

Review Article

The Human Leukocyte Antigen System ... Simplified

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INTRODUCTION

Human leukocyte antigen (HLA) system is a specialized branch of immunology. Its main role is in the presentation of peptides to the immune system and coordinating cellular and humoral immunity. HLA system has a great role to play in transplants, both hematopoietic stem cell and solid organ transplants including renal transplants. It is also important in transfusion-associated problems such as platelet refractoriness, febrile nonhemolytic transfusion reactions (FNHTRs), transfusion-related acute lung injury (TRALI), and graft versus host disease (GvHD) associated with transplants and transfusions.

It includes genetic system on human chromosome 6 and its protein products situated on white cell surface membranes. The HLA loci are part of the genetic region known as the major histocompatibility complex (MHC). The MHC has genes (including HLA) which are integral to normal function of the immune response. Since HLA antigens are present on most of the tissues of the body rather than just red cells, HLA typing is also described as "Tissue typing." As mentioned by Erik

ABSTRACT

Human leukocyte antigen (HLA) and transfusion medicine go hand in hand. Transfusion medicine experts are involved in transplants, particularly hematopoietic stem cell transplant. A lot of clinical challenges such as febrile nonhemolytic transfusion reactions, transfusion-related acute lung injury, and also graft versus host disease are caused by HLA antibodies. It is a unique genetic system located on chromosome 6 and its protein products situated on white cells. Histocompatibility in transplant scenario is not the only function of HLA antigens but the main role is to present peptides to immune system and regulate cellular and humoral immunity. HLA Class I (A, B, and C) and HLA Class II (DR, DQ, and DP) antigens are different in structure and function. Typing methods have progressed from earlier serology-based techniques to sequence-based typing to next-generation sequencing. Cross matching techniques have also changed from complement-dependent cytotoxicity (which is still considered a gold standard) to microbead-based assay to flow cytometry. Finally, HLA and its disease association has long been established, particularly so in cases of ankylosing spondylitis.

KEYWORDS: *Complement-dependent cytotoxicity, disease association, human leukocyte antigen, major histocompatibility complex, transplants*

Thorsby, histocompatibility is not the only function of HLA antigens, this complex may better be called major immune response complex!^[1]

HUMAN LEUKOCYTE ANTIGENS

HLA antigens are cell surface glycoproteins. Based on their structure and function, there are two classes, Class I and Class II.

Human leukocyte antigen Class I antigens

The cell surface glycopeptide antigens of the HLA-A, HLA-B, and HLA-C series are called HLA Class I antigens. They are expressed on the surface of most nucleated cells of the body. They are found in soluble form in plasma and are adsorbed on the surface of platelets. Only vestigial amounts remain on mature red cell, designated as Bennett-Goodspeed (Bg) antigens.^[2]

Studies indicate that HLA-B (which is the most polymorphic) is the most significant HLA Class I locus,

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followed by HLA-A and HLA-C. Class I antigens have a molecular weight of 57,000 daltons and consist of two chains – a glycoprotein heavy chain (45,000 daltons) encoded on the short arm of chromosome 6 and light chain and β 2-microglobulin molecule (12,000 daltons) encoded by a gene on chromosome 15. β 2-microglobulin is not attached to the cell membrane, it is associated with heavy chain but is not covalently bound to it [Figure 1-Class I]. Out of three α domains, α 1 and α 2 domains contain the polymorphic regions conferring the HLA antigen specificity.

The X-ray crystallography full three-dimensional picture of Class I shows that the molecule has a cleft on the outer surface which holds a peptide.^[3]

This role of Class I antigens to identify the cells which are changed (e.g., viral infection) is the reason why they need to be present on all cells.

Human leukocyte Class II antigens

The cell surface glycopeptide antigens HLA-DR, HLA-DQ, and HLA-DP are termed HLA Class II antigens. They have a molecular weight of approximately 63,000 daltons and consist of two structurally similar α - and β -glycoprotein chains, both of which are transmembrane [Figure 1-Class II].

Each chain has two amino acid domains of which the outermost domain contains the variable region of Class II alleles. Tissue distribution of Class II antigen is confined to immunocompetent cells such as B-lymphocytes, macrophages, endothelial cells, and activated T-lymphocytes. Since Class II antigens initiate a general immune response, they are present on immunologically active cells and not on all tissues. The peptide-binding groove in Class I and Class II molecules is critical for functional aspects of HLA molecules.^[4]

NATURAL KILLER CELL RECEPTORS

There are many different receptors on natural killer (NK) cells that provide activating or inhibitory signals in response to target cells. Human NK cells utilize killer cell immunoglobulin-like receptors (KIRs)

to distinguish the unhealthy targets from the healthy self.^[5,6]

MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I CHAIN-RELATED PROTEIN A AND MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I CHAIN-RELATED PROTEIN B ANTIGENS

The MHC Class I chain-related protein A (MICA) and MICB are highly polymorphic genes which are located in the MHC Class III region. The structure of the MIC molecules has the same homology as classical HLA-A, HLA-B, and HLA-C molecules except there are no binding to β 2-microglobulin and no functional peptide-binding groove. They are expressed on epithelial cells, specially of gastrointestinal tract and on fibroblasts, endothelial cells, monocytes, dendritic cells, and function as stress-induced antigens.^[7]

TERMINOLOGIES

- Splits: Improvement in serologic methods allowed antigens believe to represent a single specificity to be “split” into specificities those were serologically distinct.^[8]

For example, HLA-B5, HLA-B51, and HLA-B52. The parent is written in parentheses like “HLA-B51(5).” Some examples of broad and their split antigens are given in Table 1.

CROSS-REACTIVE GROUPS

Many epitopes are shared by different HLA antigens. Antibodies reacting with these shared determinants of antigens at one locus often cause cross-reactivity in serologic testing. The term for this group is cross-reactive group (CREG) [Figures 2-4]. For example, a cell may react with serum containing antibodies to HLA-A25, HLA-A26, and HLA-A34 and negative for pure A26 and A25. In this case, A34 can be assigned to the antibody.^[9]

PUBLIC ANTIGENS

In addition to splits and CREGs, HLA antigens show common reactivity across different specificities – “Public” as they have common amino acid sequences. Two

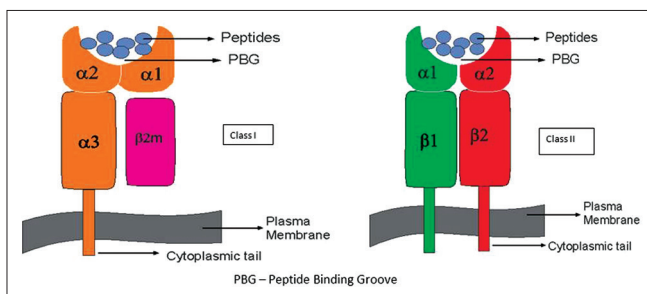


Figure 1: Structure of human leukocyte antigen Class I and Class II molecules

Table 1: Example of broad-specific antigens and their split antigens

HLA Class I						HLA Class II			
HLA - A		HLA - B		HLA - C		HLA - DR		HLA - DQ	
Broad	Split	Broad	Split	Broad	Split	Broad	Split	Broad	Split
A9	A23	B5	B51	C3	C9	DR2	DR15	DQ1	DQ5
	A24		B52		C10		DR16		DQ6

HLA: Human leukocyte antigen

well-characterized antigens are found in HLA-B series, i.e., HLA-Bw4 and HLA-Bw6 [Table 2]. Bw4 is also found on some A locus molecules.^[8]

HUMAN LEUKOCYTE ANTIGEN GENETICS

HLA complex containing 35–40 genes grouped into three regions is located on the short arm of chromosome 6. (Figure 5 - Lima-Junior JC and Pratt-Riccio LR. Major

Table 2: Bw4- and Bw6-associated specificities

Bw4: B5, B5102, B5103, B13, B17, B27, B37, B38 (16), B44 (12), B47, B49 (21), B51 (5), B52 (5), B53, B57 (17), B58 (17), B59, B63 (15), B77 (15) A9, A23 (9), A24 (9), A2403, A25 (10), A32 (19)
Bw6: B7, B703, B8, B14, B18, B22, B2708, B35, B39 (16), B3901, B3902, B40, B4005, B41, B42, B45 (12), B46, B48, B50 (21), B54 (22), B55 (22), B56 (22), B60 (40), B61 (40), B62 (15), B64 (14), B65 (14), B67, B70, B71 (70), B72 (70), B73, B75 (15), B76 (15), B78, B81, B82

histocompatibility complex and Malaria: Focus on plasmodium vivax infection. Front Immunol 2016;7:13). The genomic region is called MHC and is usually inherited *en bloc* as a haplotype.

The Class I gene encodes for the α -polypeptide chain of two Class I molecules, the β chain of the Class I molecule is encoded by a gene on chromosome 15, the β 2-microglobulin gene. Class I gene encodes for the classic transplantation molecules, HLA-A, HLA-B, and HLA-C, it also encodes for additional nonclassic genes – HLA-E, HLA-F, and HLA-G.

HLA-G is expressed by trophoblast and may be involved in the development of maternal immune tolerance of the fetus.

The Class II genes code for the α - and β -polypeptide chains of the Class II molecules. The Class III genes encode many diverse molecules including complement factors.

The proteins coded by DRA and DRB1 genes result in HLA-DR1 through HLA-DR18. The products of A and B3 genes express HLA-DR52, those of A and B4 genes express DR53 and those of A and B5 genes express DR51.^[10]

NOMENCLATURE

Progress from serology to molecular technique has led to nomenclature changes also.

For example, HLA-DR3 is the broadest description of the antigen. It can be divided into DR17 and DR18 using monospecific antisera [Figure 6].^[11,12]

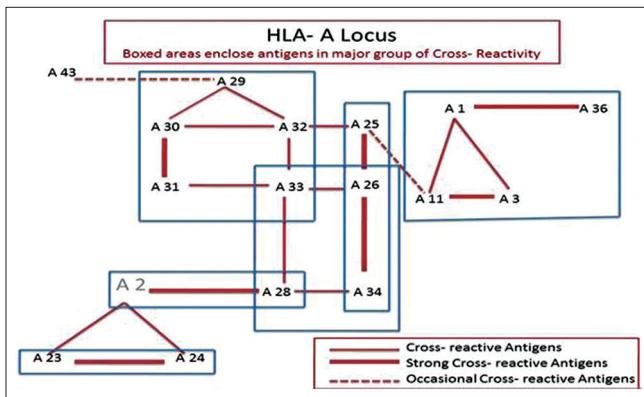


Figure 2: Cross-reactive groups in human leukocyte antigen – A locus

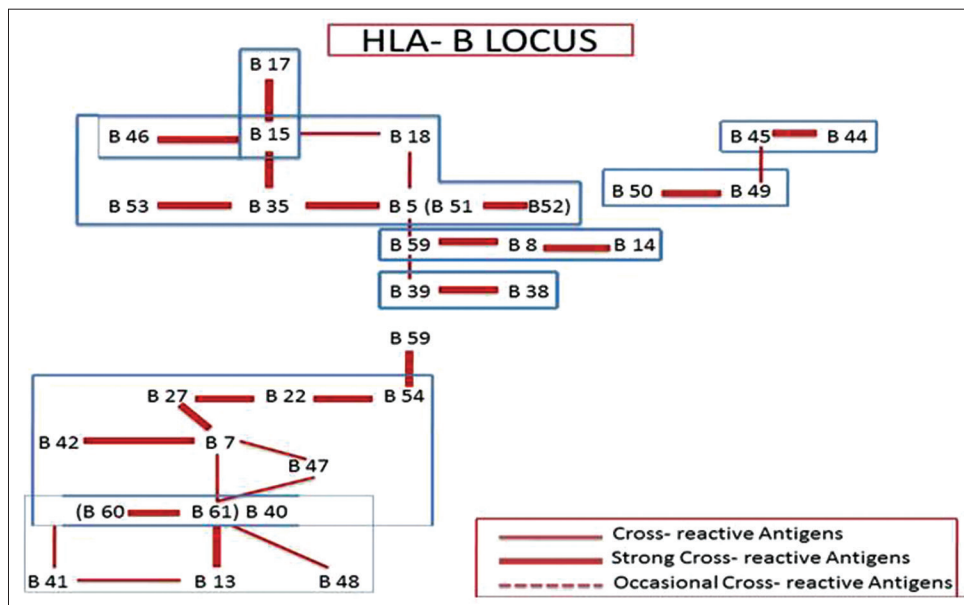


Figure 3: Cross-reactive groups in human leukocyte antigen – B locus

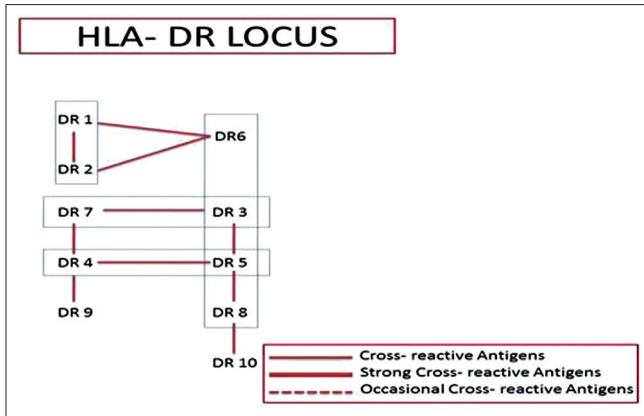


Figure 4: Cross-reactive groups in human leukocyte antigen – DR locus

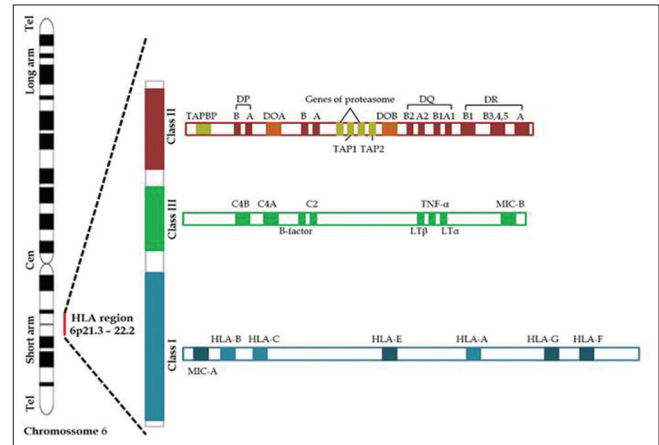


Figure 5: Location and organization of human leukocyte antigen on chromosome 6^[27]

From: *Front. Immunol*, Lima-Junior JC and Pratt-Riccio LR (2016) Major Histocompatibility Complex and Malaria: Focus on *Plasmodium vivax* Infection

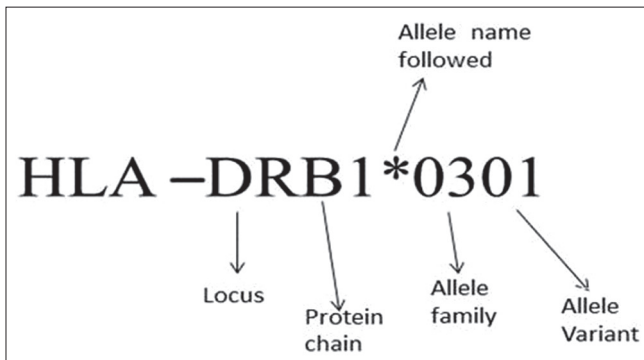


Figure 6: Human leukocyte antigen nomenclature

At DNA level, DR locus is called DRB1 (because of A and B1 genes); the antigen 03 and specific allelic variant 01, so HLA-DR17 is also called HLA-DRB1*0301.

INHERITANCE OF HUMAN LEUKOCYTE ANTIGEN

When the HLA antigens are listed as they are detected by tissue typing technique, it is known as HLA phenotype. There is no way to show which parent has passed on which antigen. However, inheritance follows the established rules of genetics. Every person has two copies of chromosome 6 and possesses two haplotypes, one from each parent. HLA genes are autosomal dominant and co-dominant, the phenotype represents the combined expression of both haplotypes [Figure 7].^[13]

POLYMORPHISM

Polymorphism at the HLA loci is extreme, it is evolved to counter all the different peptides from invading organisms. Each HLA molecule differs from the other in its amino acid sequence.

As of March 2017, the total allele number of HLA loci has reached 16755. HLA-A, HLA-B, and HLA-C have

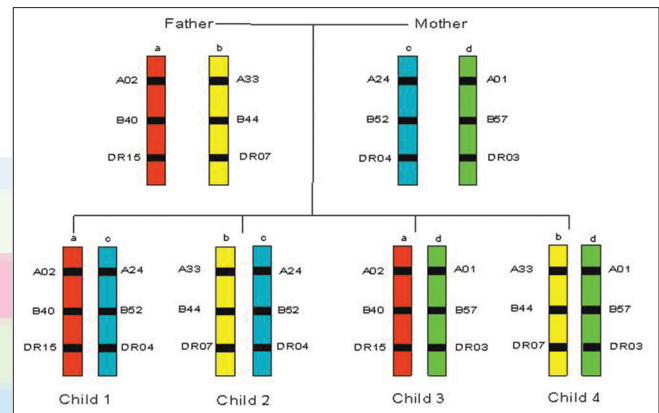


Figure 7: Segregation of human leukocyte antigen haplotypes in family

3913, 4765, and 3510 alleles, respectively. DRA1 site has 7 and DRB1 has 2311 alleles. DQA1 and DQB1 have 78 and 1079 alleles, respectively.

However, polymorphism is also population specific.

In Indian population, the common haplotypes are as follows:

- i. A26-B8-DR3
- ii. A1-B57-DR15
- iii. A2-B44-DR15
- iv. A2-B60-DR15.

Two loci haplotypes, i.e., A11-B35/A24-B40/DR3-DQ2/DR15-DQ6/DR4-DQ3 are observed with appreciable frequency. From the point of view of transplantation, it is difficult to match HLA types between two populations.^[14]

LINKAGE DISEQUILIBRIUM

Frequency of alleles at one locus should not affect the frequency of allele at another locus; however in HLA

genetics, this is not true. A classical example is A1, B8, DR3, DQ2 haplotype seen in Northern Europeans, this occurs in very high frequency than expected.

For Class II also, this phenomenon is pronounced as specific DR allele can be used to predict the associated DQ allele [Table 3]. This type of haplotype allele selection in population may be advantageous in “immunological” sense so that they have a positive selective advantage.^[15]

HUMAN LEUKOCYTE ANTIGEN CLASS I

Peptide antigens that fit into peptide-binding groove of HLA molecules are typically 8-9 amino acids in length and are derived from proteins which may be self-protein, altered self-proteins such as found in cancer cells, and viral proteins found in viral-infected cells. They are degraded by LMPs (large multifunctional proteases) and transported to endoplasmic reticulum (ER) by a transporter associated with antigen processing (TAP). Class I molecules with peptides are transported to cell surface where they are available to interact with CD8-positive T-lymphocytes. If TCR of a T-lymphocyte can bind antigenic peptide, it elicits an inflammatory response [Figure 8A].^[10]

HUMAN LEUKOCYTE ANTIGEN CLASS II

Class II molecules are synthesized in ER but peptide antigens are not inserted here in peptide-binding groove. Instead, an invariant chain (Ii) is inserted which acts as a stopper.

Class II invariant chain molecule is transported to endosome where the invariant chain is removed by

another molecule DM (its locus is also in MHC). Class II antigenic peptide is then inserted into the groove (they are normally 12 to 25 amino acids in length). These peptides are derived from exogenous proteins which may be normal self-proteins or derived from bacteria. Class II molecules are transported to cell surface where they react with CD4-positive T-lymphocytes, resulting in the production of antibodies [Figure 8B].^[10]

HUMAN LEUKOCYTE ANTIGEN TESTING

HLA laboratories perform various HLA tests to support transplant programs, these include HLA typing of recipient and prospective donor, HLA cross matching, HLA antibody screening, and HLA antibody identification.

METHODS FOR HUMAN LEUKOCYTE ANTIGEN TYPING

Serology

The complement-mediated microlymphocytotoxicity test is one of the earliest phenotyping methods.^[16] Source of reagents are sera from multiparous and multi-transfused individuals. T- and B-lymphocytes are used for typing Class I and Class II antigens, respectively. In the lymphocytotoxicity test, isolated lymphocytes are added to specific HLA typing sera. If the lymphocytes contain antigen against specific HLA

Table 3: Associations of human leukocyte antigen - DR/DQ antigens

HLA-DR antigen association with HLA-DR (other) antigens	Common HLA-DR antigen association with HLA-DQ antigens
DR1	DR1 DQ5(1)
DR4	DR4 DQ7(3) + DQ8(3)
DR7	DR7 DQ2, DQ9(3)
DR8	DR8 DQ4, DQ7(3)
DR9	DR9 DQ9(3)
DR10	DR10 DQ5(1)
DR11(5)	DR11(5) DQ7(3)
DR12(5)	DR12(5) DQ7(3)
DR13(6)	DR13(6) DQ6(1), DQ7(3)
DR14(6)	DR14(6) DQ5(1)
DR15(2)	DR15(2) DQ6(1)
DR16(2)	DR16(2) DQ5(1)
DR17(3)	DR17(3) DQ2
DR18(3)	DR18(3) DQ4

HLA: Human leukocyte antigen

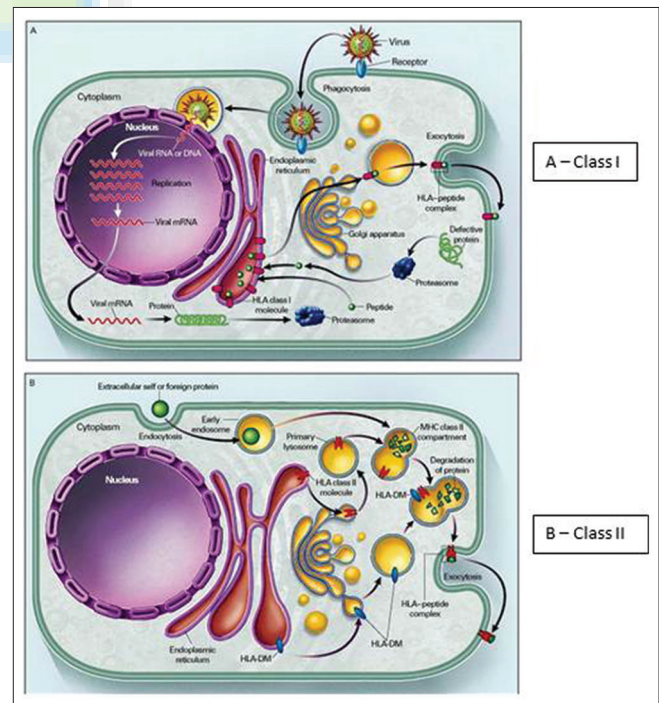


Figure 8: Schematic diagram of function of human leukocyte antigen molecules
 From New England Journal of Medicine. Reproduced with permission from - Massachusetts Medical Society

antisera (Class I or Class II), the antibody-antigen binding will take place and added complement causes membrane damage in such scenario. The damaged cells allow uptake of vital stains such as eosin. Microscopic identification of the stained cells indicates the presence of specific HLA antigen. Inherent drawbacks of serology are cross-reactivity, unavailability of monospecific antisera, difficulty identification due to weaker expression of HLA on cell surface, and possibility of false-positive and false-negative reactions. Monoclonal antibodies can circumvent the problem of cross-reactivity.^[17]

Human leukocyte antigen typing by molecular techniques

Reverse dot-blot polymerase chain reaction-sequence-specific oligonucleotide probes typing

In the reverse dot-blot, the sequence-specific oligonucleotide probes are bound to a solid surface membrane. When labeled DNA target is applied to the reverse dot-blot membrane, it will only hybridize to those oligonucleotides that are complementary in sequence. Once hybridized, biotinylated products are detected by the addition of a detector molecule, antibiotin antibody linked to streptavidin-horseradish peroxidase complex, which induces a color change in the substrate tetramethylbenzidine.^[18-20] Automated/semi-automated HLA typing systems use this method/principle.

Polymerase chain reaction – sequence-specific primers

Newton *et al.* (1989) designed sequence-specific primers on the basis of amplification refractory mutation system (ARMS) to discriminate polymorphic differences. This is based on the principle that only primers whose sequences are complementary to that of the target sequence of a DNA sample present will bind to this DNA and then amplification will take place in the polymerase chain reaction (PCR). Noncomplementary primers, on the other hand, do not bind to the DNA and there is no amplification. The amplified DNA is determined using agarose gel electrophoresis. Successful amplification generates a DNA fragment of defined length which appears as a band. This is a very rapid and sensitive method for identification of HLA alleles and has been used by many laboratories as a routine method for HLA typing.^[21,22] We are enclosing data from our center [Tables 4 and 5].

Sequence-based typing

Many PCR-based HLA typing techniques have been introduced, but sequence-based typing (SBT) is one of the best techniques which has the highest reliability in

Table 4: Human leukocyte antigen typing carried out in our center from 2001 to 2017

Year	Method	Number of tests
2002-2012	Serology typing	3503
2007-2015	Molecular PCR-SSP	2443
2013-2017	Molecular PCR-SSO	3098

PCR: Polymerase chain reaction, SSP: Sequence-specific primer, SSO: Sequence-specific oligonucleotide

Table 5: Comparison of human leukocyte antigen - ADR typing - serology versus polymerase chain reaction-sequence-specific primers

PCR-SSP versus serology	Results				
	A	B	C	DR	DQ
Completely matched alleles	62	55	52	39	26
Antigen versus blanks (blanks)	13	22	37	25	30
Antigen versus antigen (misassignment)	04	12	10	19	10
Specific variant identified	21	11	1	09	26

PCR: Polymerase chain reaction, SSP: Sequence-specific primers

defining the HLA alleles. SBT is the only technique which directly detects the nucleotide sequences of the HLA allele carried by a DNA sample and allows an exact assignment. This method not only resolves discrepancies in HLA antigens typed by other methods, but also allows the discovery of novel HLA alleles. In this technique, the first PCR amplification of the polymorphic region of the HLA loci (most commonly exon 2 for Class II and exons 2 + 3 for Class I) is carried out. Residual nucleotides and primers are removed by purification systems (e.g., columns, magnetic beads). The purified PCR product is then sequenced using multiple sequencing primers, covering the length of the template in separate sequencing reactions.^[23-26] It requires very expensive equipment and sophisticated laboratory approaches to give unambiguous high-resolution typing results. Classical direct sequencing methods cannot comprehensively elucidate the genomic makeup of HLA genes, considering the complex nature of the HLA genes.

Next-generation sequencing

Several high-throughput HLA-typing methods using next-generation sequencing (NGS) have been developed. NGS facilitates sequencing of the entire genomic DNA, provides complete information of all relevant HLA genes, minimum ambiguities, ability to identify new alleles, ability to identify null alleles, and finally a shorter turnaround time.^[27,28]

HLA null alleles are alleles for which no HLA products are expressed at the cell surface. Over 529 null alleles have been identified across HLA Class I and II, for example, A*01:01:01:02N.

This study showed that molecular technique (PCR-SSP) picks up many specific alleles and assign alleles to the so-called “blanks” by serology method.

METHODS FOR HUMAN LEUKOCYTE ANTIGEN CROSS MATCHING

Preformed antibodies directed against human leukocyte antigens (HLAs) have a major impact on allograft survival and form a significant barrier in solid organ transplantation. Detection of preformed donor-specific antibodies in recipient’s serum is done by carrying out HLA cross match test. Various cross match techniques are available.

HUMAN LEUKOCYTE ANTIGEN COMPLEMENT-DEPENDENT CYTOTOXICITY CROSS MATCH

The traditional method for assessing HLA antibodies in recipient serum sample is the complement-dependent cytotoxicity (CDC) testing. Lymphocytes from the donor are mixed with recipient serum in a multi-well plate. Rabbit complement is then added. If donor-specific antibody is present and binds to donor cells, the complement cascade will be activated via the classical pathway resulting in lysis of the lymphocytes. The read-out of the test is the percentage of dead cells relative to live cells as determined by microscopy [Table 6]. The result can thus be scored on the percentage of dead cells.^[16]

Several variations have been added to CDC assay to increase its sensitivity; it includes increasing the incubation periods and adding the secondary antibody in the form of anti-human IgG antibody. In addition, recipient’s serum treatment with Dithiothreitol (DTT) was introduced to distinguish between IgG and IgM antibodies in CDC test. The main drawback of this assay is that it is unable to detect low-titer antibodies. It is labor intensive, less sensitive, and less precise.^[16]

FLOW CYTOMETRIC CROSS MATCH

The next generation of assays introduced in 1983 utilizes flow cytometry to detect antibodies independent

of complement fixation. Three-color flow cytometry cross match (FCXM) involves adding recipient serum to donor lymphocytes and then incubating them with fluorescein-labeled antibodies against human IgG (antihuman IgG F (ab)/FITC) as well as T- and B-cell-specific cell surface proteins (CD3 for T-cells and CD19 for B-cells) to cell samples. If a donor-specific antibody (DSA) in this serum then binds to the donor lymphocytes, it will be detectable by flow cytometry at individual cell level.^[29]

Flow crossmatch has a role in pretransplant assessment. The significance of a positive result is mainly of interest when the CDC cross match is negative, this could be due to low titer of antibodies or may be due to non-complement fixing or non-HLA antibodies in some cases. In sensitized individuals, if CDC is negative and FXM is positive, it cannot be ignored.

MICRO-BEAD-BASED IMMUNOASSAY (LUMINEX -BASED CROSSMATCH)

Luminex cross match (LXM) is essentially a solid-phase immunoassay – a qualitative test which detects only antibodies directed against HLA-A, HLA-B, and HLA-DRB1. It is a cross match in which recipient sera are tested against a lysate derived from donor’s lymphocytes. The HLA molecules are isolated from these cells by treatment with beads coated with anti-human HLA Class I or II monoclonal antibodies. Therefore, hypothetically, it can detect donor-specific antibodies without the requirement for donor HLA typing. The introduction of solid-phase assays led to a second revolution in histocompatibility testing with the use of purified antigens bound to artificial surfaces rather than whole cells. These techniques augmented sensitivity and specificity to detect even low-titer antibodies to previously undetected antigens.^[30]

HUMAN LEUKOCYTE ANTIGEN ANTIBODY SCREENING

Preformed HLA antibodies can be detected by testing patient’s serum against a panel of cells with known HLA types. This can be done using various methods such as CDC, AHG-CDC, micro-bead-based assay on Luminex platform, and flow cytometric assay. This information is particularly important for the prospective organ transplant recipient to predict the chance of finding a compatible diseased donor.^[31]

PANEL-REACTIVE ANTIBODY

Patient’s serum is tested against lymphocytes of a panel of approximately 100 donors. Percentage PRA (%PRA) is the number of reactions within that

Table 6: The scoring system in lymphocytotoxicity test

Percentage of dead cells	Score	Evaluation
0-10	1	Negative
11-20	2	Doubtful/positive/negative
21-50	4	Weak positive
51-80	6	Positive
81-100	8	Strong positive

panel. If %PRA is 80%, theoretically, it means that chances of getting a compatible donor are 20% in the population.

HUMAN LEUKOCYTE ANTIGEN ANTIBODY IDENTIFICATION

Patients with positive antibody screening can be further tested for identification of the HLA antibodies (Class I and Class II). This can be also done using methods such as CDC, AHG-CDC, micro-bead-based assay on Luminex platform, and flow cytometric assay. The most common techniques used worldwide are Luminex- or flow cytometry-based assay, i.e., single antigen bead assay.^[32] It allows the precise characterization of HLA antibody specificities. In Luminex-supported SAB test, beads are coated with recombinant single HLA antigen. Approximately 100 different types of antigens are included from each class of HLA antigens.

VIRTUAL CROSS MATCHING

Virtual cross match is the determination of presence of donor-specific HLA antibodies “virtually” by comparison of the HLA antibody specificities of the recipient with the HLA typing of the donor.

CLINICAL APPLICATIONS

Human leukocyte antigen system and transplantation

HLA-A, HLA-B, and HLA-DR have long been called as major transplantation antigens; however, the role of HLA-C, HLA-DQ, and HLA-DP cannot be underestimated.^[33,34]

However, HLA molecules are not present on cells for transplant purpose only, their main role as described earlier is induction and regulation of immune responses.

In transplant scenario, T-lymphocytes recognize donor cell-derived peptides associated with HLA molecules on the graft. Antigen-presenting cells (APCs) from either donor or recipient can activate T-cells. Donor's APCs present in the graft cause “direct” activation of recipient's T-helper cells, which plays an important role in acute rejection. Recipient's APCs can acquire antigens shed from the graft, process into peptides, and present to T-helper cells for “indirect” activation, which plays role in chronic rejection.^[35]

In rejection, autoantibodies activate complement cascade and damage vascular endothelium, resulting in thrombosis and hemorrhages. Hyperacute rejection occurs in patients with ABO antibodies against group antigens and preformed HLA antibodies. HLA allo-immunization can be induced by pregnancy, transfusions, and/or transplants.

Renal transplants

In renal transplants, it is apparent that the effect of HLA matching is significant even with the highly efficient immunosuppression used.^[36] However, in renal transplant, two other major priorities, i.e., need for ABO compatibility and need for negative lymphocyte cross match reduce the chances of obtaining good HLA matches. Kidney transplantation from a living unrelated donor shows graft survival superior to deceased donor transplantation (except for 6 antigen match) despite a greater degree of HLA mismatch.^[37] This may be due to shorter ischemic period and less renal damage.

In the current scenario, recipient and donor are both tested for ABO, HLA-A, HLA-B, and HLA-DR antigens. HLA-C and HLA-DQ testing is also performed. Preferably, 48 hours before the transplant, a major cross match is required. The American Society for Histocompatibility and Immunogenetics (ASHI) standards require it to be done with more sensitive than routine testing such as prolonged incubation, use of AHG or flow cytometric cross match.^[38] A study reported 1 year graft survival from living and cadaveric donors as 93.9% and 87.7%, respectively.^[39] In India, the entire workup is done as per the Transplant of Human organ & Tissue 2014 rules. The relationship between donor and recipient needs to be confirmed by HLA laboratory as discussed in rules in Form No. 5.

Hematopoietic stem cell transplant

The HLA system is the major histocompatibility barrier in stem cell transplantation, and the degree of HLA matching is predictive of the clinical outcome. Candidate donors and recipients are typed for their HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ alleles and the optimal match being an allele-level match.^[40] Incompatibility may not only lead to rejection but also to the greater problem of GvHD in which the immunocompromised recipient is attacked.

The best compatible hematopoietic stem cells are from an identical twin and genotypically HLA-identical siblings. If such donors are not available, haplo-identical or partially mismatched donors are used, with a higher risk.^[41]

For unrelated matched donors, the National Marrow Donor Program was found in the USA in 1986 and this registry has 6–7 million donors.^[42,43]

In India also, five to six registries are active and have enrolled many HLA-typed donors. However, the number is in thousands, and in the future with awareness, this number will surely increase.

Allo-immunization has to be prevented in all potential stem cell transplant candidates and they should receive leukodepleted blood components.

PATERNITY TESTING

HLA typing has been used in paternity testing. Excluding paternity in some cases may be straightforward depending on the inheritance pattern. However, most laboratories now use along with HLA, red cell testing of mother, child and putative father together with genetic analysis such as “DNA fingerprinting.”

HUMAN LEUKOCYTE ANTIGEN AND TRANSFUSION

Platelet refractoriness

The incidence of HLA allo-immunization and platelet refractoriness in repeated transfusion recipients is as high as 20% to 71%.^[44] The failure to increase recipient’s platelet count after transfusion of suitably preserved platelets is known as platelet refractoriness.

Immune mechanism is established after exclusion of nonimmune causes such as sepsis, DIC, drug, and hypersplenism.^[2] Immune-mediated platelet refractoriness is usually caused by antibodies against HLA antigens, but antibodies against platelet-specific or ABH antigens are also involved.^[45] Leukocyte reduction to $<5 \times 10^6$ in blood product can reduce or prevent HLA allo-immunization which is achieved by third-generation leukocyte filters.

In refractory patients, it is common to find antibodies with broad reactivity rather than private specificities, particularly in patients with PRA. Finding a suitable donor is also a challenge. Platelets carry HLA Class I and platelet-specific antigens. The ideal situation is to type pools of voluntary platelet donors for HLA A and B types, and then matched or partially matched platelets can be transfused. Of course, the patient also should be HLA typed much before chemotherapy and transfusions are started. Platelet cross matching using a solid-phase red cell adherence technique has been developed.^[46] The efficacy of cross-matched platelets may be as good as HLA-matched platelets in some patients.

FEBRILE NONHEMOLYTIC TRANSFUSION REACTIONS

It is defined as a temperature rise if more than 1°C or 2°F during or strongly after transfusion. HLA antibodies as well as granulocyte- and platelet-specific antibodies have been implicated in the pathogenesis of FNHTRs.

TRANSFUSION-RELATED ACUTE LUNG INJURY

TRALI is acute noncardiogenic pulmonary edema developing in response to transfusion. It is caused by antibodies against HLA Class I and Class II or neutrophil-specific antigens.^[47]

Antibodies are usually in plasma of transfused component, less commonly in recipients.^[48]

The reaction probably activates complement cascade leading to neutrophil aggregation and sequestration in lungs. It leads to release of neutrophil granules and vascular damage.

TRANSFUSION-ASSOCIATED GRAFT-VERSUS-HOST DISEASE

When an immunosuppressed individual receives competent allogeneic T-lymphocytes from transfused blood products, these lymphocytes mount an immune attack against recipient’s cells causing TA-GvHD.^[49] It has also been observed in patients with no apparent immunosuppression. This is due to one-way HLA match (rejection direction) and one-way HLA mismatch in GvHD direction. In this case, the donor is homozygous for HLA loci while recipient is heterozygous for the same antigen as shown in Figure 9.

There is no effective treatment and the only way is the prevention.^[50] Gamma irradiation of the cellular blood products is the effective way of inactivating donor lymphocytes.

HUMAN LEUKOCYTE ANTIGEN AND DISEASE ASSOCIATION

HLA and its disease association have long being established, but the mechanisms for the same are not very clear. In case of infectious diseases, it could be that HLA antigen itself may play role by the following:

- Close similarity with the pathogen
- Poor presentation of certain antigens, viral or bacterial
- By facilitating entry of virus in the cell or by providing a binding site for virus or bacteria.

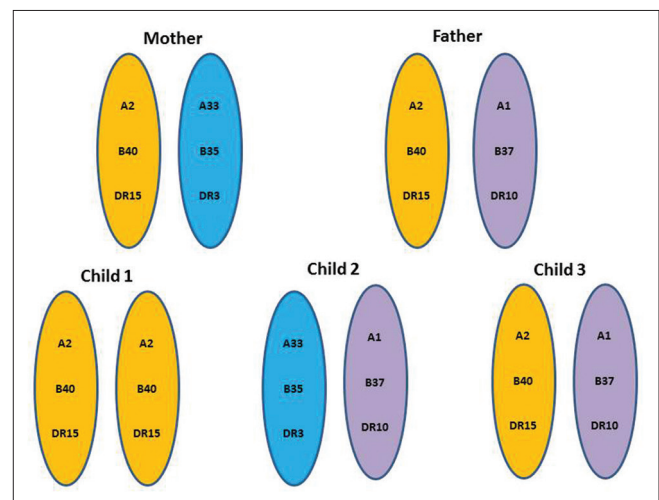


Figure 9: Human leukocyte antigen haplotype in a family at risk for transfusion-associated graft versus host disease

One of the most prominent associations is ankylosing spondylitis (AS) with HLA-B27 and celiac disease with HLA-DQ2. HLA-A1, HLA-B8, and HLA-DR17 haplotypes are frequently associated with autoimmune diseases.^[38]

The reported prevalence of HLA-B27 in AS and other spondyloarthropathies varies from 18%–94%

as compared to 1.4%–8% in the general Indian population.^[51,52] HLA-B*27:05 was the most common subtype associated with AS in the Indian population [Table 7].^[53]

With better understanding of HLA molecules and improvement in knowledge about typing and cross matching techniques, it will certainly help in greater

Table 7: List of human leukocyte antigens and their associated diseases

HLA subtype	Associated diseases
HLA Class I	
A	
HLA - A3	Hemochromatosis, multiple sclerosis
HLA - A29	Birdshot retinochoroidopathy
HLA - A2	Acute lymphoblastic leukemia, chronic myelocytic leukemia
HLA - A1	Hodgkin's disease, psoriasis
HLA - A11	Hodgkin's disease
B	
HLA - B8	Grave's disease, celiac disease, chronic hepatitis, Hodgkin's disease
HLA - B16	Multiple sclerosis
HLA - B18	Type I diabetes mellitus, chronic lymphocytic leukemia
HLA - B27	Ankylosing spondylitis, psoriatic arthritis, arthritis of inflammatory bowel disease, juvenile arthritis, reactive arthritis, reactive arthropathy including Reiter's syndrome, acute anterior uveitis
HLA - B35	Lymphoid leukemia
HLA - B51	Behcet's syndrome
HLA - B47	Congenital adrenal hyperplasia
HLA - B5	Grave's disease
HLA - B15	Hodgkin's disease
HLA - B7	Multiple sclerosis
HLA - B13	Psoriasis
HLA - B37	Psoriasis
C	
HLA - Cw6	Psoriasis vulgaris
HLA Class II	
DR	
HLA - DR1	Myasthenia gravis, rheumatoid arthritis, schizophrenia
HLA - DR2	Multiple sclerosis, hay fever, systemic lupus erythematosus, good pasture syndrome, narcolepsy, type I diabetes mellitus
HLA - DR3	Addison's disease, myasthenia gravis, systemic lupus erythematosus, Grave's disease, type I diabetes mellitus, celiac disease, dermatitis herpetiformis, sicca syndrome, idiopathic membranous nephropathy, chronic hepatitis, juvenile diabetes mellitus
HLA - DR4	Type I diabetes mellitus, rheumatoid arthritis, postpartum thyroiditis, pemphigus vulgaris, juvenile diabetes mellitus
HLA - DR5	Type I diabetes mellitus, Hashimoto's thyroiditis, pernicious anemia
HLA - DR7	Steroid-responsive nephrotic syndrome, celiac disease
HLA - DR11	Hashimoto's thyroiditis, celiac disease
HLA - DRw51	Multiple sclerosis
HLA - DR15	Multiple sclerosis
HLA - DR8	Reiter's syndrome
DQ	
HLA - DQ1	Narcolepsy
HLA - DQ2	Celiac disease, type I diabetes mellitus
HLA - DQ8	Celiac disease
HLA - DQ3	Type I diabetes mellitus
HLA - DQA1*05	Celiac disease
HLA - DQ6	Multiple sclerosis

HLA: Human leukocyte antigen

understanding of the immunity concepts and ultimately help in better transplant outcome.

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