Lung

7

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7.1 Introduction

7.1.1 Historic Perspective

Experiments with animals in the 1940 and 1950s demonstrated that lung transplantation was technically possible [33]. In 1963, Dr. James Hardy performed the first human lung transplantation. The recipient survived 18 days, ultimately succumbing to renal failure and malnutrition [58]. From 1963 through 1978, multiple attempts at lung transplantation failed because of rejection and complications at the bronchial anastomosis. In the 1980s, improvements in immunosuppression, especially the introduction of cyclosporin A, and enhanced surgical techniques led to renewed interest in organ transplantation. In 1981, a 45-year-old-woman received the first successful heart–lung transplantation for idiopathic pulmonary arterial hypertension (IPAH) [106]. She survived 5 years after the procedure. Two years later the first successful single lung transplantation for idiopathic pulmonary fibrosis (IPF) [128] was reported, and in 1986 the first double lung transplantation for emphysema [25] was performed.

Over the following years, the number of lung transplants rapidly increased, and the operation became an accepted treatment for an end-stage lung disease. Today, there are four major surgical approaches to lung transplantation: single and bilateral lung transplantation (BLT), heart–lung transplantation, and transplantation of lobes of lungs from living donors. In 2007, 2,708 lung transplantation procedures were reported worldwide to the Registry of the International Society for Heart and Lung Transplantation (ISHLT) in adults, the highest number for any year until then [21]. In the same year, 93 lung transplantations were reported in children, the majority in adolescents (12–17 years old) [6]. Although the number of single lung transplantations has been relatively stable, BLTs have continuously increased within the past 15 years. In fact, in 2007, BLT was the most common lung transplantation procedure performed with 69% of all lung transplantation procedures, largely due to transplantation for cystic fibrosis and chronic obstructive lung disease/emphysema which made up for 26.6 and 25.7% of all BLTs between 1995 and 2008 [21].

The mean age of transplant recipients has consistently increased since 1989 rising to an all time high of 50.8 years in 2008 [21].

7.1.2 Native Disease in Explanted Lungs

The most common indications for lung transplantation in adults are chronic obstructive pulmonary disease (COPD)/emphysema, IPF, cystic fibrosis and alpha-1 antitrypsin deficiency emphysema (AAT) (see Table 7.1) [21].

Indications for pediatric lung transplantation vary by age (see Table 7.1). In children over 5 years old, cystic fibrosis is the most common indication [6], followed by IPAH. In contrast, in infants and preschool children, lung transplantations are usually performed

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H. Liapis and H.L. Wang (eds.), *Pathology of Solid Organ Transplantation*,
DOI: 10.1007/978-3-540-79343-4_7, © Springer-Verlag Berlin Heidelberg 2011

Table 7.1 Distribution of diagnoses among adult and pediatric lung transplant recipients (January 1995–June 2008) [6, 21]

Diagnosis	Adult transplants (%)	Pediatric transplants	
		Age <5 years	6–17 years
COPD/emphysema	35.8	3.9	0.8
IPF	20.8	6.6	3.5
Cystic fibrosis	15.9	3.9	65.4
AAT	7.1		
IPAH	3.3	18.2	8.3
Sarcoidosis	2.6		
Bronchiectasis	2.7		1.3
LAM	1.0		
Congenital heart disease	0.7	16.0	1.4
OB	0.9	5.0	3.8
Retransplant			
OB	1.2	3.3	3.2
Non-OB	0.9	2.8	2.4
Connective tissue disease	0.8		
Interstitial pneumonitis	0.3	9.4	0.6
Cancer	0.1		
Eisenmenger syndrome		3.3	1.1
Surfactant protein B deficiency		8.3	
Bronchopulmonary dysplasia		2.2	0.6
Other	6.0	17.1	7.6

IPF idiopathic pulmonary fibrosis; *AAT* alpha1-antitrypsin deficiency; *IPAH* idiopathic pulmonary arterial hypertension; *LAM* lymphangioleiomyomatosis; *OB* obliterative bronchiolitis

for IPAH, congenital heart disease, idiopathic interstitial pneumonitis, and surfactant protein deficiency.

Well-selected patients with systemic diseases such as sarcoidosis, lymphangioleiomyomatosis, and pulmonary Langerhans' cell histiocytosis have also had satisfactory results after lung transplantation [27, 71, 91, 99, 119] as have selected patients with scleroderma [84, 110, 113].

Multiple cases of incidental T1N0M0 or even Stage IIIA non-small cell carcinoma in the excised native lungs of transplant recipients have been reported [14, 30, 124]. Although one patient with Stage IIIA poorly differentiated squamous cell carcinoma died 6 months after transplantation of a neoplastic thromboembolus, patients with T1N0M0 carcinoma are generally free of recurrence.

7.1.3 Allograft Selection and Procurement

Currently, only patients with near end-stage lung disease and a limited life expectancy should be considered for lung transplantation [95]. However, since lung transplantation is a rapidly evolving field, there are no hard and fast rules about who may be transplanted. When choosing a transplantation procedure, several issues are considered including the shortage of organ donors, the original disease, and the center's experience with graft and patient survival. General guidelines for the selection of the procedure have been proposed [36] and are based on the nature of the underlying lung disease. While BLTs are mandatory for cystic fibrosis

[35], this procedure has also become more popular for indications such as AAT, COPD, IPF, and IPAH. Single-lung transplantation is usually performed in patients with restrictive fibrotic lung disease, Eisenmenger syndrome with reparable cardiac anomaly, and older patients with COPD. Heart–lung transplantation is considered in patients with Eisenmenger syndrome with irreparable cardiac defect, pulmonary hypertension with cor pulmonale, or end-stage lung disease with concurrent severe cardiac disease [83, 89]. Transplantation of lobes from living donors is a recently developed technique involving bilateral implantation of the lower lobes usually from two blood group–compatible living donors. The procedure has been performed in patients with cystic fibrosis, although the indications have been recently broadened. The functional and survival outcomes are similar to those achieved with conventional transplantation of cadaveric lungs. Donation of a lobe decreases the donor's lung volume by an average of approximately 15%, which is not associated with long-term functional limitation.

Other factors of the recipient that must be taken into consideration on an individual basis include ventilator dependence, previous cardiothoracic surgery, and preexisting medical conditions (e.g., hypertension, diabetes mellitus, osteoporosis) since posttransplantation medical regimen can worsen these illnesses. Severe coronary artery disease is a contraindication to lung transplantation. However, coronary artery bypass grafting at the same time as lung transplantation has been performed with a reasonably good outcome in some centers, although less invasive preoperative interventions, such as percutaneous transluminal coronary angioplasty and stenting, are preferred.

Although the donor selection criteria may vary amongst centers, generally acceptable donor criteria include age of donor <65 years for lung transplantation and <45 years for heart–lung transplantation. In 2008, the average donor age was 35.5 years [21]. Other donor criteria include the absence of severe chest trauma or infection, no prolonged cardiac arrest (heart–lung transplantation only), minimal pulmonary secretions, negative screens for HIV, hepatitis C, and hepatitis B and blood type (ABO) compatibility. A close match of lung size between donor and recipient, PaO_2 > 300 mmHg on 100% fraction of inspired oxygen (FiO_2), clear chest radiograph and no history of malignant neoplasms are also required. Most transplant centers will use lungs from a cytomegalovirus (CMV)-positive donor for transplantation into a CMV-negative donor given an adequate postoperative CMV prophylaxis.

With the current techniques, satisfactory graft function can be obtained after an ischemic interval of as long as 6–8 h. For pulmonary preservation, systemic heparinization of the donor and hypothermic flush perfusion of the allograft are most commonly used in clinical practice. Most flush solutions are administered at a temperature of 4°C, while topical cooling is carried out by filling the pleural cavity with iced crystalloid solution. The harvested lungs are then immersed in crystalloid solution, packed in ice, and transported at a temperature of 1–4°C. The infusion and transport is performed during active ventilation and static inflation with O_2, respectively.

7.2 Allograft Rejection

7.2.1 Overview

Acute and chronic alloreactive injury to the donor lung affects both the vasculature and the airways [123]. Usually, rejection is evaluated on transbronchial biopsies (see below Sect. 7.3). On only rare occasions, wedge biopsies are performed. Other specimens might include explants for retransplant or autopsy specimens. Acute rejection is characterized by perivascular mononuclear cell infiltrates, which may be accompanied by sub-endothelial chronic inflammation (e.g., endotheliitis or intimitis), and also by lymphocytic bronchiolitis. In contrast, chronic rejection is manifest by fibrous scarring, involving the bronchioles and sometimes associated with accelerated fibrointimal changes affecting pulmonary arteries and veins. The presence of presumed irreversible dense eosinophilic hyaline fibrosis in airways and vessels remains the key histologic discriminator between acute and chronic rejection of lung. The histologic changes are divided into grades based on intensity of the cellular infiltrate, and the presence and absence of fibrosis.

7.2.2 Hyperacute Rejection

Hyperacute rejection occurs within minutes to a few hours after the newly transplanted organ begins to be perfused. It is a type II hypersensitivity reaction, mediated by preexisting antibodies to ABO blood groups, human leukocyte antigens (HLA) class I, or other antigens on graft vascular endothelial cells. Preexisting antibodies can result from previous pregnancies, blood transfusions, or a previous transplant. Antibody binding provokes complement and cytokine activation leading to endothelial cell damage and platelet activation with subsequent vascular thrombosis and graft destruction. The outcome is usually fatal.

In the lungs, hyperacute rejection grossly presents by edema and cyanosis of the graft. Histologically, platelet thrombi, neutrophilic infiltration, fibrin thrombi, necrosis of vessel wall, and morphologic features of diffuse alveolar damage (DAD) are observed [29].

Although hyperacute rejection is a well-known complication in kidney and heart transplantations, in lung transplantation it appears to be rather rare with only five cases reported. One patient reported presented with severe hypoxia, high fever, hemodynamic instability and developing acute renal failure 1 h after completion of the anastomoses [29]. Chest radiograph displayed a completely opacified left lung, with homogenous infiltrates. Bronchoscopy revealed abundant pink frothy fluid draining from the allograft. Mean pulmonary artery pressure increased to 29 mmHg. The patient died 24 h later. At autopsy, the vascular and bronchial anastomoses appeared patent without signs of injury. The transplanted lung showed red hepatization and a firm consistency. Microscopically, signs of acute lung injury were evident. Although a pretransplant panel-reactive antibody (PRA) was negative, flowcytometry revealed 56 and 45% reactivity against HLA class I and II, respectively with anti-A2 detected among the preformed antibodies. Three other reported patients with hyperacute rejection died within 4 h to 13 days after transplantation [11, 19, 43, 116]. Although in three of the five reported patients pretransplant PRAs were negative, crossmatch was positive in all cases with anti-A2 the most common identified antibody. Collectively, although hyperacute rejection is rare after lung transplantation, one should keep this reaction in mind given that false-negative PRAs may occur and pretransplantation cross match is not often possible [29].

7.2.3 Acute and Chronic Rejection

7.2.3.1 Overview

Acute rejection is the host's response to the recognition of the graft as foreign. Most patients develop at least one episode of acute rejection within the first 3 weeks following transplantation, typically in the first 5–10 days, with 36% of patients experiencing at least one episode in the first year [21]. Obliterative bronchiolitis (OB) is the most common late cause of mortality and morbidity after lung transplantation occurring in 28% by 2.5 years and 74% by 10 years in patients who survive at least 14 days [21]. It also has a significant negative impact on quality of life parameters. Risks for acute rejection include HLA mismatching, type of immunosuppression, infection, and recipient factors. It is generally thought that the intensity of host alloimmune response is related to recipient recognition of differences with the donor HLA antigens and that this process drives acute lung allograft rejection. A higher degree of mismatch increases the risk of acute rejection [101, 115, 141]. However, this effect is not consistent across all HLA loci or studies. Mismatches at the HLA-DR, HLA-B [115], and HLA-A [101] loci, as well as a combination of all three loci [141], appear important. In addition, the ISHLT registry has not found a correlation between HLA mismatching and survival [130]. Thus, while HLA mismatching between donor and recipient likely contributes to the immunologic basis for acute rejection, it is difficult to discern if a mismatch at a particular locus or if different degrees of mismatch significantly alter the overall risk for acute rejection.

Viral infections have been thought to modulate the immune system and heighten alloreactivity. Indeed, a high incidence of acute rejection has been found in lung transplant recipients after community-acquired respiratory tract infections with human influenza virus, respiratory syncytial virus (RSV), rhinovirus, coronavirus, and parainfluenzavirus [44, 73, 137]. Although CMV is considered a potential risk factor for OB, studies directly linking CMV infections or CMV

prophylaxis strategies with acute rejection have been inconsistent [118]. In one study, *Chlamydia pneumoniae* infection was linked to the development of acute rejection and OB [50].

Several host genetic characteristics have been suggested to modulate acute lung rejection. For instance a genotype leading to increased IL1- production may protect against acute rejection [147] and a multidrug-resistant genotype (MDR1 C3435T) appears to predispose to persistent acute rejection resistant to immunosuppressive treatment [148].

The effect of age on acute rejection appears to be bimodal, with the lowest incidence of acute rejection in infancy (<age 2) [61] and increased risk during childhood as compared with adulthood [117]. The incidence of acute rejection in older lung transplant recipients (age 65 and higher) does not seem to change [32]; in fact, an increased rate of infections in older lung transplant recipients is thought to contribute to an increased mortality detected at one center arguing for reduced immunosuppression in these patients [55].

The clinical course of acute rejection can be variable. Patients with acute rejection might present with dyspnea, fever, leukocytosis, and a widened alveolar-arterial oxygen gradient. Higher-grade rejection appears to cause more severe symptoms and can lead to acute respiratory distress [32]. In patients with rejection, pulmonary function testing may show a decrease in forced expiratory volume in 1 s (FEV_1) and vital capacity (VC). Although spirometry has a sensitivity of greater than 60% for detecting infection or rejection grade A2 and higher, it cannot differentiate between the two [133]. The usefulness of spirometry is diminished in single lung transplant recipients, as the contralateral native lung dysfunction confounds the pulmonary function test results [7]. Pulmonary function testing should therefore be used only as an adjunct to clinical evaluation.

Although in approximately half of the cases of acute rejection, the findings on chest radiograph are normal, chest radiographs may reveal ill-defined perihilar and lower lobe opacities, along with septal lines and pleural effusions. Findings on CT scan might include ground-glass opacities, septal thickening, volume loss, nodules and consolidations, and pleural effusions. Infiltrates observed on imaging studies during the first week after lung transplantation usually are caused by reimplantation response (i.e., reperfusion edema). Persistent infiltrates beyond the first week suggest acute rejection or infection. However, although early small studies attempted to demonstrate the usefulness of chest X-rays and chest CT scans in the diagnosis of rejection, more recent data show very low sensitivity for acute rejection (as low as 35%) and no discriminatory value between rejection and other processes [51].

Exhaled nitric oxide (NO) is also an attractive marker of lung injury; it has been correlated with lymphocytic bronchiolitis [31] and acute rejection [120]. Furthermore, in a study of inert gas single breath washout, the slope of alveolar plateau for helium had a sensitivity of 68% for acute rejection [133].

Although lung transplantation has come of age, the development of OB remains the biggest hurdle preventing long-term survival in many patients [136]. Because of its low sensitivity (28%) and specificity (75%), OB remains difficult to prove pathologically with transbronchial biopsy [24, 72]. As a consequence, a clinical definition, called bronchiolitis obliterans syndrome (BOS) was proposed [24]. This is based on pulmonary function criteria, initially FEV_1 evolution, and more recently, mid-expiratory flow rate ($FEF_{25–75}$) [38]. The onset of BOS-symptoms is usually insidious, with progressive exertional dyspnea, often accompanied by cough, which may be dry or productive.

The incidence of BOS is highest after the first year following lung transplantation. However, the risk of BOS increases to 60–80% 5–10 years after the lung transplantation procedure. It remains the leading cause of morbidity and death after lung transplantation, accounting for about 19–29% late mortality and some 35% of patients affected by the condition 5 years after transplantation [21].

As discussed above, an acute immunologic event (acute rejection) may trigger the onset of OB [63]. However, nonalloimmune injury such as a respiratory tract infection (CMV or non-CMV viral infection) is increasingly recognized as also having an important impact on the development of OB [41, 46]. In fact, CMV pneumonitis affects over 20% of lung transplant recipients. Despite treatment, it increases the risk for OB and death. Early detection of OB in a preclinical stage is ideal so that aggressive attempts can be made to prevent a fully developed syndrome. However, to date, no particular marker to indicate OB, either from the peripheral blood or bronchoalveolar lavage (BAL) fluid, has predicted a risk for this disease.

An association between chronic aspiration and OB after heart–lung transplantation has also been observed

[105], and there is a high frequency of gastroesophageal reflux with resultant aspiration following lung transplantation [28, 57, 97, 105, 108, 143]. For instance, one study described gastrointestinal complications including gastroesophageal reflux in 51% of patients who underwent lung transplantation [80]. Other than leading to an increase in acute rejection by exacerbating the alloimmune response, aspiration of gastric contents might act independently of the alloimmune response to promote allograft injury and the development of OB. Experiments in rats have further substantiated the association between chronic aspiration and OB [79].

Despite the common clinical impression that lymphocytic pleural effusions are a hallmark of acute rejection, published data are inconclusive [65].

Collectively, although the presentation of the patient and several ancillary studies might suggest a transplant rejection process, tissue diagnosis is necessary for definitive diagnosis.

7.2.3.2 ISHLT Classification

In 1990, the ISHLT sponsored the Lung Rejection Study Group (LRSG), a workshop to develop a "working formulation" for the diagnosis of lung rejection by transbronchial biopsy [9]. The proposed grading scheme found wide acceptance. Due to developments in the field and experience with its use, LRSG has remet twice since to assess the merits of the grading scheme. The revisions were published in 1996 [145] and 2007 [123], respectively. The grading scheme is strictly pathologic and does not consider any clinical parameters. Due to overlapping histologic features between acute rejection and infection, the grading scheme relies on the absence of concurrent infection.

The most recent classification of lung allograft biopsies is the 2007 ISHLT revised consensus classification of allograft rejection [123] (see Table 7.2). This classification was revised from the 1996 ISHLT consensus classification. There were no significant changes in the grading of acute rejection in the latest classification, but it was acknowledged that minimal acute rejection is not solely a high power diagnosis but can also be recognized at low power in an adequately alveolated biopsy. In the 1996 grading system, small airways rejection was graded in a four-tiered system, B1 through B4, respectively (see Table 7.2). The 2007 classification consolidated those into low grade and high grade small airways inflammation to make the evaluation easier. The "R" behind B1 and B2 denotes the revised 2007 classification. Chronic airways rejection is not divided into active and inactive anymore but only into present and not present. There are no differences in the classification of chronic vascular rejection between the 1996 and 2007 classification.

Table 7.2 Classification of allograft rejection according to 2007 revised and 1996 ISHLT consensus classifications of lung allograft rejection [123]

	Grade 2007		Grade 1996	
Acute rejection	A0	None	A0	None
	A1	Minimal	A1	Minimal
	A2	Mild	A2	Mild
	A3	Moderate	A3	Moderate
	A4	Severe	A4	Severe
Small airways inflammation	B0	None	B0	None
	B1R	Low grade	B1	Minimal
			B2	Mild
	B2R	High grade	B3	Moderate
			B4	Severe
	BX	Ungradeable	BX	Ungradeable
Chronic airways rejection	C0	None	C0	None
	C1	Present	Ca	Active
			Cb	Inactive
Chronic vascular rejection	D0	None	D0	None
	D1	Present	D1	Present

R denotes revised

An attempt should be made to accurately distinguish the grade of rejection since treatment is largely dependent on the histologic grade. Furthermore, an effort should be made to review the report of the previous biopsy and to comment on whether rejection is ongoing or resolving. Although in general, patients with grade A1 biopsies will not receive increased immunosuppression, surveillance might be intensified and the time to the next biopsy shortened. In contrast, A2 biopsy patients will receive an increase in immunosuppression. Grade A3 and A4 patients will be treated similar and receive more immunosuppression.

Inter- and intraobserver variability in grading can impact treatment and outcome [16, 22]. Studies have evaluated the inter- and intraobserver variability of the 1996 grading scheme. Two studies found relatively good interobserver agreements for the A-grades (kappa of 0.65 and 0.73) [16, 22], but this could not be replicated in another study in which the kappa was 0.47 in spite of dichotomization of the A-grades to A0/A1 vs. A2–4 [122]. Intraobserver agreement for acute rejection has been found to be good: kappa values of 0.65 and 0.795 [16, 122].

Infection can complicate the diagnosis of acute rejection. Viral infection in particular can cause mononuclear inflammation [126]. In addition, alveolar damage with macrophage and fibrin accumulation was found to be present in 80% of transbronchial biopsies in the first 6 months after transplantation, further increasing interobserver pathologist discordance. Therefore, in general, the LRSG recommends grading rejection only after the exclusion of infection.

In contrast to the A-grade, the interobserver variability for grading airways inflammation (B-grades) was only fair with kappas of app 0.3 [16, 122]. For this reason, the LRSG has now simplified B-grading to two possible grades. This nomenclature is to be used for grading of noncartilagenous small airways only after rigorous exclusion of infection [123].

Eosinophils can accompany acute lung rejection and are recognized in the 2007 ISHLT classification in high grade rejection (grades A3/A4) [123]. The presence of eosinophils has been suggested as a risk factor for BOS in small single-center studies [114]. Furthermore, high proportions of B cells have been shown to accompany steroid-resistant rejection in lung transplant patients [146]. The mechanisms by which B cells contribute to refractory rejection are not entirely clear, although they may reflect ongoing humoral rejection, perhaps explaining the diminished responsiveness to standard immunosuppression. Mast cells have been identified in acute rejection biopsies of increasing A-grade but their role has not been elucidated [144].

Rejection and chronic airways inflammation should be distinguished from bronchiolar associated lymphoid tissue (BALT). BALT is found in the vicinity of airways, usually contains black anthracotic pigment and presents as a rather nodular collection of chronic inflammatory cells which does not surround a vessel.

7.2.3.3 2007 ISHLT Revised Consensus Classification of Lung Allograft Rejection

Acute Rejection: A Grade

Acute rejection is defined by the presence of perivascular mononuclear cell infiltrates with or without endotheliitis. With progression, this infiltrate becomes more widespread and extends into the alveolar septa and, subsequently, into the alveoli. The majority of the mononuclear cells in acute rejection are T cells, although a few studies have described increased populations of B cells or eosinophils [104, 123, 146].

No Acute Rejection (ISHLT Grade A0)

In grade A0 normal pulmonary parenchyma is present.

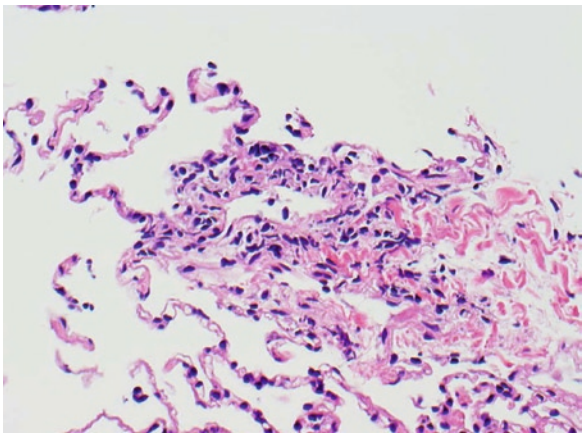

Fig. 7.1 Minimal rejection (ISHLT Grade A1). A single venule is surrounded by a thin layer of chronic inflammatory cells (H&E, magnification ×400)

Minimal Acute Rejection (ISHLT Grade A1)

Scattered infrequent blood vessels, particularly venules, in the alveolated lung parenchyma are surrounded by a relatively thin chronic mononuclear infiltrate (see Fig. 7.1). The lymphocytic rim is rather small and does not spill into the adjacent interstitium. Endotheliitis and eosinophils are absent. Although previous classification systems commented on a certain number of lymphocyte layers, the current ISHLT classification only requires a complete rim of vessels by lymphocytes. In adequately alveolated and artifact-free specimens, the lymphocytic infiltrates might be detected at low magnification.

Mild Acute Rejection (ISHLT Grade A2)

Although in mild acute rejection the perivascular infiltrate of lymphocytes is essentially confined to the perivascular adventitia without infiltrating the interstitium, there are more layers of lymphocytes surrounding the vessel and lymphocytes might focally, minimally spill into the adjacent interstitium (see Fig. 7.2). More frequent perivascular mononuclear infiltrates are seen surrounding venules and arterioles. They are readily recognizable at low magnification. These infiltrates usually consist of a mixture of small round lymphocytes, activated lymphocytes, plasmacytoid lymphocytes, macrophages, and eosinophils. There is frequently subendothelial infiltration by mononuclear cells which may be associated with hyperplastic or regenerative changes in the endothelium, i.e., endotheliitis. Concurrent lymphocytic bronchiolitis may be seen in association with mild acute rejection.

Moderate Acute Rejection (ISHLT Grade A3)

Grade A3 acute rejection shows easily recognizable cuffs of venules and arterioles by dense perivascular mononuclear cell infiltrates which are commonly associated with endotheliitis (see Fig. 7.3). Eosinophils

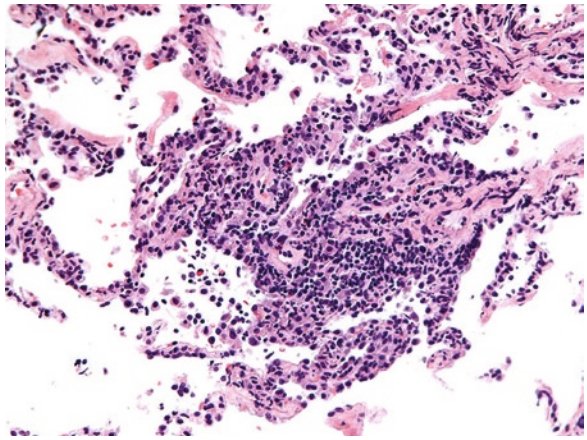

Fig. 7.3 Moderate rejection (ISHLT Grade A3). Mononuclear cells surround several small vessels and infiltrate into the adjacent interstitium. Scattered eosinophils are also present (H&E, magnification ×200)

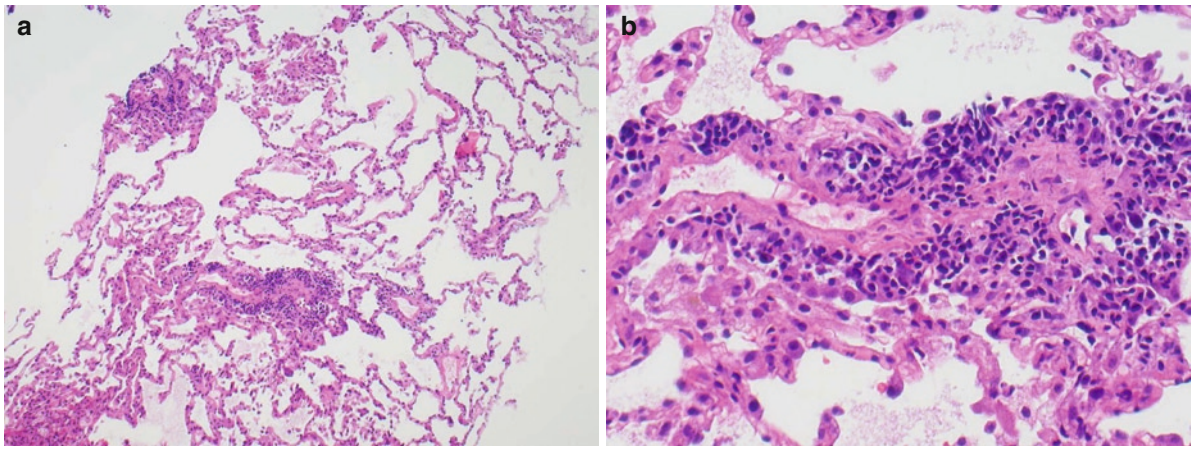

Fig. 7.2 Mild rejection (ISHLT Grade A2). (**a**) Low power view with readily apparent multiple small vessels surrounded by lymphocytes. The interstitium is normal and lacks chronic inflammation. (**b**) On high magnification, the perivascular infiltrate has a thicker cuff than in Grade 1A (H&E, magnification ×100 (**a**), 400(**b**))

and even occasional neutrophils are common. This grade is defined by the extension of the inflammatory cell infiltrate into perivascular and peribronchiolar alveolar septa which broadens the interalveolar septa and airspaces are associated with collections of intra-alveolar macrophages in the zones of septal infiltration. Type II pneumocyte hyperplasia and histologic features of acute lung injury may become apparent.

Severe Acute Rejection (ISHLT Grade A4)

In severe rejection there are diffuse perivascular, interstitial, and air space infiltrates of mononuclear cells with prominent alveolar pneumocyte damage and endotheliitis (see Fig. 7.4). This may be associated with intra-alveolar necrotic epithelial cells, macrophages, eosinophils, hemorrhage, and neutrophils and usually some evidence of acute lung injury in form of organizing pneumonia or hyaline membranes. Parenchymal necrosis, infarction, or necrotizing vasculitis might be identified; however, these features are more evident on surgical rather than transbronchial lung biopsies. It should be noted that a paradoxical diminution of perivascular infiltrates can occur as cells extend into alveolar septa and spaces where they are admixed with macrophages.

This grade can sometimes be difficult to distinguish from an infectious process, harvest/reperfusion injury, or drug toxicity, however, the presence of perivascular inflammation is helpful in establishing the diagnosis.

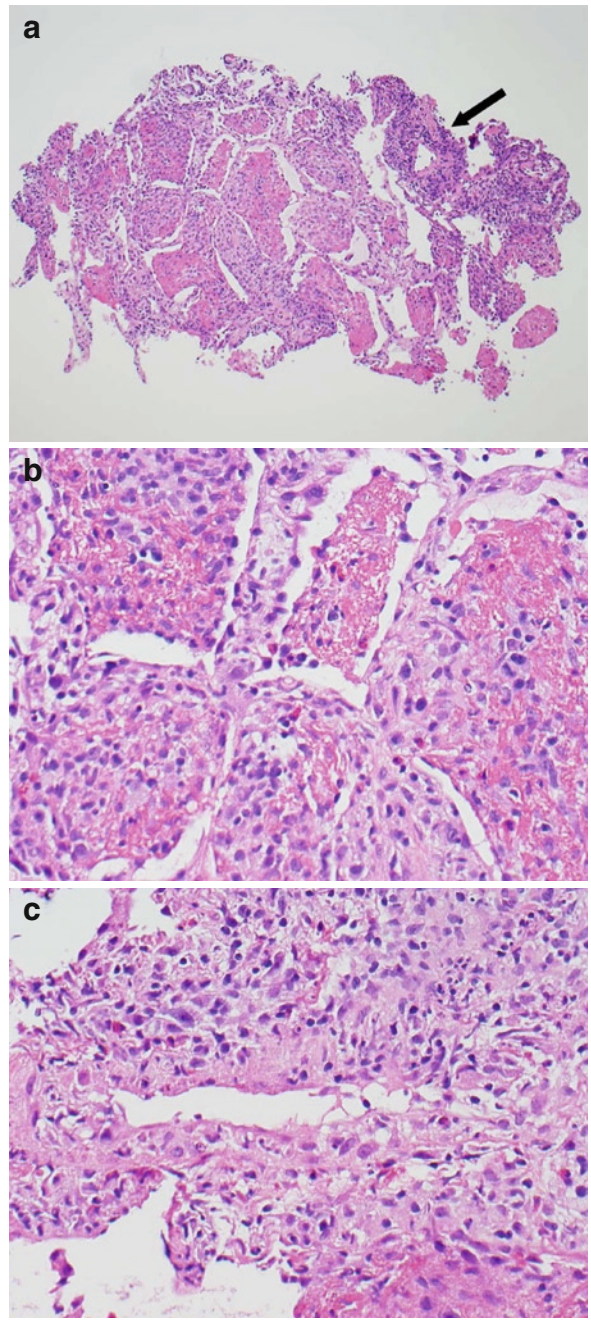

Fig. 7.4 Severe rejection (ISHLT Grade A4). (**a**) Low power view reveals perivascular lymphocytic infiltrate (*arrow*), thickening of the interstitium due to chronic inflammatory cells, and intraalveolar fibrin indicative of acute lung injury. (**b**) On high magnification, scattered eosinophils are identified. (**c**) Small vessels with features of endotheliitis characterized by the presence of lymphocytes between endothelial cells and basement membrane are also present (H&E, magnification ×100 (**a**), 400(**b**, **c**))

Acute Small Airways Rejection: B Grade

This grade applies only to small airways such as terminal or respiratory bronchioles. Bronchi, if present, should be described separately. It is important to mention in the pathology report whether or not small airways are present. The R behind grades 1 and 2 denotes the revised 2007 version.

No Airways Inflammation (ISHLT Grade B0)

The small airways appear unremarkable without evidence of bronchiolar inflammation.

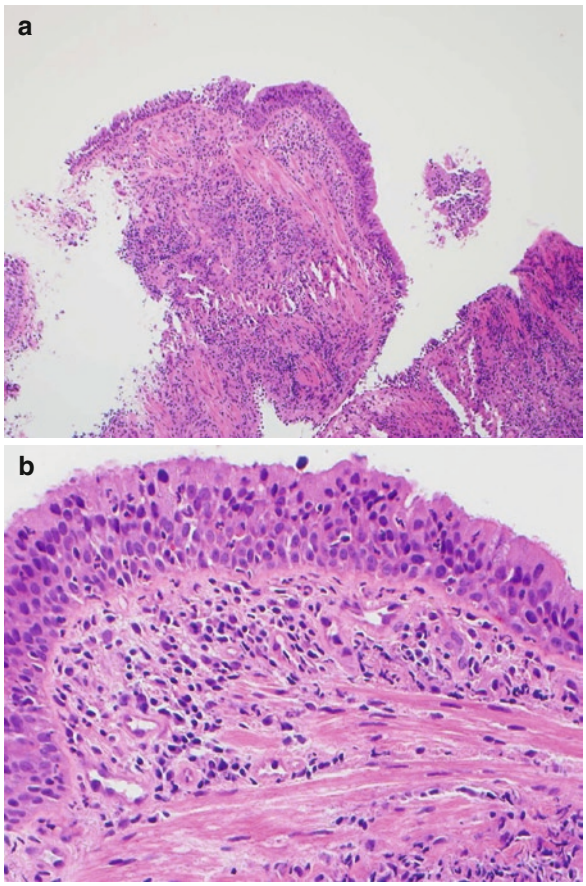

Fig. 7.5 Low grade small airways inflammation (ISHLT Grade B1R). (**a**) On low magnification a small airway is present with mild chronic inflammation of the airway wall. (**b**) Chronic inflammatory cells are in the submucosa and mucosa. Only rare neutrophils and lymphocytes are present (H&E, magnification × 100 (**a**), 400(**b**))

Low Grade Small Airways Inflammation (ISHLT Grade B1R)

Low grade inflammation is characterized by lymphocytes within the submucosa of the bronchioles (see Fig. 7.5). The lymphocytic infiltrates can be infrequent and scattered or forming a circumferential band, however, intra-epithelial lymphocytic infiltration is not present. Although occasional eosinophils may be seen within the submucosa, there is no evidence of epithelial damage, neutrophils, necrosis, ulceration, or significant amount of nuclear debris. This grade combines and replaces the 1996 working formulation B1 and B2 grades.

High Grade Small Airways Inflammation (ISHLT Grade B2R)

In high grade small airways inflammation there is marked lymphocytic infiltrate of the airway epithelium and airway wall. The mononuclear cells in the submucosa appear larger and activated with greater numbers of eosinophils and plasmacytoid cells. In addition, there is evidence of epithelial damage including necrosis, metaplasia, and marked intra-epithelial lymphocytic infiltration. In its most severe form, high grade airways inflammation is associated with epithelial ulceration, fibrino-purulent exudate, cellular debris, and neutrophils. It is important to exclude an infectious process.

Ungradeable Small Airways Inflammation (ISHLT Grade BX)

In this grade the changes are ungradeable due to sampling problems, infection, tangential cutting, artifact etc.

Chronic Airways Rejection C-Grade

The working formulation for pulmonary rejection has equated OB with one type of chronic rejection, the C-grade. The term as used in the consensus classification is restricted to submucosal and intraluminal scarring of membranous and respiratory bronchioles.

When large tissue sections of lung are examined, the process of OB is pan-lobar but patchy.

No Chronic Airways Rejection (ISHLT Grade C0)

The small airways appear similar in size to the accompanying artery with a ragged inner surface. Fibrosis is not present.

Chronic Airways Rejection (ISHLT Grade C1)

Narrowing of the airways due to fibrosis in the airway wall is seen. The fibrosis is usually eccentric (see Fig. 7.6). OB may be manifest by a histopathologic spectrum of changes depending on the acuteness of the process, the degree of organization and the amount of accompanying inflammation. In the acute phase there is usually loose myxoid granulation tissue with variable numbers of

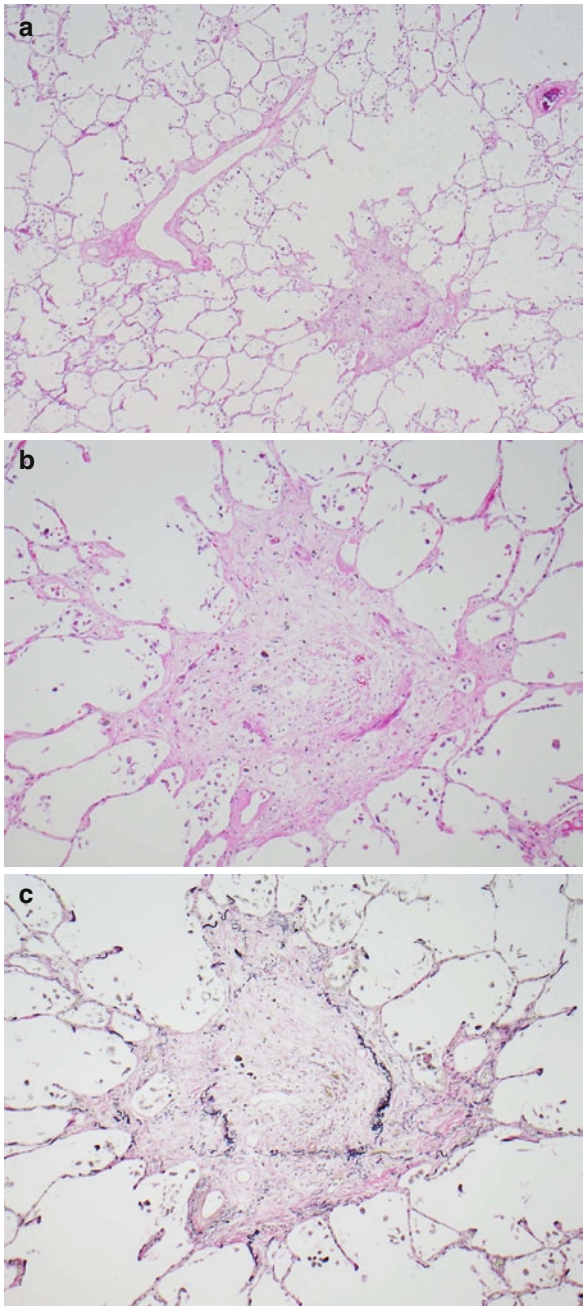

Fig. 7.6 Obliterative bronchiolitis (IHSLT C1). Autopsy slides from a patient who underwent bilateral lung transplantation 15 months prior. Her posttransplant course was complicated by PTLD, CMV and *Pseudomonas aeruginosus* pneumonia. She presented with cough and progressive dyspnea shortly before her demise. The H&E images, (**a**) and (**b**) show complete obliteration of the airway by fibrosis. A VVG stain (**c**) outlines the elastic layer indicative of the original airway diameter (H&E, magnification ×40 (**a**), 100 (**b**))

inflammatory cells filling or partially obstructing the airway lumen, resembling "organizing pneumonia." In later phases, OB may consist of eccentric, occasionally confluent plaques of dense hyalinized collagen applied to the wall of bronchioles. Metaplastic squamous or cuboidal epithelium may cover these bronchiolar scars. In other airways a slit-like lumen may remain as a result of a confluent submucosal scar or intraluminal polyps of scar tissue. Capillaries supplying these intraluminal masses of collagen are occasionally prominent. In the most severe cases the bronchiolar lumen can be entirely occluded by dense scar tissue and be recognizable only with the aid of an elastic stain, its location adjacent to an artery, and by the presence of residual circumferential smooth muscle. In the later phases, inflammation may be minimal. Usually, the scarring process is confined exclusively to respiratory bronchioles and terminal bronchioles, although it may occasionally involve adjacent alveoli.

Chronic Vascular Rejection D-Grade

No Chronic Vascular Rejection (ISHLT Grade D0)

The pulmonary arteries appear of a similar size as the accompanying airway. The intima is slender, the media not thickened.

Chronic Vascular Rejection (ISHLT Grade D1)

Chronic vascular rejection rarely is identified on biopsies since they usually lack vessels of sufficient size. Wedge biopsies, explants, or autopsy material may reveal it.

In the typical case, pulmonary arteries and more often veins are thickened by fibrointimal connective tissue (see Fig. 7.7). Also, thickening is usually concentric. Chronic vascular rejection may be patchy and typically involves smaller vascular arteries and veins. In the early stages of chronic vascular rejection, the intimal proliferation occurs on the luminal aspect of an intact elastic lamella. Subsequently, the internal elastica may become fragmented and discontinuous. Occasionally the underlying muscular wall becomes thinned. In approximately half of the reported cases a concurrent endovasculitis has been observed. The process is similar in pulmonary veins, although the

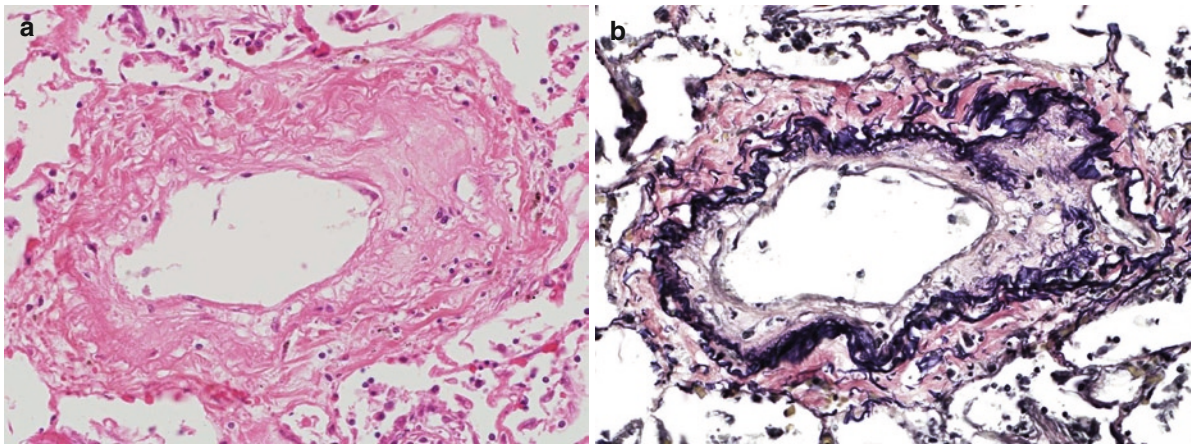

Fig. 7.7 Chronic vascular rejection (ISHLT D1). (**a**) At high power, there is a small vein with eosinophilic, waxy fibrointimal thickening as also evaluated on VVG stain (**b**) (H&E, VVG, magnification ×400)

intimal deposits may be less cellular and more waxy, eosinophilic, and sclerotic. Chronic vascular rejection should be distinguished from recanalizing thrombi.

Unlike the situation with heart transplant recipients, chronic vascular rejection in lung transplants has not resulted in graft loss; however, some patients develop pulmonary hypertension particularly those with BOS [92, 111].

7.2.3.4 Mimickers of Severe Acute Cellular Rejection

Mimickers of severe acute rejection include conditions that might present with acute lung injury or DAD. These conditions include infection, drug toxicity, antibody mediated rejection (AMR), or harvest/reperfusion injury. Therefore, careful slide review for perivascular inflammation should be performed, stains for microorganisms including Gomori-Grocott methenamine silver stain (GMS) and acid fast bacilli (AFB) might be added and careful search for viral inclusions should be undertaken.

Although perivascular mononuclear infiltrates are helpful to identify rejection, these are not entirely specific for acute rejection and many other conditions may simulate or mimic alloreactive lung injury [126]. Differential diagnostic considerations include CMV pneumonitis, *Pneumocystis jiroveci* pneumonia and posttransplantation lymphoproliferative disease (PTLD). Further differential diagnosis of perivascular and interstitial infiltrates include recurrent primary diseases.

7.2.3.5 Antibody-Mediated Rejection

AMR or humoral rejection is well established in other solid organ transplantation. It was originally recognized in kidney transplant patients who presented with acute allograft rejection, antidonor antibodies, and poor prognosis [125].

AMR is thought to be due to circulating antibodies that are either preformed because of pregnancy, blood transfusion, or previous organ transplantation or arise de novo after transplantation due to HLA-mismatch.

Although AMR is an increasingly recognized entity in lung transplantation, it is still under investigation. Early observations were based on the phenomenon of hyperacute rejection, where preexisting donor-specific antibodies lead to complement activation and rapid graft loss. Later it has been recognized that lung transplant recipients also can develop antibodies after transplantation to the allograft that might lead to AMR. Evidence suggests that AMR occurs to donor major histocompatibility (MHC) antigens, although other endothelial and epithelial antigens expressed in the lung may become antibody targets as well.

The recent development of very sensitive and specific solid phase flowcytometry and Luminex-based methodologies has allowed for accurate detection of

antibody specificities in sensitized recipients and it has become clear that more patients than previously expected present with preformed anti-HLA antibodies. Immune stimulation by prior infections or autoimmunity might contribute to the development of antibodies to alloMHC in those patients with no identifiable risk factors. These preexisting antibodies can react with donor antigens, leading to immediate graft loss (hyperacute rejection) or accelerated humoral rejection and BOS [23]. Furthermore, recent studies have consistently demonstrated an increased incidence of acute rejection (a threefold increase in one study) [48], persistent rejection, increased BOS [96] or worse overall survival [56] in patients with anti-HLA antibodies. This effect is apparent both with pretransplant HLA sensitization and with the development of de novo anti-HLA donor-specific antibodies after transplantation [96].

About 10–15% of lung transplant recipients are presensitized to HLA antigens [3]. Even though "unacceptable antigens" are avoided during the virtual crossmatch, patients with positive pretransplant PRA are at higher risk for posttransplant complications. Their posttransplant PRA can stay stable or increase via generation of either donor-specific or nondonor-specific anti-HLA antibodies. Similarly, patients that had negative PRA screening tests before transplantation can develop de novo nondonor-specific or donor-specific anti-HLA antibodies after transplantation.

The mechanisms by which antibody promotes lung allograft injury remain poorly understood. Antibody binding to alloMHC or other endothelial or epithelial targets in the lung could lead to activation of the complement cascade with complement deposits leading to endothelial cell injury, production of proinflammmatory molecules, and recruitment of inflammatory cells. Complement independent antibody-mediated mechanisms can also induce endothelial cell activation without cell injury, leading to increased gene expression and subsequent proliferation [23]. As demonstrated by in vitro studies, anti-HLA antibodies can cause proliferation of airway epithelial cells as well, producing fibroblast-stimulating growth factors [64], potentially contributing to the generation of obliterative airway lesions.

Recent studies have attempted to evaluate immunoglobulins (Ig) and complement deposits in the subendothelial space. Septal capillary deposits of Igs and complement products such as C1q, C3d, C4d, and C5b-9 have been described in association with anti-HLA antibodies [62, 90] as well as allograft dysfunction and BOS [82, 139].

The concept of specific histopathologic features associated with humoral rejection remains controversial in lung transplantation. Recent studies question the relation between complement or Ig staining and allograft rejection [112, 138]. Others demonstrate that C3d and C4d staining can occur in lung transplant recipients with nonalloimmune lung injury such as infection and primary graft dysfunction (PGD) with no evidence of anti-HLA antibodies [139]. Differences in staining techniques between different laboratories may further explain some of the inconsistencies in the published data.

The 2007 ISHLT revised consensus classification did not agree upon any histopathologic features that might be specific for AMR in lung [123]. Although capillary injury/small vessel intimitis might raise the suspicion of AMR, these are nonspecific findings that can also occur in severe acute rejection, infection, or harvest/reperfusion injury. Furthermore, although often the term "capillaritis" is mentioned, ISHLT recommends to use the term "capillary injury" which allows for a broader spectrum of morphological changes of the vessels including intimitis, whole wall thickness inflammation, neutrophilic infiltration (which should be distinguished from neutrophilic margination), thrombosis, and necrosis.

Furthermore, signs of DAD and intra-alveolar hemorrhage can occur with AMR although again, they are not specific. Given the lack of specific histologic findings of AMR in lung transplantation, a multidisciplinary approach to diagnosis is recommended that includes the following: (1) The presence of circulating antibodies (HLA antibodies, antiendothelial and antiepithelial antibodies), (2) Focal or diffuse C4d deposition (see Fig. 7.8), (3) Histologic features of acute lung injury or hemorrhage (DAD, capillary injury with neutrophils and nuclear debris) and (4) Clinical signs of graft dysfunction. If AMR is clinically, immunopathologically, or histologically suspected, one might perform immunostains for C3d, C4d, CD68, and CD31. However, these stains are extrapolates from kidney and heart transplantation and although there have been several studies advocating their use [47, 62, 123, 139], there are no large scale studies validating their use.

C4d has been studied extensively in kidney and heart transplant and has been identified as a useful adjunct in the diagnosis of AMR in these organs.

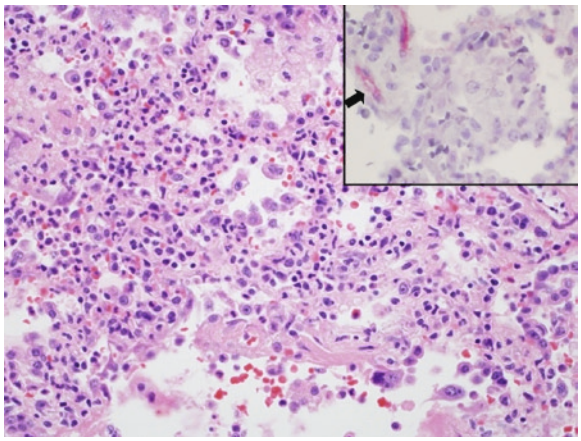

Fig. 7.8 Antibody mediated rejection (AMR). The high power view shows capillaritis in a case of AMR characterized by focally thickened interstitium due to neutrophils and nuclear debris with capillary destruction. The insert reveals C4d immunoperoxidase stain (see *arrow*) focally lining the endothelium of capillaries and small vessels (magnification × 400, insert ×600)

However, in lung that is not the case. One of the reasons for the difficulties in lung is the relatively high background that is encountered in immunohistochemistry (IHC) as well as immunofluorescence (IF). Often, C4d binds to elastic laminas or shows other nonspecific binding. Staining is commonly only focal and therefore sensitivity and specificity have not been established yet given the limited sample size of transbronchial biopsies. Only linear, continuous subendothelial staining of capillaries, arterioles, and/or venules count as positive by IHC. Also, C4d is not specific to AMR but also can be seen in infection, harvest/reperfusion injury, or even in severe acute rejection, basically in any process that is associated with complement activation.

There is no IHSLT recommendation at this time regarding to the coexistence of AMR and acute rejection.

7.3 Transbronchial Biopsy

7.3.1 Background and History

Although clinical evaluation of the patient may suggest the possibility of rejection, tissue evaluation is necessary for a definitive diagnosis. Initially, investigators were hesitant to routinely utilize transbronchial biopsy to monitor the graft as it was thought that the amount of tissue obtained would be too small to make a definitive diagnosis. There was also concern about passing a bronchoscope over the anastomosis. However, transbronchial biopsy is now a routine procedure and has become the gold standard to evaluate the graft for acute and chronic rejection, infection, and possible recurrent disease since currently, no surrogate markers have been sufficiently validated as means to reproducibly identify patients with acute rejection. Specifically, there is no serological marker to indicate rejection is occurring.

After lung transplantation the total BAL fluid cell count is constantly increased even in periods with no evidence of infection or rejection [127]. In the early posttransplantation period (first 4 weeks), there is a dominance of neutrophils in the BAL fluid (up to 25–50% of total cell count) until about 3 months later when the cell count normalizes [127]. Acute rejection has been associated with elevated CD8+ T cells, activated CD4+ T cells, a trend toward increased NK T cells, increased B cells, and decreased NK cells in the BAL [53]. Nevertheless, no study has proven the BAL cellular composition to be adequately sensitive or specific in the discrimination of rejection from infection [107].

Small studies have found a correlation between acute rejection and elevation of IL17 [134], IL15 [10], and IFN gamma in the BAL [10]. Recent advantages in genomics offer the potential for more specific means of diagnosing rejection in lung transplantation. A pilot study of gene expression in the BAL of lung transplant recipients found that gene expression signatures related to T-lymphocyte function, cytotoxic CD8 activity, and neutrophil degranulation correlate with acute rejection [98]. However, these studies have no clinical use at the present time.

Recently published studies in heart transplantation describe the use of peripheral blood gene expression profiling to identify future risk of cardiac allograft rejection [88]. A similar study is now underway in lung transplantation, known as the lung allograft rejection gene expression observational (LARGO) study. Preliminary data from almost 900 patients, similar to the CARGO results, show differential gene expression in the lymphocyte priming and neutrophil homeostasis pathways for A0 vs. ≥A2 acute lung rejection [66]. Such testing may hold promise for a noninvasive technique to monitor the status of the transplanted organs.

Another study described marked serum elevations of hepatocyte growth factor (HGF) in lung transplant

recipients with acute rejection. However, smaller elevations in HGF also occurred with lung infection, and additional studies are needed to validate specificity and sensitivity of HGF for acute rejection [1].

In conclusion, although there are a few promising noninvasive techniques, these are early studies that need to be confirmed in larger studies before being considered for widespread clinical use. Therefore, microscopic tissue evaluation remains the gold standard for diagnosing rejection.

7.3.2 Timing of Posttransplantation Biopsies

Bronchoscopy and biopsy postlung transplantation are generally performed if there is any clinical suspicion of rejection, infection, recurrent disease, or PTLD. In addition, many transplant centers now follow protocol biopsies. However, the pros and cons of surveillance bronchoscopies with biopsy are still debated and therefore, there are no guidelines established in regards to the timing of postlung transplantation surveillance bronchoscopy and biopsy. One institution reports surveillance biopsies in pediatric lung transplant recipients at 1 week and 1, 3, 6, and 12 months posttransplantation [8]. The rational for surveillance biopsies includes the occurrence of clinically silent acute rejection, inadequate surrogate markers for acute rejection, and the relatively low risk of the bronchoscopy procedure.

The yield for acute rejection by transbronchial biopsies was reported 6.1–31% and 25% or greater in studies performing surveillance transbronchial biopsies [17, 60], clinically indicated and follow-up bronchoscopies [18]. Grade A2 and higher acute rejection has been found in a relatively high percentage of asymptomatic patients, ranging from 22 to 39% [54, 131]. Silent acute rejection appears most common within the first 3 months of transplantation (24.8% at 0–3 months; 16.7% at 3–12 months; 2.7% after 1 year) [87].

The rate of unsuspected but clinically significant infection was highest between 3 and 12 months posttransplantation but a relatively high rate (18.9%) was also detected after 1 year.

One small study questioned the benefits of performing surveillance bronchoscopy with biopsy, suggesting that the benefits do not outweigh the procedural risks [132]. In this study, changes in management based on transbronchial biopsy and BAL results were significantly higher in the clinically indicated group (65%) compared with the surveillance group (13%) and were mostly related to infection. Interestingly, no silent acute rejection episode requiring treatment was detected in the surveillance group of this study. Collectively, these findings may reflect differences in programs and operators.

Although it has been shown that transbronchial biopsy is often the only mean to reveal silent acute rejection of the allograft, definitive evidence that treatment of such episodes have a positive impact on survival or prevention of BOS has yet to be demonstrated. However, based on the link between acute rejection and development of BOS, surveillance transbronchial biopsies in asymptomatic lung transplant recipients has become common practice in many large lung transplantation centers because evidence suggests that patients who have multiple episodes of low grade (A1) lesions within the first 12 months posttransplantation develop early onset BOS. However, there were no differences in overall survival in patients with and without A1 acute rejection [59]. There is also evidence that A1 rejection increases the risk of higher-grade subsequent rejections (≥A2) [13, 34]. The finding of a solitary perivascular monocytic infiltrate was followed by worsening acute rejection in four untreated patients in one study, while the treatment of such a solitary infiltrate in nine patients resulted in improvement of the rejection score [67].

The presence of severe lymphocytic bronchiolitis also has been associated with increased risk of BOS and death after lung transplantation, independent of the presence of acute rejection [49]. A study [49] in which surveillance transbronchial biopsies were performed at 3, 6, 9, and 12 weeks posttransplantation, at the time of symptoms, and for follow-up of acute rejection or CMV pneumonia showed that patients who develop acute small airways rejection within the first year after transplantation are at risk of development of BOS at 1.76, 3.3, and 5.5 years after detection of B3/B4 lesion (by 1996 ISHLT criteria, see Table 7.2), B2 lesion or B0/B1 lesion, respectively. This study strongly suggests that the main utility of surveillance biopsies may be to use lymphocytic bronchiolitis as a surrogate predictor of long-term outcome. However, the impact of therapy for lymphocytic bronchiolitis has yet to be assessed.

Although complications related to bronchoscopy occur, including a small risk of severe complications, the majority of cases have a favorable outcome. Adverse events reported with bronchoscopy in lung

transplant recipients include transient hypoxemia, bleeding, pneumothorax, arrhythmia, and anesthesia related complications [74, 87]. Bronchoscopy may have contributed to faster deterioration of one patient's already critical condition; however, in the posttransplantation setting, -bronchoscopy has no reported mortality [18, 60], although there are anecdotal reports of death related to transbronchial biopsy.

7.3.3 Specimen Adequacy and Handling

The 2007 ISHLT revised consensus classification of acute allograft rejection [123] requires the evaluation of at least five pieces of well-expanded alveolated parenchyma. However, the bronchoscopist may need to submit more than five pieces to provide this minimum number. Further biopsies may improve the detection of OB, although there is no specific number of small airways required by the ISHLT consensus classification to exclude this diagnosis. Gentle agitation in formalin to open up the alveoli may improve the histologic appearance of the fragments.

Histologic examination should include three levels from the paraffin block for hematoxylin and eosin (H&E) staining [123]. Connective tissue stains such as Trichrome or Verhoeff-Van Gieson (VVG) stain to evaluate airways for the presence of submucosal fibrosis are essential for the diagnosis of OB, and atherosclerosis. Silver stains such as GMS can be performed for fungi, including pneumocystis, but have not been routinely mandated by the 2007 ISHLT revised consensus classification, given the numerous microbiologic, serologic, and molecular techniques available for the diagnosis of opportunistic infections. BAL may be performed at the time of biopsy and is useful for the exclusion of infection, but currently has no clinical role in the diagnosis of acute rejection (see above).

7.4 Complications of Immunosuppression

7.4.1 Infection

Infection is the leading cause of death in lung transplant recipients. Factors that increase a patient's susceptibility to infection after transplantation include immunosuppression, reduced mucociliary clearance, decreased cough reflex resulting from denervation, and interruption of lymphatic drainage.

7.4.1.1 Bacterial/Viral Pneumonia

Bacterial pneumonias are the most common infections following lung transplantation [26] and occur in more than 35% of patients during the first year after transplantation (highest incidence is during the first month post-transplantation) (see Fig. 7.9). Furthermore, bacterial pneumonia remains a major infectious complication throughout the patient's life. The donor lung is affected most often. Gram-negative organisms are most common, especially *Enterobacter* and *Pseudomonas.* Bronchitis secondary to *Pseudomonas* species or *Staphylococcus aureus* infection also is observed. Bacterial pneumonia typically manifests radiographically as a lobar or multilobar consolidation.

Viral pneumonias develop in approximately 11% of patients who have undergone lung transplantation. They occur at any time following transplantation.

7.4.1.2 CMV Infection

CMV is the second most common cause of pneumonia in patients who have received lung transplants, and it

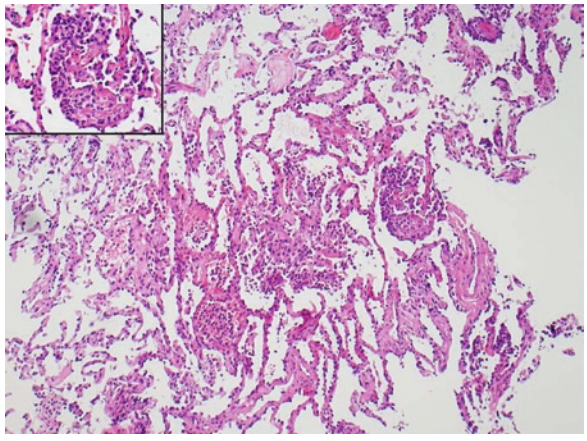

Fig. 7.9 Acute Bronchopneumonia, likely bacterial. On low magnification, some of the alveoli are filled with clusters of cells and fibrin. High magnification view (insert) reveals that the intraalveolar infiltrate is largely comprised of neutrophils, findings consistent with acute bronchopneumonia (H&E, magnification ×100, insert, ×400)

is the most common opportunistic infection (35–60% of opportunistic infection) [26]. It represents the most significant viral infection, and usually occurs 1–4 months after transplantation. Primary infection is the most serious form and is observed in 50–100% of seronegative patients who received a graft from a seropositive donor. In patients who are seropositive, secondary CMV infection develops from reactivation of latent disease following the institution of immunosuppressive therapy or from infection with a different strain of CMV.

Infected patients may be asymptomatic or may develop a fulminant pneumonia, possibly with extrathoracic findings such as retinitis, hepatitis, and gastritis. Presenting symptoms include dyspnea, fever, and cough. The most common finding on chest radiographs in patients with CMV infection is diffuse parenchymal haziness. CT scan findings include areas of ground-glass attenuation; reticulation; multiple, small, ill-defined 1- to 3-mm nodules; and, even less commonly, areas of dense consolidation. The diagnosis of CMV pneumonia can be made by bronchoscopy with lavage and biopsy. Cytopathic changes associated with CMV infection include cytomegaly, multiple small basophilic cytoplasmic inclusions, and a large nuclear inclusion surrounded by a halo with thickened nuclear membrane (see Fig. 7.10).

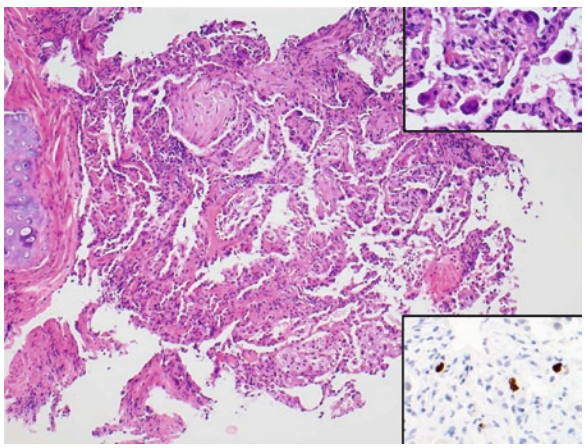

Fig. 7.10 Cytomegalovirus pneumonia. The biopsy shows intraalveolar plugs of mucopolysaccharide rich proliferating fibroblasts of organizing pneumonia and scattered large atypical cells characterized by eosinophilic nuclear and cytoplasmic inclusions (insert *right upper* corner) diagnostic of CMV infection. Immunohistochemistry highlights CMV-infected cells (insert *lower right* corner) (H&E, magnification ×100, inserts, ×600)

Prophylactic therapy with acyclovir and immune globulin has not reduced the incidence of CMV infections in patients who have undergone transplant procedures.

7.4.1.3 Herpes Simplex Virus Infection

A less common cause of viral infection includes the herpes simplex virus (HSV) infection. Patients with HSV infection present with fever, cough, and dyspnea, but they demonstrate symptomatic improvement after therapy with intravenous acyclovir. Radiographic findings may be absent or may demonstrate diffuse ground-glass opacities.

Histopathologically, the classic HSV inclusion consists of a dense, eosinophilic mass within the nucleus that is surrounded by a clear halo and peripherally marginated, beaded nuclear chromatin. HSV may form multinucleated cells.

7.4.1.4 Fungal Infections

Opportunistic fungal infections are less common than viral infections, but they are associated with higher mortality. Fungal pneumonias usually occur 10–60 days following transplantation and more commonly involve the transplanted lung. However, they also can involve the native lung in single lung transplantation cases, especially in patients with COPD. CT imaging studies most commonly reveal a combination of nodules (multiple, variable sizes, irregular margins), consolidation, and ground-glass opacification. Pleural effusions are also common (63% of cases). GMS will help to identify fungal organisms. However, even in the absence of identifiable organisms on GMS, infection might be considered and cultures and serology should be attempted.

7.4.1.5 *Aspergillus* Infection

Locally invasive or disseminated *Aspergillus* infection accounts for 2–33% of posttransplantation infections and 4–7% of deaths in patients who undergo lung transplantation. *Aspergillus* infection most commonly is characterized by local invasion of a necrotic bronchial anastomosis (i.e., ulcerative

tracheobronchitis), which typically occurs within 4 months of transplantation.

Aspergillus infection, when involving the lung parenchyma, tends to cavitate and has an upper-lobe predominance. Histopathologically, the hyphae of *Aspergillus sp* are septated and branching. They appear uniform and grow in parallel fashion with septae at regular intervals. The branching is dichotomous and usually at 45°angle.

Inhaled amphotericin B is often used in the immediate posttransplantation period to help eliminate this complication [26]. Patients are also discharged on voriconazole as daily prophylaxis for the first year.

7.4.1.6 *Pneumocystis jiroveci* Pneumonia

Patients who have undergone lung transplant procedures have an increased susceptibility to *P jiroveci* infection, but prophylaxis with trimethoprim-sulfamethoxazole is effective in preventing the infection (incidence is nearly 0%). Without prophylaxis, the incidence of *P jiroveci* infection approaches 90%.

Histologically, cysts of *P jiroveci* are round to oval, measure 5–7 μm in diameter, and often have very prominent grooves or folds. *P jiroveci* pneumonia classically consists of an intra-alveolar foamy exudate in which the organisms appear as small "bubbles" in a background of proteinaceous exudate, although a variety of other reactions can occur as well [129].

7.4.2 Posttransplant Lymphoproliferative Disorder

The incidence of PTLD is significantly higher after thoracic organ transplantation compared with any other solid-organ transplant [94]. They develop in 4–10% of lung transplant recipients, as opposed to an approximate 2% incidence in other solid organ recipients, with 60% of the PTLDs presenting in the allograft. In adults, PTLDs are the most common neoplasms at 1 year posttransplantation (peak, 3–4 months posttransplantation) [21]. In children [6], lymphomas are by far the most common posttransplantation malignancy at any time. In fact, PTLD is a major cause of morbidity and mortality in pediatric lung transplant recipients [12].

Risk factors for PTLD after solid organ transplantation include patient's age, type of organ transplanted, EBV infection status of host before transplantation, and the immunosuppressive regimen [4, 77, 93, 140].

PTLDs are a clinically, morphologically, and molecularly heterogeneous spectrum of lymphoproliferative disorders, ranging from early, EBV-driven, polyclonal, polymorphic proliferations (infectious, "mononucleosis-like") to EBV-positive or EBV-negative monomorphic tumors [15, 69, 70, 100]. PTLDs most commonly are associated with EBV infection of B-cells either by reactivation of latent virus or primary EBV, most commonly acquired from donor organs [12]. A recent study showed [37] that five of seven lung-transplanted children who developed PTLD were EBV-negative recipients who received EBV-positive lungs. In a series [81] of 988 heart and/or lung transplant recipients, 17 PTLDs were identified. Amongst the 17 PTLD cases, there were two B-cell monoclonal polymorphic PTLDs and 15 B-cell monomorphic PTLDs (see Fig. 7.11) (13 diffuse large B-cell lymphomas (DLBCL) and two Burkitt lymphomas). EBV was detected in 9 of the 17 patients. All cases showed monoclonal IGV gene rearrangements; IGV somatic hypermutation was found in 88% of cases, indicating a prevalent origin from germinal center-experienced B cells.

Given the association between EBV infection and PTLD, elevation in EBV viral load measured in peripheral blood has been proposed as a marker for the development of PTLD [109]. In addition, monitoring the degree of immunosuppression after diagnosis of PTLD with immune function assays and immunosuppressive drug levels may aid clinicians in assessing the risk for PTLD and monitoring treatment after diagnosis [45].

T-cell PTLDs tend to occur later and tend not to be associated with EBV infection. T-cell PTLDs are associated with a worse prognosis than B-cell PTLD.

Patients with PTLDs may be asymptomatic, or they may have nonspecific complaints such as fever, weight loss, dyspnea, and lethargy. Solitary or multiple pulmonary nodules ranging in size from 0.1 to 5 cm are the most common pulmonary manifestation of patients with PTLDs. Mediastinal and hilar adenopathy also can be observed in 22–50% of cases. Patients who present with a solitary pulmonary nodule have a better overall prognosis. However, most PTLDs have a rapid onset, a predilection for extranodal sites, and an aggressive clinical behavior with poor outcome [15, 69, 70, 78, 100]. As a result of the high mortality associated with PTLD, treatment often poses a challenge. The most common treatment of PTLD has included decreasing immunosuppression in combination with

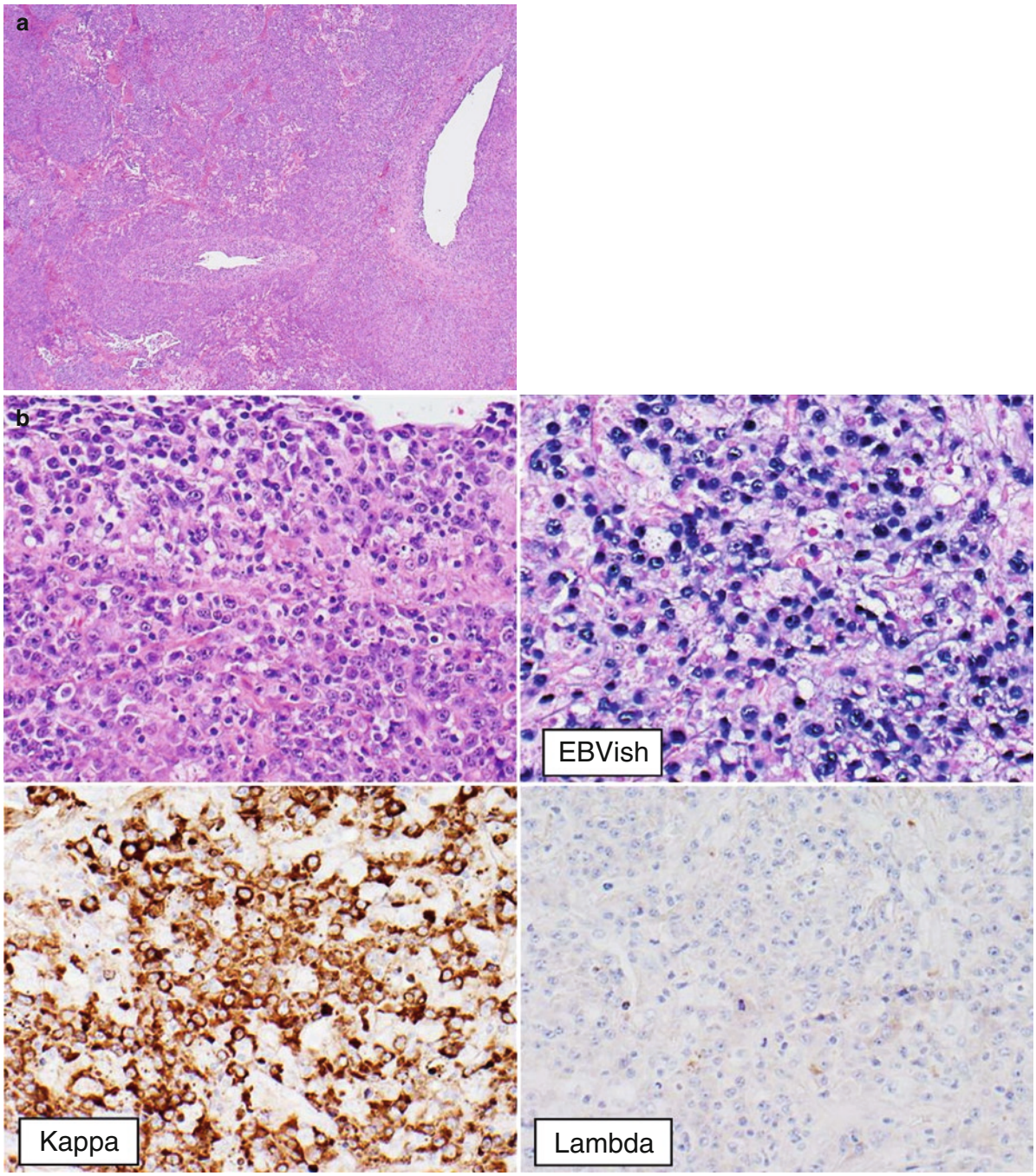

Fig. 7.11 Post transplant lymphoproliferative disorder (PTLD). Wedge biopsy from a patient who underwent bilateral lung transplant 14 months prior. (**a**) Low power view reveals a cellular infiltrate infiltrating blood vessels and focally causing necrosis. (**b**) High power view shows abnormal large lymphoid cells with rounded nuclei, dispersed chromatin, prominent nucleoli and abundant amphophilic cytoplasm. The cytoplasm is often eccentrically distributed and there are many cells with perinuclear clear zones. These atypical lymphoid cells are strongly and uniformly positive for EBV by in situ hybridization and show kappa light chain restriction. This immunoarchitecture is consistent with PTLD, monomorphous type (H&E, magnification ×40 (**a**), 400 (**b**))

surgical resection, antiviral agents, radiation, and chemotherapy [45, 103, 109]. The reduction or withdrawal of immunosuppressive therapy may result in a partial or complete regression of the PTLD [69]. A higher risk of graft rejection associated with PTLD treatment is one reason for the need for early detection of the disease [45, 102, 103, 109].

7.4.3 Solid Organ Neoplasms

In adults, malignancy remains a common complication after lung transplantation [21]. Among lung transplantation survivors, 3.5, 12.6, and 28.1% have a malignancy at 1, 5, and 10 years posttransplantation, respectively. Although lymphoproliferative disorders are most common at 1 year posttransplantation, nonmelanoma skin malignancy is the leading malignancy at 5 and 10 years posttransplantation.

In children [6], the incidence of posttransplantation malignancy is higher than in adults early after transplantation with 5.9% of children developing them within the first year after transplantation. At 5 years posttransplantation, 13.1% were found to have a malignancy. At any time posttransplantation, lymphomas are by far the most common posttransplantation malignancy in children.

The risk for cancer is higher following lung transplantation than after kidney transplantation [110, 121]. Lung transplant patients have unique characteristics, as their baseline genetic and environmental background, and immunosuppressive regimens are different from patients receiving kidney transplant, all of which may contribute to the increased risk [2].

Besides nonmelanoma skin cancer and lymphoproliferative disorders, other malignancies reported to be associated with solid organ transplantation include: Kaposi's sarcoma, anogenital cancers, oral cavity malignancies, esophageal and urinary bladder cancer, hepatocellular carcinoma, and sarcomas [110, 121].

7.4.4 Graft vs. Host Disease

Acute graft vs. host disease (Gvhd) is an uncommon and usually fatal complication of lung transplantation for which no effective therapy exists. Among the ten reported patients, eight had grade 3 to 4 acute Gvhd and died within 208 days. There is one recent case report [42] of a patient with grade 3 to 4 acute Gvhd after BLT who was successfully treated with high-dose corticosteroids after basiliximab and extracorporeal photopheresis were unsuccessful. After 26 days the patient had developed low grade fever, chills, and pruritic maculopapular rash on the chest that spread to the proximal extremities in the next 4 days. Liver function tests were elevated. A skin biopsy showed dermal inflammation, patchy loss of basal layer with vacuolar degeneration, apoptosis, and cell necrosis suggesting Gvhd. A few days later, profuse, green, watery diarrhea developed with abdominal pain and paralytic ileus. Colonoscopy showed ulceration of mucosa and destruction of intestinal crypts, features compatible with Gvhd. There was leucopenia, severe anemia, and deterioration of liver function. The diagnosis was confirmed by chimerism study that revealed a recipient/donor lymphocyte ratio of 50:50. A second case of acute Gvhd after lung transplantation was successfully treated with corticosteroids [5]. This patient presented with mild symptoms (pruritic rash and blurry vision) suggesting low-grade Gvhd. The diagnosis was confirmed when a small dose of prednisone resulted in the resolution of Gvhd within 3 weeks.

7.5 Nonrejection Related Allograft Pathology

7.5.1 Harvest/Reperfusion Injury

Lung harvest/reperfusion (ischemia/reperfusion, I/R) injury after transplantation remains the most common cause of early posttransplantation respiratory failure and manifests typically during the first 72 h after transplant [75]. Reported rates are as great as 41% [52]. The 30-day mortality of patients with I/R injury is about 40%, compared with 7% in patients without I/R injury [86].

The clinical equivalent to I/R injury is PGD. The ISHLT Working Group on PGD proposed a four-tiered grading scheme of PGD based on PaO_2/FiO_2 (P/F) ratio and radiological chest infiltrates assessed at time points up to 72 h: T (0-within 6 h of reperfusion, 24, 48, and 72 h) [20].

I/R injury usually presents with the immediate impairment in lung function after transplantation

accompanied by rapid development of pulmonary edema, increased pulmonary vascular resistance, and decreased airway compliance. Patients with I/R injury require prolonged mechanical ventilation with greater hospital stays and are at an increased risk of multiorgan failure. Lung I/R injury has long-term consequences and is a risk factor for late graft failure (OB) [39, 40].

I/R injury can occur due to prolonged ischemia during transplantation. Ischemic injury to the pulmonary vascular endothelium increases permeability and results in pulmonary edema. Histologically, I/R injury presents as acute lung injury pattern including DAD (see Fig. 7.12). Perivascular infiltrates are usually not present and distinguish it from acute rejection. However, infection can present similarly and needs to be excluded with stains, cultures, and serology.

The pathophysiology of lung I/R injury remains incompletely understood. The lungs are particularly susceptible to I/R injury, likely owing to the rich vascularity and relatively large surface area over which blood-borne components interact with the endothelium. The mechanisms of I/R injury are diverse and include generation of reactive oxygen species (ROS), leukocyte activation/recruitment, complement and platelet activation, abnormalities in pulmonary vascular tone, and increased procoagulant activity. The production of proinflammatory cytokines is increased considerably in the lung after I/R. Expression of cytokines in the lung after I/R may not only cause immediate tissue injury, but may predispose the lung allograft to rejection.

Several studies suggest that lung I/R injury is biphasic, with a distinct, acute injury characterized by macrophage activation followed by a later, neutrophil-dependent injury [39].

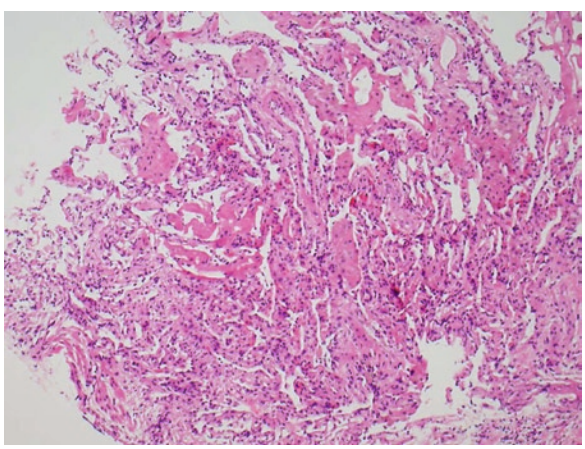

Fig. 7.12 Harvest/reperfusion injury. In this biopsy taken 2 days after transplant, there is interstitial thickening due to proliferation of mucopolysaccharide rich fibroblasts and hyperplastic type II pneumocytes. Eosinophilic hyaline membranes are also present. No perivascular inflammatory infiltrates are identified. These morphological features of diffuse alveolar damage and the history, are consistent with harvest/reperfusion injury (H&E, magnification ×100) (Case contributed by Dr. Andras Khoor)

7.5.2 Recurrent Native Disease

Although recurrent disease in the allograft has been reported in some of the transplantation cases, in general, this has not been clinically significant. Diseases for which recurrences in the allograft have been reported include giant cell interstitial pneumonia, sarcoidosis, lymphangioleiomyomatosis, Langerhans' cell histiocytosis, allergic bronchopulmonary aspergillosis, desquamative interstitial pneumonia, bronchioloalveolar carcinoma, alveolar proteinosis, and diffuse pan bronchiolitis. Morphological features are identical to the disease in nontransplanted lung.

7.5.3 Anastomotic Complications in Airways

7.5.3.1 Bronchial Dehiscence

Bronchial dehiscence is the most common anastomotic airway complication in the early postoperative period. It occurs in 2–3% of cases. Ischemia at the anastomotic site is the major factor in the development of this complication. Dehiscence probably is best assessed by bronchoscopy; however, CT scans typically demonstrate the presence of extraluminal gas, which is 100% sensitive and 72% specific for dehiscence. Patients with telescoping anastomoses also may develop small anastomotic diverticula, which appear as smooth rounded air collections at the inferior-medial aspect of the anastomosis.

7.5.3.2 Stricture

Anastomotic stricture occurs in approximately 10% of cases, and the risk for stenosis may be increased with a telescoping anastomosis. Stenoses often manifest with

progressive airflow obstruction that can be difficult to differentiate from other causes, such as acute rejection or BOS. Stricture probably is best evaluated by bronchoscopy; however, CT scans often demonstrate the area of narrowing. Treatment is stenting, typically with an expandable metallic stent. More recently, balloon dilatation has obviated the need for stents in some centers.

7.5.4 Pathology in the Remaining Native Lung

App 43% of lung transplantations between 1995 and 2008 were single lung transplants (SLT) [21]. COPD, AAT, and IPF accounted for app 85% of the indications for SLTs [21]. Advantages of SLT over BLT include a technically easier procedure, shorter surgery time, and the ability to transplant two patients from one donor. However, there are disadvantages to SLT. For instance, survival rates for SLT and BLT recipients are different, diverging most obviously in later years after transplantation. 5-year survival for SLT was 46% compared with 54% for BLT [130]. Other disadvantages of SLT include less pulmonary reserve, and the propensity for complications related to the residual native lung.

Native lung complications have previously been reported in 13.8–50% of patients. They may be associated with significant morbidity and mortality [85, 135] and may partly explain why outcomes with SLT are inferior to those of BLT. A recent study [68] reported the occurrence of pneumothoraces, malignancy, aspergilloma, pneumonia, bronchopleural fistulas, and pulmonary embolism. Some forms of advanced lung disease, including COPD and IPF, are associated with increased risk for lung cancer, a complication that can arise in the native lung after transplantation [76, 142]. Patients also often have structural damage to the native lung from their underlying disease process, which can increase the risk of other complications such as aspergilloma and pneumothoraces. The median time from transplantation to major native lung complication was 1.28 years (range, 0.04–5.1 years). In one study [68], 8 of 18 patients with native lung complications died thereof. Median posttransplant survival was lower in SLT recipient with significant native lung complications (3.2 vs. 5.3 years, $p=0.004$). Native lung pneumonectomy was performed in 11 patients. In patients with native lung complications there was a trend toward improved posttransplant survival in patients who underwent native lung pneumonectomy compared with those who did not undergo pneumonectomy. However, there was no difference in survival between patients who underwent native lung pneumonectomy to those who had no native lung complication.

7.5.5 Bronchiectasis

The majority of patients with OB also have severe bronchiectasis. By specimen bronchograms, the bronchial tree shows alternating areas of dilatation and constriction. Microscopically, the bronchiectasis may be associated with areas of mucous plugging, goblet cell hyperplasia, squamous metaplasia, denudation of the bronchial epithelium, submucosal scarring, and acute and chronic inflammation of the bronchial wall. Occasionally foreign body giant cells are present, probably representing a manifestation of aspiration. Obliteration of the terminal respiratory bronchioles is often observed distal to these areas. The bronchiectasis of lung allografts is probably the result of several factors including immune-related injury, infection, mucostasis, aspiration, and loss of innervation.

7.6 Outcomes

Despite advances in operative management, lung preservation, critical care, and immunosuppression, long-term survival in lung transplantation remains limited. In fact, outcomes for lung transplantation are the worst of any solid organ transplant [75]. The main obstacles to present day lung transplantation involve: (1) Lack of donor organs, (2) I/R injury, (3) Acute rejection, and (4) Development of OB. The increased susceptibility of the lung to injury, infection, and constant environmental exposure with local innate immune activation likely contributes to the high rates of rejection.

The ISHLT Registry reports a 1-year survival rate of 78% and 5-year survival rate of 52% [21]. Mortality is highest in the first year, which consistently decreases across subsequent time periods (see Table 7.3). In the first 30 days, graft failure, non-CMV infections, cardiovascular complications, and technical problems account for most of the mortality. After the first year,

Table 7.3 Causes of death after lung transplantation in adult lung transplant recipients (January 1992–June 2008) [21]

Cause of death	<30 days	31 days–1 year	1–5 years (%)	>5 years
OB	0.4	4.6	26.6	23.8
Acute rejection	4.3	1.8	1.3	0.6
Malignancy				
Lymphoma	0.1	2.6	2.0	2.7
Other	0.2	2.7	7.1	9.7
Infection				
CMV	0	3.0	0.7	0.1
Non-CMV	20.0	35.4	21.6	17.8
Graft failure	28.8	17.6	18.9	18.8
Cardiovascular	11.0	4.3	4.0	5.2
Technical	8.0	2.2	0.5	0.9
Other	17.3	25.9	17.3	20.4

OB obliterative bronchiolitis

BOS and non-CMV infections were the predominant causes of death. By 5 years, malignancies and cardiovascular causes account for almost 17% of reported causes of death.

However, compared with data beginning in 1988, overall survival has consistently improved by era. The improvement in survival is largely due to improvement in the 1 year survival. Long term survival has improved as well, although to a seemingly lesser degree. Evidence suggests that age, pretransplant diagnosis, and donor CMV status play important prognostic roles. For instance, the survival half-life for patients older than 65 years of age was 3.2 years compared with 6.3 years for those aged 35–49. Overall survival rates at 3 months after transplantation are lowest for IPF (86%) and IPAH (78%) and highest for CF (91%) and COPD (91%), most likely due to differences in early complications, including PGD [21]. CMV seropositivity of the donor is associated with worse survival; however, the underlying reasons for this association are not entirely clear.

Inducing a state of immune suppression is the key to successful clinical lung transplantation. The immunosuppressive regimens used for lung transplantation are based on successful protocols that have evolved for renal and heart transplantation.

Posttransplantation morbidities (see Table 7.4) present at 5 years are those commonly caused or exacerbated by immunosuppressive medicines, including hypertension, renal dysfunction, and dyslipidemia. BOS and malignancies are other common posttransplantation morbidities. For instance, BOS had developed in 28% of patients by 2.5 years after transplantation and in 74% by 10 years. However, most surviving patients reported no activity limitations at 1, 3, 5, and 10 years (>80% at each time point). Furthermore, 13% of survivors reported at least one malignancy at 5 years after transplantation, and 28% were affected by malignancies at 10 years.

Table 7.4 Morbidity after lung transplantation in surviving adult recipients (follow-up: April 1994–June 2008) [21]

Outcome	≤1 year (%)	≤5 years
Hypertension	52.4	85.2
Renal dysfunction	25.0	36.6
Creatinine <2.5 mg/dL	17.4	24.1
Creatinine >2.5 mg/dL	5.9	9.0
Chronic dialysis	1.6	3.0
Renal transplant	0.1	0.5
Hyperlipidemia	23.2	55.5
Diabetes	26.1	37.0
OB	9.5	35.3

OB obliterative bronchiolitis

Survival after pediatric lung transplantation is similar to that reported in adults with a median survival of 4.5 years for the period 1990–June 2007. But, results are clearly improving [6]. One and 5-year survival rates for pediatric recipients transplanted in the most

recent era (2002–6/2007) are 83 and 50%, respectively, compared with 67 and 43% for recipients transplanted between1988 and 1994. Graft failure, technical issues, cardiovascular failure, and infection are the most common causes of pediatric death in the early posttransplant period whereas infection, graft failure and BOS are the most common causes of late death. The prevalence of BOS steadily increases with time posttransplantation. As expected, the cumulative incidence of malignancy also increases with time after transplantation, with lymphoproliferative disorders making up the great majority of reported malignancies in children. Despite the complications, the functional status of the great majority of long-term pediatric survivors is very good, with 84% of 5-year survivors reporting no limitations in activity.

A total of 57 pediatric retransplant procedures were reported between January 1994 and June 2008. The majority of these procedure were performed >12 months after the initial transplantation. Survival over this period was slightly poorer than for primary transplantations, being 41% at 5 years.

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