Donor-Specific HLA Antibody IgG Subclasses Are Associated with Phenotypes of Antibody-Mediated Rejection in Sensitized Renal Allograft Recipients

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Donor-specific anti–histocompatibility leukocyte antigen antibodies (DSAs) have a strong and frequently considered universally deleterious effect on allografts. Preformed or de novo DSAs activate complement, induce endothelial cell proliferation, and mediate antibody-dependent cellular cytotoxicity (ADCC), resulting in progressive declines in allograft function and loss. More than 5000 renal allografts are lost each year in the United States, the majority to antibody-mediated injury.4 Thus, understanding the pathophysiology of DSA-induced antibody-mediated rejection (ABMR) and B cell activation is critical to improving the longevity of existing allografts and development of successful strategies for desensitization to prevent ABMR. With this understanding, new drugs can be developed to address relevant pathways. In addition, delineating the characteristics of DSAs aiming at a better understanding of how current assays predict antibody strength and pathogenicity would be of significance. Two of the most currently pressing unmet needs in transplant medicine involve understanding the characteristics of DSAs that confer pathogenesis and subsequently developing novel therapies to ameliorate them.

ABMR is a unique, significant, and often severe form of allograft rejection that is unresponsive to treatment with standard immunosuppressive medications, and ABMR is acknowledged as the leading cause of allograft failure in the United States and Europe.5,6 Significant advances have occurred in our ability to predict patients at risk for ABMR and to diagnose patients with ABMR. These advances include the development of newer techniques to detect complement-activating DSAs, especially those assessing C1q (complement) binding DSAs and assays for non-HLA antibodies associated with ABMR.7,8 The pathophysiology of ABMR suggests a prime role for antibodies, B cells, the complement system, and plasma cells. Recent advances in the detection of anti-HLA antibodies specific for the allograft donor (DSAs) using Luminex technology have a strong correlation with development of ABMR, and many centers currently use DSA levels as prima facie evidence for the presence of ABMR. Indeed, DSAs are emerging as the most reliable biomarker for predicting ABMR and long-term allograft survival, especially those that activate complement.4 However, the effects of DSAs on allograft pathology are protean. Often, a wide spectrum of injury ranging from no perceptible injury to severe ABMR with graft failure can be seen. For more than a decade, the Banff Conferences on Allograft Pathology have documented and formulated specific phenotypes of allograft pathology associated with DSA injury.9 Although it is now clear that DSAs are causative of ABMR,1,4,7 there are still phenotypes of ABMR in which no detectable complement deposition is seen and in which Banff scores for inflammation are low or absent when non–complement-activating DSAs are present. In more chronic forms of antibody-mediated rejection (CABMR), it is postulated that DSAs mediate injury through non–complement-mediated pathways (i.e., ADCC) or through direct interaction with endothelial cell targets with subsequent activation of endothelial cell proliferation.1–3 Of interest in this regard is the recent report of Cornell et al.,10 which showed that DSA-positive recipients who received long-term treatment with eculizumab (anti-C5, anti-complement; Alexion, Cheshire, CT) developed evidence of CABMR and transplant glomerulopathy (TG) when DSAs persisted, despite full inhibition of the complement system. This study is of importance because identical CABMR and graft survival rates were seen in patients who sustained DSAs after transplantation with or without eculizumab therapy. Thus, eculizumab failed to prevent CABMR and graft loss. In addition, TG is a known consequence of persistent DSA positivity that rapidly dissipates allograft function, resulting in graft failure and return to dialysis with attendant emotional consequences for the patients and financial consequences for the health care system.4,11–13

Serum IgG molecules can be divided into four subclasses (IgG1–IgG4) with varying capacity to activate complement (IgG1 and IgG3 > IgG2 and IgG4). Data presented by Lefaucheur et al. in this issue of JASN explored the association of DSA IgG subclasses with various phenotypes of ABMR.14,15 In this retrospective analysis of 635 consecutive kidney transplant patients performed between 2008 and 2010, the investigators identified 125 patients with DSAs detected in the first year after transplantation. Overall, 40.8% of patients had acute ABMR,
28.8% had subclinical ABMR, and 30.4% remained free of ABMR, as detected on protocol biopsies. Immunodominant donor-specific anti-histocompatibility leukocyte antigen antibodies (iDSAs; the single DSA with the highest mean fluorescence intensity [MFI]) were 672±464, and 41.6% of patients had C1q+ DSAs. After an extensive analysis of iDSAs and their subclasses was performed and related to Banff scored ABMR, as detected on protocol biopsies. Immunodominant 28.8% had subclinical ABMR, and 30.4% remained free of ABMR process.

The findings of this study are of interest to the transplantation community because clarification of iDSA characteristics can predict risk for developing different phenotypes of ABMR. This further codifies the importance of DSA monitoring in patients who might be at risk for developing ABMR as a result of previous sensitization or through development of de novo DSAs. Detection of DSAs is a sentinel event in a transplant recipient and suggests the need for allograft biopsy. Further characterization of DSAs at the time of detection could be of help in predicting the likely phenotype of ABMR and possible therapies. For example, Kamisawa et al.16 showed that patients with IgG4-related disease present with a spectrum of autoimmune disorders characterized by storiform fibrosis, phlebitis, and elevated serum IgG levels. Patients with IgG4-related disease are known to respond exceedingly well to rituximab (anti-CD20, anti–B cell; Genentech-Roche, South San Francisco, CA).17 This would be important to assess in prospective trials of anti-CD20 therapy. Patients with predominant IgG3 iDSAs and C1q+ iDSAs will likely require a combination of antibody reduction therapies and complement inhibitors to control the ABMR process.

Of course, we must be cautious and not overinterpret these retrospective data. Further confirmation in prospective trials would be important because derivative functional and subclass analysis of DSAs is confusing and expensive. Many centers feel that strength of MFI is the best predictor of DSA pathogenicity and complement-activating capacity.18 Thus, collaborative prospective analysis of DSA assays will be critical to reconcile these issues and create recommendations for best practices. Overall, we must figure out where this new information fits in our assessment of sensitized patients and whether it adds substantially to the current armamentarium of assays. This needs to be assessed in light of the limitations of currently available assays.

Two important articles were recently published, which revealed problems with the Luminex single-antigen assay that are of concern to transplant physicians.19,20 Reed et al.20 carried out a collaborative assessment of single-antigen Luminex beads used to detect anti-HLA antibodies. The study was conducted blindly and aimed at determining the reproducibility of results among 20 participating centers. Initially, it was found that the interassay variability was as high as 62%; however, when strict adherence to assay protocols was done, variability was reduced to 20%. This means that the MFI of a single test serum can vary as much as 20% from one day to the next and raises concerns regarding the ability to monitor therapeutic interventions using MFI values.

Tambur et al.19 addressed the importance of how best to determine antibody strength. The presence of donor-specific HLA antibodies before or after transplantation likely has different implications based on antibody strength. The authors felt that current approaches do not provide information regarding the true antibody strength, which is traditionally assessed by dilutional curves. In this article, the authors interrogated currently available methods to compare MFI values, C1q assay MFI values, EDTA-treated samples, and titration studies as well as peak MFI values of >7000 Luminex-based single-antigen HLA antibody determinations. Of interest, the authors found that neat MFI values do not always accurately depict antibody strength, and EDTA treatment does not always remove inhibitory factors known to complicate this assay system compared with C1q or titration studies. In this study, the authors also addressed the prozone effect (a falsely low Luminex value that becomes highly positive with dilution of serum). The prozone effect was common in patients presenting with multiple HLA antibody specificities (71%). In addition to titration studies, the C1q assay was able to address the prozone issue, because there was a high correlation with antibodies with the prozone effect and C1q positivity. However, routine use of the C1Q assay has limitations as well, because it has low sensitivity and inability to detect weak antibodies. The authors concluded that titration studies are the only method among the approaches used in this study to provide a better indication of antibody strength, and likely pathogenicity.

Despite these concerns, the article by Lefaucheur et al.15 is an important step forward because it provides new insights into the relationship between iDSA IgG subclasses and the phenotype of allograft injury. More importantly, the authors show that patients who did not develop ABMR had no C1Q+ iDSAs and no IgG3 or IgG4 iDSAs. This is also an important observation because we know that all DSAs are not created equal and if further studies show that this pattern persists, it would be of great help in counseling patients and possibly avoiding costly immunotherapy to reduce what appears to be largely benign iDSAs. Indeed, this type of analysis could be applied to organ allocation paradigms for sensitized patients, allowing more confidence that C1q-, IgG3-, and IgG4-negative DSAs detected at the time of transplantation are unlikely to result in ABMR after transplant.

Lefaucheur et al. are to be commended for this important work, which further enlightens our understanding of the natural history of iDSAs and their effect on allograft pathology and outcomes.

DISCLOSURES
None.