EDUCATIONAL COMMENTARY – THE HUMAN LEUKOCYTE ANTIGEN SYSTEM’S ROLE IN IMMUNOLOGY AND TRANSPLANTATION

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Learning Objectives

On completion of this exercise, the participant should be able to
- explain the structure and function of the human leukocyte antigen (HLA) system;
- discuss the utility of HLA matching for transplant purposes;
- differentiate between HLA typing assays used for hematopoietic cell transplant and solid-organ transplant;
- define the value of using newer sequencing technologies for HLA typing; and
- discuss the challenges with solid-phase HLA antibody immunoassays.

Introduction

The major histocompatibility complex is a region of the genome encoding a variety of molecules which play critical roles in immune function, host defense, and transplant. In humans, this complex is called the human leukocyte antigen (HLA) region and contains more than 200 genes located on the short arm of chromosome 6. Genes in the major histocompatibility complex are classified into three main groups: HLA classes I, II, and III. HLA class I and class II regions contain cell surface-expressed molecules involved in antigen presentation to T cells that drive host defense and allogeneic immune reactions, whereas the HLA class III–associated molecules are mostly proteins associated with inflammation, such as some complement factors and cytokines.

There are three main HLA class I molecules, HLA-A, HLA-B, and HLA-C, present on the surface of almost all nucleated cells and platelets. Expressed class I HLA molecules consist of one polymorphic α heavy chain associated with one β-2 microglobulin. The class I α chain contains a transmembrane region and three extracellular domains: the α1 and α2 domains form a pocket that binds peptide antigen derived from within the cell, to present the antigen to the T-cell receptor of CD8+ cytotoxic T cells.

Human leukocyte antigen class II molecules are mostly restricted to specialized antigen-presenting cells, such as macrophages, dendritic cells, and B cells. There are also three main cell surface–expressed
class II HLA molecules, HLA-DR, HLA-DQ, and HLA-DP. Human leukocyte antigens class II are heterodimeric proteins with α and β chains combining to make an intact molecule. Each chain contains two extracellular domains, with the α1 and β1 domains creating a pocket that binds peptide antigens derived from extracellular proteins for presentation to the T-cell receptor of CD4+ helper T cells.

Genes within the HLA system are among the most polymorphic in humans, with more than 15,000 HLA class I variants (HLA-A, HLA-B, HLA-C). In HLA-class II, β chains are more polymorphic than the α chains, with more than 5,000 HLA-DRB1, HLA-DQB1, and HLA-DPB1 allele variants identified to date. This variation within the HLA genes allows the immune system to recognize and respond to a wide variety of foreign substances but also complicates the nomenclature of the gene system. Traditionally, HLA molecules were defined by serologic methods with a letter indicating the locus and a number identifying the antigen specificity named in the order discovered, such as HLA-A1 and HLA-A2. With the advent of molecular methods for HLA typing (discussed in more detail below), the nomenclature system changed to incorporate an asterisk to denote that a molecular method was used (HLA-A*01) and now includes colons to separate low-resolution, or antigen-level, typing (e.g., A*02, B*57) from higher-resolution allelic identification (e.g., A*02:01, B*57:01). An additional colon, or third “field,” can be used to distinguish genetic variants that encode identical proteins, and another colon, the fourth field, identifies polymorphisms in noncoding regions of the gene (e.g., A*02:01:02:02).

The HLA genes are inherited together, in sets of linked genes called haplotypes, more frequently than would be expected based on the individual gene frequencies, owing to their chromosomal proximity to one another, a phenomenon known as linkage disequilibrium. Human leukocyte antigen genes are also codominant, meaning cells that express HLA molecules have two copies from each locus expressed on the cell surface simultaneously. Due to their high expression on a wide range of cells and their central role in immune processes, HLA molecules are the most immunogenic antigens that the immune system uses to distinguish self from nonself; consequently, they are critical for the long-term success of allogeneic transplants. Human leukocyte antigen matching is crucial to preventing rejection of solid-organ transplants or nonengraftment of hematopoietic cell transplants (HCTs). The level of HLA typing resolution and relative importance of individual HLA loci for the different transplant scenarios is discussed below.
Laboratory Assays

HLA Typing

Human leukocyte antigen matching for solid organ transplant traditionally relied on low-resolution serology-based typing. Utilizing large pools of patient serum samples specific for known HLA molecules and mixing with patient cells in the presence of complement, one could determine the HLA type of the patient cells based on patterns of cell lysis, which provided a low-resolution result (e.g., HLA-A2). However, serologic typing has many downsides, the largest being that serologic methods cannot differentiate all antigen differences that have significant effects. Consequently, the United Network for Organ Sharing (UNOS) now requires molecular-based methods for all deceased-donor HLA typing.

The molecular methods used for HLA typing have evolved significantly in a relatively short period. Although all methods require polymerase chain reaction (PCR) techniques to amplify target sequences in exons 2 and 3 of the HLA genes, there are subtle differences in the techniques used that affect their application to current clinical HLA typing. Sequence-specific priming (SSP) uses DNA primers to amplify key sequences in the HLA genes followed by gel electrophoresis for interpretation. This technique is relatively fast and requires minimal equipment, but many individual reactions are involved and therefore a large amount of DNA is needed to achieve a high resolution result. Additionally, poor quality DNA or low concentrations may lead to weak or no reactions, complicating the interpretation of results. In current practice, SSP is mostly used to help resolve typing ambiguities that arise from other molecular-based methods.

Sequence-specific oligonucleotide probe hybridization (SSOP) is another DNA-based method and is in wide use today. This technique also utilizes PCR DNA amplification, and then hybridizes the DNA to oligonucleotide-labelled probes specific for sequences that enable interpretation. The main advantage to SSOP vs. SSP is the increased throughput and the capability for semiautomated interpretation through solid-phase microarray analysis. Sequence-specific oligonucleotide probe hybridization is also fast and can be used in high-throughput settings, but it suffers from the limited available probe-hybridization sites and limitations of sequence phasing, resulting in an intermediate level of resolution. The intermediate resolution of SSOP results from its reliance on common HLA allele sequences. By contrast, SSP is capable of a higher level of resolution through allele-specific amplification.

Real-time PCR-based assays are used in most laboratories performing deceased-donor HLA typing due to the need for rapid results. This technique also utilizes PCR but can perform a large number of
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Simultaneous reactions from all the necessary loci in a 384-well format to provide a greater level of discrimination than SSP or SSOP, and in a more rapid fashion. Using reporter dye incorporation and automated real-time reading greatly reduces the need for postreaction processing and interpretation. The main disadvantage of this technique is its low resolution.

Anti-HLA Antibodies

Another critical element in the determination of histocompatibility between a donor organ and a recipient is the level of anti-HLA antibody sensitization in the recipient. On the deceased-donor waitlist there are many patients sensitized to HLA molecules through previous transplants, transfusions, or pregnancies, and UNOS considers HLA sensitization status for organ allocation. Anti-HLA antibody data is uploaded to the UNOS system and used to assign “unacceptable antigens,” for which a patient is expected to be incompatible. A numerical value based on HLA allele frequencies in the general population, called the calculated panel reactive antibody, is calculated for each patient based on their anti-HLA profile and is a relative descriptor of how sensitized individual patients are. The individual unacceptable antigens are then used to eliminate the patient from donor offers.

Laboratory detection of anti-HLA antibodies began using cytotoxic cell-based assays, where recipient serum was incubated with cells of known HLA type in the presence of complement, and cytotoxicity reactions were used to determine the presence of anti-HLA antibodies. This process was laborious, slow, and required many cell lines of known and variable HLA types. Current testing uses solid-phase bead-based flow cytometric techniques, which have revolutionized anti-HLA antibody detection. Individual latex beads (100 total beads) have unique fluorescent dye combinations that can be differentiated and identified through a flow cytometric instrument. The beads’ specificities are used to determine anti-HLA antibody specificity. Although solid-phase assays provide many advantages compared with cellular-based anti-HLA antibody detection assays, they also have some limitations. The HLA molecules used on the individual beads are derived from recombinant sources and can become unstable and express protein epitopes that are not typically seen in HLA on actual cells, leading to false-positive results. Another limitation of these assays is that the HLA density on the beads may be different from lot to lot and different from the HLA density on actual cells, which could affect the interpretation of the results.

High-Resolution HLA Typing: the Transition to Next-Generation Sequencing

To achieve high-resolution HLA typing, assays target regions beyond the antigen-binding domains of the HLA loci. The antigen binding domains are defined as exons 2 and 3 for HLA class I loci and exon 2 for HLA class II loci. Those regions harbor the majority of the diversity for the HLA region and determine the
binding capacity for a particular HLA allele. High-resolution HLA typing is performed using Sanger sequencing. Sanger sequencing assays target the antigen-binding domains of the HLA loci necessary for HCT. Those HLA loci include HLA-A, HLA-B, HLA-C, and HLA-DRB1. Some transplant centers also utilize HLA-DQA1 and HLA-DPB1. Sanger sequencing is limited owing to its inability to resolve cis/trans ambiguities, which are the result of simultaneous nucleotide incorporation for DNA templates being sequenced. In 2005, the National Marrow Donor Program established regulatory requirements for high-resolution typing of HLA-A, HLA-B, HLA-C, and HLA-DRB1, leading to many HLA laboratories spending considerable resources on resolving cis/trans ambiguities. Those ambiguities resulted in approximately 53% of cases requiring additional testing. New technology such as next-generation sequencing (NGS) has been employed to reduce ambiguities and improve sample throughput. As a result, HLA laboratories are beginning to shift away from Sanger sequencing to NGS assays.

Benefits of Next-Generation Sequencing

As previously mentioned, the wide diversity of HLA alleles requires multiple primers and probes to ensure adequate detection of HLA alleles. Many HLA laboratories use traditional Sanger sequencing; however, this approach is limited because assays target the antigen-binding domains of HLA class I and II and can lead to cis/trans ambiguities. The limited scope of Sanger sequencing assays for HLA typing leads to a continually increasing ambiguity rate due to newly discovered HLA alleles. Although the newly named HLA alleles are often rare, if they are potentially paired with a common or well-documented HLA allele, HLA laboratories are required to resolve that ambiguity per clinical regulations.

Owing to the ambiguity rate of Sanger sequencing, beginning in 2015 some HLA laboratories began using NGS for HLA typing. Next-generation sequencing is a massive parallel sequencing in which several samples are combined and sequenced simultaneously. For HLA typing, amplicons can be generated for the full gene of HLA loci and then prepared for sequencing. The library preparation process randomly generates shorter fragments, which can be stitched together after the sequencing process is complete to reconstruct the full gene. Most important, NGS enables resolution of cis/trans ambiguities that Sanger sequencing cannot. In addition, rather than a limited number of HLA loci, now all major HLA loci can be genotyped in one assay with relatively infrequent ambiguities. The result is faster and cheaper HLA genotyping overall.

Challenges of Next-Generation Sequencing

Although NGS has presented HLA laboratories with numerous benefits, NGS presents unique challenges for clinical laboratories. There are preanalytical considerations such as specimen types and DNA
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concentration; there are analytical considerations regarding validation of analysis pipelines and quality control; and there are postanalytical considerations regarding reporting of HLA typing information. Next-generation sequencing technology enables multiplexing of samples, and thus there is concern about sample mix-ups during amplification steps or DNA isolation. Given that NGS has a higher sensitivity than Sanger sequencing, there is concern that contamination may have an impact on patient results.

Analytically, NGS has the potential for amplification bias and loss of heterozygosity (allele dropout). Although many of these errors are rare (approximately 1%-3%), they still represent an opportunity for incorrect results and adversely affecting patient care.

Each laboratory approaches these challenges differently, and effective policies can be instituted to avoid misreporting patient results. Several laboratories use quality control metrics for NGS to determine the suitability of results before analysis, in addition to analysis of quality control metrics. Such a tiered approach has the advantage of detecting errors at various stages in the NGS assay workflow.

Solid-Organ Transplants

Human leukocyte antigen typing for solid-organ transplant is necessary to determine recipient-donor compatibility. Currently UNOS, the government-contracted administrator of the Organ Procurement and Transplantation Network, requires all potential kidney- or pancreas-only solid-organ transplant recipients, as well as all potential recipients of multiorgan transplants that will include a kidney, to be HLA typed at HLA-A, HLA-B, and HLA-DR loci at a minimum. Human leukocyte antigen typing of potential recipients for other single-organ transplants is not a current requirement. The United Network for Organ Sharing also requires all deceased donors to be typed at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3/4/5, HLA-DQB1, and HLA-DPB1. Kidney allograft survival has been shown to be improved with HLA matching, specifically HLA matching at HLA-DRB1 and HLA-B matches. However, although a large study using UNOS data confirmed the importance of HLA matching on graft survival, it also showed the risk of allograft failure with an HLA mismatch was independent of the locus.

Hematopoietic Cell Transplant

In patients with severe hematologic disease (benign or malignant), HCT can be a life-saving intervention. Owing to the importance of HLA and extensive polymorphisms within the HLA region, the ideal donor is a patient’s family member, particularly a sibling. Because HLA alleles are inherited as haplotypes from each parent, the possibility of a full HLA match is 25% for each sibling, and the possibility of a “haploidentical” match (single haplotype in common) is 50%. Low-resolution typing at HLA-A, HLA-B, and
HLA-DRB1 is generally sufficient to identify potential matches for related donors, owing to linkage disequilibrium.

The advent of high-resolution (allele-level) HLA typing introduced a new level of specificity to this process. Human leukocyte antigen typing by molecular methods is the only acceptable HLA typing method allowed for HCT purposes. In addition to the aforementioned SSP and SSOP typing methodologies, Sanger sequencing is an additional tool especially used to determine bone marrow donor and recipient HLA types at the allele level.

Although an identically matched related donor is ideal for HCT, such a match is only found for approximately 15% to 30% of recipients. If a matched related donor is not identified, HCT using a matched unrelated donor can be a viable option. High-resolution matching of HLA-A, HLA-B, HLA-C, and HLA-DRB1 alleles is correlated with better overall survival, with mismatches linked to increased rates of treatment-related mortality and acute graft-vs-host disease. The level of the mismatch (allele vs. antigen) has no impact on HCT outcomes. Additional studies have demonstrated that expanding HLA matching criteria to HLA-DQB1 and HLA-DPB1 can improve outcomes. However, the linkage disequilibrium between HLA-DRB1 and HLA-DQB1 means there are rare occurrences of patients who match at DRB1 but are mismatched at DQB1. Conversely, given the distance between HLA-DRB1, HLA-DQB1, and HLA-DPB1, 80% of unrelated donors will have a mismatch at DPB1.

Summary

The major role of HLA in the immune response is initiating an immune response through antigen presentation. Human leukocyte antigen molecules are classified as class I (HLA-A, HLA-B, HLA-C), class II (e.g., HLA-DRB1, HLA-DQB1, HLA-DPB1), or class III (e.g., complement, TAP1, TAP2). Regardless of type of transplant, HLA matching has been demonstrated to improve patient outcomes. The HLA loci and level of HLA matching necessary are transplant-type dependent. Assays to detect HLA antibodies have drastically improved sensitivity since introduction of the solid-phase immunoassay. The increased sensitivity enables easier detection of donor-specific antibodies and facilitates antibody-mediated rejection diagnosis. However, solid-phase immunoassays have challenges regarding stability and variable HLA expression. Human leukocyte antigen typing assays are transitioning from serology, SSP, SSOP, and Sanger sequencing to NGS-based assays. Although SSP, SSOP, and Sanger sequencing are used by HLA laboratories, the benefits of NGS technology for HLA typing have reduced the HLA typing burden. Next-generation sequencing–based HLA typing presents significant advantages over other molecular-based methods but offers additional challenges. Next-generation sequencing requires
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clinical laboratories to implement strict, evidence-based quality control parameters to ensure accurate patient results.

References


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