Antibody-Mediated Rejection in Lung Transplantation: Fable, Spin, or Fact?

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The lung transplant community continues to struggle with the diagnosis and management of antibody-mediated rejection. The four diagnostic tenets of donor-specific antibodies, C4d staining, histopathologic changes, and allograft dysfunction, which were largely derived from the early Banff meetings on renal transplantation, have somewhat arbitrarily been applied to lung transplantation. With the passage of time, it is increasingly apparent that merits of these diagnostic pillars are less robust in lung transplantation. In this article, we summarize some of the controversies and challenges surrounding the diagnosis of antibody-mediated rejection in lung transplantation.

Keywords: Lung transplantation, Antibody-mediated rejection, Humoral rejection, Chronic lung allograft syndrome.

(*Transplantation* 2014;98: 927–930)

Centuries ago, a Hans Christian Andersen fairy tale, the "Emperor's New Clothes," described the disconnection between what we think we know and what we actually know. More recently, former U.S. Secretary of Defence, Donald Rumsfeld, applied a contemporary spin describing "known knowns," known unknowns" and the "unknowns unknowns" (*1*). Both are apposite when we consider antibody-mediated rejection (AMR) in lung transplantation. In this article, we propose that an over-reliance on accepting diagnostic and therapeutic paradigms from the nonpulmonary solid organ transplant setting has muddied our thinking on how we should approach AMR in lung transplantation. In particular, the apparently rock-solid diagnostic tenets inherent to the Banff classification of AMR in renal transplantation (Table 1) may not be directly applicable to the lung allograft (*2*).

The humoral theory of rejection, put simply, states that alloantibodies against donor-specific human leukocyte antigen (HLA) drives complement activation, and the resulting tissue deposition of C4d confirms the diagnosis of AMR. Acute and chronic AMRs are clearly vascular phenomena in renal transplantation, yet many of the truly problematic chronic lung allograft dysfunction syndromes are airway and parenchymally based, without obvious vascular explanations or links (*3, 4*). To better understand AMR, we need to dissect the pathways through which immune cells are switched on to produce antibodies specifically targeted against lung epitopes, resulting in tissue damage and impaired pulmonary function. Only through an improved understanding of the immunobiology of lung AMR can we better apply our diagnostic tools with a view to delivering appropriately targeted therapeutics. We cannot simply accept that lung AMR must equal kidney AMR. It is time to take a critical view of the evidence behind the definitions of lung AMR.

DEFINING THE ANTIGENIC TARGET WITHIN THE LUNG ALLOGRAFT

After transplantation, the recipient's immune response is largely targeted against nonself major histocompatibility complex antigens, encoded by the HLA class I and class II genes, present within the lung allograft (*5*). The presence of anti-HLA donor-specific antibodies (DSA), produced by B cell or plasma cells, has been associated with both acute and chronic allograft dysfunctions (*6–11*), although the evidence in lung transplantation is not as robust as that in renal and cardiac transplantation (*12, 13*).

The alloreactive antibody response is determined not only by the antigenic load, or HLA expression, within the lung allograft, both with regard to initial B-cell priming but also subsequent complexing of antibody with antigen. Historical immunology dogma states that although HLA class I expression is universal, HLA class II expression is more selective and may not actually be present within normal lung tissue, although may be upregulated in lung injury (*14*) or in allografts with bronchiolitis obliterans syndrome (*15*). The presence of DSA is not sufficient to automatically imply injury to the allograft. In contrast, DSA may not be detectable within the blood during acute episodes of AMR, if the DSA is completely absorbed within the lung allograft complexed to its cognate ligand. Further studies are required to confirm: (i)

The authors declare no funding or conflicts of interest.

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Received 8 April 2014. Revision requested 2 May 2014.
Accepted 8 July 2014.

ISSN: 0041-1337/14/9809-927
DOI: 10.1097/TP.0000000000000392

TABLE 1. Proposed stages of antibody-mediated rejection in renal transplantation

Stage	Term	Diagnostic criteria
I	Latent AMR	Circulating DSA only (no histologic changes or graft dysfunction)
II	Silent AMR	Circulating DSA and immunologic evidence of complement activation (C4d positive)
III	Subclinical AMR	Circulating DSA, immunologic evidence of complement activation, and histologic pathology
IV	Clinical AMR	Circulating DSA, immunologic evidence of complement activation, histologic pathology and graft dysfunction

Modified from Takemoto et al ((*2*)).
AMR, antibody-mediated rejection; DSA, donor-specific antibodies.

the expression, dose, and distribution of nonself HLA within the lung allograft and (ii) the threshold with regard to both DSA titre and the stoichiometry between antigen and antibody that will "switch-on" the immune response against the lung allograft.

DEFINING DONOR-SPECIFIC ANTIBODIES

In 2014, DSA has come to be defined as the output of Luminex antigen bead testing with the mean fluorescence intensity (MFI) used as a marker of the "strength" of reactivity of immunoglobulin G antibodies. However, results actually require significant interpretation. Recipient variables, such as timing of the specimen relative to any clinical events, presence of immunoglobulin M inhibitors, and the presence or history of other biologic agents, such as blood transfusion, antithymocyte globulin, intravenous gamma globulin, and monoclonal antibodies, will alter results (*16*). Laboratory variables, such as time, dilution, batch, and the exact beads tested, will also affect results. All beads are not the same with HLA C, DQ, DP reading higher than A, B, and DR because of the differences in antigen density. Further, the test kits typically only include the 99 most common antigens, and the beads are saturated at approximately 20,000 MFI. Every article on the subject is different—the exact timing, technique, and chosen panel of classes 1 and 2 DSA, cutoffs to define positivity and thresholds for subsequent therapy are indeed arbitrary (*16*). Clinicians attempt to make decisions using Rumsfeld's "unknown, knowns."

Additionally, although the evolving development of C1q assays may yet prove helpful in defining clinically relevant DSA (*17*), discussions on AMR must also move beyond HLA as the only target. Although HLA antigens are the major transplant antigens, increasing attention is focussing on the role of other donor antigens in provoking antibody responses against the transplanted organ. These include autoantigens, such as collagen, vimentin, angiotensin-receptor and alpha-tubulin, minor histocompatibility antigens (MICA/MICB), as well antigens that are yet to be defined (*18*)—"unknown, unknowns."

DEFINING THE HISTOLOGIC FEATURES OF AMR

Unlike the situation in renal and cardiac transplantations, the histologic features of AMR in the lung currently defy accurate characterisation. The histopathologic criteria of lung AMR were not addressed in the 1990 and 1996 ISHLT classification of lung allograft rejection and were not clearly established in the 2007 revision of the same document. The 2012 update from the Pathology Council (*19*) describes "nonspecific patterns of injury that can be seen also in disorders such as severe acute cellular rejection, infection (especially bacterial and viral), graft preservation injury and drug reactions." The list of associated histopathologic patterns seen with AMR is sufficiently broad to suggest that unless the lung biopsy is normal, then AMR should be considered. If you also consider issues regarding sampling errors and that AMR can be a pauci-immune condition, whereby the complement system is activated but not driving histologic change, then even a normal lung biopsy does not rule out potentially graft-damaging AMR.

DEFINING C4D AS A BIOMARKER FOR AMR

Does immunohistochemical analysis of C4d staining add sensitivity or specificity toward a diagnosis of AMR? This diagnostic paradigm is based on the presumption that circulating antibodies recognize their cogent antigen within the transplanted organ. The surface bound antibody binds C1q, the first component of the classic pathway of the complement system, activating the complement cascade, resulting in recruitment of inflammatory cells and graft dysfunction. C4d is a complement protein that is cleaved during complement activation and binds covalently to target tissue, and its immunohistochemical detection is a tenet on which the diagnosis of AMR is found.

This reliance on using C4d as a diagnostic marker for AMR in general, and in particular in the lung allograft requires further interrogation when the following factors are considered: (i) the classic pathway of the complement system can also be activated by surface components of gram-positive bacteria, as well as by C-reactive phase protein; features more likely to be present in the transplanted lung, an organ prone to infection and inflammation (*20*); (ii) the mannose-binding lectin pathway also drives complement activation, is not dependent on the presence of alloreactive antibody, and becomes operational after lung transplantation (*21*); (iii) complement-independent antibody-mediated mechanisms may contribute to graft pathology. These pathways include endothelial and epithelial cell activations, the latter potentially being a precursor to the development of obliterative bronchiolitis (*22*). Antibodies can also lyse target cells through the low-affinity Fc receptor on the surface of natural killer cells and macrophage, a process known as antibody-dependent cell-mediated cytotoxicity; (iv) accommodation, defined as graft resistance to the pathogenic effects of alloreactive antibodies and complement fixation, may predominant despite positive C4d staining (*23*); and (v) C4d staining in the lung in often nonspecific (*24*) and nonreproducible (*25*).

DEFINING CLINICAL AMR

The lung transplant recipient presenting with an otherwise unexplained drop in lung function, anti-HLA DSA, a neutrophilic capillaritis and positive C4d staining, fulfils the Banff criteria, and is highly likely to have AMR as the cause for graft dysfunction. In such cases, a diagnosis of "Probable

AMR" is suggested (Table 2). In our clinical experience, this scenario is uncommon. Partially related to the factors discussed above, the more common clinical presentation is of a patient with an unexplained drop in pulmonary function and DSA. In these cases, lung biopsy does not suggest an alternative diagnosis, yet also does not demonstrate features that are said to represent AMR. The absence of confirmatory histology should not automatically rule out the diagnosis of AMR, and the term "Possible AMR" is appropriate. Of note, the Luminex screen for the presence of DSA may be falsely negative if the antibody is completely adsorbed within the allograft. Future epidemiologic, diagnostic, and interventional studies of AMR in lung transplantation should include patients with both "probable" and "possible" AMR.

THERAPIES

The AMR therapies are numerous, but essentially only the subject of uncontrolled case series in the setting of lung transplantation. Solid clinical evidence of efficacy is not available—particularly in terms of long-term graft outcomes. Reported treatment approaches typically involve a combination of intravenous methylprednisolone, intravenous immunoglobulin, Rituximab, and plasma exchange (*4, 6, 7, 11, 26–28*). Clinical responses and impact on DSA are variable, with a suggestion that if treatment does not reduce the MFI of the DSA, then clinical resolution is less likely (*7*). Mycophenolate mofetil, antithymocyte globulin, intravenous immunoglobulin, Rituximab, Bortezomib, Eculizumab, and plasma exchange are complex, expensive medicines or procedures with significant associated morbidity—particularly when used in combination (*29, 30*).

FUTURE DIRECTIONS

The horse must come before the cart. Before any discussion regarding the treatment and management of AMR, the lung transplant community needs to develop a robust and universally agreed definition of AMR in lung transplantation. Such a definition must incorporate some degree of recognition of the following factors:

- the role of both HLA and non-HLA DSA;
- complement-dependant and complement-independent pathways of antibody- associated immune activation;
- current limitations regarding the use of solid phase assays, such as Luminex, in defining anti-HLA and other DSA;
- pathologic correlates of AMR, with less reliance on C4d staining as a major determinant for the diagnosis of AMR;
- the presence of both clinical and subclinical phenotypes of AMR;
- long-term complications related to DSA and their association with the different chronic lung allograft dysfunction phenotypes, for example, bronchiolitis obliterans syndrome and the restrictive allograft syndrome.

Importantly, we need further epidemiologic and observational studies that describe the impact and clinical course of patients sensitized with DSA before transplantation as well as those who develop de novo DSA after lung transplantation.

TABLE 2. Proposed stages of acute antibody-mediated rejection in lung transplantation

Stage	Term	Diagnostic criteria
I	Possible AMR	Circulating DSA and graft dysfunction
II	Probable AMR	Circulating DSA, C4d positive histologic pathology (including noninfection related interstitial neutrophilia) and graft dysfunction

AMR, antibody-mediated rejection; DSA, donor-specific antibodies.

CONCLUSION

Let us be clear—the emperor's shiny expensive lung transplant AMR clothes are not actually real to a discerning practicing lung transplant clinician. The renal transplant paradigm of Takemoto's has not proven itself to be easy to apply to lung transplant, with the expensive suggested diagnostic and therapeutic strategies falling well short in terms of useful clinical outcomes. To date we have certainly not remotely seen value for clinical dollars invested in our AMR "wardrobe." In lung transplant AMR, little is known and much remains unknown.

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