
**Pulmonary Vasoconstriction and Profound Leukopenia in Two Sheep Experimental Models**

**Effects of Complement Depletion**


In the awake sheep, the acute and transient increase of pulmonary vascular resistance following either venous cardiopulmonary bypass or *E. coli* endotoxin infusion is primarily mediated by pulmonary synthesis of thromboxane. Inhibition of pulmonary vasoconstriction by pretreatment with either cyclooxygenase or thromboxane synthetase inhibitors does not prevent the leukopenia resulting from either bypass or endotoxin. Thus, certain metabolic similarities exist between these two models, although their fundamental mechanisms probably differ. Intravascular activation of complement may be central to both models since: 1) intravenous infusion of autologous plasma in which complement was activated with zymosan has been reported to produce acute eicosanoid-mediated pulmonary vasoconstriction and leukopenia in the awake sheep; 2) both bacterial endotoxins and exposing blood to polymer surfaces can activate plasma complement; 3) C5 fragments resulting from complement activation induce polymorphonuclear leukocyte aggregation in vitro. To compare the mechanisms of arachidonate activation and leukopenia, we depleted sheep of complement with cobra venom factor prior to either bypass or endotoxin infusion.

**METHODS**

Experiments were carried out in 13 awake Suffolk sheep weighing 24-34 kg. Intravascular catheterization allowed continuous measurements of pulmonary and systemic vascular pressures. Cardiac output was measured by thermodilution. ECMO was performed by partial veno-venous bypass in heparinized sheep using a new 0.8 M² spiral coil Silicone membrane lung and an occlusive roller pump. The membrane lung was ventilated with 100% oxygen and pump flow averaged 750 ml/min. *E. coli* endotoxin (LPS W, 0111:B4, Difco) 1 µg/kg was intravenously infused over 5 min. Sheep were depleted of complement over 3 days by serial intravenous injections of cobra venom factor (10 U/kg × 7, Naja Haje, Cordis, Miami, FL). Total hemolytic sheep complement (CH50) levels were measured using rabbit red cells sensitized with goat anti-rabbit red cell antibody. Sheep C5 levels were measured by a radial immunodiffusion assay using rabbit anti-sheep C5 antibody (Cappel Lab, Cochranville, PA). Plasma TxB₂ was assayed with a double antibody radioimmunoassay technique. Leukocytes were counted with a Coulter cell counter and platelets by phase microscopy.

**RESULTS AND DISCUSSIONS**

Endotoxin infusion or bypass in normal sheep decreased CH50 (25%) and C3 (30%), suggesting complement was activated. Administration of cobra venom factor reduced CH50 by >95% and C3 by >90%. Table 1 summarizes the results of complement depletion in bypass and endotoxin-treated sheep.

Complement depletion prevented an increase of pulmonary artery pressure during bypass, but did not affect endotoxin-induced pulmonary artery hypertension. Thromboxane production during bypass requires an intact complement system, while increases of plasma TxB₂ following endotoxin infusion were uninhibited by complement depletion. The transient leukopenia of bypass appears to be complement mediated, since it was absent during bypass of complement depleted sheep. Endotoxin-induced leukopenia occurred to the same extent as in normal sheep despite complement depletion. These results highlight a fundamental difference with respect to the activation of arachidonate metabolism in these two experimental models.

As shown in Table 1, several other basic differences exist between these 2 models in their normal state. The maximum levels of plasma TxB₂ and pulmonary artery pressure are much greater following endotoxin than during bypass. The leukopenia of bypass is rapid and transient lasting only 45-60 min while it is slower in onset after endotoxin and irreversible. Acute and irreversible thrombocytopenia occurs during bypass, but is not observed after endotoxin infusion. The early platelet loss following the onset of bypass may partially

### Table 1

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>VV Bypass</th>
<th>Endotoxin</th>
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<tbody>
<tr>
<td></td>
<td>mean peak</td>
<td>mean peak</td>
</tr>
<tr>
<td>Response</td>
<td>PAP (mm Hg)</td>
<td>TxB₂ (ng/ml)</td>
</tr>
<tr>
<td>Normal sheep</td>
<td>31</td>
<td>1.2</td>
</tr>
<tr>
<td>Complement depleted</td>
<td>17</td>
<td>&lt;0.1 (transient)</td>
</tr>
</tbody>
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+ present; PAP = pulmonary artery pressure; 0 absent
result from complement activation since it was reduced by complement depletion. After 2 hours of bypass there were no significant differences of platelet counts between normal and complement-depleted sheep.

In the sheep, endotoxin infusion causes increased pulmonary vascular permeability characterized by a late phase of stable pulmonary vascular pressures and an increased flow of protein-rich lymph. However, no changes in lymph flow or protein content were noted in sheep during prolonged bypass under steady state conditions. Thus, intravenous endotoxin causes lung microvascular injury in this species, whereas bypass does not. Whether the phase of increased pulmonary vascular permeability following endotoxin infusion occurs after complement depletion, requires further study.

REFERENCES


Endotoxin and Oxygen Injury to Lung Endothelium*

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Hyperoxic injury to the lung depresses pulmonary endothelial cell uptake of serotonin and norepinephrine and these alterations have been reported to be sensitive indices of oxygen-induced endothelial cell damage. Recently, bacterial endotoxin has been reported to protect against pulmonary oxygen toxicity, but the mechanism and

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FIGURE 1. Effect of 24-hr exposure to air or 100% O2 at 1 atmosphere absolute (ATA) on serotonin uptake by isolated perfused lungs of rats given endotoxin or saline (controls). Values are expressed as mean ± SE. *p<0.025 vs controls or endotoxin-treated rats exposed to air and endotoxin-treated rats exposed to oxygen.

FIGURE 2. Effect of 24-hr exposure to air or 100% O2 at 1 ATA on norepinephrine uptake by isolated perfused lungs of rats given endotoxin or saline (controls). Values are expressed as mean ± SE. *p<0.025 vs control or endotoxin-treated rats exposed to air and endotoxin-treated rats exposed to oxygen.