Pathologic Changes in Baboon Lung Allografts

Comparison of Two Immunosuppression Regimes

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THE BABOON PROVIDES A PRIMATE MODEL for experimental investigations of pulmonary allografts,[9] to serve as a background for lung transplantation in humans.[10] In the present study the pathologic changes in two groups of baboons given different immunosuppressive regimens were investigated.

Materials and Methods

Male Kenya baboons (*Papio anubis*), weighing between 25 and 35 kg., were paired by A,B,O simian blood group compatability. Exchange transplantation of the left lungs was performed under general anesthesia with fluothane and nitrous oxide and oxygen, supplemented with phencyclidine and diazepam. The left main bronchi of donor and recipient were telescoped to create an anastomosis, the left pulmonary arteries were anastomosed end-to-end, and portions of the left atria containing the left pulmonary veins were anastomosed to the atrial cuff of the recipient. In some animals an Ameroid constrictor was placed around the right main pulmonary artery.

In Group I, chilled Ringers lactate containing 5,000 units of heparin per 500 ml was perfused through the left pulmonary artery until the venous return was bloodless, beginning immediately after separation of the lung which was ventilated with room air. Each animal was given 2.5 mg/kg azathioprine* intramuscularly and 1 mg/kg methyl prednisolone† intramuscularly each day beginning the day of operation. When graft rejection was suspected 1 gram of methyl prednisolone was given intravenously followed by a 500 mg dose the next day, 100 mg the following day and then returning to the standard dose.

In Group II, 3 mg/kg of heparin was injected into the left pulmonary artery just prior to separation. Each animal received 4 mg/kg azathioprine intramuscularly daily and 30 mg/kg of methyl prednisolone intravenously every other day. The first dose of methyl prednisolone was given at the time of induction of anesthesia.

All animals in both groups received 1.2 million units penicillin and 1 gm streptomycin intramuscularly each day. Baboons were allowed to survive as long as possible.

At autopsy, in most animals, the heart-lung block was kept intact. The main pulmonary artery was cannulated with a 6 mm diameter tube and about 100 ml of a barium-gelatin-pigment injection mass with added formalin[2] was injected at 40 to 60 mm Hg pressure. Upon completion of the injection the lungs were inflated at 10 to 20 cm H_2O pressure through the tracheo-bronchial tree with 10% formalin while floating in the same solution. After several days fixation, stereoscopic radiographs of the heart-lung block were prepared. The heart and lungs were then dissected and material taken for histologic examination using hematoxylin and eosin, Verhoeff-van Gieson elastic and Masson connective tissue stains. In most animals at least one block from each lobe of both lungs and from each anastomotic site was examined. Parasitic infestation and graft rejection in the lungs were evaluated on a scale of 0 to 4+.

Submitted for publication September 13, 1972.

Supported by a grant from the John A. Hartford Foundation.

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* Supplied by the Burroughs Wellcome Company.

† Supplied by the Upjohn Company.

TABLE 1. *Baboons on Low Dose Immunosuppression Regimen*

Animal Number	Survival Days	Ameroid	Degree of Parasites	Degree of Rejection	Other Findings Right Lung	Other Findings Transplanted Lung
L72	0	0	3+	0	None	None
M17	0	0	2+	0	None	None
L39	2	+	2+	0	Not examined	Large thrombus at arterial anastamosis. Infarcts.
M18	2	0	2+	0	Extensive focal hemorrhage.	Extensive focal hemorrhage
L73	4	0	2+	0	None	Focal hemorrhage. LUL bronchopneumonia with abscesses.
M38	4	+	2+	0	Focal hemorrhages	Thrombosis of arterial anastamosis. Infarcts and hemorrhage.
L160	5	0	2+	0	Extensive hemorrhage	Extensive hemorrhage
M39	5	+			Not examined.	Not examined.
L163	7	+	3+	2+	None	Early pneumonia.
M16	10	0	2+	?	Early RUL pneumonia	LUL hemorrhage; LLL pneumonia.
L164	11	+	2+	3+	None	None
M19	12	0	3+	3+	Early bronchopneumonia	Severe necrotizing bronchitis and bronchopneumonia.
L38	17	+	2+	3+	None	Severe necrosis LUL. Thrombosis of arterial anastamosis.
L159	20	0	1–2+	4+	None	None

Results

The survival time, overall grade of graft rejection, degree of Pneumonyssus infestation, presence of Ameroid constrictor on the right main pulmonary artery, and brief description of significant pathologic findings for each lung from each animal in the two treatment groups are given in Tables 1 and 2. Baboons in treatment Group I survived up to 20 days post-transplant; and those in Group II survived from 2–31 days.

Pneumonyssus Infestation

All animals showed evidence of parasitization with lung mites. The parasites found were Pneumonyssus species and lay within bronchi (Fig. 1) or more commonly with emphysematous foci, which appear to be induced by the parasite (Fig. 2). The fecal deposits of the parasite occurred throughout the lungs and consisted of refractile granular material mostly within macrophages. The feces was associated with focal parenchymal scarring (Fig. 3) and also occurred around bronchi and blood vessels and in mediastinal lymph nodes. Particularly heavy deposits were encountered on the margins of the emphysema induced by the parasite. Bronchial wall infiltration with chronic inflammatory cells, plasma cells and eosinophils and prominence of the basement membrane and smooth muscle was found uniformly (Fig. 4). In no baboon did the degree of pathologic change in the lungs attributed to parasites seem sufficient to cause func-

TABLE 2. *Baboons on High Dose Immunosuppression Regimen*

Animal Number	Survival Days	Ameroid	Degree of Parasites	Degree of Rejection	Other Findings Right Lung	Other Findings Transplanted Lung
M82	2	+			Not examined	Not examined
M56	6	+	2+	0	Congestion. Occlusion of main pulmonary artery at Ameroid.	Bronchitis and early bronchopneumonia.
M55	7	+	2+	0	None	Disruption of bronchial anastamosis. Pneumothorax. Bronchopneumonia.
M106	7	0	2+	0	None	Thrombosis of arterial anastamosis. Massive infarct.
M107	8	0	3+	0	Extensive bronchopneumonia	Thrombosis of arterial anastamosis. Massive infarct.
M96	8	+	2+	?	Edema	Edema. Early bronchopneumonia.
M57	17	+	2+	1+	None	Large hemopericardium
M83	21	+	2+	2+	Extensive focal hemorrhage.	Extensive focal hemorrhage.
M95	29	+	3+	2+	Bronchopneumonia RLL abscess	Bronchopneumonia. Empyema.
M100	29	0	2+	?	Bronchopneumonia.	Severe necrotizing pneumonia.
M101	30	0	2+	?	Confluent bronchopneumonia.	Severe necrotizing pneumonia.
M54	31	+	2+	1+	Necrotizing bronchopneumonia	Focal hemorrhages

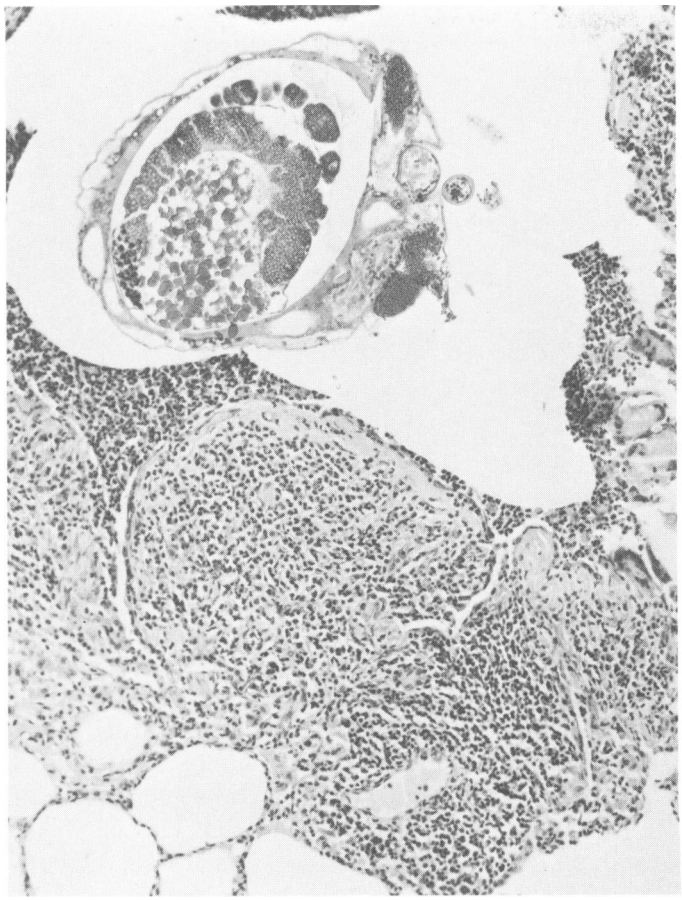

FIG. 1. Pneumonyssus parasite lying within a small bronchus which has intense mural inflammation, loss of epithelium, and intraluminal inflammatory exudate (Animal L72, H&E, 125×).

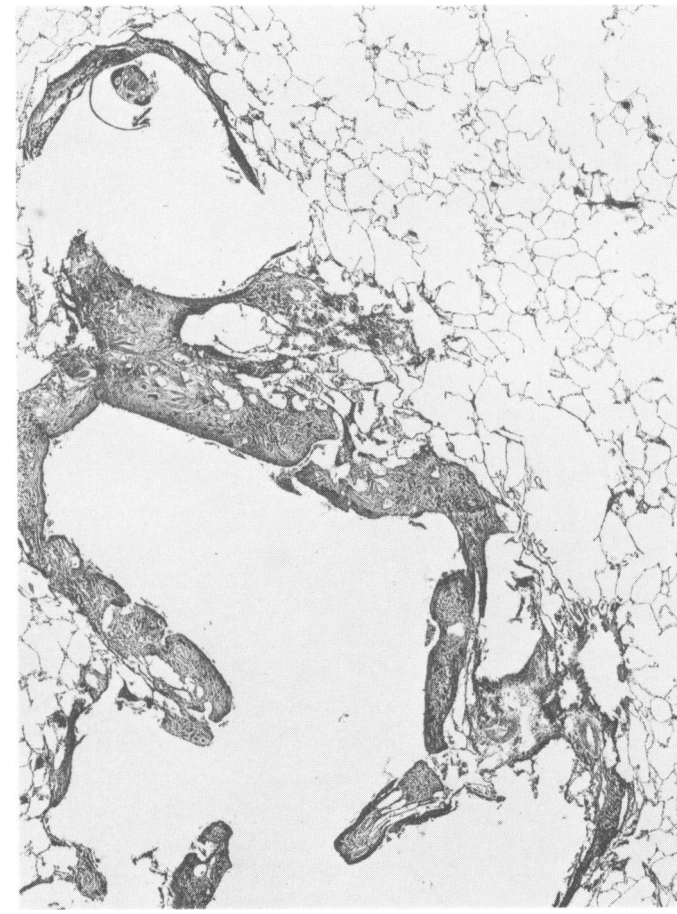

FIG. 2. Emphysematous focus with Pneumonyssus parasite (arrow). The scar tissue around the cavities contains abundant fecal matter, mostly in macrophages (M18, H&E, 30×).

tional impairment. The emphysematous foci did appear particularly susceptible to superinfection, however.

Ameroid Constrictor

Twelve animals had had Ameroid constrictors placed about the right main pulmonary artery at the time of transplantation. In most instances the vessel lumen was reduced to about 6 mm in diameter. With the exception of animal M56 where complete occlusion of the right main pulmonary artery by thrombosis had occurred at the constriction, no morphologic changes in the right lung could be attributed to the Ameroid.

Operative Complications

Two animals (L72 and M17) died at the time of operation. In one animal (M55) death occurred on the seventh post-operative day from pneumothorax following rupture of the bronchial anastomosis. Animal M57 was found at autopsy to have a hemopericardium which appeared to originate from the left atrial suture line.

Intimal thrombi were common at the line of anastomosis in both the pulmonary artery (Fig. 5) and atrium. In 4 animals the arterial thrombus produced almost complete occlusion of the lumen and infarction of the left lung (Fig. 6).

Infection

Morphologic evidence of pulmonary infection was found in 12 animals, ranging from early bronchopneumonia to severe necrotizing pneumonia with abscess formation and empyema (Tables 1 and 2). Animals in Group II, treated with higher doses of steroids and azathioprine showed a marked reduction of the inflammatory cell response. (Fig. 7). Infections spread diffusely and produced extensive parenchymal necrosis. In several animals severe pneumonia produced vascular thromboses and a marked reduction in vascular filling (Fig. 8).

Graft Rejection

In Group I, evidence of graft rejection was found in 5 animals, beginning at 7 days after operation. Morphologic changes in the transplanted lungs that were attributed to graft rejection consisted of perivascular and peribronchial inflammatory infiltrates and focal intra-alveolar accumu-

lations of proteinaceous exudate and inflammatory cells (Figs. 9 and 10). The cells of this inflammatory reaction consisted predominately of mononuclear cells, macrophages, lymphocytes and plasma cells. Numerous polymorphonuclear leucocytes and occasional eosinophils were also found. The histologic features of the inflammatory response of the graft rejection was entirely nonspecific, except that it occurred in the transplanted left lung and not in the right lung. In one animal graft rejection could not be evaluated because of pneumonia.

In Group II 4 animals showed evidence of graft rejection, beginning at 17 days. Qualitatively the inflammatory response was the same as that found in Group I (Figs. 11 and 12). The higher doses of immunosuppressive agents produced a marked quantitative reduction in the cellular reaction. However, the proteinaceous exudate was still present. In 3 animals, M96, M100 and M101, graft rejection may have been present but could not be separated from the pneumonia present throughout the transplanted lung.

In no animal was there evidence of injury to the bronchial epithelium that could be ascribed to rejection. Only one animal, L159, showed evidence of intimal inflammation and that was of a trivial nature (Fig. 10).

Discussion

The study shows that the inflammatory reaction of graft rejection in baboon lung allografts can be reduced with immunosuppressive agents. However, the overall depression of the inflammatory response exacts a high mortality through pulmonary infections. In no animal were the morphologic changes attributable to the immune response felt to be sufficiently severe to cause significant functional abnormality.

The morphologic changes attributed to graft rejection are histologically nonspecific.[4] The early features of graft rejection in the left lung and of bronchopneumonia in both lungs of the animals studied consists of an alveolitis with margination and exudation of leukocytes into the alveolar wall and the alveolar space. Proteinaceous exudation accompanies the cells. The alveolar lining cells become enlarged concommitant with the accumulation of intra-alveolar exudates. In later phases mononuclear cells are observed in increased numbers and perivascular

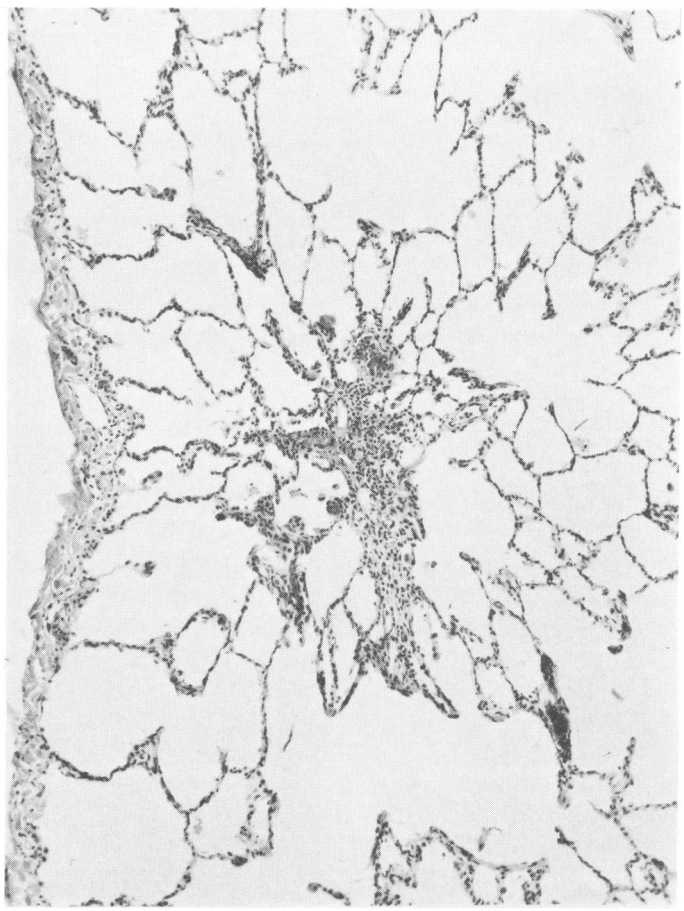

FIG. 3. Small scar caused by Pneumonyssus feces with pleural retraction and adjacent slight emphysematous change (L72, H&E, 90×).

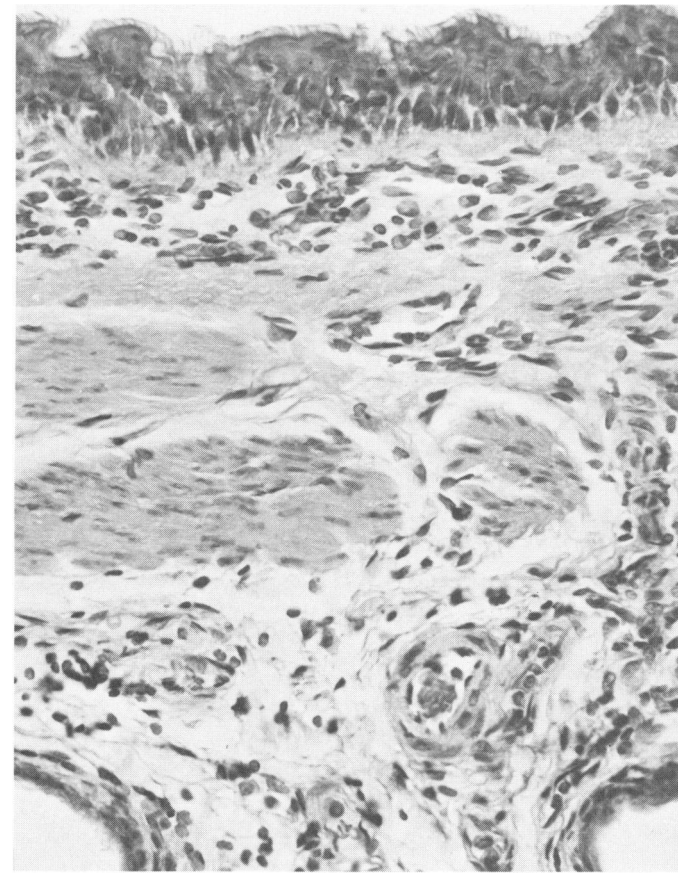

FIG. 4. Wall of segmental bronchus with extensive chronic inflammatory infiltration, including eosinophils, and prominent epithelial basement membrane and smooth muscle. These asthma-like changes apparently relate to Pneumonyssus infestation (M17, H&E, 300×).

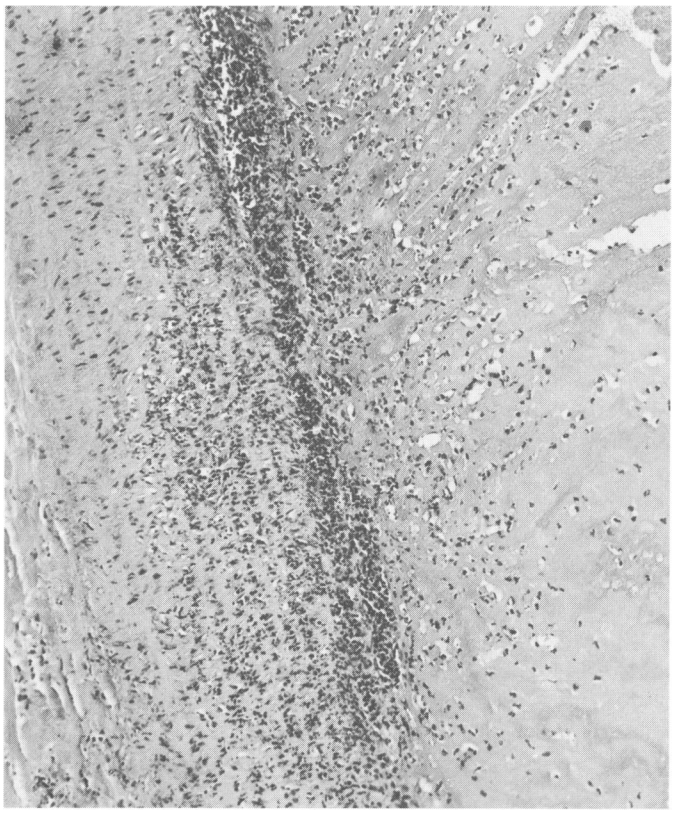

FIG. 5. Peri-anastamotic inflammation in media and intima of left main pulmonary artery (left) and overlying thrombus (right) which occluded the lumen (L38, H&E, 125×).

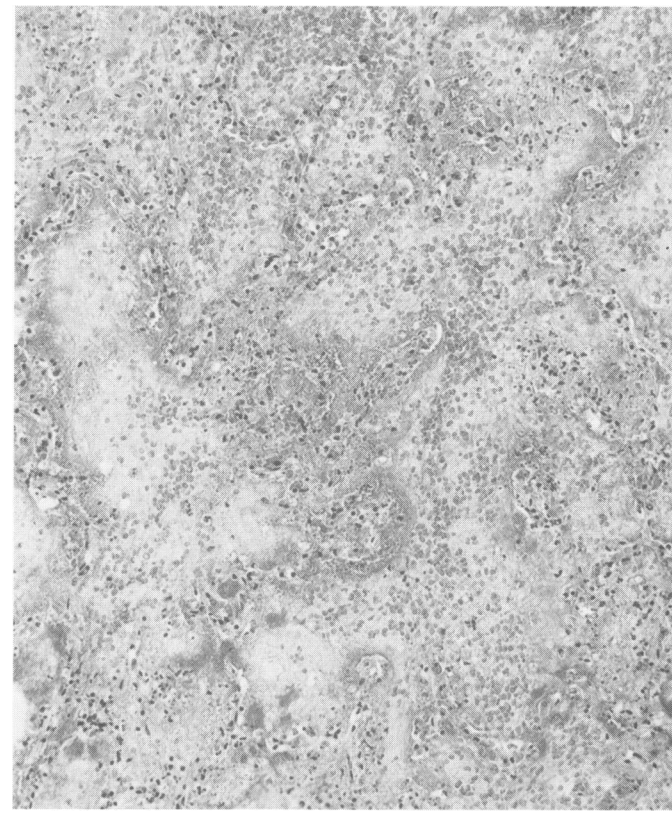

FIG. 7. Severe necrotizing pneumonia in the right upper lobe with intra-alveolar proteinaceous exudate, focal hemorrhages, alveolar wall necrosis and massive bacterial overgrowth associated with high doses of immunosuppressive therapy (M54, H&E, 140×).

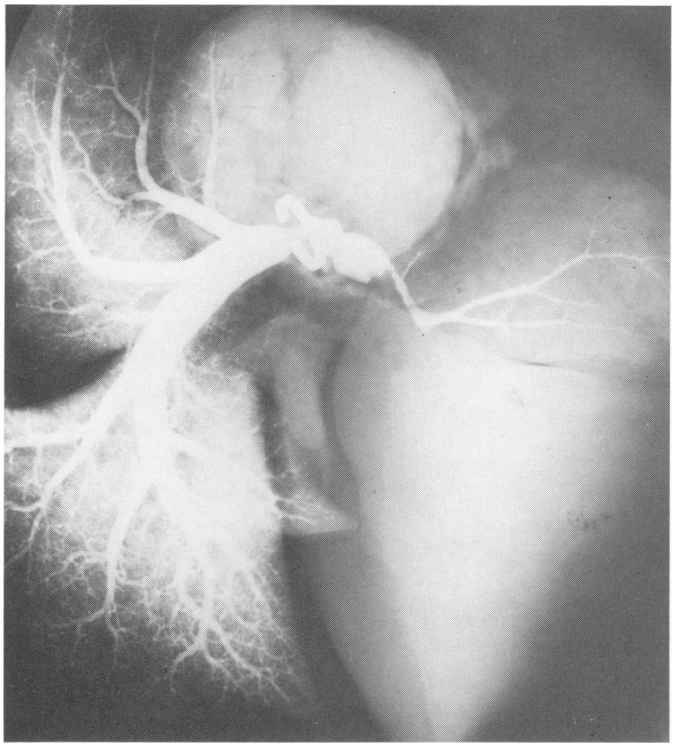

FIG. 6. Radiography of heart and lungs of M38. The left pulmonary arterial tree does not fill, due to thrombosis at the anastamosis. Note ring of ameroid constrictor on the right pulmonary artery.

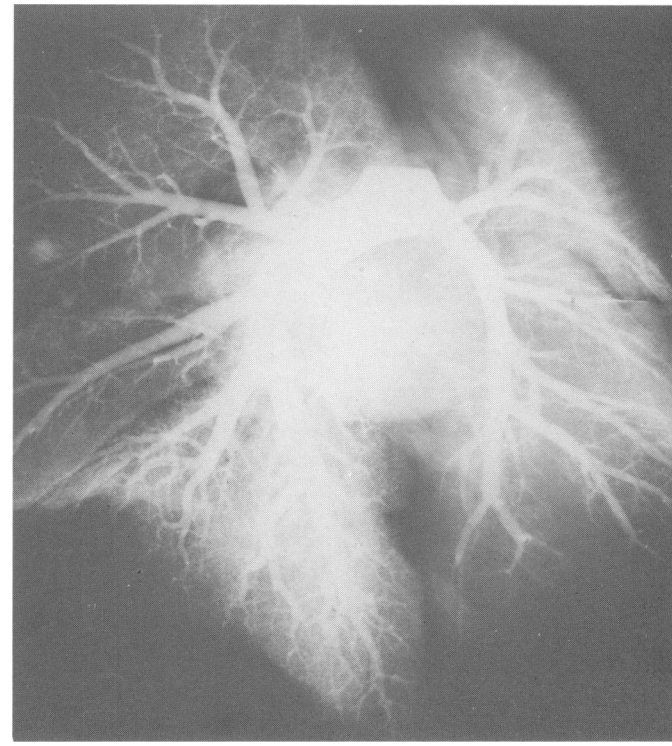

FIG. 8. Radiograph of heart and lungs of M101. The left pulmonary arterial tree fills poorly due to vasculitis and thrombosis associated with necrotizing pneumonia.

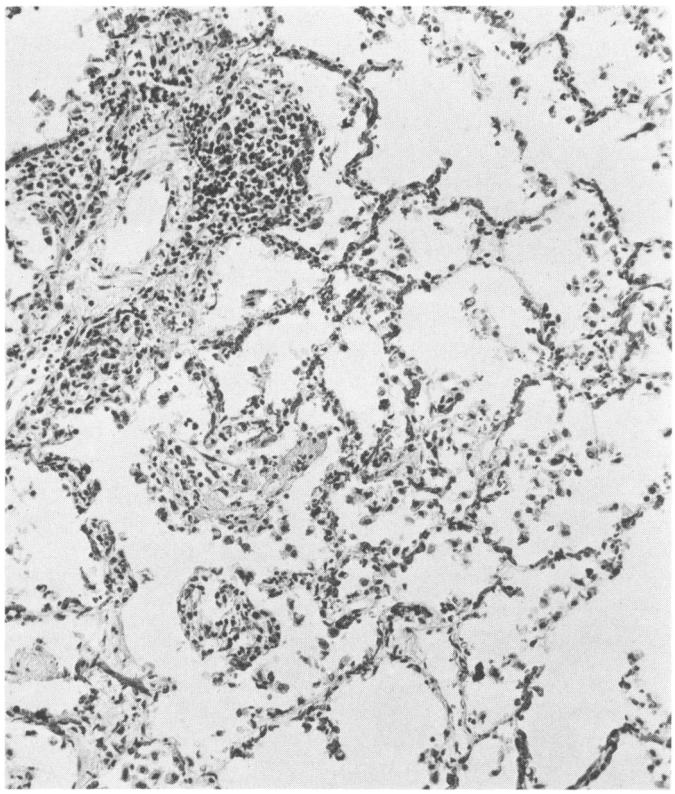

FIG. 9. Graft rejection with perivascular (upper left) and intra-alveolar (center) inflammation at 11 days post-transplant with low dose immunosuppression (L164, H&E, 140×).

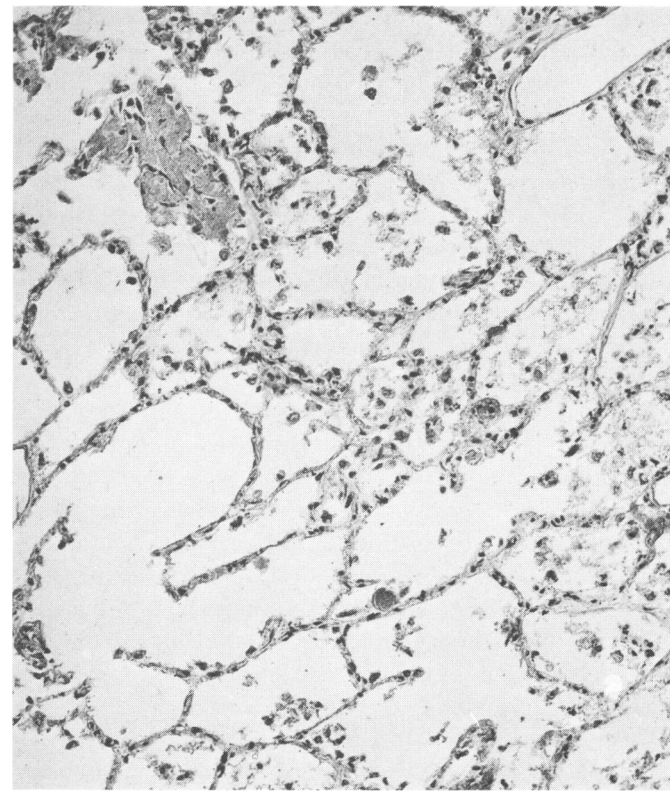

FIG. 11. Marked reduction of graft rejection with high dose immunosuppression at 17 days post-transplant (M57, H&E, 140×).

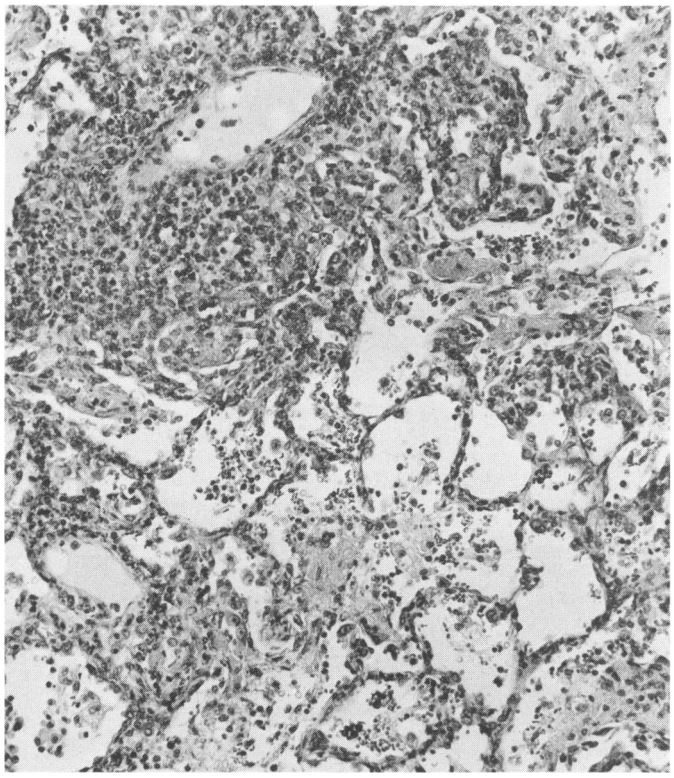

FIG. 10. Graft rejection at 20 days with low dose therapy. Note trivial intimal inflammation (upper left), severe perivascular infiltrate and organization of proteinaceous alveolar exudate (L159, H&E, 140×).

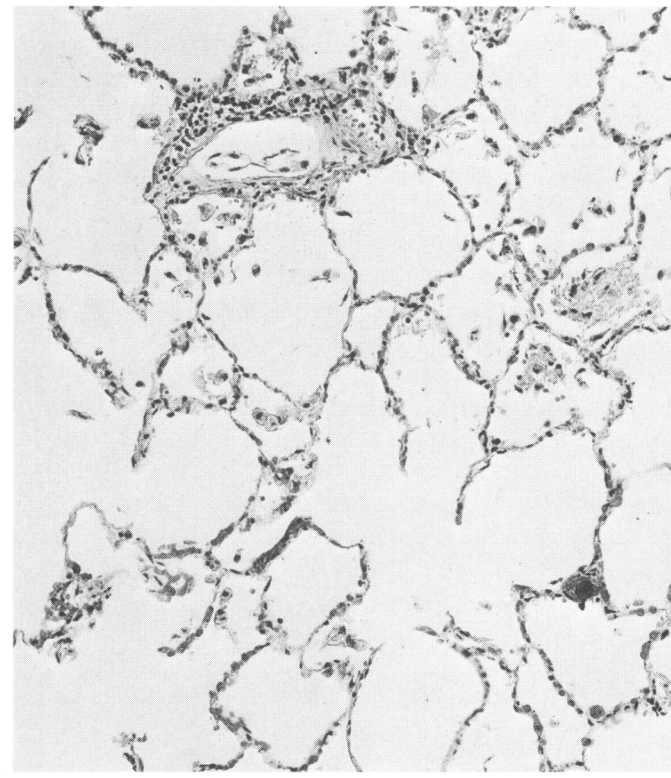

FIG. 12. Trivial perivascular (upper left) and intra-alveolar inflammation attributable to graft rejection at 31 days with high dose therapy (M54, H&E, 140×).

accumulations of inflammatory cells become prominent. The exudate within the alveoli may resolve by macrophage digestion or may become organized by fibroblastic ingrowth.

With the higher dose immunosupression regimen the cellular component of the inflammatory response disappears from both the alveolar and perivascular locations. The exudation of proteinaceous material into the alveoli seems unaffected, however. Apparently this selective suppression of the cellular component of the inflammatory reaction is what has been interpreted as indicating separate "vascular" and "alveolar" phases of graft rejection.[7]

Of particular interest is the absence of significant intimal inflammation or thrombosis related to graft rejection. The morphologic changes observed in blood vessels of heart,[8] kidney[6] and canine lung[1] allografts were not seen in this study. Except in one animal, vasculitis and thrombosis were associated either with local surgical injury or with necrotizing pneumonia. Since the bronchial circulation of the transplanted lung is not intact occlusion of pulmonary artery branches regularly produces infarction.

All lungs examined in the present study showed evidence of parasitization with the lung mite Pneumonyssus.[3,5] Fecal deposits with foci of chronic inflammation, scarring and emphysema are common. The asthma-like bronchial wall changes apparently induced by the parasite are a possible source of confusion with the alterations of graft rejection.

The observations suggest that control of the complications of surgery and of immunosuppressive therapy could produce long term survival of baboon lung allografts. The vasculitis of graft rejection seemed well controlled with the treatment used.

Summary

Thirteen pairs of baboons underwent left lung allografts and treatment on either a low or a high dose regimen of azathioprine and methyl prednisolone. Animals survived up to 31 days. Pathologic studies showed that graft rejection, including vasculitis, could be effectively controlled. There was a high incidence of severe necrotizing pulmonary infections with the larger doses of immunosuppressive agents. Thrombosis at the arterial anastomosis leading to pulmonary infarction occurred in animals in both groups. Inflammatory reactions due to lung parasites must be distinguished from graft rejection in baboons. The overall results suggest that long term success with lung transplantation is possible with control of the complications of treatment.

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