

XIV. HLA AND TRANSPLANTATION MEDICINE

A. Introduction

1. The HLA system includes a complex array of genes and their molecular products that are involved in immune regulation and cellular differentiation.
2. Antigens of the HLA system have been variously designated *histocompatibility locus antigens*, *human leukocyte antigens*, transplantation antigens and tissue antigens.
3. The HLA gene products are glycoprotein antigens found on the surface membranes of all *nucleated cells* of the body, including those of solid tissues and most of the circulating blood cells; namely lymphocytes, granulocytes, monocytes and platelets.
4. Mature *nonnucleated red cells usually lack HLA antigens* demonstrable by conventional methods, but immature, nucleated rbc's exhibit HLA reactivity.
5. The antigens of the HLA system are second in importance only to the ABO antigens in influencing the survival of transplanted solid organs.
6. Immunologic recognition of differences in HLA antigens is probably the first step in the rejection of transplanted tissue.
7. The antigens of the HLA system are determined by genes present in the major histocompatibility complex (MHC) on the short arm of chromosome 6.
8. Genes of the MHC contribute to recognition of non-self, to coordination of cellular and humoral immunity, to synthesis of several circulating proteins and, quite possibly, to genetic susceptibility to various diseases.
9. HLA antigens are important in tissue and organ transplantation, platelet transfusions, disease associations and very useful in parentage testing.

B. Discovery

1. The MHC that encodes cell surface antigens was first *discovered in skin graft rejection* experiments with mice. When the antigens were matched between donor and recipient, the ability of a graft to survive was remarkably improved.
2. By the early 1960's, an association between human leucoagglutinating antibodies and tissue transplantation was inferred from observations of *accelerated skin graft rejection* in recipients who were preimmunized with peripheral blood leukocytes from the prospective donor.
3. The presence of HLA in humans was first recognized when multiply transfused patients experienced transfusion reactions despite proper crossmatching.
4. It was discovered that these reactions resulted from *leukocyte antibodies* rather than antibodies directed against RBC antigens.

C. General Considerations

1. The MHC is the *most complex immunogenetic system* presently known in humans.

2. The MHC includes several loci closely linked. Each of these loci involves numerous alleles, having at least 10 to 40 alleles per locus that control the production of their corresponding antigens.
3. Some groups of these antigens exhibit cross-reacting characteristics that further increase the complexity of the system.
4. Although HLA was originally identified by its role in transplant rejection, it is now recognized that proteins encoded in this region are involved in many aspects of immunologic recognition, including interaction between different lymphoid cells and between lymphocytes and antigen-presenting cells.
5. The products of HLA genes play a crucial role in our immune system. The HLA genes encode for three classes of molecules (**MEMORIZE**):
 - a. **Class I** major transplantation antigens are serologically defined. This class includes the main HLA-A, B, and C antigens.
 - b. **Class II** immune response gene region antigens are encoded in the HLA-D region and can be subdivided into three families, called HLA-DR, DC (DQ), and -SB (DP).
 - c. **Class III** molecules bear no clear relation to class I and II molecules aside from their genetic linkage. Class III molecules are involved in immunologic phenomena because they represent components of the complement pathways.
6. Both class I and Class II gene products are biochemically distinct, although they appear to be distantly related through evolution.
7. Both class I and class II antigens function as targets of T lymphocytes that regulate the immune response.
 - a. In an infection, the invading microorganism infects cells or is engulfed by immune cells such as monocytes.
 - b. Class I or class II molecules act as receptors for fragments of these microorganisms.
 - c. Once antigenic fragments have bound to the cell surface, receptors on T lymphocytes interact with the antigen-MHC complex triggering both humoral and cellular immune responses.
 - d. T cell-specific cell surface molecules, CD8 and CD4, act to strengthen this cellular interaction and to transmit activation signals.
 - e. Other cell adhesion molecules (CD2, LFA-3 or LFA-1) also act to increase the affinity of cellular interactions.
 - f. The same pattern of interactions between a cell bearing an antigen-MHC complex and a T lymphocyte occur not only in the recognition of infecting microorganisms but also in the recognition of foreign histocompatibility molecules (alloantigens) found on an allograft and in the recognition of self antigens (autoantigens) during autoimmune disease.
7. The impact of studies of the human MHC on clinical medicine was first felt in tissue transplantation, the area that gave the MHC its name.
8. Because foreign MHC molecules are recognized as antigens by the graft recipient, it is beneficial to ensure that donor and recipient share HLA alleles.

9. Since the MHC is extremely polymorphic, this is often a difficult task and requires close collaboration among the surgeon, the clinical HLA typing laboratory, and large organ-sharing networks.

D. HLA and Disease

1. More recently, studies of the MHC have been important in understanding many autoimmune diseases that have immunologic imbalance or disturbances of immune regulation with an MHC associated genetic component.
2. For some conditions, an association exists between HLA phenotype and occurrence of clinical disease.
3. Statistically significant associations have been noted between HLA antigens and a number of diseases.
4. The use of HLA profiles to identify individuals at risk is useful for some diseases in which an absolute association in individual families between transmission of these diseases and inheritance of certain HLA haplotypes has been proven.

E. Techniques for Detection of HLA Antigens

1. **Lymphocytotoxicity** (AKA microlymphocytotoxicity test)

- a. The standard technique used to detect HLA-A, -B, -C, -DR and -DQ antigens is the microlymphocytotoxicity test.
- b. A living lymphocyte suspension from peripheral blood or lymphatic tissue is mixed with antisera and complement.
- c. In the presence of the corresponding antigen, complement is fixed and the cells are killed.
- d. Cell death is determined by staining. A stain such as trypan blue penetrates dead cells but not living ones.
- e. Unaffected cells remain brilliantly refractile when observed microscopically.

2. **Mixed Leukocyte Culture (MLC) Tests**

- a. The HLA-D system is determined by cellular events in MLC.
- b. In the MLC, lymphocytes from two individuals are cultured together, allowing mutual exposure to cell surface antigens.
- c. If the HLA-DR and -DQ antigens are not completely identical, stimulation with lymphocyte enlargement and proliferation occurs within 5 days.
- d. This blast transformation and DNA synthesis is measured by adding a radiolabeled material after the 5-7 days incubation.
- e. Uptake of the radiolabeled material is proportional to DNA synthesis in the dividing cells and is indicative of an immune response.

3. Donor-Recipient Testing

- a. MLC is used to determine class II compatibility between a recipient and prospective organ donor, the donor cell line is pretreated with a substance to prevent proliferation in response to the recipient's antigens.
- b. A unilateral reaction thus occurs in which the treated donor cells act only as stimulator cells and the noninhibited lymphocytes of the recipient respond to foreign antigens present on the stimulating cells.
- c. Kidney transplants from recipient/donor pairs with low reactivity in the MLC have better survival than those showing high reactivity.
- d. The MLC takes several days to perform and is used to select living-related, prospective kidney donors, but it is not practical for selecting cadaveric transplant donors.
- e. The MLC is also used to select donors for bone marrow transplantation.

4. Primed Lymphocyte Typing (PLT)

- a. PLT is a faster method for cellular compatibility testing, but is more expensive and more complex than the routine MLC test.
- b. If cells in an MLC are continued in culture for 9-14 days, the stimulator cells die and the responder cells, although still viable, cease to proliferate actively.
- c. These "primed" responder cells undergo very rapid proliferation if again exposed to the DR, DQ and also, in this case, the DP antigens present on the original stimulating cell.
- d. This technique gives results within 24 hours rather than 4-7 days, but the results, although closely associated with MLC results, are not identical.
- e. The PLT system defines the system of antigens, designated DP, which do not cause sufficient proliferation to be detected in primary MLC tests.

F. HLA Testing and Transplantation

1. Introduction

- a. Transplantation of foreign tissue induces both humoral and cellular immune responses in the recipient, which leads to graft rejection or, for bone marrow transplantation, graft vs host disease (GVHD).
- b. The severity of rejection or GVHD may be reduced by:
 - 1) HLA-matching between donor graft and the host,
 - 2) giving steroids to decrease the humoral antibody response and,
 - 3) inhibiting the cellular immune response with cyclosporin A, antithymocyte or antilymphocyte globulin, azathioprine or monoclonal antibody to the T3 cell receptor.

2. Antigen Testing

- a. Both recipient and donor are ordinarily tested in most solid organ transplants for ABO, HLA-DR, HLA-B and HLA-A antigens; listed in decreasing order of importance.

- b. Anti-HLA-C testing is sometimes performed.
- c. The DQ and DP systems are under evaluation for their usefulness in predicting transplant survival for solid organs but are known to be important in bone marrow transplantation.
- d. A positive T-cell crossmatch by microlymphocytotoxicity testing indicates preexisting antibodies and contraindicates performing a kidney transplant since patients can continue to be maintained on dialysis.
- e. Incompatibility between donor and recipient in the ABO system and preexisting antibodies in the HLA-A and -B systems pose the possibility of hyperacute rejection.
- f. HLA-A and-B typing is important in matching donors for recipients with preexisting HLA antibodies, and also contributes to the likelihood for long-term kidney graft survival.

G. Blood Transfusion in Kidney Transplantation

1. In the early 1970's, many potential kidney recipients were not transfused, or were transfused with D-RBCs in order to minimize HLA immunization from exposure to white cells and platelets.
2. Interestingly, however, kidney graft survival was poorer in these patients than in patients repeatedly transfused with WB or RBCs.
3. As a result, some investigators deliberately transfused potential kidney transplant patients with nonfrozen components that contained abundant HLA-reactive material, and observed increased kidney graft survival.
4. Some current protocols to select living, related kidney donors include transfusing RBCs from the potential donor in an attempt to improve graft survival.
5. These donor specific transfusions (DSTs) are examples of using a designated donor. If the recipient produces HLA antibodies against the donor cells, transplantation does not occur. This spares both donor and recipient major surgery.
6. If the donor does not produce antibodies following the DSTs, the likelihood of success in the subsequent renal transplantation is high.
7. The mechanism by which blood transfusions enhance kidney graft survival remains an enigma.

H. Red Blood Cell Antigens and Kidney Transplantation.

1. ABO compatibility is probably the most important factor determining the immediate survival of organ and tissue transplants other than bone marrow.
2. An interesting exception to the requirement for ABO compatibility is the observation that A2 donor kidneys survive as well as O donor kidneys in group O recipients.
3. Since ABO antigens are expressed in varying amounts on all cells of the body, transplanted ABO-incompatible tissue comes into continuous contact with the recipient's ABO antibodies.
4. For bone marrow transplants, unlike other organ transplants, HLA identity between donor and recipient is more important than ABO.

I. Liver Transplants

1. A clinical liver transplant program presents one of the greatest challenges to the donor center and hospital transfusion service.
2. The demands of a liver transplant program require that the support be maximal in terms of preparedness, supply and responsiveness.
3. Institutes that begin a liver transplant program must make a major commitment to this support.
4. Cooperation and communication are required between the hospital administration, staff of the operating room, intensive care unit, respiratory therapy, radiology, gastroenterology, anesthesiology, coagulation laboratory, transfusion service and donor center.
5. The institutional commitment must extend 24 hours a day and on weekends and holidays since advance notice of a liver transplant may be minimal and may extend only for a few hours.
6. The surgical procedure frequently takes place at night or on weekends because of the availability of the donor organ and in order not to disrupt the set operating room schedule. The transplant procedure may take 6-24 hours and involve several surgical teams.
7. When the organ arrives the institution may have more than one patient waiting for a liver transplant, therefore, the blood bank may have to crossmatch more than one patient.
8. This could involve patients with different ABO and D types (liver must be ABO compatible):
 - a. One protocol involves Type and Screen and crossmatching the patient with 20 units of RBCs using the coombs crossmatch procedure.
 - b. All additional units are released after an IS crossmatch only, if the antibody detection test is negative.
 - c. The protocol also involves the provision of the first 10 units of RBCs as D= for D= patients. Subsequent units issued may be D+ until near the end of the procedure.
 - d. The last 10 to 15 units are provided as D=.
 - e. An attempt to provide all D= blood for younger female adults and children.
 - f. The same policy has been used for patients with specific clinically significant alloantibodies; the first 10 are antigen negative-next, additional units are antigen untested and the last 10-15 units are antigen negative.
 - g. Additionally, 10-20 units of FFP are thawed for initial transplant availability.
9. Adults use approximately 20 units of RBCs, 24 units of FFP, 25 units of PCs and 8 units of CRYO. Children may use up to one-half of these amounts.

J. Heart Transplants

1. Blood bank support for cardiac transplantation is very similar to that routinely used for other types of open-heart surgery in which cardiopulmonary bypass is employed.
2. The heart must be ABO compatible with the recipient.
3. If two patients are waiting for a heart, the heart is given to the patient with the better HLA crossmatch.

K. Pancreas Transplants

1. Pancreatic transplants have lesser transfusion requirements.
2. Either a Type and Screen or the complete crossmatching of a few RBC units should be established as a routine.
3. Pancreas must be ABO compatible.

L. Autologous Bone Marrow Transplantation

1. Autologous bone marrow transplantation (BMT) is used in patients with malignancies of the marrow itself or of solid tumors elsewhere in the body.
2. Preservation of the patient's harvested marrow allows the use of an otherwise lethal dose of radiation and/or chemotherapy followed by reinfusion of the stored marrow to "rescue" the patient.
3. In patients with leukemia, the marrow may be collected during remission for storage or it can be treated to remove the leukemic cells after harvesting and before storage.
4. Experimental methods to eradicate the leukemic cells in vitro have included methods that depend upon physical, chemical and immunological differences between the leukemic cells and normal hematopoietic.
5. The marrow is harvested by aspiration from multiple sites in the iliac crest under general anesthesia.
6. The marrow is processed and may be stored at 4C for up to 3 days or frozen at -196C for long term storage.

M. Homologous Bone Marrow Transplantation

1. Introduction
 - a. Transfusions prior to homologous BMT are known to be detrimental since they compromise the success of engraftment, particularly in patients with aplastic anemia.
 - b. Unfortunately, since most BMT candidates have aplastic anemia or leukemia, transfusions are oftentimes necessary.
 - c. Family members in particular, should be avoided as blood donors prior to transplant since one of them may be used as the bone marrow donor.
 - d. Following BMT, the recipient must receive transfusion support until engraftment occurs and blood cell production is adequate.

- e. RBCs are usually given to maintain the hemoglobin at 9-10 mg/dL. This usually requires 6-20 units in the 10 week posttransplant patient.
 - f. All blood components (except buffy coats from the donor) must be irradiated to prevent GVHD and be anti-CMV negative (CMV-) if the recipient and donor are CMV-.
 - g. Platelet support may also be needed for BMT recipients and should be HLA identical, although this is not always possible and random donor platelets are often used.
 - h. Since an HLA-identical donor is usually required for bone marrow transplantation, the donor (usually a sibling) may not have the same ABO and D identity as the patient. ABO and D identity or compatibility between the donor and the recipient are not required for success of the BMT, but incompatibilities may pose special problems for the blood bank.
2. Major ABO incompatibility
- a. If the donor and recipient have a major ABO incompatibility (ie, donor is group A, recipient is group O), then the group A red cells in the aspirated bone marrow would be rapidly hemolyzed when the marrow is transfused into the recipient and could cause significant problems.
 - b. The problem is resolved by processing the aspirated marrow to deplete it of contaminating red cells.
 - c. Stem cells do not express A or B antigens.
 - d. The group O recipient transplanted with group A marrow may continue to produce anti-A and anti-B for 3-4 months.
 - e. The grafted marrow will begin to produce a new population of blood cells. Group A red cells will appear in the circulation when the recipient's anti-A disappears.
 - f. If transfusion is necessary, washed group O RBCs, CMV-, can be given. All blood components transfused to BMT recipients should be irradiated to prevent GVHD.
3. Minor Side ABO Incompatibility
- a. When marrow from a group O donor is to be transfused into a non-O recipient, the plasma should be removed to reduce the amount of incompatible alloantibody given to the recipient.
 - b. The marrow transfusion then is analogous to a unit of group O RBCs used as a universal donor unit.
4. Chimerism
- a. In spite of the intensive pretransplant chemotherapy and irradiation, some of the host's hematopoietic cells may survive and subsequently co-exist with the transplanted donor's cells.
 - b. This results in what has been termed mixed hematopoietic chimerism and thus the recipient may have a dual cell population.
 - c. This phenomenon does not mean that the recipient will necessarily have a recurrence of the hematologic malignancy.

5. National Bone Marrow Donor Registry

- a. This registry was established for those patients in need of a bone marrow transplant who do not have a suitably matched family member to serve as a donor.
- b. HLA-A, -B (and/or -DR) typed volunteers are included in the central registry of potential bone marrow donors for unrelated patients.
- c. After preliminary HLA-A, B matching, -DR typing and MLC tests are performed as indicated.
- d. When a match is found, and the volunteer wishes to proceed, the donor may have his/her bone marrow aspirated locally.
- e. The aspirated marrow is then sent by airplane to the transplant center of the recipient.
- f. Access to the registry is through participating transplant centers.

N. Hematopoietic Stem and Progenitor Cells

1. Replacing bone marrow transplants.
2. Hematopoietic stem cells are capable of self renewal and of differentiation into any blood cell lineage.
3. Progenitor cells are committed to a lineage pathway and do not have the capacity for infinite self renewal.
4. Hematopoietic stem and progenitor cells can be collected from bone marrow, circulating peripheral blood and umbilical cord blood. Collection is performed by cell separator instruments and involves several collections to ensure adequate numbers of cells are obtained.
5. Three types of transplant options:
 - a. Allogeneic, infusion of hematopoietic stem cells from an individual with genetic constitution different from the recipient's.
 - b. Syngeneic, transplant from an identical twin.
 - c. Autologous, patient donates their own, then is subjected to intensive chemotherapy and/or radiation therapy to cure malignancy, then the autologous donation is infused to rescue the patient.
6. Umbilical cord blood is an alternative to bone marrow or peripheral blood as a source of hematopoietic cells.
 - a. One study estimated that a single cord blood collection can provide enough stem cells for transplantation and hematopoietic reconstitution of an adult.
 - b. The cord blood progenitor cells grow well in culture suggesting the possibility of using in-vitro techniques to provide more stem and progenitor cells.