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Transplantation Pathology

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The first human lung transplant was carried out in 1963 by Hardy and colleagues,[1] and subsequently this radical therapeutic modality has been applied to patients with terminal vascular, interstitial, and obstructive lung disease.[2–6] This experience plus the results of experimental work, particularly by Veith and colleagues,[2,3] have provided much insight into the surgical problems, immunosuppressive complications, and rejection phenomena associated with the procedure.[3,7] Between 1963 and 1980, over 40 lung transplants were performed, but no long-term clinical successes were observed except for two patients surviving 6 and 10 months, respectively.[7]

In 1981, coinciding with the introduction of cyclosporine, investigators at Stanford University, under the leadership of Reitz and Shumway,[8–10] performed the first heart-lung transplantation (HLT). Since then, over 200 HLT operations have been carried out, primarily for patients with irreversible pulmonary hypertension due to Eisenmenger complex and primary pulmonary hypertension.[11] HLT is indicated in patients with terminal heart and lung disease, such as in advanced pulmonary hypertension; it is also the therapeutic choice in patients with such diseases as severe emphysema, cystic fibrosis, and pulmonary lymphangioleiomyomatosis.[12]

Single-lung transplantation (SLT) or bilateral sequential SLT,[13] however, may be a better indication in patients with lung disease unassociated with cardiac disease or infection. It is indicated in patients younger than 60 years of age with end-stage pulmonary disease, including all types of interstitial fibrosis, emphysema due to α_1-antitrypsin deficiency, and the adult respiratory distress syndrome.[14] SLT has been shown to be effective in the treatment of bilateral pulmonary disease, because the transplanted lung can accommodate the entire cardiac output with easily tolerable pulmonary artery pressures.

SLT may be preferable to HLT because it obviates replacing a normal heart with a transplanted heart, and because the initial problems of bronchial anastomotic healing associated with this procedure have been overcome with newer techniques.[14] HLT, on the other hand, offers some major advantages, including relative technical simplicity, secure healing of the tracheal anastomosis, elimination of all of the diseased lung tissue, and provision of a maximal amount of normal lung parenchyma.[14]

TECHNICAL CONSIDERATIONS

For HLT, the recipient's vital functions are maintained by a cardiopulmonary bypass machine, and the heart and each lung are removed from the chest with the airways amputated above the carina. The ascending aorta is severed, the intact aortic arch and thoracic aorta remaining within the recipient's chest; both venae cavae attached to a portion of the right atrium are also severed and left in place. For on-site transplants, the donor organs (*i.e.*, the heart and lung) are removed *en bloc* in an adjacent operating suite. They are placed within the recipient's chest and anastomosed to the trachea, ascending aorta, and right atrium.

Of relevance to subsequent clinical complications and pathologic findings are the following points:

- Ligation of bronchial arteries of the recipient and donor: the bronchial arteries are ligated at their origin from the aorta and are not anastomosed; for that reason they are a frequent source of postoperative bleeding.
- Interruption of lymphatic routes: pulmonary parenchymal drainage is impaired until the hilar lymphatics are able to regenerate and anastomose with one another.
- Denervation of the bronchial tree: the loss of nervous connections results in impaired cough reflex and pulmonary toilet. This leads to mucostasis and the need for exhaustive pulmonary toilet in order to prevent aspiration of secretions and subsequent pulmonary infections.
- Because the donor's peribronchial, hilar, and mediastinal lymph nodes are not removed from the heart-lung block, they act as immunogens.

Postoperative immunosuppressive therapy includes intravenous methylprednisolone supplemented with oral cyclosporine and azathioprine for 21 weeks.[11,15] Azathioprine is then stopped and immunosuppression is maintained with prednisone and cyclosporine. Episodes of cardiac or pulmonary rejection are treated with bolus intravenous methylprednisolone, prednisone, or antilymphocyte globulin.

REIMPLANTATION RESPONSE

From 2 days to 2 weeks following transplantation, the patient may experience a phenomenon referred to as the reimplantation response.[9,14,15] This response reflects the morphologic, roentgenographic, and functional changes due to surgery, ischemia, denervation, and lymphatic interruption of the transplanted lung. The reimplantation response mimics pulmonary rejection, and it is manifested by alveolar infiltrates and perihilar shadows of pulmonary edema. Histologically, the lungs demonstrate alveolar and interstitial edema primarily in the perihilar regions. Small numbers of neutrophils may also be seen within alveoli or alveolar septa. Dehydrating therapy usually resolves the edema; once the lymphatics regrow, intrinsic elimination of the fluid takes place.

REJECTION OF THE LUNG ALLOGRAFT

Since the pioneering studies of Veith and colleagues,[2,3] the histopathologic manifestations of pulmonary rejection have been well described, with the initial work in human allografts confirmed and expanded in animal models. Although histologic features are widely accepted in broad concept, only recently have they been categorized and presented in a standardized form. The Society for Heart and Lung Transplantation has provided a working formulation for the diagnosis and grading of pulmonary rejection based on human studies and animal models.[16] Their grading scheme for pulmonary rejection is divided into two forms, acute and chronic rejection.[16–20] This classification was designed to be exclusive of clinical information and focused solely on the histologic findings, primarily in transbronchial biopsy specimens.[16] Because it is recognized that infection and rejection often occur together, the exclusion of infection is essential for the proper interpretation of all biopsy specimens; reproducible grading of rejection is not feasible in a setting of pulmonary infection.

Evaluation of the airways and vasculature is necessary to diagnose acute and chronic rejection of the lung. Acute rejection is characterized by both perivascular mononuclear infiltrates and a lymphocytic bronchitis or bronchiolitis. Chronic rejection is manifested by the development of bronchiolitis obliterans (BO) and accelerated arteriosclerosis. Although these changes probably represent a continuum, they have been divided into grades in this classification (Display 71-1).

To use this classification, the pathologist should label the process with the appropriate capital letter and numerical designation and designate the presence or absence of airway inflammation by a lower-case letter.[16] For example, mild acute rejection with bronchiolar injury would be classified A2a; moderate acute rejection without evidence of small airway injury would be classified A3b. In some instances, acute rejection may be superimposed on chronic rejection, such as moderate acute rejection with airway damage superimposed on preexisting small airway scarring, which would be classified A2a and C1a.

DISPLAY 71-1. WORKING FORMULATION FOR CLASSIFICATION AND GRADING OF PULMONARY REJECTION

A. Acute rejection
  0. No significant abnormality
  1. Minimal acute rejection
    a. With evidence of bronchiolar inflammation
    b. Without evidence of bronchiolar inflammation
    c. With large-airway inflammation
    d. No bronchioles present
  2. Mild acute rejection
    a. With evidence of bronchiolar inflammation
    b. Without evidence of bronchiolar inflammation
    c. With large-airway inflammation
    d. No bronchioles to evaluate
  3. Moderate acute rejection
    a. With evidence of bronchiolar inflammation
    b. Without evidence of bronchiolar inflammation
    c. With large-airway inflammation
    d. No bronchioles to evaluate
B. Active airway damage without scarring
  1. Lymphocytic bronchitis
  2. Lymphocytic bronchiolitis
C. Chronic airway rejection
  1. Bronchiolitis obliterans—subtotal
    a. Active
    b. Inactive
  2. Bronchiolitis obliterans—total
    a. Active
    b. Inactive
D. Chronic vascular rejection
E. Vasculitis

From Yousem SA, Berry GJ, Brunt EM, et al. A working formulation for the standardization in the diagnosis of heart and lung rejection: lung rejection study group. J Heart Lung Transplant 1990;9:53.

ACUTE REJECTION

If a lung is allotransplanted in a patient who is not receiving immunosuppressive therapy, it undergoes a series of clinical, radiographic, functional, and morphologic disturbances that are recognized as manifestations of acute pulmonary rejection.[3,19,21] These findings appear to be comparable in experimental animals such as rats, mongrel dogs, baboons, and rhesus monkeys. In humans, they occur in both lung transplants and HLT. In the latter group, it was initially thought that lung and heart rejection occurred simultaneously. However, clinical and experimental studies have shown that although heart rejection is almost uniformly associated with pulmonary abnormalities, lung rejection can occur with a normal endomyocardial biopsy.[22–25] These findings may be a result of quantitative differences in the expression of HLA antigens in the two organs.[26–28] Furthermore, the addition of cyclosporine to the immunosuppressive protocol has allowed a more expeditious reversal of the rejection crisis.[29]

The precise incidence of early pulmonary rejection in transplant recipients is unknown, although more than one half of the recipients usually have a rejection episode within the first 2 to 3 weeks.[30] For the most part, patients experience fever, dyspnea, and cough, without serologic, culture, or histologic evidence of pulmonary infection; other patients may be asymptomatic. Chest roentgenograms reveal progressive bilateral infiltrates, predominantly in the lower lobes, that are unresponsive to antibiotic therapy over a 1- to 2-day interval.

Blood gas studies show progressive hypoxemia, which coincides with an increase in pulmonary vascular resistance and the presence of transformed lymphocytes in peripheral blood smears. Bronchoalveolar lavage reveals lymphocytosis, and the lymphocytes show high levels of cell-mediated lympholysis.[31,32] Because the transplanted lungs have their full complement of bronchial-

associated lymphoid tissue, they undergo repetitive interactions with the immunocompetent host lymphocytes.[33,34] An *in vivo* mixed lymphocyte reaction results, and lavage studies have revealed that during the first 7 weeks after transplantation, the majority of donor lymphocytes and macrophages are replaced by those of the recipient. This phenomenon is an integral part of the early rejection period.

Acute rejection may be divided into four grades (see Display 71-1), which probably represent a spectrum of lesions: minimal (grade A1), mild (grade A2), moderate (grade A3), and severe (grade A4). By convention, classification of acute rejection into these four categories is determined by the nature and extent of the perivascular infiltrates.[35,36] Minimal acute rejection (grade 1) is not obvious at scanning magnification, but at higher magnification, subtle perivascular infiltrates of small, round, angulate, and transformed lymphocytes and histiocytes can be seen, particularly within the interlobular septa and perivenular zones (Fig. 71-1). When these infiltrates are readily identifiable at scanning magnification, mild rejection (grade 2) is diagnosed (Fig. 71-2). Endothelial cell hyperplasia with subendothelial infiltration by lymphocytes (*i.e.*, endothelialitis), and occasional eosinophilia may be observed (Fig. 71-3).

Moderate rejection (grade 3) is diagnosed by intense perivenular, peribronchiolar, and periarterial cuffing by lymphocytes and immunoblasts with endothelialitis. The inflammatory infiltrates percolate within the alveolar septa proper, resulting in an interstitial pneumonitis with large numbers of activated lymphocytes (Fig. 71-4 and 71-5). The inflammatory process extends to the pleura. Neutrophils may also be identified.

As the inflammatory process progresses to severe acute rejection (grade 4), changes of acute alveolar septal injury develop, with necrosis of alveolar pneumocytes, formation of hyaline membranes, and exudation of fluid into the alveolar spaces (Fig. 71-6). Further progression is characterized by fibrinoid necrosis and thrombosis of veins and arteries, with hemorrhage and necrosis of lung parenchyma. There is marked acute suppurative bronchiolitis with widespread desquamation of epithelial cells, with organizing plugs of granulation tissue within the air spaces (Fig. 71-7). Alveolar pneumocytes appear hyperplastic and atypical, and prominent infiltrates of neutrophils and macrophages in air spaces are also present.

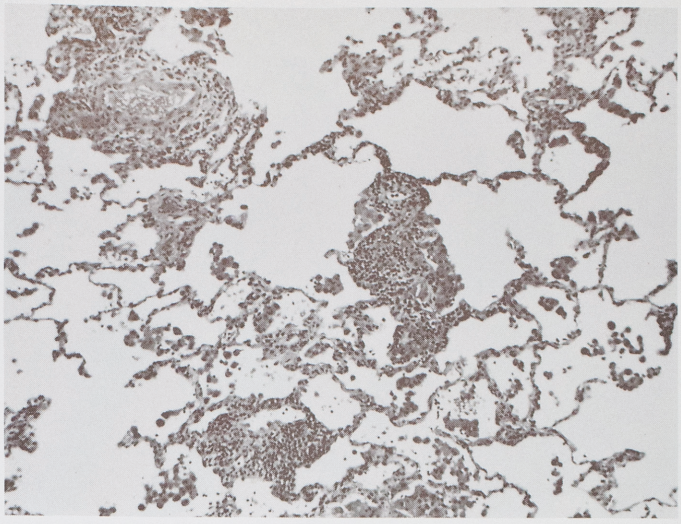

FIGURE 71-2. In mild acute rejection, obvious cuffing of veins and arteries by mononuclear cells is seen. (H & E stain; low magnification.)

Injury to the conducting air passages is extremely important because it is largely responsible for the pulmonary functional abnormalities afflicting graft recipients. In fact, airway injury in acute rejection has been found to be an independent predictor of the subsequent development of BO (see Chronic Rejection).[37] Airway injury in acute rejection is usually associated with perivascular lymphocytic infiltrates.

Lymphocytic Bronchitis or Bronchiolitis

Lymphocytic bronchitis shows a mixture of mononuclear cells in the mucosa and submucosa of bronchi, whereas lymphocytic bronchiolitis involves the terminal and respiratory bronchioles (see Chap. 30).[16] Submucosal gland and epithelial injury with single cell necrosis and squamous metaplasia may be evident. There is no associated fibrous scarring or perivascular infiltrates.[16]

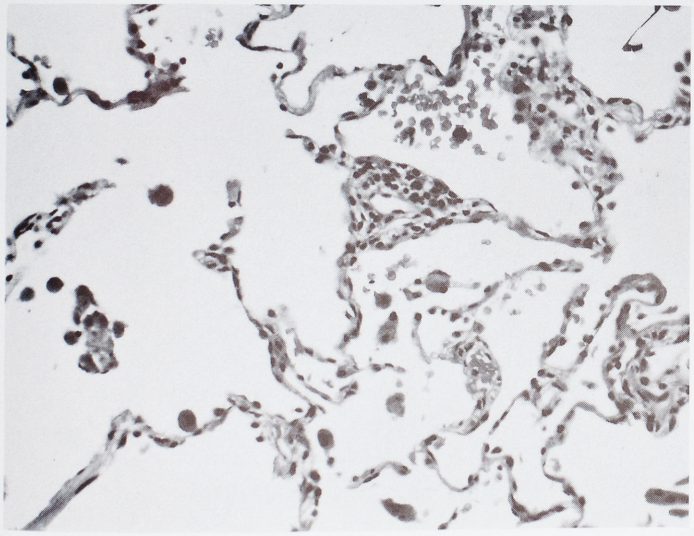

FIGURE 71-1. Minimal acute rejection. At this magnification a narrow band of lymphocytes and histiocytes cuffing small venules is barely noticeable. (H & E stain; low magnification.)

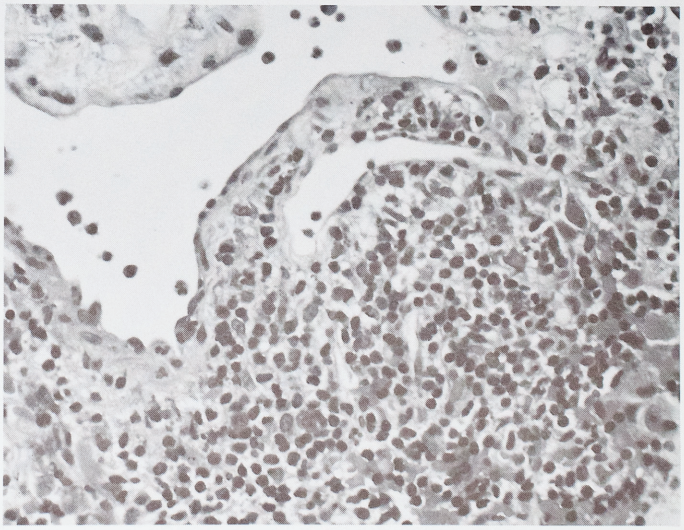

FIGURE 71-3. In mild acute rejection, lymphocytes move to the perivascular adventitial zones and percolate beneath the endothelium. (H & E stain; low magnification.)

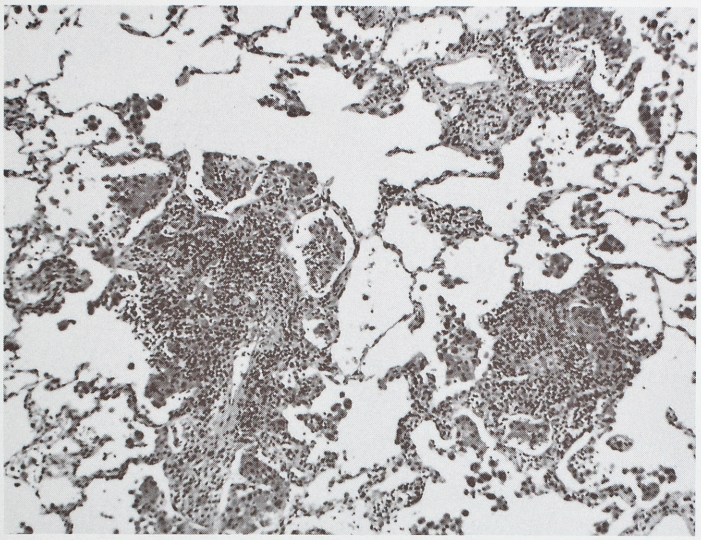

FIGURE 71-4. In moderate acute rejection, mononuclear cells extend from the perivascular cuffs into the alveolar septa. (H & E stain; low magnification.)

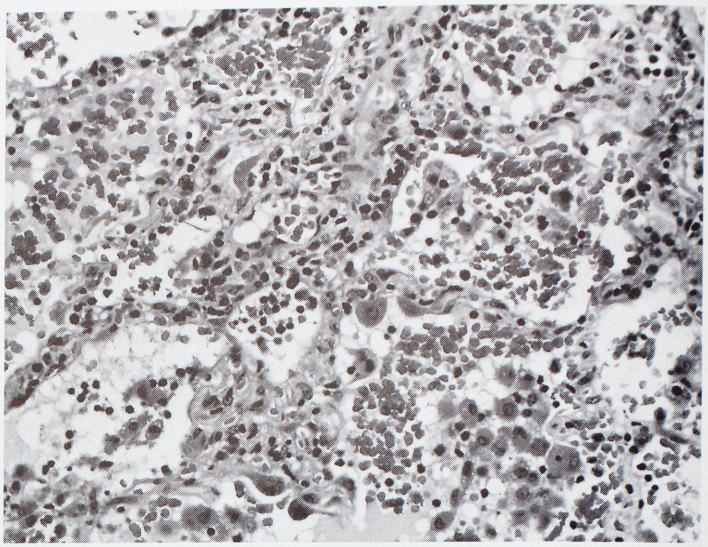

FIGURE 71-6. In severe acute rejection, an intense interstitial infiltrate expands the septa in a diffuse fashion, and lymphocytes and neutrophils fill air spaces along with hyaline membranes and desquamated pneumocytes. (H & E stain; low magnification.)

Diagnosis of Acute Rejection

Clinical evaluation is the most important method of diagnosing acute rejection. It should be suspected in a transplant recipient with fever, leukocytosis, and bilateral lung infiltrates.[23,30,35] Bronchoscopy and lavage should be used primarily to isolate infectious agents, because cell differential counts show no consistent correlations with rejection episodes.[35–38] Recent studies have shown that increased lymphocyte cytotoxicity in lavage and peripheral blood lymphocytes is associated with early rejection and may be a helpful adjunct to diagnosis. Tissue diagnosis of acute rejection by either transbronchial or open biopsy is extremely difficult because many viral and idiopathic reactions are accompanied by perivascular infiltrates.

The features of acute rejection previously described, combined with negative special stains and cultures for microorganisms, and the clinical information are necessary for a definitive diagnosis. Reversal of radiographic, clinical, and functional abnormalities by intravenous boluses of methylprednisolone provides the ultimate confirmation. One myth that should be discarded is that lung rejection cannot occur without simultaneous cardiac rejection.

CHRONIC REJECTION

Chronic rejection is also characterized by airway, vascular, and interstitial changes. The most prominent changes affecting the airways is BO, also known as obliterative bronchiolitis.[2,3,5]

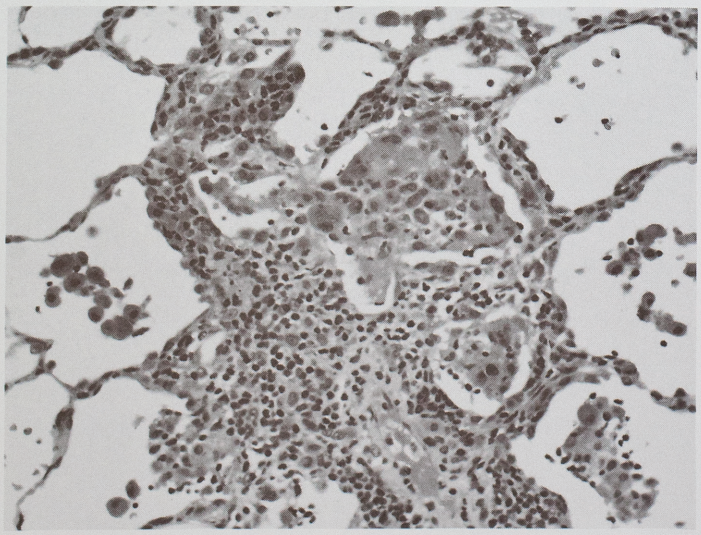

FIGURE 71-5. In moderate acute rejection, a cellular interstitial infiltrate is seen within thickened septa associated with intraalveolar collections of macrophages. (H & E stain; low magnification.)

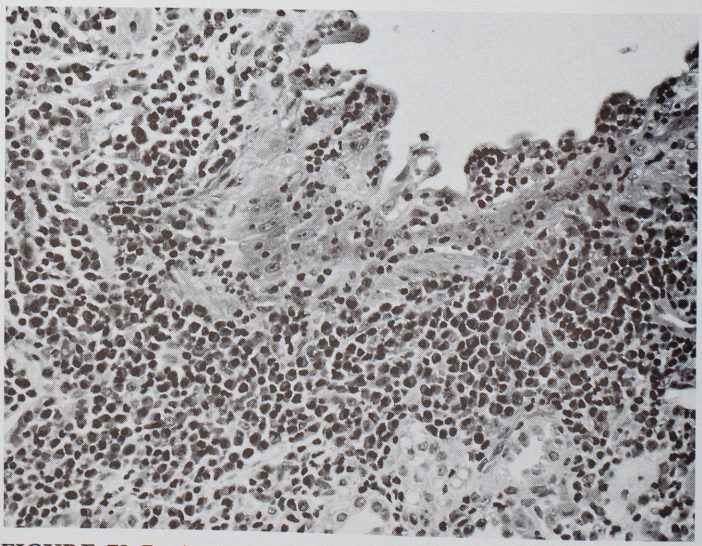

FIGURE 71-7. As part of acute rejection, small airway injury is a harbinger of subsequent obliterative bronchiolitis. (H & E stain; low magnification.)

Bronchiolitis Obliterans

BO is a nonspecific response to airway injury resulting in a fibrosing inflammatory process usually involving terminal and respiratory bronchioles. If persistent, BO may lead to progressive small airway obstruction (see Chap. 30). The presumed sequence of injury is denudation and ulceration of the respiratory epithelium with sloughing of necrotic debris, fibrin, and macrophages into the lumen. With time, ingrowth of fibroblasts from the exposed submucosa results in intraluminal plugs of acid mucopolysaccharide–rich myxoid tissue (*i.e.*, Masson bodies). As the tissue organizes, concentric sheets of more mature collagen envelop the central core of necrotic debris, lymphocytes, plasma cells, and macrophages. Progressive scarring results in a thickened fibrotic submucosa lined by attenuated respiratory epithelium or metaplastic squamous mucosa, creating a rigid bronchiole. Frequently, dense submucosal and peribronchiolar fibrosis replaces the muscular wall, resulting in a reduction of the luminal diameter (Figs. 71-8 and 71-9). With time, the only residual finding may be an eccentric submucosal scar and disrupted elastica.

BO may also be classified as active or inactive, depending on the degree of mononuclear cell infiltration of the submucosa, and as total or subtotal, depending on whether there is complete or incomplete obliteration of the airway lumen.[16] In subtotal BO, the scar tissue may be concentric or eccentric and associated with destruction of the smooth muscle wall, and there may also be extension of the fibrosis into the peribronchiolar interstitium. The lumen in total BO is completely obliterated by dense scar tissue and may be associated with smooth muscle loss and peribronchiolar fibrosis. With controlled rejection or with persistent low-grade allograft injury, progressive scarring and fibrosis may produce obliteration of the airways and airflow obstruction.[16,19]

Depending on the severity of injury and its chronicity, several outcomes are possible[15]:

The injury may resolve and the submucosa reepithelialize, leaving an intact bronchiole of normal diameter.

An eccentric or concentric submucosal scar may form with reepithelialization of the mucosa and luminal narrowing; this sequel may be obvious only with elastic tissue stains that highlight the disrupted bronchiolar elastica.

The entire bronchiole may be obliterated and completely replaced by dense scar tissue and be identifiable only by its location next to pulmonary arterioles, its residual smooth muscle layer, and its elastic tissue lamina.

Peribronchiolar fibrosis may result in extrinsic compression of the lumen, the constrictive form of BO.

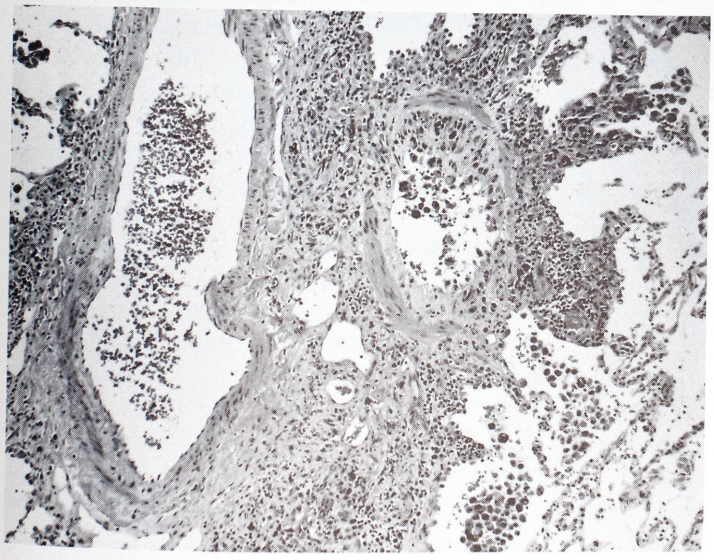

FIGURE 71-8. Chronic rejection with obliterative bronchiolitis. Subtle submucosal fibrosis of the small airway to the right (*arrow*) is present, with a coexistent chronic inflammatory cell infiltrate. (H & E stain; low magnification.)

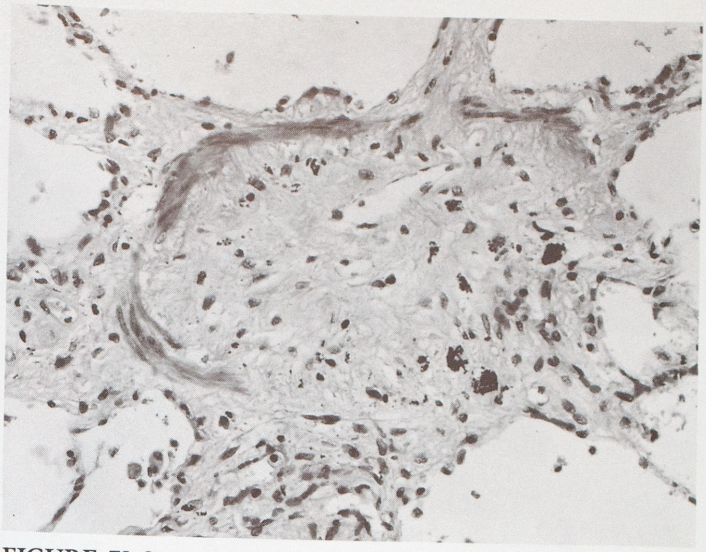

FIGURE 71-9. Chronic rejection with obliterative bronchiolitis. The lumen of this respiratory bronchiole is obliterated by dense fibrous scar tissue and is reduced to a slitlike space. (H & E stain; low magnification.)

BO, which causes destruction or constriction of small airways, is associated with an unusual finding in the major airways: cylindrical bronchiectasis and mucostasis. Large bronchi may show acute and chronic inflammation with extensive squamous metaplasia. The submucosa is densely fibrotic and scarred, and the smooth muscle layer is focally absent and replaced by fibrovascular connective tissue. This loss of bronchial smooth muscle and supporting matrix results in a fusiform dilatation of the large cartilaginous airways on specimen bronchograms.[19] The acute peripheral tapering and pruning of the bronchi correlate with the obliteration and luminal reduction of the terminal and respiratory bronchioles. Recent studies have suggested that large airway inflammation may be a good marker for small airway disease in these patients.

Patients with BO frequently develop coexistent accelerated coronary arteriosclerosis.[39] The vascular disease may be manifested by a concentric fibroelastosis occurring on the inner aspect of an intact internal elastica; an active endovasculitis has also been observed.[15] Myocardial infarcts may be seen, and some patients develop patchy areas of myocardial fibrosis of unknown etiology. The coronary arterial changes are closely related to the vascular disease of the pulmonary arteries and veins and have been attributed to chronic graft rejection.[17]

Pulmonary Arteries and Veins

The pulmonary arterial changes of chronic graft injury consist of intimal fibrous hyperplasia in arteries and arterioles and mild muscular hypertrophy of arterioles (Fig. 71-10).[15] Plexiform and

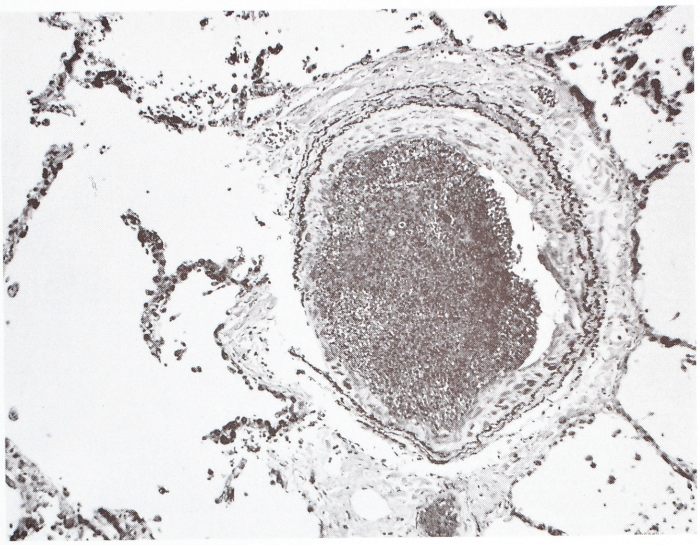

FIGURE 71-10. Chronic rejection with graft atherosclerosis. Fibrointimal thickening of the arteries and veins is a manifestation of chronic vascular rejection. (H & E stain; low magnification.)

angiomatoid lesions are not usually present. The intimal changes may be accompanied by a mononuclear cell infiltrate, frequently T cells, and the proliferating cellular components of the mesenchyme (*i.e.*, fibroblasts, myofibroblasts, and occasionally smooth muscle cells).[39]

The pulmonary veins show intimal thickening similar to that seen in the arteries and arterioles; however, unlike the arterial lesions, the venous thickening has a waxy, sclerotic appearance similar to the changes seen with aging. When fibrosis affecting both arteries and veins is severe, perivascular fibrosis is present as well.

Interstitium

The interstitial changes in chronic rejection are usually mild, patchy, and without functional significance.[15] They usually represent diffuse subtle peribronchial and perilobular fibrosis, particularly in the subpleural regions. Prominent alveolar septal fibrosis and interstitial scarring are not seen.

Visceral Pleura

Although one would expect extensive pleural fibrosis due to implantation of a lung or a massive heart-lung block, pleural adhesions are not that prominent.[15] They occur as a uniform adherence, similar to fibrothorax, only rarely, and in patients who are not functionally incapacitated. The adhesions usually form over the posterobasal portions of the lung and seem to correlate with the degree of operative hemorrhage. Pleural scars frequently extend down the interlobular septa, giving the lung a latticeworklike pattern of coarse scarring.

Infections

Both the Stanford and Pittsburgh groups have reported infectious complications in over 85% of heart-lung recipients.[37,40] Between 67% and 89% of them involved the lung or thoracic cavity, and they were overwhelmingly bacterial (71%).[37] Gram-negative bacilli, particularly *Serratia*, *Pseudomonas*, *Bacteroides*, and *Haemophilus* species, constituted 66% of these pulmonary infections.[37] The infectious episodes usually occurred within the first 6 weeks after surgery.

Forty-two percent of patients with transplants also developed cytomegalovirus (CMV) infection, whereas 8% had episodes of cutaneous herpes simplex infection.[40] In the Pittsburgh experience, a much higher incidence of *Pneumocystis pneumonia* was observed,[30,41] and all recipients not receiving antibiotic prophylaxis apparently developed pneumocystosis. Two thirds of infections were also diagnosed unexpectedly during routine bronchoalveolar lavage. This incidence is in marked contrast to the Stanford experience, in which pneumocystosis was a rare occurrence.[37]

In one autopsy study of heart-lung recipients, *Candida* pneumonia and tracheitis were noted in three patients, and herpes tracheobronchitis was found in two others.[17] One patient died of aspergillosis, another of coccidioidomycosis, and a third of pneumocystosis. Except for two patients, long-term survivors died of progressive unrelenting BO.

Heart-lung recipients also appear to be at increased risk for Epstein-Barr virus–induced lymphoproliferations.[42,43] Also at Pittsburgh I have seen four patients develop pseudoaneurysms of the aorta at the anastomotic site, leading to rupture and fatal exsanguination.

ATYPICAL FORMS OF PULMONARY REJECTION

In addition to the classic scheme of acute and chronic pulmonary rejection, several atypical forms of rejection have been identified. Veith proposed that a pattern of diffuse alveolar damage (DAD) in the pulmonary parenchyma represented an atypical form of pulmonary rejection.[44] My experience indicates that most cases of DAD are a consequence of preservation or harvesting injury in the immediate perioperative period, or a result of oxygen toxicity or infection and sepsis in later periods.[45,46]

Also, some cases of rejection are dominated by intense eosinophilic infiltrates.[15] These infiltrates are usually exquisitely responsive to steroid therapy. Other causes of these infiltrates include allergic-hypersensitivity reactions, particularly to drugs, as well as infections. Agents that are known to cause pulmonary parenchymal eosinophilia include *Aspergillus*, *Coccidioides*, and *Pseudomonas* species, and some viruses (*e.g.*, coxsackievirus).

Another form of atypical rejection resembles cryptogenic organizing pneumonia (*i.e.*, bronchiolitis obliterans organizing pneumonia).[47] In these cases, young plugs of granulation tissue are present within airways and air spaces and are accompanied by perivascular mononuclear inflammatory infiltrates. Although this is recognized as an atypical rejection reaction, other causes of cryptogenic organizing pneumonia in a lung allograft recipient should be identified. This includes harvesting injury in the first month after transplantation, which commonly lacks the perivascular mononuclear infiltrates, and infection, particularly bacterial in type.

A variety of other histopathologic observations have been seen in patients with lung rejection. In particular, treatment for acute rejection leaves several characteristic abnormalities. First, there are frequently large numbers of plasma cells and small round

lymphocytes left in the perivascular zones after the angulated and transformed lymphocytes have disappeared because of intense immunosuppressive therapy. In moderate acute cellular rejections, vascular injury may be significant and may result in exudation of blood, converted to hemosiderin, into the air spaces. This alveolar hemosiderosis is a marker of previous rejection and may be accompanied by metaplastic bone formation and calcification.

Several points need to be made in the setting of these histologic abnormalities. First, acute and chronic lung rejection can be diagnosed on the basis of transbronchial biopsies. There is a strong correlation between clinical manifestations of acute rejection and biopsy findings, as well as the finding of significant airway injury with pulmonary function abnormalities indicative of airflow obstruction. Nonetheless, there are cases that do not fit this classic scenario, and in those instances open lung biopsy may be indicated.

Second, it should be recognized that there are many different causes of peribronchiolar and perivascular mononuclear infiltrates. These include infections, such as CMV and pneumocystis, and it is my policy not to make a diagnosis of rejection in the setting of active pulmonary infection. This is particularly difficult in patients with end-stage chronic rejection when persistent bacterial infections of the graft make this distinction difficult; special stains to exclude microbial infestation are clearly necessary.

Third, routine hematoxylin and eosin stains are insufficient to identify the characteristic scarring of the anatomic compartments of the lung. For this reason, Masson trichrome stain, elastic tissue stain, methenamine silver stain, and Ziehl-Neelsen stain are necessary on all transbronchial biopsy specimens. Other stains, such as Gram, Warthin-Starry, immunoperoxidase stains for CMV and herpes simplex virus, lymphocyte cell-surface markers, and class II antigens, may also be helpful.

If the biopsy represents a follow-up to one that had previously shown an acute rejection or active chronic rejection episode, the following terminology should be used:

Ongoing rejection refers to no significant change in the histology of the current biopsy specimen as compared with the previous biopsy specimen

Resolving rejection consists of a reduction but not an elimination of the cellular infiltrates noted in the previous biopsy specimen

Resolved rejection represents complete resolution of the infiltrates.

The optimal number of transbronchial biopsy specimen fragments required to evaluate a lung transplant is unknown; however, I recommend a minimum of five samples containing alveolar tissue.

The histologic findings described in this review reflect my current interpretation of acute and chronic allograft rejection. Such morphologic observations have been confirmed by functional studies using the prime lymphocyte test,[48] and the empiric use of steroid therapy. Although not specific to alloreactive injury, they appear to be predictable in their response to clinical therapy and consistent with the observations made in other solid organ transplants.

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