Antibody-mediated rejection in lung transplantation: Myth or reality?

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Whether antibody-mediated rejection after lung transplantation exists as an entity is debated by immunologists, histopathologists, and clinicians, without a strong consensus regarding diagnostic characteristics despite an increasing body of evidence that attests to a significant role in other solid organ transplant disciplines. Evidence for and against the protean manifestations of antibody-mediated rejection after lung transplantation is discussed, with special reference to hyperacute pulmonary allograft rejection as well as acute and chronic pulmonary allograft rejection, emphasizing the potential role of complement and antibodies to human leukocyte antigens and anti-endothelial antigens. A well-described clinical phenotype exists for hyperacute pulmonary allograft rejection with low-level evidence for efficacy of therapy with intravenous immunoglobulin, plasmapheresis, and anti-CD20 monoclonal antibodies plus supportive care, if instituted early in the evolution of the process. The clinical phenotype of acute antibody-mediated rejection is now better defined, if not widely diagnosed, and a similar treatment protocol appears effective. The role of antibody-mediated rejection in the development of chronic pulmonary allograft rejection remains an exciting area for further study based on some compelling preliminary work to date. Antibody-mediated rejection after lung transplantation remains a major area for research. In the clinical domain, experience suggests antibody-mediated rejection should be considered a potential cause of graft dysfunction, whether concomitant acute cellular rejection is diagnosed or not, and especially where resistance to corticosteroid therapy is encountered.

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The concept of antibody-mediated rejection (AMR) of the pulmonary allograft is not new. Almost 40 years ago, Veith and Hagstrom described a pattern of alveolar capillary damage thought to be due to rejection, which of late has become a key histopathologic discussion point. Hence, it is timely to examine the evidence for the existence and significance of AMR after lung transplantation. Several lines of evidence will be assessed, commencing with the rationale for considering the possibility of AMR.

Essentially, AMR is recognized to a greater or lesser degree as a significant cause of graft dysfunction in other solid organ transplants and, some would postulate, the major cause of late graft loss, especially in kidney transplants. However, the first 2 iterations of the deliberations of the Lung Rejection Study Group (LRSG) of the International Society for Heart and Lung Transplantation (ISHLT) do not describe AMR. After a vigorous debate, the 2007 revision discussed AMR in some detail, and it was agreed that although controversial, the term capillary injury should be used in preference to capillaritis, with the caveat that it can represent a morphologic spectrum from infection to severe acute cellular rejection (ACR). This usage has not yet permeated the literature, and capillaritis is still in common use. In addition, careful study of the true sensitivity and specificity of a diagnosis of AMR was advised by the LRSG, but is still awaited.

If AMR does exist, then a plausible immunologic pathogenesis should include the ability to demonstrate that an
elevated pre-formed panel reactive antibody (PRA) to potential donor human leukocyte antigens (HLA) would be associated with graft dysfunction and a survival risk. Indeed, data from the United Network for Organ Sharing (UNOS) Standard Transplant Analysis and Research files from 1987 to 2005 show that survival decreased with increasing PRA and was significant when PRA exceeded 25%. Multivariable analysis showed PRA was associated with increased 30-day (hazard risk, 2.6) and overall mortality (hazard risk, 1.3). Importantly, this effect was not seen when a cohort from 1998 to 2005 was analyzed, perhaps reflecting changes in immunosuppressive therapy.3

Hyperacute rejection

Evidence for the concept of AMR presenting with hyperacute rejection, albeit a rare phenomenon, is more compelling. In 1996 Frost et al8 described the first convincing case of clinical hyperacute rejection, which was corroborated by a positive, donor–antigen-specific immunoglobulin (Ig) G-mediated lymphocytotoxic crossmatch, and compatible histopathologic, immunofluorescent, and electron microscopic features. Features of diffuse alveolar damage, neutrophilic infiltrates, and endothelial and epithelial damage with IgG-fluorescent staining within alveolar spaces and septae were demonstrated. They suggested that lymphocytotoxic crossmatch should be performed routinely before lung transplant in all patients with a high PRA.6

Other cases followed. In the third patient, oxygen desaturation and increased airway pressure developed due to acute pulmonary edema associated with hemodynamic instability, thrombocytopenia, and coagulopathy. The retrospective crossmatch was positive, and a donor-specific IgG HLA antibody to A2 was identified. Treatment included plasmapheresis and anti-thymocyte globulin treatment as well as cyclophosphamide to decrease the antibody level and production. A repeat crossmatch showed significantly decreasing anti-donor reactivity.9

Even low titer pre-formed donor-directed antibodies may be associated with fatal early graft dysfunction after lung transplantation, especially in women.10 The fifth patient received a lung allograft after 2 negative pre-transplant PRA results, but acute pulmonary edema developed 1 hour after the vascular clamps were released. Multiple organ failure developed and the patient died after 24 hours. The repeat PRA was positive at 24%. The complement-dependent cytotoxicity crossmatch was negative, but flow cytometry was positive for both HLA-I (56%) and HLA-II (45%). Further investigation detected an anti-HLA A2 in the recipient serum, and the donor had an A2 antigen.11 The tools are important.

Acute rejection

Evidence for the role of AMR as a potential modality of acute rejection is conflicted and relies on the histopathologic definition of changes that can be ascribed to AMR vs those that are deemed compatible with ACR. Capillaritis, or more correctly, capillary injury, appears to be one of the most definitive findings suggestive of AMR in the literature, the concerns of the current LRSG notwithstanding. Of course, the level of concordance between pathologists in grading AMR is untested. In 1996 Saint Martin et al12 prospectively studied frozen sections from 55 lung transplant recipients using direct immunofluorescence for C3, IgM, and IgG antibodies and compared the results with 13 explanted lungs, 1 donor lung, and 2 controls. Of 106 samples, 89 (84%) contained alveolated lung parenchyma and arterioles or venules, but there was no demonstrable immunofluorescence in the wall of the blood vessels or in the lung parenchyma in any sample. They concluded that transbronchial biopsies and wedge biopsies provide adequate material to evaluate AMR, but despite the large population studied, the satisfactory material obtained, and the wide range of histologic diagnoses, they could not demonstrate the occurrence of AMR in the lung.12 This report sits at odds with the subsequent literature, perhaps reflecting selection of cases and immunologic tools.

In 1998 Badesch et al13 reported 5 patients with pulmonary capillaritis that had a histologic appearance distinct from typical ACR. Four patients had histologic evidence of alveolar hemorrhage, and 2 had frank hemoptysis. Three patients were fulminant, and 2 died. All were treated with augmented immunosuppressive therapy. Plasmapheresis use coincided with temporary improvement in 2 patients. Two had recurrent biopsy-proven ACR within 6 weeks of treatment, and 1 had recurrent severe pulmonary hemorrhage that abated with total lymphoid irradiation. This report suggested that pulmonary capillaritis in lung transplant recipients could be an acute, fatal illness with the potential for recurrence in survivors.13

In 2005 Astor et al,14 from the same group, reviewed 40 lung transplant recipients with biopsy-proven pulmonary capillaritis. Patients presented with a clinical syndrome characterized by dyspnea, hypoxemia, abnormal chest X-ray, and a decrease in forced expiratory volume in 1 second (FEV1). Hemoptysis was present in 25% and fulminant respiratory failure in 18%. Therapy with intravenous corticosteroids resulted in clinical improvement in 17 patients (43%). A response to plasmapheresis was seen in 12 of 18 patients (67%) refractory to corticosteroids. There were 5 deaths within 3 months of diagnosis. Of 11 lung allograft recipients who presented with capillaritis within 4 weeks after transplantation, 9 (82%) were alive at 1 year; all but 1 patient achieved expected FEV1 values. Only 3 of 21 patients (14%) who presented with capillaritis >1 month after transplant had a >20% decrease in the FEV1 after 12 months. These results suggest that post-transplant pulmonary capillaritis is a form of acute allograft rejection that is clinically and histologically distinct from typical ACR, less responsive to corticosteroid therapy, and possibly not associated with long-term adverse effects on allograft function.14
logic role in the insidious onset and progression of graft dysfunction categorized as chronic rejection, which in the lung is manifest as bronchiolitis obliterans syndrome (BOS). A number of authors have postulated that BOS is the result of humoral and cellular immune responses developed against major histocompatibility complex molecules expressed by airway epithelial cells of the lung allograft, perhaps aggravated by alloimmune-independent mechanisms such as ischemia-reperfusion and infection.18

So, how sound is the evidence? In 1986 the Stanford group19 postulated a potential role between the degree of HLA-A locus and the severity of obliterative bronchiolitis (OB), suggesting that a better match might have a salutary effect on long-term results and that OB may be at least partly a result of chronic rejection. Risk factors for OB were subsequently analyzed in a single-center population of 152 lung transplant recipients. The only significant HLA risk factor for OB was mismatch at the HLA-A locus (p = 0.01).20 In another single-center retrospective study, the number of combined HLA-A and HLA-B mismatches was strongly associated with the stage of BOS at 4 years (p < 0.01).21

These preliminary studies raised the question of mechanism (ACR vs AMR), which was explored in a further retrospective analysis that showed that the development of antibodies to HLA after transplantation correlated significantly with BOS (p = 0.02), but not survival (p = 0.33), unless they were detected within 2 years of transplant (p = 0.04).22 Similarly, the interplay of HLA mismatch and development of anti-HLA antibodies was examined in a study that showed in multivariate analysis that only HLA locus mismatch and development of anti-HLA antibodies were significant independent predictors of the development of BOS.23

It follows that a strong causal association should be able to be demonstrated from a large study population. Accordingly, the influence of HLA matching on survival and the development of rejection and OB after lung transplantation was examined using data from the UNOS/ISHLT registry. Multivariate logistic regression demonstrated that the number of mismatches at the HLA-A and HLA-DR loci predicted 1-year mortality, with odds ratios of 1.18 (p = 0.03), respectively. No significant association was found between HLA mismatching and the development of OB. The authors concluded that the number of HLA mismatches at the HLA-A and HLA-DR loci predicted 1-year mortality, and the total number of mismatches predicted 3- and 5-year mortality after lung transplantation; however, the effect of each covariate was small in this multicenter study of 3,549 patients.24

### Chronic rejection

In concert with other solid organ transplants, it remains probable that AMR is indeed a lead candidate for an etio-

### Table 1: Stages of Antibody-Mediated Rejection of the Pulmonary Allograft

<table>
<thead>
<tr>
<th>Stage of humoral rejection</th>
<th>Circulating antibody</th>
<th>Lung biopsy specimen</th>
<th>Graft dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Latent</td>
<td>Yes</td>
<td>Normal</td>
<td>No</td>
</tr>
<tr>
<td>II: Silent</td>
<td>Yes</td>
<td>C4d</td>
<td>No</td>
</tr>
<tr>
<td>III: Subclinical</td>
<td>Yes</td>
<td>C4d + tissue pathology</td>
<td>No</td>
</tr>
<tr>
<td>IV: Clinical</td>
<td>Yes</td>
<td>C4d + tissue pathology</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*After Takemoto et al.2

*Presence of circulating antibody to human leukocyte antigen or other donor antigens.

Subsequent reports warn of worse outcomes. In a 2005 report,15 2 further patients exhibited (1) donor-specific anti-HLA antibodies at multiple times after transplantation; (2) continuous, linear, and sub-endothelial C4d deposition concomitantly with detection of anti-HLA antibodies; and (3) biopsy-proven rejection that was refractory to augmented immune suppression. Pulmonary function deteriorated in both patients, ultimately ending with allograft failure. The presence of donor-specific anti-HLA antibodies in the context of vascular C4d deposition and refractory acute rejection is highly suggestive of AMR.15

The first pediatric case of post-transplant pulmonary capillaritis was reported in 2009, also by Astor et al.16 who described an 8-month-old infant with evidence of C4d deposition and B-lymphocytes in the allograft, donor anti-HLA antibodies in the serum, and a clinical and immunohistochemical response to anti-CD20 monoclonal antibody therapy. These findings strongly support the hypothesis that pulmonary capillaritis may represent a form of AMR in the lung allograft that is less common than, and clinically and histologically distinct from, typical ACR.16

Also in 2009, Morrell et al17 described the pathologic findings and clinical course of acute AMR in a 31-year-old man with cystic fibrosis. Acute graft dysfunction developed 1 month after bilateral lung transplant. Lung biopsy specimens showed acute pneumonitis with capillary injury, neutrophilic infiltration, and nuclear dust. Immunostaining for C4d demonstrated endothelial cell deposition, and circulating donor-specific HLA antibodies were identified. Despite severe hypoxemic respiratory failure, he responded well to methylprednisolone, plasma exchange, intravenous Ig, and anti-CD20 monoclonal antibody therapy. Donor-specific HLA antibodies remain detectable. This case fulfills all of the consensus diagnostic criteria (Table 1) and raises the probability that AMR could be an under-recognized cause of acute graft dysfunction.2,17

### Non-HLA antibodies

If HLA mismatch and anti-HLA antibodies demonstrate a small effect, it is possible that non-HLA antibodies may have a significant effect. An early, exciting report in 1995 by Smith et al25 from Harefield showed that anti-epithelial...
cell antibodies (AECAs) detectable in a microcytotoxicity assay (but not by Western blotting) before single-lung transplantation were associated with a decrease in 1-year graft survival of 56% for AECA-positive recipients vs 78% for AECA-negative recipients \( (p = 0.01) \). Subsequent work by Abdul-Karim et al\(^\text{26} \) revealed that AECAs were actually anti-cytomegalovirus (CMV) IgM antibodies related to infection of the cell line by CMV. No further publications on AECAs have been forthcoming.

Additional support for a potential role of non-HLA antibodies was delayed till 2002, when Magro et al\(^\text{27} \) described post-transplant septal capillary injury syndrome in 22 patients in the absence of a positive PRA. All presented with deterioration in respiratory function and all had elevated factor VIII levels that were significantly greater than pre-transplantation values \( (p < 0.03) \). Biopsy specimens were remarkable for septal capillary necrosis with significant septal capillary deposition of C1q, C3, C4d, and/or C5b-9, along with Ig, including IgG, with variable endothelial cell localization. The degree of septal capillary necrosis was significantly less in post-transplant patients who were clinically well \( (p < 0.01) \), as was the degree of C1q, C3, C4d, and C5b-9 \( (p < 0.05) \). Indirect antiendothelial cell antibody studies were positive in most patients. After plasmapheresis, follow-up biopsy specimens showed a reduction in the degree of septal capillary injury \( (p < 0.01) \) and also in the amount of C1q, C3, C4d, and C5b-9 deposition \( (p < 0.05) \). They concluded that septal capillary injury accompanied by direct and indirect immunofluorescent evidence of humoral immunity is a frequent finding on transbronchial biopsy specimens. These novel findings suggested that humoral immunity to endothelial-based alloantigen is a common occurrence in lung grafts and may be a critical factor in chronic graft dysfunction.\(^\text{27} \)

Further work by Magro et al\(^\text{28} \) in 2003 found that C4d, a stable marker of classic comple ment activation, is deposited in lung allografts and correlated with clinical rejection and parenchymal injury. They hypothesized that the antigenic target may be the endothelium in recurrent acute rejection, whereas components of the bronchial wall may be important in chronic graft dysfunction. Accordingly, Magro et al sought to establish whether there was a role for antibodies with HLA specificity in pulmonary AMR. Flow cytometric and enzyme-linked immunosorbent assays (ELISA) to assess donor-specific antigens were conducted on sera from 25 lung transplant recipients who had experienced 1 or more episodes of clinical rejection; in addition, serum samples were tested for evidence of antiendothelial cell antibody activity. Morphologically, each patient had biopsy specimens showing septal capillary injury with significant deposits of immunoreactants with microvascular localization and positive indirect immunofluorescent antiendothelial cell antibody assay. Results of PRA testing were negative, as was an ELISA-based crossmatch for donor-specific major histocompatibility complex class I/II–specific antibodies. They concluded AMR could occur in the absence of antibodies with HLA specificity and that antigenic targets may be of endothelial cell origin.\(^\text{28} \)

Also in 2003, Magro et al\(^\text{29} \) explored the hypothesis that humoral immunity was involved in the evolution of BOS. Fresh frozen tissue from 13 single-lung transplant patients was analyzed for deposition of C1q, C4d, C5b-9, and IgG, IgM, and IgA. An indirect immunofluorescent assay was also conducted with patient serum against cytospins of the pulmonary endothelium. In each case, the tissue samples showed a microvascular injury syndrome involving the bronchial wall characterized by 1 or more of hemorrhage, fibrin deposition, and endothelial cell necrosis. Other features included bronchial epithelial and chondrocyte necrosis. The end-stage lesion was a thinned bronchial epithelial lining mural fibrosis. Immunofluorescent analysis showed deposition of C1q, C3, C4d, C5b-9, and Ig in the bronchial epithelium, chondrocytes, basement membrane zone of the bronchial epithelium, and bronchial wall microvasculature. The indirect antiendothelial cell antibody assay result was positive in all tested. They concluded that AMR may be involved in the pathogenesis of BOS and that the antigenic targets included the bronchial wall microvasculature, the bronchial epithelium, and chondrocytes.\(^\text{29} \)

At the time of writing, this intriguing body of work stands alone as a potential explanation of the role of AMR in the development of BOS. Confirmation from other workers in the field is needed before general acceptance, but the pathway from myth to reality is becoming clearer.

The role of comple ment

AMR is a better-defined cause for cardiac and renal graft dysfunction, where C4d deposition, a stable component of complement activation, inversely correlates with graft survival. Magro et al\(^\text{30} \) examined biopsy specimens from 23 lung transplant recipients in an attempt to correlate clinical status and morphologic findings with the pattern of C4d deposition. In patients with symptomatic acute rejection, all specimens showed light microscopic and immunofluorescent evidence compatible with AMR. The level of C4d deposition was correlated with the degree of parenchymal injury, and the light microscopic hallmark was septal capillary necrosis. C4d deposition was more strongly associated with parenchymal injury and clinical status \( (p < 0.01) \) than C1q, C5b-9, and Ig deposition. Deposits of C4d and other immunoreactants were found in the bronchial wall of patients with BOS, but the only statistically significant finding in BOS was bronchial wall deposition of C1q. There was no association with the presence of HLA antibodies; hence, they suggested that the antigenic target resulting in complement deposition might not be histocompatibility related.\(^\text{30} \)

These findings have not been replicated, but in 2004 Miller et al\(^\text{31} \) reported a lung allograft recipient with circulating antibodies to donor HLA who responded to therapy for AMR after treatment for ACR rejection failed. Bronchoalveolar lavage fluid showed elevated C4d in the absence of infection. Analysis of bronchoalveolar lavage fluid from 25 additional lung allograft recipients showed that C4d
concentrations >100 ng/ml were correlated with anti-HLA antibodies ($p < 0.01$) but were also observed with infection and in asymptomatic patients.\textsuperscript{31}

Others found no evidence of lung deposition of C4d. In 2005 Wallace et al.\textsuperscript{32} used immunohistochemical staining techniques to determine whether there was any specific staining pattern for C4d in 68 lung allograft biopsy specimens with or without the diagnosis of acute or chronic cellular or humoral rejection. Positive staining in a variable, focal non-specific pattern was observed, and there was no consistent staining pattern within the different diagnostic groups. They concluded that C4d staining of paraffin-embedded lung allograft biopsies, using currently available techniques, does not identify acute or chronic cellular or humoral rejection in lung allograft tissue.\textsuperscript{32}

In 2006 Magro et al.\textsuperscript{33} examined the potential value of C3d deposition in lung allograft specimens using frozen and paraffin-embedded tissue. Intermediate and extensive C3d staining using immunofluorescence and immunohistochemical methodologies correlated with chronic graft dysfunction and immunofluorescence C3d deposition was associated with septal and bronchial wall fibrosis ($p < 0.01$). Weak or absent C3d staining with immunofluorescence and immunohistochemistry correlated with clinical stability ($p < 0.01$), but higher levels of C3d by immunohistochemistry were more sensitive for BOS than C3d levels by immunofluorescence.\textsuperscript{33}

Further support for a potential role of complement deposition was provided in 2008 by Westall et al.,\textsuperscript{34} who described a retrospective analysis of allograft C3d and C4d deposition performed by an experienced histopathologist (Magro) blinded to clinical outcomes. Biopsy specimens were graded 0 to 3 based on the extent of septal capillary complement staining. They concluded that complement activation, as judged by lung allograft deposition of C3d/C4d, is common early after lung transplantation and may be triggered by primary graft dysfunction or airway infection, or both, and may be involved in the development of early BOS.\textsuperscript{34}

**Conclusion**

Although some questions are unanswered regarding the true incidence, severity, and significance of AMR after lung transplantation, biologically plausible clinical phenotypes have been described for hyperacute and acute AMR. Other causes of acute lung injury such as aspiration pneumonitis and hemorrhagic viral infection must be excluded in the acute circumstance, but prompt action to manage potentially life-threatening graft dysfunction is critical for the practitioner, who must be guided by the practical realities of time constraints and available laboratory expertise. Furthermore, the relationship among AMR, ACR, and graft dysfunction is likely a dynamic one and is perhaps best illustrated in a simple Venn diagram (Figure 1). Multiple insults may cause and perpetuate graft dysfunction, but not all are immunologic. Conversely, not all rejection events cause detectable graft dysfunction. Some do, and it is likely that more severe ACR events are temporally associated with AMR.

This relationship may change with the passage of time after transplantation, with AMR assuming a more important, albeit insidious, role as the cause of airflow limitation that manifests as BOS and leads to graft loss. In this regard, the specter of AMR will continue to haunt the seemingly well lung transplant patient who remains at risk of a subtle but progressive loss of graft function, newer diagnostic, and therapeutic modalities notwithstanding. The challenge remains to demonstrate the efficacy of a prospective management strategy to prevent and treat the protean manifestations of AMR after lung transplantation.

**Disclosure statement**

Allan R. Glanville is the global principal investigator for the CeMyLungs Investigator Driven study, for which his institution will receive grant support to conduct the study from Novartis Pharmaceuticals (not to exceed €2.4 million).

**References**


