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Introduction

Before a patient can be safely transplanted several aspects of the match between the donor and recipient must be evaluated. These include the ABO blood group match, the major histocompatibility (human leukocyte antigen, HLA) antigen match, and an assessment of the presence, in the serum of a patient, of anti-HLA antibodies that might cause a transplanted kidney to fail. Highly accurate laboratory techniques for determining the suitability of the match are now available in most tissue typing laboratories. Outcomes of kidney transplantation have steadily improved over the years because of improvements in these matching techniques as well as the more commonly cited improvements in immunosuppression and patient care.

The most important immunologic barrier to a successful transplant is the ABO blood group antigen system. Anti-ABO A and B antibodies are naturally made by humans who do not express one or both of those antigens. ABO-incompatible kidneys can be rejected immediately by these antibodies if present in a large amount at the time of transplant. The second important immunologic barrier is the presence of anti-donor HLA antibodies in the serum of a potential recipient. These are not naturally occurring but will be made as a result of exposure to HLA antigens via blood transfusions, pregnancy, and tissue transplants. If present in a high amount at the time of transplant anti-HLA antibodies will cause hyperacute rejection. Mismatched HLA antigens are the next important immunologic barrier to a successful transplant. The fewer HLA antigens on the donor tissue that

are mismatched by the recipient, the better the outcome of transplant. Minor histocompatibility antigens are the final immunologic barrier and explain why recipients of HLA-identical sibling kidney transplants still require some, although reduced, immunosuppression. Minor histocompatibility antigens are not well defined but we know that they exist and we strongly suspect that they can be targets of the immune response. The purpose of this chapter is to describe the immunologic barriers to a successful transplant, and the different laboratory methods available to evaluate the compatibility of a donor for a potential recipient.

Barriers to Successful Transplantation

ABO Incompatibility

ABO blood group incompatibility is the initial and most important barrier to a successful transplant. Table 2.1 lists frequencies of ABO blood types within the US population as a whole and their acceptable ABO blood type donors.

ABO blood group B frequencies are higher among individuals of African, Asian, and Central European descent. As an example, approximately 20 % of African Americans type as blood group B. In contrast, native Americans rarely express the blood group B allele. When ABO-incompatible blood is transfused into individuals a severe immune reaction can occur, including acute lung injury and fatal complement-mediated hemolysis. ABO-incompatible organs can be rejected immediately due to the presence of circulating preformed anti-A and/or anti-B antibodies. However in certain circumstances, transplantation across ABO disparate blood groups is possible. Individuals who are ABO blood group B or O may receive a kidney from an ABO A2 donor if their anti-A antibody titer is low ($\text{IgG} \leq 1:2$) [1–4]. The A2 antigen is less reactive with anti-A isoagglutinin and is expressed in lower amounts on the surface of red blood cells and tissue cells [3]. Waiting times and outcomes for A2 kidneys transplanted into O or B recipients between

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Table 2.1 Recipient blood groups, their relative frequencies in the population, and compatible blood group donors

Recipient blood group	Percent of population (%)	Donor blood group compatible with recipient
A	42	A, O
B	10	B, O
AB	4	A, B, AB, O
O	44	O

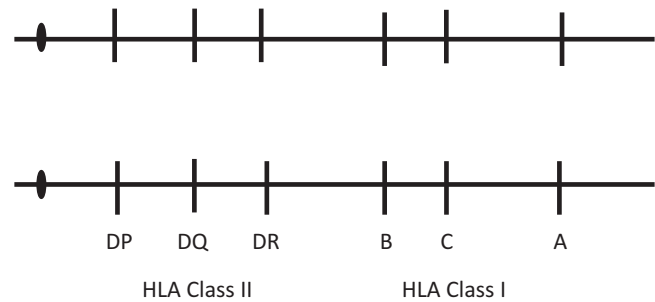
Note: Rh factor status (positive or negative) is not an issue in transplantation

1995 and 2006 were examined using the United Network for Organ Sharing (UNOS) database [5]. There were 150,118 first kidney transplant recipients and among these 113 were O recipients of A2 kidneys and 125 were B recipients of A2 kidneys. These recipients had shorter waiting times than their counterparts who received ABO-compatible kidneys (A2 into O median wait time was 0.7 vs. 1.63 years and A2 into B median wait time was 0.74 vs. 1.9 years) [5]. In addition, there was no significant difference in graft or patient survival between the recipients of A2 compared with the ABO-compatible kidneys.

Several groups have developed protocols to transplant kidneys across major ABO barriers [6–11]. These protocols employ methods to reduce naturally occurring anti-A or anti-B antibodies. A variety of techniques and drugs have been used to achieve antibody reduction including plasmapheresis, intravenous immunoglobulin (IVIG), splenectomy, and rituximab [6–11]. These methods of antibody reduction have helped to expand the number of patients who may receive a kidney from a living donor. However, the growing participation of patients in paired donor exchanges may reduce the need for using ABO-incompatible donors.

Major Histocompatibility Complex

Major histocompatibility complex antigens (HLA) pose the next most important barrier to successful transplantation. HLA antigens are encoded by genes located on the short arm of the sixth human chromosome. This region, spanning an area of 3.6 mega base pairs, includes over 100 genes that are involved in the regulation of immunity. These genes are divided into three groups or classes. Class I and II genes encode for HLA molecules that are important in transplantation. Class III genes encode for other proteins related to the immune system, including heat shock proteins, complement factors, and cytokines. Class I HLA molecules consist of a heavy chain with three domains (alpha1, alpha2, alpha3) and an invariable light chain called beta-2 microglobulin (coded on chromosome 15). The alpha3 domain anchors the molecule into the cell, while the alpha1 and

**Fig. 2.1** Major histocompatibility complex/human leukocyte antigen on the short arm of chromosome 6 and their relative orientation on the chromosome

alpha2 domains form a peptide binding groove. Class II HLA molecules consist of two chains, an alpha and a beta chain (both coded on the sixth chromosome). Each chain has two domains, with the alpha1 and beta1 domains forming a peptide binding site.

The importance of HLA molecules hinges on their ability to present peptides to T cells. T cells, via their T cell receptor, are only capable of recognizing peptides (self or non-self) when these are presented in the peptide binding regions of HLA molecules [12–14]. T cells are continuously engaging HLA molecules to assess the nature of peptides. T cells will kill cells whose HLA molecules express nonself-peptides, such as viral peptides. This is how a person prevents viruses from spreading from cell to cell. Donor (allo) HLA molecules elicit a very strong immune response. Up to 15 % of all T cells are able to recognize nonself (allo)-HLA and initiate an effector response to a transplanted organ. It is because T cells are constantly surveying HLA molecules that HLA antigens are considered the major histocompatibility antigens.

HLA Class I genes include A, B, and C. HLA Class II genes include DP, DQ, and DR. HLA genes are co-dominantly expressed. Therefore, there are two HLA A molecules, two Bs, and so on. We inherit one number 6 chromosome from each of our parents. One set of HLA genes derived from one number 6 chromosome is called a haplotype. The probability that a sibling has inherited the same two haplotypes as a brother or sister who is in need of a transplant is 25 %, or 1–75 % (the probability that he/she inherited either one, 50 %, or inherited neither, 25 %). The probability that one of the several siblings is HLA identical (a two-haplotype match) is determined by the formula $1 - (0.75)^n$, where n is the number of siblings. Figure 2.1 illustrates the orientation of the HLA genes on chromosome 6. Figure 2.2 demonstrates the inheritance pattern of the HLA genes.

HLA genes are highly polymorphic, with more than 1,980 unique alleles. These alleles are not randomly distributed, and certain alleles are more frequent than others. In fact, allele

Mother		Father	
HLA A2 B7 DR4		HLA A11 B18 DR6	
HLA A24 B8 DR51		HLA A36 B40 DR8	
Sibling 1	Sibling 2	Sibling 3	Sibling 4
HLA A2 B7 DR4	HLA A2 B7 DR4	HLA A24 B8 DR51	HLA A2 B7 DR4
HLA A11 B18 DR6	HLA A36 B40 DR8	HLA A11 B18 DR6	HLA A11 B18 DR6

Fig. 2.2 The figure demonstrates the inheritance pattern for HLA genes. Each child is a one-haplotype match with its parent. Sibling 1 is a one-haplotype match to siblings 2 and 3, and a two-haplotype match with sibling 4. Sibling 2 is a one-haplotype match with siblings 1 and 4, and a 0 match with sibling 3. Sibling 3 is a one-haplotype match with siblings 1 and 4. Sibling 4 is a one-haplotype match with siblings 2 and 3. The probability that one of the several siblings is HLA identical (a two-haplotype match) is determined by the formula $1 - (0.75)^n$, where n is the number of siblings

frequencies differ among different human populations [15]. Each HLA allele is unique in its ability to bind amino acids. The restricted nature of peptide binding favors having several different HLA molecules so many different peptides can be presented. If a person does not possess the ability to present peptides of a virus he/she will die because that virus will escape T cell-mediated elimination. The large number of HLA alleles (capable of presenting peptides) that exist in a population protects the population from extinction due to a specific viral infection. It is overwhelmingly likely that some members of the specific population will express HLA alleles that are capable of presenting peptides of all viruses to T cells. The fact that an individual has 12 distinct genes that code for HLA molecules makes it more likely that at least some peptides from a virus will bind in the peptide grooves of the HLA molecules to be presented to T cells, allowing T cells to kill virally infected cells.

HLA Class I is expressed on the surface of all nucleated cells while HLA Class II is expressed on antigen presenting cells (mononuclear phagocytes, B lymphocytes, dendritic cells) as well as some endothelial cells and thymus epithelium. Of note, HLA Class II is expressed on the endothelial cells of glomeruli and peritubular capillaries [16]. HLA of the transplanted organ can activate the recipient's T cells via the direct and indirect pathways of T cell activation [17]. Recipient T cells residing in lymph nodes can be directly activated by donor passenger cells from the allograft migrating to local draining lymph nodes. The direct pathway is dominant early after transplantation. The indirect pathway is the classic pathway of T and B cell activation used by the immune system to combat microorganisms. Recipient antigen presenting cells process donor antigen first and then present donor peptides to recipient immune cells. This pathway is responsible for rejection episodes that occur later after transplant, after the passenger donor cells are no longer around to directly activate recipient immune cells.

Preformed Anti-HLA Antibodies

The presentation of donor HLA via the direct or indirect pathway can lead to the development of anti-donor HLA antibodies. Anti-HLA antibodies do not occur naturally (as do anti-ABO antibodies). There are three ways that an individual can be exposed to HLA antigens and subsequently develop anti-HLA antibodies. The first is via blood product transfusions. The second is via pregnancy and the third is via tissue transplantation. Preformed anti-HLA antibodies represent another major barrier to a successful transplant either by limiting the number of compatible donors or, worse, by causing early graft failure if transplantation occurs despite the presence of donor-specific anti-HLA antibodies. Donor-specific antibodies (DSAs), if present in high amounts, will cause immediate (hyperacute) graft loss and if present in small amounts will limit the survival of an allograft.

Influence of Mismatched HLA Antigens on Transplant Outcomes

Mismatched donor HLA antigens become a target of the immune response by a recipient. Unfortunately, not all recipients will have the opportunity to receive a 0-antigen-mismatched kidney. The outcomes of this type of transplant are superior as demonstrated by data tracked by the Scientific Registry of Transplant Recipients (SRTR) illustrated by Fig. 2.3 [18].

The best opportunity for finding a minimally mismatched donor is among family members and specifically among siblings. However, for patients who do not have a living donor the national 0-antigen mismatch sharing program provides an opportunity to improve graft survival from a deceased donor. Patients who benefit the most from this program are those who possess anti-HLA antibodies and are therefore limited to a small pool of compatible donors. To qualify for this sharing program a patient must have a calculated panel reactive antibody (cPRA) (see below) of $\geq 20\%$ [18]. Not all patients will qualify for this program and even for those who do, if they possess a rare HLA phenotype, it is unlikely that a 0-antigen-mismatched donor will ever be identified. There is good evidence that long-term allograft survival is directly related to the number of HLA mismatches at the time of transplantation. A study of over 30,000 first deceased donor allograft recipients transplanted between 1984 and 1990 showed that 0-antigen mismatch kidneys had 1- and 5-year survivals of 84.3 % and 65.4 %, respectively. The 1- and 5-year survivals for 6-antigen-mismatched kidneys were 76.1 % and 52.3 %, respectively. There was also a stepwise decrease in survival with each mismatched HLA antigen [19]. Another study of nearly 136,000 recipients from 363

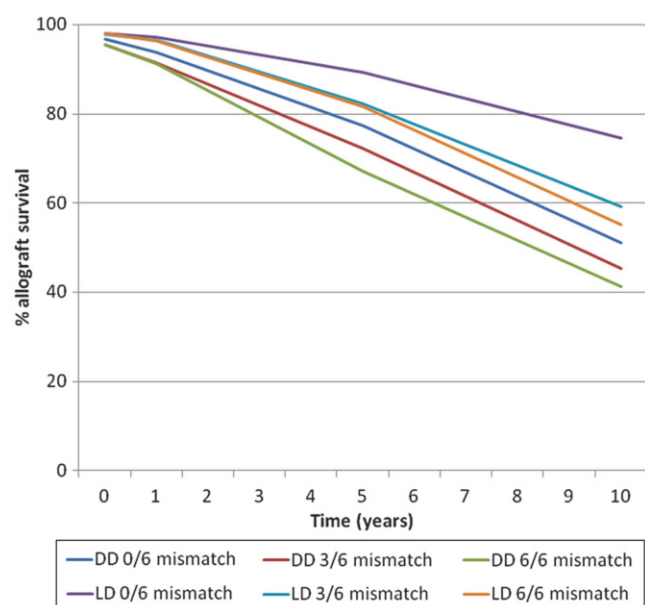


Fig. 2.3 Survival of deceased donor (DD inclusive of non-expanded criteria donor and expanded criteria donor) and living donor (LD) allograft kidneys according to their HLA mismatches with the recipient. Data for this figure was obtained from the OPTN/SRTR 2010 Annual Data Report (ADR) Table 5.10c, d as of October 1, 2010

centers, transplanted between 1985 and 1994, reported 5-year allograft survivals of 69.9 % and 54.3 % for a 0- and 6-antigen mismatch, respectively [20]. This study also noted a persistent importance of HLA mismatching for recipients transplanted between 1995 and 2004, well after the introduction of superior immunosuppression, and also a stepwise decrease in 5-year allograft survival with an increasing HLA antigen mismatch [20]. Other studies have shown that greater levels of HLA mismatch are associated with higher rates of rejection [21, 22].

Pretransplant Assessment of Anti-HLA Antibody Status

The pretransplant assessment for anti-HLA antibodies consists of determining the breadth and strength of anti-HLA antibodies that are present and performing a crossmatch prior to transplant to be certain that there are no DSAs.

There are several reasons for determining if anti-HLA antibodies are present in a patient's serum prior to transplant. First, it is known that patients with a high level of anti-HLA antibodies have a higher incidence of rejection [23, 24]. Most transplant programs will give stronger immunosuppression to patients who are sensitized (possess high levels of anti-HLA antibodies). Second, patients who have anti-HLA antibodies receive additional points in the allocation scheme and are prioritized for transplantation with a deceased donor [18]. Third, by determining to which specific HLA

Table 2.2 Time (years) to transplantation according to percentage of panel reactive antibodies (PRAs)

	Peak PRA			
	Total patients	0–9 %	10–79 %	≥80 %
10th percentile (TT)	0.29	0.31	0.43	0.52
25th percentile (TT)	0.94	0.93	1.32	1.97
50th percentile	3.50	3.18	4.86	Not enough patients to calculate

Data for this table was obtained from the OPTN/SRTR 2010 Annual Data Report (ADR) Table 5.2 as of October 1, 2010. The data is for patients who were registered onto the transplant waiting list in 2005. The panel reactive antibodies (PRAs) of these patients are the peak PRAs. The time to transplantation (TT) is denoted in years. The percentiles represent the time to transplantation for the total population waitlisted, for the time to transplantation for 10 % of the population of waitlisted individuals, time to transplantation for 25 % of the population of waitlisted, and the time to transplantation for 50 % of the waitlisted population

antigens a patient has antibodies, it is now possible to list those on the national computer (UNet, the computer program operated by the UNOS, the national organ procurement and transplantation network) as unacceptable antigens [18, 25–28]. Their policies and guidelines regarding the listing of unacceptable antigens can be found on their website. If listed in UNet as an unacceptable antigen for a specific patient, kidneys from donors with that antigen will not be offered to that patient. Moreover, the only way to obtain allocation points for being sensitized is to list unacceptable antigens in UNet (more later). Fourth, and finally, it is very useful for the physicians caring for a patient who is on the waiting list to know if a patient is sensitized. The more sensitized a patient, the longer it will take to find a compatible donor, as illustrated by SRTR data in Table 2.2 [18]. This is important information for both the patient and his/her physician.

The techniques for determining the presence of anti-HLA antibodies have evolved. The commonly used term to describe the breadth of antibodies is PRA. PRA stands for panel reactive antibody and is expressed as a percent. The techniques for the determination of an individual's PRA are illustrated in Fig. 2.4.

The classical method for determining the PRA used a panel of individuals who together possessed as many of the known HLA antigens as possible [29]. Cells from these individuals were put on a tray, each well containing the cells of one individual. Serum from a patient was added to the tray to determine if antibodies were present, the readout being cytotoxicity in the presence of complement. The PRA percent was calculated by simply dividing the number of wells with dead cells by the total number of wells (e.g., 24 wells with dead cells and a total of 48 wells=PRA of 50 %). The cytotoxicity method is less sensitive than techniques that are currently used but the term PRA has endured. Current techniques are bead—instead of cell—based. Polystyrene or latex beads are coated with HLA molecules that are obtained from

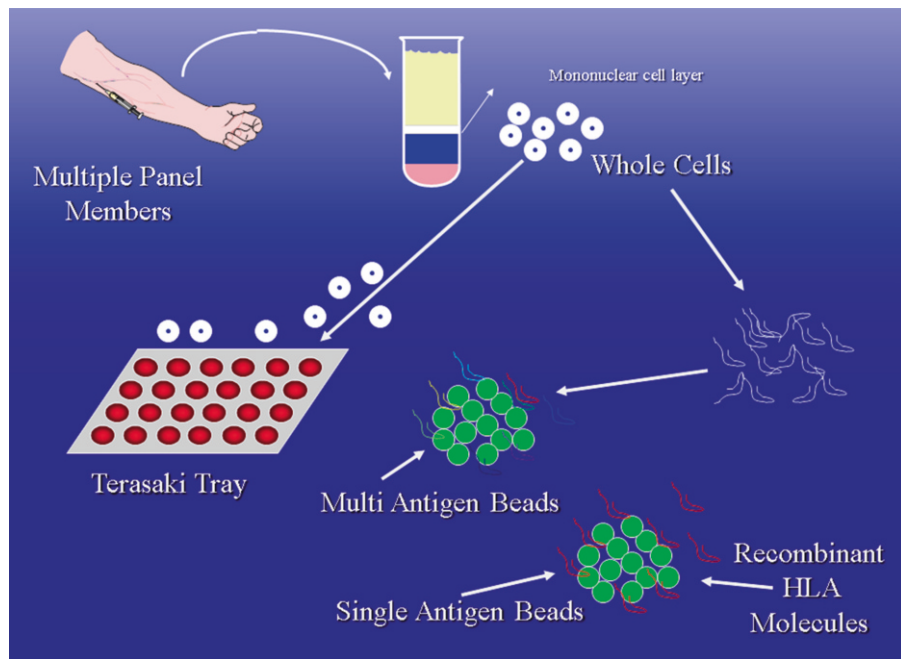


Fig. 2.4 Panel reactive antibodies. Two different techniques: cytotoxicity method and bead-based method. The cytotoxicity method involves the use of whole cells from a panel of donors who together possess as many known HLA antigens as possible. Each donor has his/her cells placed into one well of a tray and mixed with the recipient serum and complement. Cytotoxicity as evidenced by dead donor cells in a tray well represents reactivity of the recipient sera with the donor. The percent PRA is the number of “reactive” wells, or wells with dead cells, divided by the total number of wells. Example: 12 wells with dead cells and a total of 48 wells=PRA of 25 %. The current techniques are bead based. Polystyrene or latex beads are coated with HLA molecules that are obtained from either digested cells from multiple donors

(multi-antigen beads) or recombinant techniques (single antigen beads). A mix of either Class I or II beads is chosen to represent as many HLA antigens as possible. The “flow PRA” uses multi-antigen beads, patient serum, fluorochrome-tagged anti-human immunoglobulin antibodies, and a flow cytometer to detect anti-HLA antibodies. In the assay, beads that have antibodies attached indicate that the patient possesses anti-HLA antibody to the HLA bound to the bead. The “reactive beads” emit photons from laser activation of fluorochromes on the antibodies. The PRA percent is calculated by counting the number of beads that have antibodies attached and dividing by the total number of beads present. For instance, if 30 % of the beads have antibodies attached the flow PRA is 30 %

either digested cells (multi-antigen beads) or recombinant techniques (single antigen beads). The “flow PRA” uses multi-antigen beads, patient serum, fluorochrome-tagged anti-human immunoglobulin antibodies, and a flow cytometer to detect anti-HLA antibodies. Multi-antigen beads have either HLA Class I or Class II antigens attached [30, 31]. A mix of either Class I or II beads is chosen to represent as many HLA antigens as possible. The mix does not represent the frequency of HLA antigens in the population because this varies by demographic group. In the assay, beads that have antibodies attached (indicating that a patient has an anti-HLA antibody(ies) that recognizes the HLA antigen(s) that is (are) bound to that bead) emit photons from laser activation of fluorochromes on the antibodies. The PRA percent is calculated by counting the number of beads that have antibodies attached and dividing by the total number of beads present. If 10 % of the beads have antibodies attached the flow PRA is 10 %. Another technique using multi-antigen beads (quick screen) can be used to determine if a patient has anti-HLA antibodies, but the breadth of antibodies (PRA) is not calculated [32]. When either the flow PRA or quick screen is positive a single antigen bead assay is generally run to identify the specific

antibodies causing the positive results. Single antigen bead assays use a Luminex platform that in addition to recognizing the presence of antibodies attached to beads can identify intrinsic color of the beads [33, 34]. Polystyrene beads with multiple (up to 1,000) different colors are used in these assays. The advantage of this platform is the ability to attach specific recombinant HLA molecules to beads having a unique color. For example, HLA B7 adherent beads will have a different color from HLA A2 adherent beads. Luminex assays can determine if antibodies are bound to the HLA A2 beads, to the HLA B7 beads, to both, or to multiple other beads with unique colors and unique single HLA antigen specificities.

For patients who are waitlisted on the national computer for a deceased donor a PRA is calculated (cPRA) by entering unacceptable antigens into UNet. The computer is programmed to calculate a cPRA based on the frequency of HLA Class I and Class II antigens expressed by 12,000 US organ donors [18, 35]. The higher the cPRA, the greater the number of points offered to a patient via the allocation scheme. A high cPRA can result from a small number of common HLA antigens (e.g., HLA A2) or from a large number of less common HLA antigens. It is the responsibility of

each transplant program to enter unacceptable HLA antigens for each of their waiting patients into UNet. Most patients will have none. Some will have one or more and a few will have many unacceptable antigens. An unacceptable HLA antigen may be defined differently by different programs and currently there is no national standard. HLA antigens will be listed if they exceed a threshold based on the strength or amount of antibody measured. The measure of strength in the Luminex single antigen bead assay is mean fluorescence intensity (MFI). Increasing fluorescence intensity measured by photons emitted by specific beads bound with specific HLA molecules correlates with increasing amounts of antibody to that specific HLA antigen. Each program, in consultation with their tissue typing laboratory director, chooses an MFI threshold above which an HLA antigen will be listed as unacceptable. If the MFI threshold is low, more HLA antigens will be listed and if the threshold is high fewer will be listed. At present MFI thresholds chosen by programs range from as low as 1,000 to as high as 10,000. One distinct advantage of listing unacceptable HLA antigens in UNet is that when a donor kidney is offered to a patient it is highly likely that the final pretransplant crossmatch (see below) will be compatible. However, this is much more likely if the unacceptable MFI threshold is set low and much less likely if the MFI threshold is set high.

When considering a living donor, knowing about antibody specificities can help determine compatibility. A DSA with a high MFI indicates an incompatible match (virtual crossmatch, see below).

The Pretransplant Crossmatch

Before a kidney transplant is performed it is essential that the recipient is found to be devoid of any antibodies that can cause hyperacute rejection or early graft failure. A pretransplant crossmatch is performed using techniques that can detect the presence of DSAs. The tissue typing laboratory generally considers the crossmatch to be the most important test that it performs. Crossmatching techniques have evolved to become substantially more sensitive. Several crossmatching assays are

currently available for use and tissue typing laboratories differ regarding which are used. Figure 2.5 provides a list of the different techniques used when performing a crossmatch between a potential donor and a recipient. Each technique is described in the following sections.

Standard Complement-Dependent Cytotoxicity or NIH Crossmatch

The complement-dependent cytotoxicity test (CDC) or standard crossmatch developed in 1964 by Terasaki and McClelland has been used as the standard crossmatch for several decades, shown in Fig. 2.6 [25].

Buffy coat containing lymphocytes from the donor is combined with serum (in several dilutions) from the recipient in a multiwell tray and incubated at room temperature for 30 min followed by the addition of complement and further incubation at room temperature for 60 min. A vital dye (usually eosin) is added along with formalin (permanently fixes cells). Eosin enters cells that have been damaged by complement activation and can be easily identified under an inverted phase microscope. Live cells remain small and refractile and exclude eosin. In their original publication that appeared in the New England Journal of Medicine, Patel and Terasaki conclusively demonstrated the specificity of this technique [36]. Patients were transplanted and a retrospective crossmatch was performed. Patients were divided into two groups based on the presence or absence of anti-HLA antibodies. Among patients with antibodies and with a positive crossmatch immediate graft failure occurred in 80 % and only 10 % of kidneys survived for more than 3 months. This confirmed the need for a crossmatch with the specificity of the standard cytotoxic crossmatch before every transplant. However, among patients with antibodies and a negative crossmatch immediate graft failure occurred in 15 % but among patients without antibodies only 2.4 % failed immediately. These findings indicated that while highly specific (grafts will fail if the test is positive) this test lacked adequate sensitivity. This led to the development of more sensitive crossmatching techniques.

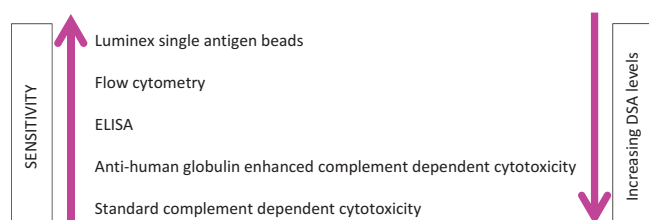
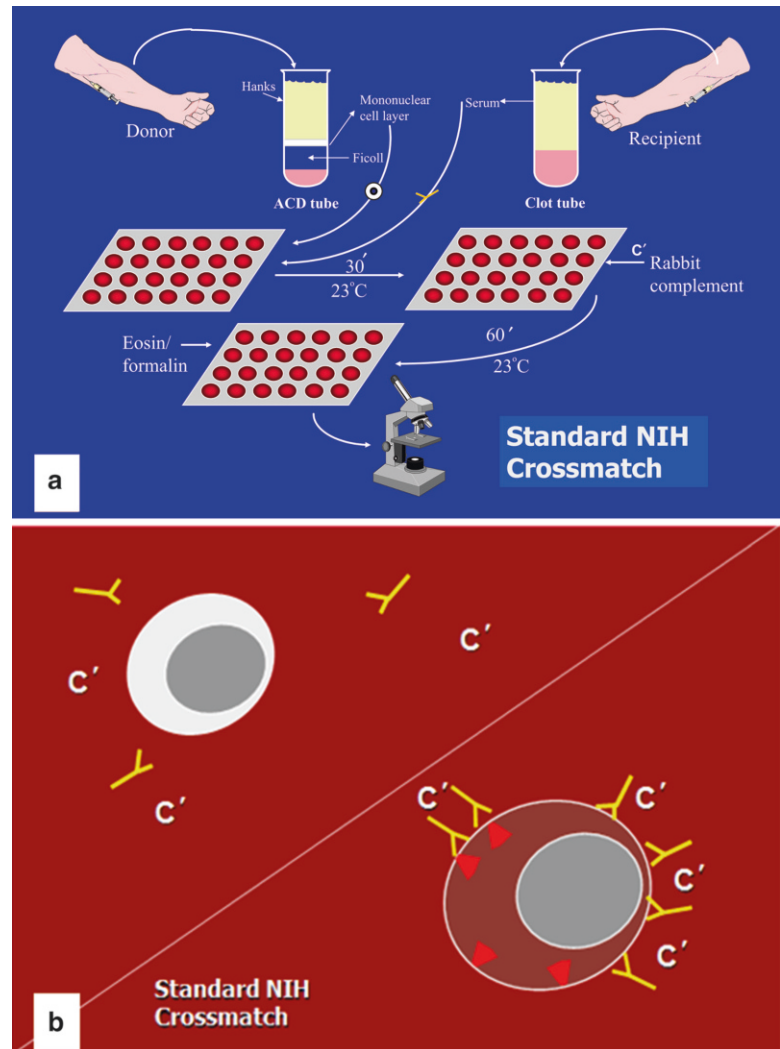


Fig. 2.5 This figure illustrates the increasing sensitivity of immunologic evaluation tests for detecting donor specific antibody (DSA) in the recipient serum. The most sensitive is the single antigen beads, while the least sensitive is the standard complement-dependent cytotoxicity test

Anti-human Globulin-Enhanced Crossmatch

Adding several washes and increasing incubation times increase the sensitivity of the standard crossmatch [37, 38]. However, the most effective way to increase sensitivity of the cytotoxic crossmatch is with the use of anti-human globulin [39, 40]. The anti-human globulin crossmatch technique is similar to the standard CDC crossmatch, with one additional step. Anti-human globulin is added prior to the addition of complement to augment the cytotoxicity reaction. Complement activation requires cross linking of antibodies.

Fig. 2.6 (a) Standard NIH Crossmatch. Donor lymphocytes are collected and placed into wells. Recipient serum is then added to the wells and incubated with the lymphocytes. Rabbit complement is then added to this mixture and incubated. After the second incubation, eosin dye and formalin are added to fix the cells and evaluated under the microscope. (b) If donor specific antibodies are present, then the combination of donor lymphocytes, recipient sera, and complement leads to a reaction in which the antigen/antibody complex recruits complement that then will form membrane attack complexes that destabilize the cell membrane, allowing eosin dye to enter the cell and lead to cell swelling, which is discernible under phase contrast microscopy



If non-complement binding antibodies (IgG4) or low levels of antibodies are present cytotoxicity might not be seen even if antibodies are donor specific. Anti-human immunoglobulin can create cross linking and complement activation and reveal the presence of a DSA. Figure 2.7 demonstrates the steps in performing the anti-human globulin CDC crossmatch.

In a study that compared the standard complement-dependent microcytotoxicity test, the standard test with the addition of antiglobulin, and the standard test with doubled incubation periods 52 of 56 sera tested (93 %) were positive when adding antiglobulin compared with 28/56 (50 %) when the incubation time was extended and 13/56 (23 %) when the standard technique was used [40].

B Cell Cytotoxic Crossmatch

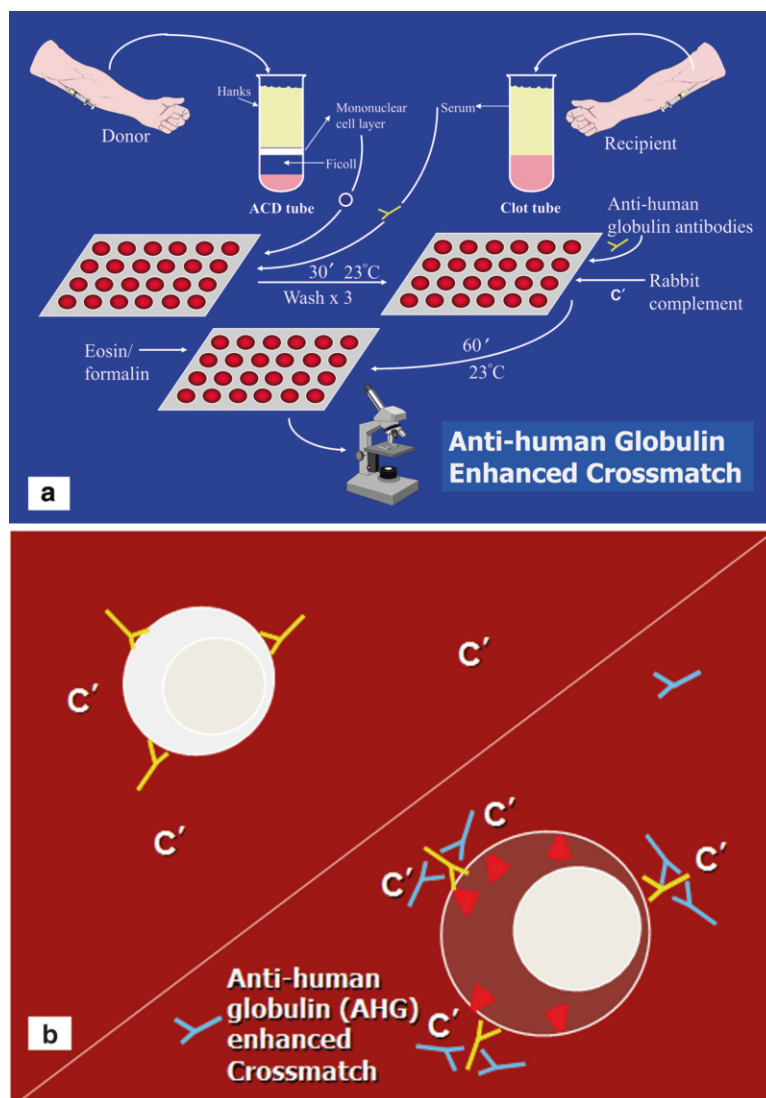
Peripheral blood lymphocytes (PBL) are used for the standard CDC crossmatch and PBL are mostly T lymphocytes (80–85 %). T lymphocytes do not express HLA Class II

molecules but B lymphocytes do express Class II. The B cell crossmatch uses PBL that have been enriched for B cells and therefore is able to detect anti-HLA Class II anti-donor antibodies. Moreover, B cells express higher amounts of Class I molecules. Therefore, the B cell crossmatch can also detect low levels of anti-Class I antibodies even when the standard crossmatch might not. The B cell crossmatch is more technically difficult than the standard crossmatch for several reasons. B cells must be enriched using immuno-magnetic beads, incubation is done at 37 °C, fluorescent dyes are used, incubation is longer, and B cells might be damaged by the enrichment technique [41].

Flow Crossmatch Test

The flow crossmatch test is the most sensitive of all of the tests. Donor lymphocytes are mixed with recipient serum followed by the addition of a fluorochrome-tagged anti-human immunoglobulin. After washing, donor cells are run through

Fig. 2.7 Anti-human globulin-enhanced CDC crossmatch. (a) The anti-human globulin-enhanced CDC crossmatch is similar to the CDC crossmatch, except for additional wash steps and the addition of anti-human globulin, which is thought to bind to the recipient HLA-bound antibodies, thereby improving the ability for the immunoglobulins to fix complement. (b) The additional anti-human globulin binds to the human immunoglobulin that binds to the recipient HLA. Anti-human globulin increases the sensitivity of the CDC crossmatch



a flow cytometer where cells are counted individually. Cells that are bound by antibodies are identified by laser activation of the fluorochrome. This technique allows detection of very small amounts of DSAs and it can differentiate between T and B cells and between IgM and IgG antibodies [42, 43]. It can also be done with donor cells that have been damaged and could not be used in a cytotoxic crossmatch which requires live cells. Figure 2.8 shows the steps involved in a flow crossmatch.

B cells can nonspecifically bind immunoglobulin via FcγR receptors, which bind to the Fc region on IgG antibodies [44]. Addition of the enzyme pronase, which cleaves FcγR receptors, has led to significant improvement in the sensitivity and specificity of the flow crossmatch [44, 45]. While the general understanding is that a positive standard or antiglobulin

crossmatch is a contraindication to transplantation, a positive flow crossmatch may indicate a lower risk of rejection if the standard and antiglobulin crossmatches are both negative. Each transplant center decides which crossmatches to use and how to weigh the importance of each. Table 2.3 lists the different crossmatch test combinations possible and the interpretations of these combinations.

IgM Antibodies and Autoantibodies

IgM antibodies are not considered pathogenic in kidney transplantation [23, 46–48]. However, IgM antibodies can cause a positive cytotoxic crossmatch. Therefore, techniques that remove IgM are necessary. One such technique is the use

Fig. 2.8 Flow crossmatch illustration. In this test, donor cells are incubated with recipient serum. Fluorochrome-tagged anti-human globulin antibodies are added to the mixture which is then placed into the flow machine. Each cell travels through the machine and undergoes evaluation by two lasers. One laser measures the fluorochrome tag differentiating T cells from B cells. The other laser detects the fluorochromes as they emit their signature within the spectrum that identifies the cells with antibody attached to the HLA

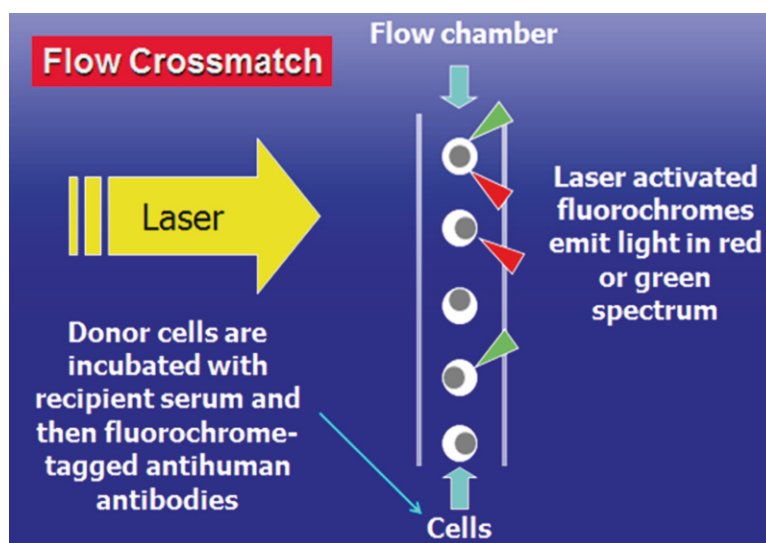


Table 2.3 Various combinations of immunologic tests and possible interpretations

Cytotoxic crossmatch			Flow crossmatch		Interpretation of crossmatch results
Standard	AHG	B cell	T cell	B cell	
+	+	+	+	+	Serum contains significant amount of antibodies to the donor HLA. High risk for hyperacute rejection. Transplantation contraindicated
+	+	0	+	0	Probably not anti-Class I antibodies as B cell crossmatch should also be positive. Perform further antibody testing for antibody specificity
0	0	0	+	+	Probably with a low titer of anti-Class I antibodies and requires further testing. Some risk of hyperacute rejection likely
0	0	+	0	+	Anti-Class II antibody present, or low titer anti-Class I antibody. Check for titer for anti-Class II as this may lead to hyperacute rejection
0	0/+	+	0	0	There is likely an autoantibody, IgM, which is low risk for rejection. Treat with DTT or auto-absorb to remove IgM antibody. May be early sensitizing event prior to class switch from IgM to IgG. If class switch occurs, will be at risk for rejection
0	0	0	0	0	No anti-HLA antibodies present. Low risk for hyperacute rejection

The cytotoxic crossmatch tests include the standard CDC, AHG, and B cell. The flow crossmatch includes the T cell and B cell
AHG anti-human globulin, 0 negative reaction, + positive reaction

of dithiothreitol (DTT) [49]. DTT reduces the disulfide bonds of IgM antibodies, destroying the tertiary structure and reactivity without affecting the IgG reactivity. IgM antibodies, which are reactive at 4 °C (39.2 °F), can also be removed by increasing the incubation temperature to 55 °C (131 °F). It is generally considered important to evaluate the sera of patients who are sensitized to determine if IgM antibodies are present. The flow crossmatch allows the differentiation of IgG from IgM by the reagents used. The fluorochrome-tagged anti-human immunoglobulin used in the assay is specifically directed to IgG only, ignoring IgM.

Occasionally the flow B cell crossmatch is positive but the flow T cell crossmatch is negative. Both T and B lymphocytes express HLA Class I but only B lymphocytes express Class II. The discrepant T and B cell results could be

due to the presence of a donor specific anti-HLA Class II antibody (such as an anti-HLA DP, DQ, or DR antibody). However, IgM or IgG autoantibodies might also cause this due to nonspecific binding on B cells. If a donor specific anti-HLA antibody is not found and if treatment with pronase to cleave FcR from the surface of B cells does not reduce the reaction, an autoantibody might be present. Performing an auto-flow crossmatch (recipient lymphocytes and recipient serum) can clarify these findings. An autoantibody will cause a positive flow B and sometimes flow T cell crossmatch. In the absence of a DSA and in the presence of a positive auto-crossmatch it is generally considered safe to disregard the positive crossmatch against a kidney donor. Many kidney transplants have been performed safely under these circumstances.

The Virtual Crossmatch

Because very sensitive techniques are now available to detect (and specifically identify) the presence of anti-HLA antibodies in the serum of a potential kidney transplant recipient it has become reasonable to consider transplant without a physical pretransplant crossmatch. However, several conditions are necessary to allow the safe application of a virtual crossmatch. If no anti-HLA antibodies are present in a patient's serum it would be considered safe to bypass a physical crossmatch if the most sensitive single antigen bead assay was used to detect antibodies. When anti-HLA antibodies have been detected, the donor HLA type is known, and there are no DSAs, a physical crossmatch can also be bypassed. Further, if a high level of a DSA is present it would be permissible to rule out a donor without performing a physical crossmatch. In most other circumstances including if a highly sensitized patient is being considered or if a patient appears to have a low level DSA a physical crossmatch must always be performed. Moreover, whenever a deceased donor is available several potential recipients are considered and among these there are often sensitized patients. So, unless a kidney is being offered for a specific patient who has no anti-HLA antibodies a physical crossmatch must always be performed. It is always prudent to have backup recipients available even in a directed-donor circumstance in case the recipient is ruled out for some reason. Since the backup recipients are not donor directed a physical crossmatch would need to be performed. It may be possible to more fully use the virtual crossmatch in the future but the most sensitive techniques would need to be used to detect anti-HLA antibodies and the donor would need to be fully HLA typed (A, B, C, DP, DQ, and DR). Further cautioning the use of a virtual crossmatch is the possibility that a potential recipient has a non-HLA antibody that might cause rejection [50–54]. These antibodies would not be detected using only HLA beads and there are no bead-based techniques that detect non-HLA antibodies. However, further complicating issues is the fact that non-HLA antigens that might be important in kidney transplantation (e.g., MICA) are not expressed on lymphocytes, possibly rendering the crossmatch useless in their detection anyway.

Donor-Specific Antibodies

Following transplantation patients can make DSA and these can cause acute antibody-mediated rejection and chronic rejection. Noncompliance with immunosuppressive drugs or chronic under-immunosuppression is likely a cause of the development of DSA. Several studies have shown a significant adverse effect of DSA on graft survival [24, 33, 55, 56].

One study evaluated the outcomes over a 30-year period of patients who were previously transplanted and then re-transplanted with donor allografts of the same HLA A and B antigens as the first allograft. These patients were at increased risk for rejection and early graft loss [55]. Another study found that patients with preformed DSA even with low anti-donor HLA antibody levels suffered from decreased allograft survival [56]. Another found that recipients who developed de novo anti-HLA antibodies to their allografts were more likely to develop acute rejection than those who did not have DSA (29 % vs. 9.5 %) as well as lower 2-year allograft survival (83 % vs. 98 %) [24]. There is probably a level below which DSAs are not pathogenic but that level has not been exactly identified. Certainly, low levels of DSA will not cause a hyperacute rejection. It is generally assumed that the MFI of a DSA must be greater than 10,000 for it to cause a positive standard cytotoxic crossmatch (the one that is associated with 80 % immediate graft loss). It is for this reason that a living donor might be considered acceptable even if a DSA is present if there are no other donors available and if the MFI is <2,000. Techniques and drugs (plasmapheresis, IVIG, rituximab, bortezomib) are available and have been used successfully to reduce or eliminate anti-HLA antibodies. Since most patients who take their immunosuppressive drugs as prescribed do not make DSA it is likely that standard immunosuppression might also help to decrease low levels of DSA present at transplant.

Conclusions

The practice of transplantation has improved tremendously over the last half century. These improvements have been partly due to the recognition of the importance of HLA and ABO blood group matching. Methods to detect antibodies directed against potential donor HLA have improved, and we now possess the ability to detect very low levels of anti-HLA antibodies. Information gathered from the detection of anti-HLA antibodies have helped in donor allocation and the ability of highly sensitized patients to be offered donor kidneys.

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