REVIEW ARTICLE

Understanding HLA, non HLA, DSA and PRA: Transplant Essentials.

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Introduction

Transplantation of cells, tissues or organs reinstates physiological function and prolongs life being superior to any other alternative treatment. Immune compatibility between the donor organ and recipient is the major barrier for a successful transplant. Incompatibility can result in allograft rejection which can be T cell or antibody mediated. The Human Leucocyte Antigens (HLA), with genes located on chromosome 6 in humans are pivotal in immune recognition and hence allograft rejection.

In the last 50 years there have been major advancements in HLA antigen and antibody recognition assays leading to changes in modern practice. Hence, this review outlines the concepts of HLA antigens, antibodies, DSA (donor specific antibodies) and PRA (panel reactive antibodies) along with the progression and utilization of HLA antibody detection assays in the current transplantation era.

What is HLA?

The Major Histocompatibility Complex (MHC) is the group of genes found in higher vertebrates that encode the cell surface molecules responsible for both antigen presentation and immunological identity. The human MHC is known as the HLA complex. Due to the huge variation exhibited by the HLA genes, HLA tend to vary between individuals and the response against them is instrumental in rejecting grafts expressing non-self HLA antigens. There are two broad classifications of HLA molecules known as class I (A,B,C) and II (DP,DQ,DR). Class I molecules are present on all nucleated cells, while class II are found expressed constitutively on antigen presenting cells such as B cells, macrophages, dendritic cells and Langerhans cells.

What are HLA antibodies?

Antibodies against the above mentioned HLA antigens are known as anti-HLA antibodies. In relation to organ transplantation antibodies can be pre-formed or de-novo. HLA antibodies occur due to a sensitization event like pregnancy, blood transfusion or a previous transplant. The objective of screening transplant recipients for HLA antibodies prior to transplantation is to detect pre-existing anti-HLA antibodies, define their specificity (which HLA antigens they react against), and determine their relative strength.

What are Donor Specific Antibodies (DSA)?

DSA are HLA antibodies directed against the HLA antigens expressed by the donor. Presence of HLA-DSA in a recipient is detrimental to the transplant outcome. Preformed DSA can cause early rejection, such as hyperacute rejection, accelerated antibody mediated acute rejection and graft loss. Further, de novo HLA DSA developed after transplant are associated with late acute antibody-mediated rejection, chronic antibody-mediated rejection, and transplant glomerulopathy,. Nonetheless, “benign” DSAs exist that may not be clinically associated with ABMR or graft failure.

HLA antibody detection

Since the introduction of the complement dependent cytotoxicity (CDC) technique as the initial crossmatch methodology between recipient and donor pairs, novel, more sensitive and reproducible assays have been introduced. Below we describe the assays used in HLA antibody detection.

1.Complement-Dependent Cytotoxicity

Upon introduction of this test for crossmatching by Terasaki in 1964, it quickly became established as a crucial and non-negotiable pre-transplant test predicting hyperacute rejection, with a negative result enabling renal transplantation to proceed.

This test consists of incubating patient's serum with potential donor lymphocytes separated from peripheral blood or spleen with subsequent addition of rabbit serum as a source of complement. If recipient serum contains HLA-DSA, they will bind to antigens on lymphocytes resulting in cell lysis via the classical complement pathway. The percentage of dead cells can then be scored via two-colour fluorescence microscopy.

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CDC is less expensive and effectively avoids hyperacute rejection upon transplantation. However, it lacks sensitivity compared to novel techniques for detecting HLA-DSA. Furthermore, viability of donor cells is vital for accurate CDC assay. However, optimal viability may not be achievable in case of deceased donors. As well as clinically relevant IgG antibodies, the test detects IgM, auto antibodies and non-HLA antibodies where corresponding antigens are expressed on donor cells. Due to these inherent disadvantages and its general lack of sensitivity to identify lower levels of HLA-DSA, CDC has gradually been replaced by novel assays.

Modifications were made to the assay to enhance the specificity by using 1,4-dithiothreitol (DTT), which breaks down IgM, although this can also result in some loss of IgG antibody,. Addition of antihuman globulin (IgG) to enhance DSA binding, lengthening of incubation time and extra wash steps to remove anti-complement factors, which may give false negatives, can also be employed to increase sensitivity,.

2.Flow cytometry

Garavoy et al introduced the flow cytometry crossmatch (FCXM) into modern clinical practice. During this test, donor cells are incubated with recipient serum followed by addition of a fluorescent -labeled second anti-human IgG immunoglobulin which fixes to recipient's antibodies bound to donor cells. Subsequently, the test is read on a flow cytometer with the degree of positivity being expressed as a channel shift.

FCXM is a more sensitive than CDC for detecting HLA-DSA. Further, FCXM detected antibodies have shown a predictive value regarding post-transplant rejection and graft loss. Positive FCXM with negative CDC is seen in approximately 15% of primary and 30% of second transplants correlated with a higher incidence of early graft loss (<3 months), rejection and poorer 1-year allograft survival for both primary, and second transplants.

The significance of a positive FCXM result with a negative CDC could be due to presence of low-level HLA-DSA, presence of non-complement fixing antibodies or presence of low level non-HLA antibodies. To confirm the presence and specificity of HLA antibodies a solid phase assay like Luminex testing is often used. Many studies show that, if no HLA-DSA is detected using Luminex, then a positive FCXM has no impact on allograft survival .

One important limitation in FCXM based crossmatching is that there is considerable inter-laboratory variability in methods and cut off values making results between laboratories discordant.

With the introduction of solid phase assays, particularly single antigen bead (SAB) technology which detects low levels of HLA antibodies, FCXM is used in many centers to assist clinical decision making. For example, in the presence of a weak HLA-DSA detected by SAB, the decision to proceed transplant may be influenced by the FCXM result, depending on the degree of immunological risk warranted for a particular patient.

3.Solid Phase Antibody Detection Assays

3.1.Enzyme-Linked Immunosorbent Assay (ELISA)

In this assay, HLA antigens are immobilized in the wells of microtiter trays. Recipient serum is added followed by addition of an enzyme attached anti IgG antibody and a chromogenic substrate. Presence of HLA antibodies will result in a color change of the wells.

The ELISA technique is more sensitive than CDC or FCXM in detecting HLA antibodies. ELISA identifies HLA-specific antibodies and further differentiates between anti-HLA class I and II antibodies. Moreover, it allows retrospective crossmatch using frozen sample, especially in deceased donations.

The inability to differentiate between CB and non-CB antibodies is a potential shortcoming of this assay. Although, this technique was utilized effectively for detecting pre and post-transplantation antibodies, currently its mostly superseded by the SAB technology,.

3.2. Luminex Bead Technology

In the late 1990s, a new solid phase assay using fluorescent labeled bead technology began to be introduced, transforming HLA antibody testing. Currently available commercial kits (One Lambda, Immucor) consist of beads impregnated with different ratios of two fluorochromes generating a unique signal for each set of beads. Depending on the kits used, beads have either a single or several types of HLA molecules attached.

In order to perform this bead based testing, firstly the recipient's serum is incubated with the beads. In case of presence of HLA antibodies, the serum will react with the bead expressing the corresponding HLA molecule. Upon washing of excess serum, the incubation of beads is done with a secondary antibody, usually a phycoerythrin-labeled anti-human IgG . Then, a Luminex fluorocytometer with dual laser beams is employed to identify either presence of HLA

antibody (positive/negative for HLA class I and II) if using 'screening' kits or to give the HLA specificity and a measure of the antibody level in the sample, if using single antigen bead kits. The degree of fluorescence is stated as mean fluorescence intensity (MFI), which is normalized based on the degree of fluorescence perceived with an antibody negative serum and beads with no HLA molecules attached.

The definitive Luminex assay utilizes SAB technology where beads bound with single HLA molecules are produced by recombinant technology which enable characterization of a patient's HLA antibody profile in a few hours. This technology is the most sensitive means of detecting HLA antibodies and currently regarded mandatory for the pre-transplant testing of sensitized patients.

In 2008, Billen et al. introduced beads for donor-specific cross-matching using Luminex assay. Yet, to date, the published literature doesn't support the Luminex cross match as a stand-alone method for organ allocation.

Interpretation challenges of Luminex SAB

Although, Luminex SAB is considered as the gold standard HLA antibody detection assay, there are many inherent challenges in interpretation of results.

First of all, with SAB assays being very sensitive, this raises problems in interpretation in the context of a negative FCXM. The clinical relevance of these HLA antibodies in rejection in organ transplantation is debated,. Secondly, there is no consensus on "cut off" value for MFI positivity. Hence, it is obligatory for each laboratory to establish their own MFI "cut off" levels based on the experience gained over time. Thirdly, SAB express denatured molecules in addition to native molecules as a result of the assay manufacturing process which may confuse assay interpretation. The denatured molecules could express cryptic epitopes which are not typically accessed by antibody molecules. Antibodies reacting with these exposed cryptic epitopes on denatured molecules have been spotted in individuals inclusive of non-sensitized males. It has been proven that the antibodies to denatured epitopes have no clinical effect in renal or heart transplantation. Fourthly, the difficulty in differentiation of complement versus non complement biding antibodies raises a question of clinical relevance. In 2010, Chin and colleagues modified the assay enabling detention of complement binding antibodies eliminating this obstacle. However, the clinical utility of using complement modified Luminex assays is still debated, with many studies indicating that the ability of HLA antibodies to give a positive signal in these assays is often just a function of the strength of the antibody.

Finally, another technical challenge of using SAB is the prozone effect. Here, a diluted serum gives a greater MFI than the undiluted serum, signifying an inhibitory effect which is removed by dilution. Inhibitory effect of IgM or complement component, C3 is postulated to be the cause. This can be overcome by heating serum or adding ethylenediaminetetraacetic acid (EDTA) to destroy complement or DTT to eliminate IgM.

The Virtual Cross-match

The utilization of highly specific and sensitive methods like Luminex SAB allows the characterization of a complete picture of the HLA immunization status of an individual patient. The "virtual crossmatch" (VXM) was hence established to predict the outcomes of a final crossmatch. Bielman et al. depicted that there was 86% concordance between the VXM and FXCM. VXM predicts the potential cross match negative donors, based on the HLA antibody profile of a patient and donor typing information. This methodology was successfully incorporated in to the kidney transplantation allowing transplants to proceed quickly with saving costs for testing and staffing.

The use of a VXM approach reduces laboratory workload, improves allocation efficiency and increases the rate of transplantation for sensitized patients and helps facilitate national paired exchange programs.

The potential challenge for VXM is the novel appearance of a DSA after a sensitization event, which was absent at the time of data gathering for VXM. Further, potential donors can be mistakenly excluded because of VXM false positives due to weak HLA DSA. In contrast, false negative VXMs can occur as solid-phase assays cannot necessarily accommodate all potential HLA antigens .

PRA and c PRA

PRA was used historically as a measure of the broadness of sensitization in transplant recipients . Traditionally, the recipient sera were tested against cells from a panel of HLA-typed donors using CDC. PRA was calculated as the percentage of positive reactions in the cell panel. A high PRA suggested that a candidate will immunologically react against a large section of the population. Bray et al. illustrated that PRA levels per se were not associated with allograft survival as long as HLA-DSA was not present which has subsequently been shown in other studies.

Furthermore, the introduction of SAB lead to a more precise definition of HLA antibody specificities in a recipient,

making the use of PRA obsolete and leading to development of a new formula known as calculated PRA (cPRA), or in the UK, calculated Reaction frequency (cRF)

The cRF is calculated as the proportion of donors which will be incompatible with the listed unacceptable HLA antigens of a prospective recipient. This is calculated against a pool of 10 000 actual deceased donors, and thereby gives an estimation of being allocated a suitable donor. The use of cPRA or cRF is a standardized method of estimating the possibility of the recipient having DSA via measuring the difference between antibody specificities and the prevalence of HLA alleles in a target population.cPRA reflects the broadness of sensitization more precisely and has a lower center-to-center variability matched with PRA.

As precise determination of DSA can be done, SAB technology has also revolutionized pre-transplantation risk assessment. This questions the utilization of broadness of sensitization hence, cPRA to predict the occurrence of rejection and allograft loss in an era with modern DSA assignment. Despite this ambiguity, cPRA is still widely used when deciding the choice of immunosuppression. But the new evidence suggests that cPRA values per se do not confer an immunological risk in absence of DSA. Whereas, HLA-DSA can be regarded as the most significant immunological pre-transplantation risk factor, the cPRA is mainly utilized to heighten access of sensitized patients to appropriate organs allocation systems.

Non HLA antibodies

Even though the main targets of allograft immune response are the highly polymorphic HLA antigens, the contribution of non-HLA antigens is also implicated as evidenced by the development of ABMR in HLA matched organ transplantation between HLA matched siblings,. Non-HLA antibodies can be autoantibodies or alloantiboidies. Human Neutrophil antibodies (HNA), MHC Class I-related Chain A (MICA) Antibodies, Anti-endothelial cell antibodies (AECA), Antibodies Against Glomerular Basement Membrane, Peroxisomal Trans-2-enoyl-CoA Reductase (PECR), Phospholipase A2 Receptor (Pla2R) are known non-HLA antibodies.

Most of the well-studied non-HLA target antigens are not expressed on lymphocytes, hence are not detected on traditional lymphocyte (CDC/FCXM) crossmatch. Therefore, positive crossmatches in the absence of Luminex detected HLA-DSA may not be immunologically relevant and should not preclude a transplant.

Although there are numerous studies demonstrating role of non HLA antibodies in rejection and adverse graft outcomes, currently there is no consensus in the transplant community as to which patients to screen, the frequency of testing, when and how to desensitize or treat in situations of Non-HLA antibody identification . Further research is required to define these in future.

Conclusion

The field of transplant immunology has advanced immensely over the past few decades. The presence of development of de novo DSA plays a central role in causation of graft loss hence characterization and quantification is of foremost importance. Multidisciplinary approach with the involvement of transplant physician, surgeon and clinical immunologist should be employed in careful Interpretation of different immunological tools and deciding on immunological risk of proceeding with transplantation.

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