Antiallograft antibodies: relevance, detection, and monitoring
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Purpose of review

Solid-phase immunoassays increase the accuracy of assessing pretransplant immunologic risk and facilitate posttransplant prediction and diagnosis of antibody-mediated rejection (AMR). This review will describe methods available for antibody analyses, discuss the types of targets of AMR and the characteristics of pathogenic alloantibodies, and provide guidelines for the application of antibody tests in the prediction of rejection risk and diagnosis of rejection.

Recent findings

The presence of human leukocyte antigen-specific antibodies increases the risk of AMR, but the clinical relevance of low antibody levels is questioned with reports of stable graft function in their presence. Posttransplant monitoring has been shown to provide early diagnosis of AMR permitting preemptive intervention. Antibodies to other alloantigens and autoantigens are being implicated as potential targets for both acute and chronic AMR. Certain limitations and interfering factors have also been recognized that should be recognized in the interpretation of solid-phase antibody assay results.

Summary

Contemporary technology is clearly advancing the detection of various antibodies that can contribute to AMR, but continued work is needed to elucidate the relevance of very low levels of human leukocyte antigen-specific antibody and the importance of antibodies to other alloantigens and autoantigens.

Keywords

alloantibodies, antibody-mediated rejection, autoantibodies, donor-specific antibodies, human leukocyte antigen-specific antibodies

Introduction

The clinical relevance of donor human leukocyte antigen (HLA) class I- and/or class II-specific antibodies (DHSA) at levels sufficient to yield a positive cytotoxicity cross-match is well established and has been considered historically as a contraindication to transplantation without some therapeutic intervention such as desensitization. Solid-phase immunoassays (SPAs) that use purified HLA targets permit the detection and characterization of alloantibodies with much greater sensitivity and specificity than do cytotoxicity assays. Similar techniques have been applied to other donor antigens such as the HLA class I-related chain A (MICA) and some autoantigens. Utilizing SPAs, recent studies [1[*],2,3[**]] have shown that the presence of pretransplant DHSA at levels below those detectable by cytotoxicity is associated with reduced renal graft survival, and antibodies developing de novo in the first posttransplant year are associated with increased incidence of chronic rejection [4,5]. It is now widely accepted in renal transplantation that HLA-specific antibodies contribute to the pathogenesis of both acute and chronic antibody-mediated rejection (AMR)

and the presence of donor-specific antibody is included as one of the criteria in the Banff classification of renal allograft pathology for antibody-mediated changes [6[*],7]. Alloantibodies have also been implicated in the survival of other organ transplants [8,9,10[*]], and the role of alloantibodies in organ transplantation was a major topic of discussion at the most recent Tenth Banff Conference on Allograft Pathology (August 2009). The relationship of low levels of DHSA to subclinical rejection in renal transplant recipients [11] and the possible impact of non-HLA-specific antibodies in all types of organ transplants are two issues that indicate the need for continued antibody detection and monitoring.

Methods for alloantibody analysis

The detection and characterization of HLA-specific antibodies have been the subject of extensive recent reviews [12–14]; therefore, only the major features, applications, strengths, and weaknesses of different methods will be given here. The targets of antibody assays can be cells or purified alloantigens. Cell-based assays are performed using variations of the basic complement-dependent

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cytotoxicity (CDC) method with modifications employed to enhance sensitivity or specificity by the addition of antiglobulin, wash steps, changes in incubation time or temperature. Flow cytometric assays are more sensitive than CDC-based methods, and additionally permit identification of cell types, as well as immunoglobulin isotypes and subclasses. Additionally, flow cytometry can be used for cells that are not amenable to CDC tests such as for crossmatch tests of endothelial cell precursors [15••]. Since the advent of SPAs, cell-based methods are most often used for crossmatches.

SPAs use purified HLAs that are derived either by solubilization from cell membranes or from transfected cell lines. These purified HLA molecules are employed in the following three formats: antigens pooled from multiple individuals, panels of individual class I or class II HLA phenotypes, and single HLA. Assays using pooled antigens are generally used in screening for the presence of HLA-specific antibodies, whereas the phenotype and single-antigen methods are used to determine antibody specificity and strength. SPAs differ in their platforms (ELISA or fluoroanalyzers), matrices (microtiter plates, polystyrene beads, or glass chips), reporter molecules (fluorochromes and enzymes þ substrate), and detection methods (colorimetric or fluorescence). Percentage panel reactive antibody can be determined from phenotype panels; however, this value reflects more the composition of the panel, which is usually limited in size, than the likelihood of a positive crossmatch with a random donor. However, specificity and strength can be used to identify antibodies of a strength considered as a contraindication to transplantation, which, in turn, provides the information for defining unacceptable antigens and performing a virtual crossmatch. Use of a virtual crossmatch has been shown to expand the geographic area for organ recovery and provide a substitute for crossmatch testing during posttransplant monitoring [16,17•,18•]. Accurate identification of antibody specificity and strength is necessary for the virtual crossmatch and also for determining acceptable mismatches [19,20].

The strengths, weaknesses, and applications of various antibody testing methods are given in Table 1 [21–23,24•,25–27]. Interference by cytoreductive agents can occur in any of the assays, although the effects vary among the assays. Variations in antigen concentrations [9,26] and substances inherent in the serum, such as high levels of immunoglobulin (Ig)M and immune complexes [21,22], can lead to false positive or negative reactions in any of the assays. To optimize the amount and quality of information obtained, it is desirable to have more than one type of SPAs available, as each has unique deficiencies. The impact in the amount and condition of individual specificities can drastically effect correlations between the results of

Table 1 Comparisons of antibody testing assays

Assay	Strengths	Weaknesses	Applications	References
CDC	Inexpensive, extensive experience	Requires viable cells, not specific for HLA, low sensitivity	Donor-recipient crossmatch	[12,13]
Flow cytometry (cell targets)	Sensitivity > CDC, can differentiate cell types and immunoglobulin classes/subclasses	Expensive reagents and equipment, not specific for HLA	Donor-recipient crossmatch	[12,13]
SPA – in general	Very high sensitivity and specificity, high throughput, low-volume serum requirement, no need for viable cells	Subject to interference from various serum factors	See below	[21–23]
SPA – pooled antigens	Least expensive SPA	No specificity	Antibody detection and monitoring	[12–14]
SPA – phenotype panel	Emulates real life, correlates well with crossmatch	Some antibodies may be 'masked' by others; inadequate detection of antibodies to Cw, DQ, and DP	Determine antibody strength and specificity, monitoring, identify unacceptable antigens	[12–14,16,24•,25]
SPA – single antigens	Identifies all antibodies present; improved detection of antibodies to Cw, DQ, and DP; identifies epitope and allele-specific antibodies	Antigens represented by single bead may be missed, variable amount of antigen and distorted molecules, increased susceptibility to interference, no proven way to assess collective strength of multiple antibodies	Confirm presence/absence of DHSA	[9,16,17•,18•,19,20,24•,25,26]

CDC, complement-dependent cytotoxicity; DHSA, donor HLA-specific antibody; HLA, human leukocyte antigen; SPA, solid-phase immunoassay. Adapted with permission from [12,27].

single-antigen bead assays and crossmatch tests. For example, the level of reactivity in a single-antigen assay that correlates with a positive flow cytometric crossmatch for DQ antigens can be twice that for DR [18]. However, despite these limitations, with thorough and experienced analysis, SPAs greatly improve antibody detection and characterization.

Antibodies to nonhuman leukocyte antigens

There have been tremendous advances in the definition of antibodies to all the major HLA antigens and alleles, including SPAs for HLA-DP, MICA/B, and some auto-antigens, as well as the previously mentioned cytometric test for endothelial cell reactive antibodies [15]. However, detection of antibodies to other non-HLA antigens has remained primarily in the research arena. Nonetheless, there is growing awareness of the importance of non-HLA-specific antibodies, both alloreactive and auto-reactive, in transplant outcomes (reviewed in [28,29]). Alloantibodies to antigens other than HLA may be evoked by ABO blood group antigens, MICA/B, and other, as yet unidentified endothelial cell antigens. Aside from ABO isohemagglutinins, the best evidence for an impact of antibodies directed toward non-HLA, poly-morphic alloantigens is for the MICA/B molecules. MICA and MICB are expressed on endothelial cells and monocytes, and antibodies to MICA have been associated with increased graft loss in renal recipients who were otherwise well matched for HLA [30,31]. An increased expression of MICA antigens has been demonstrated in endomyocardial biopsies in MICA antibody-positive patients experiencing acute cardiac allograft rejection [32]. A more recent study [33] examining MICA-specific antibodies as a marker for chronic heart allograft failure revealed significant associations of anti-MICA antibodies with cardiac allograft vasculopathy (CAV) and acute cellular rejection. In contrast to these positive associations, Smith et al. [34] have reported no effect of pretransplant or posttransplant production of MICA antibodies on acute rejection episodes in the first posttransplant year or on incidence of CAV at 3 and 5 years, and Scornik et al. [35] found no correlation of antibodies to MICA with C4d^P renal rejection.

There are several nonpolymorphic autoantigens expressed on endothelial cells that are plausible targets for AMR. Potential antigens that have been investigated as antibody targets in renal and cardiac allografts include the angiotensin type 1 receptor; intercellular adhesion molecule-1; and the cytoskeletal elements vimentin, actin, tubulin, and cytokeratin [28,29,36]. Antibodies against glutathione-S-transferase T1 may also be involved in liver and kidney rejection [37]. Delineation of a pathogenic role for non-HLA antibodies is hampered by the lack of readily available antibody assays, the number and variety of possible target antigens, and the frequent cooccurrence of HLA-specific antibodies. A recent multicenter trial [15] examined endothelial cell reactive antibodies in a crossmatch test using endothelial cell precursors isolated from peripheral blood. Among the patients with endothelial cell antibodies, 37% also had HLA-specific antibodies, which is consistent with earlier work indicating increased prevalence of these antibodies among patients who are also sensitized to HLA [29]. The prevalence of non-HLA-specific antibodies, in the absence of HLA-specific antibodies, is not clear. In contrast to the incidence noted in the trial of the endothelial cell precursor assay, two other reports [38,39] found incidences of only 2.3 and 9.5%. It appears that there is a causal relationship between antibodies to HLA and those to autoantigens. Fukami et al. [40] have shown in a murine lung transplant model that passive transfer of MHC class I-specific antibodies induced interleukin-17-dependent formation of antibodies to K-a1 tubulin and collagen V, as well as a histology consistent with chronic rejection in distal airways. Further clarification of the role of non-HLA-specific antibodies in allograft rejection is clearly needed, but will depend upon both the development of appropriate antibody assays and better characterization of the nature and cellular distribution of non-HLA antigens.

Antibody characteristics

Antibody characteristics that can be defined with SPAs and that affect their clinical relevance include antigen specificity, ability to activate complement, immunoglobulin isotype, and titer. Perhaps most critical are antigen specificity and titer, with antibodies directed toward mismatched donor HLA antigens (DHSA) being the most important [12–14]. The occurrence of AMR due to antibodies to HLA-A, -B, -Cw, -DRB1, -DRB3 –5, and -DQB1 is well established. Further, antibodies for HLA class II antigens are associated with the development of transplant glomerulopathy in kidney allografts [41]. Antibodies to HLA-DP and the alpha chain of DQ molecules can be distinguished with SPAs, but their potential impact on transplant outcomes will require further study [42–44]. The ability of DHSA to fix complement has been assessed in two recent studies using modifications of SPAs on the Luminex^{TM} platform (Luminex Corporation, Austin, Texas, USA). Rose and Smith [10] correlated C4d deposition with 1 year cardiac graft survival and found significantly lower graft survival with C4d^P– DHSA as compared with both C4d^P non-DHSA and C4d –DHSA. Wahrmann et al. [45], in contrast, did not observe any differences in renal graft outcomes between C4d^P–DHSA and C4d^P non-DHSA but noted that this difference was likely due to peritransplant immunoadsorption of their sensitized patients. SPAs are also being used to address the relevance of antibody isotype. The

pathogenic impact of IgM DHSA has been unclear since the introduction of calcineurin inhibitors. Preliminary results from Stastny et al. [9] indicate that pretransplant IgM DHSA is predictive of transplant rejection in renal recipients and the development of transplant coronary artery disease in heart transplants. Combining both isotype determination and complement fixation, Arnold et al. [46] investigated the prevalence of IgG and IgA noncomplement-binding HLA-specific antibodies and observed a high prevalence of donor-specific, noncomplement-binding antibodies in retransplant candidates.

Although the relevance of non-IgG and noncomplement fixing antibodies needs further study, the level or titer of DHSA that is clinically relevant is currently the most debated question. The sensitivity of SPAs in some reports is greater than that of a flow cytometric crossmatch. Some consider any level of DHSA as an unacceptable level of patient risk [23]. However, other reports [1•,47–54] indicate that low levels of pretransplant DHSA, detectable only by SPAs, may increase the risk of AMR, but do not adversely impact graft survival. Much of the current debate stems from variability among centers in the level of reactivity that defines the presence of DHSA or the level of antibody that is clinically relevant. It has been suggested that the major problem with using SPAs is the lack of standardization in interpretation [55]. However, as there are no standard reference reagents, standardization in the manner employed in other areas of laboratory medicine is not possible. Standardization is also confounded by the inherent variability in tests of high sensitivity and variance in acceptable levels of risk at different transplant centers. Determination of the threshold for antibody presence in SPAs requires consideration of multiple factors, including the validity of the assay as measured by results of positive and negative controls, the variability in the reaction strengths of different antigens, the clustering of phenotypes bearing the antigen of interest in tests of phenotype panels, recognition of the presence of interfering factors, and clear definition of the target of interest [18•]. Simply reporting test results, whether expressed as mean fluorescence intensity, optical density ratio, or molecules of equivalent soluble fluorochrome, is insufficient without consideration of these factors. Regarding level of risk, it is generally agreed that the degree of immunologic risk decreases with antibody strength, the highest risk conferred by high CDC titers and the lowest with levels detectable only by SPAs [12,48]. Determination of the threshold for clinically relevant antibodies is dependent on a correlation between SPA test results, crossmatch results, clinical outcomes, and each center's clinical protocols. Considering all these factors, several recent reports [17•,18•,20,24•,56] illustrate excellent correlations with this approach.

Application of solid-phase antibody tests in prediction and diagnosis of antibody-mediated rejection

Reports generated during the last year alone clearly support the value of SPAs in assessing the risk for and early detection of AMR. As part of the Banff criteria, detection of DHSA is an important adjunct to the pathological diagnosis of AMR, but periodic monitoring can provide early detection of increases in DHSA that permits therapeutic intervention and even avoidance of invasive biopsies [8,12,17•,57•]. Eng et al. [58] have demonstrated the value of detecting low levels of de-novo HLA class II-specific antibodies that are associated with renal transplant glomerulopathy and predictive of later graft loss. The sensitivity of SPAs is also proving useful in the interpretation of focal C4d deposition [57•]. As the interest and need for these antibody assays grows, there are legitimate considerations for their cost-effective use. In general, the need and frequency of testing should correlate with the immunologic profile of a patient. Pretransplant and posttransplant test results should be integrated with the history of sensitization, donor risk factors, such as repeat HLA mismatches, and clinical treatment protocols. Highly sensitized patients and those undergoing desensitization require much more intensive monitoring than nonsensitized patients or those receiving well matched grafts [25]. For highly sensitized patients undergoing desensitization, the first few weeks after transplant are critical. Sequential testing can be extremely informative, particularly in patients in whom low levels of DHSA are detected below the threshold levels for positive crossmatch results. As low DHSA levels may exist with stable graft function [52–54], isolated single determinations are insufficient to warrant intervention. However, if serial samples demonstrate a progressive increase in DHSA levels, then preemptive treatment may be indicated to forestall an AMR episode. The potential for increases in HLA-specific antibodies following allosensitizing events, such as transfusion or pregnancy, is well recognized. Among sensitized patients, it has been demonstrated that nonspecific, proinflammatory events, such as trauma or infection, can induce significant increases in both the level and breadth of HLA-specific antibodies [59•]. Therefore, for high-risk patients, any potentially sensitizing event may warrant consideration of antibody monitoring. Guidelines for applications of SPAs before and after transplantation will certainly improve, as more experience and clinical correlations are accrued. Given the explosive increase in published reports during the last year alone, additional data should not be long in forthcoming.

Conclusion
The advances in the technology to detect and characterize DHSA facilitate the diagnosis of AMR and permits

early detection of the development or increase in DHSA after transplant. Detection of very low levels of DHSA is helping to define subclinical rejection and focal C4d deposition. Correlation of antibody test parameters with crossmatch results, clinical outcomes, and center-specific clinical protocols provides a basis for defining clinically relevant levels of antibody that can be used for post-transplant monitoring and performance of virtual cross-matches. The relevance of low levels and different types of DHSA is still under debate and will require further investigation, including the potential role of both IgG and IgM antibodies directed toward non-HLAs.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
•   of special interest
•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 131–132).

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