EDITORIAL

Clinical Trial Registry - India (CTR-I): A meaningful initiative. How to take it forward?
Bavdekar SB

ORIGINAL ARTICLES

Detection of Rh antibodies using two low ionic diluents: Extension of the incubation time and the number of Rh antibodies detected
Skaik YA

Immunophenotypic characterisation of peripheral T lymphocytes in pulmonary tuberculosis
Al Majid FM, Abba AA

Relationship between N-terminal pro-B type natriuretic peptide and extensive echocardiographic parameters in mild to moderate aortic stenosis
Cemri M, Arslan U, Kocaman SA, Çengel A

Relative efficiency of polymerase chain reaction and enzyme-linked immunosorbant assay in determination of viral etiology in congenital cataract in infants
Shyamala G, Sowmya P, Madhavan HN, Malathi J

Stomaplasty—anterior advancement flap and lateral splaying of trachea, a simple and effective technique
Trivedi NP, Patel D, Thakkar K, Iyer S, Kuriakose MA

CASE REPORTS

Rhodotorula mucilaginosa as a cause of persistent femoral nonunion
Goyal R, Das S, Arora A, Aggarwal A

Repeated fracture of pacemaker leads with migration into the pulmonary circulation and temporary pacemaker wire insertion via the azygos vein
Udyavar AR, Pandurangi UM, Latchumanadhas K, Mulasari AS

Recurrent respiratory papillomatosis complicated by aspergillosis: A case report with review of literature
Kuruvilla S, Saldivar H, Joseph LD

Citrobacter freundii infection in glutaric aciduria type I: Adding insult to injury
Mukhopadhyay C, Dey A, Bairy I

IMAGES IN RADIOLOGY

Chordoma: A rare presentation as solitary ivory vertebra
Kumar S, Hasan R

IMAGES IN PATHOLOGY

Intracystic papillary carcinoma associated with ductal carcinoma in situ in a male breast
Dragoumis DM, Tsiftsoglou AP
REVIEW ARTICLE
Implications of HLA sequence-based typing in transplantation
Shankarkumar U, Pawar A, Ghosh K

DRUG REVIEW
Ramelteon: A melatonin receptor agonist for the treatment of insomnia
Devi V, Shankar PK

STUDENTS CORNER
The internet: Revolutionizing medical research for novices and virtuosos alike
Jethwani KS, Chandwani HS

VIEW POINT
Documenting indications for cesarean deliveries
Kushtagi P, Guruvare S

CLINICAL SIGNS
Cherry-red spot
Suvarna JC, Hajela SA

LETTERS
Central retinal vein occlusion associated with thrombotic thrombocytopenic purpura/hemolytic uremic syndrome
Author’s reply
Simultaneous umbilical hernia repair in patients undergoing laparoscopic cholecystectomy: Is obesity a risk factor for recurrence?
Authors’ reply
Snap sound and detumescence: Fracture penis
Paraphenylene diamine-induced acute renal failure: Prevention is the key
Inadequate awareness of the role of erythrocytic parameters in the detection of beta-thalassemia minor
Model for end-stage liver disease and outcome of portosystemic encephalopathy
Aortic thrombus during invasive aspergillosis in a kidney transplant recipient
Castleman’s disease in interpectoral lymph node mimicking mammary gland neoplasia
Bacterial endocarditis due to Group C streptococcus
Postpartum Group B streptococcal meningitis
Implications of HLA sequence-based typing in transplantation

Shankarkumar U, Pawar A, Ghosh K

ABSTRACT

Serology-based conventional microlymphocytotoxicity HLA typing method, which has been regarded as the gold standard in organ and hematopoietic stem cell transplantation, has been replaced now by DNA-based typing. Many laboratories all over the world have already switched over to molecular methods. Microlymphocytotoxicity-based tissue typing was done using commercial sera, while the molecular typing by genomic DNA based. DNA quality and its quantity obtained using various DNA extraction protocols was found to be an important factor in the molecular method of tissue typing in transplant outcome. Many polymerase chain reaction-based molecular techniques have been adopted with far reaching clinical outcome. The sequence-based typing (SBT) has been the ultimate technique, which has been of the highest reliability in defining the HLA alleles. The nonavailability of specific HLA antisera from native populations, large number of blank alleles yet to be defined and comparable low resolution of HLA alleles in SSP or SSOP technique, suggests that highly refined DNA-based methods like SBT should be used as an adjunct to HLA serology and/or low/intermediate/high resolution HLA typing in order to achieve a better transplant outcome.

KEY WORDS: HLA-A antigens, sequence analysis, transplantation

The major histocompatibility complex (MHC), a group of closely linked genes on chromosome 6, encodes the class I (HLA-A,-B,-C) and class II (HLA-DR,-DQ,-DP) HLA molecules, which, in concert with T-cell receptors, make possible the immune recognition of foreign antigens. HLA molecules are also alloantigens that can trigger immune recognition and graft rejection in unmatched transplant recipients.[1] An enormous effort to define and characterize new HLA alleles has culminated in a large number of alleles and continues to grow at a sustained pace, making the polymorphism of the linked genes of the HLA complex truly astounding. As of October 2007, a total of 2941 alleles 2009 (of class I), 932 (of Class II) and 97 (other loci) comprising of 617 HLA-A, 960 HLA-B, 335 HLA-C, 9 HLA E, 21 HLA F, 28 HLA G, 12 HLA H, 9 HLA J, 6 HLA K, 5 HLA L, of class I while, 626 HLA-DRB1, 34 HLA-DQA1, 87 HLA-DQB1, 23 HLA-DPB1, 127 HLA-DPBI, 4 DMA, 7 DMB, 12 DOA, 9 DOB, of Class II alleles have been described.[2] Apart from this, 61 MICA and 30 MICB alleles have been described. HLA Null alleles have been described in HLA A (43), HLA B (32), HLA C (7), HLA G (1) in class I while DRB1 (8), DQAI (1), DQBI (1), DPBI (2), DOA (1) in class II and in MICB (2). Further 21 different HLA-class I expression variant alleles; 12 are HLA-A locus alleles and 9 are HLA-B locus alleles have been identified. These alleles have high sequence homology with wild-type alleles, but because of mutations in their nucleotide sequence are not expressed on the cell surface (null alleles) or are expressed at levels that make them undetectable by routine serologic (and possibly cellular) methods. Theoretically, the alleles of different loci can combine to produce over 11 million different HLA haplotypes and billions of possible diploid combinations.

HLA Null Allele

The occurrence of null alleles has important implications in the strategies that a laboratory adopts to select HLA typing methods to be used for routine testing. Most of these expression variants were identified by discrepant results between serologic and DNA-based testing methodologies and serendipitously in other instances by the identification of novel DNA polymorphisms in the HLA genes. It must be stressed that there may be important biological differences between low-expression (L) and null (N) alleles. Examples of low expression and null alleles have been identified in the A*24 group (A*2402102L, A*2409N, A*2411N A*2436N, A*2440N, A*2445N, A*2448N, A*2460N, and A*2483N). While the low-expression alleles may be poor targets for antibody and T-cell recognition, these alleles probably have sufficient expression levels in the thymus and periphery to result in tolerance to wild-type alleles (with higher levels of expression and with identical or similar amino acid sequence as one of the low-expression alleles). In contrast, individuals carrying a null allele are able to mount an immune response against the expressed alleles with similar nucleotide sequence.

Serology—Molecular Technique a Comparison

The usefulness of conventional serologic assays for HLA typing has been limited by the availability of allele-specific sera [Figure
As antibodies identify structural differences on the surface of HLA molecules, protein structure differences caused by single or limited nucleotide polymorphism particularly within the peptide-binding groove of the HLA heavy chain are not detectable by these techniques. However, these differences are of functional significance as they determine the specificity and affinity of peptide binding[3,4] and therefore, T-cell recognition of self as well as allogeneic target cells.[5,6] For this reason, functionally significant high-resolution typing of HLA is only achievable through molecular methods. However, serologic techniques and reagents cannot reveal all currently known HLA molecular variants. Whole families of alleles whose HLA products share serologic markers have been found to encode distinct molecular variants. They can be distinguished by high-resolution techniques, including direct sequencing of the corresponding alleles and other sequence-based methods.[7] Current DNA-based methods that are in use for HLA typing are polymerase chain reaction-sequence-specific priming (PCR-SSP).[8,9] Polymerase chain reaction-sequence-specific oligo hybridization (PCR-SSO).[10] and sequence-based typing (SBT).[11] The PCR-SSO and -SSP are powerful methods for detecting genetic variability by identifying sequence motifs [Figure 2]. However, to maintain the high accuracy of these methods, the number of probes and primers has to keep up with the rapidly increasing allelic diversity. Direct sequencing of genomic DNA or cDNA is being used increasingly in routine diagnostic methods. For clinical procedures such as bone marrow transplants, detailed genotype information on both the recipient and the donor is required. The SBT is the most comprehensive method for characterizing human leukocyte antigen gene polymorphisms. The SBT involves PCR amplification of specific coding regions of HLA genes and sequencing of the amplicons. A detailed interpretation of HLA alleles is possible by comparing nucleotide sequences of the HLA gene to an online database of possible allelic combinations[12] (http://www.ncbi.nlm.nih.gov/mhc/sbt.cgi?cmd=main).

Most SBT typing strategies currently employed use the exons 2 and 3 sequences for HLA class I analysis and exon 2 alone for HLA class II analysis. Sequencing strategies used in SBT differ between laboratories and can generate either heterozygous sequences, haploid sequences (after allele separation of the sample) or a combination of heterozygous and haploid sequences for each typed sample [Figure 3]. While SBT permits the highest resolution of genotypes, it has its limitations. One of the problems with SBT is interpretation of ambiguous allele combinations that can occur due to several reasons.[13]

Since both the alleles are amplified and sequenced, it is difficult to determine which two alleles were responsible for the sequence results. Two or more different allele combinations combine to produce identical sequences due to the heterozygous base pair combinations. For example, in the class I region HLA B*070201, 3503 would have the same nucleotide sequence as HLA B*0724, 3533 in positions 559 and 560 as a result the interpretation of the high-resolution typing cannot be made because it is not known which allele combination is correct. Ambiguity may result from a nucleotide difference outside of the region amplified or when the entire sequence of the amplified region is not known. Resolving the ambiguity can be difficult and laborious. When two alleles differ by a single nucleotide, it may or may not be necessary to resolve the ambiguity because many single nucleotide changes do not affect the function of the HLA molecule. If the ambiguity is a result of an identical heterozygous sequence, a group specific primary amplification,[13] cloning,[14] reference strand conformational analysis (RSCA),[15] pyrosequencing[16] and denaturing high performance liquid chromatography (DHPLC)[17] to produce an unambiguous homozygous sequence can be followed. Some ambiguous allele combinations due to heterozygosity can be resolved by sequence-specific primers (SSP) or sequence specific oligonucleotide probes (SSOP) and may be a more viable approach for laboratories already performing these technologies.

### Transplantation Outcome

High-resolution HLA typing is increasingly in demand in clinical and experimental settings. In allogeneic transplantation, it is important to know if HLA antigens are expressed or not thus necessitating the need for accurate HLA typing for donor and recipient matching.[18] The HLA matching between the donor and the recipient improves the success of unrelated hematopoietic stem cell transplantation (HSCT). The HSCT with phenotypically matched unrelated donors is associated
with an increased rate of post-transplant complications mainly
due to serologically undisclosed HLA incompatibilities.\cite{18}
Retrospective studies have also shown that HLA disparities were
not necessarily associated with post-transplant complications,
even though an overall beneficial effect of HLA classes I and II
compatibility is determined by DNA typing methodology.\cite{19}
It has been shown that the use of high-resolution tissue typing
to obtain the best possible HLA match has resulted in an
improved transplant outcome.\cite{20} The HPCT involving partially
mismatched or unrelated donor-recipient pairs require a high-
resolution typing, but those involving HLA identical siblings
may not. Recently, multivariate analysis in 334 patients from 12
French transplant centers for HLA A, B, C, DRB1, and DQB1
loci mismatches revealed that the number of mismatches is
strongly associated with overall survival.\cite{21} Due to the important
role that HLA molecules play in antigen presentation and the
stringency of the relationship between epitope and associated
HLA allele, high-resolution typing is increasingly requested for
appropriate enrollment of patients into immunization protocols
aimed at the enhancement of T-cell responses.\cite{22} Molecular
HLA identity between unrelated donors and recipients of the
HPCT, approaching that, which exists between HLA-identical
siblings, seems to provide the best chance for avoiding graft
rejection and other serious complications of the HPCT.\cite{23,24}
These remarkable observations suggest new strategies for the
selection of bone marrow donors that could improve the odds
of engraftment by categorizing single HLA class I mismatches
according to their implications for the risk of rejection. First,
if the recipient is homozygous for an HLA allele, donors with
a single HLA mismatch who are also homozygous for the same
allele are preferable. Second, when the single mismatch involves
only an allele (e.g., patient type, A*0202, donor type, A*0203),
the mismatch predicts no increase in the odds of rejection and
this donor could be accepted as if fully matched. By contrast,
a single antigen mismatch (e.g., patient type, A*0202, donor
type, A*0302) does increase the risk of rejection and a donor
with no antigen mismatches should be sought.

In the allogeneic HPCT settings that utilize unrelated donors
or donors and recipients that are not identical by descent,
distinguishing null alleles from the expressed alleles (rather than
those of low-expression alleles) is of great importance. If only
DNA typing methods are used to type donors and recipients
and the methods applied cannot distinguish the expressed vs.
the null alleles (ambiguous), it is possible to mis-classify donors
and recipients that are mismatched in the expressed alleles. If
the donor carries a null allele and the recipient an expressed
one, then the transplanted immune system of donor origin will
be able to mount a response against the recipient’s tissues and
organs (graft vs. host disease). In cases in which the recipient
carries a null allele and the donor an expressed one, the recipient
may have preformed antibodies against the donor expressed
HLA antigens; the presence of donor-specific alloantibodies in
the patient’s serum may increase the risk for rejection of the
transplanted marrow. HLA Class I sequence-based typing (HLA-
SBT) is intended to identify HLA allelic polymorphisms at the
level of individual nucleotides using genomic DNA amplified
by PCR. HLA Class I SBT should be used as an adjunct to HLA

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**Figure 3:** HLA A SBT typing results

Shankarkumar, et al.: Implications of HLA SBT in transplantation
Class I serology and/or low/intermediate resolution molecular typing using either SSP or SSOP technology. For clinical purposes, this test should be used whenever possible to identify unrelated allogeneic bone marrow donors who are HLA-A, B and C genotypic matches with the intended recipient.

**Conclusion**

Recent advances in technology have created the ability to provide high-resolution HLA typing at a high throughput level in a routine laboratory,[27] but there are many aspects, which need to be contemplated by a laboratory before a decision is made to implement this technology. The implications of being able to provide high-resolution HLA allele typings are far reaching - not only for the knowledge that will be provided to the HLA community, but also for the potential clinical benefits of such information. Due to the strong connection between HLA and immunological response, high-resolution HLA typing is important for vaccine trials, unrelated bone marrow transplant and many other areas of clinical interest.

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