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Anti-HLA antibody testing in hematology patients

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Abstract

Anti-human leukocyte antigens (HLA) antibodies can adversely impact the care of hematology patients. In particular, HLA antibody testing provides important information for optimal stem cell and platelet donor selection in the management of stem cell recipients and platelet refractory patients. Current testing methods for HLA antibodies are briefly reviewed, with particular emphasis on laboratory and clinical issues associated with solid-phase multiplex assays.

Introduction

Antibodies against human leukocyte antigens (HLA) can adversely impact the care of patients requiring platelet transfusion support and/or hematopoietic progenitor cell (HPC) recipients. In thrombocytopenic patients requiring platelet transfusion support, HLA alloantibodies can lead to platelet refractoriness [1]. Failure to provide HLA compatible platelets can result in morbidity and mortality [2]. One approach for provision of compatible allogeneic platelets is to identify HLA antibody targets and select platelet donors lacking the specific HLA antigens.

Similarly, the presence of HLA antibodies against allogeneic HPC donor HLA antigens can lead to graft failure with different types of donors [3–6]. Optimal donor selection for sensitized stem cell candidates also requires avoidance of donors who express target HLA antigen or desensitization of the recipient to decrease HLA antibodies [7].

HLA antibody testing can provide clinically relevant information in the management of platelet refractory patients and HPC transplant candidates/recipients. Here, we briefly present HLA antibody testing methods with emphasis on solid-phase multiplex testing.

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Conflict of interest

Nothing to report

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HLA Antibody Testing

There are a number of methods for HLA antibody testing. Broadly, they fall into cell-based or solid-phase assays. Testing methods can be used as a screen (i.e., *Is there antibody?*) or to identify the antibody specificity (i.e., *Which HLA antigen is the antibody targeting?*) or even more specifically, determine whether the antibody can cause a positive cross-match reaction (i.e., *Can the antibody bind the intended donor cells?*).

Cell-based assays were first applied to selection of solid organ donors suitable for transplantation to HLA allosensitized individuals [8,9]. Cell-based cross-match assays are performed by incubating donor leukocytes with recipient serum. Lymphocyte subsets (e.g., T cells and B cells) are used as target cells following enrichment or selection to allow separate detection of antibodies against HLA Class I (HLA-A, -B, and -C) and/or Class II (HLA-DR, -DQ, and -DP) antigens [10]. If donor-specific antibody (DSA) is present in the patient serum, antibody binds its antigen target on the donor lymphocyte.

The earliest HLA antibody assay was the lymphocytotoxic test, also referred to as complement-dependent cytotoxicity (CDC), which can be used for either antibody screens (using a panel of HLA typed cells as targets) or for donor-specific cross-matches. Antibody–antigen interaction leads to fixation of exogenous complement onto target lymphocytes, and results in cell death. Addition of a secondary antibody (anti-human globulin, AHG) can improve the sensitivity of the CDC [11].

The cross-match can also be performed by flow cytometry [12]. In the flow cytometric cross-match (FCXM), donor cells are stained with fluorophore-conjugated antibodies that distinguish T cells from B cells (usually using anti-CD3 and anti-CD19, respectively). A third antibody (fluorphore conjugated-AHG) is used to detect cell-bound HLA antibodies. The degree of cytotoxicity (for CDC) or fluorescence signal (for FCXM) is proportional to the amount of HLA antibodies. For technical and logistical reasons, cell-based assays are not commonly performed for HLA antibody testing for HPC transplantation and transfusion support. In particular, solid-phase assays are more sensitive in detecting HLA antibodies and additional donor cells are not readily available for cross-matching.

In contrast, solid-phase assays utilize immobilized HLA antigens as antibody targets. Solid-phase assays for detecting anti-HLA antibodies include enzyme-linked immunosorbent assays and multiplex bead-based testing either by Luminex™ or conventional flow cytometry. The remainder of this review will focus primarily on the Luminex-based multiplex bead assay as this is the most commonly used assay for stem cell and platelet donor selection.

In Luminex™ assays, purified HLA molecules are bound to the surface of fluorescent beads. Currently, there are two vendors (One Lambda/Thermo-Fisher and Lifecodes/Immucor) offering commercially available HLA antigen-coated beads for clinical testing by Luminex™. Patient serum is combined with HLA antigen-coated beads. If present, HLA antibodies will bind the HLA antigens on the beads. The beads are washed to remove unbound antibody and a secondary reporter antibody (usually anti-human IgG conjugated

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with phycoerythrin) is added. The beads undergo further washing and are analyzed in a Luminex™ instrument.

Luminex™ instruments are similar to flow cytometers, but the flow stream is optimized to separate 5.6 μm diameter polystyrene beads instead of cells.

The instrument distinguishes one bead from another by the emission color of each bead after laser excitation. The earliest iterations of Luminex™ instruments distinguish up to 100 different colored beads in a single reaction tube. By coupling specific HLA antigens (or combination of antigens) onto specific colored beads, one can identify the specificities of anti-HLA antibodies present in patient serum. The signal emitted by the phycoerythrin-conjugated secondary antibody, following excitation by a second laser, is used to measure relative abundance of HLA antibodies bound to HLA molecules on the beads. This is reported as mean fluorescence intensity (MFI). Together, the bead color and MFI are used to identify the HLA antigen target(s) and provide semi-quantitative assessment of antibody strength. Notably, current bead kits are not Food and Drug Administration-approved for quantitative analysis.

There are three formats of HLA beads available for use on the Luminex™ platform. The differences among bead kits lie in the antigens coupled to the bead surface. “Mixed” or “screen” beads, are screening tests for the presence of HLA antibodies. Multiple Class I or Class II HLA antigens are coupled to the bead surface. The presence of any HLA antibody in the patient serum will result in binding to the HLA molecules. Because several HLA antigens are present on any one bead, specific HLA antigen targets cannot be identified.

The second bead kit is called the “PRA” or “ID” or “phenotype” beads. Each bead color has either Class I or Class II antigens derived from a single individual donor. These kits can serve as both a screening test and to determine how broadly reactive the antibody is (i.e., to determine % panel reactive antibody, PRA). In some cases, they can also be used to identify the specific targets of anti-HLA antibodies. In both the mix/screen and the PRA/ID kits, the antigens are solubilized from B cell lines and separated into Class I and Class II molecules by affinity purification.

The final bead type is called the “single antigen” bead. As the name implies, each bead color is coupled with only a single HLA antigen (e.g., an A1 bead is covered only with HLA-A1 molecules). The antigens used to coat these beads are recombinant. These beads are the most helpful for determining specific HLA antigen targets, including identification of antibodies against HLA-DP antigens [13].

Challenges in Anti-HLA Antibody Testing

Histocompatibility personnel are challenged with interpretation of solid-phase multiplex testing results for clinical use. Select salient issues in antibody testing interpretation are briefly discussed.

Although Luminex™ technology allows multiplexing, there are limitations to the number of distinctly colored beads when compared with the polymorphisms in the HLA system.

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Currently, there are >11,000 alleles representing 164 distinct HLA antigens, with each antigen having several epitopes. Thus, interrogation for the presence of a particular HLA antibody is restricted to the range of antigens (and epitopes) represented on the beads. In general, the more commonly encountered HLA antigens are used in commercial bead kits.

It has been recognized that reactivity to bead-bound HLA antigens can be due to HLA molecule conformational changes during manufacturing. Single antigen beads, in particular, are susceptible to this false-positive reactivity because the coupling process of recombinant protein to the beads leads to unmasking or creation of neo-epitopes [14]. Histocompatibility laboratories are able to identify such false-positive reactivity in some, but not all, instances.

Once the specificity of anti-HLA antibodies is determined, the clinical significance of such antibodies must be established. Solid phase assays are more sensitive than cell-based assays [15]. We know from experience that strong anti-HLA antibodies are seen in platelet refractory patients [2] and that weak antibodies are less likely to be significant [16]. Provision of compatible platelets can be achieved by matching platelet donor and recipient Class I HLA, platelet cross-matching, or by selecting platelet donor units that do not express HLA antigens targeted by recipient antibodies [17].

However, it is less clear what level of antibody is sufficient to cause delayed engraftment and/or failure in HPC transplantation. In unrelated donor transplants, the National Marrow Donor Program recommends selecting an 8/8 HLA match (at HLA-A, -B, -C, and DRB1) for optimum HLA matching [7]. One might question the clinical utility of anti-HLA antibody testing when donors are matched at this level. In fact, only ¾ of Caucasian HPC transplant candidates will find an 8/8 matched donor and in other ethnic groups, this can be as low as 16% [18]. In addition, it has been shown that 75–86% of 8/8 matched donor–recipient pairs will be mismatched at HLA-DPB1 [4,19]. Though no data exist for HPC transplantation, in solid organ transplant candidates, the frequency of antibodies against HLA-DP existing as the sole HLA Class II antibody is about 6% [20]. Therefore, despite attempts at HLA matching, the potential for anti-HLA antibodies adversely affecting HPC transplant outcomes is still a reality.

Many studies have used different MFI cut-offs to assign clinical significance to HLA antibodies in HPC transplantation. In one study, patients who failed to engraft had anti-DPB1 antibodies of at least intermediate strength (i.e., MFI > 1,500) [4]. In haploidentical donors, intermediate strength DSA was seen in recipients with graft failure [21]. DSA with MFI > 1,000 was associated with double cord transplant engraftment failure [5] while MFI > 2,000 was associated with graft failure in mismatched donors [6]. One problem with using MFI alone to assign clinical significance is that bead arrays display high variation in MFI values (up to 62%), which can be improved (to about 25%) with inter-laboratory standardization [22]. Sources of this wide variation in MFI are inter-operator technique as well as differences in antigen density on beads. There should be discussion between the clinical team and HLA laboratory regarding MFI cut-offs, if any, to be used in donor selection.

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It has been recommended that all patients undergoing HPC transplant be screened for HLA antibodies to aid in donor selection [23]. However, the frequency of repeat screening remains unclear. Because HLA sensitization may occur following pregnancy, transplantation, and/or blood transfusion, it is important to monitor changes in HLA antibody status following sensitizing events prior to HPC transplant.

In contrast, no anti-HLA antibody screening (or repeat screening) recommendations for platelet refractoriness exist despite the cost associated with HLA alloimmune platelet refractoriness [2]. Targeted screening of patients at risk for HLA alloimmunization may be one approach, but further studies need to be undertaken to determine what role, if any, *a priori* testing is in predicting HLA alloimmune-mediated platelet refractoriness. However, there may be some role for repeat antibody testing of highly-sensitized patients who require extended platelet transfusion support due to myelosuppression (e.g., HPC transplant patients undergoing chemotherapy) as HLA antibodies can wane (e.g., following chemotherapy-associated immunosuppression). Therefore, transfusion of antigen-positive platelet units may result in satisfactory platelet count increments if repeat testing shows that a specific antibody has decreased.

Unfortunately, these solid-phase tests are expensive and repeat testing incurs higher costs. While cost determination for HLA antibody testing is beyond the scope of this manuscript, one can get a general sense by review of the most recent Center for Medicare and Medicaid Services (CMS) laboratory test fee schedule. Reimbursement fees for some HLA antibody solid-phase testing ranks in the top 70 (of over 1300 tests) by CMS.

Should HLA antibody testing be performed serially, it is important to ensure that the same platform is utilized to enable the laboratory to adequately interpret changes in antibody identity and relative strength. Finally, although HLA antigens are the most important targets of alloantibodies leading to graft failure and platelet refractoriness, it is noteworthy that other antigens have been implicated in stem cell engraftment failure [24] and platelet refractoriness [25].

In summary, the presence of anti-HLA antibodies can result in engraftment failure in HPC transplantation as well as alloantibody-mediated platelet refractoriness. HLA antibody testing in hematology patients by multiplex bead array provides increased information for management of patients, but requires expert interpretation. At a minimum, patients at risk for HLA allosensitization (i.e., history of pregnancy, blood transfusion, or prior transplantation) should be screened prior to HPC transplant to aid in allogeneic donor selection. HLA antibody screening for predicting platelet refractoriness requires further studies. In select instances, repeat testing should be considered following additional HLA sensitization events, or following immunosuppression. There should also be consideration for utilizing methods that detect antibodies against HLA-DP. Importantly, dialogue between the treating physicians and the histocompatibility laboratory director is essential to establish the most appropriate center- and patient-specific antibody testing approaches.

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Summary Table

When is anti-HLA antibody testing indicated?

Anti-HLA alloantibodies play an important role in several pathologic processes. In certain hematology patients, anti-HLA antibody testing is important for appropriate selection of allogeneic HPC or platelet donors. The presence of donor-specific HLA antibodies in an HPC candidate is associated with graft failure. Similarly, alloantibodies against HLA molecules can lead to unresponsiveness to allogeneic platelet transfusion. Therefore, patients who are allogeneic HPC transplant candidates and patients exhibiting HLA alloimmune platelet refractoriness should be tested for the presence of HLA antibodies.

Who is at risk for developing HLA antibodies?

Anti-HLA alloantibodies are formed following exposure to foreign HLA antigens. Risk factors for development of HLA antibodies include women who have been pregnant, patients who have received blood transfusion or transplant recipients.

How is HLA antibody testing performed?

Tests for anti-HLA antibodies may be cell-based or solid phase. Cell-based assays are not generally performed for hematologic applications (they are performed for solid organ transplantation purposes) because cell-based testing requires donor cells, which are not readily available. The most commonly used method is the solid phase bead multiplex assay (i.e., by Luminex instrument). Luminex antibody testing can be performed using different types of beads: screen/mix, PRA/phenotype and single antigen beads. The major difference among the three is the number of distinct HLA antigens immobilized on the bead surface. Patient serum is incubated with beads. If anti-HLA antibodies are present, the antibody binds HLA antigens on the bead surface. A secondary reporter antibody (antihuman globulin) with a fluorphore is added and binds anti-HLA antibodies attached to cognate antigens. The bead-antigen-antibody-reporter complex is analyzed by Luminex, a flow-cytometer-based instrument. Information derived from Luminex testing include the presence of HLA antibodies, the HLA target of antibodies and the relative strength of the antibody.

What are the issues with HLA antibody testing?

Interpretation of solid-phase antibody testing can be challenging. Not every HLA antigen can be detected. In addition, there may be false-positive/non-specific reactivities which may deny a potential recipient of a donor (either HPC or platelet). Further studies are required to identify what level of antibody (i.e., titer) is of clinical significance. The frequency of testing is also not clear, but it would be reasonable to test patients after potentially HLA allosensitizing events such as pregnancy or transfusion.