A correlation of radiographic, functional, and morphologic changes in baboon lung allografts

A comparison of two immunosuppressive regimens

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Transplantation of the lung has been extensively investigated in the experimental laboratory for the past two decades, and within the past decade at least 31 clinical cases have been reported. The clinical results have been discouraging. The majority of patients who received lung allografts died within 30 days. Only 2 patients survived for longer than 5 months, and both of these patients died within 10 months. On the basis of this experience, further clinical trial is not indicated until better methods of managing rejection have been developed and the radiographic, functional, and morphologic changes produced by the transplantation procedure have been more clearly defined.

The experiments described in this paper were designed to study the radiographic, functional, and morphologic changes which occur in two groups of baboons treated by different immunosuppressive regimens. One group was treated with relatively low doses of immunosuppressive drugs. High doses of immunosuppressive drugs were employed in the second group of animals.

Methods

The studies were performed in Kenya baboons weighing 25 to 35 kilograms. The animals received left lung allografts from donor animals with similar simian ABO blood groups. The lung allografts were inserted in all animals by a technique similar to that described by Veith. All animals were treated with penicillin and streptomycin postoperatively. Serial chest x-ray films, inhalation scintiscans, and perfusion scintiscans were obtained at 1 to 3 day intervals until the animals died. The regional ventilation and perfusion studies were performed by means of a scintillation camera in conjunction with an Image Display Analysis (IDA) system. The regional ventilation study which measured alveolar volume was performed with xenon-133 radioactive gas. The animals were allowed to breathe room air to wash out the radioactive gas from their lungs. During equilibration and wash out, images (posterior view) of the distribution of concentration of the radioactive gas in the lungs were recorded on magnetic...
Table I. Group I—low doses of immuno-suppressive drugs

<table>
<thead>
<tr>
<th>Baboon No.</th>
<th>Ameroid constrictor</th>
<th>Survival (days)</th>
<th>Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not present</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Present</td>
<td>20</td>
<td>4+</td>
</tr>
<tr>
<td>3</td>
<td>Not present</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Present</td>
<td>11</td>
<td>2+</td>
</tr>
<tr>
<td>5</td>
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<td>?</td>
</tr>
<tr>
<td>6</td>
<td>Present</td>
<td>12</td>
<td>3+</td>
</tr>
</tbody>
</table>

tape in the IDA system. The animals were then given 1 to 2 mCi 99mTc-labeled albumin microspheres intravenously. A posterior view of the perfusion of both lungs was recorded with the subject in the same position relative to the scintillation camera as for the ventilation study. To assess the contribution of the transplanted lung to total lung function, the total activity of the left lung for both alveolar volume and perfusion was expressed as the ratio of activity in the left lung to that obtained in both lungs (left/left + right).

Group I. Group I consisted of 6 baboons. Following removal of the allograft from the donor animal but before its insertion into the recipient, the lungs were ventilated and the pulmonary artery was perfused with cold (4°C) Ringer’s lactate. In 3 animals of this group a 9 mm. Ameroid constrictor was placed on the right pulmonary artery so that changes in perfusion of the allograft could be studied in the presence of progressive impairment of blood flow to the right lung. All 6 of these animals were treated daily with 1 mg. per kilogram of methylprednisolone and 2.5 mg. per kilogram of azathioprine (supplied by Burroughs Wellcome & Co., Inc., Tuckahoe, N. Y.).

Group II. This group consisted of 7 animals. In these animals both donor and recipient animals received 3 mg. per kilogram of heparin intravenously immediately prior to removal of the graft. The lung allografts were then immediately inserted into the recipients. The allografts were not perfused or ventilated in this group of animals. In 3 of these 7 baboons, a 9 mm. Ameroid constrictor was placed on the right pulmonary artery. These baboons received 30 mg. per kilogram of methylprednisolone on the day of operation and every other day thereafter. In addition, they were treated with 4 mg. per kilogram of azathioprine which was administered daily.

Results

Group I. Survival in this group of 6 baboons correlated with the operative procedure, and autopsy findings are summarized in Table I.

Chest x-ray films. A perihilar infiltrate was noted within 3 days of operation. By 6 to 8 days after operation the left lower lung field was opaque and a prominent air bronchogram was present. Consolidation of the allograft increased until the entire allograft was opaque in those animals that died at 10 and 12 days. In the animal that died at 20 days (No. 2), there was partial clearing of the allograft by 14 days after operation. Increasing consolidation was again noted 2 to 3 days prior to death (Fig. 1).

Inhalation scintiscans. Relative alveolar volume of the allograft was decreased in the immediate postoperative period, and this decreased aeration of the allograft persisted until 4 of the 5 animals died. The animal surviving for 20 days (No. 2) demonstrated a maximum decrease in alveolar volume of the allograft by 6 to 8 days after operation. Alveolar volume subsequently improved by 13 days after operation and then further deteriorated prior to death (Fig. 2).

Perfusion scintiscans. In those animals without an Ameroid constrictor on the right pulmonary artery there was a precipitous decline in blood flow to the allograft which persisted until the animals died (Fig. 3). Two animals with Ameroid constrictors on the right pulmonary artery (Nos. 4 and 6), had less initial impairment of perfusion of the allograft. In these animals a relatively balanced perfusion between the two lungs persisted for at least 7 days after operation (Fig. 4). In the remaining animal (No. 2), perfusion of the allograft was initially depressed but subsequently returned toward
control values, and a relatively balanced perfusion was present until at least 5 days prior to the baboon's death (Fig. 5).

Autopsy. At autopsy the allografts were dark red and consolidated. The bronchial anastomoses were intact. There was no evidence of thrombus formation at the site of the vascular anastomoses. The Ameroid constrictors had narrowed the right pulmonary artery to an internal diameter of approximately 6 mm. There was no evidence of rejection in the animals dying at 4 and 5 days. The remaining animals had extensive histologic evidence of rejection manifested by perivascular and peribronchial inflammatory infiltrates and focal areas of intra-alveolar edema containing inflammatory cells and desquamative pneumocytes.

Group II. Survival in this group of 7 baboons correlated with the operative pro-
Fig. 2. Relative alveolar volume of the left lung allograft compared to total alveolar volume in animal No. 2 (Group I).

Fig. 3. Perfusion ratio of relative blood flow to the left lung allograft in an animal without an Ameroid constrictor on the right pulmonary artery, animal No. 5 (Group I).

Fig. 4. Perfusion ratio of relative blood flow to the left lung allograft in an animal with an Ameroid constrictor on the right pulmonary artery, animal No. 6 (Group I).

Fig. 5. Perfusion ratio of relative blood flow to the left lung allograft in an animal with an Ameroid constrictor on the right pulmonary artery which survived for 20 days, animal No. 2 (Group I).

Table II. Group II—high doses of immunosuppressive drugs

<table>
<thead>
<tr>
<th>Baboon No.</th>
<th>Ameroid constrictor</th>
<th>Survival (days)</th>
<th>Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Present</td>
<td>17</td>
<td>1+</td>
</tr>
<tr>
<td>2</td>
<td>Present</td>
<td>21</td>
<td>2+</td>
</tr>
<tr>
<td>3</td>
<td>Present</td>
<td>31</td>
<td>1+</td>
</tr>
<tr>
<td>4</td>
<td>Not present</td>
<td>8</td>
<td>?</td>
</tr>
<tr>
<td>5</td>
<td>Not present</td>
<td>29</td>
<td>?</td>
</tr>
<tr>
<td>6</td>
<td>Not present</td>
<td>30</td>
<td>?</td>
</tr>
<tr>
<td>7</td>
<td>Not present</td>
<td>31</td>
<td>?</td>
</tr>
</tbody>
</table>

procedures and autopsy findings as summarized in Table II.

Chest x-ray films. By 3 days after operation there was consolidation of the central one third of the lung evidenced by the presence of an air bronchogram. The central pulmonary vessels could not be identified. For the next 3 to 5 days this alveolar process continued and progressed. There was a loss of lung volume with elevation of the left hemidiaphragm and a shift of the mediastinum to the left side. The consolidation usually involved the entire lung by 6 days after operation. By 8 days after operation the alveolar process began to diminish in 3 of the 7 baboons (Nos. 1, 3, and 7). During the next 3 to 5 days, gradual clearing of the alveolar density finally resulted in a chest radiogram that approached normal in these 3 animals. The chest radiograms remained clear for 3 to 5 days; several days before death alveolar densities again appeared in the left lung. Similar alveolar densities were present in the right lung in 2 animals. Finally, consolidation of the left lung was evident shortly before death (Fig. 6). In distinct contrast, a progressive pattern of left lung consolidation was present in the remaining 3 animals (Nos. 2, 5, and 6). By 8 to 10 days
after operation there was consolidation of the entire left chest, mediastinal shift to the left, and elevation of the left hemidiaphragm. In 2 animals these findings never cleared before the animals' death (Nos. 5 and 6), while in the other animal the consolidation partially cleared prior to death (No. 2).

Inhalation scintiscans. Alveolar volume of the left lung compared to the total alveolar volume gradually declined in the first week in all 7 animals. By the ninth to tenth day after operation there was improvement in the inhalation lung scintiscans in 3 animals (Nos. 1, 3, and 7), manifested by an increase in alveolar volume of the left lung and a greater contribution of that lung to total pulmonary function (Fig. 7). In the remaining 3 animals, alveolar volume of the allograft either improved slightly (No. 2) or remained severely depressed until the animals died (Nos. 5 and 6).

Perfusion scintiscans. The perfusion ratios demonstrated a prompt and sustained increase in perfusion of the allograft in 2 of the 3 animals with Ameroid constrictors on the right pulmonary artery (Nos. 1 and 3). The perfusion of the allograft returned to preoperative control values in 1 animal, but increased perfusion of the allograft persisted in the second animal until it died (Fig. 8). In contrast, in 1 animal (No. 2) with an Ameroid constrictor on the right
pulmonary artery there was an initial decline in perfusion of the allograft despite the Ameroid constrictor. The relative decrease in perfusion of the allograft improved somewhat but remained below preoperative control values until this baboon died. Two animals (Nos. 5 and 7) without Ameroid constrictors on the right pulmonary artery demonstrated a balanced perfusion between the right and left lungs. A persistent decrease in perfusion of the allograft was present in the remaining animal (No. 6) (Fig. 9).

Autopsy. The bronchial anastomoses were intact in all 7 animals. There was no evidence of thrombus formation at the site of the vascular anastomoses. The Ameroid constrictors had again narrowed the internal diameter of the right pulmonary artery to approximately 6 mm. Histologic evidence of rejection was most prominent in animal No. 2 and was manifested by an organizing alveolar exudate. Two other animals also showed minimal evidence of graft rejection manifested by scattered areas of a mild perivascular and peribronchial cellular infiltrate. A severe necrotizing pneumonia was present in the allografts of the remaining animals, and this disease process obscured any other histologic findings. In all 3 of these animals there was little evidence of an inflammatory response on the part of the host. The right lung in 5 of the 7 animals had extensive foci of pneumonia.

Discussion
A number of radiographic findings following lung allografting have been described. Seigelman, in a series of experiments with dogs receiving lung allografts and no immunosuppression, described an initial perihilar alveolar infiltrate which progressed until the entire graft was totally consolidated. In dogs treated with immunosuppressive drugs, Veith described a patchy alveolar infiltrate which subsequently cleared by 7 days. Increasing consolidation was again noted when the dogs began to reject the allografts. Tsai has reported that 23 of 24 lung allografts in baboons became completely opaque on chest x-ray within 4 to 34 days following grafting; in 1 animal partial clearing of the consolidation occurred. Our findings on chest x-ray study are in general agreement with those reported by Seigelman and Veith but considerably different from those reported by Tsai. The initial infiltrates seen on chest x-ray films are due to alveolar edema and focal areas of atelectasis which occur as a result of the operative procedure. If rejection is not controlled, the allograft rapidly becomes consolidated because of an increasing cellular exudate within the alveoli. If rejection is partially controlled, partial clearing becomes evident on chest x-ray; if rejection is completely controlled, the allograft begins to clear within 6 to 8 days after operation and is totally clear by 15 days postoperatively.
This temporal sequence of events corresponds remarkably well with Eraslan's demonstration of lymphatic regeneration in lung autografts, and we have therefore assumed that lymphatic regeneration is responsible for the clearing of the lung on chest x-ray films in animals with effective immunosuppression.

The results of the inhalation scintiscans correlated well with the x-ray findings. Consolidation on the chest film was associated with a marked decrease in ventilation of the allograft, and clearing on the chest x-ray was associated with a significant increase in ventilation of the allograft. The subsequent improvement in ventilation in some animals has not been described before. Isawa described a persistent deterioration in ventilation of lung allografts following operation with no subsequent improvement. We would again attribute the improvement in ventilation of the allograft to lymphatic regeneration.

Previous experiments from this laboratory with unmodified lung allografts in calves, in which an Ameroid constrictor was placed on the opposite pulmonary artery, demonstrated a prompt and sustained decrease in blood flow to the allograft even in the presence of the Ameroid constrictor. We were unable to demonstrate a morphologic lesion in the calves as the cause of the decrease in blood flow to the allograft. Our present experiments with baboons confirmed these earlier experiments in calves, but we are again unable to find a morphologic lesion to account for the decrease in blood flow. Animals in Group I, which had increasing consolidation of the allograft and progressive deterioration of ventilation of the allograft, also had a progressive decline in blood flow to the grafted lung. The lessening of blood flow to the allograft occurred earlier and was more precipitous in animals without an Ameroid constrictor on the opposite pulmonary artery, but it also occurred 3 to 4 days later in those animals with an Ameroid constrictor on the right pulmonary artery. We could not detect histologic evidence of rejection in those animals dying at

4 and 5 days, nor could we detect any morphologic evidence of a vascular injury. Histologic evidence of rejection was present in 3 of the 4 animals that died after 10 days, but we were again unable to demonstrate a specific morphologic lesion in the vasculature of the allograft. Rejection appeared to be partially controlled in only 1 animal from the group of baboons given low doses of azathioprine and prednisone. In this animal, consolidation of the allograft partially cleared, and, although perfusion was initially severely depressed, perfusion did return toward control values and was maintained at reasonable levels until 5 days prior to death.

In contrast to the results of the perfusion scintiscans in Group I animals, the majority of baboons from Group II, on high doses of immunosuppressive drugs, demonstrated either an increased perfusion of the allograft in the presence of an Ameroid constrictor on the right pulmonary artery or a balanced perfusion of the allograft in the absence of the Ameroid constrictor. Only 2 animals from this group had impaired perfusion of the allograft, and in both of these animals impaired perfusion was associated with persistent consolidation on the chest x-ray film and decreased alveolar volume of the allograft. One of these animals with decreased perfusion of the allograft also had the most convincing histologic evidence of rejection at autopsy; in the other baboon a severe necrotizing pneumonia obscured any evidence of rejection.
On the basis of these experiments we have concluded that increasing consolidation of the lung allograft on chest x-ray films associated with decreased ventilation of the allograft is not necessarily a manifestation of rejection. If adequate perfusion of the allograft, as measured by a scintiscan technique, is maintained in the presence of consolidation on chest x-ray study, significant rejection has probably not occurred. However, if x-ray evidence of increasing consolidation is associated with decreased perfusion of the allograft, significant rejection has probably occurred and the dose of immunosuppressive drugs should be increased. The changes on chest x-ray films, inhalation scintiscans, and perfusion scintiscans can also, of course, be due to infection, and the results of these studies must be carefully correlated with serial examinations of the sputum for bacteria and other manifestations of infection.

Summary

A series of experiments which studied the radiographic, functional and morphologic changes in baboon lung allografts is described. If rejection was effectively controlled with immunosuppressive drugs, perfusion of the allograft was maintained in spite of initial x-ray evidence of consolidation and decreased ventilation of the allograft. Uncontrolled rejection of the allograft was associated with decreased perfusion as well as an increasing consolidation and decreased ventilation of the allograft. On the basis of these experiments, we have concluded that increasing consolidation of the lung allograft on chest x-ray study associated with decreased ventilation of the allograft is not necessarily a manifestation of rejection. If adequate perfusion of the allograft as measured by a scintiscan technique is maintained in the presence of consolidation on chest x-ray films, significant rejection has probably not occurred.

REFERENCES