

Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection

Susan Stewart, FRCPath, Michael C. Fishbein, MD, Gregory I. Snell, MD, Gerald J. Berry, MD, Annette Boehler, MD, Margaret M. Burke, FRCPath, Alan Glanville, MD, F. Kate Gould, FRCPath, Cynthia Magro, MD, Charles C. Marboe, MD, Keith D. McNeil, FRACP, Elaine F. Reed, PhD, Nancy L. Reinsmoen, PhD, John P. Scott, MD, Sean M. Studer, MD, Henry D. Tazelaar, MD, John L. Wallwork, FRCS, Glen Westall, MD, Martin R. Zamora, MD, Adriana Zeevi, PhD, and Samuel A. Yousem, MD

In 1990, an international grading scheme for the grading of pulmonary allograft rejection was adopted by the International Society for Heart and Lung Transplantation (ISHLT) and was modified in 1995 by an expanded group of pathologists. The original and revised classifications have served the lung transplant community well, facilitating communication between transplant centers with regard to both patient management and research. In 2006, under the direction of the ISHLT, a multi-disciplinary review of the biopsy grading system was undertaken to update the scheme, address inconsistencies of use, and consider the current knowledge of antibody-mediated rejection in the lung. This article summarizes the revised consensus classification of lung allograft rejection. In brief, acute rejection is based on perivascular and interstitial mononuclear infiltrates, Grade A0 (none), Grade A1 (minimal), Grade A2 (mild), Grade A3 (moderate) and Grade A4 (severe), as previously. The revised (R) categories of small airways inflammation, lymphocytic bronchiolitis, are as follows: Grade B0 (none), Grade B1R (low grade, 1996, B1 and B2), Grade B2R (high grade, 1996, B3 and B4) and BX (ungradeable). Chronic rejection, obliterative bronchiolitis (Grade C), is described as present (C1) or absent (C0), without reference to presence of inflammatory activity. Chronic vascular rejection is unchanged as Grade D. Recommendations are made for the evaluation of antibody-mediated rejection, recognizing that this is a controversial entity in the lung, less well developed and understood than in other solid-organ grafts, and with no consensus reached on diagnostic features. Differential diagnoses of acute rejection, airway inflammation and chronic rejection are described and technical considerations revisited. This consensus revision of the working formulation was approved by the ISHLT board of directors in April 2007. *J Heart Lung Transplant* 2007;26:1229-42. Copyright © 2007 by the International Society for Heart and Lung Transplantation.

The original 1990 working formulation for the classification of pulmonary allograft rejection resulted from an International Society for Heart and Lung Transplantation (ISHLT) workshop to develop a standardized grading system for the pathologic diagnosis of rejection in transplant lung biopsies.¹ A core group of pathologists developed a grading scheme for pulmonary allograft rejection that allowed data to be compared between institutions as a result of uniformity of grading. The grading system was intended to be simple, easily taught, and readily reproducible, and was adopted at the majority of institutions performing lung transplantation at the time.

In 1995, an expanded group of international pathologists convened to revise the original 1990 proposal in response to developments in the field and their experience with using the working formulation.² On this occasion, the lung rejection study group critically assessed the merits of the initial working formulation and improved it on the basis of both published data and practical experience across many centers. The goal was again to maintain a uniform description and grading scheme for lung rejection, to improve communication between clinicians and investigators, to enable comparison of treatment regimes and outcomes between transplant centers, to facilitate multi-center clinical trials, and to promote further studies to determine the clinical significance of the various histologic patterns. The revised classification was based on histologic findings of acute and chronic lung rejection by primarily using transbronchial biopsies for allograft monitoring in both adults and children. It was emphasized that all biopsy data needed to be interpreted in an integrated clinical context to allow optimum patient management and clinical decisions. It was also noted that infection/rejection often occur together and can be confused histologically and that infection needs to be rigorously

From the Papworth Everard Pathology Department, Papworth Hospital, Cambridge, UK.

Submitted October 29, 2007; revised October 30, 2007; accepted October 31, 2007.

Reprint requests: Susan Stewart, FRCPath, Papworth Everard Pathology Department, Papworth Hospital, Cambridge CB3 8RE, UK. Telephone: 44-1480-364-304. Fax: 44-1480-364-777. E-mail: susan.stewart@papworth.nhs.uk.

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excluded for the accurate and reproducible interpretation of pulmonary allograft biopsies.

The 1996 revision was itself widely adopted by the lung transplant community and has served it well for over a decade.^{3,4} The revised working formulation represented a simplification of the original classification scheme, but it also highlighted some unresolved and complex issues such as the diagnosis and significance of airway inflammation. In 2004, again under the direction of the ISHLT, a multidisciplinary review of the cardiac biopsy grading system was undertaken to address challenges and inconsistencies in its use and also to address recent advances in the knowledge of antibody-mediated rejection. The revised consensus classification was accepted by the board of directors and published in 2005.⁵ It was clear that the success of the multidisciplinary approach could be usefully adopted for a further revision of the diagnosis of lung rejection to take into account a decade of developments in the clinical, pathologic and immunologic fields. Toward this end, a multi-disciplinary consensus meeting was held at the ISHLT 2006 meeting in Madrid and its conclusions form the basis of this consensus report. The multidisciplinary task forces examined the histopathology of cellular rejection, humoral (antibody-mediated rejection) and clinical issues and future research.

Comments solicited from the ISHLT membership at large and from the transplant pathology community were also taken into account. Compared with the numerous responses from ISHLT members in 2004 regarding the cardiac grading system, only a small number of responses were received concerning lung grading. This was interpreted as most likely reflecting an overall higher level of satisfaction with the existing scheme compared with the 1990 cardiac working formulation. The present study reports on the consensus of revisions to the pathologic classification (Table 1) and is supplemented by the consensus of lung transplant physicians and surgeons focusing on the clinical viewpoint.⁶

HISTOLOGIC GRADING OF PULMONARY ALLOGRAFT REJECTION

The histopathology task force again recognized that alloreactive injury to the donor can affect both the vasculature and the airways in acute and chronic rejection. Acute rejection is characterized by perivascular mononuclear cell infiltrates, which may be accompanied by sub-endothelial infiltration, so-called endothelialitis or intimitis, and also by lymphocytic bronchitis and bronchiolitis.^{1,2,7} However, chronic rejection is manifest by fibrous scarring, which is often dense and eosinophilic, involving the bronchioles and sometimes associated with accelerated fibrointimal changes affecting pulmonary arteries and veins. As in the original and revised classifications, the histologic changes have been

Table 1. Revised Working Formulation for Classification and Grading of Pulmonary Allograft Rejection

A: Acute rejection
Grade 0—none
Grade 1—minimal
Grade 2—mild
Grade 3—moderate
Grade 4—Severe
B: Airway inflammation
Grade 0—none
Grade 1R—low grade
Grade 2R—high grade
Grade X—ungradeable
C: Chronic airway rejection—obliterative bronchiolitis
0—absent
1—present
D: Chronic vascular rejection—accelerated graft vascular sclerosis

“R” denotes revised grade to avoid confusion with 1996 scheme.

divided into grades based on the intensity of the cellular infiltrate and the presence or absence of fibrosis. The presence of presumed irreversible dense eosinophilic hyaline fibrosis in airways and vessels remains the key histologic discriminator between acute and chronic rejection of the lung.

A. ACUTE REJECTION

A diagnosis of acute rejection is based exclusively on the presence of perivascular and interstitial mononuclear cell infiltrates. The intensity of the perivascular mononuclear cell cuffs and the distribution of the mononuclear cells, including extension beyond the vascular adventitia into adjacent alveolar septa, form the basis of the histologic grade. Acute rejection usually affects more than one vessel (particularly in adequate transbronchial biopsy samples) but is occasionally seen as a solitary perivascular infiltrate. This finding should be evaluated with the same criteria as those applied to multiple infiltrates as outlined in what follows. In the setting of multiple foci of rejection, the grade reflects the most advanced pattern of rejection rather than the predominant pattern. The infiltrates surrounding small vessels in the sub-mucosa of airways are again interpreted as part of the spectrum of airway inflammation rather than being diagnostic of acute rejection, Grade A.

Grade A0 (No Acute Rejection)

In Grade A0 acute rejection, normal pulmonary parenchyma is present without evidence of mononuclear cell infiltration, hemorrhage or necrosis.

Grade A1 (Minimal Acute Rejection)

In Grade A1 acute rejection, there are scattered, infrequent perivascular mononuclear infiltrates in alveolated lung parenchyma (Figures 1, 2 and 3). Blood vessels,

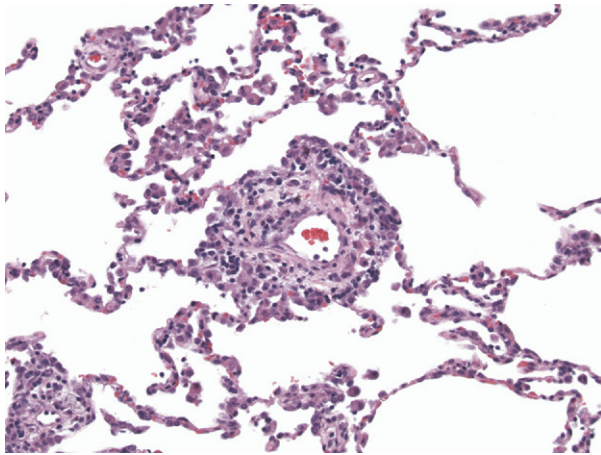


Figure 1. Minimal acute cellular rejection (A1). The characteristic feature of minimal acute cellular rejection is circumferential infiltration of the perivascular interstitium by mononuclear cell inflammatory infiltrate. This typically involves the small veins and consists of scattered mononuclear cells within loose perivascular connective tissue. No significant expansion of the perivascular interstitium or extension of mononuclear cells into adjacent alveolar septa is present. Haematoxylin and eosin (H&E).

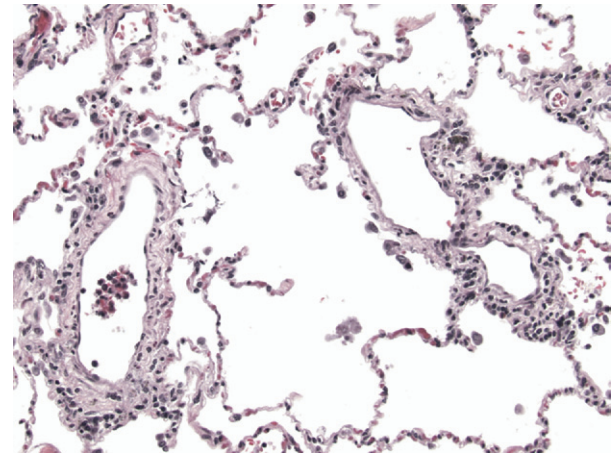


Figure 3. Minimal acute cellular rejection (A1). In this example of A1 rejection, the vessel at right displays a perivascular mononuclear infiltrate which in one segment of this vessel appears to be circumferential therefore warranting a diagnosis of minimal acute rejection. At left the vessel contains an ill-defined noncircumferential infiltrate of mononuclear cells of low intensity which would be regarded as a nonspecific morphologic finding. H&E.

particularly venules, are cuffed by small, round plasmacytoid and transformed lymphocytes forming a ring of two or three cells in thickness within the perivascular adventitia. This cuffing may be loose or compact and is generally circumferential. Eosinophils and endothelialitis are not present. The previous grading schemes suggest that these minimal infiltrates are not obvious at low magnification, but it was believed that this criterion can be misleading. Grade A1 infiltrates can be seen at scanning magnification if the specimen is

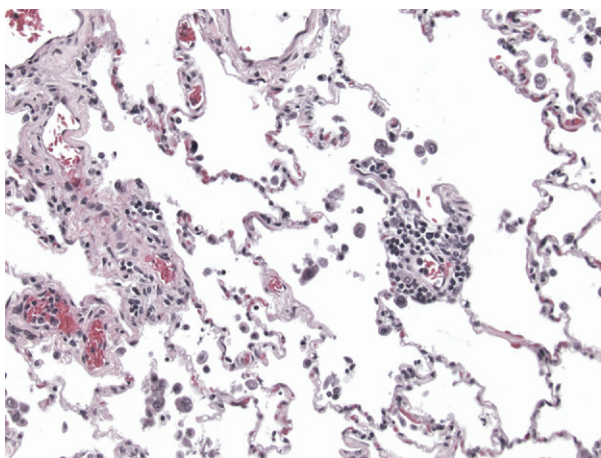


Figure 2. Minimal acute cellular rejection (A1). A sparse mononuclear cell infiltrate is present in the perivascular zones in this example of A1 minimal rejection at right. At left is a nonspecific mononuclear inflammatory infiltrate which fails to show the circumferential cuffing of vessels or the density of mononuclear cells that is sufficient for a diagnosis of minimal acute cellular rejection. H&E.

adequately alveolated and free from artifact. The consensus was that evidence of infrequent perivascular infiltrates at low-power (scanning) magnification is not a reliable discriminator between Grade A1 and A2 acute rejection.

Grade A2 (Mild Acute Rejection)

In Grade A2 mild rejection, more frequent perivascular mononuclear infiltrates are seen surrounding venules and arterioles and are readily recognizable at low magnification (Figures 4, 5, 6 and 7). They may be densely compacted or loose. These infiltrates usually consist of a mixture of small, round lymphocytes, activated lymphocytes, plasmacytoid lymphocytes, macrophages and eosinophils. Eosinophils are not a feature of Grade A1 minimal rejection. In Grade A2 rejection there is frequently sub-endothelial infiltration by mononuclear cells, which may be associated with hyperplastic or regenerative changes in the endothelium, that is, endothelialitis. In making the distinction between Grade A2 and higher grade acute rejection it is important to note that the perivascular interstitium can be expanded by mononuclear cells in A2 rejection but there is no obvious infiltration by mononuclear cells into the adjacent alveolar septa or air spaces. Concurrent lymphocytic bronchiolitis (see later) may be seen in association with mild acute rejection (Grade A2), but is less common with minimal acute rejection (Grade A1).

Mild acute rejection is therefore distinguished from minimal acute rejection by the presence of unequivocal mononuclear infiltrates, which are more easily identified at scanning magnification. In addition, endotheliali-

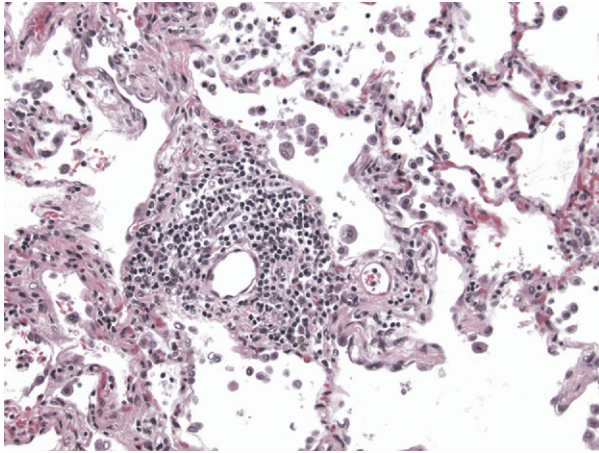


Figure 4. Mild acute cellular rejection (A2). In mild acute cellular rejection, the perivascular interstitium of small vessels, venules and arterioles, demonstrates significant circumferential expansion of the perivascular interstitium by mononuclear inflammatory infiltrate. The infiltrate consists largely of mononuclear cells with occasional activated lymphocytes and plasmacytoid lymphocytes. The mononuclear inflammatory infiltrate within the perivascular zones may be accompanied by alveolar macrophages. No infiltration of adjacent alveolar septa by the mononuclear infiltrate is present. H&E.

tis, the presence of eosinophils and co-existent airway inflammation favor mild Grade A2 over minimal A1 acute rejection.

Grade A3 (Moderate Acute Rejection)

Grade A3 acute rejection shows easily recognizable cuffing of venules and arterioles by dense perivascular mononuclear cell infiltrates, which are commonly asso-

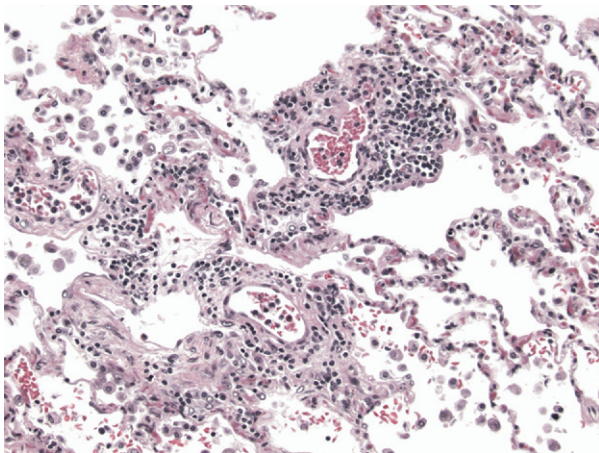


Figure 5. Mild acute cellular rejection (A2). In this example of mild acute cellular rejection, a tortuous small vessel in the lung parenchyma is cuffed by an inflammatory cell infiltrate which expands and tracks along the perivascular interstitium. The infiltrate remains associated with the perivascular interstitium without infiltration or expansion of adjacent alveolar septa by mononuclear cells. Subendothelial lymphocytic infiltration is also noted in upper right and such endothelialitis is a common finding in mild acute cellular rejection. H&E.

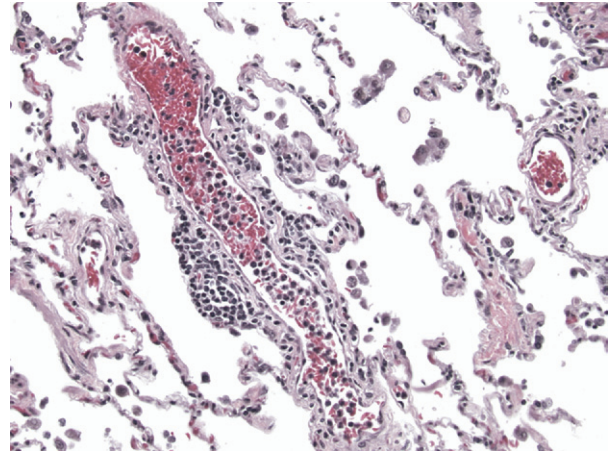


Figure 6. Borderline minimal-mild acute cellular rejection (A1-A2). In this example, a blood vessel is cut along its long axis. For the most part, the lymphoplasmacytic infiltrate is loosely distributed within the perivascular connective tissue. In only one focus is there expansion of the perivascular interstitium by the mononuclear infiltrate and therefore this case would be classified as mild acute cellular rejection even though the majority of this vessel shows minimal changes. H&E.

ciated with endothelialitis (Figures 8, 9, 10 and 11). Eosinophils and even occasional neutrophils are common. This grade is defined by the extension of the inflammatory cell infiltrate into perivascular and peribronchiolar alveolar septa and airspaces, which may be associated with collections of intra-alveolar macrophages in the zones of septal infiltration and Type 2 alveolar cell hyperplasia. The interstitial infiltration can take the form of cells percolating singly into alveolar walls or more sheet-like infiltration with corresponding expansion of the septa. There is continuity with the

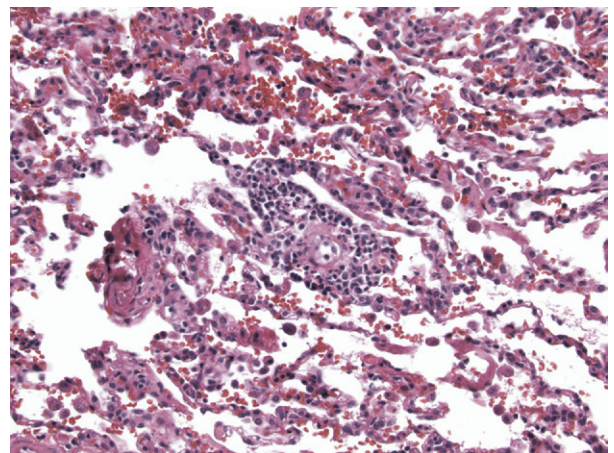


Figure 7. Borderline minimal-mild acute cellular rejection (A1-A2). This solitary perivascular infiltrate is associated with endothelial cell hyperplasia and expansion of the perivascular interstitium by mononuclear cells. The expansion, however, is rather slight and not pronounced and such a case would fall along the borderline of minimal-mild acute cellular rejection. H&E.

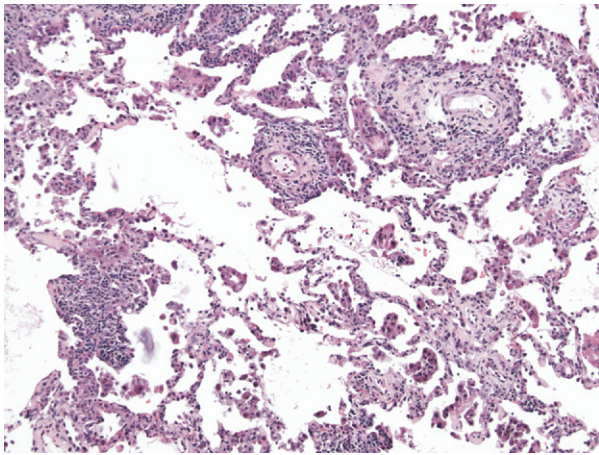


Figure 8. Moderate acute cellular rejection (A3). At scanning power, the perivascular mononuclear inflammatory infiltrate within the lung is easily identified. In addition, mononuclear cells are seen to percolate from the perivascular interstitium of small vessels into the alveolar septa where they are accompanied by alveolar pneumocyte hyperplasia. Mononuclear cells percolate into the perivascular airspaces where they are accompanied by a pronounced intraalveolar macrophage reaction. H&E.

perivascular infiltrates. True interstitial infiltration characterizing moderate acute rejection should be distinguished from the expansion of the potential space of the perivascular adventitia in mild acute rejection.

Grade A4 (Severe Acute Rejection)

In Grade A4 severe rejection there are diffuse perivascular, interstitial and air-space infiltrates of mononuclear cells with prominent alveolar pneumocyte damage and endothelialitis (Figures 12, 13 and 14). These

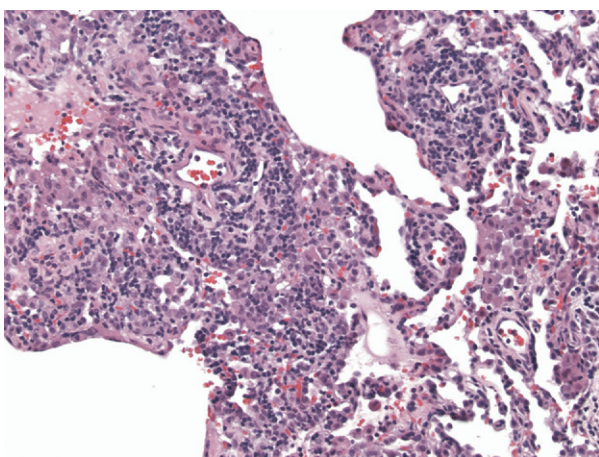


Figure 9. Moderate acute cellular rejection (A3). In this transbronchial biopsy, the lung parenchyma appears rather collapsed and atelectatic and yet, the perivascular mononuclear inflammatory infiltrate, with plasmacytoid lymphocytes and occasional eosinophils, expands the perivascular interstitium and extends into alveolar septa resulting in an "interstitial pneumonitis". Alveolar pneumocytes are prominent and endothelialitis is readily identified. H&E.

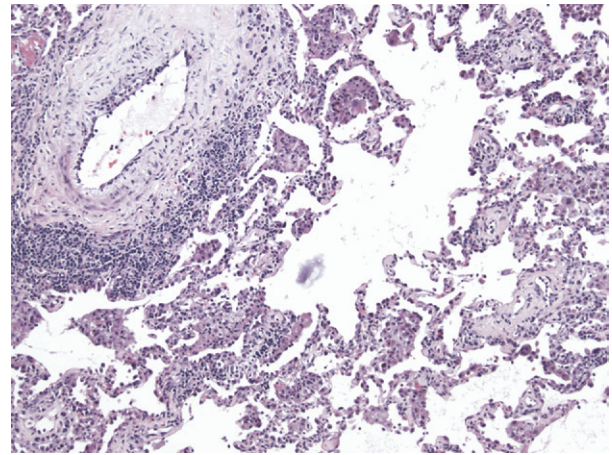


Figure 10. Moderate acute cellular rejection (A3). The characteristic feature of moderate acute cellular rejection is the expansion of the perivascular interstitium by mononuclear cells and the extension of the same cells into adjacent perivascular alveolar septa. Such cells may extend into the airspaces resulting in collections of macrophages and lymphocytes within alveoli. H&E.

may be associated with intra-alveolar necrotic epithelial cells, macrophages, hyaline membranes, hemorrhage and neutrophils. There may be associated parenchymal necrosis, infarction or necrotizing vasculitis, although these features are more evident on surgical rather than transbronchial lung biopsies. There may be a paradoxical diminution of perivascular infiltrates as cells extend into alveolar septa and spaces where they are admixed with macrophages.

Grade A4 acute rejection must be distinguished from post-transplantation acute lung injury by the presence of numerous perivascular and interstitial mononuclear cells, which are not a feature of reperfusion-related damage.

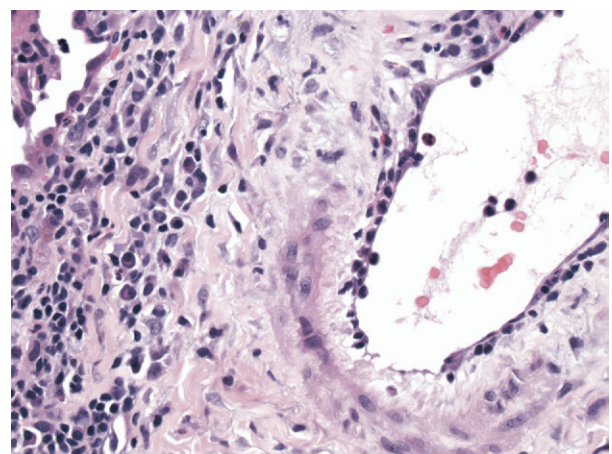


Figure 11. Moderate acute cellular rejection (A3). In almost all cases of moderate acute cellular rejection, subendothelial infiltrates of small round and plasmacytoid lymphocytes are characteristic, often accompanied by eosinophils i.e. endothelialitis or endothelialitis. H&E.

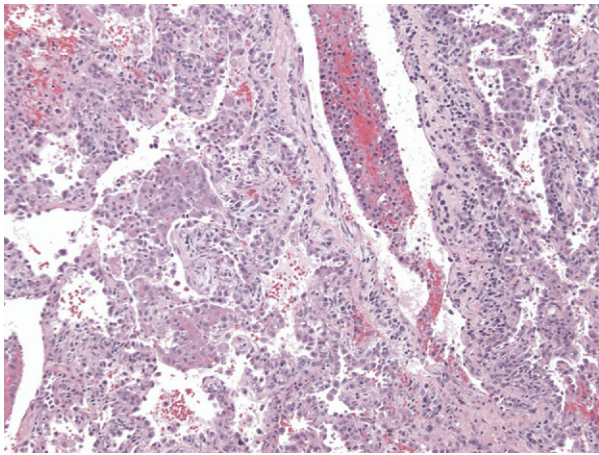


Figure 12. Severe acute cellular rejection (A4). At low magnification, the perivascular spaces and alveolar septa are expanded by a mononuclear inflammatory infiltrate, which paradoxically seems less intense than that seen in moderate acute cellular rejection. Percolation of mononuclear cells into the alveolar septa is readily identified and in severe rejection is accompanied by such pronounced alveolar pneumocyte injury that airspace fibrin and hyaline membranes form, with varying degrees of organization. This is accompanied by a nonspecific neutrophilic infiltrate. Such injury to the alveolar septa with fibrin exudation and neutrophil infiltration is characteristic of severe acute cellular rejection. Endothelialitis is almost uniformly seen in these cases. H&E.

In summary, the diagnosis of acute rejection is based on the presence of perivascular and interstitial mononuclear cell infiltrates. After much debate about the merits or otherwise of collapsing the A1 to A4 grades into fewer grades, the consensus was to retain the existing 5-point system while recognizing that, in most pathologists' experience, Grade A4 is uncommon. The nature of the tissue damage in Grade A4, however, was

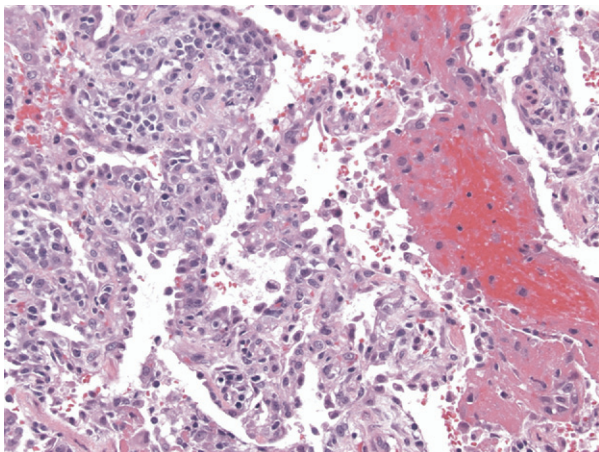


Figure 13. Severe acute cellular rejection (A4). In this example of severe acute rejection, perivascular mononuclear infiltrates are seen surrounding a small vessel at upper left and within alveolar septa. Injury to alveolar septa has resulted in hemorrhage and airspace fibrin undergoing varying degrees of organization. H&E.

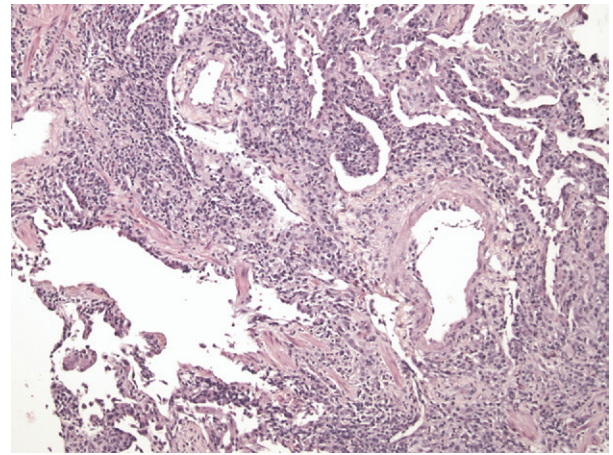


Figure 14. Severe acute cellular rejection (A4). In this example of severe acute cellular rejection two additional features are worthy of note. In addition to the perivascular and alveolar septal mononuclear infiltrate, there is significant injury to a small airway at lower left with an intense peribronchiolar mononuclear infiltrate. At upper right, airspace organization is noted with fibrin and hyaline membranes undergoing transition to granulation tissue. Such a finding can be a marker of the prior alveolar septal injury observed in moderate and severe acute rejection. H&E.

identified as having a potential relationship with an antibody-mediated form of acute rejection (see later) and therefore potentially useful in contributing to further understanding of lung rejection in the future, albeit in an infrequently diagnosed grade. The histopathology task force also recommended that perivascular infiltrates related to acute rejection should be truly circumferential and that incomplete vascular cuffing is unlikely to represent acute rejection. It is advised that further samples, deeper serials or levels into the tissue block should be obtained when the infiltrates are equivocal to discriminate both between rejection and non-rejection pathology and between the various grades of acute cellular rejection.

The participants also noted that the transbronchial biopsy diagnosis of acute rejection represents but one component of an integrated approach to the assessment of lung allograft recipients. The diagnosis of acute lung rejection therefore requires integration with clinical and particularly microbiologic data.⁸ In relation to the treatment of acute rejection the task force noted that different clinical groups have different therapeutic algorithms and that, since the 1996 working formulation, the potential long-term significance of Grade A1 minimal acute rejection has emerged.⁹⁻¹¹ It was decided to retain this minimal grade for further evaluation in light of better guidance for its recognition.

B: AIRWAY INFLAMMATION: LYMPHOCYTIC BRONCHIOLITIS

The 1996 working formulation allowed airway inflammation to be graded from B0 (no inflammation) to B4

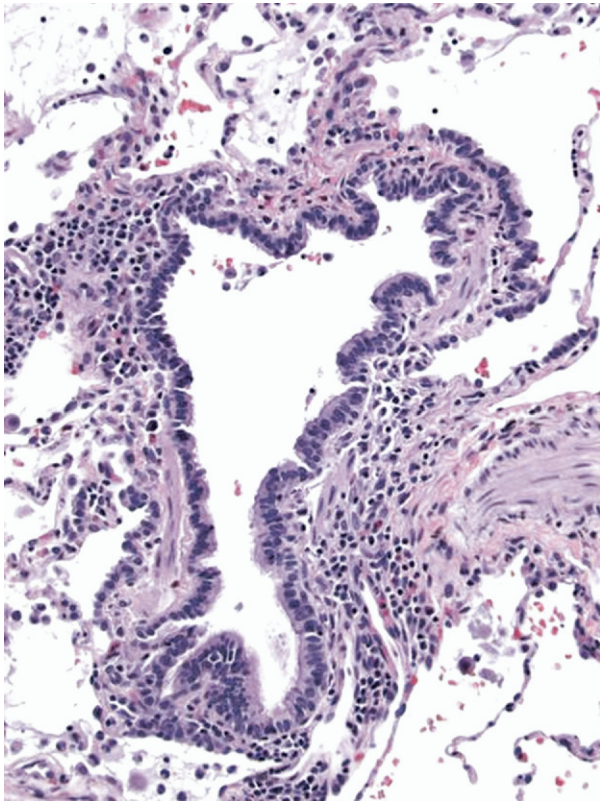


Figure 15. Low grade lymphocytic bronchiolitis (B1R). In this example the bronchiole shows a mild patchy peribronchiolar mononuclear cell infiltrate which spares the respiratory epithelium and is unassociated with epithelial injury. The infiltrate forms an incomplete circumferential band in places. There is no evidence of fibrosis in lymphocytic bronchiolitis in comparison with obliterative bronchiolitis. H&E.

(severe airway inflammation).² The earlier 1990 formulation recommended airway inflammation co-existent with Grade A acute rejection to be recorded as present or absent, but did not reflect the intensity of the inflammatory infiltrates.¹ The 1996 grading of airway inflammation was not accepted by all members of the lung rejection study group for several reasons, including the lack of convincing evidence that airway inflammation could be used solely to grade rejection because of its frequent co-existence with airway infection. Also, there are frequent problems with adequate sampling of small airways in transbronchial biopsies and with technical issues such as tangential cutting, etc. An ungradeable category was designated for those biopsies limited by sampling problems, infection, tangential cutting, etc. It was accepted that the scientific and clinical usefulness of airway inflammation grades would need revisiting over the course of time.¹² However, the format of Grades A and B in the 1996 classification emphasized the need to retain perivascular infiltrates as the primary

focus in the histologic classification of acute lung rejection.

At the 2006 consensus meeting, the majority of pathologists felt that the criteria for separating four grades of airway inflammation were poorly defined and difficult to discriminate on transbronchial biopsy. Previous studies of reproducibility of the 1996 working formulation both in terms of inter- and intra-observer variability had shown significant problems with the airway inflammation B grades in comparison to the acute rejection A grades and it was recognized that new recommendations must improve reproducibility.^{3,4,13} The revision of the B grades has collapsed the four previous grades into two and retained B0 (no airway inflammation) and BX (ungradeable for reasons just stated). The B grade designation applies only to small airways, that is, bronchioles, and the description of inflammation in cartilage-containing large airways is covered later. It is recognized that airway inflammation can be present in the absence of perivascular infiltrates and that rigorous exclusion of infection is necessary before ascribing the features to acute rejection of the airway.

Grade B0 (No Airway Inflammation)

In Grade B0 there is no evidence of bronchiolar inflammation.

Grade B1R (Low-grade Small Airway Inflammation)

In Grade B1R there are mononuclear cells within the sub-mucosa of the bronchioles, which can be infrequent and scattered or forming a circumferential band (Figures 15 and 16). Occasional eosinophils may be

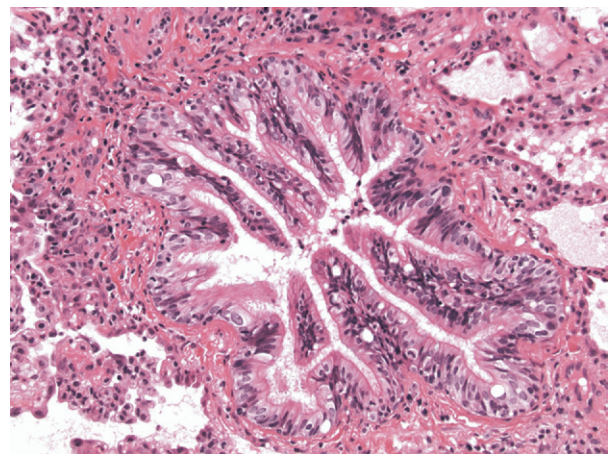


Figure 16. Low grade lymphocytic bronchiolitis (B1R). This terminal bronchiole shows epithelial hyperplasia and some epithelial undulation but is accompanied by a very sparse mononuclear inflammatory infiltrate which does not home to the basement membrane or injure the mucosal epithelium. H&E.

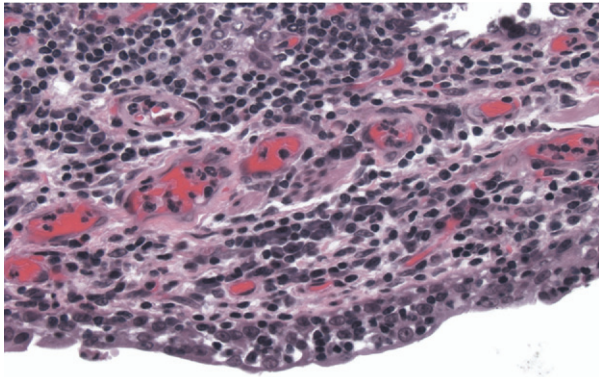


Figure 17. High grade lymphocytic bronchiolitis (B2R). In high grade lymphocytic bronchiolitis, in contrast to the low grade variant, mononuclear cells expand the submucosa and home to the epithelial basement membrane where they percolate through the basement membrane into the overlying respiratory epithelium. Epithelial cell necrosis and apoptosis is observed. H&E.

seen within the sub-mucosa. There is no evidence, however, of epithelial damage or intra-epithelial lymphocytic infiltration. This grade combines and replaces the previous B1 and B2 grades.

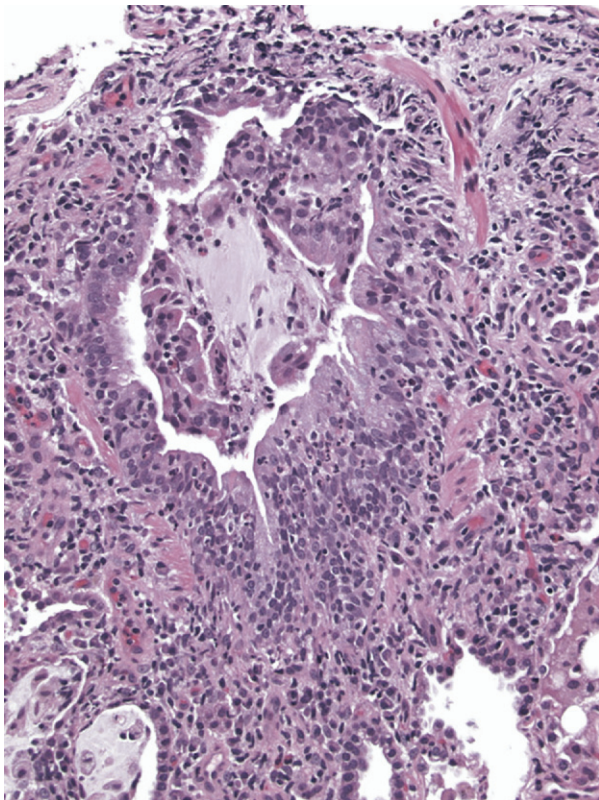


Figure 18. High grade lymphocytic bronchiolitis (B2R). This small bronchiole shows an intense mucosal and peribronchiolar mononuclear cell inflammatory infiltrate involving the epithelium with focal epithelial damage. Neutrophils are present in the epithelium and should not be confused with infectious bronchiolitis if correlation with microbiology is undertaken. H&E.

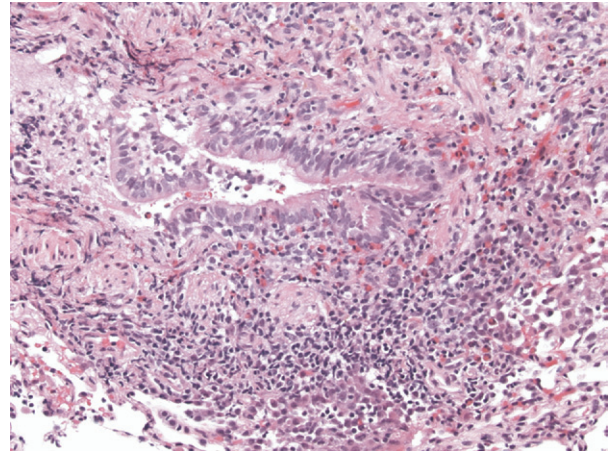


Figure 19. High grade lymphocytic bronchiolitis (B2R). In this example of a small bronchiole in a transbronchial biopsy, the mononuclear inflammatory cell infiltrate is accompanied by an intense eosinophilic infiltrate with eosinophils and lymphocytes traversing the epithelium accompanied by epithelial cell necrosis. Infection should be excluded as a cause of the eosinophilia. H&E.

Grade B2R (High-grade Small Airway Inflammation)

In Grade B2R the mononuclear cells in the sub-mucosa appear larger and activated, with greater numbers of eosinophils and plasmacytoid cells (Figures 17, 18 and 19). In addition, there is evidence of epithelial damage in the form of necrosis and metaplasia and marked intra-epithelial lymphocytic infiltration. In its most severe form, high-grade airway inflammation is associated with epithelial ulceration, fibrino-purulent exudate, cellular debris and neutrophils. The presence of a disproportionate number of neutrophils within the epithelium and sub-mucosa in relation to the numbers of sub-mucosal mononuclear cells is highly suggestive of infection rather than rejection. Any accompanying lavage or aspirate may also be purulent and/or show evidence of organisms.

Grade BX (Ungradeable Small Airways Inflammation)

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

The consensus group recommended that the diagnosis of acute rejection with co-existent airway inflammation be in the same form as the 1996 formulation—that is, acute rejection grade with airway inflammation grade. For example, moderate acute cellular rejection in which there is intense small airways inflammation would be designated moderate acute rejection, Grade A3, with airways inflammation being Grade B2R. The category of lymphocytic bronchiolitis is graded as A0, B1R or A0, with B2R depending on the severity of the airway inflammation.

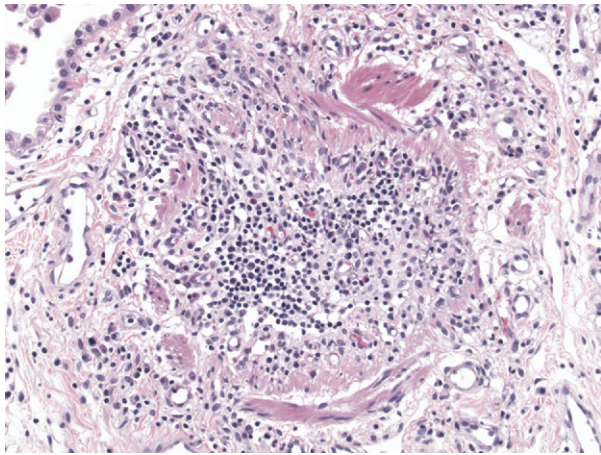


Figure 20. Obliterative bronchiolitis. In this example of obliterative bronchiolitis, the entire airway lumen has been obliterated by scar tissue and mononuclear cells, with the circumference of the small airways defined by an interrupted layer of smooth muscle bundles. H&E.

C: CHRONIC AIRWAYS REJECTION: OBLITERATIVE BRONCHIOLITIS

Obliterative bronchiolitis describes dense eosinophilic hyaline fibrosis in the sub-mucosa of membranous and respiratory bronchioles, resulting in partial or complete luminal occlusion (Figures 20, 21, 22, 23 and 24). This tissue can be concentric or eccentric and may be associated with fragmentation and destruction of the smooth muscle and elastica of the airway wall. It may extend into the peri-bronchiolar interstitium. Mucostasis and/or foamy histiocytes in the distal air spaces are commonly associated with obliterative bronchiolitis and may be observed in transbronchial biopsies in the absence of bronchiolar occlusion or any bronchiolar tissue.

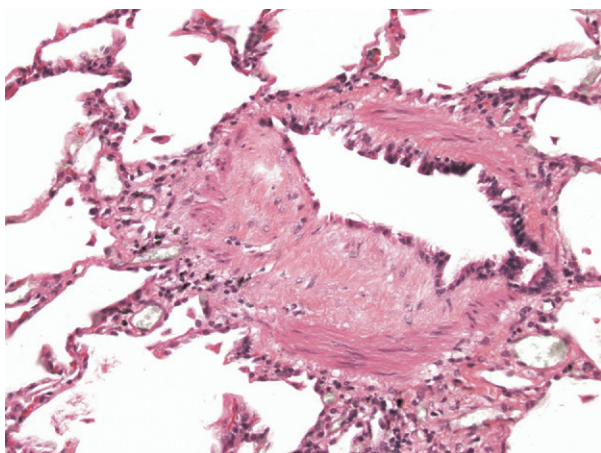


Figure 21. Obliterative bronchiolitis. This small bronchiole shows eccentric scarring of the submucosa of the small airway associated with an inconspicuous peribronchiolar mononuclear infiltrate. The overlying epithelium appears attenuated, while the lumen of the airway is distorted. Such partial occlusion of the small airways may be responsible for significant increases in airflow resistance. H&E.

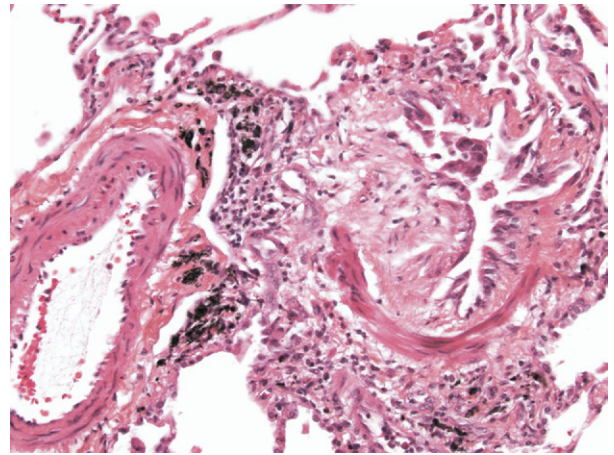


Figure 22. Obliterative bronchiolitis. In this transbronchial biopsy, an eccentric polypoid plaque of dense eosinophilic scar tissue is superimposed between attenuated respiratory epithelium and the smooth muscle wall of the airway. Such focal scarring of the airways is classified as obliterative bronchiolitis. H&E.

The 1996 working formulation concluded that the 1990 distinction between sub-total and total forms of obliterative bronchiolitis was not useful, but retained the designation of active vs inactive, depending on the presence and degree of accompanying inflammation.² The consensus in 2006 was that the distinction between active and inactive obliterative bronchiolitis is no longer useful and the condition should be designated merely as C0, indicating a biopsy with no evidence of obliterative bronchiolitis, and C1, indicating that obliterative bronchiolitis is present in the biopsy. Transbronchial biopsy is an insensitive method for detecting obliterative bronchiolitis and the clinical use of bron-

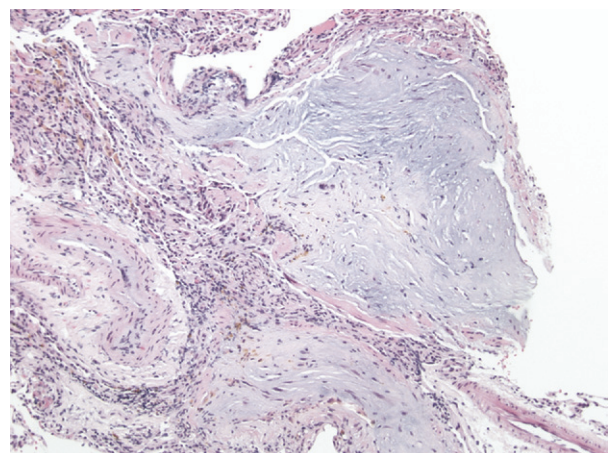


Figure 23. Obliterative bronchiolitis. In this distorted transbronchial biopsy, the scar tissue which is obliterating the airways has a loose myxoid quality but still shows dense lamellae of irreversible fibrous scar tissue in the airways. Once again the location of these scars adjacent to pulmonary arteries and the residual smooth muscle within the walls of these airways alert the pathologist to small airway disease. H&E.

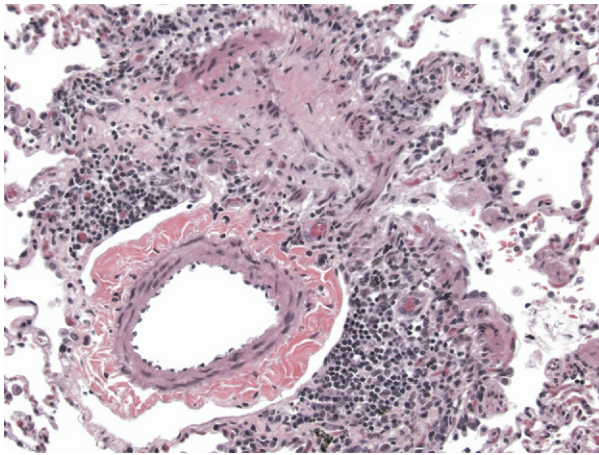


Figure 24. Obliterative bronchiolitis. The hint to underlying obliterative bronchiolitis in this case is the interrupted cords of smooth muscle forming a tubular structure associated with dense scar tissue in a position adjacent to a pulmonary artery. H&E.

chiolitis obliterans syndrome (BOS) with its functional grading is the preferred means of diagnosing and monitoring chronic airway rejection.¹⁴

D: CHRONIC VASCULAR REJECTION

In chronic vascular rejection/accelerated graft vascular sclerosis there is fibrointimal thickening of arteries and veins, which is similar to coronary artery disease in transplanted hearts (Figure 25). In the veins, the histologic appearance is usually of poorly cellular hyaline sclerosis and it is recognized that the use of older donors is associated with a higher incidence of this phlebosclerosis in biopsy material. Chronic vascular rejection is not applicable to transbronchial biopsies but may be noted on open biopsy material.

Acute Antibody-mediated (Humoral) Rejection

Acute humoral rejection is now recognized as a clinical entity in heart and renal transplants, although it remains controversial with a highly varied incidence between different centers.¹⁵⁻¹⁷ There is no consensus on its recognition and diagnosis either histopathologically or immunologically, nor on its significance and treatment. The 2004 ISHLT cardiac rejection meeting reviewed evidence from histopathology, immunopathology and clinical task forces and was able to suggest diagnostic criteria in specific clinical circumstances so that further assessment of this entity could be encouraged.⁵ Pathologists can follow the guidance in that consensus report if they intend to investigate the possibility of antibody-mediated rejection as a cause of cardiac dysfunction. Recommendations were published to allow incorporation, as required, into the revised working formulation for heart rejection. It was noted that acute antibody-mediated rejection is associated with worse graft sur-

vival and is observed in allosensitized patients, including those with previous transplantation, transfusion or pregnancy, and those with prior use of a ventricular assist device.¹⁵

The diagnosis and recognition of antibody-mediated rejection of the lung is more controversial and less well developed than for other solid-organ grafts.¹⁶⁻¹⁸ However, the presence of serum anti-HLA antibodies and the deposition of complement in alveolar tissue after transplantation suggest a role for humoral immune responses in lung transplantation.¹⁹ A significant portion of the lung consensus meeting was devoted to reviewing evidence for antibody-mediated acute lung rejection. Pulmonary transplant recipients with evidence of sensitization, as demonstrated by elevated titers of panel-reactive antibodies, have significantly more ventilator days post-operatively compared with non-sensitized patients.²⁰ Humoral immune responses are also implicated in the pathogenesis of obliterative bronchiolitis, possibly due to anti-HLA antibodies contributing to the development of scarring fibrosis via stimulation of epithelial cells within the airway.²¹

Historically, acute antibody-mediated rejection of the lung has been associated with “hyperacute rejection,” which is clinically manifested by primary graft failure occurring very early after transplantation in the setting or pre-formed antibodies to donor HLA antigens or endothelial cells.¹⁶ Morphologically, this is associated with fibrin thrombi in alveolar septa, fibrinoid necrosis of alveolar septal walls and hemorrhage. In 2006, no histologic features for antibody-mediated rejection in the lung were agreed upon. However, there was a consensus that, although pulmonary capillaritis has been described as possibly related to acute lung rejection, it is not recognized in transbronchial biopsies in

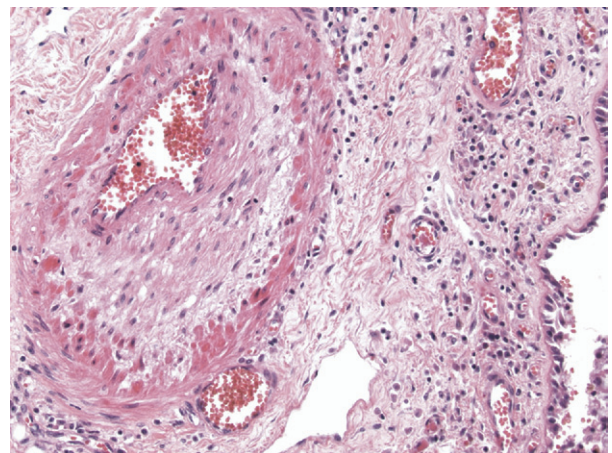


Figure 25. Graft atherosclerosis. In this example of accelerated vascular atherosclerosis due to alloreactive injury the pulmonary arteries adjacent to airways show fibro-intimal thickening of the subendothelial zones with atrophy of the media. H&E.

the majority of institutions performing pulmonary transplants and this term should not be used to indicate the histologic hallmark of antibody-mediated rejection.²² Pulmonary capillaritis should also be distinguished from neutrophil margination and congestion. It was agreed that the term capillary injury is more useful as it can indicate a morphologic spectrum of capillary damage, although it can be a non-specific finding occurring in infection, diffuse alveolar damage and severe cellular rejection.²³

Extrapolating from other solid-organ descriptions of antibody-mediated rejection, it was agreed that small vessel intimitis could raise the suspicion of humoral rejection. It was also agreed, on an empirical basis, that, should antibody-mediated rejection be suspected clinically, immunopathologically or with histologic evidence of capillary injury, immunohistochemistry could be performed on the transbronchial biopsies for C3d, C4d, CD31 and CD68. This extrapolates from experience in heart and kidney grafts. The use of broad immunofluorescence panels and electron microscopy was not recommended. It was emphasized that antibody-mediated rejection in the lung is not as well developed as an entity as in the heart and kidney and more work is required for its evaluation.

The use of agreed-upon immunohistochemical markers may prove helpful in understanding the diagnosis. The use of C4d staining in particular may allow the humoral response to a lung graft to be interpreted along the lines of the NIH recommendations from the 2003 national conference (Table 2). However, recent studies of C4d staining of pulmonary allograft biopsies have shown conflicting results with immunohistochemistry by indicating positive staining in a variable, focal, non-specific pattern without a consistent staining pattern within different diagnostic groups.²⁴ Specifically, C4d deposition has been variably demonstrated as

present or absent in the microvasculature of lung biopsies in patients with acute and chronic rejection.^{25,26} Specific immunohistochemical sub-endothelial C4d deposition has been suggested as a marker for the involvement of HLA antibodies in lung allograft rejection.¹⁹ However, the patchy nature and low sensitivity and specificity of the C4d staining suggested limited clinical use in protocol biopsies, but raised the possibility of specific C4d deposition serving as a marker of co-existent antibody-mediated rejection in patients with refractory acute cellular rejection.

No recommendations could be made on the diagnosis of concomitant acute cellular rejection and antibody-mediated rejection at this time, although it is likely to occur by extrapolation from other solid-organ grafts. The true specificity and sensitivity of a diagnosis of antibody-mediated rejection (with and without concomitant acute cellular rejection, infection or even primary graft dysfunction) requires further careful study. Caution is urged in the diagnosis of acute antibody-mediated rejection in the lung until this evidence is forthcoming and a multidisciplinary approach is again recommended in view of the wide differential diagnosis and the potential toxicity of treatments.

GENERAL RECOMMENDATIONS

Adequacy of Specimens

Transbronchial biopsy has been the mainstay of lung allograft evaluation. It was again the uniform opinion of the consensus meeting that at least five pieces of well-expanded alveolated lung parenchyma are required for an assessment of acute rejection. The bronchoscopist may need to submit more than five biopsies to provide this minimum number of adequately alveolated pieces, and possibly further biopsies if small bronchioles are required to be present. A strip of bronchus may be attached to the alveolated parenchyma and this should be distinguished from bronchiolar tissue. Specimens can be gently agitated in formalin to inflate the fragments and require tender handling in the laboratory to avoid crush artifacts that can render interpretation difficult or nearly impossible.

Histologic Examination

Histologic examination should include a minimum of sections from three levels of the paraffin block for hematoxylin and eosin (H&E) staining with connective tissue stains to evaluate any sub-mucosal fibrosis, essential for the diagnosis of bronchiolitis obliterans and arteriosclerosis. Silver stains can be performed for fungi, including pneumocystis, but have not been absolutely mandated by the group in view of the numerous microbiologic, serologic and molecular techniques presently in use for the diagnosis of opportunistic infections in these patients. Beyond this minimum H&E

Table 2. Putative Stages of Humoral Response to an Organ Graft (From Reference¹⁶)

I: Latent humoral response
Circulating antibody ^a alone (but without biopsy findings or graft dysfunction)
II: Silent humoral reaction (accommodation vs pre-rejection state)
Circulating antibody ^a + C4d deposition (but without histologic changes or graft dysfunction)
III: Sub-clinical humoral rejection ^b
Circulating antibody ^a + C4d deposition + tissue pathology (but without graft dysfunction)
IV: Humoral rejection
Circulating antibody ^a + C4d deposition + tissue pathology + graft dysfunction

^aCirculating antibody to HLA or other antigens expressed on donor endothelial cells.

^bMay differ among organs, as the ability to detect particularly mild degrees of graft dysfunction varies among organs.

and connective tissue stain work-up, investigators may wish to augment their evaluation with histochemical, immunohistochemical and in situ hybridization studies. Bronchoalveolar lavage may be performed at the time of biopsy and is useful for the exclusion of infection and for research investigations, but it has no clinical role in the diagnosis of acute rejection.

Differential Diagnosis of Perivascular and Interstitial Infiltrates

Perivascular mononuclear infiltrates are not specific for acute rejection and many other conditions may simulate or mimic alloreactive lung injury.²⁷ Differential diagnostic considerations include cytomegalovirus pneumonitis, *Pneumocystis jiroveci* (previously *P carinii*) pneumonia and post-transplantation lymphoproliferative disease, which can itself range from pneumonitis to active lymphoproliferation with tumor nodules. These conditions have been described elsewhere. Cytomegalovirus (CMV) pneumonitis often shows disproportionate alveolar septal cellular infiltrates as compared with any perivascular cuffing, and may include perivascular edema. In addition to infected cells with intranuclear and intracytoplasmic viral inclusions, the presence of abundant neutrophils with the formation of microabscesses and marked atypia of alveolar pneumocytes may also contribute to the diagnosis.

Molecular and serologic methods for monitoring and diagnosing CMV disease are also extremely helpful in suggesting the diagnosis. Transbronchial biopsy, however, remains the only standard for assessing concomitant CMV infection/pneumonitis and acute rejection. Although pneumocystis can exactly mimic acute rejection with perivascular and interstitial infiltrates, it can also manifest atypical histologic reactions, including granulomatous inflammation, diffuse alveolar damage and foci of necrosis. Granulomatous inflammation is not a feature of acute rejection and should always raise the possibility of mycobacterial or fungal, including pneumocystis, infection. Punctate zones of necrosis should also raise the possibility of mycobacteria, fungi or herpesvirus infections rather than acute rejection. Further differential diagnoses of perivascular and interstitial infiltrates include recurrent primary disease such as sarcoidosis and, in the early post-transplant period, reperfusion injury, although the latter is more often associated with neutrophils and evidence of acute lung injury.

OTHER NON-REJECTION BIOPSY FINDINGS

Aspiration

The pulmonary allograft is not protected by a cough reflex and patients are highly predisposed to recurrent aspiration. Helpful features in making this diagnosis include the identification of exogenous material with associated foreign-body giant-cell reaction within the

Table 3. Other Pathologic Features to Note in Transbronchial Biopsies

Infection
Aspiration
Organizing pneumonia
Post-transplant lymphoproliferative disorder
Large airway inflammation
Bronchus-associated lymphoid tissue
Smoker's-type respiratory bronchiolitis
Diffuse alveolar damage
Recurrent native disease
Hemosiderosis

airways and parenchyma (Table 3). Large lipid droplets and/or macrophages with large vacuoles are helpful markers of aspiration. Distal organizing pneumonia can also be seen. Since the last revision of lung rejection grading, aspiration has emerged as a significant cause of chronic allograft dysfunction, which may be ameliorated by treatment.^{28,29} It can occur early or late after transplantation and is therefore within the differential diagnosis throughout the post-operative period.

Organizing Pneumonia

Organizing pneumonia with intra-alveolar fibromyxoid tissue associated with variable interstitial inflammation is another common finding in biopsies from lung allografts.³⁰ It can occur in a variety of clinical contexts and requires microbiologic correlation where infection is suspected. Organizing pneumonia can be seen as a sub-acute form of infectious lung damage. Patchy organizing pneumonia may also represent reperfusion/ischemic injury where there may have been evidence of primary graft failure. The histologic pattern of organizing pneumonia can also be seen in association with acute rejection of Grade A3 severity and greater where there is alveolar extension of the acute inflammatory response with subsequent organization. Idiopathic/cryptogenic organizing pneumonia can also manifest identical histologic features in biopsies from a lung transplant recipient, but many other causes must be excluded before the reaction is attributed to an idiopathic origin.

Large Airway Inflammation

The importance of distinguishing large and small airways inflammation was again the subject of much discussion and dissent.^{7,31} No definite evidence was produced to support a separation of small and large airway inflammation as useful in the diagnosis of acute rejection. Large airway inflammation is most commonly associated with infection and aspiration (see earlier). Scarring can be seen in the large airway in addition to the bronchiolar scarring of bronchiolitis obliterans, but this feature is regarded as so non-specific as to not

warrant a separate comment. However, the presence of large airway scarring, like the presence of intra-alveolar, foamy macrophages, can alert the pathologist to the possibility of obliterative bronchiolitis and the need to examine further sections.

Bronchus-associated Lymphoid Tissue

Bronchus-associated lymphoid tissue consists of subepithelial mucosal lymphoid follicles that are distributed along the distal bronchi and bronchioles. It is scattered throughout the lung in adults, tending to be most prominent at the bifurcation points of airways. The lymphoid follicles contain mainly B lymphocytes and normally lack true germinal centers. These follicles are associated with specialized bronchial and bronchiolar epithelium, which is composed of modified cuboidal, non-ciliated, non-mucinous cells allowing for the trans-epithelial migration of antigens and cells.³² Attention to these histologic features and recognition of the often prominent vascularity should enable distinction to be made between bronchus-associated lymphoid tissue (BALT) and rejection-related airway inflammation.^{32,33} BALT is often well circumscribed and may contain macrophages with particulate matter. There should be no evidence of epithelial injury, neutrophils or eosinophils in a BALT collection. BALT aggregates can trail off into fibrovascular septa and should not be confused with perivascular or interstitial infiltrates.

Smokers'-type Respiratory Bronchiolitis

In respiratory (smokers') bronchiolitis, biopsies show an accumulation of tan-colored alveolar macrophages around respiratory bronchioles. Macrophages may contain flecks of brown or black material and show Prussian blue positivity. There may be associated interstitial thickening and variable accompanying chronic inflammation. There may be other features of chronic obstructive pulmonary disease with goblet-cell metaplasia, mucostasis and bronchiolar metaplasia. This appearance should be distinguished from rejection-related inflammation and BALT. The incidence of smokers'-type respiratory bronchiolitis in transbronchial biopsies from lung transplants has increased with the expansion of the donor pool to include smokers' organs. Occasionally, dust macules/nodules are seen of donor origin. The persistence of smokers' macrophages in the donor lung should not be confused with recipient smoking.

Alveolar Septal Fibrosis

Some members of the consensus group had observed fibrotic thickening of the alveolar septal walls in transbronchial biopsies from pulmonary allografts and noted the clinical entity of upper-lobe fibrosis, which has been described as a newly identified late-onset complication after lung transplantation.^{34,35} However, due to

the lack of specificity and the difficulty in interpretation of interstitial fibrosis in transbronchial biopsy specimens it was considered to be an unhelpful observation.

CONCLUSIONS

This multidisciplinary review of the classification of lung allograft rejection has taken place more than a decade since the previous revision.² There was continued support for retaining the previous acute rejection grades and for collapsing of the previous lymphocytic bronchiolitis (B) grades. The consensus group concluded that more detailed descriptions of the various grades and differential diagnoses, mainly in the form of additional photomicrographs, would enhance the usefulness of the 2006 revision and thereby improve reproducibility. The group also tackled the contentious issue of antibody-mediated rejection in the lung and reviewed the available literature. The consensus was that the available evidence supports the possibility of antibody-mediated rejection after lung transplantation but that more studies are required to determine which of the previously described pathologic lesions could be the histologic counterparts of this form of acute rejection.

Proposals for a standardized approach to investigating possible antibody-mediated rejection have been suggested to focus research endeavors in this difficult field. The consensus meeting again emphasized the importance of amalgamating the clinical, histologic, radiologic, immunologic and microbiologic data in a multidisciplinary setting to achieve the most accurate diagnosis for a particular patient episode. As always, the working formulation is regarded as a live document that will no doubt require further modification in the future with the advent of further molecular and other diagnostic refinements for the diagnosis and management of this complicated group of allograft recipients.

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APPENDIX: PARTICIPANTS BY TASK FORCE

Chair of consensus meeting: Susan Stewart, FRCPath.

Histopathology

Chair: Samuel A. Yousem, MD. *Participants:* Gerald J. Berry, MD, Margaret M. Burke, FRCPath, Michael C. Fishbein, MD, Charles C. Marboe, MD, Henry D. Tazelaar, MD.

We also acknowledge the invaluable contribution of the following international lung transplant pathologists who answered the questionnaire on the re-evaluation of the 1996 working formulation: Philip Cagle, MD, Belinda Clarke, FRCPA, Aliya Husain, MD, David Hwang, MD, Alberto Marchevsky, MD, N. Paul Otori, MD, Jon Ritter, MD, Dani S. Zander, MD.

Immunopathology

Chair: Michael C. Fishbein, MD. *Participants:* Cynthia Magro, MD, Elaine F. Reed, PhD, Nancy L. Reismoen, PhD, Adriana Zeevi, PhD.

Clinical Lung Transplantation

Chair: Gregory I. Snell, MD. *Participants:* Annette Boehler, MD, Alan Glanville, MD, F. Kate Gould, FRCPath, Keith D. McNeil, FRACP, John P. Scott, MD, Sean M. Studer, MD, John Wallwork, FRCS, Glen Westall, MD, Martin R. Zamora, MD.