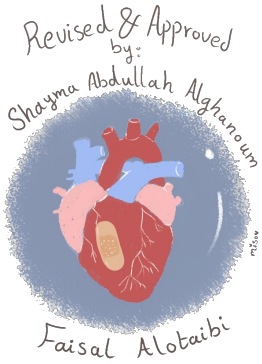


Transplantation

Color index

Important

Extra information

Notes

Slide reference

IMMUNOLOGY

TEAM 439

Objectives

- To understand the diversity among human leukocyte antigens (HLA) or major histocompatibility complex (MHC).
- To know the role of HLA antigens in transplant rejection.
- To be familiar with types of immune responses mediating transplant rejections and importance of tissue matching.
- To understand the principles of management after transplantation.

Editing File

Click here!
Please check frequently

Please do not be frightened by the slide number or notes. The lecture is easy and simple. We did our best to explain it in the clearest way possible.

WE RECOMMEND STUDYING THIS LECTURE BEFORE PATHOLOGY

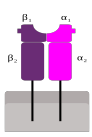
GOOD LUCK!

Major Histocompatibility Complex & Transplantation

- The ability of our immune system to recognize its **own cells** and distinguish those cells **from foreign bodies** depends on a group of protein markers found on cell membranes called Major Histocompatibility Complex (**MHC**). The human version of MHC is called Human Leukocyte Antigens (HLA). (They're the same)
- MHC proteins were discovered for the first time with the advent of tissue transplantation, it's essential for organ transplants.
- The success of tissue and organ transplantation depends upon the donor's and recipient's (HLA).
- Alloantigens are antigens in an a allograft (transplant) which are made up of proteins. (Pathology) Alloantigens could be **HLA** or **blood group (ABO) antigens,** both are important when speaking of compatibility.
- **Transplant rejection** is a process in which a recipient's immune system attacks the transplanted/donated tissue. This should not be confused with **Graft Versus Host Disease (GVH)** which occurs when immune cells in the donated tissue attack recipient's body cells. (Both will be discussed)

Major Histocompatibility Complex (Major HLA antigens)

- Classified into: MHC Class I, MHC Class II, MHC Class III (complement system related, not our interest in this lecture)
- Play a major role in graft rejection
- Present antigens to T cells



- Encoded by genes in the short arm of chromosome 6

Minor Histocompatibility Antigens (Minor HLA antigens)

- Not encoded by genes, do not present antigens to T cells.
- Only play a role in chronic rejection of a graft.
- There are no laboratory tests to detect minor antigens
- Weak antigens, when recognized by immune system they stimulate a weak immune response

*Mechanism of action of minor HLA antigens is not known

*After HLA Typing, any difference later appearing in proteins between recipient and donor may be considered as minor antigen and induce chronic rejection, that is why it is difficult to match recipient and donor for minor antigens because we don't know how to detect them.

Major Histocompatibility Complex

MHC class I	MHC class II
Both play a role in rejection but **MHC II is mainly the major cause**	
Encoded glycoproteins found on the surface of virtually all the nucleated cells (all body cells except RBCs).	Encoded glycoproteins found on the surface of **A**ntigen **P**resenting **C**ells (macrophages, B cells, and dendritic cells), **APC's** are the only cells that contain MHC I & MHC II on their surface.
Present endogenous peptide antigens (virus infected cells, tumor cells, pathogen infected cells) to T cytotoxic CD8 cells for clearance	Present exogenous peptide antigens (bacterial toxins) to T helper CD4 cells for clearance.
T Cytotoxic cell kills virus infected cells in association with MHC class I proteins (MHC is recognized by T cytotoxic through T cell CD8 receptor)	**T Helper cell** recognize an antigen in association with MHC class II proteins (MHC is recognized by T helper through T cell CD4 receptor)
Encoded by HLA-A, HLA-B, and HLA-C genes	Encoded by **HLA-D** genes

MHC class	MHC **class I**			MHC **class II**			MHC class III	
Region	A	B	C	DP	DQ	DR	C4, C2, BF	
Gene products	HLA- A	HLA-B	HLA-C	HLA-DP	HLA-DQ	HLA-DR	C' proteins	TNFα TNFβ
Polymorphisms	47	88	29	**More than 300 HLA-D**				

- Each individual has two "haplotypes" i.e, two sets of these genes, one paternal and one maternal
- Polymorphism means there are many types of gene forms that could be expressed on the MHC of individuals, resulting in genetic differences.
- MHC class III has no relevance regarding transplantation, but it is important for complement protein expression and its activity

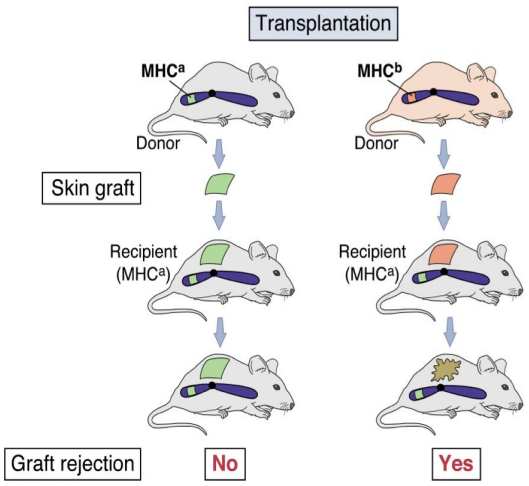


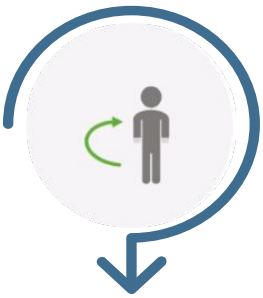
Figure illustrates importance of MHC genes matching between donor and recipient.

Left side: MHC of the donor is identical to recipient's, graft is accepted.

Right side: MHC of the donor is different from the recipient's, graft is rejected.

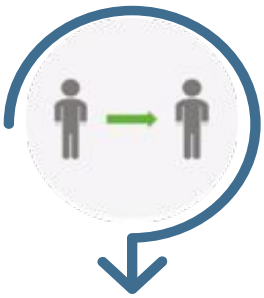
The immune system of host recognizes donor cells as foreign and initiates an immune response leading to the transplant rejection.

Types of Transplants



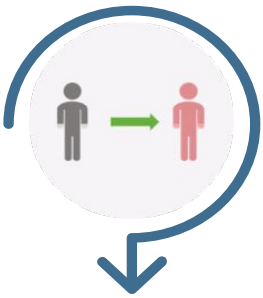
1. Autografts, Autologous grafts

- Donor and recipient are same individual, (self-tissue is transferred from one body site to another)
- Common in skin (e.g. burns) and bone marrow grafting (stored in Bio Banks and can be used in case of Leukemia).
- Commonly accepted



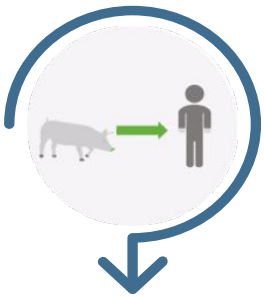
2. Isograft grafts (Syngeneic)

- Donor and recipient are genetically identical (syngenic).
- Animal models (inbred to be genetically similar), identical twins
- Commonly accepted



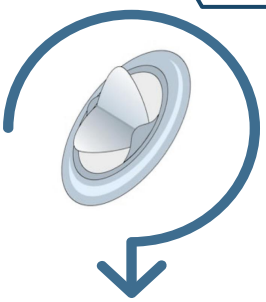
3. Allogeneic grafts

- Donor and recipient are same species, but genetically unrelated (ex. from one human to another).
- Common heart, lung, kidney, liver graft
- Most common type
- Commonly rejected



4. Xenogeneic grafts

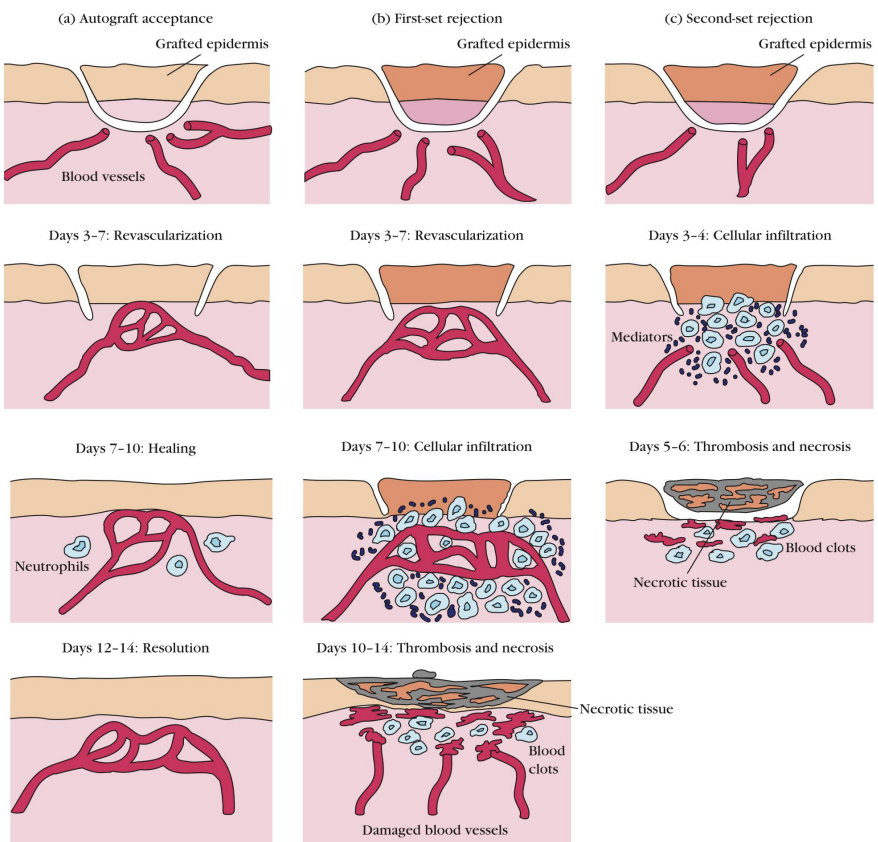
- Donor and recipient are different species
- Commonly rejected
- e.g. planting human tumors in rats to observe drugs effects on them. If you're interested in more details click here, or Check [KUBY] P.548



5. Artificial grafts

Synthesized
E.g. Heart Valves

1st Set Vs. 2nd Set Rejection



T cells play primary role in 1st and 2nd set rejection reactions

(a): Autograft is accepted, no necrosis.

(b): 1st set rejection of the graft tissue happens if the graft was not previously sensitized / introduced to the recipient.

(c): 2nd set rejection happens if the 1st set rejection happened & then the same graft is introduced again to the same recipient.

Necrosis in (c) will happen faster than the first time (b), this is due to the immunity developed against the graft due to presence of **memory cells.**

Both sets (b) & (c) are considered **acute rejection**. Notice how Cellular Infiltrate & Necrosis is faster in 2nd set rejection.

Skin grafts are generally rejected faster than other tissues, such as kidney or heart. [KUBY]

Importance of T cells

Two experiments have been performed to see the effect of T cells in transplants.

1

To see the degree of involvement of the T cells in rejection, nude mice were used to see whether rabbit skin (non-matching HLA) grafts would be rejected. The experiment revealed that graft is accepted. **Why?** Due to absence of thymus, therefore no maturation of T cells. This means T cells play a **primary role in graft rejection.**

What if mice were B cell deficient? Will the mice accept the non-matching HLA graft like in the case of nude mice? **No.** Graft will be rejected as the mice **possesses** T cells.

We interpret from this that B cells play a minor role in rejection, unlike the role of T cells which is major. (B cells will have a greater role in hyperacute rejection which will be discussed later, yet still not greater than T cells)

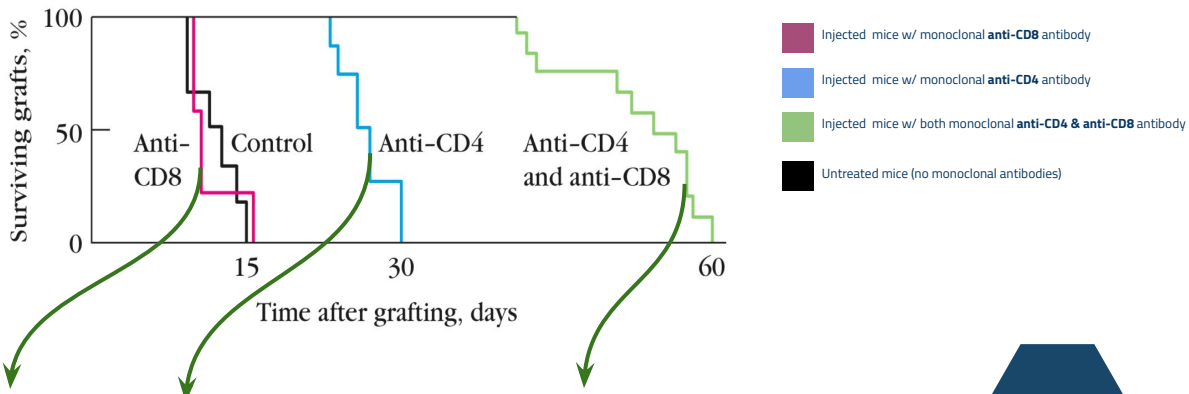
a special strain bread without a thymus

Nude mouse with a transplant of rabbit skin

2

Another experiment was done to see which T cell (CD4 or CD8) has more influence in graft rejection. In this experiment, mice were transplanted with non-matching HLA grafts (a graft which we know will be rejected) and were injected with monoclonal antibodies to deplete each type of T cell, (anti-CD4 antibodies and anti-CD8 antibodies). The experiment revealed when we block CD4, graft survives for longer time. Therefore, **CD4** effect is **more important** compared to CD8.

Graph: Role of $CD4^+$ versus $CD8^+$ T cells In Rejection



Mice injected with only **anti-CD8** monoclonal antibody showed **little improvement** in survival of graft **from untreated control mice**.

Mice injected with only **anti-CD4** monoclonal antibody showed **significant improvement** in survival of graft **from untreated control mice**.

Mice injected with both **anti-CD4 & anti-CD8** monoclonal antibody showed **most significant improvement** in survival of graft **from untreated control mice**.

Classic Adaptive/Acquired Immune Response in Rejection

Adaptive immunity is unique in its properties as it has the ability to induce a response against **specific antigens,** and forms a **memory** against this antigen so upon repeated exposure response will be rapid. So how do we see this in rejection?

(To understand this you have to imagine a scenario with 3 hamsters: **Hamtaro, Bijou, and Sandy**)

Hamtaro
Donor

Bijou
Donor

Sandy
Recipient

1

Memory

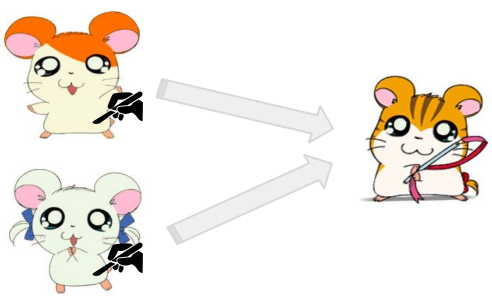
Immunologic memory is demonstrated when Sandy (a previously engrafted hamster) receives a **second** graft from **Hamtaro**. In this situation, Sandy would have formed memory cells from the first graft she has taken from Hamtaro. Due to the memory cells, second graft gets rejected faster than the first. (This shows the secondary set rejection or secondary response)

What are memory cells? Specialized B lymphocytes that will release anti-HLA antibodies upon second transplantation.

2

Specificity

Specificity can be demonstrated by grafting skin to Sandy from both Hamtaro and Bijou **at the same time** (Remember, Sandy has already received a graft from Hamtaro but never from Bijou). Rejection of **Bijou's** graft will be **slower** (**first-set** rejection or primary response,) whereas **Hamtaro's** graft will be rejected in an **accelerated second-set fashion. Why?** because Sandy's immune system has recognized the Hamtaro's graft and not Bijou's making it "Specific".

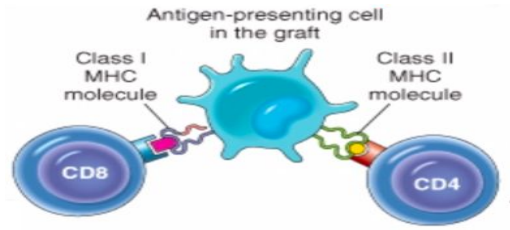


Mechanism Involved in Graft Rejection

(Recognition mechanism of MHC molecules)

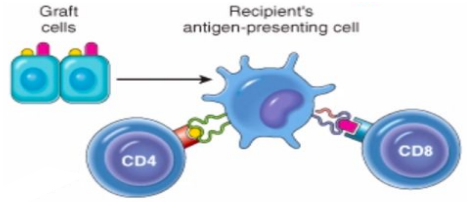
Direct Pathway

Unprocessed antigen coming from **graft APC** and binds with host T cells for recognition.



Indirect Pathway

Graft cell is processed by **host APC,** and coat themselves with antigen of the graft and presents it to host T cells for recognition.



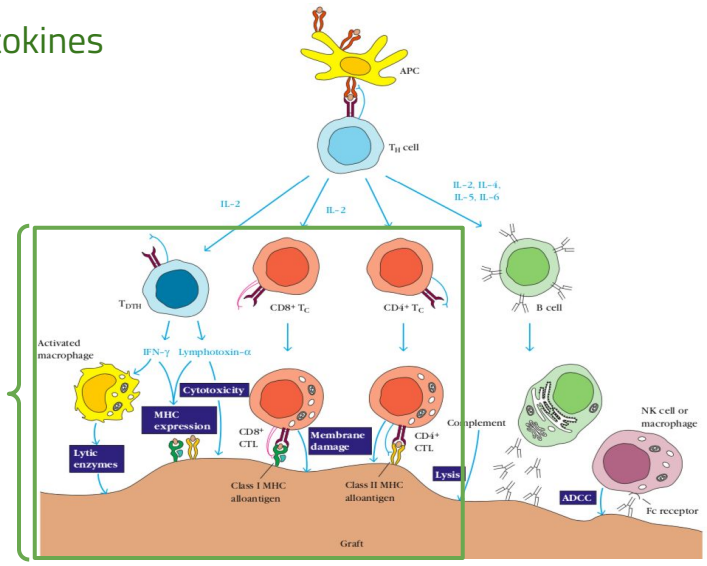
The only difference is that in the direct pathway APC are from Graft NOT recipient.

Rejection Response

- Here we see activated T cells produce cytokines such as in any immune reaction of CMI.
- Humoral immunity has very little effect.

"The left side of the diagram represents the various functions of T cells, meaning CMI plays a major role in the rejection response".

"On the right side, you can see B cells have a role in the rejection response but not as important".



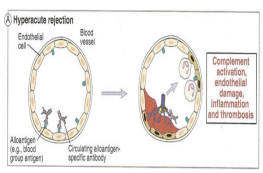
IL-2 and **IFN-γ** produced by Th1 cells have been shown to be important mediators of graft rejection. These two cytokines promote:

- T-cell proliferation (including cytotoxic T cells)
- DTH responses
- Synthesis of IgG by B cells
- Resulting complement activation

Clinical Manifestations of Graft Rejection

I. Hyperacute Rejection

Onset	Mechanism
Very quick, Immediate (minutes- less than 24hr)	Consists of **pre-existing** host serum antibodies specific for graft antigens; were in the body **before** the transplant that are carried to the graft where they will cross-react with graft tissue and it will be rejected. Source could be: 1. Previous rejection (attempted transplant) 2. Wrong blood transfusions 3. Women with multiple pregnancies (blood of the infant goes to the maternal blood and antibodies are produced). To avoid this type of rejection, we must make sure there's no pre-existing antibodies before grafting. (methods are discussed later)



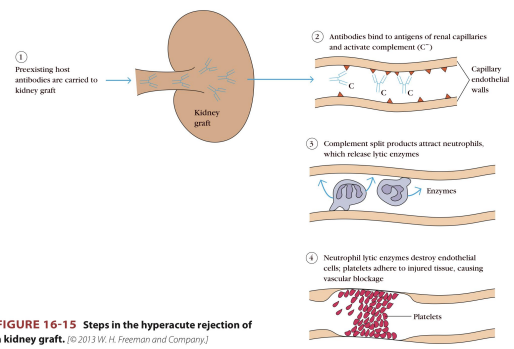
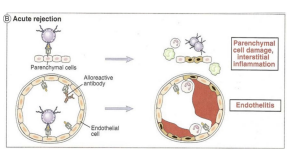


FIGURE 16-15 **Steps in the hyperacute rejection of a kidney graft.** *(© 2013 W. H. Freeman and Company.)*

II. Acute Rejection

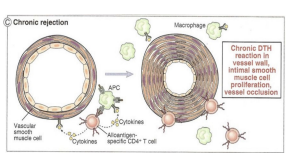
Onset	Mechanism
~10 days (Weeks to months)	First-set & second-set rejections are acute rejections. (This means there's massive infiltration of macrophages and lymphocytes at the site of tissue destruction, suggestive of TH -cell activation and proliferation) [KUBY] Involves **CMI** and Humoral Immunities. However, Acute Antibody-Mediated Rejection is the cause of only 20% to 30% of acute rejection cases. [KUBY] Can be prevented by immunosuppressive therapy.



To sum up, what you need to know about pre-existing anti-donor specific antibodies is that **their presence will cause graft rejection**, whether its acute or hyperacute, its too complicated to discuss. To distinguish between them look at the time until rejection.

III. Chronic Rejection

Onset	Mechanism
Months to years after engraftment	Idiopathic cause (thought to be due to Minor HLA mismatch). Involves both CMI and Humoral Immunities. Main pathologic finding: **Atherosclerosis** of the vascular endothelium.



Graft-Versus-Host (GVH) Reaction

- T cells of the transplanted graft attack the recipient's body cells resulting in severe organ dysfunction. (Not like rejection, where the graft is attacked and it's the only part affected)
- Organs commonly affected by GVH are: Skin (maculopapular rash), Liver (jaundice and hepatosplenomegaly), intestines (diarrhea).
- Specially seen in ⅔ of bone marrow transplants. **Why?** Bone marrow contains hematopoietic stem cells that differentiate into all types of blood cells **including immune cells** specifically, T cells.
- **Donor's cytotoxic T cells** play a major role in destroying the recipient's cells.
- GVH reactions usually end in infections and death.

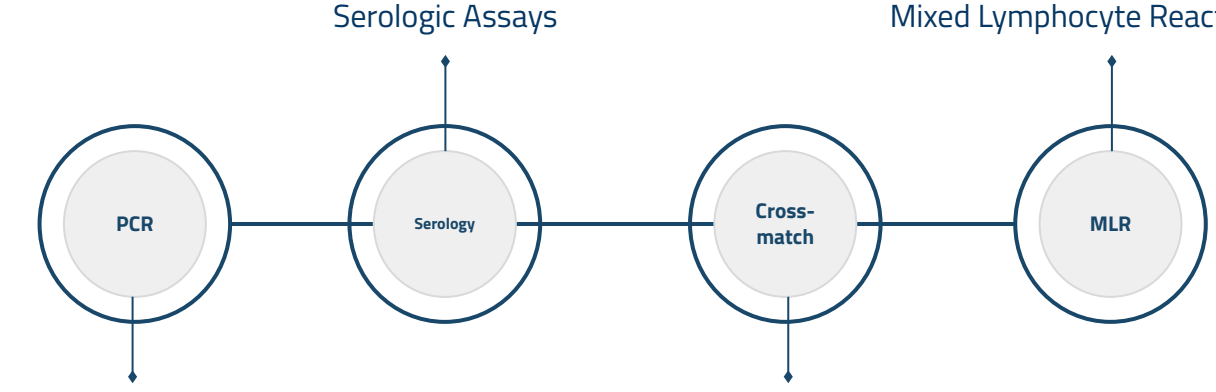
Three key elements to this reaction:

1. Immune system of recipient **must** be compromised giving graft immune cells time to attack.
2. Host's HLA must be **different** than donor's so the host's HLA proteins would appear as foreign to donor's and it would attack it.
3. **Graft** must contain immune cells.

HLA Typing in Laboratory

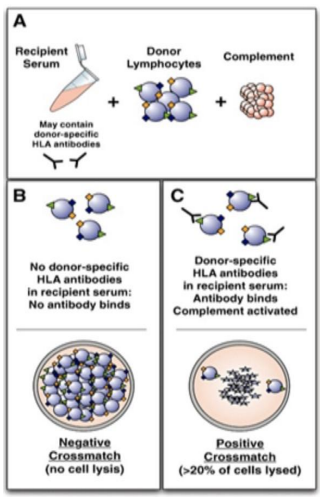
- Prior to transplantation laboratory test commonly called as **HLA typing** or **tissue typing** is done to determine the closest MHC match between the donor and recipient.

Methods:

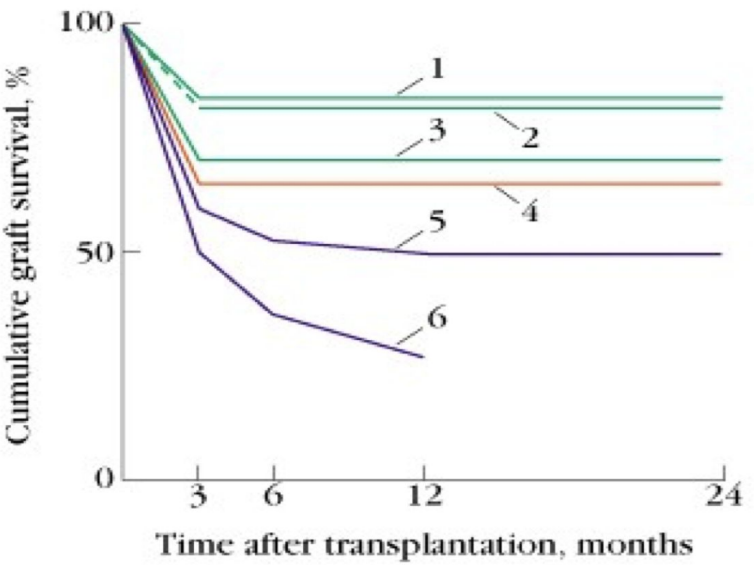


= Recipient's Serum + Donor's lymphocytes + Complement

See diagram!



Transplant Matching



Graph shows importance of MHC II in comparison to MHC I

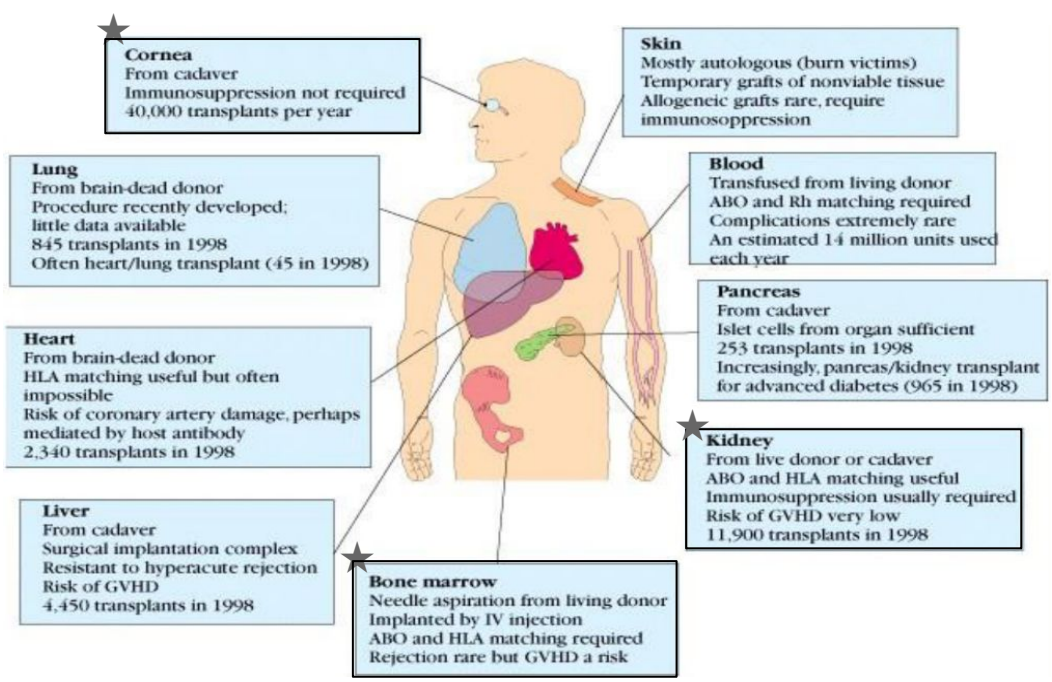
Number of HLA mismatches		
MHC I	MHC II	Curve number
0	0	1 "Control group"
1 or 2	0	2
3 or 4	0	3
0	1 or 2	4
1 or 2	1 or 2	5
3 or 4	1 or 2	6

Recall: CD4 is more important than CD8 in rejection. And **MHC II** presents antigens to **CD4**, while **MHC I** presents antigens to **CD8.**

This graph shows effect of HLA class I & II matching on survival of kidney grafts.

As seen in curve no. 2, slight mismatch in MHC I only has **little** effect in decreasing the survival rate of the graft in comparison to curve no. 1 which is the control group (no mismatch of MHC's). However, in curve no. 4, a slight mismatch in MHC II only has **significant** effect in decreasing the survival rate of the graft in comparison to control group.

Just read



EXTRA INFO

Do all transplantations require tissue matching? Surprisingly, no.

Bone marrow:
Tissue matching is **required** to minimize chance of developing GVHD

Kidney:
Tissue matching is N**OT required BUT useful**

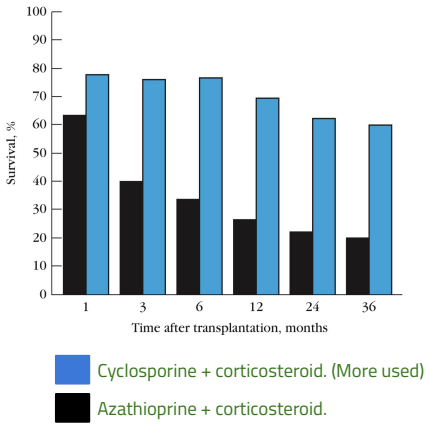
Cornea:
Tissue matching **NOT required** because it's NOT vascularized, therefore risk of rejection is ABSENT.

- *Team435*

General Immunosuppression Therapy

Immunosuppressants are drugs used to prevent aggressive immune responses from the body in order to lower the body's ability to reject a transplanted organ.

1. **Corticosteroids:** suppresses T cell mediated immune response and reduces inflammation
2. **Mitotic inhibitor:** Azathioprine (pre & post surgery) it affect the cell cycle at (S) phase (which is responsible for synthesis or replication of DNA), affects proliferation of T-cell, used with corticosteroids.
3. **Cyclosporins:** Inhibits transcription of IL-2 thus affecting T-cells and used with corticosteroids
4. **Total lymphoid irradiation:** radiation of primary and secondary lymphoid organs for 4 weeks before surgery, thus when transplantation takes place the immune system will be suppressed.



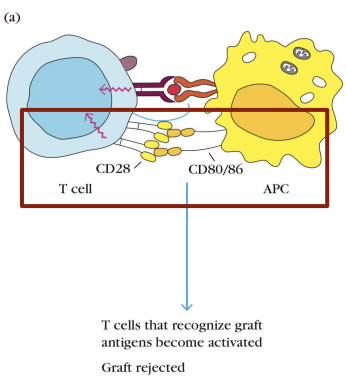
Specific Immunosuppression Therapy

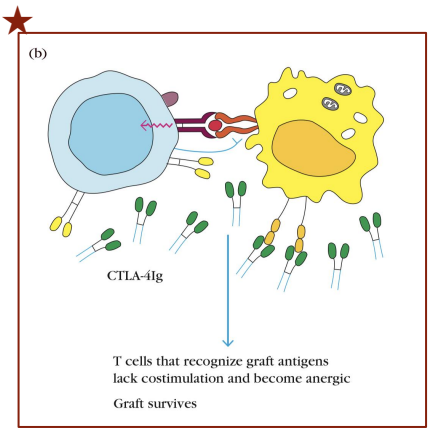
1. Monoclonal antibodies against *T cell* components or *Cytokines*
2. Agents blocking co-stimulatory signal

In order to activate T-cells **2** signals are required:

1. MHC II on APC binds to TCR on T-cell.
2. **Co-stimulation signal**; B7 on APC binds to CD28 on T-cell.

If patient is given co-stimulatory blockers, the signal is **broken,** the T-cell becomes **anergic** (deactivated) fulfilling its purpose and allows the graft to survive.





Downsides of Immunosuppression Therapy

- Must be maintained for life
- Toxicity
- Susceptibility to infections
- Susceptibility to tumors

Summary

MHC class I	MHC class II
Both play a role in rejection but MHC II is mainly the major cause	
Found on the surface of virtually all the nucleated cells (all body cells except RBCs).	Found on the surface of Antigen Presenting Cells
Helps T Cytotoxic cell to recognize an antigen	Helps T Helper cell to recognize an antigen
Encoded by HLA-A, HLA-B, and HLA-C genes	Encoded by HLA-DP, HLA-DQ, and HLA-DR genes
has a **little** effect on the transplant matching	has a **significant** effect on the transplant matching

Types of Transplants

- **Autografts** self-tissue
- **Isograft grafts** genetically identical twins
- **Allogeneic grafts** same species, but genetically unrelated
- **Xenogeneic grafts** different species
- **Artificial grafts**

Importance of:

T cell	T cells play a major role	B cells play a minor role
CD4	CD4 effect is more important	CD8 effect is less important

Rejection
Recipient's immune system attacks the transplanted tissue.

Set of rejection	1st set rejection if the graft was not previously introduced	2nd set rejection: if the 1st set rejection happened & same graft is introduced again
Adaptive immunity	**Memory** second graft gets rejected faster than the first	**Specificity** A graft that is recognized before will be rejected faster
Recognition mechanism of MHC molecules	Direct Pathway (by graft's APC)	Indirect Pathway (by host's APC)
Clinical manifestations of rejection	- **Hyperacute Rejection**, Very quick, less than 24hr, pre-existing host serum antibodies specific for graft antigens - **Acute Rejection,** 10 days (Weeks to months) - **Chronic Rejection**, Involves both CMI and Humoral Immunities. Months to years after engraftment	

HLA typing (Tests)

PCR , Serologic Assays, Mixed Lymphocyte Reaction (MLR), and Cross-match Test

Immunosuppression Therapy

General Immunosuppression Therapy	Specific Immunosuppression Therapy
Corticosteroids, Mitotic inhibitor, Cyclosporins, Total lymphoid irradiation	- Monoclonal antibodies against *T cell* components or *Cytokines* - Agents blocking co-stimulatory signal

Take home messages

HLA or MHC molecule **mismatch** can stimulate humoral and cell mediated immunity which is the **main cause of rejection of transplants**

Cell mediated immune responses play a **major** role in transplant rejection

Tissue matching particularly for HLA-**D** antigens is **important for successful transplantation**

Immunosuppressive therapy is usually required **after** transplantation

Extra

MHC

Osmosis - GVHD

HLA Testing

QUIZ

Q1) Atherosclerosis of the vascular endothelium associated with which type of rejection:							
A	Hyperacute rejection	B	Acute rejection	C	Chronic rejection	D	Both A & B
Q2) Most important cell in rejection reactions:							
A	Plasma cell	B	B cell	C	T Helper	D	T cytotoxic
Q3) Which of the following genes encode for Class II MHC:							
A	HLA-A	B	HLA-B	C	HLA-C	D	HLA-D
Q4) Which of the following play a primary role in rejection reaction?							
A	Cytokines	B	B cells	C	T cells	D	APC's
Q5) Which one of the following is <u>not</u> considered as a general Immunosuppression therapy?							
A	Mitotic inhibitor	B	Corticosteroids	C	Mitotic inducer	D	Cyclosporin
Q6) Which of the following is a characteristic of Hyperacute reaction?							
A	Takes 10 days	B	Slow	C	Due to pre-existing anti-HLA	D	Atherosclerosis of endothelium
Q7) Genes for HLA proteins are clustered in the MHC complex located on:							
A	short arm of chromosome 16	B	short arm of chromosome 6	C	long arm of chromosome 16	D	long arm of chromosome 6
Q8) The success of tissue and organ transplantation depends upon the donor's and recipient's ?							
A	HLA matching	B	Blood type	C	Age	D	Absence of APC's
Q9) Which one of the following is a type of specific Immunosuppression therapy?							
A	Co-stuimulatory signal inducers	B	Co-stuimulatory signal blockers	C	T cell proliferation inducer	D	Corticosteroids
Q10) Which type of transplant is between genetically identical individuals?							
A	Autograft	B	Syngeneic	C	Allogeneic	D	Xenogeneic

Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
C	C	D	C	C	C	B	A	B	B

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