Lec(7) Immunotechnology

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**Transplantation**

Transplantation is a potentially lifesaving treatment for end stage organ failure, cancers, autoimmune diseases, immune deficiencies, and a variety of other diseases. Over 28,000 solid organ(kidney, pancreas, liver, heart, lung, small intestine)transplants in particular the role of human leukocyte antigens (HLA) and the development of pharmacological agents that interfere with various components of the immune system to promote sustained graft survival .The HLA system is the

largest immunologic barrier to successful allogeneic organ transplantation. It consists of cell surface proteins that play a central role in immune recognition and initiation of immune responses. Because of the ubiquitous presence of these proteins on the surface of nucleated cells and their extensive degree of polymorphism, an allogeneic response may result in graft rejection in solid-organ and stem cell transplantation or graft-versus-host disease in stem cell

transplantation.

**HISTOCOMPATIBILITY SYSTEMS**

The classical (transplant) HLA antigens, also known as **major histocompatibility antigens,** include the class I (HLA-A, B, and C) and class II (HLA-DR, DQ, and DP)proteins. HLA proteins are encoded by a set of closely linked genes on the short arm of chromosome 6 in the major histocompatibility complex (MHC). The HLA genes are inherited as **haplotypes** from parental chromosomes **(Fig1)**. Offspring receive one maternal and one paternal HLA haplotype. Based on this Mendelian inheritance, there is a 25 percent chance that any two siblings will inherit the same two haplotypes (i.e., are HLA identical), a 50 percent chance of being HLA haplo identical (i.e., share one of two HLA haplotypes), and a 25 percent chance of being HLA non identical (i.e., share neither HLA haplotype).

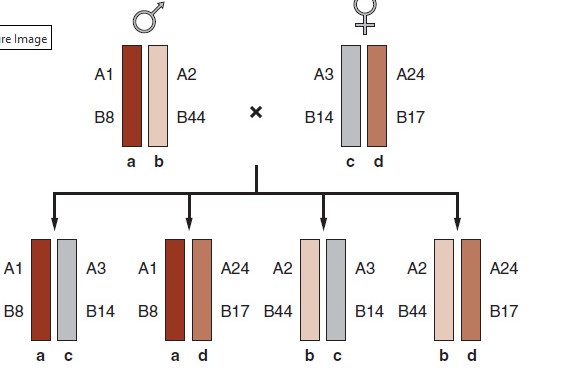


Fig1: HLA genes are linked and inherited in Mendelian fashion as haplotypes. One paternal (a or b) and one maternal (c or d) haplotype is passed to each offspring. Four different combinations of haplotypes are possible in offspring. Elucidation of haplotype sharing between siblings is an important assessment in the search for a transplant donor.

**Minor Histocompatibility Antigens**

A second set of transplantation antigens was identified based on studies in mice and humans demonstrating tissue rejection in MCH-identical transplants and based on outcomes of human stem cell transplants between HLA-identical siblings in whom graft-versus-host disease has developed .Early experimental studies documented a “slower” rejection pace mediated by these transplantation antigens, thus their name—minor histocompatibility antigens (mHAs).mHAs are non-HLA proteins that demonstrate polymorphism in amino acid sequence within a species. Both X-linked and autosomally encoded mHAs have been identified.Introducing a polymorphic variant of one of these proteins from one individual into another individual who possesses a different polymorphic variant (via transplantation of tissue or cells) can induce a recipient immune response to the donor variant. The immune response is mediated by CD4 and/or CD8 T cells recognizing a variant protein in the context of the recipient MHC molecule. This response is analogous to the reaction to a foreign microbial antigen. Several different types of mHAs have been identified, including proteins encoded by the male Y chromosome, proteins for which the recipient has a homozygous gene deletion, proteins that are

autosomally encoded, and proteins that are mitochondrial DNA encoded.

**MIC Antigens**

The MHC class I–related chain A (MICA) encodes a cell surface protein that is involved in gamma/delta T-cell responses. MICA is polymorphic with over 50 allelic variants .MIC proteins are expressed on endothelial cells ,keratinocytes, fibroblasts, epithelial cells, dendritic cells, and monocytes, but they are not expressed on T or B lymphocytes.As such, MICA proteins could serve as targets for allograft immune responses. Antibodies to MICA antigens have been detected in as many as 11 percent of kidney transplant patients. MICA antibodies have been associated with rejection episodes and decreased graft survival.

**ABO Blood Group Antigens**

The ABO system is the only blood group system that impacts clinical transplantation. Anti-A or anti-B antibodies develop in individuals lacking the corresponding blood group antigens. ABO blood group incompatibility is a barrier

to solid-organ transplantation, because these antibodies can bind the corresponding antigens that are expressed on the vascular endothelium. Binding activates the complement cascade, which can lead to **hyperacute rejection** of the transplanted organ. As such, recipient–donor pairs must be ABO identical or compatible to avoid this adverse outcome For example, an individual of blood group A will possess anti-B antibodies and can thus receive an organ only from an ABO-A or O donor. Likewise, a B-expressing individual has anti-A antibodies and can receive an organ only from an ABO-B or O donor. Recently, approaches using plasma exchange and intravenous immunoglobulin administration have allowed successful transplantation of kidneys from ABO-incompatible donors by lowering ABO antibody to levels that allow transplantation to proceed without risk of hyper acute rejection.

**ALLORECOGNITION**

Transplantation of cells or tissues between two individuals is classified by the genetic relatedness of the donor and the recipient. An

1-**autograft:** is the transfer of tissue from one area of the body to another of the same individual.

2- **syngeneic graft :**is the transfer of cells or tissues between identical twins.

3- **allograft:** is the transfer of cells or tissue between two individuals of the same species

4-**xenograft :**is the transfer of tissue between two individuals of a different species.

Most transplantation falls into the category of allo grafting. As stated earlier, HLA disparity between donor and recipient will result in a vigorous immune response to the foreign MHC antigens and is the primary stimulus of graft rejection. The response to foreign MHC antigens is characterized by strong cellular and humoral immune responses The recipient immune system recognizes foreign HLA proteins via two distinct mechanisms—direct and indirect allo recognition **(Fig. 2).**

**direct allorecognition,**recipient T cells bind and respond directly to foreign

(allo) HLA proteins on graft cells. Although an individual T lymphocyte can recognize self-HLA + peptide, foreignHLA proteins may mimic a self-HLA +peptide complex due to similarities in structure of the allo-HLA protein itself

or to structural similarities of allo-HLA protein \_ peptide.Either way, direct allorecognition is characterized by a high frequency (up to 2 percent) of responding T cells compared to the responder frequency in a typical T-cell response to a foreign antigen.The high frequency of responding T cells may be due to several factors, including 1-recognition of multiple amino acid disparities by multiple T-cell clones; 2-the presence of multiple different peptides on an allogeneic cell that are each recognized by different T-cell clones; and the presence of many foreign molecules per cells, resulting in activation of T cells with low affinity, which normally would not be stimulated. The **mixed lymphocyte response (MLR)** is an in vitro correlate of direct allo recognition.

**Indirect allorecognition** is the second -pathway by which the immune system recognizes foreign HLA proteins. Indirect allorecognition is analogous to the normal mechanism of recognition of foreign antigens, as it involves the uptake, processing, and presentation of foreign HLAproteins by recipient antigen-presenting cells to recipient T cells. Indirect allorecognition plays a predominant role in acute and chronic rejection.

The effector responses against transplanted allogeneic tissue include direct cytotoxicity, delayed-type hypersensitivity responses, and antibody-mediated mechanisms.Antibody may mediate antibody-dependent cellular cytotoxicity

reactions and may fix complement, resulting in cell death. Rejection episodes vary in the time of occurrence and the effector mechanism that is operative.



Fig2: Direct versus indirect allorecognition. *(A)* In direct allorecognition, cytotoxic T cells bind directly to foreign HLA proteins on graft cells. *(B)* In indirect allorecognition, foreign MHC

antigens are presented by phagocytic cells, and CD4\_ T cells respond.

**TRANSPLANT REJECTION**

**Hyperacute Rejection( Humoral immune response)**

Hyperacute rejection occurs within minutes to hours after the vascular supply to the transplanted organ is established.This type of rejection is mediated by preformed antibody that reacts with donor vascular endothelium. ABO, HLA,

and certain endothelial antigens may elicit hyperacute rejection.Binding of preformed antibodies to the alloantigens activates the complement cascade and clotting mechanisms and leads to thrombus formation. The result is ischemia and

necrosis of the transplanted tissue.Hyperacute rejection is seldom encountered in clinical transplantation. Donor–recipient pairs are chosen to be ABO identical or compatible, and patients awaiting transplantation are screened for the presence of preformed HLA antibodies. In addition, the absence of donor HLA specific antibodies is confirmed prior to transplant by the performance of a cross match test.

**Acute Cellular Rejection(Humoral &cellular immune response)**

Days to weeks after transplant, individuals may develop**acute rejection.** This is a cellular-type rejection but may involve antibody as well. Acute rejection is characterized by parenchymal and vascular injury. Interstitial cellular infiltrates

contain a predominance of CD8-positive T cells as well as CD4 T cells and macrophages. CD8 cells likely mediate cytotoxic reactions to foreign MHC-expressing cells, while CD4 cells likely produce cytokines and induce

delayed-type hypersensitivity (DTH) reactions. Antibody may also be involved in acute graft rejection by binding to vessel walls, activating complement, and inducing transmural necrosis and inflammation as opposed to the thrombosis typical of hyperacute rejection. The development and application of potent immunosuppressive drugs that target multiple pathways in the immune response to

alloantigens has improved early graft survival of solid-organ transplants by reducing the incidence of acute rejection

**Chronic rejection ☹Cellular immune response)**

**Months to years**

results from a process of graft arteriosclerosis characterized by progressive fibrosis and scarring with narrowing of the vessel lumen due to proliferation of smooth muscle cells. Several predisposing factors impact the development of chronic rejection, including prolonged cold ischemia, reperfusion, acute rejection episodes, and toxicity from immunosuppressive drugs. Chronic rejection is also thought to have an immunologic component, presumably a delayed-type hypersensitivity reaction to foreign HLA proteins. This is indicated in studies employing animal

models of graft arteriosclerosis in which mice lacking IFN gamma do not develop graft arteriosclerosis. In addition ,similar studies support an important role for CD4

T cells and B cells in this process. Cytokines and growth factors secreted by endothelial cells, smooth muscle cells,and macrophages activated by IFN gamma stimulate smooth muscle cell accumulation in the graft vasculature.

**GRAFT-VERSUS-HOST DISEASE**

Stem cell transplants (and less commonly lung and liver transplants) are complicated by a unique allogeneic response—**graft-versus-host disease (GVHD).** Recipients of stem cell transplants for hematologic malignancies typically have depleted bone marrow prior to transplantation as a result of the chemotherapy used to treat the malignancy.Next, donor bone marrow or, more commonly, peripheral blood stem cells are infused. The infused products often contain some mature T cells. These cells have several beneficialeffects, including promotion of engraftment, recon stitutionof immunity, and mediation of a graft-versus-leukemia effect. However, these mature T cells may also mediate

GVHD.

**Acute GVHD** occurs during the first 100 days post infusion and targets the skin, gastrointestinal tract, and liver.In mismatched allogeneic stem cell transplantation, the targets of GVHD are the mismatched HLA proteins, while inmatched stem cell transplantation, minor histocompatibilityantigens are targeted. The infused T cells can mediate GVHD in several ways, including a massive release of cytokines due to large-scale activation of the donor cells by MHC mismatched proteins and by infiltration and destruction of tissue.The incidence and severity of GVHD is related to the match status of the donor and recipient as well as other factors. In efforts to reduce the incidence and severity of GVHD, several approaches are taken, including immunosuppressive therapy in the early post-transplant period removal of T lymphocytes from the graft. T-cell reduction is very effective in lowering the incidence of GVHD, but it can also reduce the graft-versus-leukemia (GVL) effect of the infused cells and increase the incidence of graft failure.Beyond 100 days post-transplant, patients may experience chronic GVHD. This condition resembles autoimmune disease, with fibrosis affecting the skin, eyes, mouth, and other mucosal surfaces.

**IMMUNOSUPPRESSIVE AGENTS**

There is a growing list of agents that are employed to suppress ant igraft immune responses in solid-organ and stem cell transplantation. **Immunosuppressive agents** are used in several ways, including induction and maintenance of immune suppression and treatment of rejection. Combinations of different agents are frequently used to prevent graft rejection. However, the immunosuppressed

state (and graft survival) induced by these agents comes at a price of increased susceptibility to infection, malignancies, and other associated toxic side effects. There are several classes of immunosuppressive agents, which are

**Corticosteroids**

Corticosteroids are potent anti-inflammatory and immunosuppressive agents used for immunosuppression maintenance. At higher doses, they are used to treat acute rejection episodes.Steroids act by blocking production and secretion of cytokines, inflammatory mediators, chemoattractants, and adhesion molecules. These activities decrease macrophage function and alter leukocyte-trafficking patterns. However,long-term use is associated with several complications, including

hypertension and diabetes mellitus.

**Antimetabolic Agents**

Anti-metabolic agents interfere with the maturation of lymphocytes and kill proliferating cells. Azathioprine was the first such agent employed. It has been replaced in large part by mycophenolate mofetil, which has a more selective effect

on lymphocytes compared to azathioprine and thus fewer side effects.

**Calcineurin Inhibitors**

Cyclosporine and FK-506 (tacrolimus) are compounds that block signal transduction in T lymphocytes, resulting in impairment of cytokine syntheses, including IL-2, 3, 4, and interferon-gamma. Inhibition of cytokine synthesis blocks

the growth and differentiation of T cells, impairing the antigraft response. Rapamycin (sirolimus) is an agent that inhibits T-cell proliferation by binding to specific intracellular proteins, including mammalian target of rapamycin (mTOR).

**Monoclonal Antibodies**

Several monoclonal antibodies that bind to cell surface molecules on lymphocytes are used as induction agents and to treat severe rejection episodes. OKT3 is a mouse monoclonal antibody the binds to the CD3 receptor on human lymphocytes. Binding of OKT3 to the CD3-positive T-cell surface has several outcomes. Binding may modulateCD3 from the cell surface, rendering the affected T cells

nonfunctional. Higher doses of antibody deplete T cells from the circulation via complement-mediated lysis oropsonization for removal by phagocytic cells.

A problem with monoclonal antibody preparations administered to patients is the development of anti-mouse antibody.

**Polyclonal Antibodies**

Two polyclonal anti-T-cell antibody preparations are used to treat severe rejection. Thymoglobulin is an anti thymocyte antibody prepared in rabbits, and ATGAM is a polyclonal antiserum prepared from immunization of horses. Both are potent immunosuppressive agents that deplete lymphocytes from the circulation. The development of these anti-mouse antibodies can interfere with the activity of the monoclonal antibody. A drawback associated with administration of polyclonal antibody preparations is the development of serum sickness due to antibody responses to the foreign immunoglobulin