FUNCTIONAL AND MORPHOLOGIC CHANGES
AFTER LUNG ALLOGRAFTING IN BABOONS

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The clinical results after pulmonary transplantation have been discouraging. The majority of the 31 patients who received lung allografts have died within 30 days. Only two patients survived for longer than five months; both died within ten months. The deaths of these patients have been attributed to a variety of causes, including bacterial as well as viral infections and rejection as well as imbalances between perfusion and ventilation of the allograft and the remaining lung. Lesions produced by rejection, particularly if modified by immunosuppression, remain obscure, and the functional changes produced by these morphologic lesions have not been completely defined. These experiments were designed to correlate the early morphologic changes which occur in lung allografts in baboons with serial roentgenographic changes of the chest and serial ventilation changes of the allograft.

METHODS

These studies were performed in nine Kenya baboons, weighing between 25 and 35 kilograms, that received left lung allografts from donor baboons with similar simian ABO blood groups. Each baboon was treated with 30 milligrams per kilogram of methylprednisolone sodium succinate during the induction of anesthesia. The same dosage of methylprednisolone was repeated every other day postoperatively. In addition, the baboons received 4 milligrams per kilogram of azathioprine on the first postoperative day and at daily intervals thereafter. Penicillin and streptomycin also were administered daily. The lung allografts were inserted in all baboons by a technique similar to that described by Veith and his colleagues (7). Immediately prior to removal of the graft, both donor and recipient baboons received 3 milligrams per kilogram of heparin intravenously. The lung allografts were not perfused or ventilated but were immediately inserted into the recipients.

Two of the nine baboons received left lung allografts, and serial biopsies of the allograft and serial roentgenograms of the chest were obtained at one to three day intervals postoperatively. The baboons were studied until they died. Five baboons had a 9 millimeter constrictor placed on the right pulmonary artery immediately after insertion of the allograft to simulate the increased vascular resistance that would be encountered clinically in a patient with pulmonary hypertension. The remaining two baboons received left lung allografts but a constrictor was not placed on the right pulmonary artery. All seven of these baboons were studied at one to three day intervals with serial roentgenograms of the chest and serial inhalation scintiscans being made. The serial inhalation scintiscans which measured alveolar volumes were obtained with the baboon in the supine position and after the insertion of an endotracheal tube which was attached to a spirometer containing 133Xe, 7 millicuries per liter. Images were obtained with the scintillation camera in conjunction with an image display and analysis system. After equilibration, the baboons were allowed to breath room air to wash out the radioactive gas from the lungs. During equilibration and wash out, images of the distribution and concentration of the radioactive gas in the lungs were recorded on magnetic tape in the image display analysis system. To assess the contribution of the transplanted lung to the total lung function, the total activity in the left lung allograft was expressed as the ratio of activity in the left lung to that obtained in both lungs.

RESULTS

The various operative procedures correlated with the length of survival and findings at autopsy are
summarized in Table I. The results of the serial roentgenograms of the chest, serial inhalation scintiscans, serial biopsies, and more detailed data obtained at autopsy will be described.

*Roentgenograms of the chest.* On the first postoperative day, an increase was noted in the soft tissue density on the left side of the chest as well as an indentation of the lateral border of the left lung. These changes were attributed to the operative procedures. By the third postoperative day, there was consolidation in the central one-third of the allograft, evidenced by the presence of an air bronchogram. The central pulmonary vessels could not be identified. Increasing consolidation of the allograft continued for the next three days with loss of lung volume, elevation of the left hemidiaphragm, and a shift of the mediastinum to the left side. By six days after operation, the lower two-thirds of the allograft were consolidated (Fig. 1a). Two distinct roentgenographic patterns were seen after six to eight days. In three baboons, 5, 6, and 8, the consolidation of the allograft began to clear nine to 11 days after operation. The clearing of the consolidation began in the periphery of the allograft and extended toward the central portion. By 13 days after operation, a roentgenogram of the chest showed almost complete clearing (Fig. 1b). Roentgenograms of the chest in these baboons continued to show a clearing of the consolidation until three to seven days prior to death, when alveolar densities again appeared in the left lung. The alveolar densities in the left lung were associated with similar densities in the right lung. In one baboon, a large cavity was present in the right lung on the day prior to death (Fig. 1c).

In distinct contrast in the remaining baboons, 2, 7, and 9, the initial consolidation seen on the roentgenogram of the chest did not clear. Consolidation of the allograft persisted after six to eight days, and consolidation was present until the baboons died (Fig. 2).

*Serial inhalation scintiscans.* The serial inhalation scintiscans revealed a decrease in alveolar volumes of the allograft on the first postoperative day. This decline in alveolar volumes of the allograft continued for the first week after operation. In those baboons in which there was a clearing of the consolidation on the roentgenograms of the chest, relative alveolar volumes in the allograft began to improve after eight to ten days, and, by 12 to 15 days after operation, alveolar volumes of the left lung had returned to preoperative control values. The alveolar volumes in these baboons remained balanced between the right and left lungs until shortly before death (Fig. 3). In the baboons in
which clearing was not demonstrated on the roentgenogram of the chest, relative alveolar volumes of the left lung allograft remained depressed in comparison to the right lung until they died (Fig. 4).

Pathologic findings. The initial serial biopsies obtained three days after operation revealed minimal perivascular cuffing and acellular edema fluid within the alveoli. Similar edema fluid was present within the dilated lymphatic vessels of the allografts (Fig. 5). Histologic examination of the allograft from baboon 1, which died at eight days, revealed acellular edema fluid within the alveoli and focal areas of bronchopneumonia, characterized by an interstitial infiltrate of polymorphonuclear cells and large numbers of polymorphonuclear cells and edema fluid within the alveolar spaces. By the eighth postoperative day, specimens taken from surviving baboon 2 revealed a moderate mononuclear perivascular cuffing and a definite cellular component to the alveolar exudate (Fig. 6). Pathologic changes in the specimen from this baboon on the tenth postoperative day were generally the same as those observed two days earlier. By the twenty-fourth day, a severe necrotizing pneumonia was present, and empyema also was present by the twenty-seventh postoperative day. At autopsy, foci of bronchopneumonia were present in the allograft. Histologic evidence of rejection, if present, was obscured by the extensive bronchopneumonia. Histologic study of the material obtained at autopsy from baboons 3 and 4 that died at six and seven days after operation revealed bacterial bronchopneumonia in the upper lobes of the left lung and focal areas of acellular edema fluid within the alveoli of the lower lobe of the left lung. The lymphatics of the allograft were greatly dilated and filled with edema fluid. Histologic examination of the right lung of the baboon with complete obstruction of the right pulmonary artery revealed severe capillary and venous engorgement but no evidence of infarction. Histologic findings of the right lung of the baboon which died at seven days after disruption of the bronchial anastomosis were normal. The autopsies of the remaining five baboons revealed bilateral scattered areas of necrotizing bronchopneumonia and bilateral focal areas of hemorrhage. The inflammatory cellular response, usually encountered in bacterial pneumonia, was never present in these baboons. There was no evidence of rejection other than severe necrotizing bronchopneumonia in three of these five baboons. Definite histologic evidence of rejection was present in baboon 7 and was manifested by an organizing exudate in the alveoli and moderate perivascular cuffing by mononuclear cells. Baboon 5 also had histologic evidence of minimal perivascular cuffing by mononuclear cells.

Discussion

On the basis of the serial biopsies and the material obtained at autopsy of the baboons dying at six and seven days, we have concluded that the increasing consolidation seen on the initial roentgenograms of the chest and the functional changes detected by the inhalation scintiscans are due to a combination of interstitial and intra-alveolar edema associated with focal areas of bacterial pneumonia and capillary engorgement. Perivascular cuffing by mononuclear cells and a cellular alveolar exudate were present in only one of four baboons in which serial biopsies or material from an autopsy was available. Intra-alveolar edema was present in the remaining three baboons, but it had no cellular component, and we could not determine if this intra-alveolar edema fluid represented a trans-
update in response to operation or an exudate in response to rejection. Results at autopsy of the five baboons dying 17 to 31 days after operation revealed definite histologic evidence of rejection in only two baboons. The necrotizing pneumonia seen in the remaining baboons was compatible with severe bacterial pneumonia in baboons in which defense mechanisms have been severely depressed by azathioprine and methylprednisolone.

To our knowledge, the roentgenographic pattern of increasing consolidation of lung allografts for the first six to eight days after operation, followed by complete clearing of the consolidation within 15 days after operation, has not been described before. These roentgenographic changes are not compatible with the reimplantation response described in dogs by Siegelman and his associates (3), since they persist too long and involve too much of the lung. Tsai and his colleagues (6) have reported roentgenographic evidence of dense consolidation in 24 baboon lung allografts within an average of 11 days after grafting. In 23 of these baboons, the graft remained opaque. In one baboon, there was partial clearing of the consolidation by 14 days after operation.

The changes on the inhalation scintiscans, an initial decline in relative alveolar volumes of the allograft, but a subsequent return to preoperative control values also have not been reported before. An early and sustained decline in ventilation of unmodified lung allografts in dogs has been reported by Strieder and her colleagues (5). Isawa and his co-workers (2) have described an early decrease in ventilation of lung allografts in dogs given immunotherapy, and this initial decrease in ventilation of the allograft was followed by a continued progressive decline in ventilation during the third and fourth weeks after operation. A similar early sustained decrease in ventilation of lung allografts in man has been reported by White and his associates (9) and Stevens and his colleagues (4). Veith and his co-workers (8) have attributed this impairment to...

TABLE I.—CORRELATION OF OPERATIVE PROCEDURES, LENGTH OF SURVIVAL, AND FINDINGS AT AUTOPSY

<table>
<thead>
<tr>
<th>Baboon No.</th>
<th>Procedure</th>
<th>Survival, days</th>
<th>Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left lung allograft</td>
<td>8</td>
<td>Right lung, edema; left lung, early bronchopneumonia</td>
</tr>
<tr>
<td>2</td>
<td>Left lung allograft</td>
<td>29</td>
<td>Right lung, edema; left lung, bronchopneumonia and empyema</td>
</tr>
<tr>
<td>3</td>
<td>Left lung allograft + constrictor RPA</td>
<td>6</td>
<td>Right lung, occlusion of RPA at constrictor; congestion; left lung, bronchitis, alveolar edema, bronchopneumonia</td>
</tr>
<tr>
<td>4</td>
<td>Left lung allograft + constrictor RPA</td>
<td>7</td>
<td>Right lung, normal; left lung, disruption of bronchial anastomosis, alveolar edema, bronchopneumonia</td>
</tr>
<tr>
<td>5</td>
<td>Left lung allograft + constrictor RPA</td>
<td>31</td>
<td>Right lung, necrotizing bronchopneumonia; left lung, 1+ rejection and focal hemorrhage</td>
</tr>
<tr>
<td>6</td>
<td>Left lung allograft + constrictor RPA</td>
<td>17</td>
<td>Right lung, normal; left lung, alveolar edema</td>
</tr>
<tr>
<td>7</td>
<td>Left lung allograft + constrictor RPA</td>
<td>22</td>
<td>Right lung, focal hemorrhage; left lung, 2+ rejection and extensive focal hemorrhage</td>
</tr>
<tr>
<td>8</td>
<td>Left lung allograft</td>
<td>29</td>
<td>Right lung, bronchopneumonia; left lung, severe necrotizing pneumonia</td>
</tr>
<tr>
<td>9</td>
<td>Left lung allograft</td>
<td>30</td>
<td>Right lung, confluent bronchopneumonia; left lung, severe necrotizing pneumonia</td>
</tr>
</tbody>
</table>

RPA, Right pulmonary artery.
ment of ventilation of lung autografts to the presence of an alveolar exudate which they detected in both dogs and man. They attributed the alveolar exudate to rejections. Results of our experiments indicate that the intra-alveolar fluid may not be an exudate and may not be due to rejection. It would seem unlikely that extensive roentgenographic evidence of consolidation due to rejection would clear without any change in the dosage of immunosuppressive drugs. Similarly, it also would be unlikely that infection would account for the roentgenographic changes, since these baboons given intensive immunosuppressive therapy probably would die of bacterial or viral infection rather than clear the infection.

The results of our experiments indicate that the roentgenographic, functional, and morphologic changes which we have observed during the first 15 days after operation probably are due to the inability of the baboons to clear edema fluid from the allografted lung. Clearing of the consolidation present on roentgenograms of the chest and subsequent improvement of inhalation scintiscans most likely is due to regeneration of the lymphatics on the left side of the chest. Eraslan and his group [1] have demonstrated morphologic evidence of lymphatic regeneration in lung autografts within 12 days of the grafting procedure and functional evidence of lymphatic regeneration within seven days of reimplantation of the lung, a temporal sequence of events which corresponds remarkably well with the clearing of the consolidation on the roentgenograms of the chest and the improvement of the alveolar volumes in the autografts demonstrated in our series of experiments. Failure of complete clearing of the consolidation, in one baboon at least, appeared to be due to rejection. Subsequent roentgenographic changes of the chest in baboons in which the consolidation initially cleared appeared to be due to scattered foci of pneumonia that appeared in both lungs. The inhalation scintiscans did not reflect any significant changes in alveolar volumes, since these scintiscans compared the relative alveolar volumes in the allograft with the relative alveolar volumes in the right lung and since both lungs usually were involved by the pneumonia.

**SUMMARY**

In this series of experiments, nine baboons received left lung autografts.Serial biopsies were obtained in two baboons and serial roentgenograms of the chest and inhalation lung scintiscans were obtained in the remaining seven. All nine demonstrated roentgenographically an increasing consolidation of the allograft and also a progressive decline in alveolar volumes of the allograft for the first six to eight days after operation. In three baboons clearing of the allograft was demonstrated roentgenographically, and there was a return of alveolar volumes in the allograft to preoperative control values by 15 days after operation. In three baboons, consolidation failed to clear, and relative alveolar volumes in the allograft remained depressed.

The serial biopsies and the findings at autopsy revealed morphologic evidence of rejection in three of nine baboons. We have attributed the initial increasing consolidation of the allograft on the roentgenograms of the chest and the associated decline in ventilatory function to the operative procedures, primarily interruption of the lymphatic drainage. When lymphatic regeneration began to occur, three baboons had a clearing of the consolidation, roentgenographically, and the serial inhalation scintiscans detected a similar improvement in ventilatory function.

**REFERENCES**