Immunology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

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In this sheet we are going to discuss the MHC molecules, protein antigens, and transplantation immunology.

Recognizing how molecules were discovered helps us to better understand and acquire most information about these molecules.

MHC (major histocompatibility complex):

At the early beginnings of transplantation procedures, inflammation was recognized among patients who have undergone kidney transplants or blood transfusion. It's been revealed that antibodies of the receiver of transplantation recognized the cells of the donor. Even multiparous women had circulating antibodies that recognized the paternal cells.

They named the antigens **as HLA (Human Leukocyte Antigens)**, thinking that they only exist on the surface of the leukocytes. They were addressed to leukocytes due to the experiment that lead to their discovery. In that experiment, they put leukocytes of the donor with the antibodies found in the recipients' body. The result was that the antibodies did bind to the leukocytes, and stimulated the production of more antibodies. Later on, scientists discovered that these antigens exist on so many other types of cells in the donor's body. The antigens where then renamed as **MHC (Major Histocompatibilty Complexes)**. Both terms (**MHC**) and (**HLA**) are used interchangeably.

In another study, scientists noticed a variability in responding to a specific antigen between different strains of mice. Two mice were injected with a pathogen. Certain mice had good immune response against the particular polypeptide antigens, while other mice didn't respond probably, mounting a less effective immune response. This difference in response is due to inherited genes, because each strain progenies had the same response. The gene responsible for this validity is called MHC gene, whose products can bind peptides derived from these antigens.

The MHC molecules are glycoproteins encoded in the large cluster of genes. Their most striking structural feature is a cleft running across their outermost surface, in which a variety of peptides can be bound, forming **peptide-MHC complexes** that can be recognized by helper & cytotoxic T cells.

Each class of MHC molecules (class I and class II) has a single peptide-binding cleft that binds one peptide at a time, but each MHC molecule can bind many different peptides;(MHC I usually binds small peptides with 8-10 amino acids while MHCII binds a larger 13-17 amino acid).

MHC molecules show great genetic variation in the population, and each individual carries up to 12 of the possible variants, which increases the range of pathogen-derived peptides that can be bind. Molecular sequencing has shown that a single serologically defined HLA allele actually consists of multiple variants that differ slightly. This means that MHC I, for example, has different alleles (variants) for each individual. Different alleles will have the same general constitution of units as a protein, but they'll differ in the cleft shape, which affects the way in which antigen peptides would bind to it, producing different complexes recognized differently by the T lymphocytes.

The cause of variation is still unknown. One hypothesis is that when a disease attacks people, some people must survive, because they're better responders to pathogens with an effective immune response and that's due to the specific way their MHCs bind to peptides.

Displaying of Proteins on MHC

Cytosolic proteins, whether viral, bacterial, or cell related, will be expressed on MHC by being broken down into peptides by proteasomes. Peptides than enter the ER with the help of TAP (transporter for antigen presentation), where they bind to the MHC molecules and are then displayed on the cell surface.

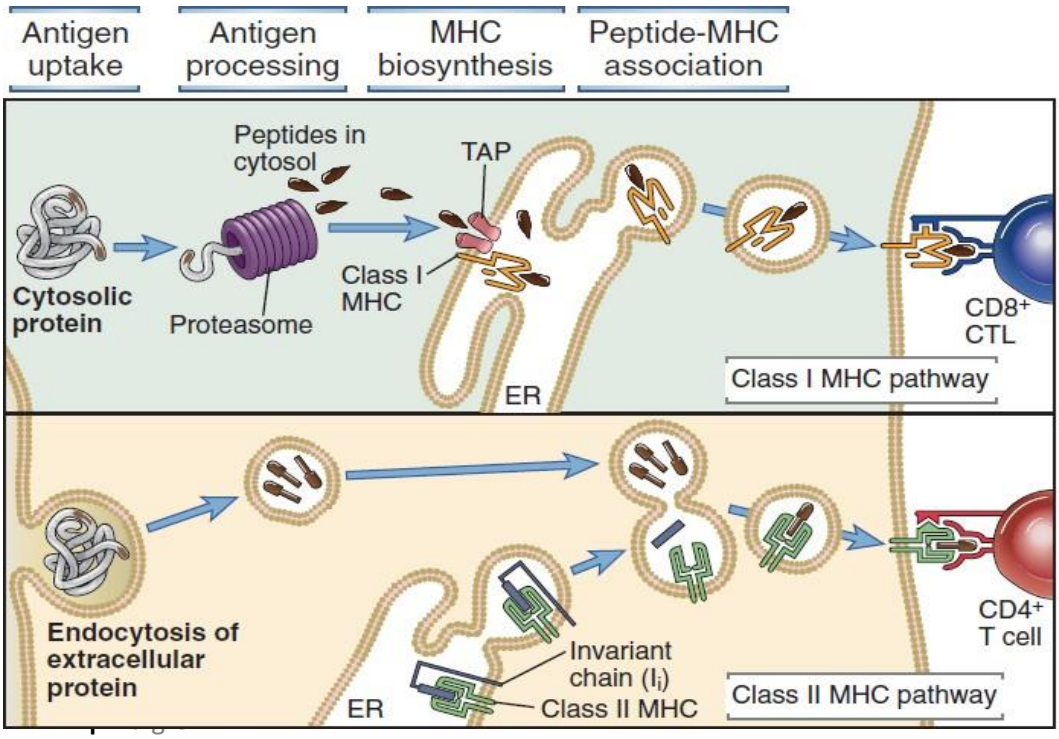


FIGURE 6–14 Pathways of antigen processing and presentation. In the class I MHC pathway *(top panel)*, protein antigens in the cytosol are processed by proteasomes, and peptides are transported into the endoplasmic reticulum (ER), where they bind to class I MHC molecules. In the class II MHC pathway *(bottom panel)*, extracellular protein antigens are endocytosed into vesicles, where the antigens are processed and the peptides bind to class II MHC molecules. Details of these processing pathways are in Figures 6-16 and 6-17.

-The type of MHC molecules on which peptides will bind, depends on the pathway by which the pathogenic protein enters the cell:-
-if the protein was a cytosolic protein, it would be presented on MHCI thus CD8+ will recognize it.
-if the protein was an extracellular protein, it would be presented on MHCII & thus CD4+ T cells will recognize it.
NOTE: Remember that MHCII is usually present on professional antigen presenting cells. Some endothelial and epithelial could also present MHC2.

Recognition of MHC

T-cells' receptors are not only specific to foreign peptides, but also to a unique combination of a peptide antigen and a particular MHC molecule. This kind of recognition is known as **MHC restriction** (MHC+Protein = Complex that is recognized).

MHC Restriction:
-In an experiment, mice of two different strains were infected with a certain virus. After a period of time, (the several days we wait in order for the adaptive immune response to activate the T lymphocytes), the activated T lymphocytes of one mouse (mouse1) where extracted.
-The T Lymphocytes were then co-cultured with target cells of the two infected strains in two separate petri dishes.

*Petridish1(mouse1 Tcells + mouse 1 infected cells) and petridish2 (mouse1 Tcells+ mouse2 infected cells)

Why did we do this (Cytotoxic assay)?

In order to see whether T lymphocytes will kill (induce apoptosis in) mouse2's infected cells.
The whole experiment was performed to see the reaction of T Lymphocytes of an individual to different individual's MHC+protein complex (MHC restriction).
In petridish1, Mouse1 lymphocytes with Mouse1 infected cells reacted properly, inducing apoptosis of cells. This happened because MHC complex was recognized.
On the other hand, in petridish2, Mouse1's T Lymphocytes with Mouse2's infected cells didn't react properly. T Lymphocytes didn't recognize the MHC complex of Mouse2, therefore, no apoptosis was induced.

Cross presentation(cross-priming):

The only cells able to convert naïve T lymphocytes to the active form are dendritic cells. Dendritic cells ingest infected cells and present their antigens to naïve T lymphocytes by a process called cross presentation; in which dendritic cells perform pinocytosis (endocytosis) to uptake antigens in order to process them then present them extracellularly to naive T lymphocytes.

Dendritic cells have the ability to capture and to ingest virus-infected cells or tumor cells and present the viral or tumor antigens on ***MHC1*** to activate CD8+ T cells.

-After ingesting the infected cell, dendritic cells will proceed in one of the following pathways (two):

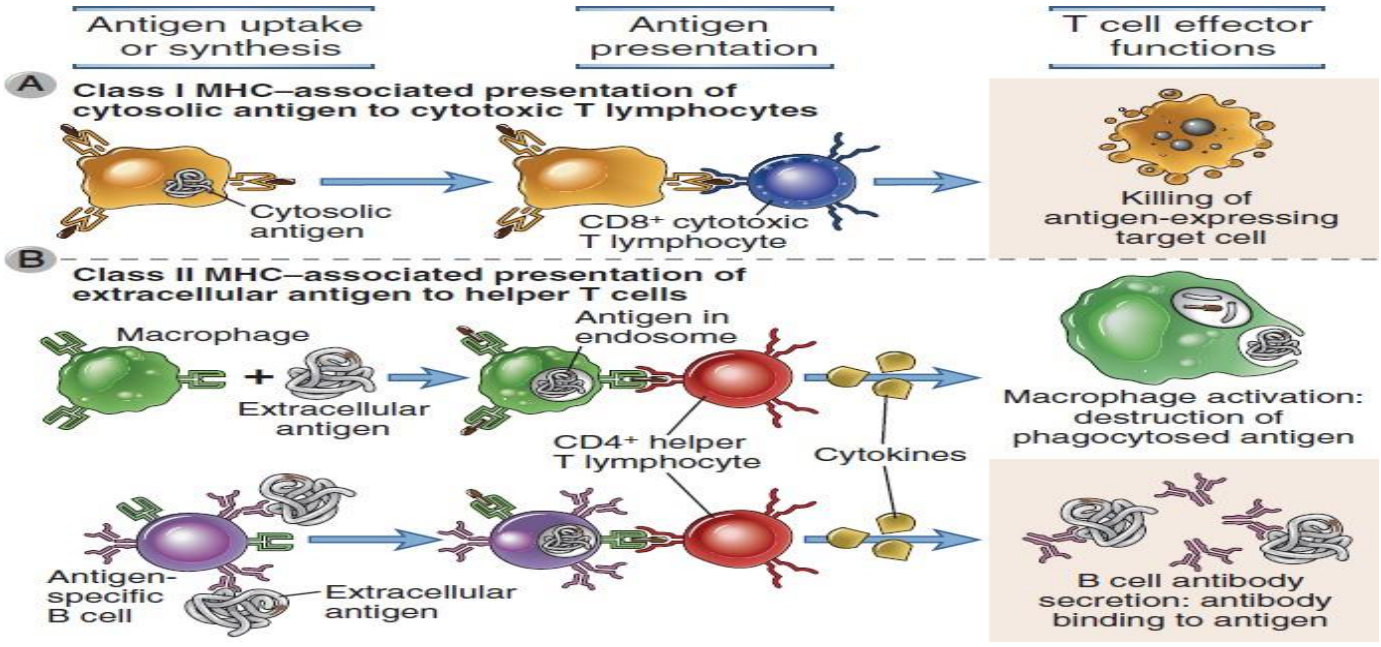
1- Dendritic cells will present the antigens on MHCII (mainly activating CD4 helper T cells).

2-In a not well defined pathway, dendritic cells will present the antigens on MHCI activating CD8+ T cells.

NOTE:

1) Post endocytosis of infected cells, dendritic cells might represent the antigen on MHC1; thus cross presentation is the ability of a cell to represent antigens on MHC1 and MHC2.

2) B cells & macrophages can't activate T lymphocytes although they are antigen presenting cells; they can only increase the activity of an already activated one increasing the effectivity of its function.



Ways of antigen presentation:-

1) MHC1 presentation after antigen uptake => cytosolic antigen presented for CD8+ T cells by dendritic cells. T lymphocytes then induce apoptosis of the infected cell.

2) MHC2 presentation by =>

a. Macrophages: cells like macrophages ingests microbes & can't kill them, so they present the antigen and a CD4 helper T cell, which is already activated (from dendritic -antigen presenting-cell), recognizes the macrophage carrying the same antigen and secretes cytokines helping the macrophage to become activated & kill the antigen.

b. B cell receptors: recognition of helper T cells(CD4) of an antigen presented on B cell receptor which was either, digested or, encountered, and then presented. This stimulates CD4 cells to release cytokines which would activate B cells proliferation and differentiation(sheet9).

❖ This Whole table is important but we've explained a few things that aren't clear in it.

TABLE 3-3 COMPARISON OF ANTIGEN RECOGNITION BY T CELLS AND B CELLS

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

Notes on the previous table:-

1) Modes of activation

-binary complex: antigen bound to B cell receptor.

-Ternary complex: antigen bound to MHC (MHC complex) + T cell bound to it to recognize it.

2) Chemical nature of antigens

- B cells can recognize proteins, polysaccharides and lipids as epitopes.
-The epitopes of complex proteins that elicit the strongest T cell responses are the peptides that are broken down by proteolysis in APCs, then, presented most avidly to MHC molecules.

3) Epitope properties:

The shape of the epitope in the antigen (the special arrangement of amino acids it's composed of) can influence the binding of T & B lymphocytes receptors to it.

- B cells mainly use **conformational determinants**, meaning that the tertiary and final structure of the antigen contains the epitope that the antibody of b cells binds to. If the structure of the antigen gets broken down or disrupted, B cells' antibodies won't be able to bind the epitope. This enables B cells to uptake soluble antigens and display them.

-T cells usually use **linear determinants**, which are a stretch of peptides on the epitope. This stretch of peptides(epitope) doesn't depend on the structure the of the antigen. **Sometimes** the antigen must be denatured, or broken down for the T cell to recognize it on MHC molecules.

Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Molecules that stimulate immune responses are called **immunogens.**

So detriments could be accessible and immediately recognized or they need to be denatured.

The linear detriments need to be denatured, cleaved beforehand & presented on MHC molecules in order for T cells to recognize it.

Neoantigenic detriments(neoepitopes):

The antigen doesn't have an epitope, but by the actions of proteases, the protein is spilt in a certain way in which a detriment is created due to the conformational changes and a neoepitope is produced.

Remember //these are the different ways in which T / B cells detect antigens & respond to them.

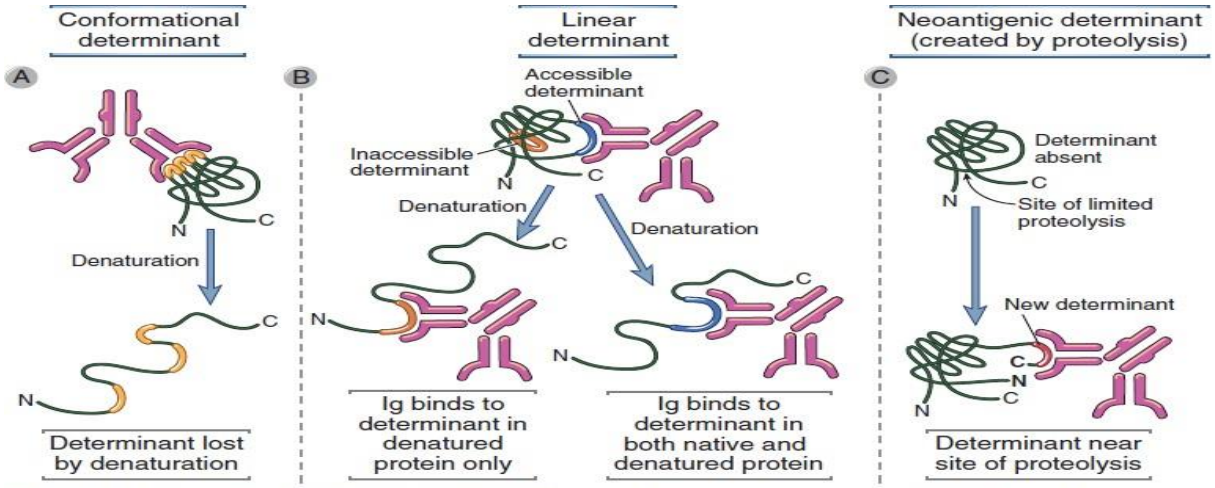


FIGURE 5–12 The nature of antigenic determinants. Antigenic determinants (shown in orange, red, and blue) may depend on protein folding (conformation) as well as on primary structure. Some determinants are accessible in native proteins and are lost on denaturation **(A)**, whereas others are exposed only on protein unfolding **(B)**. Neodeterminants arise from postsynthetic modifications such as peptide bond cleavage **(C)**.

Immunodominant epitopes

The majority of the responding T cells are specific for only one or a few linear amino acid sequences of the antigen. These are called the **immunodominant epitopes** or determinants. Immunodominant epitopes can mount a strong immune response against it.

Understanding the basis for this can help in vaccine production. Some vaccines mount good immune responses thus a higher level of protection & others produce weak and inadequate immune responses. This difference in reaction of different vaccines seems to be related to the structure of the antigens' epitope. If we know the antigen's structure, we can produce better vaccines → better protection.

Note: vaccination is introducing a systematic antigen(weakened) to the body to mount an immune response against it. The next time the body encounters this antigen, it recognizes it faster, creating a better response in the case of a real infection.

Transplantation:

Transplantation is the process of moving cells, tissues or organs from one site to another, for the purpose of replacing or repairing damaged or diseased organs and tissues. With it we can beat chronic diseases of the heart, liver, kidney...etc.

The immune system poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another. **Rejection** is caused by the immune system identifying the transplant as a foreign object, triggering a response that will ultimately destroy the transplanted organ or tissue.

The donor and the recipient are carefully **matched** prior to transplantation to minimize the risk of rejection. The best donor is the one that have the most similar genetic material matched with the recipient.

Even if we find the best donor, there will always be a need to give immunosuppressive drugs that work on certain parts of the immune system to prevent the rejection of the transplanted organ by dampening the overall immune response.

Immunosuppressive drugs are given in two phases; an initial induction phase involving a high dose, and a later maintenance phase which involves using the drug in the long term at a lower dose. The combination of drugs and the dosage given will vary depending on the type of transplant and the chosen treatment regime.

Examples include: The calcineurin inhibitors cyclosporine and tacrolimus, steroids, target of Rapamycin Inhibitors, Azathioprine.

- Research on the immunological mechanisms of rejection will help improve cross matching, diagnosis and treatment as well as facilitating the discovery of novel strategies for preventing rejection.

Note: the most frequently transplanted organs are kidneys then livers.

Types of transplantation:

Autograft –Transplantation of cells, tissues or organs between sites within the same individual e.g. skin grafts in burnt patients.

Allograft – Transplantation of organs or tissues from a donor to a non-genetically identical individual of the same species. Allografts are the most common type of transplant; the most common one.

Isograft - Transplantation of organs or tissues from a donor to a genetically identical individual (i.e. identical twin). It's the best type of transplantation because the donor and the recipient have identical genetic material. We give immunosuppressive drugs for a small period of time => easily accepted.

Xenograft – Transplantation of an organ or tissues between two different species. 'Pig valves', for example, are commonly used to repair or replace a defective heart valve in humans.

Allogeneic MHC molecules (MHC molecules of the donor that are available on the transplanted organ/tissue) of a graft may be presented for recognition by the T cells of the recipient in two fundamentally different ways, called direct and indirect alloantigen presentation.

The MHC variability between individuals causes T cells to recognize them as foreigners producing an alloreactive T cells.

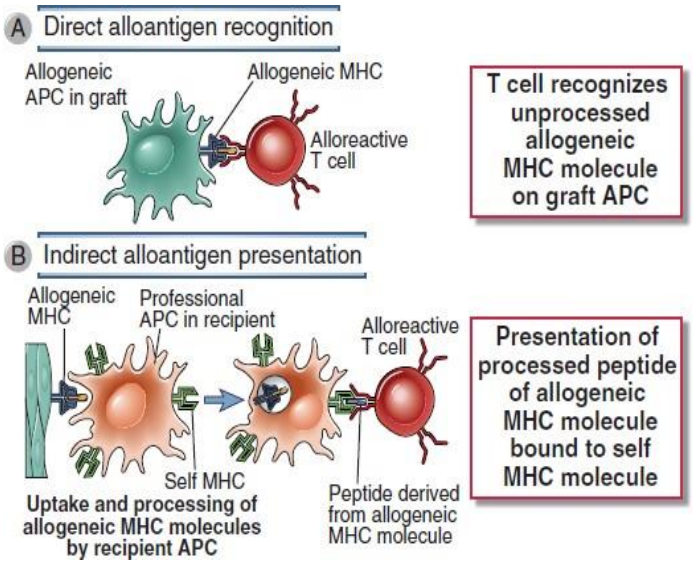


FIGURE 16–4 Direct and indirect alloantigen recognition. A, Direct alloantigen recognition occurs when T cells bind directly to an intact allogeneic MHC molecule on a graft (donor) antigen-presenting cell (APC). **B,** Indirect alloantigen recognition occurs when allogeneic MHC molecules from graft cells are taken up and processed by recipient APCs and peptide fragments of the allogeneic MHC molecules containing polymorphic amino acid residues are bound and presented by recipient (self) MHC molecules.

-***Direct Alloantigen Recognition:***

T cells of the recipient recognize MHC complexes that are foreign, causing the initiation of an immune response.

-Indirect Alloantigen Recognition:

Dendritic cells of the recipient recognize and uptake the foreign MHC complex of the donor. Then, dendritic cells represent the uptaken MHC complex to T cells which mount an immune response against the donor cells.

Rejection of graft (types/stages):

Hyperacute rejection occurs immediately within minutes or hours after a transplantation and is caused by the presence of preexisting antibodies of the recipient that match the foreign antigens of the donor (eg. Blood groups), triggering an immune response against the transplant by the innate immune system; usually circulating alloantigen's specific antibodies react with a preformed antibodies in the recipient circulation.

The antibodies react with cells in the blood vessels of the graft, causing blood clots to form, which leads to the death of the organ and blood vessels by preventing blood supply from reaching the graft which results in immediate rejection of the transplant.

The binding of the antigen will activate the complement system and will draw WBC into the endothelium, damaging the blood vessel, causing thrombosis and clots.

So the recipient already has antibodies against the antigen of the donor. ex.: BGA in the endothelial cells.

Acute rejection usually takes several days-weeks, and occurs within the first 6 months after transplantation by the dominance of adaptive immune response. Some degree of acute rejection will occur in all transplantations, except between identical twins.

When the organ is transplanted, alloantibodies are newly formed by B-cells. Cytotoxic T lymphocyte will recognize the antigen (either directly or indirectly) and they'll harm parenchymal cells of the organ causing them to die. This will injure endothelial cells because they carry the antigen.

Parenchymal & endothelial (endothelialitis) damage and interstitial inflammation include the participation of CD4 helper T cell, cytotoxic T lymphocyte and the production of antibodies which also injure the graft.

Giving immunosuppressive drugs could help overcome the acute rejection. Problems occur when we have an inflammatory response (which probably exists), the inflammatory response because, due to immunosuppressive drugs, the immune response is not efficiently active. Meaning that the mechanism of acute rejection is taking place in the background and in a less harmful manner; the environment is inflamed but we are trying to suppress it.

Repeated episodes of acute rejection can ultimately lead to **Chronic(Graft) Rejection** of the graft and failure of the transplant. Chronic rejection commonly manifests as scarring of the tissue or organ, which can occur months to years after acute rejection has subsided.

We don't know what causes the chronic rejection clearly; although one theory says that the production of cytokines (which both, proliferate and induce inflammation of the tissue) affects the intimal smooth muscle cells causing their proliferation and the

occlusion of blood vessels in a chronic inflammation. Therefore, cells keep proliferating until they occlude the blood vessels resulting in ischemic damage.

Compatibility testing (matching)

Rejection can be **minimized** by carefully matching the donor and recipient for compatibility prior to transplantation. The better matched the donor and recipient are the more successful the transplantation is likely to be. Several tests are commonly done including:

ABO blood group compatibility – The donor and recipient are tested for compatible blood groups.

Tissue typing – A blood sample is taken from the recipient to identify the HLA antigens present on the surface of their cells to help find a compatible donor. **Siblings** offer the best donors usually.

Cross matching – Blood samples are taken from both the recipient and the donor, and the cells of the donor are mixed with the blood serum of the recipient. If the recipient's antibodies attack the donor cells, they are considered a positive match and transplantation will not be suitable due to increased risk of hyper-acute rejection.

Panel reactive antibody test – The blood serum of patients awaiting transplantation are tested for reactive antibodies against a random panel of cells. The more HLA antibodies present, the higher the panel reactive antibody (PRA) level denoted to the patient, and the greater the chance of graft rejection.

Graft vs host disease (GVHD)

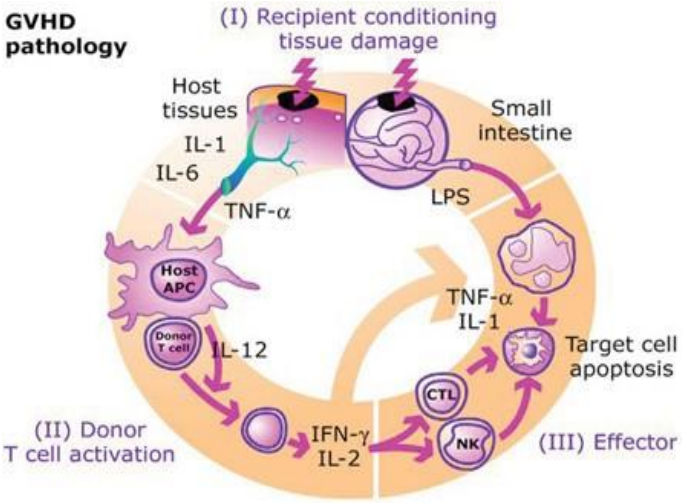
Allogeneic hematopoietic stem cell transplantation (HSCT) is used for treatment of several hematological malignancies as well as immune disorders.

GVHD is initiated by mature $CD4^+$and/or $CD8^+$ **T cells** that accompany **allogeneic HSCT**.

GVHD can occur in **HLA identical** individuals, due to differences in **minor** histocompatibility antigens (miHA). Many miHA are encoded on the Y chromosome.

Diagnosis of GVHD is based on signs and symptoms the affected tissue.

***Note*,GVHD** wasn't discussed by Dr Anas.



قد تورق الأشجار بعد ذبولها

و يخضرّ ساق النبت و هو هشيم

-محمود البارودي.