Acute Antibody-mediated Rejection After Lung Transplantation

Matthew R. Morrell, MD,a G. Alexander Patterson, MD,b Elbert P. Trulock, MD,a and Ramsey R. Hachem, MDa

The role of humoral immunity after lung transplantation remains unclear. In this report, we describe the pathologic findings and clinical course of a case of acute antibody-mediated rejection (AMR) after lung transplantation. After an uncomplicated early course, a 31-year-old man with cystic fibrosis developed acute graft dysfunction 1 month after bilateral lung transplantation. Lung biopsies showed acute pneumonitis with capillary injury, neutrophilic infiltration and nuclear dust. Immunostaining for C4d demonstrated endothelial cell deposition, and circulating donor-specific human leukocyte antigen (HLA) antibodies were identified. Despite severe hypoxemic respiratory failure, he responded well to a regimen consisting of methylprednisolone, plasma exchange, intravenous immunoglobulin and rituximab therapy. He completely recovered clinically although donor-specific HLA antibodies have remained detectable. The incidence of acute AMR after lung transplantation is unknown, but this case fulfills all of the consensus diagnostic criteria, and we suggest that AMR could be an under-recognized cause of acute graft dysfunction. J Heart Lung Transplant 2009;28:96 –100. Copyright © 2009 by the International Society for Heart and Lung Transplantation.

Lung transplantation has become the standard treatment for patients with end-stage lung disease, but long-term outcomes remain disappointing. The median survival after lung transplantation is approximately 5 years, and the leading causes of death are infection and rejection.1 The principle proposed pathway for lung allograft rejection has focused on a T-cell–mediated process, manifesting as a perivascular lymphocytic infiltrate in acute rejection, and progressive fibrosis of the airways in chronic rejection, known clinically as bronchiolitis obliterans syndrome.

With the emergence of detection of antibodies to donor HLA antigens after transplantation, antibody-mediated rejection has recently surfaced as a potential form of graft dysfunction. Recently, guidelines have been published for the diagnosis and treatment of antibody-mediated rejection in solid-organ transplantation.2 This type of rejection is an accepted form of allograft failure in other solid-organ transplants; however, in lung allografts, antibody-mediated rejection remains enigmatic. We report the case of a patient who developed fulminant allograft dysfunction in the presence of newly formed HLA antibodies 1 month after lung transplantation.

CASE REPORT

A 31-year-old man underwent bilateral lung transplantation for cystic fibrosis–related bronchiectasis on January 30, 2008. Pre-transplantation sputum cultures were positive for oxacillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa. The recipient’s haplotype was HLA-A21, 29; -B8, 44; -DR7, 3; -DQ2, 2, and no HLA antibodies were detected on several screening assays (Luminex, Austin, TX) over 6 months before transplantation. After transplantation, the immediate post-operative course was uncomplicated. There was no primary graft dysfunction (PGD Grade 0), and he was weaned successfully from ventilatory support on post-operative day (POD) 1. The donor haplotype was HLA-A2, 26; -B44, -DR4, 11; -DQ7, 8, and the direct crossmatch between donor and recipient was negative. The recipient was treated with equine anti-thymocyte globulin (Pfizer, New York, NY) for induction (10 mg/kg/day for 3 doses) and tacrolimus (Astellas Pharma, Deerfield, IL), azathioprine and prednisone for maintenance immunosuppression. Post-operatively, he had a persistent pneumothorax with an air leak that required thoracotomy and creation of a pleural tent on POD 6. He was discharged from the hospital on POD 14 in good clinical condition without supplemental oxygen. At his first outpatient office visit on POD 20, he was doing well; his chest radiograph was clear (Figure 1a), and his spirometry showed a forced vital capacity of 2.92 liters (53% predicted) and a forced expiratory volume in 1 second of 2.84 liters (62% predicted).

The first surveillance bronchoscopy was performed on POD 23; transbronchial lung biopsies showed mild
acute cellular rejection with lymphocytic bronchitis (Grade A2B1R). In addition, an HLA antibody screen (Luminex, Austin, TX), which is routinely performed with surveillance bronchoscopy at our center, was positive for Class II donor-specific antibodies (DSA) to HLA-DQ7 and -DQ8. An immunostain for C4d demonstrated positive endothelial cell staining, but other histologic features of acute antibody-mediated rejection, such as capillary injury and nuclear dust, were absent. Microbiologic studies on the bronchial washings and bronchoalveolar lavage fluid were negative for bacteria, fungi and mycobacteria and *Pneumocystis jiroveci*.

Treatment for rejection was planned, but the patient developed high fever, malaise and a cough 3 days after the bronchoscopy. A follow-up chest radiograph demonstrated bilateral pulmonary infiltrates (Figure 1b). Because of a concern for superimposed pneumonia, treatment for rejection was withheld, and the patient was hospitalized for antibiotic therapy on POD 28. Over the next few days, he remained febrile and developed worsening pulmonary infiltrates with hypoxemic respiratory failure that required mechanical ventilation on POD 33 (Figure 1c).

After stabilization with ventilatory support, another bronchoscopy with transbronchial lung biopsies was performed on POD 33. The biopsies showed acute pneumonitis with fibrin deposition, intra-alveolar hemorrhage, neutrophilic interstitial infiltration and capillary injury with pericapillary neutrophils and nuclear dust (Figure 2a-c). Notably, findings of acute cellular rejection were not apparent. Immunostaining for C4d was positive for diffuse capillary endothelial cell staining (Figure 2d); a cytomegalovirus immunostain was negative. Cultures of the bronchoscopy specimens were positive for *Pseudomonas aeruginosa*, but no other potential viral, fungal or mycobacterial pathogens were identified. Overall, we interpreted these findings as acute antibody-mediated rejection.

He was treated with combination therapy consisting of methylprednisolone 1,000 mg/day intravenously for 5 days, plasma exchange on an alternate-day basis for a total of 5 treatments, low-dose intravenous immunoglobulin (IVlg 100 mg/kg) after each plasma exchange treatment with a large dose (1,000 mg/kg) after the last treatment, and a single dose (375 mg/m²) of intravenous rituximab (Genentech, San Francisco, CA) after the last plasma exchange treatment. In addition, mycophenolate mofetil (Roche Pharmaceuticals, Nutley, NJ) was substituted for azathioprine. His clinical course improved significantly during treatment. He was weaned from mechanical ventilation on POD 45 and discharged from the hospital 33 days after admission. He did not require supplemental oxygen. A
follow-up chest X-ray obtained 1 week after discharge was clear (Figure 1d).

His most recent spirometry on POD 98 showed a forced vital capacity of 4.06 liters (74% predicted) and a forced expiratory volume in 1 second of 3.66 liters (81% predicted). A subsequent transbronchial lung biopsy on POD 77 showed no evidence of cellular or antibody-mediated rejection, and an immunostain for C4d was negative. However, subsequent HLA antibody screens have remained positive for DSA, and he has been maintained on monthly infusions of IVIg (500 mg/kg).

DISCUSSION

Although diagnostic criteria and treatment regimens have been established in kidney transplantation, acute antibody-mediated rejection after lung transplantation remains a conundrum. The detection of HLA antibodies among lung transplant recipients has been recognized as a significant risk factor for persistent and high-grade acute rejection and for the development of bronchiolitis obliterans syndrome (BOS). In addition, the role of pre-formed donor-specific antibodies in hyperacute rejection after lung transplantation has been well recognized. However, acute antibody-mediated lung rejection has not been well documented. In the latest International Society for Heart and Lung Transplantation revision of the nomenclature of lung rejection, there was no consensus on the histologic hallmarks of antibody-mediated rejection.

The working group from the National Conference to Assess Antibody-Mediated Rejection in Solid Organ Transplantation proposed a general classification of humoral immune responses to facilitate research in this area. In addition, putative stages of humoral responses to allografts were proposed (Table 1). According to this proposal, the 4 necessary features of antibody-mediated rejection include: (1) detection of circulating HLA or other donor-specific antibodies; (2) evidence of C4d deposition in the allograft; (3) compatible tissue pathology; and (4) graft dysfunction. The present case meets these criteria, and it highlights the unique progression from silent humoral reaction or sub-clinical humoral rejection to overt humoral rejection. At the time of the first transbronchial lung biopsy (POD 23), the patient had donor-specific HLA antibodies, C4d deposition and

<table>
<thead>
<tr>
<th>Stage</th>
<th>Circulating antibody</th>
<th>C4d deposition</th>
<th>Tissue pathology</th>
<th>Graft dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Latent humoral response</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II: Silent humoral reaction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III: Sub-clinical humoral rejection</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>IV: Humoral rejection</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*From Takemoto et al.*

*Circulating antibody to HLA or other antigens expressed on donor endothelial cells.*
acute cellular rejection, but the histologic features of antibody-mediated rejection and clinical evidence of graft dysfunction were not present. This pattern could represent either silent humoral reaction with concomitant cellular rejection or sub-clinical humoral rejection, if cellular rejection is considered a manifestation of humoral-mediated tissue pathology. Regardless, this constellation of findings progressed to overt humoral rejection as the patient developed clinical graft dysfunction and had transbronchial biopsies that were consistent with antibody-mediated injury.

In other solid-organ allografts, a positive capillary stain for C4d has been associated with active antibody-mediated rejection. However, the significance of positive C4d staining in lung allografts has been somewhat controversial, and this has recently been linked to primary graft dysfunction and airway infection. C4d deposition in a non-specific diffuse pattern has been seen occasionally on surveillance biopsies in the absence of other features of graft dysfunction, but the clinical significance of this finding remains uncertain. However, in patients with declining lung function or refractory acute cellular rejection, a relatively higher incidence of C4d deposition has been found in a continuous sub-endothelial capillary pattern, and the specificity of a positive C4d stain is further increased by the presence of donor-specific antibodies. The transbronchial biopsies on our patient did demonstrate capillary sub-endothelial positive staining for C4d. This finding, accompanied by severe allograft dysfunction, the presence of donor-specific antibodies, and significant clinical improvement after treatment for antibody-mediated rejection, strongly supports a diagnosis of antibody-mediated rejection. In addition, subsequent surveillance biopsies lacked C4d positivity, suggesting pathologic resolution of his prior antibody-mediated rejection, which has not been documented previously in cases suggestive of antibody-mediated rejection.

The absence of cellular rejection on the second biopsy (POD 33) without any intervening treatment was somewhat unexpected. It is possible that this was due to sampling error, but the biopsies included 6 pieces of alveolated parenchyma that were sufficient for the diagnosis of antibody-mediated rejection. In addition, multiple small vessels were visible but did not demonstrate features of cellular rejection. Nonetheless, the tissue injury caused by antibody-mediated rejection could have obscured those of cellular rejection. Alternatively, the acute cellular rejection could have resolved with ongoing maintenance immunosuppression in the 10 days between the procedures.

It is noteworthy that the donor-specific antibodies have remained detectable even though the patient is doing well clinically. It is possible that the treatment regimen has reduced the DSA titer below some critical threshold; however, the antibody assay is not quantitative and this remains speculative. In addition, IVIg may have a neutralizing effect on DSA by blocking their binding to target cells and by inhibiting their ability to fix complement. This neutralizing effect is supported by the absence of C4d deposition on the follow-up lung biopsy at POD 77. Because the presence of DSA has been strongly associated with chronic graft dysfunction, we have instituted a screening protocol in our program for DSA that includes a Luminex-based assay at 1, 2, 3, 6 and 12 months after transplantation and when clinically indicated. Our treatment regimen for positive DSA screens includes a single dose of rituximab and 6 monthly doses of IVIg. However, this regimen remains empirical and we are currently evaluating its efficacy and safety.

The incidence of acute antibody-mediated rejection after lung transplantation is not known. It is possible that less severe cases may be clinically occult, similar to some cases of minimal and mild cellular rejection. This case confirms that acute antibody-mediated rejection occurs after lung transplantation, and we suggest that it should be considered in the differential diagnosis of acute graft dysfunction. Clearly, a systematic study of this complication will be necessary to better understand the role of humoral immunity after lung transplantation.

REFERENCES


