Tissue typing for kidney transplantation for the general nephrologist

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ABSTRACT:
Tissue typing is the process by which an individual’s human leukocyte antigens (HLA) are determined. In transplantation, this vital process allows the immunologic or rejection risk of a donor–recipient pairing to be assessed through reviewing their HLA matching and whether any anti-HLA antibodies present in recipient serum are donor specific. Tissue typing has increased in sophistication over time which has allowed a deeper appreciation of the antigenically important parts of HLA and increased the complexity of determining immunologic risk.

SUMMARY AT A GLANCE
This review explains the complexity and shortcomings of serological and molecular human leukocyte antigens (HLA) typing, which are critical in the assessment of immunological risks for potential kidney transplant candidates. Consequently, the determination of HLA structure using molecular typing has enabled a more accurate assessment of HLA compatibility between donors and recipients.

Determining the tissue type (human leukocyte antigens (HLA) profile) of the donor and recipient is integral to estimating the immunological risk of that pairing for transplant. Closely matched pairs have a lower risk of rejection and improved long-term graft survival compared with poorly matched pairs.1,2 Additionally, avoidance of poor HLA matching reduces the risk of recipient sensitization to mismatched HLA which might affect their prospects for future repeat transplantation should it be required.3,4 An individual’s tissue type can be assessed rapidly at relatively low cost by serological typing. While the costs, time requirements and expertise required in interpreting the results of modern sophisticated molecular typing can be greater, the enhanced precision and detail obtained make molecular typing the preferred option. The method used in different jurisdictions will be determined by the urgency for the result, such as deceased donor relative to living donor transplantation and the resources and expertise available.

Improvements in tissue typing have led to an explosion in the number of recognized HLA and a growing appreciation of HLA structure. It has become apparent that subunits of HLA molecules, called epitopes, rather than the entire molecule, are antigenically important in transplantation.5–7 Furthermore, as each HLA molecule contains multiple epitopes, the number of antigenically important epitopes which are potentially relevant to a single transplant pairing can be more than 100. Clearly, this makes for a substantially more complex assessment than was required in the era of 6-antigen (HLA-A, B and DR) tissue typing. This review aims to explain the evolution of tissue typing to the
current era to the general nephrologist with the aid of a case study.

**Case study – serological tissue typing**

A 25-year-old man with end-stage kidney disease due to IgA nephropathy is being assessed for a living donor transplant from a work colleague. They are both O blood group but they ask you if their tissue typing is compatible:

**Recipient:** A2,24 B39,45 DR4,14

**Donor:** A24,25 B39,45 DR4,14

**Interpretation:** The 6-antigen serological typing for this pair reveals a 5/6 match with the only mismatch being the donor HLA A25. This is excellent matching for unrelated individuals.

**Human leukocyte antigens**

Human leukocyte antigens are considered as the primary alloantigens relevant to transplant rejection. HLA proteins are encoded by genes on the short arm of chromosome 6. Half of each gene is inherited en bloc from each parent and is termed a haplotype. HLA are clustered into loci and divided into class I (HLA-A, B and C) and class II (HLA-DR, DQ and DP) based on function. They are named by the letter of their loci followed by a number designating their serological type, for example, HLA A24, HLA DR7.

**Serological tissue typing**

Serological typing has been relied upon for HLA determination for many years. It uses well-defined anti-HLA antibodies (antiserum) which are available commercially. Antibodies specific for a single HLA are mixed with lymphocytes of the individual to be typed. T-cells are used to define class I HLA and B-cells class II HLA. The process is similar to traditional complement-dependent cytotoxicity cross-matching in that complement is added to the reaction so that if antisera binds the lymphocyte, complement-mediated lysis occurs, signifying presence of that HLA on the lymphocyte. Multiple antisera are required to determine the complete serological tissue type profile of an individual.

Serological typing is relatively quick and inexpensive to perform, however, it has some limitations. It allows definition of the broad antigenic family but not the subtype of that HLA. For example, serological typing can identify HLA A2 but cannot differentiate between subclasses of HLA A2 such as HLA A2:01 and HLA A2:04 which may have important antigenic differences (Figure 1). Some antisera cross-react with multiple HLA impairing the ability to definitively assign tissue type. Additionally, HLA which is only expressed at low levels such as some C and DP antigens may not be detected serologically. The number of defined HLA types now exceeds 17 000 (https://www.ebi.ac.uk/ipd/imgt/hla/stats.html) which means that thousands of antisera would be required for full serological typing. Many antisera are no longer available and some loci have never been characterized fully serologically. The sum of these limitations has meant that serological typing is no longer considered a suitably accurate tissue typing methodology with the focus moving to molecular typing.

**Molecular tissue typing**

Molecular typing describes an individual’s HLA profile according to their genetic code rather than which antibodies bind the molecule. The sophistication of the techniques used for molecular typing continues to increase over time. First-generation molecular typing is based on DNA amplification by polymerase chain reaction (PCR) (Sequence-Specific Oligonucleotide and Sequence-Specific Primers techniques) with subsequent sequencing of the amplified gene product to define HLA type. Next- or second-generation sequencing also uses PCR gene amplification after first digesting the DNA into short fragments and amplifying the fragments from multiple points to generate vast numbers of DNA copies that are read and virtually reconstructed using computer algorithms. This improves the accuracy of the typing. Third generation or single-molecule typing continues to develop but seeks to increase speed, simplicity and accuracy of typing by avoiding the need for DNA amplification (PCR). The techniques used are complex but in simple terms make use of panels of ‘barcoded’ molecules that can bind directly to the HLA gene. The molecules which bind the individual’s DNA have their barcode read to determine the HLA type. These techniques are highly efficient requiring only a very small DNA sample.
Molecular typing has led to the understanding that the genes encoding HLA are the most polymorphic in humans.1 The degree of polymorphism has made accurate molecular typing challenging as design of the reagents used frequently requires knowledge of the possible DNA sequences present. However, as additional HLA types have been defined, understanding has been gained as to why rejection can occur between donor and recipients with the same serological type (explained below and Fig. 1). Part of this understanding relates to defining the genetic polymorphism while advances in definition of HLA protein structure have also assisted.

Case Study (continued) – molecular tissue typing

Molecular typing is undertaken to gain a better understanding of the HLA matching:

**Recipient:** HLA Class 1: A*02:01,*24:01; B*39:01,*45:04; C*07:02,*12:03.
HLA Class 2: DRB1*04:01,*14:02; DQB1*03:01,*01:01; DQA1*01:02,*04:01; DPB1*03:01,*04:01.

**Donor:** HLA Class 1:A*24:04 *25:01; B*39:01,*45:04; C*07:02,*12:03.
HLA Class 2: DRB1*04:01,*14:02; DQB1*03:01,*01:01; DQA1*01:02,*04:01; DPB1*03:01,*04:01.

**Interpretation:** Molecular typing provided typing on 14 HLA antigens for both donor and recipient and reveals matching at all loci except HLA A. Donor HLA A25 is mismatched with the recipient and whilst serologically matched at HLA A24, molecular typing demonstrates the antigen is mismatched as the donor is HLA A*24:04 and the recipient HLA A*24:01. Hence, there are differences in the coding regions between the donor and recipient for HLA A24 which are associated with differences in the HLA protein expressed. The nomenclature used is standardized and begins with the HLA loci, followed by an ‘*’ to designate molecular typing and then the serological group followed by a ‘.’ and the serological subtype.

**Epitopes and eplets**

An increased knowledge of HLA coding, based on molecular typing, coupled with the use of crystallography, has allowed further definition of the structure of HLA molecules, making it possible for tissue type to be assessed at the protein level.5–7 Each HLA molecules has multiple antigenically important subunits, termed epitopes, on its surface. In transplantation, epitopes are parts of the molecule which stimulate an immune response. T-cell epitopes interact with the T-cell receptor and stimulate T-cell responses and are beyond the scope of this review. B-cell epitopes are targets for anti-HLA antibodies. Donor specific (anti-HLA) antibodies (DSA) do not bind the whole HLA molecule but rather these epitopic subunits.14 Donor epitopes which are the same as the recipients will not elicit antibodies while those which differ may.7 Small amino acid clusters within the epitope, termed eplets, are thought to define the antigenic specificity of the epitope.5 These have been described based on crystal structures of HLA proteins. Freely available software, HLAMatchmaker (www.epitopes.net), can be used to provide the eplet profile of an individual based on their molecular typing.

Each HLA molecule contains multiple epitopes, meaning multiple antibodies specific for different epitopes can all bind the same HLA. Conversely, as each epitope can exist on multiple different HLA molecules, an antibody specific for a single epitope can bind multiple different HLAs (Fig. 1). In the first instance, multiple antibody specificities may be seen as a single DSA while in the second instance, a single antibody may be seen as multiple DSA. This is relevant to tissue typing in that the eplet or eplet profile of a donor and recipient can be compared to determine the degree of similarity. Because different epitopes can be present within subgroups of the same HLA, donor–recipient pairs who are well matched on the basis of serological HLA typing can have a significant number of differing epitopes. Conversely, because different HLA can express the same epitopes, it is possible for poorly matched pairs to have quite similar epitope profiles (Figure 1). This is useful knowledge as an increasing number of eplet mismatches is associated with an increased risk of rejection, HLA sensitization and graft loss.15–18

Case Study (continued) – eplet analysis of a single HLA

Eplet matching for the donor and recipient HLA A24 subtypes was undertaken with the eplets for each listed:

**Recipient:** HLA A*24:01 62EE 65GK 80I 82LR 138MI 166DG
**Donor:** HLA A*24:04 62EE 65GK 76ANT 79GT 127 K 138MI 144KR 150AAH 166DG

**Interpretation:** At HLA A24 the donor has 5 eplets (red) which differ from the recipient. This means that the recipient may already have or may generate in the future, antibodies against HLA A24:04 at the mismatched epitopes.

**Interpreting tissue typing results**

Once donor tissue typing results are received the clinician must determine their significance in the context of the tissue
type and anti-HLA antibody profile of the recipient. Due to time constraints in the deceased donor transplant setting it is not uncommon to only have incomplete or serological (not high-resolution molecular) donor typing. This will preclude complete eplet matching which relies on molecular typing results. Additionally, it will not be possible to determine if an antibody directed against an HLA serotype subset, for example, HLA A24:01 is donor specific or not, if the donor has only had serological typing and is found to be HLA A24. In this instance the antibody could only be reported as ‘potentially donor specific’ until the HLA A24 serological subtype is determined.

The tissue typing results will also be viewed in conjunction with cross-match results between donor and recipient. A ‘virtual crossmatch’ will be possible if the anti-HLA antibody profile of the recipient is known. The antibodies can be compared with the donor tissue typing to determine if any of the antibodies are donor specific. This will allow the immunological risk of the transplant to be judged so that a decision on whether to proceed can be made. Cross-matching techniques and assessment of immunological risk are covered in detail in a previous review article for the General Nephrologist in this journal. Additionally, tissue typing is used in deceased donor kidney allocation where good HLA-matching of a waiting list patient to a donor frequently gives them priority to be allocated a kidney from that donor. This is performed to increase the likelihood that the kidney will last longer than if it was allocated to a recipient with a poorer match to the donor.

Tissue typing provides the opportunity to reduce the immunological risk and future HLA-sensitization for a transplant recipient. Serological tissue typing has led to the current grouping of HLA loci and provided the basic nomenclature but is not suitable to fully determine HLA type. Molecular typing has advanced our understanding of the variability in HLA and allowed definition of many new HLA types. Additionally, we are now using ‘virtual serology’ through examination of the epitope expression of an individual, derived from analysis of HLA protein structure, to assess the degree of donor-recipient matching.

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