55
Histopathology of Lung Transplantation

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INTRODUCTION

The increasing number of transplant centers has resulted in providing lung transplantation as a therapeutic option for many patients with end-stage pulmonary diseases. However, despite improvements in immunosuppression, surgical techniques, and diagnostic accuracy, post-transplant complications remain problematic. One of the key elements to patient survival is the prompt and appropriate intervention of allograft dysfunction[1]. While there are a number of ways to monitor the recipient, tissue examination still remains the mainstay in assessing allograft alterations[2–5]. Perhaps it is important to distinguish between rejection and non-rejection processes such as infection, since treatment is often opposite. Graft syndromes typically occur in their particular context, and it is the understanding of the adaptation of the lung allograft to the host environment which is critical in arriving at the correct diagnosis. The intent of this chapter is to review the histopathology and pathophysiology of lung allograft rejection and other non-rejection processes which may also contribute to graft dysfunction. The efficacy of types of biopsies in specific situations will also be discussed.

EARLY POST-TRANSPLANT ALLOGRAFT COMPLICATIONS

During the first week post-transplant, virtually all allografts are subject to the so-called 're-implantation response' characterized by bilateral opacification on chest radiograph and histologic demonstration of interstitial and alveolar edema and margination of neutrophils (Figure 1)[6]. The process is thought to be related to fluid overload secondary to disruption of the hilar lymphatics, organ ischemia during harvesting and transport, and division of nerves and bronchial arteries[7]. It usually resolves by the end of the first week after transplantation, before acute cellular rejection generally takes place.

Following the immediate post-transplant period a variety of other complications are encountered, many of which are related to the donor organs. Preservation (harvest) injury manifests pathologically as diffuse alveolar damage (DAD) with interstitial edema, hyaline membranes, and granulation tissue (Figure 2)[8,9]. While the process is thought to be secondary to organ ischemia, we have seen DAD in cases with minimal ischemic times in living-related transplants, thus implicating other etiologic factors. In contrast to the usual DAD is the occasional development of a temporally homogeneous patchy (as opposed to diffuse)

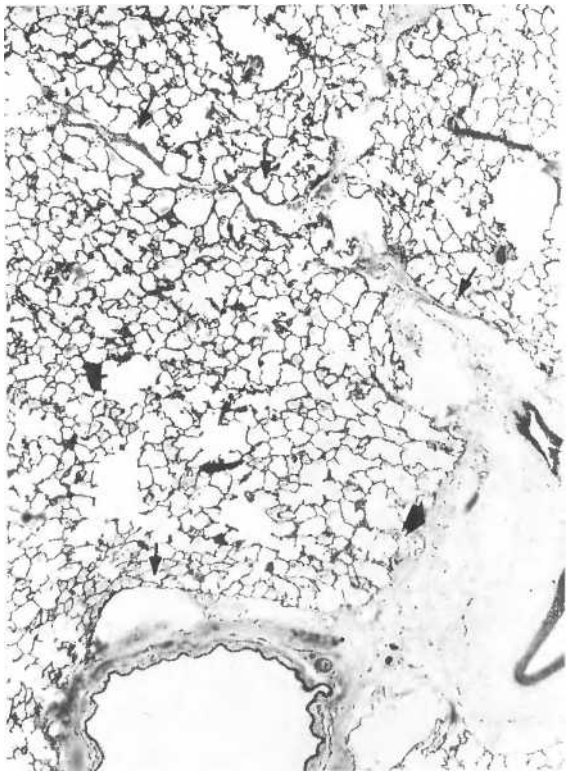

Figure 1 Reimplantation response. The pulmonary interstitium is edematous, as reflected in the perivascular pallor (large arrow) and marked dilatation of lymphatic channels (small arrows)

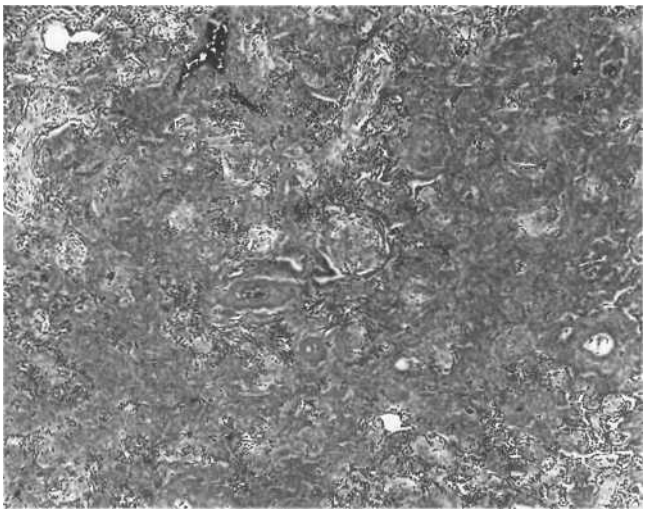

Figure 2 Acute harvest injury manifesting as organizing diffuse alveolar damage. Plugs of myxoid granulation tissue are seen diffusely in the airspaces as well as the airways

Figure 3 Thromboembolic disease. Massive thromboembolus seen adjacent to a large area of infarct resulted in organ failure in this case

process[10]. Clinically, its distinction from acute cellular rejection is the main differential diagnosis. This is not difficult in most cases with mild to moderate degrees of reversible DAD. However, in severe or prolonged cases, uncertainty in the clinical impression often necessitates a biopsy. Pathologically, severe DAD demonstrates extensive injury, to involve not only the interstitium but also the airways to produce acute bronchitis and bronchiolitis with luminal ingrowth of loose granulation tissue[10,11]. Although some cases may demonstrate concurrent DAD and rejection, attempts should be made to distinguish features of DAD from alveolar damage secondary to severe acute cellular rejection (see below) and chronic airway rejection. While the intraluminal granulation tissue of DAD has often been referred to as 'bronchiolitis obliterans', it differs from the chronic rejection-related bronchiolitis obliterans, which exhibits dense eosinophilic collagen characteristic of irreversible intraluminal scar[10,12].

Early in the history of heart–lung transplantation, tracheal dehiscence was a relatively common complication[1,13,14]. Due to improved surgical techniques this complication is now a rarity. While the acute complications of tracheal dehiscence are now under control, chronic bronchomalacia, involving the main stem bronchi and their branches due to the sacrificed bronchial artery circulation, is still a problem[15–18].

Other causes for early post-transplant complications include donor organ infection and thromboembolic disease. Sources of the embolic material include the brain, bone marrow, cartilage, and deep venous thrombi[19]. The consequences of embolic disease are probably as varied as in the non-transplant setting. Reports of rapidly fatal embolic diseases are noted at one end of the spectrum, while small incidental thromboemboli are not uncommonly found in biopsy specimens (Figure 3). Finally, a progressively downhill respiratory course lacking a demonstrable etiology is classified as primary graft failure[9]. At our institution the incidence of primary graft failure has been approximately 6% since 1982.

ACUTE LUNG REJECTION

In solid organ allografts, rejection may take the form of hyperacute, acute or chronic rejection. Hyperacute rejection is an immediate rejection response following implantation, and results in graft failure. While it has been reported in the animal lung transplant model[20], rigorous documentation in human lung transplants has not been made. Morphologic findings by themselves are not specific and therefore an integrated approach with clinical findings, histology, serology, and immunofluorescence is required. Specifically, the following are the considered criteria for diagnosis: (a) early graft failure without alternative etiology; (b) consistent gross, histologic, and immunofluorescence findings; (c) a high percentage of panel-reactive antibodies prior to transplantation; and (d) demonstration of donor-specific antibodies in the eluate of the failed allograft[21].

Acute cellular rejection (ACR) typically manifests after a week post-transplant and is one of the main clinical differential diagnoses of graft dysfunction along with harvest injury and infection. It should be noted, however, that ACR may occur any time post-transplant, especially when there is an alteration in the effectiveness of immunosuppression. ACR is mediated by an immunologic mechanism targeting the donor histocompatibility antigens expressed on bronchial-associated lymphoid tissue (BALT), bronchial epithelium, and vascular endothelium[22–25]. The relationship between the infiltrating cellular population and MHC class II antigen expression is somewhat unclear. HLA-DR and DQ expression is found in the transplanted bronchial epithelium[26,27], but there is no correlation between the level of expression and episodes of rejection. Furthermore, normal pulmonary epithelium and endothelium may also express MHC class II antigens[28]. The major infiltrative cell population consists of T lymphocytes with occasional B cells[29] of recipient origin as demonstrated by Y chromosomal probe analysis[30,31]. In early ACR, most of the infiltrating T lymphocytes belong to $CD4^+$ (helper) phenotype whereas, later, the population of $CD8^+$ (suppressor/cytotoxic) T cells increases[29,32]. Recently the role of B cells in persistent and immunosuppression-resistant ACR has

been appreciated. When comparing rejection episodes responding and not responding to solumedrol in the early transplant period, the number of infiltrating B cells was significantly larger in the non-responder group than in the responder group[33]. Furthermore, another study has documented the formation of nodular B cell aggregates reminiscent of lymphoid follicles in early bronchiolitis obliterans[34]. Since the number of episodes of ACR has been correlated with the subsequent development of chronic rejection (bronchiolitis obliterans), the involvement of a humoral mechanism in ACR may implicate another pathway for long-term graft compromise.

ACR is characterized by a perivascular mononuclear cell (lymphocyte and plasma cell) infiltrate primarily surrounding pulmonary veins, but also involving arteries and lymphatics, depending on the severity (Figure 4)[8,35,36]. The cuff of infiltrating mononuclear cells undermines the endothelium to produce reactive changes in the endothelial cells ('endothelialitis') (Figure 5). The airway mucosa, particularly the BALT, is also targeted early in acute rejection. The resulting depletion of the donor BALT has been postulated to play a role in the increased susceptibility to graft infection due to the loss of mucosal immunity[22]. With increasing airway inflammation the infiltrate insinuates into the overlying airway mucosa, inducing cytotoxic effects on bronchial epithelial cells (apoptosis). Over time the peribronchiolar and perivascular mononuclear cell cuffs result in disruptions of the laminin and type IV collagen basement membrane components, as demonstrated immunohistochemically[37]. These alterations probably contribute to irreversible remodeling in the long-term allograft.

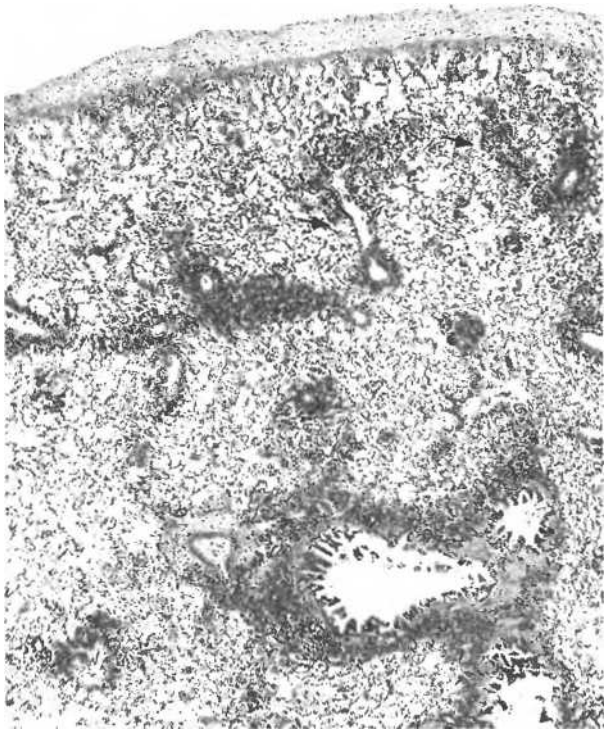

Figure 4 Acute lung rejection. A marked inflammatory infiltrate cuffs the pulmonary veins running in the pleura and interlobular septa (arrows). Concentric cuffing of bronchioles and arterioles is seen at lower right

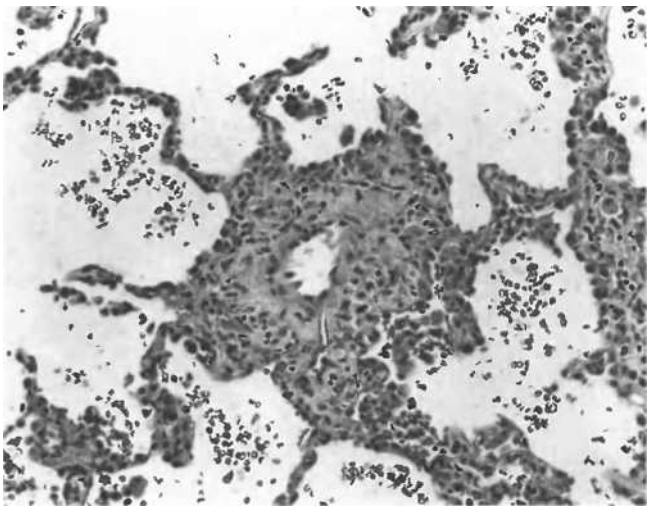

Figure 5 Acute cellular rejection. Concentric perivascular cuffing by mononuclear cells (lymphocytes, plasma cells, and macrophages) with endothelialitis

Grading of ACR by the Working Formulation for the Diagnosis of Lung Rejection[38] is based on the intensity, distribution, and quantity of the mononuclear cells. The lowest degree of rejection response is characterized by the subtle, two-to-three-cell-layer cuffing of small vessels by small, round, plasmacytoid, and transformed lymphocytes (minimal ACR, grade A1). Bronchial and bronchiolar involvement by mononuclear cells is not commonly seen in this grade. In mild ACR (grade A2) there is a significant, five-to-seven-cell-layer perivascular cuffing, which is obvious at low-power examination. The infiltrate commonly also involves the peribronchial/bronchiolar areas. Extension of the infiltrate into the interstitium and air spaces qualifies for moderate ACR (grade A3). With this degree of rejection, airway involvement is seen in most cases and additional histologic features of eosinophilia, neutrophilia, and airspace collections of lymphocytes and macrophages are common (Figure 6). With severe ACR (grade A4), the infiltrate diffusely permeates the lung parenchyma as it involves vascular, airspace and interstitial components, and produces parenchymal damage manifested by alveolar damage, necrosis, hyaline membrane formation and neutrophilic and macrophage infiltrates[39,40]. Localization of the mononuclear infiltrates to the perivascular and peribronchial/bronchiolar areas is lost and other inflammatory cell types, including large numbers of neutrophils and macrophages, are attracted. The resulting injury produces a picture similar to diffuse alveolar damage, and its distinction from other processes such as preservation (harvest), infectious, chemical, drug, and physical injuries is important.

Evaluation of airway alterations is a difficult task in TBB (transbronchial biopsy) interpretation, since inflammation involving the airways is less specific than perivascular inflammation when considering rejection as a diagnosis. In contrast to other solid-organ transplants the lung is constantly exposed to the external environment so low-level chronic inflammation involving the large airways often represents non-specific inflammation. Some long-term patients have airway inflammation due to large airway

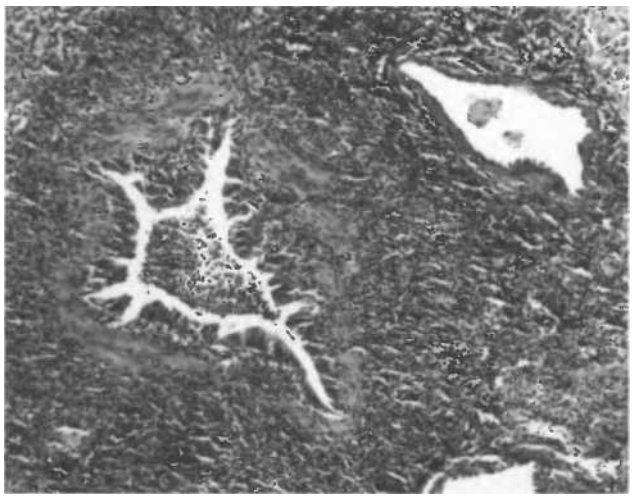

Figure 6 Moderate acute cellular rejection with intense mononuclear cell infiltrate involving the arteries and the bronchioles. The intervening interstitium and airspaces are also involved

alterations such as bronchiectasis, bronchomalacia, and persistent bacterial colonization (e.g. *Pseudomonas* species in cystic fibrosis patients). Small airway inflammation, particularly when involved primarily by a mononuclear cell population, may indicate rejection. However, one should keep in mind that similar appearances may be produced by infections; therefore attributing airway inflammation to rejection is a diagnosis of exclusion[41]. In most instances of ACR the vessels as well as the airways are involved, but there are situations when the biopsies only demonstrate airway inflammation with activated mononuclear cells typical of rejection. The term 'lymphocytic bronchitis/bronchiolitis (LBB)' (grade B) is used to describe this type of inflammation involving the airways exclusively (Figure 7)[41]. It should be recognized that the diagnosis of LBB lacks specificity, and an infectious etiology should be considered as well as a rejection process. When infection is ruled out, the possible reasons for LBB include: (a) treatment of ACR with resolution of the perivascular but not the airway inflammation, (b) inadequate sampling of the perivascular component, (c) bronchocentric ACR, or (d) chronic airway inflammation of unknown significance[41,42]. The decision to treat for rejection would depend more on the clinical parameters.

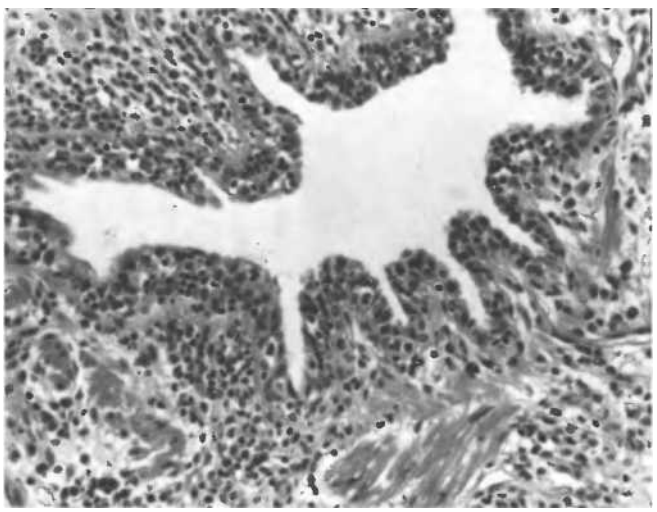

Figure 7 Lymphocytic bronchiolitis. An active lymphocytic cell infiltrate in the submucosa extends into the overlying respiratory epithelium, resulting in focal areas of necrosis

Histopathologic assessment is the most informative diagnostic method in assessing rejection. While thoracoscopic or open lung wedge biopsies are considered the gold standard, the associated morbidity and the intensive labor to obtain the tissue preclude routine use. As an alternative, transbronchial biopsies are commonly utilized. Perhaps the most important point in evaluating transbronchial biopsies is the assessment of adequacy. Since rejection and other allograft syndromes tend to be patchy and focal in nature, transbronchial biopsies should sample multiple areas to obtain alveolated parenchyma with small airways (terminal and respiratory bronchioles). Furthermore, since the features of ACR (such as perivascular and airway inflammation) are not entirely specific, adequate sampling must be obtained to identify histologic features indicating non-rejection processes, particularly infection and lymphoproliferative disorders[43,44].

It is generally agreed that five or more pieces of alveolated lung tissue provide adequate sampling[5,38,45]. Fragments of large airway wall representing the entry point of the biopsy forceps should not be counted in the assessment for adequacy, since they are not as diagnostically informative. In situations in which the transbronchial biopsy findings do not correlate with the clinical presentation, a thoracoscopic or open lung wedge biopsy may be necessary for histopathologic assessment.

Once the diagnosis of rejection is made, enhanced immunosuppression (e.g. bolus doses of solumedrol) is administered. Histologic response is initially seen with the diminution of perivascular infiltrates while the peribronchiolar and interstitial infiltrates may persist. Clinical response often precedes histologic resolution, which may take up to 4 weeks and, even after complete resolution, biopsies may show evidence of previous injury (e.g. interstitial scarring)[46,47].

CHRONIC REJECTION

Chronic rejection represents the development of an irreversible injury to the allograft with permanent functional compromise. In lung allografts, chronic rejection manifests as small airway scarring (bronchiolitis obliterans, OB), large airways bronchiectasis and graft atherosclerosis[48–50]. Injury to the small airways begins with a mucosal mononuclear cell infiltrate which, over time, produces luminal occlusion with granulation tissue and dense hyalinized scar (Figure 8). Like ACR, OB appears to be immunologically mediated and is associated with a $CD8^+$ T cell infiltrate in the peribronchial areas with heightened expression of MHC class I and II antigens in the airway of the allograft[51,52]. Recent studies have also demonstrated the possible role of humoral immunity with B cell aggregates recognized in developing OB[34]. ACR is often seen concurrently with OB, and the recognition of a B cell component in refractory ACR, as well as developing OB, leads one to speculate whether humoral immunity is a common denomi-

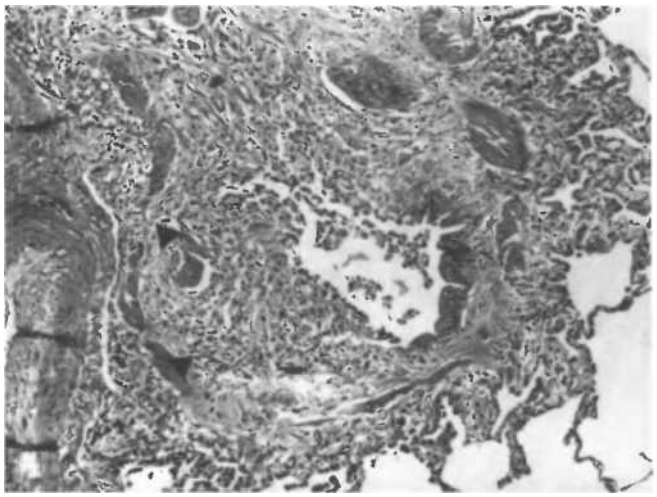

Figure 8 Subtotal active bronchiolitis obliterans. An intraluminal plug of granulation tissue (arrows) entraps metaplastic epithelial cells

nator in ACR and this form of progressive airway injury. The patchy and segmental distribution of OB also suggests a link to ACR[8,53]. Regardless of the precise mechanism, repeated insults to the airway mucosa contribute to disruption of the bronchiolar basement membrane, epithelial cell necrosis, myofibroblastic ingrowth, loss of smooth muscle, and eventual scarring[37,49,50,53–55]. In the final phase of OB the bronchiolar lumen is replaced by a dense hypocellular scar (Figure 9). Since OB proceeds in a temporally heterogeneous manner[8,56], obstructed bronchioles are often seen adjacent to actively inflamed, as well as relatively normal, airways. Although OB requires the exclusion of other causes of airway fibrosis, including infection, aspiration, and ischemia, this patchy, predominantly bronchocentric injury and scarring are highly characteristic of immunologically related airway rejection process. Clinically, the pulmonary function abnormalities are obstructive early in the course of OB, and later become restrictive. In contrast to chronic rejection of the liver, the diagnosis of OB does not portend imminent organ failure, and the rate of functional deterioration is variable.

While the small airways scar are obliterated as a consequence of chronic airway rejection, the inflamed large airways scar and paradoxically develop bronchiectatic changes. This alteration may be seen in non-rejection processes such as chronic infection and aspiration, and therefore lack the specificity to be attributed solely to an airway rejection process[8,53].

In addition to the airway damage, many long-term survivors show graft arteriosclerosis (GAS) characterized by a myofibrointimal proliferation and collagen deposition[48]. These vascular lesions are patchy, segmental and circumferential, although asymmetry is occasionally noted. The degree of proliferation corresponds to grade 2 in the Heath–Edwards classification of pulmonary hypertension (Figure 10). However, the clinical significance of these vascular lesions is unclear, since these patients rarely develop pulmonary hypertension and the development of GAS does not necessarily correlate with the onset of OB[53].

Since the clinical significance of GAS is uncertain, and the large airway alterations are non-specific, the diagnosis of chronic rejection depends largely on the identification of OB. This can been a challenge to both the clinician and the pathologist. The histologic diagnosis of OB requires the demonstration of dense submucosal scarring of the small airways that may be eccentric, concentric, or associated with total obliteration of the bronchiolar lumen[38]. The trichrome stain is particularly helpful in this assessment. Transbronchial biopsy may establish the diagnosis of OB, and the sensitivity and specificity are 87% and 99%, respectively[57]. Nevertheless, the bronchoscopist occasionally encounters a patient with scarred and fibrotic lungs, which are difficult to biopsy due to the lack of compliance. In these cases, despite multiple biopsies, the pieces obtained tend to be minute and small airways are not often sampled. This may further necessitate an open lung or thoracoscopic wedge biopsy to assess the possibility of OB.

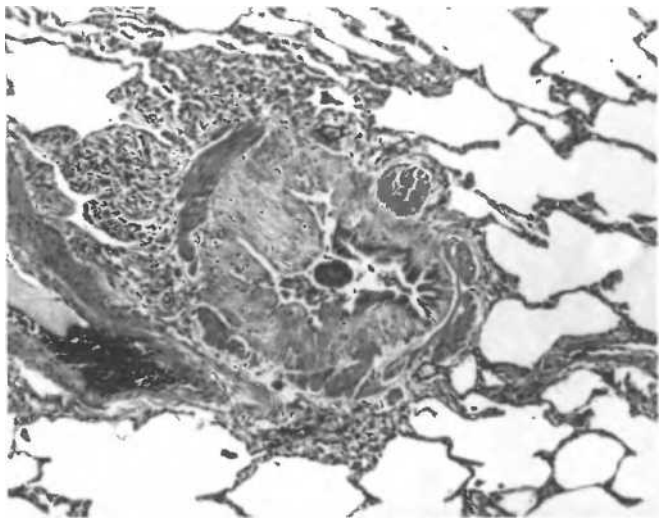

Figure 9 Subtotal inactive bronchiolitis obliterans. Diminution of the mononuclear cell infiltrate leaves an eccentric old scar tissue in the bronchiolar lumen (arrows)

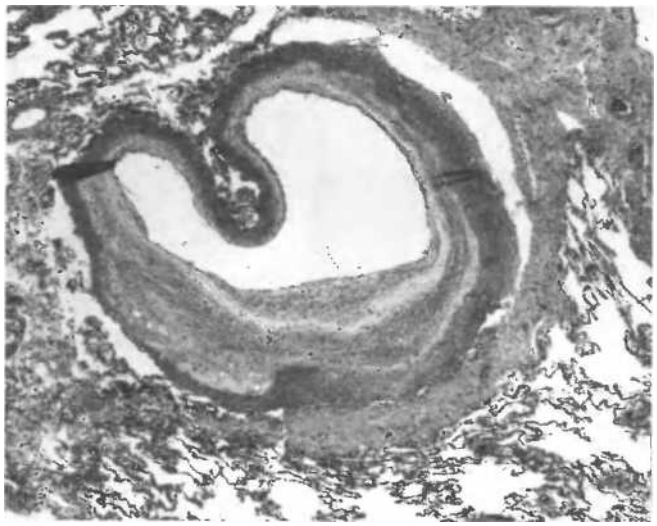

Figure 10 Graft atherosclerosis. Pulmonary artery branch with an eccentric fibromyxoid plaque and a mild mononuclear cell infiltrate produces an endovasculitis

Due to these difficulties, diagnostic terms have been defined to describe the manifestations of OB. The term bronchiolitis obliterans (OB) is reserved for histologically proven lesions either by biopsy (transbronchial or wedge) or at autopsy. Bronchiolitis obliterans syndrome (BOS) is a clinically defined entity of allograft deterioration secondary to progressive airway disease with no other known cause[58]. A pulmonary function test measuring the forced expiratory volume in one second (FEV_1) is utilized in grading the severity of the airway lesion. BOS does not require histologic confirmation, but patients must demonstrate less than 80% of baseline FEV_1 value for this diagnosis to be made. Following the diagnosis of OB, patients are treated with enhanced immunosuppression in an attempt to quell the active cellular component of OB to recover some of the pulmonary function deficits.

INFECTION

The allograft environment is ideal for the proliferation of opportunistic microorganisms. In addition to enhanced immunosuppression there are a multitude of reasons for the susceptibility, some of which are unique to the lung allograft. During the terminal course of the donor, aspiration resulting in bacterial and fungal contamination contributes to a lower 1-year survival of 35% (in contrast to 67% for those without early infection)[59]. The lung transplantation procedure involves anastomoses of the major airways and pulmonary arteries, but not the bronchial arteries and the peripheral nerves, which are sacrificed. Consequently, the vascular supply to the large airways is dependent on the collaterals from the pulmonary arteries. With the denervation there is loss of mucociliary clearance and cough reflex[60,61]. Another reason for early infectious susceptibility is the loss of the bronchial-associated lymphoid tissue (BALT) secondary to ACR targeting the MHC class II antigens on the donor BALT lymphocytes. BALT normally provides secretory IgA-mediated humoral defense along the airway mucosa, and its compromise and constant bombardment by external pathogens through the airways increase the chances of early allograft infection[22]. During the mid and late post-transplant course, additional factors contribute to graft susceptibility. In single lung transplants the remaining native lung may become a nidus of infection and seed the allograft. Patients with the primary diagnosis of cystic fibrosis are known to have their upper airways and sinuses colonized by *Pseudomonas* species (*aeruginosa* and/or *cepacia*), which subsequently infects the allograft lung downstream[62–64]. Unfortunately, these *Pseudomonas* species are often resistant to currently available antibiotics and therefore difficult to control. Finally, the parenchymal alterations following chronic rejection result in remodeling, manifesting as interstitial, septal and subpleural scarring and cylindrical bronchiectasis which alter air flow and decrease mucus clearance[8,53]. These airways are readily colonized by Gram-negative rods, particularly *Pseudomonas*. Under these compromised circumstances, acute bronchitis and pneumonia is not uncommon.

Specific types of infections are often encountered in the typical clinical context mentioned above. Bacterial pneumonia is the most common infection in lung transplant recipients, manifesting early (within the first 2 months) or late in the post-transplant course[59,65,66]. The common types of bacteria include *Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Enterococcus*, *Streptococcus pneumoniae*, *Acinetobacter*, *Hemophilus*, and *Klebsiella*[67]. The early infections are related to aspiration by the donor, whereas the later infections are due to parenchymal remodeling, bronchiectasis, mucus inspissation and primary disease such as cystic fibrosis.

Bronchoalveolar lavage (BAL) is the most efficacious method for isolating and speciating bacteria as well as fungal and viral organisms. Biopsies are less sensitive and specific, and speciation is not possible. Nevertheless, histologic identification of bronchopneumonia may be made before culture results are available, allowing empiric therapy to be instituted. When considering infectious processes, determination of the significance of isolated microorganisms is an important issue. This depends on multiple factors including type of species isolated, colony count, and clinical manifestation. The diagnosis of bacterial pneumonia depends on the documentation of new fevers, infiltrates on chest radiograph, and isolation of significant numbers of the organism (generally greater than 100 000)[68].

Nocardiosis is less common, but is nevertheless an important bacterial infection in the transplant population. These Gram-positive aerobic, filamentous rods infect the immunocompromised or others with underlying medical conditions[69]. Eighty-five percent of nocardiosis is by *N. asteroides* and the manifestations include bronchopneumonia, abscess formation, cavitation, and empyema (Figure 11). Furthermore, the infection may metastasize to the brain, bone, skin, and subcutaneous tissue. The irregularly branching, thin, beaded, filamentous rods are characteristic of *Nocardia* although *Actinomyces* and *Streptomyces* should also be considered in the differential diagnosis (Figure 12). *Nocardia* may be seen on Gram and Grocott, as well as Fites (modified Ziehl–Nielsen) stain, which has been shown to be particularly useful. Since *Nocardia* abscesses often manifest as localized lesions, TBB may be ineffective in obtaining diagnostic tissue. Under such circumstances, fine-needle aspiration biopsy is often

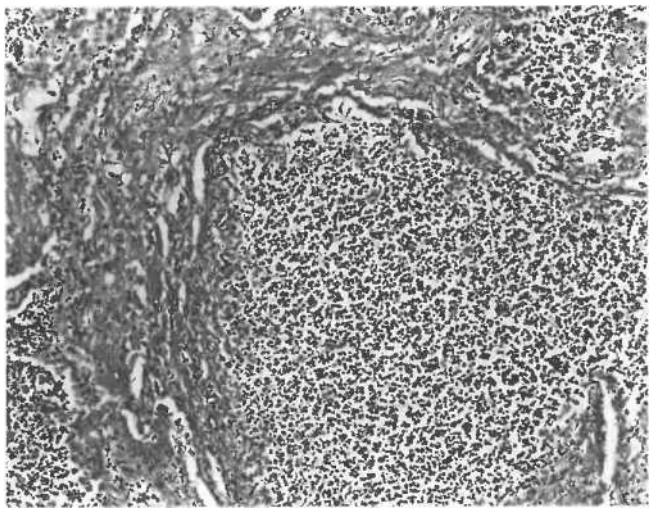

Figure 11 *Nocardia* abscess. Along with bronchopneumonia and empyema, abscess formation is one of the common manifestations of nocardiosis.

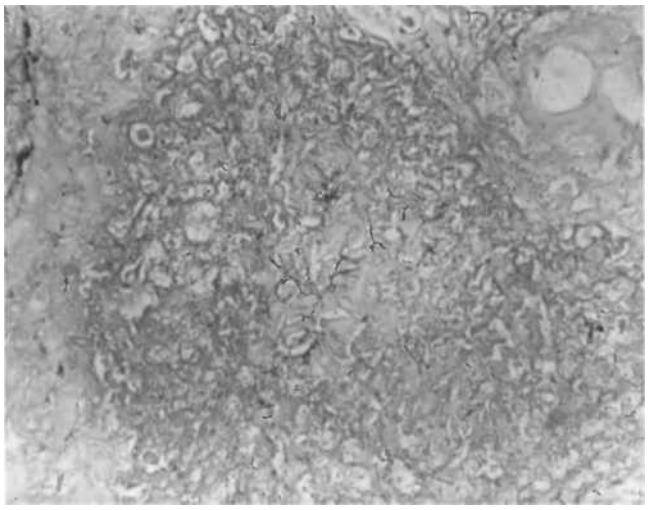

Figure 12 Grocott stain of a *Nocardia* abscess. Thin, irregularly branching, beaded filaments are noted. These organisms are also Gram-positive and stain with the Fite's modification of the acid-fast stain

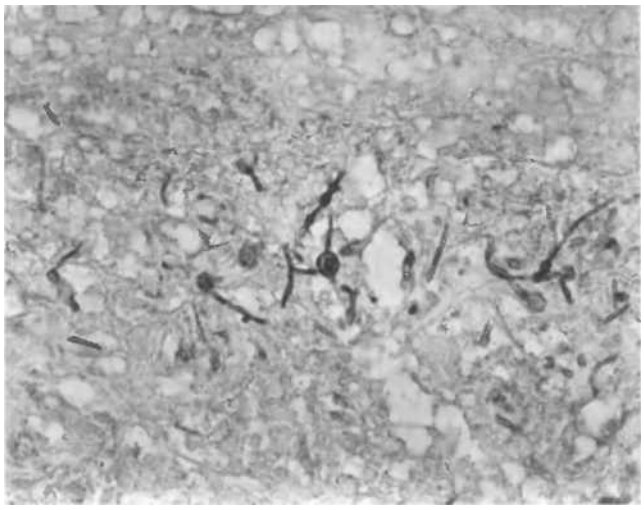

Figure 13 *Pseudallescheria boydii* may colonize cavities or produce invasive pneumonia and empyema. Although they resemble *Aspergillus* species, the identification of thin-walled vesicles and less-acute-angle branching are helpful features in recognizing *P. boydii*

more effective in sampling the centrally necrotic material which harbors the organisms.

Fungal infections are also most common in the early post-transplant period, but may occur any time afterwards. *Candida* and *Aspergillus* are common offenders and their identification must be taken in the context of their invasiveness[67,70]. *Candida* mainly infests the upper tracheobronchial tree with less chance of dissemination. However, the isolation of *Aspergillus* may represent colonization, allergic fungal response, or invasive disease involving the deep parenchyma. Although highly sensitive and specific, the BAL culture has a low predictive value[68] and in consideration of the high fatality from invasive aspergillosis, many cases representing contaminant and colonization are probably overtreated. Nonetheless, the current antifungal regimen has been effective in decreasing the morbidity and mortality from fungal disease.

Pseudallescheria boydii is ubiquitous in the environment and produces an opportunistic infection which mimics aspergillosis both clinically and pathologically[69,71]. Like *Aspergillus* infections, the isolation of *P. boydii* needs to be correlated with the setting where it is found. Colonization commonly occurs in the remodeled pulmonary parenchyma and cavities. On the other hand, invasive necrotizing pneumonia with abscess formation and pleural involvement with empyema may be associated with hematogenous dissemination to the brain, kidney, heart, and thyroid. Manifestation as an allergic bronchopulmonary fungal disease has been also recently reported. Morphologically, *P. boydii* and *Aspergillus* are similar, with both showing narrow (2–5 μm) septate hyphae with acute angle branching. The hyphae of *P. boydii* may show thin-walled vesicles and terminal conidia and these features are helpful in distinguishing it from *Aspergillus* (Figure 13). This distinction has clinical importance as amphotericin which is usually used for aspergillosis is not effective in pseudallerscheriasis, whereas miconazole or ketoconazole may be effective.

Among the viral infections, cytomegalovirus (CMV) is the most common and important[72–74]. Unfortunately, due to the various clinical presentations and methods to detect CMV, identification must be correlated with disease presentation. CMV-related illnesses may be subdivided into CMV infection, recognizing only the presence of the virus with or without associated clinical/pathological manifestations, and CMV disease, with recognizable pulmonary manifestations (i.e. pneumonitis) due to the virus[68].

To assess the appropriate risk, both the recipient (R) and donor (D) are tested for circulating CMV antibodies[68]. The risk for CMV infection, disease, and related deaths varies depending on the combination of the R/D serologic status. The highest risk for significant disease and death occurs in R–D+ patients and requires the most aggressive anti-CMV prophylactic regimen. While the risk for significant CMV infection and disease is lowest in R–D– patients, the risk of death is approximately 8%. This is in contrast to the R+D– and R+D+ patients who may have a higher incidence of infection and disease but whose risk for CMV-related death is lowest, approximately 1–2%, perhaps due to acquired immunity[68]. Significant CMV disease occurs most commonly in the first 2–3 months post-transplant, although occasional presentation may occur afterwards. Histologically, the manifestation of CMV pneumonitis ranges from a subtle patchy interstitial mononuclear cell infiltrate with rare inclusions to diffuse interstitial and perivascular neutrophilic and mononuclear cell infiltrates with alveolar damage and numerous CMV inclusions[75,76]. The identification of CMV in biopsies should be placed in context of the patient's risk of developing significant disease, as discussed above. The inflammatory background may be distributed in a perivascular pattern, mimicking ACR[75,77]. This reiterates the importance of obtaining adequate sampling to demonstrate the diagnostic inclusions. When an isolated CMV is found in a background lacking inflammation, the interpretation depends on the clinical context. It may represent the earliest manifestation of a developing pneumonitis or the detection of a latent virus; close follow-up is warranted. The detection of CMV by

culture or Shell-vial assay alone, without clinical disease or histologic confirmation, indicates CMV infection without disease. On such occasions the decision for treatment would depend on the clinical situation[68]. With the current antiviral regimen, mortality from CMV pneumonitis has markedly decreased. CMV involvement has also been associated with an increased risk for the development of chronic airway rejection (bronchiolitis obliterans)[78]. The up-regulation of HLA class II antigens following CMV infection has been postulated as a mechanism for its development. Such associations cloud the distinction between rejection and infection.

Due to prophylactic acyclovir, the incidence of and morbidity from herpes simplex pneumonia have diminished. Nevertheless, those susceptible present commonly in the first post-transplant month, and the lung may be the only site of infection[79]. An association with herpes tracheitis and prolonged intubation has been noted. The histologic findings of HSV pneumonia are similar to those occurring in other immunocompromised patients[80,81]. The pneumonia tends to be florid with extensive necrosis and presence of infected cells with intranuclear ground glass inclusions and occasional Cowdry type A inclusions. Multinucleated giant cells with similar nuclear changes are also common features. Rapid treatment following its detection is critical as the disease may be rapidly fatal if left unchecked.

Adenovirus (ADV) infections have been reported sporadically in the lung transplant literature[67,82]. The manifestations range from an acute bronchitis/bronchiolitis to diffuse alveolar damage. Even in cases of DAD a bronchocentric accentuation of severe necrosis is often noted (Figure 14). In our series most of the patients belonged to the pediatric age group[83]. They acquired the infection within the first $1\frac{1}{2}$ months post-transplant, and experienced a rapidly fatal course. Smudgy basophilic nuclear inclusions are characteristic of ADV infections and, in cases which are equivocal, the use of immunohistochemical stain or *in-situ* hybridization probe for ADV may be helpful (Figure 15). An indeterminate number of patients may carry ADV subclinically without ever developing disease. The relatively high incidence in the pediatric population, in contrast to the adult population, suggests that ADV pneumonia represents a primary infection rather than a reactivation. Those who develop antibodies may acquire lasting immunity.

The depressed cellular immunity also provides an opportune setting for *Pneumocystis* infection and, early in the history of lung transplantation, *Pneumocystis carinii* pneumonia (PCP) was a common problem[84,85]. However, with the institution of PCP prophylaxis (sulfonamides), the incidence of PCP has markedly diminished[86,87]. Nevertheless, some patients are allergic to sulfonamides and in rare instances prophylaxis may not prevent the infection.

The pattern of PCP in the lung transplant recipient is similar to that of other immunosuppressed settings. The gross appearance of the lung appears as bronchopneumonia or diffuse consolidation. Histologically there is a range of tissue responses from minimal alterations to granulomatous response to florid diffuse alveolar damage. Foamy alveolar exudates are characteristic findings in H&E sections, although this appearance may be mimicked by alveolar fibrin, macrophages and other cellular debris. Therefore, the Grocott stain is indispensable in assessing the possibility of PCP, and should be a component of every BAL cytology and lung biopsy work-up. The typical Grocott morphology shows cup-shaped cysts with central intracystic bodies. The differential diagnoses include *Candida*, *Torulopsis*, *Coccidioides*, *Histoplasma*, and *Cryptosporidia*.

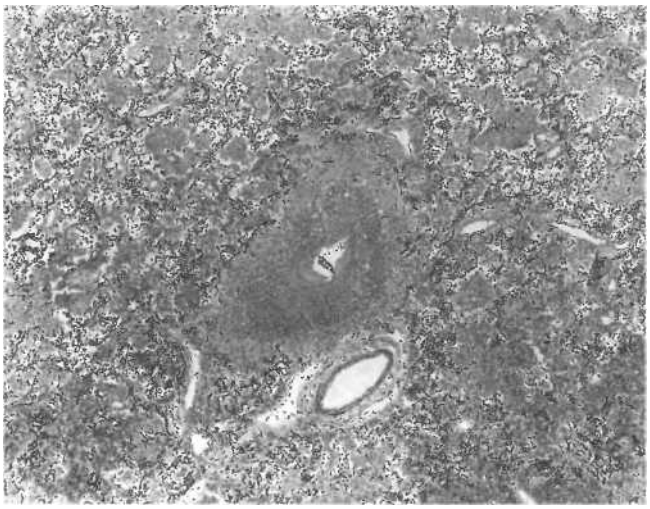

Figure 14 Adenovirus pneumonia typically manifests as a necrotizing bronchocentric pneumonia. In this severe case the background shows diffuse alveolar damage

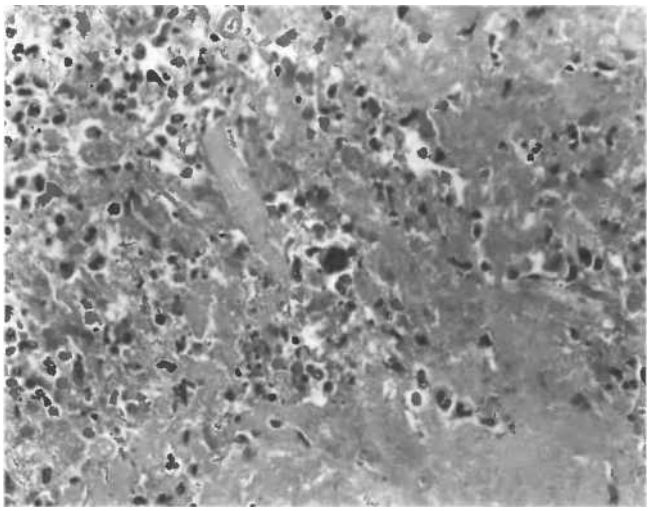

Figure 15 Adenovirus-infected cell with smudgy basophilic nuclear inclusions. In contrast to CMV, cytomegalic changes and intranuclear inclusions are not seen

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD)

PTLD arising in lung transplant patients is morphologically similar to those found in other solid organ transplants[88]. It consists of a proliferation of atypical lymphocytes (usually of B cell origin) arising in the background of overimmunosuppression, and has a strong association with primary Epstein–Barr virus infection, not reactivation. PTLD occurs early in the post-transplant course, generally in the first 3 months. Lung transplant patients

have a relatively high incidence of PTLD occurring in the allograft. At our institution, PTLD developed in approximately 7% of lung transplant recipients and, of these, approximately 60% occurred in the allografted lung[89,90]. This may be due to: (a) the allograft lung being the primary site of EBV infection, (b) the high level of immunosuppression as compared to other organ transplants, and (c) donor BALT acting as 'homing' sites for EBV-infected host B cells.

Morphologically, PTLD produces a mass-like lesion with some cases showing angioinvasion. Histological classification into monomorphous (uniform population of transformed large cells and immunoblasts) and polymorphous (representing the entire spectrum of B cell differentiation with small lymphocytes, plasma cells, large lymphoid cells and immunoblasts) subtypes has some correlation with monoclonality in the former and polyclonality in the latter (Figure 16). With expansion of the mass, foci of necrosis appear, leaving viable lymphoid cells at the periphery (Figure 17). When these areas are biopsied transbronchially, distinction from acute cellular rejection may be difficult. In these instances, demonstrating the presence of Epstein–Barr virus latent membrane protein (EBV-LMP) by immunohistochemistry or Epstein–Barr virus encoded RNA (EBER) by *in-situ* hybridization has been shown to be useful in establishing the diagnosis of PTLD. Specifically, perivascular lymphocytes marking with EBV-LMP are found at the peripheral edges of PTLD, whereas the perivascular lymphocytes of acute cellular rejection are negative[91]. While EBER *in-situ* hybridization studies are also informative, it should be cautioned that, due to the high sensitivity of the study, positive interpretation should be made only when EBER positivity is found in large atypical lymphocytes. Similar principles apply to the interpretation of polymerase chain reaction studies, which may detect very small quantities of EBV genomes in patients without evidence of PTLD[92].

DISEASE RECURRENCE

In contrast to most lung transplants, for disease primarily limited to the lungs, transplants for systemic diseases are at risk for recurrence. Of these, sarcoidosis and lymphangioleiomyomatosis (LAM) have been documented to recur[93–95]. In sarcoidosis, the diagnosis of recurrence is first suspected by the identification of non-caseating granulomas, negative for infectious organisms by special stains. Other etiologies for granulomas must be ruled out clinically. The granulomas found on the transbronchial biopsies tend to be very small and focal; often they may not be present on deeper levels of histologic sections. The significance of these recurrent granulomas is at present uncertain, since functional compromise attributable to recurrent disease has not been shown. Recurrent LAM was seen in a female recipient who had received an allograft from a male donor[95]. Interestingly, *in-situ* hybridization Y-probe analysis demonstrated the donor origin of the recurrent smooth muscle proliferation, thus suggesting the possibility of a circulating factor promoting the growth of myocytes in the pathogenesis of LAM. Due to its rarity, the clinical significance of recurrent LAM is also uncertain.

Early recurrence of diffuse panbronchiolitis (DPB) 10 weeks after transplantation has also been reported[96]. Clinical deterioration was attributed to the recurrent DPB, and the patient was treated with erythromycin, which resulted in resolution of symptoms over a few weeks. Rare case reports of giant cell interstitial pneumonia (GIP) have been documented in single-lung transplant recipients[97,98]. Since GIP is now thought to be a form of pneumoconiosis secondary to occupational hard metal exposure, recurrence suggests the possibility of residual hard metal in the remaining recipient lung 'seeding' the donor lung or the hard metal precipitating a persistent autoimmune reaction in recipient lymphocytes/monocytes.

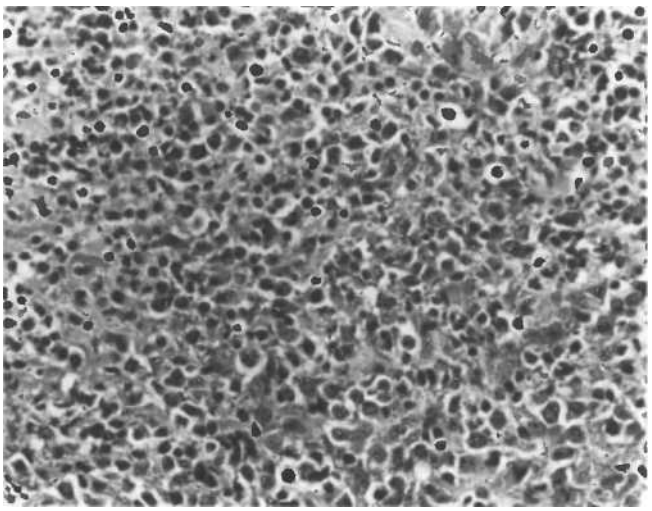

Figure 16 Polymorphous PTLD with a mixed population of small round, plasmacytoid, large, and occasional immunoblastic lymphocytes

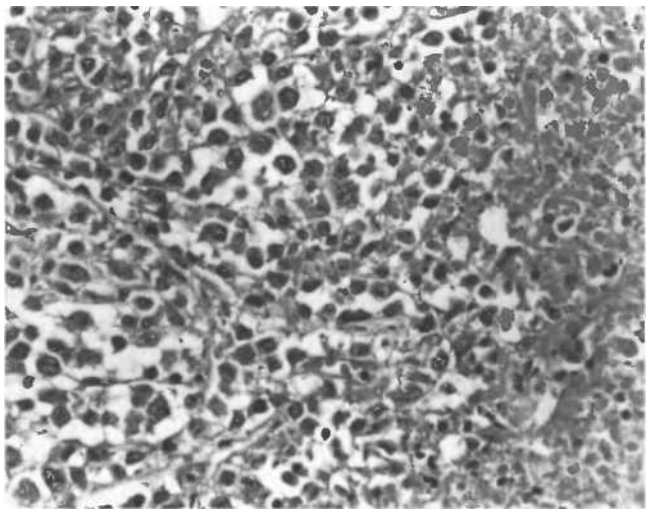

Figure 17 Monomorphous PTLD adjacent to area of necrosis. The proliferating cell population is uniformly large with a complex chromatin pattern. Nucleoli are also readily identified

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