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Acute Allograft Rejection: Cellular and Humoral Processes

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KEYWORDS

- Lung transplantation • Acute rejection • Humoral rejection
- Antibody-mediated rejection • Histocompatibility

Acute allograft rejection remains a prevalent and serious problem in lung transplantation, with an incidence of 36% in the first year after transplant according to the latest report from the registry of the International Society for Heart and Lung Transplantation (ISHLT).¹ Although acute lung rejection in itself is rarely fatal, its indirect consequences have considerable adverse effects on transplant outcomes. Treatment of acute rejection with increased immunosuppression increases the risk for many post-transplant infections. Furthermore, despite treatment, cellular rejection and humoral rejection constitute the major risk factors for bronchiolitis obliterans syndrome (BOS). BOS is a condition of progressive airflow obstruction thought to reflect a manifestation of chronic lung transplant rejection. Most post-transplant deaths beyond the first year occur directly or indirectly as a result of BOS.²

Compared with other solid organs, the lung appears to be at particularly high risk for rejection. Although the reasons are not entirely clear, increased lung vulnerability to early ischemic injury, recurrent infections, and constant environmental exposures might contribute to the high rates of lung rejection. In this article, the authors present the immunologic basis for acute lung allograft rejection, describing the clinical and pathologic features of acute cellular perivascular (A-grade) rejection and acute cellular airway (B-grade) rejection also known as lymphocytic bronchiolitis (**Figs. 1** and **2**). In addition, the authors discuss the emerging understanding of the importance of humoral rejection in lung transplantation, focusing on the role of anti-HLA antibodies, which can be present before or develop de novo after transplantation (see **Figs. 1** and **2**). Current strategies will be highlighted for the

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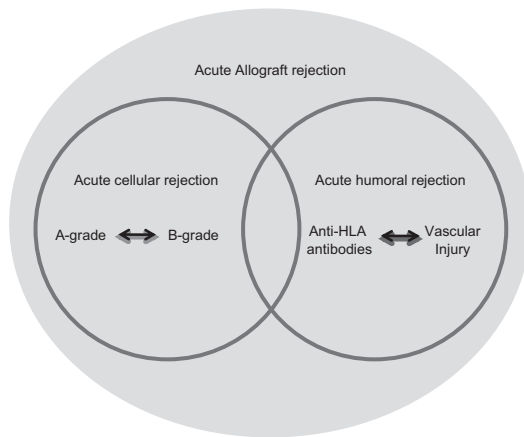


Fig. 1. Venn diagram representing the relationship between acute cellular rejection (grade A and grade B) and humoral rejection manifest by presence of anti-HLA antibodies or histologic findings.

prevention and treatment of both cellular and humoral allograft rejection.

MECHANISMS OF ACUTE REJECTION

In the absence of immunosuppression, the transplant recipient develops a robust response to the allograft, predominantly driven by T-cell recognition of foreign major histocompatibility complex (MHC) proteins, called human leukocyte antigens (HLA) in humans. Foreign MHC, expressed on transplanted tissue cells, is first presented directly to recipient T-cells by donor dendritic cells in the graft (direct pathway). As donor antigen presenting cells (APCs) die out or are destroyed, recipient dendritic cells process and present alloantigens to recipient T-cells (indirect pathway).³

HLA genes are located on the short arm of human chromosome 6 and are traditionally divided into two classes based on historic differentiation. The classical HLA class 1 genes include A, B, and Cw loci, which are expressed on most nucleated cells. The classical HLA class 2 genes include DR, DQ, and DP genes, which are expressed constitutively on B-cells, monocytes, dendritic cells, and other APCs, but can be upregulated on various other cells under inflammatory conditions. The extraordinary diversity of HLA polymorphisms creates a considerable barrier to transplantation, as the donor organ is quickly recognized as nonself on the basis of HLA differences with the recipient.³

The common pathway of acute cellular rejection involves the recruitment and activation of recipient lymphocytes (predominantly effector T-cells) to the lung allograft, which can result in allograft injury and loss of function.³ Consequently, successful outcomes after lung transplantation did not

become a possibility until the widespread introduction into clinical practice of the calcineurin inhibitor cyclosporine, which permits a highly effective blockade of T-cell activation and proliferation.^{4,5} However, in spite of intensive T-cell suppressive strategies, lung transplant patients continue to experience high rates of rejection. This process of allorecognition is likely augmented by local innate immune activation through endogenous tissue injury and exogenous infection. Innate immune activation can promote alloantigen presentation, costimulation, and T-cell activation.

Humoral responses following lung transplantation have only recently been appreciated due to the advent of modern highly sensitive solid-phase antibody detection techniques. It is now clear that some patients present for transplantation with preformed anti-HLA antibodies, which are usually acquired through prior pregnancy, transfusions, or transplantation. Immune stimulation by prior infections or autoimmunity might contribute to the development of antibodies to allo-MHC in those patients with no identifiable risk factors. These pre-existing antibodies can react with donor antigens, leading to immediate graft loss (hyperacute rejection) or accelerated humoral rejection and BOS.⁶ In addition, some lung transplant recipients appear to mount a humoral response to the allograft after transplantation. Most evidence suggests that this humoral response occurs to donor MHC antigens, although other endothelial or epithelial antigens expressed in the lung may become antibody targets as well. T-cells activated through indirect presentation provide help for B-cell memory, antibody class switching, and affinity maturation in the presence of appropriate cytokines and costimulatory factors. Acute and chronic humoral rejection have been well described in renal transplantation.⁶ Furthermore, histologic features of antibody-mediated rejection can be found on lung biopsy in the absence of measurable anti-HLA antibodies.⁷

The precise immune mechanisms and their complex interactions leading to stimulation of cellular or humoral immunity and ultimately to lung rejection remain to be fully elucidated. Nevertheless, acute cellular rejection, acute humoral rejection resulting in vascular injury, and the presence of anti-HLA antibodies are processes that overlap clinically and may potentiate each other (see **Fig. 1**).

ACUTE CELLULAR REJECTION

Clinical Presentation and Diagnosis

Acute lung allograft rejection can be asymptomatic at the time of pathologic diagnosis. When present,

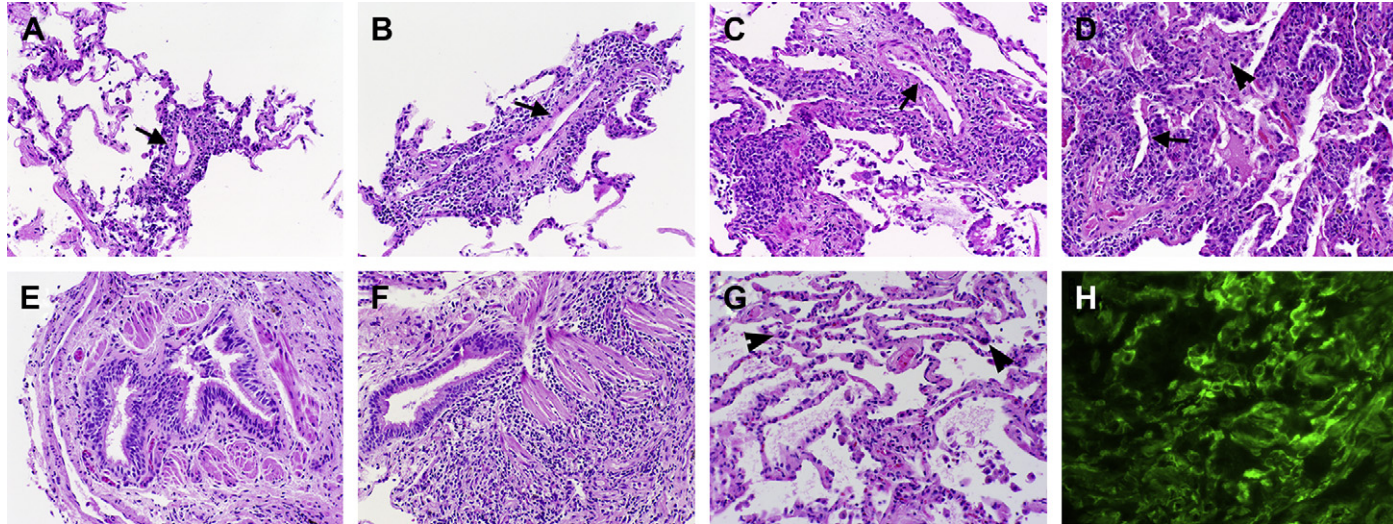


Fig. 2. Examples of acute lung allograft rejection pathology. (A–D) Grade A acute cellular rejection; arrows indicate vessel lumina. (A) Grade A1 acute rejection with rare perivascular lymphocytes, H&E. (B) Grade A2 acute rejection with a prominent perivascular mononuclear infiltrate, H&E. (C) Grade A3 acute rejection with extensive perivascular infiltrate extending into interstitial spaces, H&E. (D) Grade A4 acute rejection with a diffuse mononuclear infiltrate with lung injury, including fibrinous exudate (*arrowhead*), H&E. (E) Grade B1R (low-grade) lymphocytic bronchiolitis with small numbers of bronchiolar mononuclear cells, H&E. (F) Grade B2R (high-grade) lymphocytic bronchiolitis with dense bronchiolar mononuclear infiltrate and epithelial involvement, H&E. (G) Neutrophilic capillaritis consistent with humoral rejection (*arrowheads* indicate neutrophils), H&E, with (H) associated immunofluorescence on frozen lung tissue, demonstrating ring-shaped profiles of C4d staining in alveolar septal capillaries, Immunofluorescent staining. All images are at 200× magnification.

symptoms range from dyspnea, cough, or sputum production to acute respiratory distress, with physical findings that may include fever, hypoxia, and adventitious sounds on lung auscultation.⁸ Because of the nonspecific nature of symptoms and signs, emphasis should be placed on objective data, mainly pulmonary function testing, in identifying patients at risk for rejection. Spirometry has been found to have a sensitivity of greater than 60% for detecting infection or rejection grade A2 and higher, but it cannot differentiate between the two.⁹ Radiographic imaging of lung transplant patients is useful in identifying specific causes of symptoms or decreased pulmonary function, such as focal infections or neoplasms. Findings of ground glass opacities, septal thickening, volume loss, and pleural effusions on high-resolution chest computed tomography (CT) scans suggest acute rejection. Although early small studies attempted to demonstrate the usefulness of chest radiographs and chest CT scans in the diagnosis of rejection, more recent data show very low sensitivity for acute rejection (as low as 35%) and no discriminatory value between rejection and other processes.^{10,11} Given the poor specificity of pulmonary function tests and radiographic studies, the authors discourage empiric treatment of rejection and recommend histopathologic analysis of lung tissue to diagnose and grade acute lung rejection. The incidence of acute rejection is highest within the first year after transplant, arguing for a high clinical suspicion during this time period.

Bronchoscopy, with bronchoalveolar lavage (BAL) and transbronchial biopsies, is the most important diagnostic modality for acute allograft rejection and should be considered in any lung transplant recipient with allograft dysfunction. It allows acute rejection to be distinguished from other potential etiologies of allograft dysfunction such as airway stenosis or infection. Most transbronchial biopsies are performed in the lower lobes, a practice that seems reasonable in light of data showing that different lung lobes have similar rejection grades and that if rejection is present, the grade is usually worse in the lower lobes as compared with the upper lobes.¹² The Lung Rejection Study Group (LRSG) now recommends 5 pieces of well-expanded alveolated lung parenchyma to provide adequate sensitivity to diagnose rejection.¹³ Adverse events reported with bronchoscopy in lung transplant recipients are relatively low and include transient hypoxemia (10.5%), bleeding greater than 100 mL (4%), clinically significant pneumothorax (0.6–2.5%), arrhythmia (0.57%–4%), possibly postprocedural pneumonia (8%), and ventilation support (0.32%), but no reported mortality.^{14–17}

While there is widespread agreement on the benefit of clinically-directed bronchoscopy in lung transplantation, the role of surveillance bronchoscopy in asymptomatic patients remains disputed. Many centers perform scheduled bronchoscopies at about 1 month, 3 months, 6 months, and annually after transplant, in addition to the clinically-indicated and post-rejection follow-up bronchoscopies.¹⁸ The rationale includes the occurrence of clinically silent acute rejection, inadequate surrogate markers for acute rejection, and the relatively low risks of the bronchoscopy procedure. Grade A2 and higher acute rejection has been found in up to 18% to 39% of asymptomatic patients,^{14,19,20} with occasional presence of late-onset acute rejection beyond 1 year after transplant.²¹ Disputing this approach, one group showed that 3-year outcomes in patients who underwent only clinically-indicated bronchoscopies were comparable to outcomes in patients who underwent surveillance bronchoscopies,¹⁵ as well as to the ISHLT database outcomes.²² A randomized trial would be helpful to determine the benefit of surveillance bronchoscopies in lung transplant recipients.

In an attempt to obviate the need for surveillance biopsies, many reports have focused on less invasive surrogates of acute lung rejection. Multiple studies have assessed BAL cells and proteins as possible correlates of acute rejection, but many of these studies were small and have not been replicated.²³ Acute rejection has been associated with elevated CD8 T-cells, activated CD4 T-cells,²⁴ activated NK cells,²⁵ elevated interleukin (IL)-17,²⁶ IL-15,²⁷ and interferon-gamma in the BAL.²⁸ A pilot study of gene expression in the BAL fluid of lung transplant recipients found that gene expression signatures related to T-lymphocyte function, cytotoxic CD8 activity, and neutrophil degranulation correlate with acute rejection.²⁹ Additional studies are needed to validate these findings and establish whether BAL microarray determinations of acute rejection signature could be cost-effective and provide information that supplements or replaces biopsy results.

Even more attractive are studies of noninvasive means of diagnosing acute rejection without bronchoscopy. Although no effective serum biomarkers are currently in use in clinical lung transplant, many have been studied, and some, such as the hepatocyte growth factor, have been shown to correlate with acute rejection in small single-center studies.³⁰ In 2002, the Cylex Immune Cell Function Assay (Immuknow; Cylex, Incorporated, Columbia, MA, USA) was approved by the US Food and Drug Administration to measure global immune function in solid organ transplant

recipients. This assay measures the *in vitro* production of adenosine triphosphate (ATP) by the patient's peripheral blood CD4 T-cells in response to stimulation by phytohemagglutinin-L. Several studies in kidney, liver, heart, and small bowel allograft recipients have demonstrated that low ATP levels (≤ 225 ng/mL) correlate with infection, while high levels (≥ 525 ng/mL) are associated with rejection.³¹ Two studies that evaluated this assay in lung transplant recipients demonstrated that low ATP levels correlated with infection,^{32,33} but association with acute rejection was not assessed. Preliminary data published in abstract form showed that 87% of lung rejection episodes occurred in the setting of low-to-moderate ATP levels.³⁴ Additionally, exhaled breath analysis studies have shown some promising results. Exhaled nitric oxide (NO) has been correlated with lymphocytic bronchiolitis³⁵ and acute rejection,³⁶ and, in a study of inert gas single-breath washout, the slope of alveolar plateau for Helium (S_{He}) had a sensitivity of 68% for acute rejection.⁹ In summary, no surrogate markers

have been sufficiently validated as a means to reproducibly identify patients with acute rejection with adequate specificity, and none supplant direct histopathological examination of lung tissue. Nevertheless, further studies in this arena will likely provide valuable information about underlying mechanisms of rejection and better explain clinical heterogeneity of the disease.

Histology and Cellular Infiltration of Acute Lung Rejection

The histologic appearance of acute lung allograft rejection and the grading rules for acute cellular rejection (A-grade), airway inflammation (B-grade), chronic airway rejection or bronchiolitis obliterans (C-grade), and chronic vascular rejection or accelerated graft vascular sclerosis (D-grade) are outlined in the Working Formulation published by the Lung Rejection Study Group (LRSJ) of the ISHLT.¹³ The grading scheme and its key features are summarized in **Table 1**, and illustrative images from the authors' institution are shown in **Fig. 2**.

Table 1
Pathologic grading of lung rejection

Category of Rejection	Grade	Severity	Histologic Appearance
Grade A: acute rejection	0	None	Normal lung
	1	Minimal	Inconspicuous small mononuclear perivascular infiltrates
	2	Mild	More frequent, more obvious, perivascular infiltrates; eosinophils may be present
	3	Moderate	Dense perivascular infiltrates, extension into interstitial space, can involve endothelialitis, eosinophils, and neutrophils
	4	Severe	Diffuse perivascular, interstitial, & air-space infiltrates with lung injury. Neutrophils may be present.
Grade B: airway inflammation	0	None	No evidence of bronchiolar inflammation
	1R	Low grade	Infrequent, scattered, or single-layer mononuclear cells in bronchiolar submucosa
	2R	High grade	Larger infiltrates of larger and activated lymphocytes in bronchiolar submucosa; can involve eosinophils and plasmacytoid cells
	X	Ungradable	No bronchiolar tissue available
Grade C: chronic airway rejection—obliterative bronchiolitis	0	Absent	If present describes intraluminal airway obliteration with fibrous connective tissue
	1	Present	
Grade D: chronic vascular rejection—accelerated graft vascular sclerosis		No grading	Fibrointimal thickening of arteries and poorly cellular hyaline sclerosis of veins; usually requires open lung biopsy for diagnosis

Abbreviation: R, revised.

Data from Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007;26(12):1229–42.

The typical A-grade acute cellular rejection of the lung allograft manifests as perivascular mononuclear inflammatory cell infiltrates with or without interstitial mononuclear cells. Most of these mononuclear cells are T-cells, with a preponderance of CD8 T-cells,³⁷ although a few studies have described increased populations of B-cells or eosinophils.^{13,38,39} Increasing thickness of the mononuclear cell cuff around vessels with increasing mononuclear invasion into the interstitial and alveolar spaces determines the A-grade (see **Table 1** and **Fig. 2A–D**). While the intra-reader agreement for acute rejection has been found to be good (kappa 0.65–0.795),^{40,41} the inter-reader reliability of this grading scheme has ranged from good to suboptimal (kappa as high as 0.73 and as low as 0.47).^{40–42} Confounding features, such as concurrent infection or alveolar damage early after transplant, may additionally blur the picture and contribute to the inter-reader pathologist discordance.⁴² In general, the LRSG recommends grading rejection only after the exclusion of infection.

The B-grade airway mononuclear inflammation is clearly part of the spectrum of acute cellular rejection (see **Fig. 2E, F**), but grading remains inconsistent due to frequent lack of airway tissue on biopsies, susceptibility to tissue artifacts, and confounding by concurrent infections. Because of low inter-reader reliability of the prior 5-grade (0–4) grading schema for B-grade rejection,^{40,41} the LRSG has simplified the B-grading to 3 grades (0–2) (see **Table 1**).¹³ This nomenclature is to be used for grading noncartilaginous small airways only after rigorous exclusion of infection.

Clinical Significance of Acute Rejection

Multiple studies have demonstrated that acute rejection is the major risk factor for the development of chronic airflow obstruction: a single episode of acute rejection as well as increased frequency and severity of acute rejection increase the risk for BOS.^{2,43} An area of controversy has been the significance of minimal acute rejection (A1) or of a solitary perivascular infiltrate. In the early years of lung transplantation, A1 rejection was usually discounted and not treated. Studies have since found that minimal acute rejection (grade A1) increases the risk of subsequent higher-grade rejections (grade \geq A2)^{44,45} and of subsequent BOS⁴⁶ and that an untreated solitary perivascular monocytic infiltrate may lead to worsening acute rejection.⁴⁷ Furthermore, based on multiple studies, grade B lymphocytic bronchiolitis is now also known to be an important risk factor for BOS⁴⁸ and death, independent of acute vascular

rejection.^{2,48} Although lymphocytic inflammation is frequently seen on endobronchial biopsies of large cartilaginous airways, its clinical and prognostic significance remain unclear, and there is no demonstrated link between lymphocytic inflammation seen on endobronchial biopsies and lymphocytic bronchiolitis or bronchitis seen on transbronchial biopsies.^{49–52} Although eosinophils,⁵³ B-cells,³⁸ and mast cells⁵⁴ have been identified in acute rejection biopsies and have been correlated with worse prognosis, their exact clinical significance remains unclear.

Risk Factors for Acute Rejection

While many risk factors for acute lung allograft rejection have been studied, this article will focus on those that have been found to be significant and categorize them as allorecognition-related, immunosuppression-related, recipient-related, and infectious.

Allorecognition-related risk factors

It is generally thought that the intensity of the host alloimmune response is related to recipient recognition of differences with the donor antigens and that this process drives acute lung allograft rejection. Consistent with this idea, several single-center studies have shown that an increasing degree of HLA mismatch, especially at the HLA-DR, HLA-B, and HLA-A loci, increases the risk of acute rejection.^{55–57} Additionally, the ISHLT registry data show a correlation between HLA matching and gender-matching and 5-year survival.¹ Although not very well understood, multiorgan transplantation is generally believed to provide an immunologic advantage and lead to lower rates of rejection due to dampening of allorecognition through a high burden of foreign HLA antigens. Decreased rejection has been shown for grafted kidney, liver, and heart in combined heart–kidney, liver–kidney, and heart–lung transplant recipients,^{58,59} although this benefit does not seem to translate into prolonged graft or recipient survival. The data regarding lung rejection in the presence of a second organ remain inconclusive.^{1,59–61}

Immunosuppression-related risk factors

While it is clear that adequate immunosuppression is necessary for lung allograft maintenance, the optimal regimen has not been defined. Standard immune suppression includes a calcineurin inhibitor, a cell-cycle inhibitor, and a corticosteroid. Several studies suggest that there may be lower incidence of acute rejection with tacrolimus as opposed to cyclosporine.^{62,63} One randomized double-blind trial showed decreased rejection with everolimus as opposed to azathioprine.⁶⁴

The self-reported ISHLT registry data support the idea of decreased acute rejection episodes with tacrolimus and MMF as compared with cyclosporine and azathioprine.¹ Surprisingly very few studies have directly examined the link between levels of immunosuppression and acute rejection. High titers of Epstein-Barr virus (EBV) in peripheral blood, a surrogate marker of a high overall level of immunosuppression, have been found to correlate with lower incidence of acute rejection.⁶⁵ Furthermore, one episode of early high-grade acute rejection appears predictive of additional acute rejection episodes within the first year after lung transplant, suggesting that more aggressive immunosuppression should be used in these patients.⁴⁵

Recipient-related risk factors

Genetic polymorphisms have also been considered as potential independent risk factors for rejection.⁶⁶ A genotype leading to increased IL-10 production may protect against acute rejection,⁶⁷ while a multidrug resistance genotype (MDR1 C3435T) appears to predispose to treatment-resistant acute rejection,⁶⁸ and a copy number variation in the CCL4L chemokine gene is associated with susceptibility to acute rejection.⁶⁹ Additionally, the idea has been developed that genetic variation in innate pattern recognition receptors modulates the development of acute rejection after lung transplantation. In this regard, the authors have found reduced acute rejection in association with a variant in toll-like receptor 4 (TLR4) that blunts the innate immune response, and increased rejection with a CD14 variant that augments the innate response.^{70,71} Although these studies suggest that polymorphic variants outside of the HLA region also influence the risk for acute rejection, larger multicenter efforts are needed to fully validate these findings and also test for gene-gene and gene-environment interactions among relevant polymorphisms.

Collectively these genetic studies provide considerable support for the overall hypothesis that the constant interplay between the environment and pulmonary innate immunity modulates adaptive alloimmunity after lung transplantation. Consistent with this paradigm, gastroesophageal reflux disease also has been associated with increased rates of acute rejection.⁷² Furthermore, as will be discussed, rejection has been appreciated following a number of respiratory infections.

The effect of age on acute rejection appears to be bimodal, with lowest incidence of acute rejection in infancy (younger than age 2)⁷³ and increased risk during childhood as compared with adulthood.⁷⁴ The incidence of acute rejection

in older lung transplant recipients (age 65 or higher) appears to be similar to that of younger adults,⁷⁵ while their rate of infections appears higher, potentially contributing to increased mortality.⁷⁶ However, more studies are needed to determine the true effect of age on rejection before a strategy of reduced immunosuppression can be advocated in older recipients.

Infectious risk factors

Infectious etiologies have been given a lot of attention as potentiators of adaptive immunity in solid organ transplantation. Viral infections have long been thought to modulate the immune system and heighten alloreactivity. Indeed, a high incidence of acute rejection has been found in lung transplant recipients following community-acquired respiratory tract infections with rhinovirus, parainfluenza virus, influenza virus, human metapneumovirus, coronavirus, and respiratory syncytial virus (RSV),⁷⁷⁻⁸⁰ although respiratory viruses do not appear to be associated with acute rejection during the acute phase of infection.⁸¹ Studies on the role of other herpes viruses and polyoma viruses are being conducted, with no evidence of association with acute rejection to date.⁸²⁻⁸⁴ Studies directly linking cytomegalovirus (CMV) infection or CMV prophylaxis strategies with acute rejection have been inconsistent,² and a recent randomized trial of CMV prophylaxis did not identify a correlation between CMV incidence and acute rejection rates.⁸⁵ In one study, bacterial infection with *Chlamydia pneumoniae* was linked to the development of acute rejection and BOS.⁸⁶

Treatment of Acute Lung Rejection

Treatment of acute lung allograft rejection consists of increased immunosuppression. There has been clear consensus that grade A2 and higher-grade rejection episodes require treatment. However, in light of recent evidence that grade A1 rejection and lymphocytic bronchiolitis are major risk factors for BOS, treatment seems prudent for those entities as well. The mainstays of treatment for acute lung rejection are pulse steroids. Several studies from the 1990s showed successful resolution or improvement of acute rejection after high-dose steroid treatment.^{38,87} There are no data to clearly guide dosing of the pulse steroids; a standard dose is 500 mg of methylprednisolone intravenously,⁴ although centers use doses that range from 125 mg up to 1000 mg per day. Duration of treatment also varies but typically includes at least 3 doses, followed by an oral prednisone taper.

Response to steroids is variable, but early post-transplant rejection seems to respond better than

late rejection.⁸⁸ A major challenge in lung transplantation has been the treatment of persistent or recurrent rejection. A repeat course of corticosteroids is one option. Several studies support switching from cyclosporine to tacrolimus for treatment of persistent acute rejection.^{89,90} Many centers use alternative immunosuppressive agents such as polyclonal antithymocyte globulin (ATG), anti-IL-2 receptor (IL2R) antagonists, or muromonab-CD3 (OKT3).⁹¹ A recent report demonstrated the utility of alemtuzumab, an anti-CD52 monoclonal antibody, in the treatment of refractory acute rejection in a small cohort of patients who previously failed treatment with ATG.³⁹ Other therapies that have been considered include inhaled cyclosporine,^{92,93} extracorporeal photopheresis,⁹⁴ and total lymphoid irradiation.⁹⁵

The relationship between acute rejection, its current treatments, and the eventual occurrence of BOS is an area of considerable interest. Although acute rejection appears to be a major risk factor for BOS, it remains unclear how its treatment impacts long-term allograft function and patient survival.

HUMORAL REJECTION

Antibody-mediated allograft rejection is an increasingly recognized entity in lung transplantation. Early observations were based on the phenomenon of hyperacute rejection, where pre-existent donor-specific antibodies led to complement activation and rapid graft loss. With the advent of improved crossmatching before transplant, the incidence of hyperacute rejection in all organs has decreased. However, acute or chronic antibody-mediated lung rejection is an emerging and controversial subject. With the development of improved antibody detection and identification techniques, allograft-specific antibodies have been implicated in both acute and chronic kidney as well as heart rejection, and recent data have expanded the concept to lung transplantation.

The mechanisms by which antibody promotes lung allograft injury remain poorly understood. Antibody binding to allo-MHC or other endothelial or epithelial targets in the lung could lead to activation of the complement cascade with complement deposits leading to endothelial cell injury, production of pro-inflammatory and fibroblast-stimulating molecules, recruitment of inflammatory cells, and increased gene expression and subsequent proliferation,^{6,96} potentially contributing to the generation of obliterative airway lesions.

This section will discuss emerging issues in humoral lung rejection, including humoral sensitization both before and after lung transplantation,

as well as pathologic features of humoral rejection, which can occur with or without the presence of detectable antibodies in the serum.

Detection of anti-HLA Antibodies

The original methodology for HLA serologic typing, antibody screening and identification, and direct crossmatching was the complement-dependent cytotoxicity (CDC) assay. The assay is based on the specific reactivity between serum antibody and cell surface antigen that activates complement, causing cell death, which can be identified under the microscope using vital dyes for cell staining.

The CDC assay has now been replaced at most institutions with the more sensitive and specific solid-phase technologies that use a solid matrix coated with purified HLA antigens obtained from either cell lines or recombinant technology. These assays have the ability to detect both complement-fixing antibodies and noncomplement-fixing antibodies. Screening for antibodies is usually achieved by flow cytometry using a panel of 30 populations of beads coated with HLA antigens extracted from 30 individual donors (**Fig. 3**). This assay determines the panel reactive antibody (PRA), which is the percentage of beads or lymphocytes from the given panel that are recognized by patient's anti-HLA antibodies. Once a patient's PRA is determined to be positive, the actual HLA specificity of a recipient's anti-HLA antibodies is determined using a single antigen bead assay with beads coated with recombinant HLA single antigens.⁹⁷ The most recently developed solid-phase methodology for single-antigen detection is the Luminex single-antigen bead array assay (Luminex Corporation, Austin, TX, USA), which can simultaneously detect a maximum of 100 different colored beads in suspension with a different HLA antigen bound to each colored bead (**Fig. 4**).

In spite of these technological advances, antibodies may still be present at a level of detection below the sensitivity of the methodology or against antigens not represented by the screening reagents. However, it is believed that antibodies that remain undetected by current methods are mostly weak antibodies and may be clinically irrelevant. Nevertheless, the most definitive compatibility test remains the real-time crossmatch of the recipient serum with the potential donor cells. Flow crossmatch, whereby actual donor cells are incubated with recipient serum and bound antibodies are then tagged with secondary fluorescent anti-immunoglobulin (Ig)G antibodies, has been proven to be up to 10 to 250 times more sensitive than a CDC crossmatch.⁹⁸

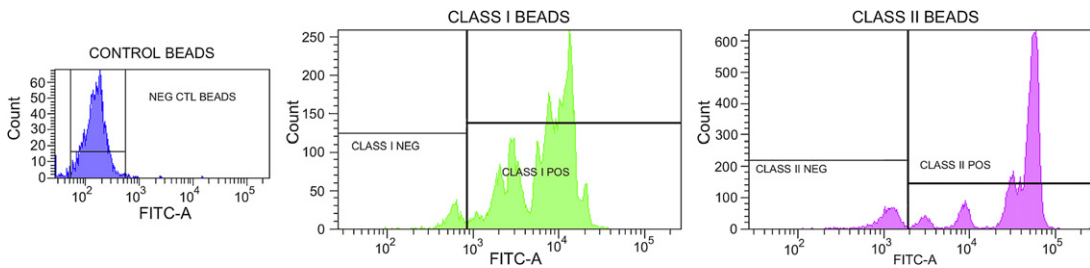


Fig. 3. Flow cytometric antibody screening for measurement of panel reactive antibody (PRA). FlowPRA beads are coated with purified HLA antigens. After incubation with patient serum and subsequent staining with FITC-labeled antihuman immunoglobulin (Ig)G, FlowPRA beads were analyzed on a flow cytometer. Beads with antibody binding have greater fluorescence intensity as represented by the rightward channel shift compared with the negative control. A percentage value of PRA is calculated based on the area of peak shifted. This patient demonstrated a PRA of 95% for HLA class 1 and a PRA of 94% for HLA class 2. The multiple peaks in the positive flow histogram are due to different bead populations emitting fluorescence of different intensity. The negative control was generated using uncoated beads. FITC, fluorescein isothiocyanate.

Pre-transplant Considerations for Sensitized Patients

One of the major goals in donor selection is to avoid HLA antigens, against which the potential recipient has preformed antibodies. About 10% to 15% of lung transplant recipients are presensitized to HLA antigens.⁹⁹ Antibody-detection technologies identify unacceptable donor antigens that should be avoided at the time of transplant. When a donor becomes available, information about the donor HLA antigens and the recipient antibodies is compared, constituting a virtual crossmatch and allowing for the real-time prospective crossmatch to be waived. This virtual cross-match approach has significantly shortened the waiting time for presensitized recipients, and correlates highly with cross-match results performed at the time of transplant.^{100,101} A high number of anti-HLA antibodies can significantly decrease the donor pool and increase waiting time for a lung transplant candidate. In these instances, interventions to remove or decrease the production of these antibodies may be considered before transplantation.

Post-transplant Considerations in Sensitized Recipients

Even though unacceptable antigens are avoided during the virtual crossmatch, patients with positive pre-transplant PRA (ie, circulating anti-HLA antibodies) are at higher risk for post-transplant complications. Their post-transplant PRA can remain stable or increase via generation of either donor-specific or nondonor-specific anti-HLA antibodies. Similarly, patients who had negative PRA screening tests before transplant can develop de novo nondonor-specific or donor-

specific anti-HLA antibodies after transplant. Using modern sensitive antibody detection techniques, recent studies have consistently demonstrated increased incidence of acute rejection,¹⁰² persistent rejection, increased BOS,¹⁰³ and worse overall survival¹⁰⁴ in patients with anti-HLA antibodies. This effect is apparent with both pre-transplant HLA sensitization as well as with the development of de novo donor-specific anti-HLA antibodies after transplantation.¹⁰³

The importance of donor specificity and target antigens in humoral rejection is not well understood. The risk of poor outcome may be heightened in the setting of donor-specific antibodies and positive retrospective crossmatches.¹⁰⁴ However, patients with positive PRA, with negative crossmatches and without specificity to mismatched donor HLA antigens also have been found to be at increased risk for poor outcome. On the one hand, nondonor-specific antibodies that are present might cross-react with the donor HLA, or antibodies specific to donor HLA might be rapidly absorbed in the lung allograft precluding their detection in the sera. Alternatively, other non-HLA antibodies could contribute to graft injury. For example, de novo autoimmunity after lung transplantation against type V collagen¹⁰⁵ and K-alpha1 tubulin expressed on airway epithelial cells have been shown to predispose to BOS.¹⁰⁶ Another study demonstrated the presence of anti-endothelial antibody directed against donor antigens in the absence of anti-HLA antibodies.⁷

It remains unclear exactly how often post-transplant PRAs should be measured and to what extent humoral rejection occurs among lung transplant recipients. Additional research is needed to more precisely define the significance

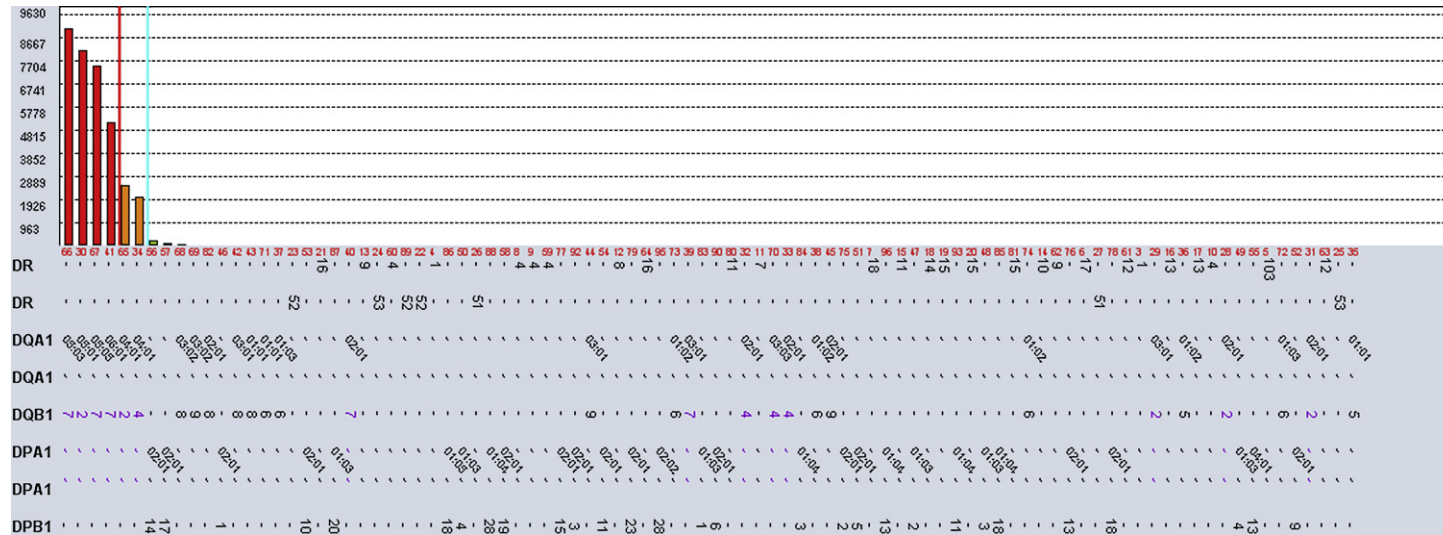


Fig. 4. Standard Luminex single antigen (SA) bead assay results for detection of specific anti-HLA antibodies. SA bead numbers are listed in red on the x-axis. Each SA bead is coated with multiple copies of a single recombinant HLA antigen. The mean fluorescence intensity (MFI), which represents the strength of antibody binding to the beads, is plotted on the y-axis: the color of the bar represents the score of the antibody reactivity strength. The specific HLA antigens tested are listed in the gray chart below the graph. For this patient, positive antibody reactivities were assigned to the 6 beads DQB1*03:01/DQA1*05:03, DQB1*02:01/DQA1*05:01, DQB1*03:01/DQA1*05:05, DQB1*03:01/DQA1*06:01, DQB1*02:01/DQA1*04:01, and DQB1*04:01/DQA1*04:01 based on the cutoff established in the laboratory. Therefore, the patient has specific antibodies against HLA DQA 1 chains encoded by DQA1 alleles DQA1*05:03, DQA1*05:01, DQA1*05:05, DQA1*06:01, and DQA1*04:01. The presence of antibodies against DQB1 chains encoded by alleles DQB1*03:01, DQB1*02:01, and DQB1*04:01 can be excluded based on the negative reactivities with other beads, which also carry the DQB1 chains/antigens encoded by these alleles.

of antibody to donor HLA, to third-party HLA, or to self-antigens after lung transplantation.

Pathologic and Clinical Patterns of Humoral Lung Rejection

Although uncommon due to the use of cross-match screening, hyperacute rejection, caused by pre-existing recipient antibodies against donor HLA antigens, has been described. Hyperacute rejection usually occurs within hours of transplantation and manifests with acute pulmonary decompensation, profound hypoxemia, diffuse pulmonary edema, and alveolar hemorrhage. Such patients may respond to aggressive antihumoral therapy, but mortality is high.¹⁰⁷

More recently, the concept of acute (distinct from hyperacute) humoral rejection, occurring later (weeks to years) in the post-transplant course, has evolved. However, the notion of a specific histopathological syndrome associated with acute humoral rejection remains controversial. Post-transplant vascular injury with pulmonary capillaritis has been described as an atypical form of rejection that may be resistant to steroids but in several cases responsive to plasmapheresis, suggesting that it may represent an antibody-mediated process. The clinical presentation of this form of pulmonary capillaritis typically includes dyspnea, hypoxemia, and pulmonary infiltrates on chest radiograph, mimicking acute cellular rejection.¹⁰⁸ Frank hemoptysis, reflecting underlying diffuse alveolar hemorrhage, has been described in a subset of recipients with antibody-mediated capillaritis and should prompt consideration of this entity.^{108,109}

More recent studies have attempted to evaluate immunoglobulin and complement deposits in the subendothelial space as possible manifestations of antibody-mediated rejection. Septal capillary deposits of immunoglobulins and complement products such as C1q, C3d, C4d (see **Fig. 2H**), and C5b-9, as well as elevation of C4d in the BAL, have been described in association with circulating anti-HLA antibodies.^{110,111} Similar pathologic findings have also been identified in the setting of treatment-resistant cellular rejection,¹¹² decreased pulmonary function tests, or BOS.^{113,114} However, other studies have not found evidence of antibody deposits or complement activation in the setting of allograft rejection or vascular injury.¹¹⁵⁻¹¹⁷ Others have demonstrated that C3d and C4d staining can occur in lung transplant patients with nonalloimmune lung injury such as infection and primary graft dysfunction with no evidence of anti-HLA antibodies, although this staining does appear to be an

independent risk factor for BOS.¹¹⁴ Differences in staining techniques between laboratories and subjective interpretation of results by pathologists may further explain some of the inconsistencies in the published data.

The LRSG report on the working formulation for the diagnosis of lung rejection remains very cautious in defining the pathologic appearance of humoral rejection. The consensus is that capillary injury can be detected on lung allograft biopsies (see **Fig. 2G**), although it can be a nonspecific finding. Findings of small vessel injury with intimitis or endothelialitis along with immunohistochemical demonstration of complement deposition should raise the suspicion for acute humoral rejection.¹³ Although such pathologic findings have been reported without evidence of circulating anti-HLA antibodies and *visa versa*, the presence in one patient of both circulating anti-HLA antibodies and characteristic pathologic findings should be seen as strong evidence for acute humoral rejection.

Prevention and Therapy for Antibody-Mediated Rejection

Plasmapheresis is the mainstay for antibody removal from the circulation and has been shown to lead to clinical improvement in lung transplant recipients with pulmonary capillaritis unresponsive to steroids.¹⁰⁸ However, it is usually reserved for severe cases of suspected humoral rejection, given its relatively invasive and cumbersome nature. Intravenous immunoglobulin (IVIG) is one of the most common therapies used to decrease antibody-mediated immunity, with a relatively low adverse effect profile. IVIG causes B-cell apoptosis, reduces B-cell numbers, blocks binding of donor-reactive antibodies, and may inhibit complement activation. The peri-transplant use of IVIG and plasmapheresis at the authors' institution in presensitized patients led to elimination of antibodies in 6 of 7 patients with class I anti-HLA antibodies and 1 of 3 patients with class II anti-HLA antibodies. As a group, those presensitized patients who received this regimen demonstrated a significant decrease in acute rejection episodes and a trend toward greater freedom from BOS compared with a cohort of presensitized patients who did not receive desensitization therapy.⁹⁹ Rituximab, an anti-CD20 monoclonal antibody that causes B-cell depletion, has been proven effective in the treatment of presensitized renal transplant recipients in conjunction with IVIG.^{6,118} In a recent study of 61 lung transplant recipients with newly acquired post-transplant donor-specific antibodies, a regimen of IVIG combined with rituximab (44 patients) or

administered alone (17 patients) led to clearing of antibodies in 62%.¹¹⁹ Notably, freedom from BOS and survival were better in the group of patients who cleared their donor-specific antibodies than those with persistent antibodies. Bortezomib, a selective inhibitor of the 26S proteasome that causes plasma cell apoptosis, is a new therapy that appears useful in the reversal of alloantibody-mediated rejection in renal transplant recipients.¹²⁰ Its use in lung transplantation has been described in one case report.¹²¹

Despite new highly sensitive measures to screen for anti-HLA antibodies and evidence that such antibodies are detrimental to the allograft, optimal monitoring, treatment parameters for humoral rejection, and the benefits of preemptive strategies to deplete these antibodies remain uncertain. Further studies are needed to determine whether IVIG, plasmapheresis, rituximab, or bortezomib alter the risk for chronic allograft dysfunction in sensitized patients.

SUMMARY

Acute cellular rejection affects greater than one-third of lung transplant recipients. Alloreactive T-lymphocytes, responding directly or indirectly to donor antigen, constitute the basis of lung allograft rejection, as diagnosed by well-established histopathological criteria that reflect the severity of perivascular or peribronchial inflammation in the lung allograft. Recent evidence supports a more complex immune response to the allograft with involvement of humoral mechanisms, characterized by circulating antibody to donor HLA and specific patterns of lung injury, occurring in parallel with T-cell-based rejection. Emerging evidence further suggests that the interaction between recipient genetics, immunosuppression therapies, and allograft environmental exposures, including pulmonary infection, contributes to high rejection rates after lung transplantation. A greater understanding of the heterogeneous mechanisms of lung rejection is critical to developing effective therapies that target the precise pathophysiology of the disease and ultimately improve long-term lung transplant outcomes.

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