CCTC Clinical Chemistry Trainee Council | AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: "HLA: Basic Terminology and Nomenclature."

PRESENTER: Gizem Tumer, MD

Slide 1:

Hello, my name is Gizem Tumer. I am an Assistant Professor in Laboratory Medicine and Pathology and one of the medical directors of the HLA laboratory at the University of Minnesota. Welcome to this Pearl of Laboratory Medicine on "HLA: Basic Terminology and Nomenclature."

Slide 2:

What is the definition of HLA or Human Leukocyte Antigen?

HLA refers to a gene complex that encodes the major histocompatibility complex (MHC) proteins in humans. In humans, MHC is named HLA for Human Leukocyte Antigens because these gene products were first described as proteins expressed on leukocytes. These proteins were causing agglutination reactions when leukocytes were incubated with serum from multiparous women or people that have been transfused (similar to the erythrocyte agglutination observed on ABO typing assay). These proteins are cell surface glycoproteins expressed in almost all cells in the body. The HLA gene loci are located on the short arm of the chromosome 6.

Slide 3:

What are the characteristic features of HLA?

HLA is highly polygenic meaning that it is composed of many genes, which broadly can be divided into three categories: Class I, Class II, and Class III.

HLA is also highly polymorphic. Polymorphism is a term that refers to the multiple variations of antigens or alleles. As an example: HLA B*27:05 vs B*27:08 are 2 distinct alleles. HLA class I and class II antigens have the most highly polymorphic structural genes found in humans. This

means that the sequence of amino acids in any given HLA molecule varies slightly from one person to the next. Literally, hundreds of different sequence variants are seen in the human population. This variation generates distinct HLA types and also causes an allograft rejection when tissues are transplanted.

It also has many functions. In other words it is Pleiotropic. The main function is the distinction of self from non-self. T lymphocytes interact with peptide antigens when Complementarity-Determining Region 3 (CDR3) of T-cell receptor (TCR) engages both the HLA molecule and the antigenic peptide present within the antigen-binding groove of the HLA molecule. Normally, TCRs recognize foreign antigenic peptides presented on the HLA molecule, but not self-antigens. TCRs can recognize peptide antigens only if they're presented on particular MHC molecules, which is known as "MHC restriction. Thus, MHC functions as a major player in the immune response such as immune surveillance, vaccines, autoimmunity and transplantation with orchestration of T cells, B cells and macrophages.

Slide 4:

The MHC genes that act as transplantation antigens are referred to as classical HLA genes. The classical HLA class I genes (HLA-A, -B and -C) are expressed in most of the somatic cells in the body. Classical HLA Class II genes (HLA-DR,-DQ, -DP) are expressed on antigen presenting cells such as B- cells, Activated T cells, Macrophages, Dendritic cells and Thymic epithelial cells. If interferon is present, other cell types can also express class II HLA molecules. The Class I molecule is composed of one polypeptide chain and a B2 microglobulin chain. Whereas, the class II molecule has 2 polypeptide chains. Alpha1 and alpha2 domains form the peptide binding site for class I; alpha1 and beta1 domains form the peptide binding site for class II.

One important function of the HLA class I molecule is to display peptides that result from the degradation of cytosolic proteins to the cell surface where they can be recognized by the CD8+T cells. CD8 binds to the alpha3 region of the class I molecule and presents peptide antigens to CD8+T cells.

HLA class II molecules display peptides that result from the degradation of *endocytosed* proteins to the cell surface where they can be recognized by the CD4+ T cells. CD4 binds to the beta2 region of the class II molecule.

Class I and class II molecules also differ in the number of amino acids that they can accommodate in the peptide-binding groove.

Slide 5:

This cartoon image shows the structure of Class I and Class II molecules. The B2 microglobulin chain of the class I molecule is encoded by a gene on chromosome 15. The alpha polypeptide of the class I molecule is encoded by the class I genes. Class I and Class II molecules are structurally and functionally different. The alpha chain has 5 domains: 2 peptide-binding domains (alpha1 & alpha2), one alpha3 domain, the transmembrane region, and the cytoplasmic tail. The alpha3 domain penetrates the cell membrane, whereas B2 microglobulin does not. Alpha1 and alpha2 domains contain the majority of polymorphic regions conferring HLA antigen specificity, which are the outermost domains.

The class II genes encode for the alpha and beta polypeptide chains of the class II molecules. Each alpha and beta chain of the class II molecule has four domains: the peptide-binding domain (either alpha1 or beta1), either alpha2 or beta2 domain, the transmembrane region, and the cytoplasmic tail. Both alpha and beta chains traverse the membrane. The extramembranous portion of each chain has two amino acid domains, of which the outermost domain contains the variable regions of the Class II alleles

Slide 6:

After this brief review of the HLA molecule, let's start understanding the historical nomenclature.

- Initially serology methodology was used to describe the polymorphism of the class I and class II loci.
- New HLA antigens were defined at the international workshops with the extensive exchange of typing reagents between laboratories and HLA scientists exhaustively examining the reactivity of antisera, which were submitted as defining a particular specificity.
- When an HLA antigen was established as reproducibly defined, it was given a formal designation to be used by all laboratories which consisted of a number preceded by a workshop "w" designation.
- When sufficient antisera were available for a particular specificity and most laboratories in the world had gained experience in accurately defining the specificity, the "w "designation was removed from all loci.
- The "w" designation was also removed from the C locus alleles but will remain part of the antigen nomenclature to avoid confusion with the complement factors in the most current nomenclature.

Slide 7:

What are the challenges of converting serologic typing into molecular typing?

Due to the improvements in serologic techniques, some of the antigens defined as combinations of closely related antigens, which could be distinguished serologically. For example, HLA-B15 consists of 8 closely related specificities, HLA-B62, B63, B70, B71, B72, B75, B76, and B77.

Serologic supertypes are the broad specificities. Eg: B15

"Splits" or Subtypes are the finer specificities that comprised the supertype. Eg: B62.

As the splits were discovered, they were given number designations that again give no indication as to the supertype to which they belong. Eg: Molecular B*15:12 is equivalent of HLA-B76.

Slide 8:

The assigned numbers reflect the chronological order of discovery rather than a systematic numbering system.

To the uninitiated, the HLA numbering system is not something that can be deduced by studying the designated numbers of the supertypes and subtypes, and to those who work in the field, it is something that must be learnt.

Slide 9:

Let's now move on to learn more about the current HLA nomenclature. All HLA antigens are uniformly named starting with the locus, antigenic specificity, and molecularly typed allele group. The asterisk "*" sign indicates that typing is performed by a molecular method and the colon ":" is a field separator. Now, I will walk you through this example. A*03 is low resolution typing by molecular method. In most cases this is equivalent to the antigen.

Slide 10:

A*03:01 is the allele and the 1st field (A*03) refers to a group of alleles that encode for the A3 antigen. 2nd field (:01) refers to an allele, which encodes a unique HLA protein (A*03:01). Next we will describe the 3rd field designation. This 3rd field refers to a synonymous mutation. This is a change in the DNA sequence that codes for amino acids in a protein sequence, but does not change the encoded protein. A*03:01:01 vs A*03:01:02 difference is due a synonymous (silent) mutation.

Slide 11:

The 4th field is reserved for non-coding regions. Finally, expression modifiers follow after the 4th field. An example includes Null alleles, which are not expressed are noted as capital N.

Slide 12:

We finished discussing the nomenclature. Now let's learn about a few common serologic terms utilized to identify the HLA antibodies and define their serum reactivity.

Epitope (Antigenic determinant): is the minimum structural unit composed of a few amino acids in the HLA antigen that can be recognized by a B or T cell receptors.

Private Epitopes: Epitopes that are present only on a single gene product such as HLA-A2.

Public Epitopes: Refers to epitopes that are shared by more than one HLA antigen. HLA antibodies will show reactivity with the antigens that contains the public epitope.

Cross-reactive groups (CREGs): is basically a group of antigens that share a public epitope, as demonstrated by the ability of a specific antibody to react with all of them.

Slide 13:

HLA is an important barrier for hematopoietic stem cell (HPC) transplantation. Therefore, HLA matching and compatibility between the donor and the recipient is required for a successful HPC transplantation. HLA antigens also have role in graft- versus-host disease (GVHD), a potentially serious complication of allogeneic stem cell transplantation. GVHD occurs when donor T cells react to host antigens on antigen-presenting cells (APCs) and attack host tissues, with sequential activation of donor T cells and monocytes/macrophages.

In solid organ transplantation, HLA antibodies play an important role and HLA matching is not as important. When patients are exposed to foreign HLA antigens due to pregnancy or blood transfusions or transplantation, they can make antibodies against epitopes of those foreign HLA molecules. This is called HLA alloimmunization.

Presence of HLA antibodies against graft can cause antibody mediated rejection and graft loss.

HLA alloimmunization can also causes platelet refractoriness. This condition creates difficulty in finding compatible platelet units especially in transfusion dependent HPC transplant patients. In platelet refractoriness HLA Class I antibodies especially antibodies against HLA-A and HLA-B play an important role.

Slide 14: References

1. Klein J, Sato A. The hla system. First of two parts. Engl J Med 2000;343:702-9.
2. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, et al. Nomenclature for factors of the hla system, 2010. Tissue Antigens 2010;75:291-455.
3. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG. The ipd and imgt/hla database: Allele variant databases. Nucleic Acids Res 2015;43:D423-31.
4. Fung MK, Eder AF, Spitalnik SL, Westoff CM, editors. Technical Manual. 19th Ed. Bethesda (MD): AABB Press; 2017. p.435-44.
5. Rodey GE. HLA Beyond Tears: Introduction to Human Histocompatibility. 2nd Ed. Durango (CO): De Nova; 2000. p.17-25.
6. Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai Cellular and molecular immunology updated 6th Ed page 102.
7. B.D. Tait The ever-expanding list of HLA alleles: changing HLA nomenclature and its relevance to clinical transplantation. Transplantation Reviews 25 (2011) 1–8

Slide 15: Disclosures

Slide 16: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "HLA: Basic Terminology and Nomenclature."