

HLA Antibody Analysis

Sensitivity, Specificity, and Clinical Significance in Solid Organ Transplantation



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Abstract

The clinical relevance of humoral allosensitization has gained a lot of attention in the last few years. An increasing number of studies have demonstrated adverse graft survival in patients who have either preformed or post-transplant-developed anti-HLA antibodies. The detection of HLA antibodies and the specificity analysis have evolved over time from primarily cell-based to solid-phase methods, including the availability of single-HLA antigen preparations. These technological advances combined with a better understanding of the epitope structure of HLA antigens have provided a more efficient, structurally based strategy to determine HLA compatibility. In conclusion, these emerging approaches can be reliably used to predict crossmatch results in highly sensitized patients and also to monitor the development of clinically relevant anti-HLA antibody after transplantation.

Key Words

HLA
Alloantibody
Crossmatch
Transplantation
Antibody-mediated rejection
HLAMatchmaker

Introduction

Humoral sensitization to human leukocyte antigens (HLA) is an important barrier for solid organ transplantation. The exposure to HLA antigens can occur during pregnancy, blood transfusion, or previous transplants. The HLA system has multiple antigen-encoding highly polymorphic loci. There are anti-

genic epitopes expressed on individual HLA molecules while many others are shared among various HLA antigens (1,2). This complexity of the HLA system constitutes a significant challenge for the histocompatibility laboratories in order to develop sensitive and specific methods to analyze the repertoire of anti-HLA antibodies in transplant candidates. Furthermore, anti-HLA antibodies can appear

Table 1. Screening for Anti-HLA Antibodies

	Cell based	Solid phase
Sensitivity of the method	CDC < CDC+AHG<Flow	ELISA < Flow beads
HLA molecules	Natural configuration on cell surface	Isolated proteins bound on artificial surface
HLA antigens	HLA phenotypes	Pooled HLA antigens, phenotypes and single antigens
False-positive reactions	Non HLA-specific antibody	Reactions with cryptic epitopes on denatured HLA molecules.
False negative reactions	Antibody level below detection	Loss of epitope expression on isolated molecules

CDC = Complement-dependent cytotoxicity; AHG = Anti-human immunoglobulin.

following transplantation in previously unsensitized individuals and the efficiency to detect, characterize, and remove these antibodies may influence the long term outcome of solid organ transplantation (3).

The detection of circulating anti-HLA antibodies and the specificity analysis have evolved over time, from primarily cell-based to solid-phase immunoassays, using solubilized and recombinant HLA molecules (4,5). Furthermore, the application of HLA Matchmaker, a computer-based algorithm to determine donor–recipient HLA compatibility at the structural level, has enhanced our ability to interpret the patterns of anti-HLA antibodies in sensitized transplant recipients (6–9). In this publication, we will discuss the various methods for antibody detection and specificity analysis. We will also address the clinical impact of anti-HLA antibodies before and after transplantation in various solid organs, a topic that has been extensively reviewed in recent publications.

Assays to Detect Anti-HLA Antibodies

The screening for anti-HLA antibodies has evolved from complement-dependent methods [complement dependent cytotoxicity (CDC) and CDC with anti-human globulin (AHG)] to the more sensitive flow-based assays (10,11).

These methods are referred to as “cell-based” or “membrane-dependent” assays, as the target for the antibody in patient’s serum is the HLA antigen expressed on intact cell membrane of lymphocytes. Advances in the purification technology of HLA antigens have facilitated the development of solid-phase assays, whereby the HLA antigens can be bound to a solid matrix.

The “membrane-independent” assays include ELISA and flow technology using beads coated with HLA antigens (12,13). In Table 1 we summarize the pros and cons of these two approaches. Cell-based assays are considered clinically relevant having the HLA molecules displayed in their natural configuration. However, these assays may lack specificity because positive reactions (false positive) may occur in the presence of non-HLA antibodies and auto-antibodies. Different cytolytic therapies applied pre- and post-transplantation, such as anti-CD3 antibody, polyclonal anti-thymoglobulin, anti-CD20 antibody, may interfere with the cell-based techniques for the detection of anti-HLA antibodies. Solid-phase methods are more specific than cell-based assays because the HLA molecules can be purified and bound to the plates (ELISA) or beads (flow) and these assays are not influenced by lymphocytotoxic immunosuppressive drugs. However, there are

potential problems with the isolation of HLA antigens, whereby new cryptic molecules from the denatured HLA antigen can be exposed and bind to patient's sera (false-positive reactions), or antigenic epitopes may be lost as a consequence of changes in configuration of the isolated HLA molecules (false-negative reactions). The analysis of antibody specificity has improved with the availability of single antigen-coated plates/beads. For the highly sensitized patients awaiting transplant, it was very difficult to identify their anti-HLA antibody specificity and to find a suitable donor. Currently, with the new reagents, it is possible to define the HLA antigens that are considered unacceptable and/or acceptable mismatches. Furthermore, knowledge of patient's history of sensitizing events such as previous grafts, blood transfusions, or pregnancy is required for the laboratory assessment of HLA sensitization. The characterization of anti-HLA antibody should be based on a combination of cell-based and solid-phase assays with single antigens.

Interpretation of Serum Screening Results

The so-called PRA (Panel-Reactive Antibody) represents a semiquantitative estimate of the degree of HLA sensitization. It is calculated as the percentage of an HLA panel that reacts with a serum. Patients with >80% PRA are considered highly sensitized and for them it is difficult to find crossmatch-negative donors. The accumulation of highly sensitized patients on transplant waiting lists represents a growing problem.

The analysis of serum reactivity of transplant candidates has two goals. Most commonly used is the identification of unacceptable HLA antigens that should be avoided on donor organs. This system is designed to identify donors who must be excluded, but it does not necessarily mean that all other HLA antigens would be compatible for a patient. The other goal is to

determine HLA antigens that are acceptable mismatches. This strategy represents a direct approach of finding a compatible donor for a sensitized patient (14,15).

The analysis of serum reactivity patterns with HLA phenotyped panels in cell-based and solid-phase assays is primarily done with 2×2 table statistical methods such as chi square to identify antigens and epitopes with significant correlations. Unfortunately, this method is of limited value for >80% PRA sera. The use of single HLA antigens in ELISA and Luminex assays permits a better interpretation of antibody reactivity patterns. An important consideration is that each HLA antigen carries multiple epitopes that can be structurally defined by amino acid residues in polymorphic positions of the HLA molecule. Stereo chemical modeling of crystallized HLA antigens has visualized these rather extreme structural polymorphisms. Figure 1 shows examples of three class I molecules: HLA-A2, HLA-B27, and HLA-Cw3.

The molecular surface around the bound peptide (see top view) has similar numbers of exposed polymorphic positions on the $\alpha 1$ helices of HLA-A and HLA-B antigens but more polymorphic positions are visible on the $\alpha 2$ helices of HLA-A antigens. The α helices of HLA-C antigens have much fewer polymorphic positions.

In contrast, HLA-C antigens have more polymorphic positions in the membrane-proximal region, which becomes visible upon side viewing. HLA-A antigens have also more surface-exposed polymorphic positions in that region than HLA-B antigens. It should be noted that the sequence positions in the membrane-proximal domain of HLA-B are all monomorphic.

Class II HLA antigens have similarly complex structural polymorphisms (not shown). This applies to all DRB, DQB, and DPB chains. DQA chains have more structural

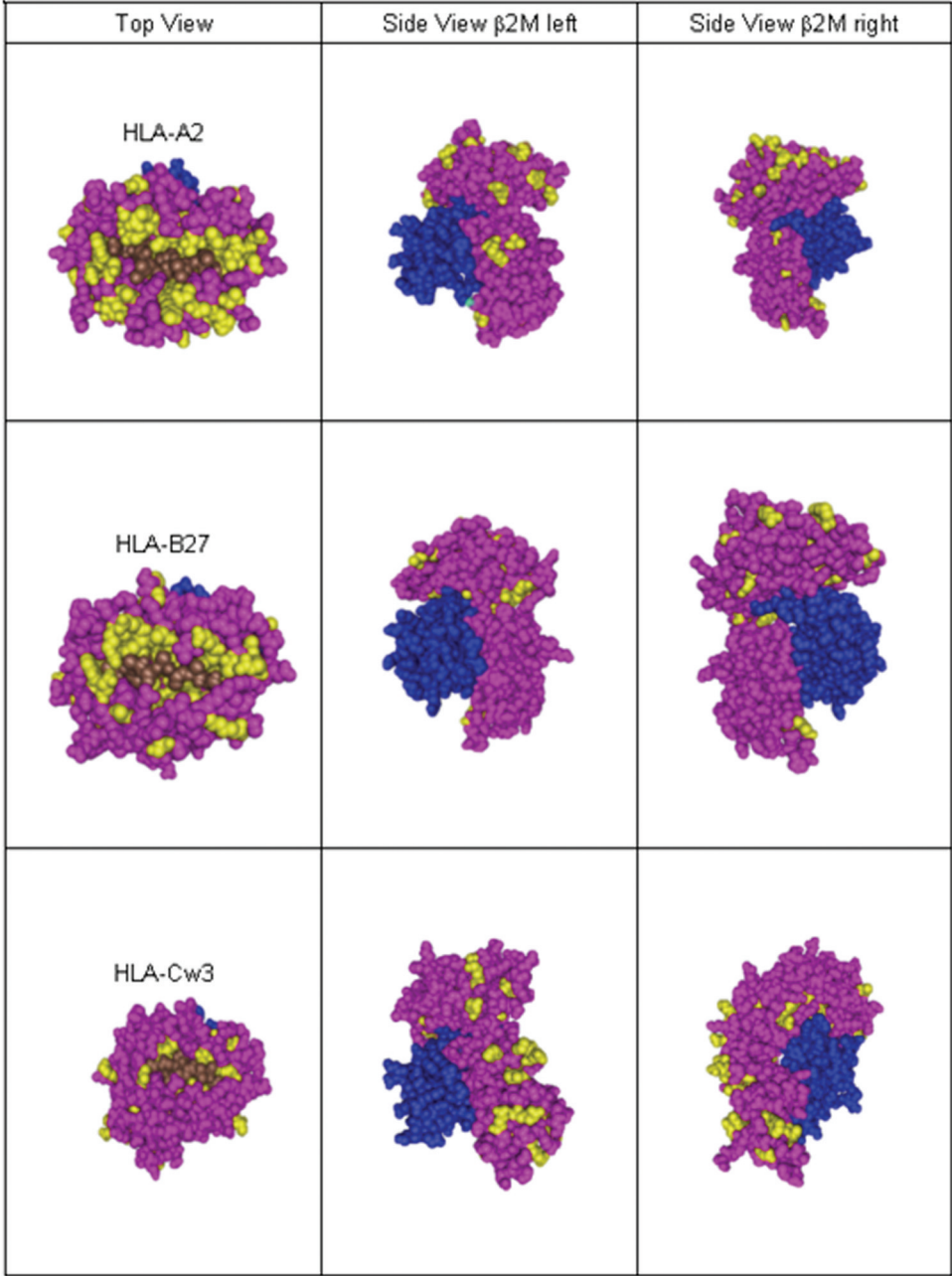


Fig. 1. Polymorphic residues on class I molecules controlled by HLA-A, B, and C loci ($\beta 2M = \beta 2$ microglobulin).

polymorphisms than DPA chains whereas DRA chains are primarily monomorphic.

Considering the high number of HLA antigens (and alleles) and their extensive poly-

morphisms, one can expect that HLA antibody formation in transplant patients is extensive and complex. A better understanding of the epitope structure of HLA antigens is

important not only for the characterization of HLA-specific antibodies but also will permit a more efficient, structurally based strategy to determine HLA compatibility.

HLAMatchmaker is a matching program that considers the structural basis of epitopes on class I HLA antigens (7). Each HLA antigen can be viewed as a string of short sequences (triplets) involving polymorphic amino acid residues in antibody-accessible positions; they are considered key elements of epitopes that can induce the formation of specific antibodies. The patient's HLA phenotype represents the repertoire of self-triplets to which no antibodies can be made and HLA-Matchmaker determines for each mismatched HLA antigen, which triplets in corresponding sequence positions are different. HLAMatchmaker-based matching improves transplant outcome (9,16,17), and is useful in serum analysis and the identification of acceptable mismatches for alloimmunized kidney transplant candidates (6,15,18–23) and refractory thrombocytopenic patients requiring matched platelet transfusions (24).

The number of amino acid triplet differences between patient and donor correlates with the development of anti-HLA antibody following pregnancy and kidney transplantation (25). Certain patients become highly sensitized following exposure to a single mismatched HLA antigen. The following two cases illustrate how HLAMatchmaker can explain this.

The first case was a patient who after removal of a rejected kidney graft 7 mo post-transplant developed a serum PRA of about 90% due to class I antibodies (22). This kidney was a one antigen mismatch, namely, HLA-B13, but patient's serum reacted not only with HLA-B13 but also with a large number of other HLA-A and HLA-B antigens. An HLA-Matchmaker-based analysis showed antibody specificity to the 144tQl triplet unique to HLA-B13 and the 76En, 80rTa, 82aLr triplets

shared between HLA-B13 and other HLA antigens such as HLA-A9, HLA-B17, HLA-B27, and many more. (The triplet notation system uses the amino acid letter code and the number represents the sequence position of the residue in capital letters.) The 76En, 80rTa, 82aLr carrying antigens must be considered unacceptable mismatches although the patient might have never been exposed to them. The reason why they become unacceptable was that they share one or more epitopes with the immunizing HLA-B13.

The second example is a high anti-class II antibody activity following sensitization to a one HLA-DR antigen mismatch. This patient typed homozygous for HLA-DR7 and had rejected a kidney transplant with a HLA-DR11 mismatch. Patient's serum reacted with all DRB1 antigens except HLA-DR7. HLA-Matchmaker identified a mismatched triplet 14ER on HLA-DR11 that is shared with all DRB1 antigens except HLA-DR7, which has 14QK. Thus, the high reactivity of this patient's serum might be due to antibodies to a single epitope defined in this case by 14ER. This conclusion is consistent with descriptions of monoclonal antibodies reacting with all DRB1 antigens except HLA-DR7 (26). Exposure to single HLA-DQ mismatches may also lead to antibody reactivity to all DQB1 antigens except self-DQB1 and corresponding structurally defined DQB1 epitopes can readily be identified.

It should be noted that HLA antibody responses are generally restricted to a limited number of epitopes. High PRA sera reflect the presence of antibodies against high-frequency epitopes. The HLAMatchmaker-based interpretation of serum reactivity incorporates patient's HLA type determined preferably by DNA methods at the 4-digit allele level. HLA information of the immunizer (i.e., a previous transplant) will identify structurally defined epitopes the patient has been exposed to. This

facilitates the interpretation of serum screening results and the determination of mismatch acceptability for prospective donors.

Clinical Impact of Anti-HLA Antibodies

Humoral rejection is emerging as a leading cause of graft failure and is associated with all forms of allograft rejection: hyperacute, acute, and chronic (4, 27–29). Although circulating antibodies were found in patients rejecting their allografts, the lack of histological evidence of antibody-mediated process hampered the diagnosis of antibody-mediated rejection (AMR). Detection of complement activation in tissue by staining for C4d and the recognition of other clinical features, such as graft dysfunction that does not respond to standard therapies, greatly facilitated the diagnosis of AMR (28,30,31).

HLA antibodies are associated with acute and chronic rejection in kidney, kidney–pancreas, heart, and lung transplant recipients and are present after almost all kidney failures (32,33). Donor-specific HLA antibodies not found prior to graft removal became detectable after transplant nephrectomy in most patients (22). This study supports the concept that the allograft may remove the high affinity circulating donor-specific antibodies and upon re-transplantation those HLA antigens recognized by the antibodies should be avoided. Furthermore, the serum analysis performed with the HLAMatchmaker program can identify additional HLA antigens that may share antibody-reactive epitopes with the immunizing donor and therefore should also be avoided (22).

The deleterious effect of anti-HLA antibodies developed post-transplantation was addressed in a large collaborative study of 2231 kidney recipients from 23 centers followed for 2 yr (34). Among those patients who were pre-Tx antibody negative and developed

de novo HLA antibodies, 16.7% ($n=233$ antibody positive) failed within the 2-yr follow-up, while only 6.5% of antibody negative patients ($n=1331$) lost their grafts. When the patients were further divided by the serum creatinine levels, at the time of antibody testing, a progressive decline was observed in patients who had antibody and increased serum creatinine (34).

The incidence of allosensitized patients on cardiac transplant waiting lists is on the rise, owing to the use of left ventricular assist devices, blood transfusions, and the increasing number of re-transplants. Cardiac recipients with a history of sensitization have an increased incidence of antibody-mediated rejection. AMR has been shown to predispose heart transplant recipients to coronary vasculopathy (29,35,36). Furthermore, patients who develop AMR after cardiac transplantation progress to transplant-associated coronary artery disease earlier and at increased frequency compared with controls (37,38).

AMR in the heart, like the kidney, may occur in combination with cellular rejection. The incidence of AMR in biopsies with cellular rejection has been reported to be 23%, while the prevalence of AMR without cellular rejection is about 15% (35).

We studied the role of pre-formed and de novo developed anti-HLA antibodies in lung transplantation (39). HLA antibodies detected primarily by solid-phase ELISA were associated with severe forms of acute allograft rejection that required multiple treatments (persistent and recurrent acute perivascular rejection, ACR-PR, Table 2). As depicted in Table 2, the relative risk for ACR-PR in patients with circulating HLA antibodies, was fivefold higher than in patients who did not develop anti-HLA antibodies. Moreover, there was no influence of the immunosuppressive protocol on the frequency of de novo anti-HLA antibody production.

Table 2. Increased Risk of Persistent Lung Allograft Rejection in Patients with Circulating Anti-HLA Antibodies

Therapeutic Protocol	HLA antibody		No HLA antibody		<i>p</i> <	Relative risk
	<i>N</i>	ACR-PR	<i>N</i>	ACR-PR		
Triple drug (CsA, Ster, Aza)	12	11/12 (90%)	35	10/35 (34%)	0.005	5
Pre-transplant thymoglobulin	14	10/14 (71%)	23	3/23 (13%)	0.001	5.5

ACR-PR = Persistent / recurrent acute cellular rejection; CsA = Cyclosporine A; Ster = steroids; Aza = Azathyoprine.

We, and others, have observed a significant association between anti-HLA antibodies and chronic lung allograft dysfunction referred to as bronchiolitis obliterans syndrome (BOS) (40,41). A multivariate risk factor analysis for the development of BOS in 51 lung transplant recipients followed for 4.2 + 1.6 yr, showed significant associations between the development of de novo antibodies and lymphocytic bronchiolitis, which is considered the airway rejection form (Fig 2), as well as between antibodies and BOS (40). The HLA antibodies preceded the development of BOS by more than 1 yr, and both anti-class I and anti-class II HLA antibodies were associated with worse outcomes (40).

The hallmark of complement activation due to antibody-mediated processes in allograft tissue is C4d deposition (30). Specific immunostaining patterns are considered when continuous, linear, subendothelial deposition is detected in microcirculation (capillaries, arterioles, and venules) (42). Lung transplant recipients who rejected their allografts and also had circulating antibodies, had higher frequency of C4d deposition than histological matched patients without anti-HLA antibodies (42). Circulating HLA antibodies in lung transplant recipients during ACR episodes were also associated with increased levels of soluble C4d in bronchoalveolar lavage fluids (43). These results support the notion that humoral immunity may contribute to allograft dysfunction, and improved methods of anti-

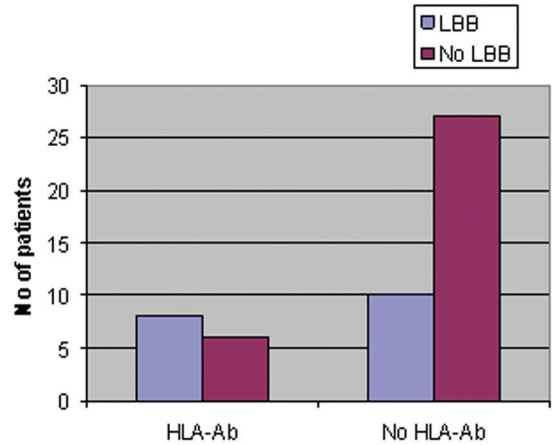


Fig. 2. Higher prevalence of lymphocytic bronchiolitis (LBB) in lung transplanted patients with anti-HLA antibodies (HLA-Ab).

body detection pre- and post-transplantation, combined with complement deposition staining and novel treatment protocols, should be incorporated in the clinical management of transplant recipients.

Virtual Crossmatch

The impact of sensitization on transplant outcome has been recognized since the first reports of antibody-mediated hyperacute rejection in renal transplant recipients. Sensitized patients, those with PRAs > 10%, currently comprise 33% of the patients on the waiting list, and the proportion of sensitized women (PRA ≥ 10) is twice that of men (3). There is also disparity in rates of sensitization

among different racial groups with the highest percentage of highly sensitized patients (PRA ≥ 80) observed among African Americans (3).

The level of sensitization significantly prolongs waiting time and prospective CXM have to be performed pre-transplant to avoid the deleterious effects of antibody-mediated rejection. Owing to the need to minimize organ ischemia time, especially for thoracic organs, pre-transplant CXMs are performed with donor samples prior to organ recovery. This approach limits the pool of donors for highly sensitized thoracic transplant candidates because it precludes the use of donors obtained at a distant site.

With the application of newer, specific, and sensitive techniques for detection and characterization of anti-HLA antibodies, the clinical laboratory can determine an appropriate donor without the actual CXM test. By using a virtual CXM, we exclude donors that express those HLA antigens, or epitopes that are recognized by the patient's antibody (unacceptable antigens), while donors that carry the acceptable HLA antigens can be considered. The use of the virtual CXM has improved the ability to transplant sensitized heart and lung transplant recipients, and it resulted in a significant decrease in their waiting list time (44,45).

We also implemented the virtual CXM at our institution for sensitized thoracic transplant candidates. The pre-transplant antibody screening and specificity analysis is performed with solid-phase assays including single HLA antigen preparations. Following transplantation, the next working day, the actual CXM is done to confirm the negative virtual CXM.

Based on this approach, two sensitized lung transplant recipients were successfully transplanted. Both patients had multiple sera samples for antibody analysis collected over a period of 6 mo pre-transplantation (6, 4, 2 mo), including sera obtained within 2 wk of the allograft. Based on the antibody reactivity pattern we

could identify the unacceptable antigens. Furthermore, the application of the HLAMatchmaker program provided additional information regarding the acceptable HLA mismatches, and increased our predictability of a negative virtual CXM. Although both patients were sensitized, we could find a compatible donor in a timely fashion so they could proceed to transplantation. Both patients had an uneventful post-transplant course and did not develop de novo anti-DSA within the 2–3 mo of follow-up.

This virtual CXM relies on complete information of sensitizing events and patient history. In any case whereby the transplant candidate had a sensitizing event, such as blood transfusion or exposure to left ventricular device (heart transplant candidates), the antibody analysis needs to be reassessed. The predictive value of the virtual CXM is highly dependent on the accuracy of the latest antibody analysis.

Summary

There is good evidence that pre-formed and de novo production of anti-HLA class I and class II antibodies contribute to graft deterioration at all times after transplantation. Furthermore, recent studies suggest that monitoring for anti-HLA antibodies post-transplantation is prognostic of allograft outcome and can provide a useful measure of therapeutic efficiency. Combination of solid-phase and cell-based methods should be used to identify low levels and clinically significant anti-HLA antibodies. The analysis of antibody reactivity patterns incorporating the HLAMatchmaker program is a valuable tool to determine the acceptable mismatches and provide important information of donor compatibility for highly sensitized transplant candidates.

Acknowledgments

This work was partially supported by grants AI 55933 and 1P50 HL074732.

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