothelial injury from oxygen can occur even in the absence of neutrophils. We therefore designed experiments to see if neutrophil depletion would prevent or reduce the severity of lung injury caused by prolonged oxygen breathing. We found that neutropenia, induced by nitrogen mustard, had no effect on survival time or lung water content of adult rabbits that continuously breathed pure oxygen at 1 atmosphere of pressure.

We used 38 New Zealand white rabbits that we divided into 4 groups. We pre-treated 11 rabbits (group 1) with nitrogen mustard intra-arterially, 1.5 mg/kg body weight every third day, until the circulating neutrophil count decreased to <50 cu mm of blood, after which we placed the rabbits in oxygen. Nine other rabbits (group 2) received no nitrogen mustard and had normal leukocyte counts in their peripheral blood during oxygen breathing. We killed 18 additional control rabbits that had no supplemental oxygen: 9 were neutropenic from nitrogen mustard (group 3) and 9 were untreated (group 4).

We placed a catheter in the aorta of all the rabbits so that we could give drugs and measure systemic blood pressure and partial pressures of oxygen and carbon dioxide in arterial blood. We also placed a Swan-Ganz catheter in the pulmonary artery of 3 rabbits in each group so that we could measure their pulmonary arterial pressure. All rabbits received antibiotics daily. We studied them one at a time in a Lucite chamber, through which flowed either oxygen (partial pressure >710 mm Hg) or air. While the rabbits breathed oxygen, we made daily measurements of vascular pressures, circulating neutrophils, arterial pH and partial pressures of oxygen and carbon dioxide in arterial blood. When the rabbits died, we removed their lungs for measurement of extravascular water, and we froze a block of tissue in liquid nitrogen for microscopy. In addition, we fixed in formalin a piece of lung from four rabbits in each group, and we made thin sections for determining the number and distribution of neutrophils in the lung.

There was no significant difference between neutropenic and non-neutropenic oxygen-treated rabbits with respect to vascular pressures, arterial pH and partial pressures of oxygen and carbon dioxide in arterial blood. Table 1 is a summary of results. All oxygen-treated rabbits died of respiratory failure from pulmonary edema. Neutrophil depletion decreased the number of neutrophils in the lungs of rabbits that breathed oxygen, but it had no significant effect on survival time or lung water content. Air-breathing rabbits, treated with nitrogen mustard and killed after

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Table 1—Summary of Results for Neutrophil-Depletion Studies in Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Nitrogen Mustard Rx</th>
<th>Inspired Gas</th>
<th>Circulating Neutrophils/mm³</th>
<th>Neutrophils in Lung</th>
<th>Extravascular Lung Water</th>
<th>Survival Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>O₂</td>
<td>17 ± 19</td>
<td>6 ± 7</td>
<td>6.7 ± 1.5</td>
<td>75 ± 10</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>O₂</td>
<td>422 ± 2119</td>
<td>62 ± 30</td>
<td>6.7 ± 1.3</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>Air</td>
<td>29 ± 26</td>
<td>0</td>
<td>3.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>Air</td>
<td>7633 ± 2967</td>
<td>2 ± 3</td>
<td>3.7 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Numbers are mean ± 1 standard deviation.

64 ± 21 hours of neutropenia had no pulmonary edema.

We conclude that neutrophil depletion does not prevent or reduce the severity of oxygen-induced lung injury in adult rabbits.

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REFERENCES


Pulmonary microembolization is a characteristic feature of the adult respiratory distress syndrome. The microemboli consist of fibrin clots and of leukocytes and platelets trapped in small pulmonary vessels. Previous studies have characterized the effects of pulmonary microembolization on lung fluid and protein exchange. It is clear now that embolization by whatever means increases the pulmonary vascular permeability to proteins indicating that pulmonary microembolization leads to lung vascular injury. This phenomenon is observed in both sheep and dog lymph preparations.

In this report, we summarize the effects of thrombin-induced pulmonary microembolization on lung fluid and protein exchange and our results dealing with the mechanisms by which these alterations are induced. Thrombin was chosen as a method for inducing pulmonary microembolization because it converts fibrinogen directly to fibrin, which in turn results in the activation of plasmin, and finally the activation of the complement system. The lung vascular injury after pulmonary microembolization is the complex effect of activation of these systems. In these studies we have examined the independent effects of some of the key blood-borne factors in the mediation of lung vascular injury. Studies were made in intact sheep in which pulmonary lymph was collected.

**ROLE OF BLOOD COMPONENTS IN MEDIATING LUNG VASCULAR INJURY AFTER PULMONARY VASCULAR THROMBOSIS**

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**PLATELETS**

We examined the effect of platelets in mediating the increase in lung vascular permeability after pulmonary microembolization by depleting circulating platelets. The platelet count was decreased by 97% of its baseline value in sheep using anti-platelet serum prepared against sheep platelets. Despite the thrombocytopenia, pulmonary lymph flow increased after thrombin infusion and there was no change in the lymph-to-plasma protein concentration ratio (Table I). This response was similar to that observed in the control group after thrombin (Table I). Platelet depletion did not prevent the thrombin-induced increase in lung vascular permeability since raising the pulmonary microvascular pressure (Pmv) by inflation of a left atrial balloon produced a relatively large increase in lymph flow without a change in the lymph-to-plasma protein concentration ratio; this response was the same as observed in control thrombin group after the increase in Pmv. Therefore, platelet depletion per se did not prevent the thrombin-induced increase in lung vascular permeability, suggesting that platelet aggregation did not mediate the response.

**GRANULOCYTES**

The role of granulocytes was examined by selectively depleting the circulating granulocyte levels with hydroxyurea (200 mg/kg per day for 4 days). Hydroxyurea did not