

HLA Diversity: Detection and Impact on Unrelated Hematopoietic Stem Cell Donor Characterization and Selection

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Abstract

Matching of patient and unrelated donor for HLA molecules significantly decreases the probability of graft rejection, graft vs. host disease, and transplant-related mortality in hematopoietic stem cell transplantation. A significant challenge in the identification of matched donors is the diversity of the HLA system. Almost 1500 alleles have been identified at 12 HLA loci. Significant progress has been made in the application of DNA-based testing to identify this diversity in patients and unrelated volunteer donors; however, the resolution of registry testing remains limited by the need to test many donors inexpensively. Thus, the transplant center must predict which donor might be a match for their patient using incomplete typing information. Design of a typing strategy based on knowledge of allele and haplotype frequencies is critical to speed donor identification. A further challenge is to compare patient HLA assignments to the over 7.7 million volunteer donors on registries carrying both DNA and serologic assignments. The links between alleles and serologic specificities remain unclear in many cases and complicate the design of computer algorithms used to match patients and donors. Finally, since few patients will find donors who are allele matched for all HLA loci, studies are underway to understand which of the HLA loci are most critical to match and to define rules of permissive mismatching to achieve an acceptable outcome.

Matching of patient and unrelated donor for HLA molecules significantly decreases the probability of graft rejection, graft vs. host disease, and transplant-related mortality and enhances immune reconstitution in hematopoietic stem cell (hsc) transplantation [1,2]. A significant challenge in the identification of matched donors is the diversity of the HLA system [3]. Diversity was originally observed with serologic testing using human alloantisera to discriminate HLA types. However, using DNA-based testing methods, it is now clear that this diversity is more extensive and that each serologically defined antigen (e.g., DR11) can be specified by one of many alleles (DRB1*1101-DRB1*1143). Today, nearly 1500 alleles have been identified at 12 HLA loci as powerful DNA typing technologies have been applied to study large populations through International Histocompatibility Workshops [4] and hsc volunteer donor registries. Most of the loci are extremely polymorphic; the HLA-B locus has over 470 alleles and HLA-DRB1,

over 300 alleles. Some loci have fewer identified alleles (e.g., HLA-DRB4 has 11 alleles) but are still polymorphic. The majority of the alleles differ by substitutions which alter the amino acid sequence of the antigen binding groove of the HLA molecule [5] and, thus, potentially affect allorecognition.

In spite of the extensive diversity observed in population studies, only a portion of the HLA alleles and haplotypes occur at reasonable frequencies. For example, within individuals from the United States who carry alleles of DR11, 98% of these individuals carry one of only four alleles, DRB1*1101-DRB1*1104 [6]. Six other alleles of the remaining 40 DRB1*11 alleles make up the final 2%. In general, only about 25% of DRB1 alleles are usually detected in population studies. The alleles differ also in frequency in different populations. Thus, the search for a DRB1*0302 matched donor, for example, should be focused on populations of direct African origin since this allele is extremely rare in

Caucasian or Oriental populations. These frequencies must be taken into account as patients are evaluated for unrelated donor transplantation in order to estimate their probability of finding an allele matched donor and to design a search strategy. One useful strategy is to perform an initial search of the Bone Marrow Donors Worldwide database to evaluate how many donors worldwide might potentially match a patient being considered for an unrelated transplant [7].

Significant progress has been made in the application of DNA-based testing to identify HLA diversity in patients and unrelated volunteer donors; however, the resolution of unrelated volunteer donor registry testing remains limited by the need to test many donors inexpensively. Since transplant centers match the patient and unrelated donor for HLA-A, -B, and -DR specificities, volunteer donors are typed for these loci. Initially most donors were typed for only HLA-A,-B since testing reagents for other HLA loci were limited and testing less robust. As the importance of DR matching was demonstrated [8] and as DNA based typing for DR became more routine, typing strategies changed to include DR. Today, the U.S. National Marrow Donor Program's (NMDP) registry is 64% HLA-A,-B,-DR typed and >98% of the transplants are facilitated through the HLA-A, -B, -DR typed pool of donors showing the effectiveness of this typing strategy. Volunteer donor typing is at low (serologic equivalent) resolution at a minimum. This means that typing narrows down the volunteer's HLA assignments to, for example, DRB1*11,*08 but does not identify which one of the >40 possible DRB1*11 alleles and >20 DRB1*08 alleles the volunteer carries. Since many transplant centers attempt to find allele matches for their patients, additional reagents have been added into the NMDP registry testing system to narrow down the allele possibilities even further (intermediate resolution testing producing a typing such as DRB1*1101 or DRB1*1104 but not DRB1*1102 or DRB1*1103).

In the United States with its diverse population, the use of DNA-based testing to assign HLA-A, -B, -DR types to new volunteer donors was essential to increase the accuracy of HLA assignments [9]. To achieve high volume testing at low cost, the laboratories performing registry typing under contract with the NMDP apply polymerase chain reaction amplification to isolate specific HLA genes followed by testing with a panel of oligonucleotide probes (sequence specific oligonucleotide probe hybridization or SSOPH). Guidelines for testing donors and patients have been described [10].

Allele/haplotype frequency information is critical to facilitate donor selection. Because the typing of volunteers is low to intermediate resolution, transplant centers searching for allele matches for their patients must predict which donor might be a match for their patient using the incomplete, low resolution typing information found in the registry. To facilitate rapid donor selection, it is helpful for the registry to use a new volunteer donor typing strategy based on knowledge of allele and haplotype frequencies. For example, where possible, typ-

ing reagents should discriminate between common high frequency alleles or separate rare from frequent alleles. The strategy for registry typing for the NMDP has been reported [11,12].

The institution of donor registries in many countries world-wide has been important in facilitating the search for matched donors. This not only has increased the number of volunteer donors to 7.7 million but has increased the diversity of HLA types represented in registries. Facilitated by the World Marrow Donor Association [7], over 50 registries in 37 countries participate in international exchanges involving patients in one country receiving hsc from a donor in another country. In the NMDP network, almost 1500 patients have received hsc from foreign donors and an equal number of US donors have provided hsc for patients in other countries. Within each country, targeted recruitment can aid in increasing the diversity of the registry. Evaluation of the HLA assignments and haplotypes represented in the registry [13] coupled with calculations of the percent of patients finding matched donors can assist in measuring the success of these efforts.

A further challenge resulting from the HLA system is to compare patient HLA assignments to the over 7.7 million volunteer donors on registries carrying either DNA or serologic assignments. The links between alleles and serologic specificities remain unclear in many cases [14] and complicate the design of computer algorithms used to match patients and donors. Some alleles have not been characterized at the serologic level while other alleles have designations that do not relate to their serologic assignment. For example, allele DRB1*1415 specifies an HLA molecule which serologically types as DR8, not DR14 as its name implies. Thus, individuals tested as DRB1*1415 should consider donors typed serologically as DR8 as potential matches. Further high resolution HLA testing of potentially matched donors is used to select allele matches. Accommodating all of the nuances of the HLA nomenclature system makes upkeep of the registry matching algorithms complicated and time consuming and confuses transplant centers attempting to select the best potential matches. In these cases, it is critical to include an HLA expert in designing the registry search algorithm and in assisting with potential donor selection.

Finally, since few patients will find donors who are allele matched for all HLA loci, studies are underway to understand which of the HLA loci are most critical to match and to define rules of permissive mismatching to achieve an acceptable outcome. The NMDP has begun to evaluate the allele level disparity in donor-recipient pairs transplanted through its network since the program began in 1988. Cells from each pair have been stored in a repository and the alleles carried by each individual are being retrospectively identified [15]. The matching is being correlated to transplant outcome and loci critical to outcome are being defined [16]. Still other NMDP studies focus on the impact of specific HLA mismatches in donor selection. That is, when confronted with two equally mismatched donors, what

guidelines should be used to decide which HLA mismatch might be less alloreactive. A recent study evaluates the impact of selection of a mismatch falling within a cross reactive epitope group (CREG) compared to a mismatch outside of a CREG on outcome [17]. No differences in outcome were found between CREG matched and CREG mismatched transplants thus increasing the number of potential mismatched donors available to a patient. Non-HLA factors, specifically donor age, also have a significant impact on outcome and should be considered in donor selection [18].

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