

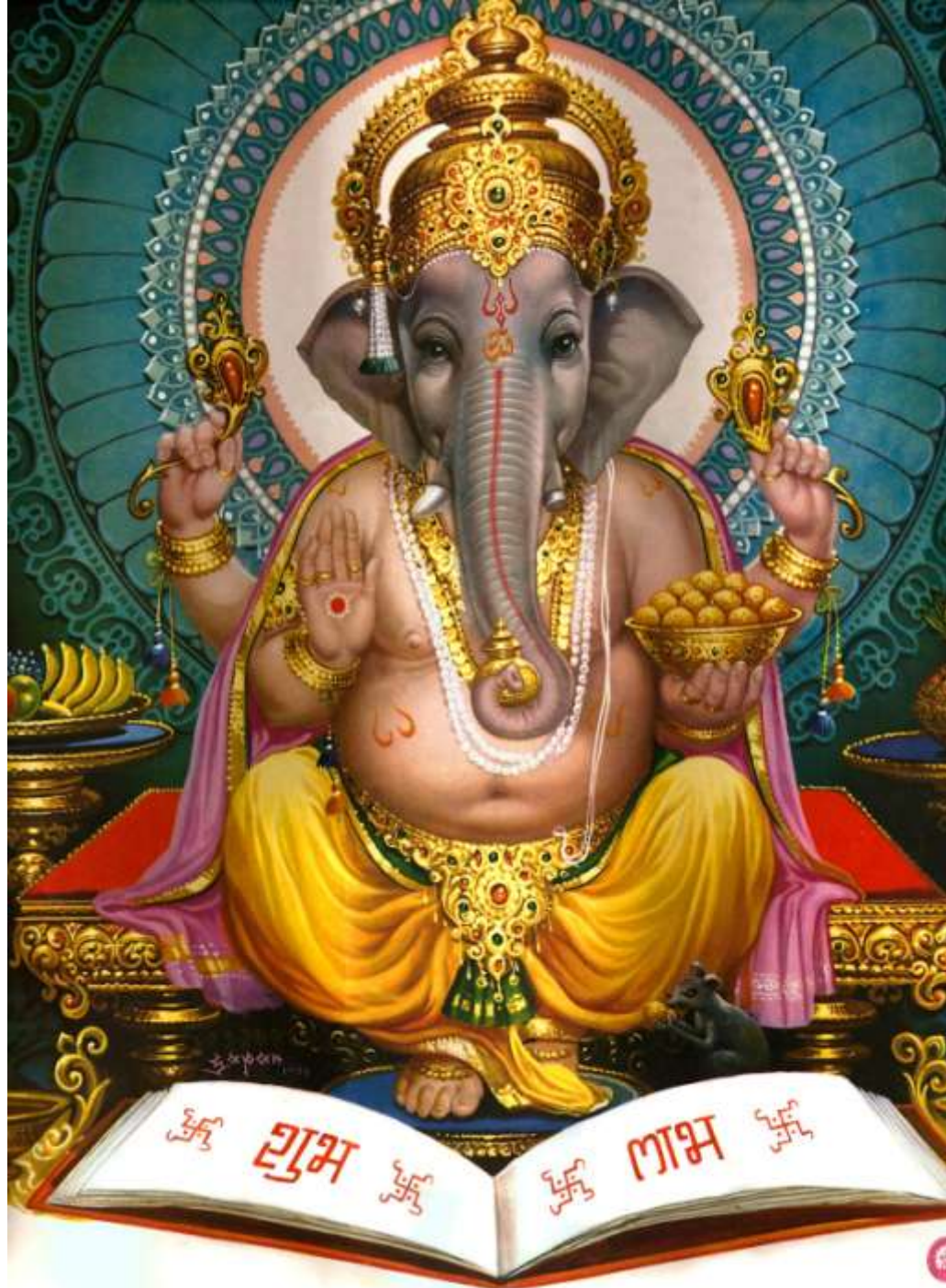
# *Transplantation Immunology*

*Sudarsan Agarwal*

# History

- To start with,,,, oldest known transplantation,,

Lord Ganesha represented with human body & an elephant head & multiple limbs



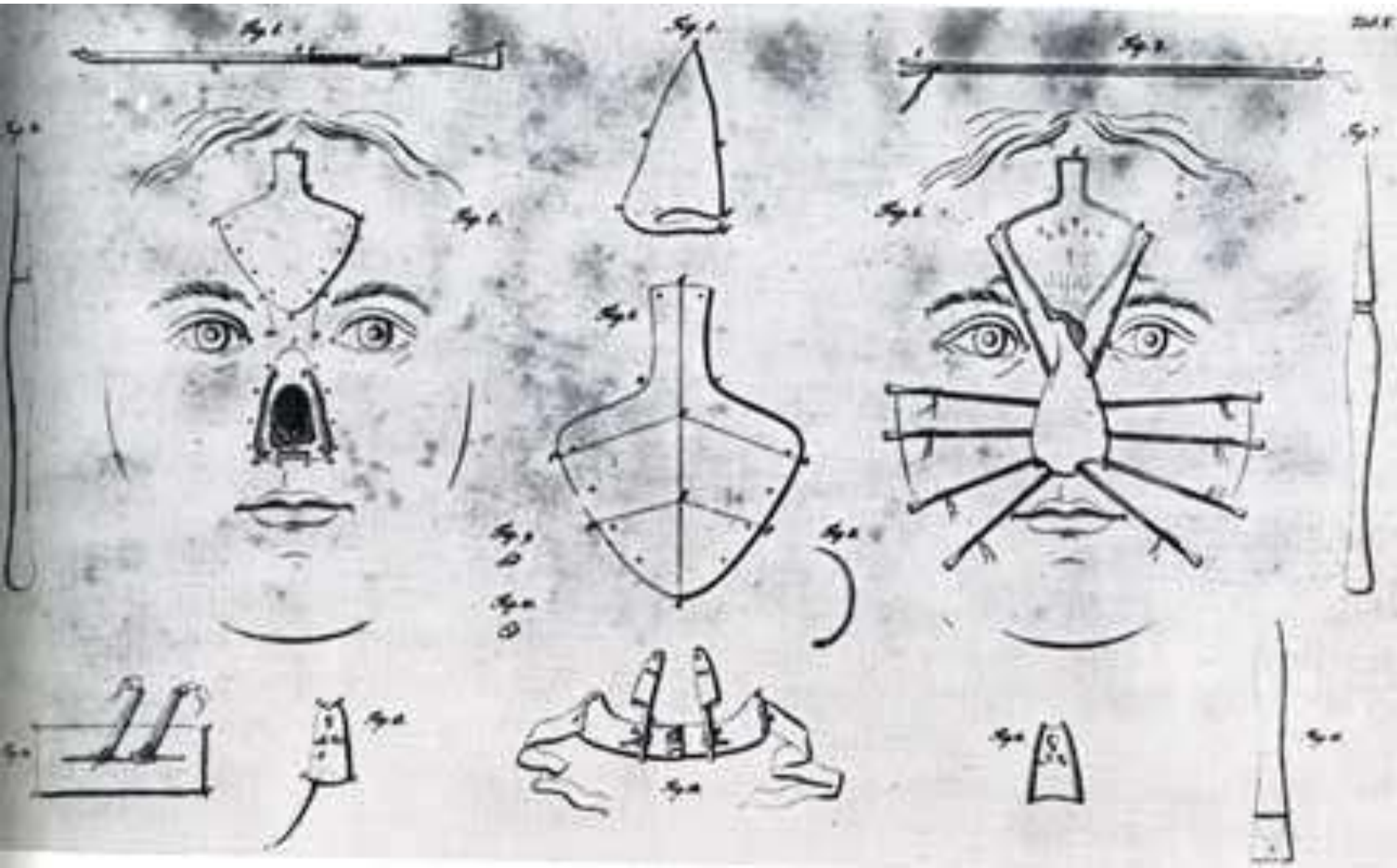
# Sushrutha



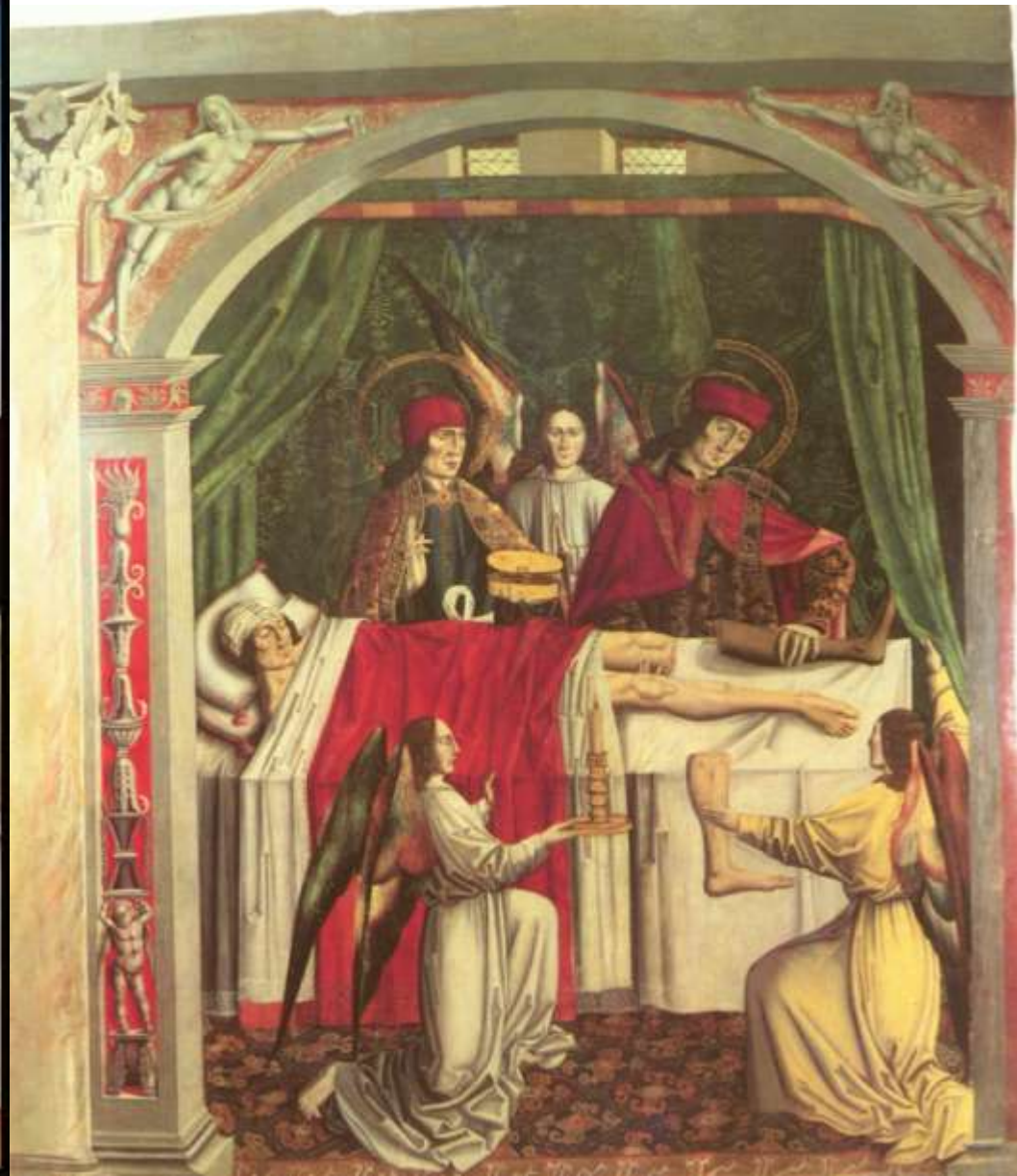
- **600 BC THE INDIAN SURGEON SUSHRUTA DESCRIBED RECONSTRUCTION OF DISFIGURING FACIAL WOUNDS BY TRANSPLANTATING SKIN AND CARTILAGE**



# Sushrutha



# The legend of the “Miracle of the Black Leg”



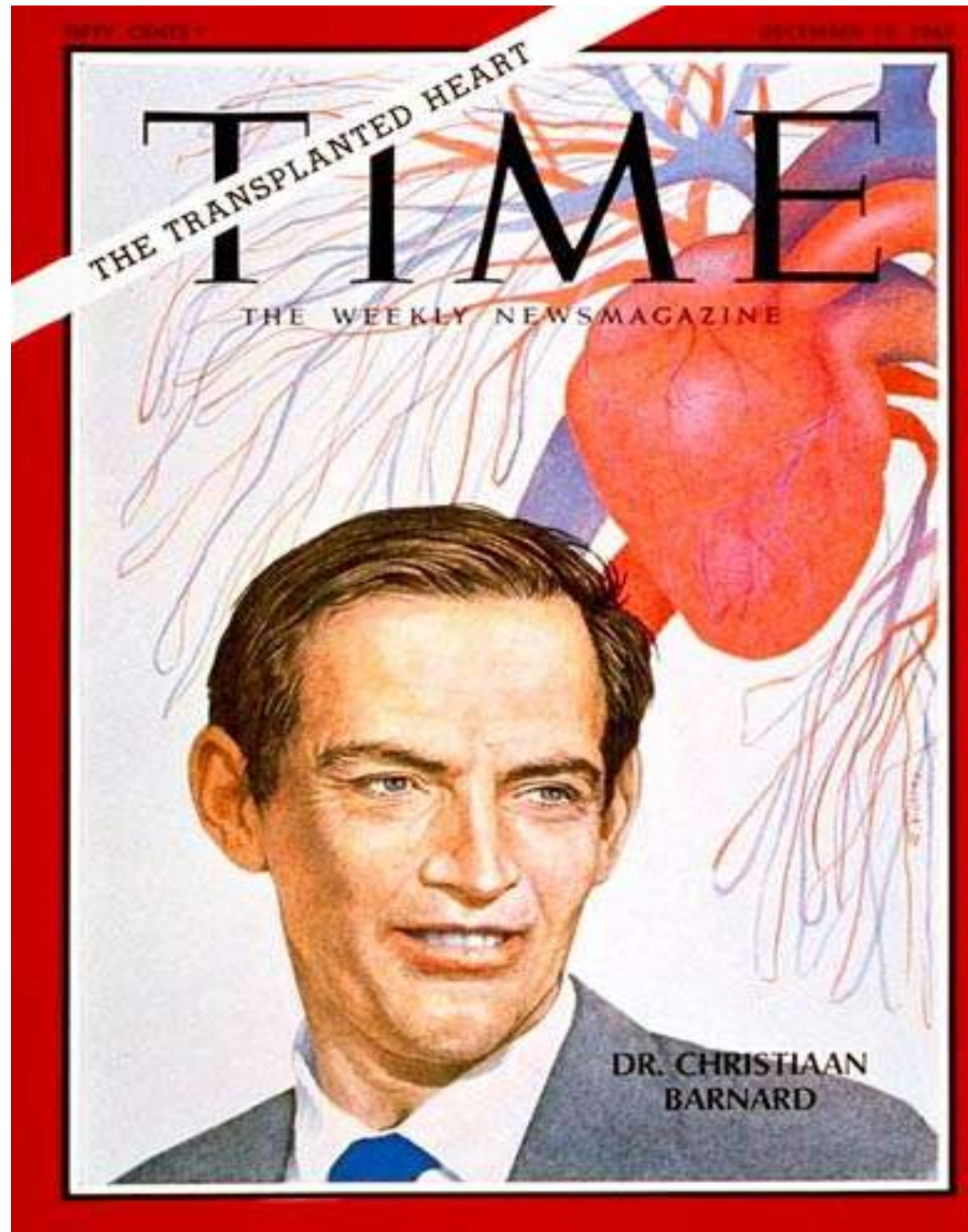
**Nobel Prize in  
Physiology or  
Medicine 1912**

**Alexis  
Carrel**





In 1967, the first human heart transplantation was done in Cape Town, South Africa



**Nobel Prize in  
Physiology or  
Medicine 1960**

**Peter Brian  
Medawar**



# Nobel Prize in Physiology or Medicine 1980

**George D. Snell**



**Jean Dausset**



**Nobel Prize in  
Physiology or  
Medicine 1988**

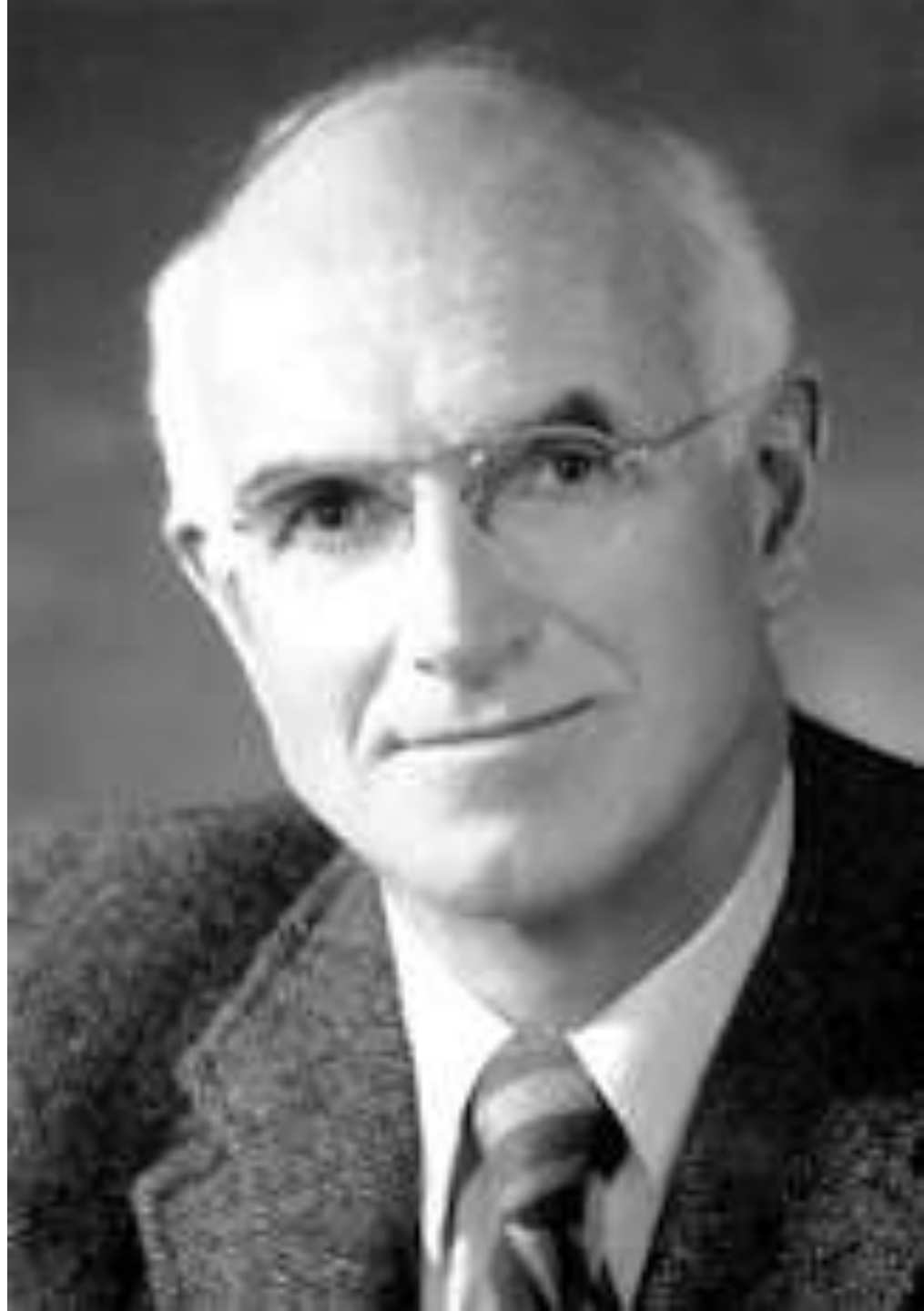
**Gertrude B.  
Elion,  
Hitchings**





**Nobel Prize in  
Physiology or  
Medicine 1990**

**Joseph E.  
Murray**



# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

*Part III Classification and effector mechanisms of allograft rejection*

*Part IV Prevention and treatment of allograft rejection*

*Part V Exnogtransplantaion*

# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

*Part III Classification and effector mechanisms of allograft rejection*

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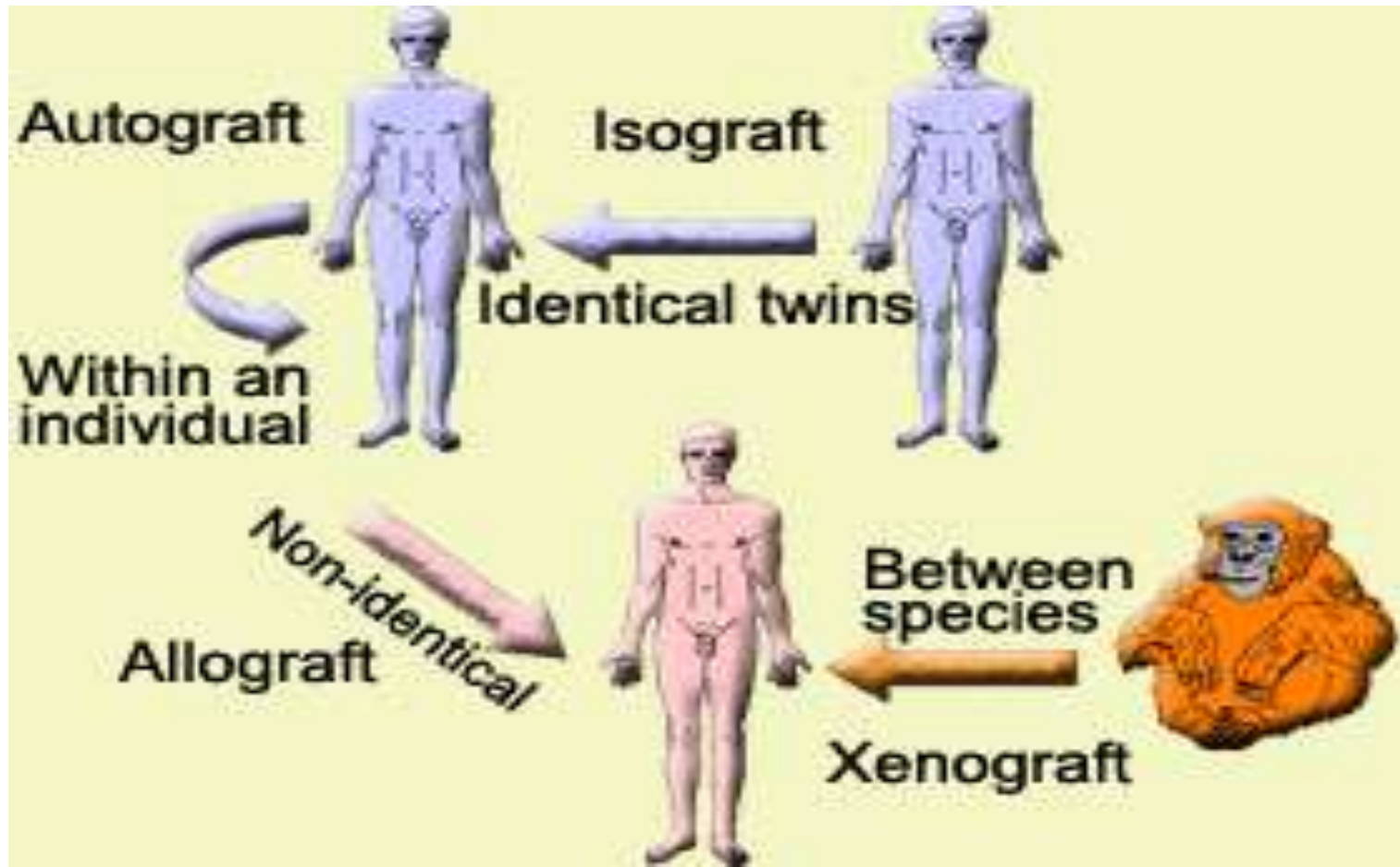
*Part V Exnogtransplantaion*

# *Part I Introduction*

- **Transplantation:** The process of taking cells, tissues, or organs from one individual and placing them into a different individual or different site of the same individual.
- **Graft:** Transplanted cells, tissues, or organs.
- **Donor:** The individual who provides the graft.
- **Recipient:** The individual who receives the graft. Also called the host.



- Autologous graft (Autograft):
- Syngeneic graft (Isograft ):
- Allogeneic graft (Homograft):
- Xenogeneic graft (Heterograft ):



## Autografting

The transfer of self tissue from one body site to another in the same individual

Due to the genetic homology of the tissue, the immune system does not respond to it

Use: synthetic implantation

skin grafts

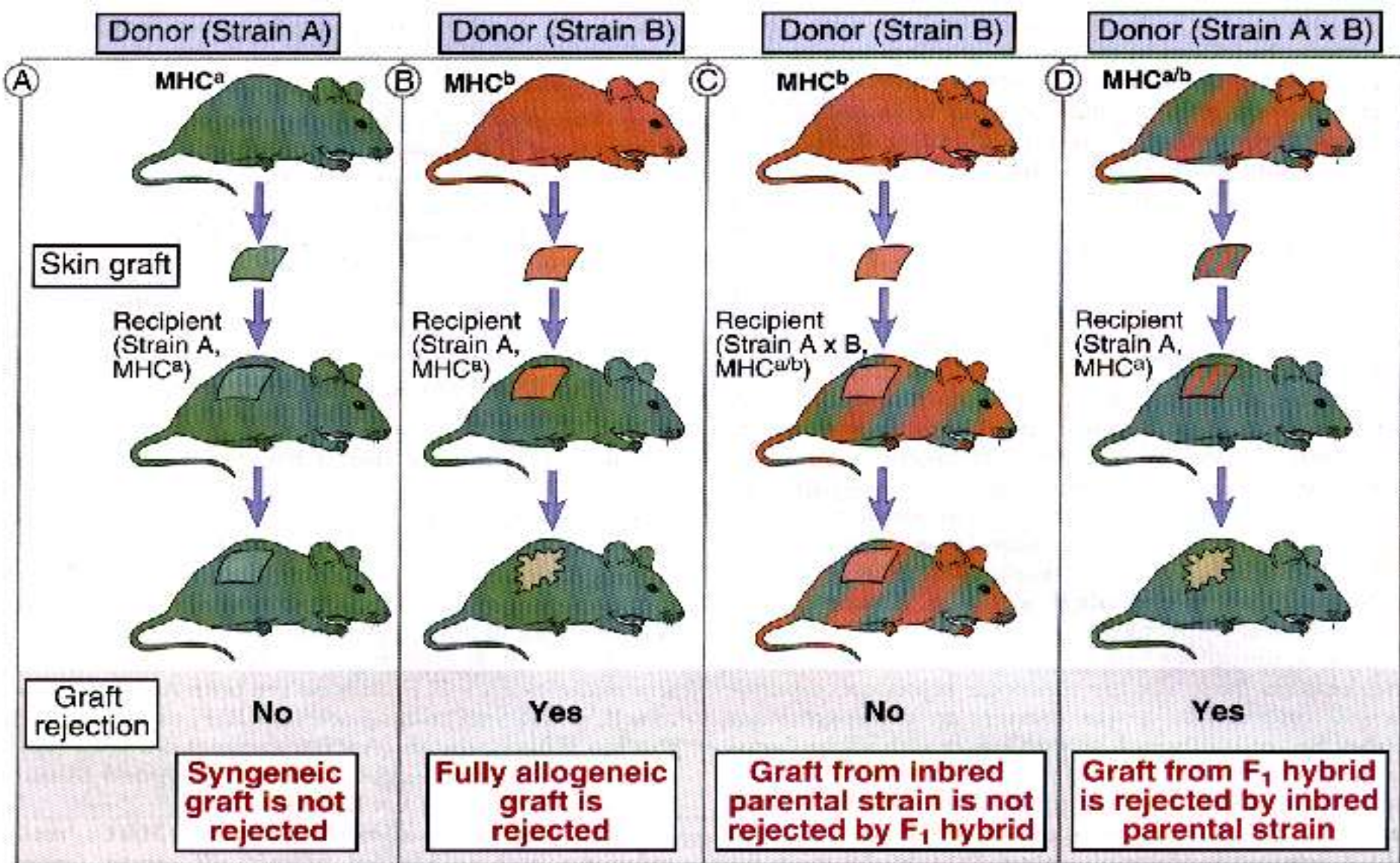
bone marrow transplantation

hair



- **Grafts rejection is a kind of specific immune response**
  - Specificity
  - Immune memory
- **Grafts rejection**
  - First set rejection
  - Second set rejection

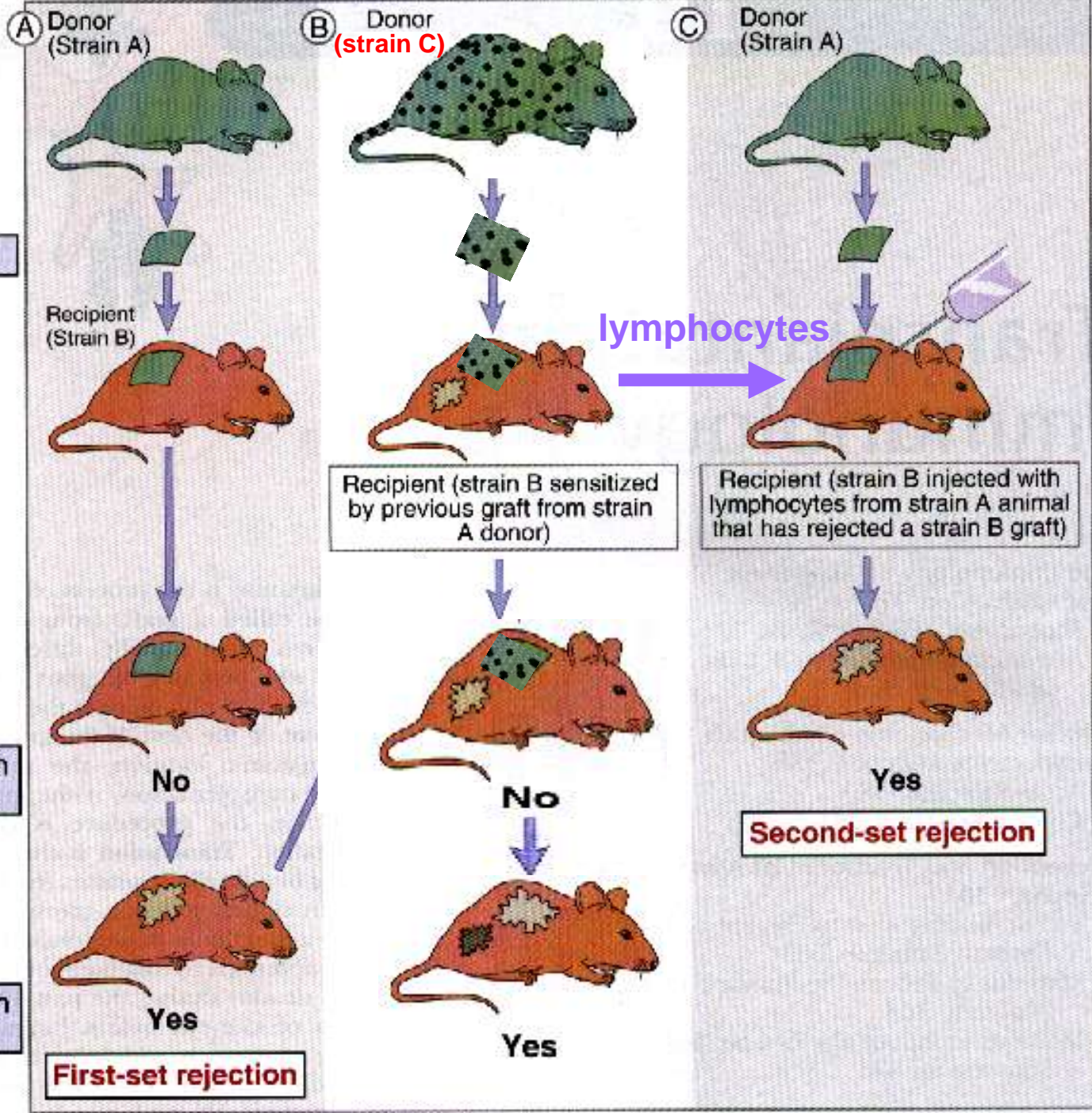




**Figure 16-2 The genetics of graft rejection.**

In the illustration, the two different mouse colors represent different MHC haplotypes. Inherited MHC alleles from both parents are codominantly expressed in the skin of an A × B offspring, and therefore these mice are represented by both colors. Syngeneic grafts are not rejected (A). Allografts are always rejected (B). Grafts from a parent of an A × B mating will not be rejected by the offspring (C), but grafts from the offspring will be rejected by either parent (D). These phenomena are due to the fact that MHC gene products are responsible for graft rejection; grafts are rejected only if they express an MHC type (represented by a color) that is not expressed by the recipient mouse.





Skin graft

Recipient (Strain B)

Recipient (strain B sensitized by previous graft from strain A donor)

Recipient (strain B injected with lymphocytes from strain A animal that has rejected a strain B graft)

Graft rejection Day 3

Graft rejection Day 10

**First-set rejection**

lymphocytes

**Second-set rejection**



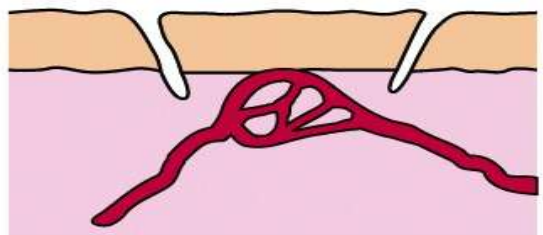
**(a) Autograft acceptance**

Grafted epidermis

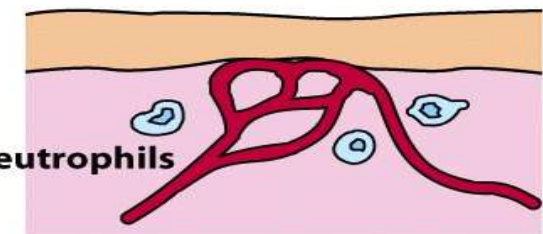


Blood vessels

Days 3-7: Revascularization

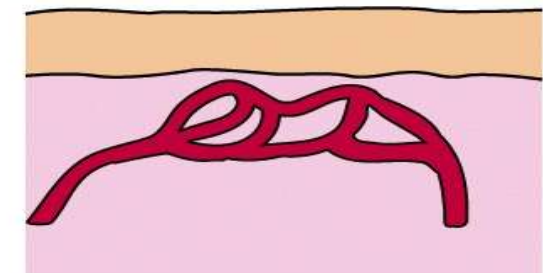


Days 7-10: Healing



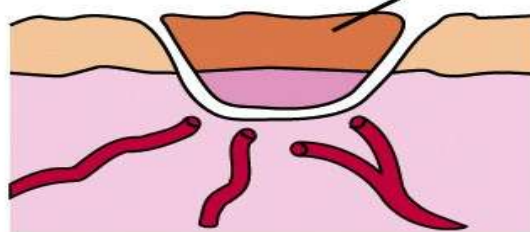
Neutrophils

Days 12-14: Resolution



**(b) First-set rejection**

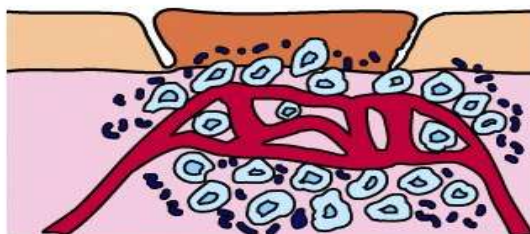
Grafted epidermis



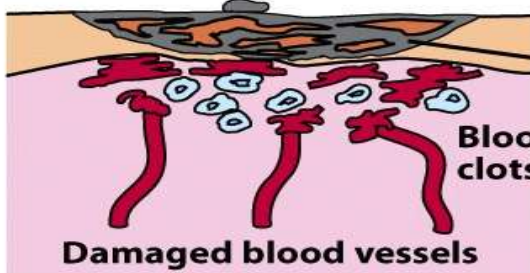
Days 3-7: Revascularization



Days 7-10: Cellular infiltration



Days 10-14: Thrombosis and necrosis



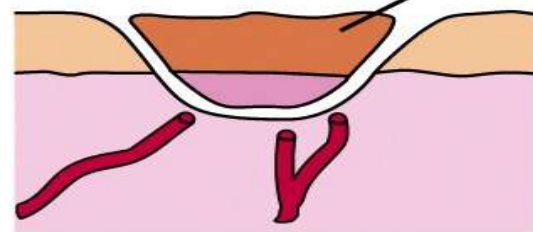
Necrotic tissue

Blood clots

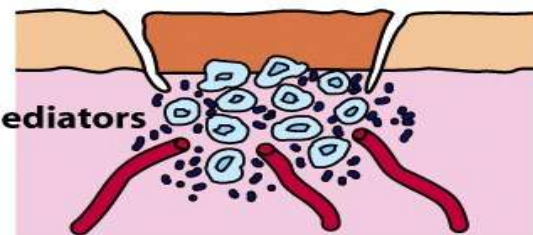
Damaged blood vessels

**(c) Second-set rejection**

Grafted epidermis

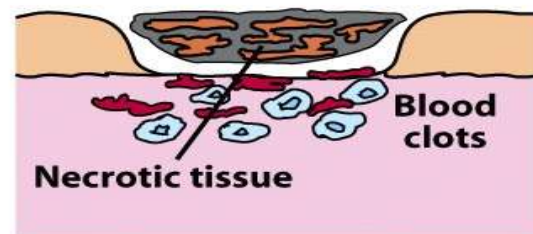


Days 3-4: Cellular infiltration



Mediators

Days 5-6: Thrombosis and necrosis



Necrotic tissue

Blood clots

# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

*Part III Classification and effector mechanisms of allograft rejection*

*Part IV Prevention and treatment of allograft rejection*

*Part V Exnogtransplantaion*

# *Contents*

*Part I Introduction*

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*Part III Classification and effector mechanisms of allograft rejection*

*Part IV Prevention and treatment of allograft rejection*

*Part V Exnogtransplantaion*

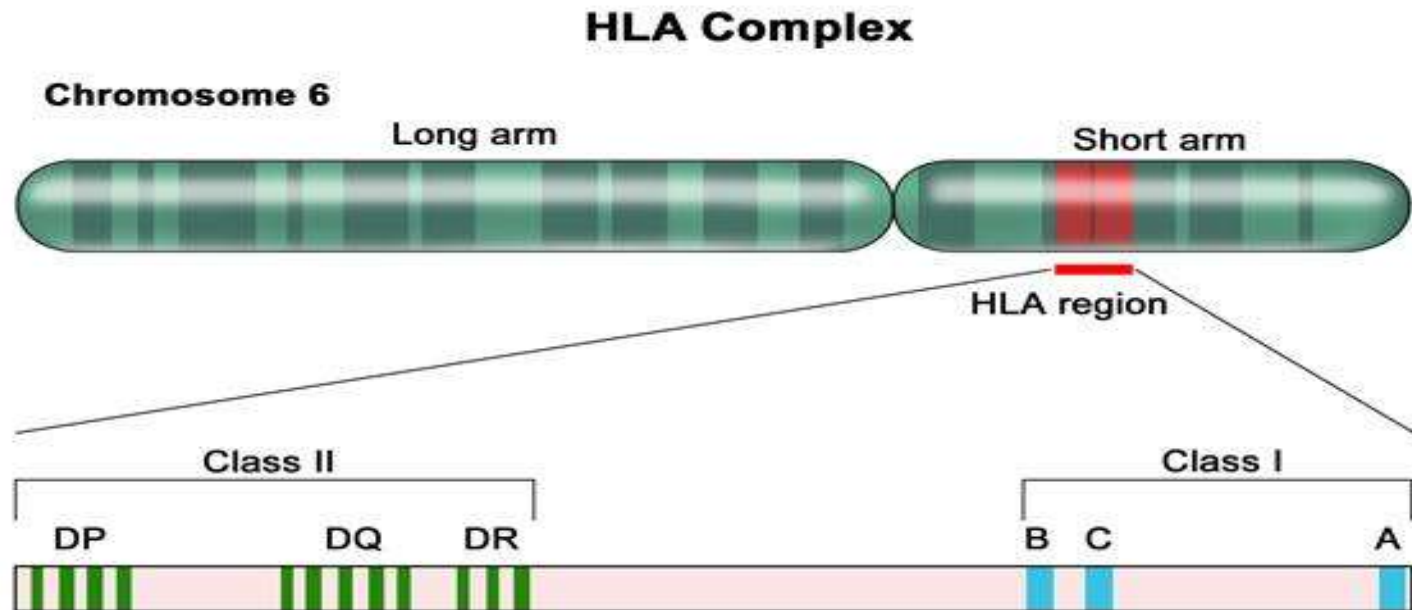
## *Part II Immunologic mechanism of allogeneic transplantation rejection*

- **Transplantation antigens**
- **Mechanism of allograft rejection**

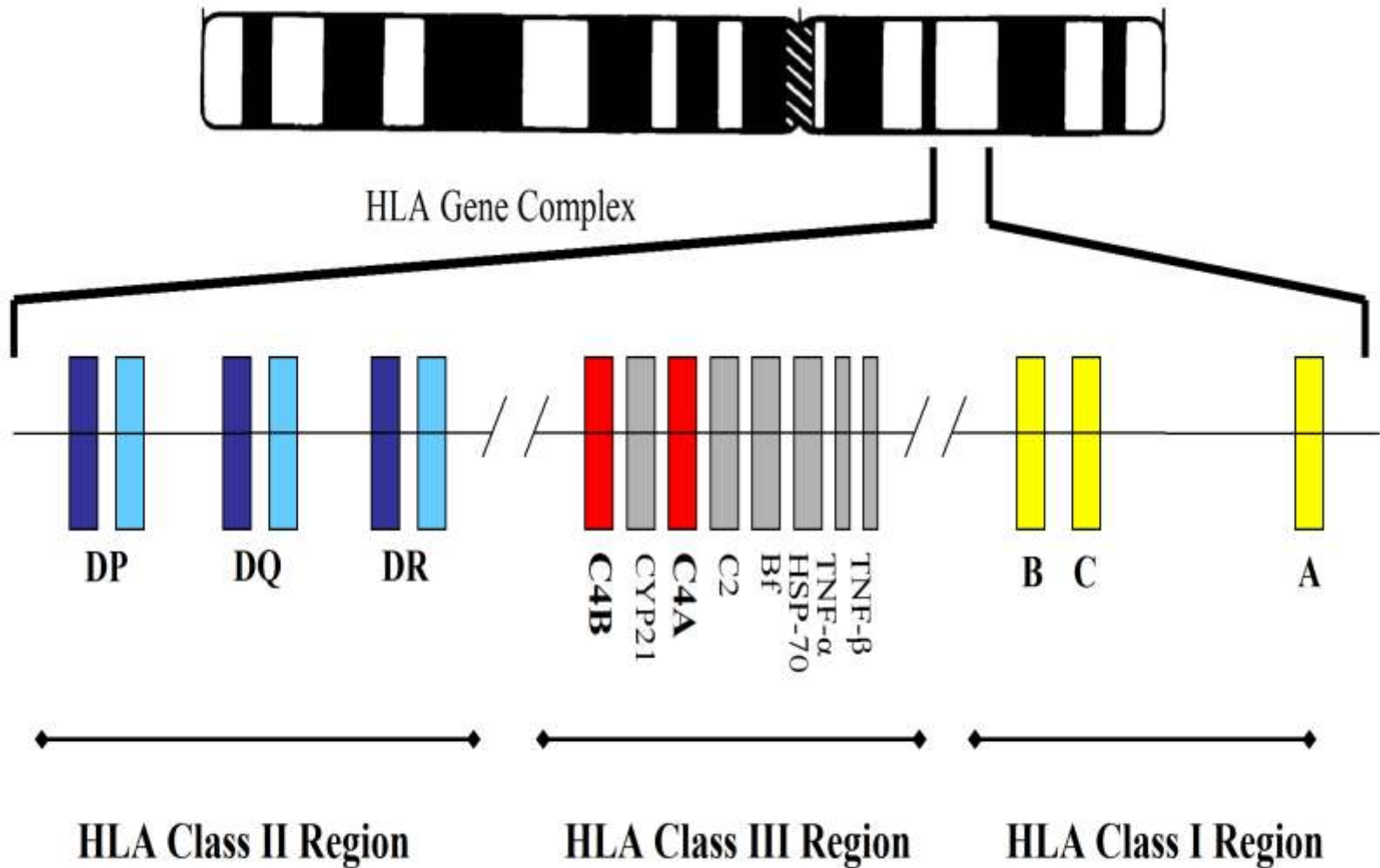


# 1. Transplantation antigens

- Major histocompatibility antigens (MHC molecules)
- Minor histocompatibility antigens
- ABO Blood group antigens
- Rh grouping is not needed at all



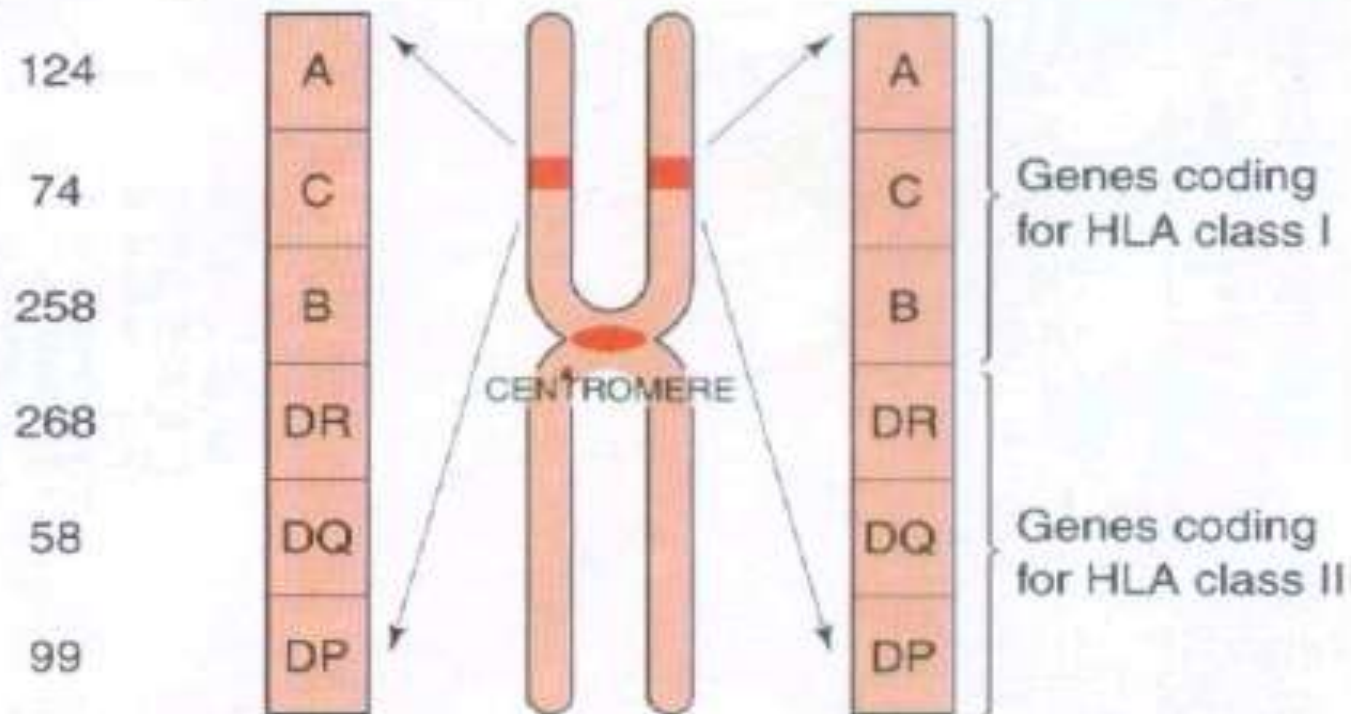
# Chromosome 6



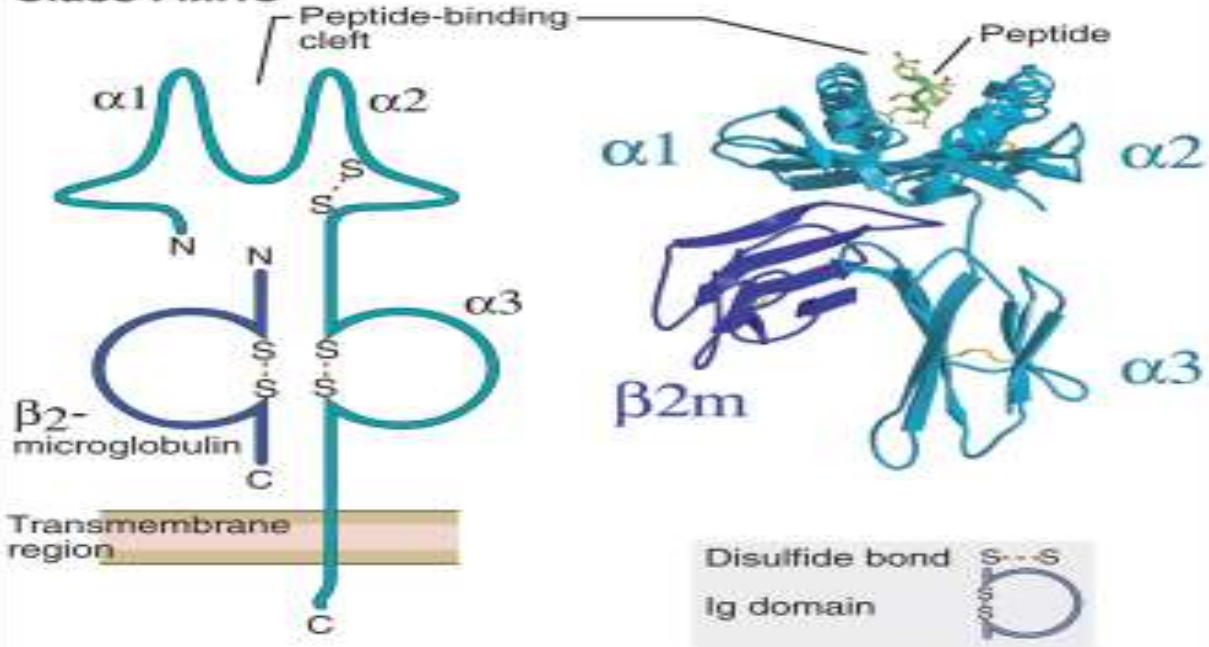
HLA I expressed in all nucleated cells

HLA II expressed in B cells, Dendritic cells, Macrophages

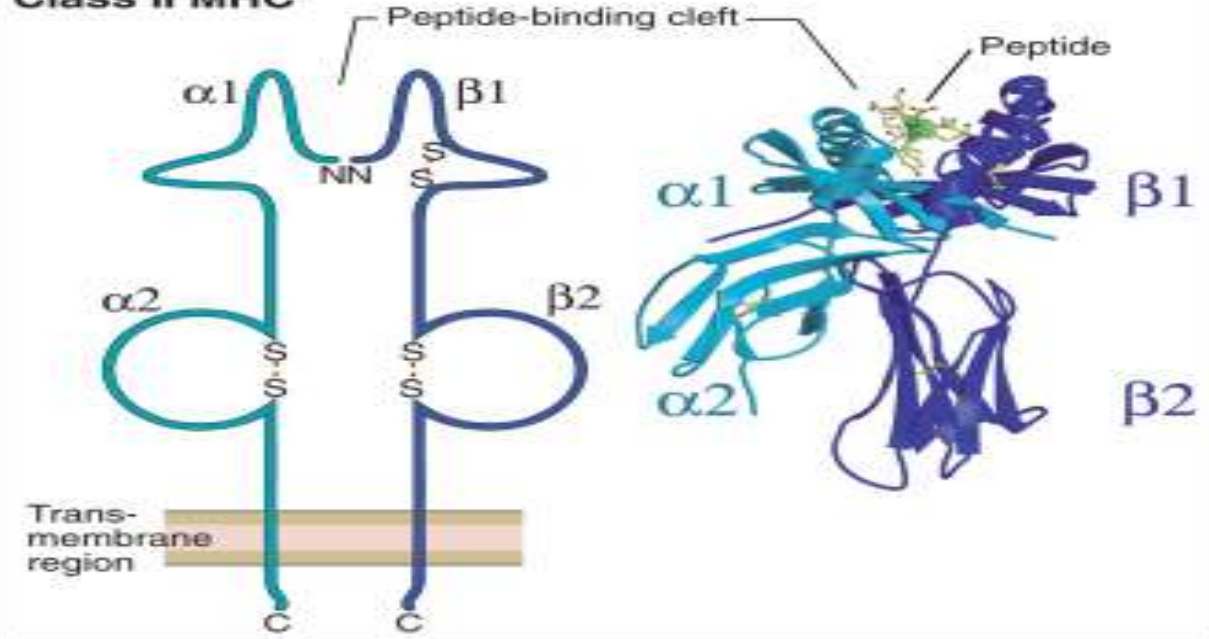
Approximate number  
of alleles defined  
by molecular typing



### Class I MHC



### Class II MHC



They are inherited from both parents as MHC haplotypes and are co-dominantly expressed

## **2. Mechanism of allograft rejection**

**The immune responses in allogeneic transplantation:**

- T cell mediated rejection of allograft**
- Antibody mediated rejection of allograft**
- NK cell mediated rejection of allograft**



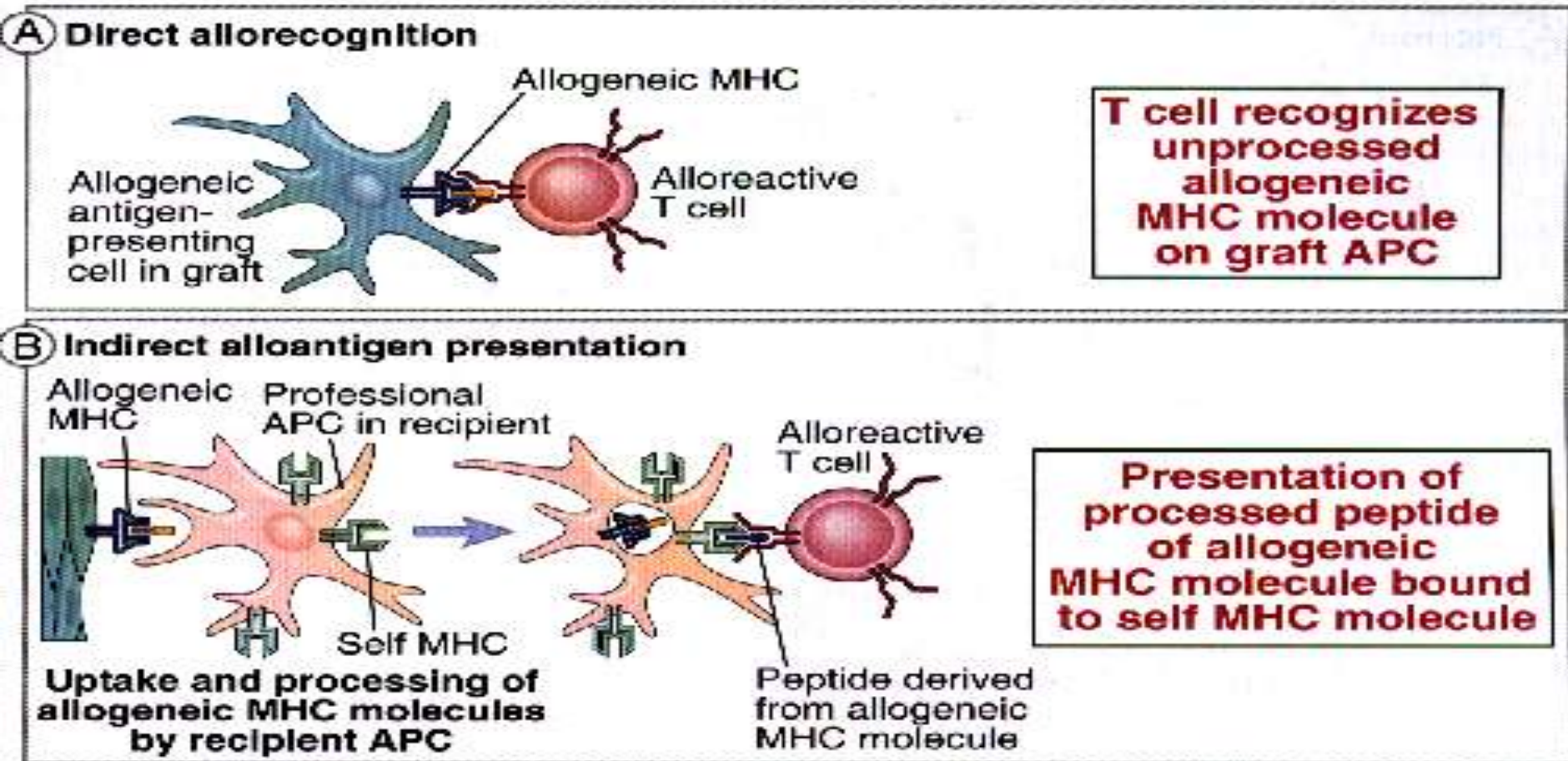
# **(1) T cell mediated rejection of allograft (mechanism of cellular immunity)**

**1) Recognition of alloantigens**

**2) Activation of T cells and rejection of allograft**

# 1) Recognition of alloantigens

- Direct recognition -----acute rejection
- Indirect recognition -----chronic rejection



## 2) Activation of T cells and rejection of allograft

- CD4<sup>+</sup> differentiate into cytokine producing effector cells
  - Damage graft by reactions similar to Delayed Hypersensitivity reaction
- CD8<sup>+</sup> cells activated by direct pathway kill nucleated cells in the graft
- CD8<sup>+</sup> cells activated by the indirect pathway are self MHC-restricted

# Difference between direct and indirect allorecognition

	Direct allorecognition	Indirect allorecognition
MHC	Intact allogeneic MHC	Antigenic peptide of allogeneic MHC
APC	Recipient APC not necessary	APC of recipient
Activated T cell	CD4+T or CD8+ T	CD4+T (or CD8+ T)
Functions in graft rejection	Acute graft rejection	Chronic graft rejection
Degree of rejection	Very strong	weak

## (2) Antibody-mediated rejection of allograft (mechanism of humoral immunity)

- Important role in hyperacute rejection  
(Preformed antibodies)
  - Complements activation
  - ADCC (Antibody Dependent Cell-mediated Cytotoxicity)
  - Opsonization
  - Enhancing antibodies / Blocking



### (3) NK cell mediated rejection of allograft

- KIR(Killer cell Immunoglobulin-like Receptor) can't recognize allogeneic MHC of graft
- Cytokines secreted by activated T helper cells can promote Natural Killer cell activation.

# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

*Part III Classification and effector mechanisms of allograft rejection*

*Part IV Prevention and treatment of allograft rejection*

*Part V Exnogtransplantaion*

# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

*Part III Classification and effector mechanisms of allograft rejection*

*Part IV Prevention and treatment of allograft rejection*

*Part V Exnogtransplantaion*

## *Part III Classification and effector mechanisms of allograft rejection*

- **Host versus graft reaction (HVGR)**
  - ① **Hyperacute**
  - ② **Acute**
  - ③ **Chronic**
- **Graft versus host reaction (GVHR)**

# Types of Rejection

1. Chronic Rejection: Mixed CD4 and antibody – “DTH like” (3mt-10 yrs)
2. Acute Rejection: CD4 controlled CD8 mediated (8-11 days)
3. Hyperacute Rejection: pre-existing antibodies to donor tissue (7 min)
4. Xenograft Rejection: pre-existing antibodies to donor tissue (7 min)



# Hyperacute Rejection

- Characterized by thrombotic occlusion of the graft
- Begins within minutes or hours after anastomosis
- Pre-existing antibodies in the host circulation bind to donor endothelial antigens
- Activates Complement Cascade
- Xenograft Response
- Immune suppression is not effective

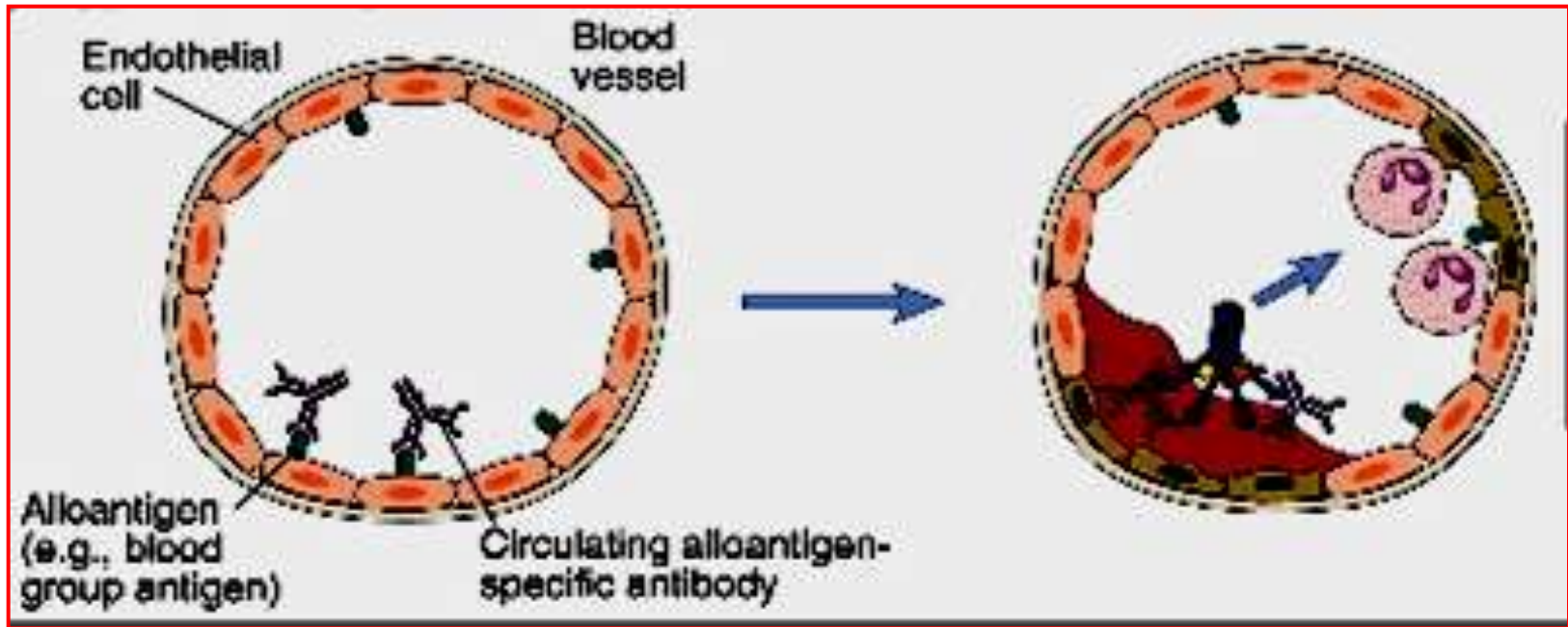
# Hyperacute Rejection: Early days

- Mediated by pre-existing IgM alloantibodies
- Antibodies come from carbohydrate antigens expressed by bacteria in intestinal flora
  - ABO blood group antigens
- Not really a problem anymore

# Hyperacute Rejection: Today

- Mediated by IgG antibodies directed against protein alloantigens
- Antibodies generally arise from
  - Past blood transfusion
  - Multiple pregnancies
  - Previous transplantation

# Hyperacute Rejection



1. Preformed Ab,
2. Complement activation,
3. Neutrophil margination,
4. Inflammation,
5. Thrombosis formation

## 2. Acute rejection

### *Occurrence time*

- Occurs within days to 2 weeks after transplantation,
- 80-90% of cases occur within 1 month

### *Pathology*

- Acute humoral rejection
  - Acute vasculitis manifested mainly by endothelial cell damage
- Acute cellular rejection
  - Parenchymal cell necrosis along with infiltration of lymphocytes

## *Mechanisms*

### Vasculitis

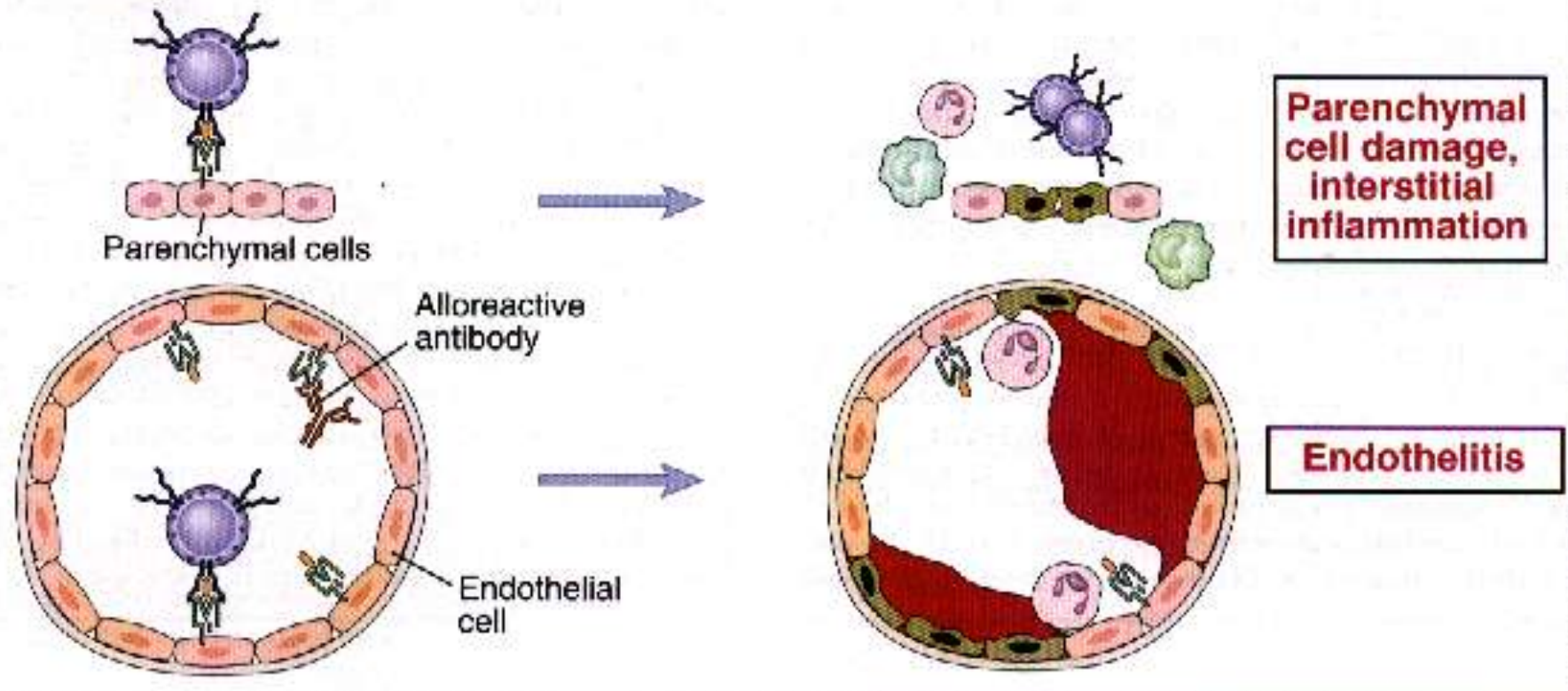
- IgG antibodies against alloantigens on endothelial cell

### Parenchymal cell damage

- Delayed hypersensitivity mediated by CD4+Th1
- Killing of graft cells by CD8+Tc



**B Acute rejection**



1. T-cell, macrophage and Ab mediated,
2. Myocyte and endothelial damage,
3. Inflammation

## 3. Chronic rejection

*All types of transplant are susceptible to chronic rejection, and it is the major cause of allograft failure.*

### *Occurrence time*

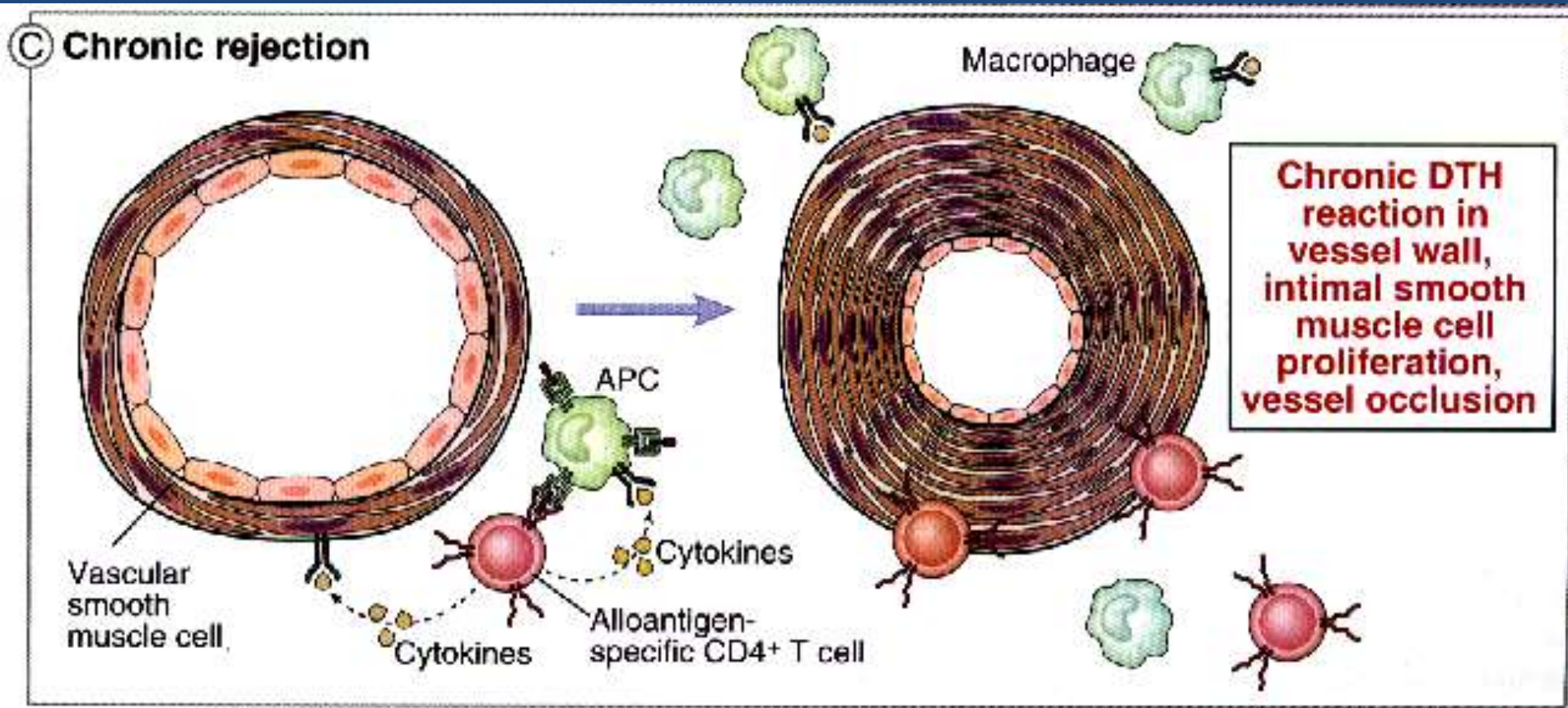
- Develops months or years after acute rejection reactions have subsided

### *Pathology*

- Fibrosis and vascular abnormalities with loss of graft function

## *Mechanisms*

- Not clear
- Extension and results of cell necrosis in acute rejection
- Chronic inflammation mediated by CD4+T cell
- Organ degeneration induced by non immune factors



1. Macrophage – T cell mediated
2. Concentric medial hyperplasia
3. Chronic DTH reaction

# **Graft versus host reaction (GVHR)**

- **Bone marrow transplantation**
- **Thymus transplantation**
- **Spleen transplantation**
- **Blood transfusion of neonate**

**In most cases the reaction is directed against minor histocompatibility antigens of the host**

# Occurs if

- Graft is not rejected by host
- Enough immune competent cells in grafts
- Immuno compromised host
- Histocompatibility differences between host and graft



# 1. Acute GVHD

- Endothelial cell death in the skin, liver, and gastrointestinal tract
- Rash, jaundice, diarrhea, gastrointestinal hemorrhage
- Mediated by mature T cells in the grafts



- **Acute graft-versus-host reaction with vivid palmar erythema**

## **2. Chronic GVHD**

- **Fibrosis and atrophy of one or more of the organs**
- **Eventually complete dysfunction of the affected organ**

- **Both acute and chronic GVHD are commonly treated with intense immunosuppression**
- **Often Fatal**

# Runt disease

- Presents clinically as growth retardation, diarrhea, hepatosplenomegaly, lymphoid atrophy, anemia and finally death

# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

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*Part IV Prevention and treatment of allograft rejection*

*Part V Exnogtransplantaion*



# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

*Part III Classification and effector mechanisms of allograft rejection*

*Part IV Prevention and treatment of allograft rejection*

*Part V Exnogtransplantaion*

# *PartIV Prevention and treatment of allograft rejection*

- ① **Reducing the immunogenicity of allografts  
(tissue typing)**
- ② **Immunosuppression**
- ③ **Inducing donor-specific tolerance**

## ① Reducing the Immunogenicity of allografts:

- Donors and Recipients Are Typed for ABO Blood grouping and MHC Antigens.
- Rh specificity is not needed.

- HLA-A, B and HLA-DR is important for predicting outcome
- A **000** mismatch is a 'full house' or 'complete match'
- A **012** mismatch is *matched at HLA A loci*, has *one mismatched HLA B* antigen and is *mismatched for both DR antigens*.
- Size is not a deal for kidney transplantation. Adult kidneys can be used for pediatric recipients and vice versa

# Tissue typing

---

- Microcytotoxicity assay
  - Known antibody to WBCs of donor / recipient
  - Complement mediated lysis if Ab present on cell surface
- Mixed lymphocyte culture (MLC)
  - Irradiated donor lymphocytes (stimulants)
  - Incubated with recipient lymphocytes
- Flow cytometry cross typing
- DNA analysis
  - Genomic typing (very precise, many subtypes)

Tissue typing laboratory carries out three key tasks

1. Determine HLA type of blood of both by PCR
2. Cross matching is done to exclude presence of clinically significant circulating antibodies. Flow cytometric tests are preferred over cytotoxic cross match tests
3. Determine HLA specificity of circulating anti HLA antibodies in recipients before and after organ transplantation to guide immuno suppressive therapy



## ② **Immunosuppression:**

Immunosuppression can be brought about by 3 different ways

:-

- Surgical ablation
- Total Lymphoid Irradiation
- Immunosuppressive drugs

# Immunosuppressive Drugs

## Three main immunosuppressant drugs

- **Cyclosporins** act by inhibiting T-cell activation, thus preventing T-cells from attacking the transplanted organ.
- **Azathioprimines** disrupt the synthesis of DNA and RNA and cell division.
- **Corticosteroids** such as prednisolone suppress the inflammation associated with transplant rejection.

## **I. Inhibitors of cytokine (IL-2) production or action:**

### **1) Calcineurin inhibitors**

- Cyclosporine
- Tacrolimus (FK506)

### **2) Sirolimus (rapamycin).**

**Everolimus**

## **II. Inhibitors of cytokine gene expression**

- Corticosteroids

## **III. Cytotoxic drugs**

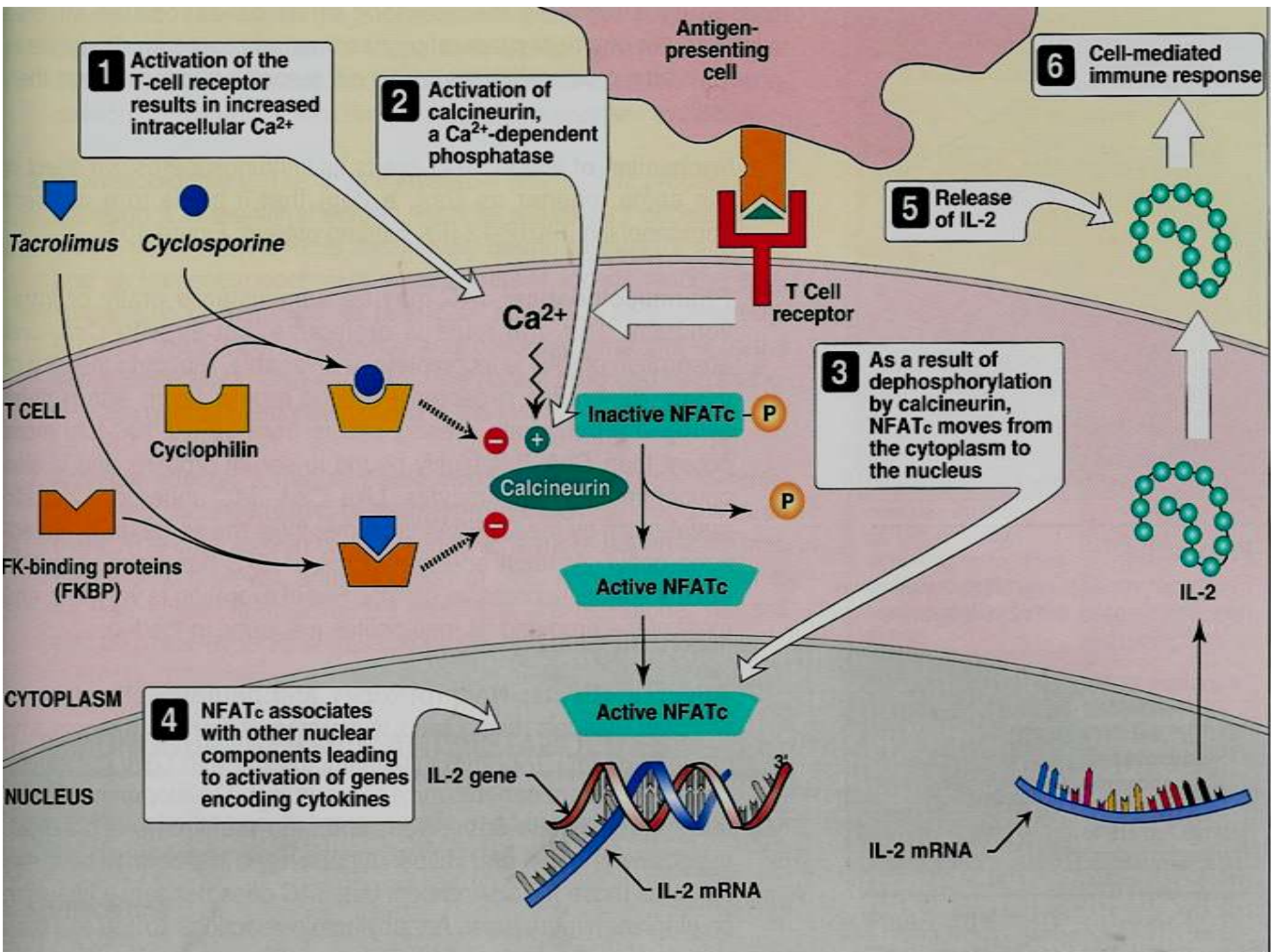
- ☐ **Inhibitors of purine or pyrimidine synthesis:**
  - Azathioprine
  - Mycophenolate Mofetil
  - Leflunomide
  - Methotrexate
- ☐ **Alkylating agents**
  - Cyclophosphamide**

## **IV. Immunosuppressive antibodies that block T cell surface molecules involved in signaling Ig**

- antilymphocyte globulins (ALG).
- antithymocyte globulins (ATG).
- Rho (D) immunoglobulin.
- Basiliximab
- Daclizumab
- Muromonab-CD3

## **V. Interferon**

## **VI. Thalidomide**



**1** Activation of the T-cell receptor results in increased intracellular  $Ca^{2+}$

**2** Activation of calcineurin, a  $Ca^{2+}$ -dependent phosphatase

**5** Release of IL-2

**6** Cell-mediated immune response

**3** As a result of dephosphorylation by calcineurin, NFATc moves from the cytoplasm to the nucleus

**4** NFATc associates with other nuclear components leading to activation of genes encoding cytokines

Tacrolimus Cyclosporine

T CELL

FK-binding proteins (FKBP)

CYTOPLASM

NUCLEUS

Antigen-presenting cell

T Cell receptor

$Ca^{2+}$

Inactive NFATc - P

Calcineurin

Active NFATc

Active NFATc

IL-2 gene

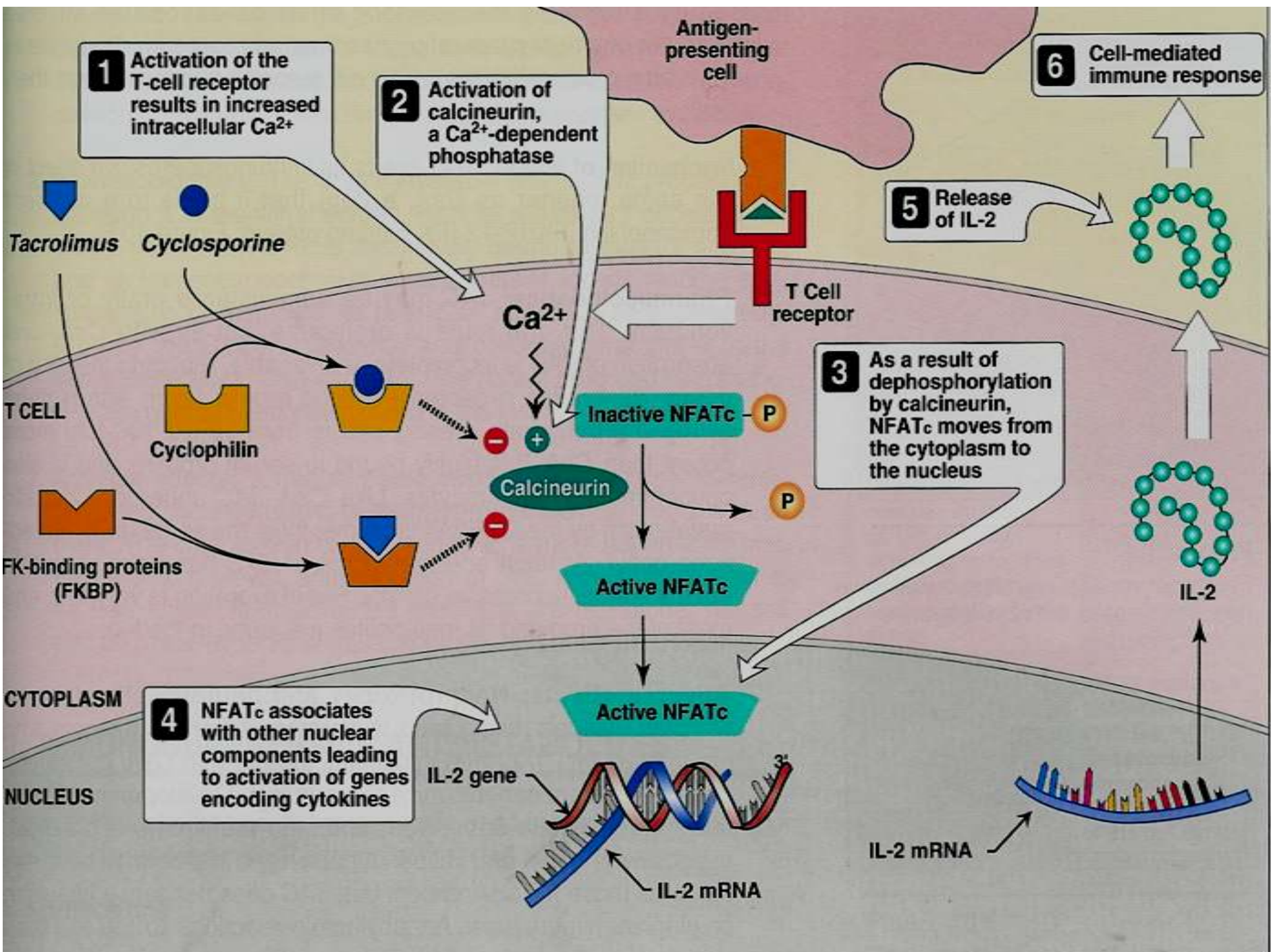
IL-2 mRNA

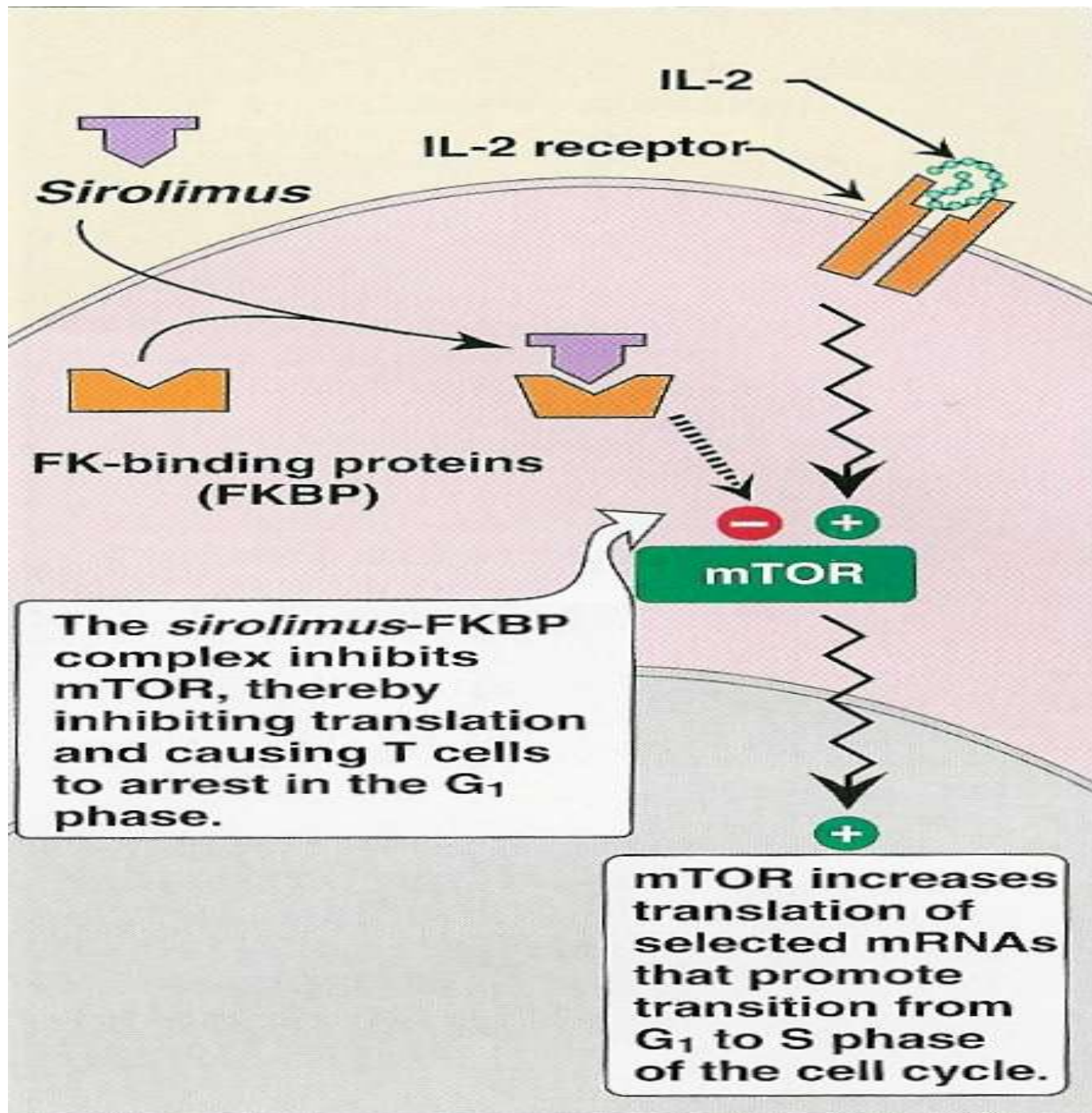
IL-2 mRNA

IL-2

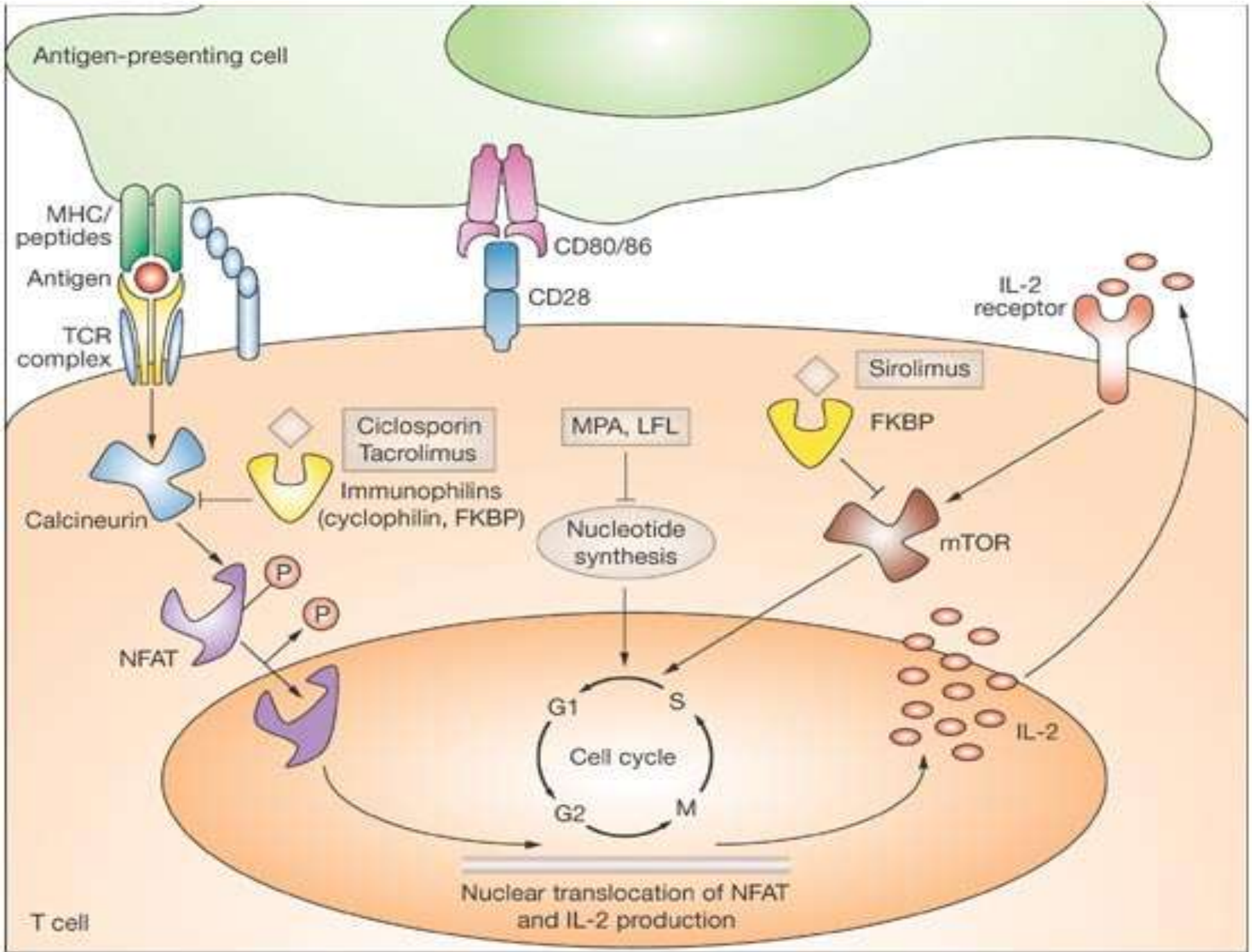
Cyclophilin



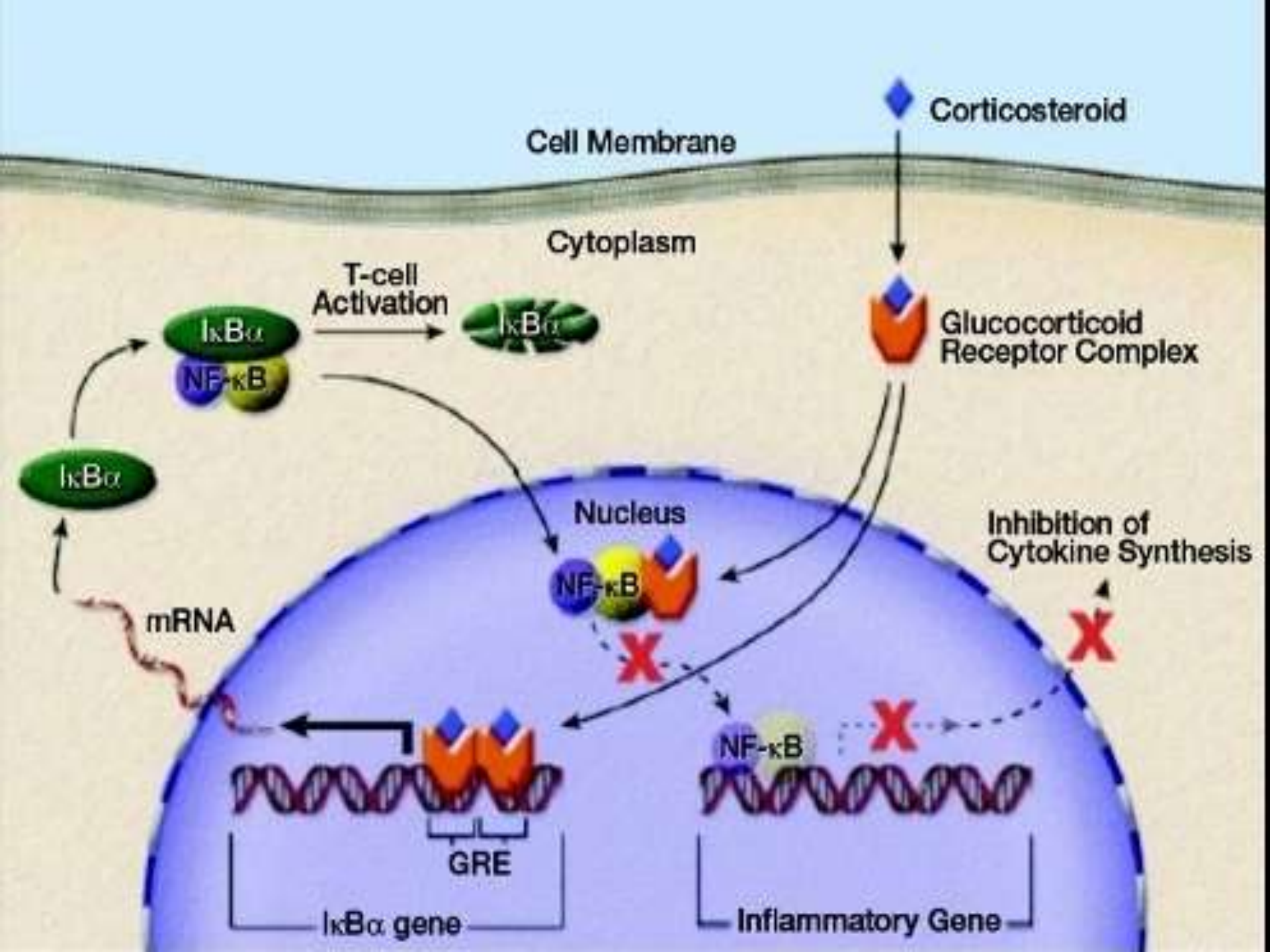












Corticosteroid

Cell Membrane

Cytoplasm

Glucocorticoid Receptor Complex

T-cell Activation

IκBα

NF-κB

IκBα

IκBα

mRNA

Nucleus

NF-κB

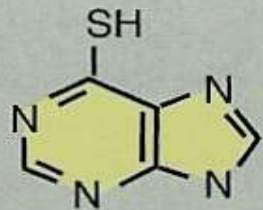
Inhibition of Cytokine Synthesis

GRE

IκBα gene

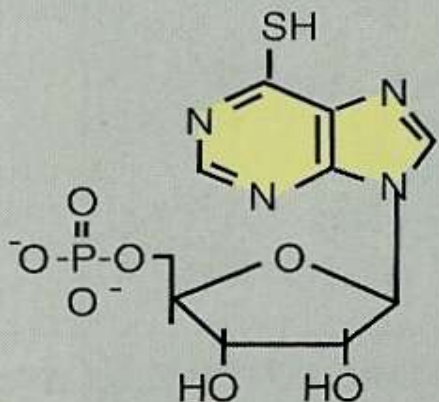
NF-κB

Inflammatory Gene

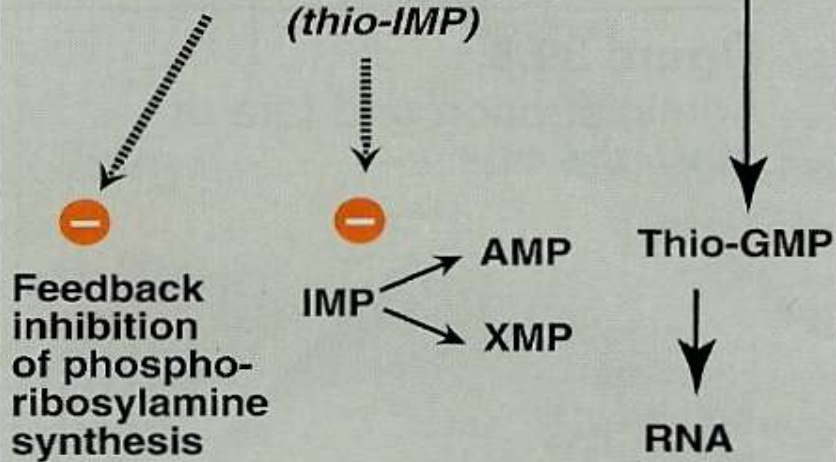


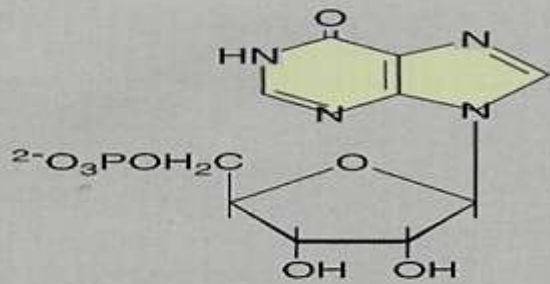
**6-Mercaptopurine**

Hypoxanthine-guanine  
phosphoribosyl  
transferase



**6-Thioinosinic acid  
(thio-IMP)**



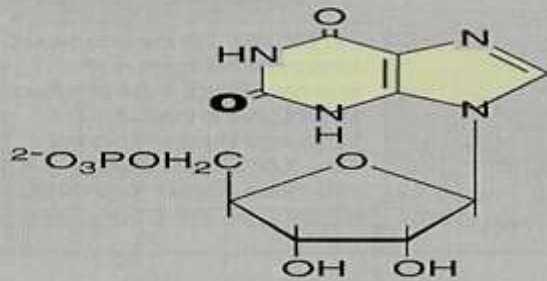


Inosine monophosphate



← *Mycophenolate*

IMP  
dehydrogenase



Xanthosine monophosphate



GMP



Blocking the formation of GMP deprives rapidly proliferating T and B cells of a key precursor required for nucleic acid synthesis.

## Monoclonal antibodies

### Infliximab

- a chimeric human-mouse IgG
- Directed against TNF- $\alpha$
- Is approved for ulcerative colitis, Crohn's disease & rheumatoid arthritis

### Omalizumab

- a humanized monoclonal IgE
- Directed against Fc receptor on mast & basophils
- Is approved for asthma in steroid-refractory patient

# INTERFERONS

Three families:

Type I IFNs ( IFN- $\alpha$ ,  $\beta$  ):

- acid-stable proteins; act on same target cell receptor
- induced by viral infections
- leukocyte produces IFN- $\alpha$
- Fibroblasts & endothelial cells produce IFN- $\beta$

Type II IFN (IFN- $\gamma$ ):

- acid-labile; acts on separate target cell receptors
- Produced by Activated T lymphocytes.

## Interferon Effects:

### IFN- $\gamma$ : Immune Enhancing

- increased antigen presentations with macrophage, natural killer cell, cytotoxic T lymphocyte activation

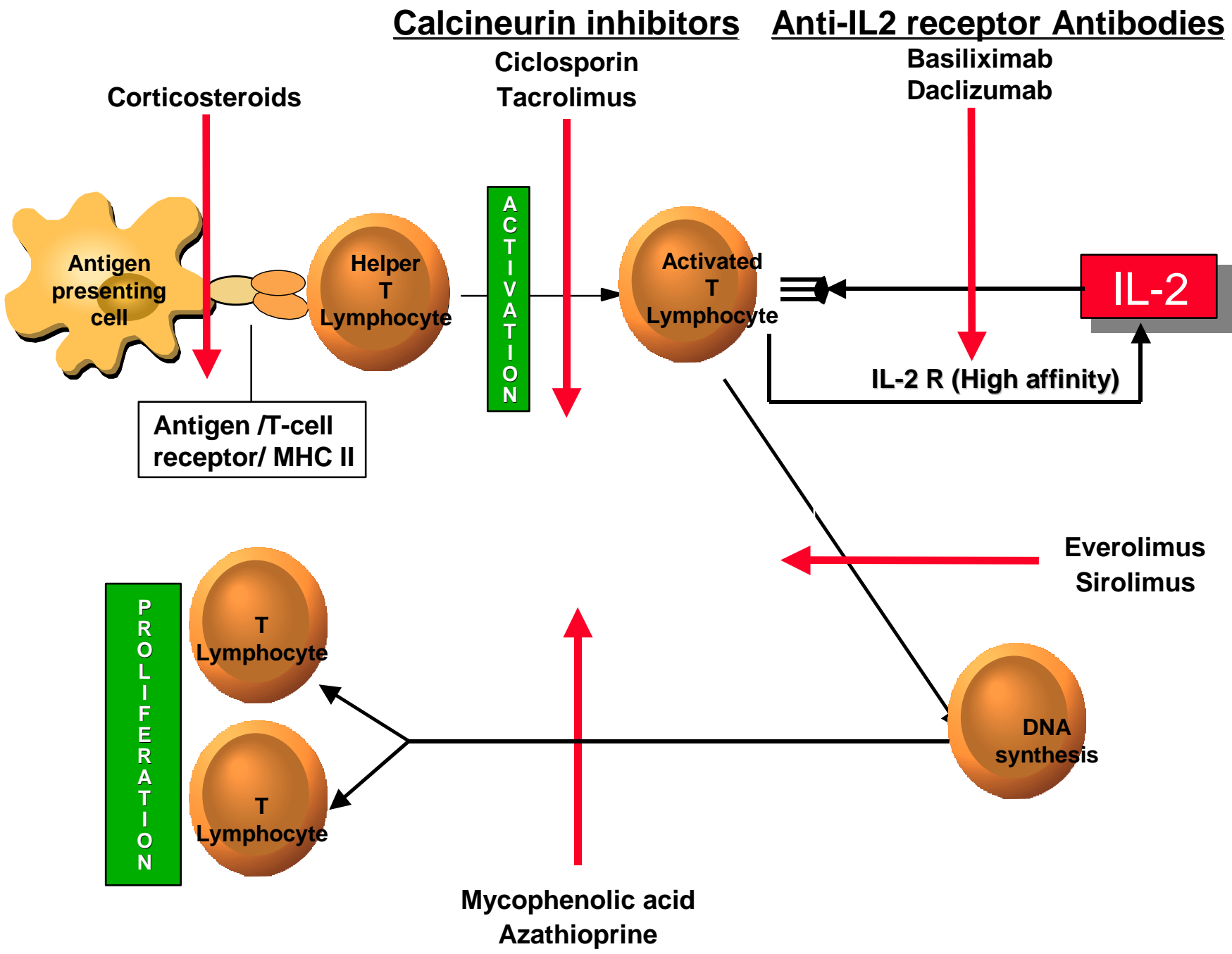
### IFN- $\alpha, \beta$ :




- effective in inhibiting cellular proliferation  
(more effective than IFN-  $\gamma$  in this regard)

## THAMLIDOMIDE

- A sedative drug.
- Teratogenic (Class-X).
- Can be given orally.
- Has immunomodulatory actions
- Inhibits TNF- $\alpha$
- Reduces phagocytosis by neutrophils
- Increases IL-10 production





DRUG	ACTION	ADVERSE EFFECTS
 <p>Antigen</p>	<p><i>Antithymocyte globulins</i> <i>Muromonab-CD3</i></p>	<p>Profound immunosuppression Cytokine release syndrome</p>
 <p>T-cell receptor</p>	<p><i>Cyclosporine</i> <i>Tacrolimus (FK506)</i></p>	<p>Nephrotoxicity, neurotoxicity, hepatotoxicity Nephrotoxicity, neurotoxicity, diabetes</p>
<p>Activated calcineurin</p>		
<p>Dephosphorylation of NFATc</p>		
<p>IL-2 gene promotion</p>		
<p>IL-2</p>		
 <p>IL-2 receptors</p>	<p><i>Basiliximab</i> <i>Daclizumab</i></p>	<p>Gastrointestinal disorders Gastrointestinal disorders</p>
<p>Progression into cell cycle</p>		
 <p>Cell proliferation</p>	<p><i>Sirolimus</i></p>	<p>Hyperlipidemia, thrombocytopenia, leukopenia, headache, nausea</p>
<p>Cell proliferation</p>	<p><i>Azathioprine</i> <i>Mycophenolate mofetil</i></p>	<p>Bone marrow suppression, hepatotoxicity, thrombocytopenia, anemia, neoplasia GI upset, nausea, diarrhea, leukopenia, tumors, increases susceptibility to infection</p>
<p>Cell proliferation</p>		

### ③ **Inducing donor-specific tolerance:**

- **Inhibition of T cell activation**
  - Soluble MHC molecules
  - CTLA4-Ig
  - Anti-IL2R mAb
- **Th2 cytokines**
  - Anti-TNF- $\alpha$ , Anti-IL-2, Anti-IFN- $\gamma$  mAb
- **Microchimerism**
  - The presence of a small number of cells of donor, genetically distinct from those of the host individual

# Why is fetus not rejected by the mother?



A/B

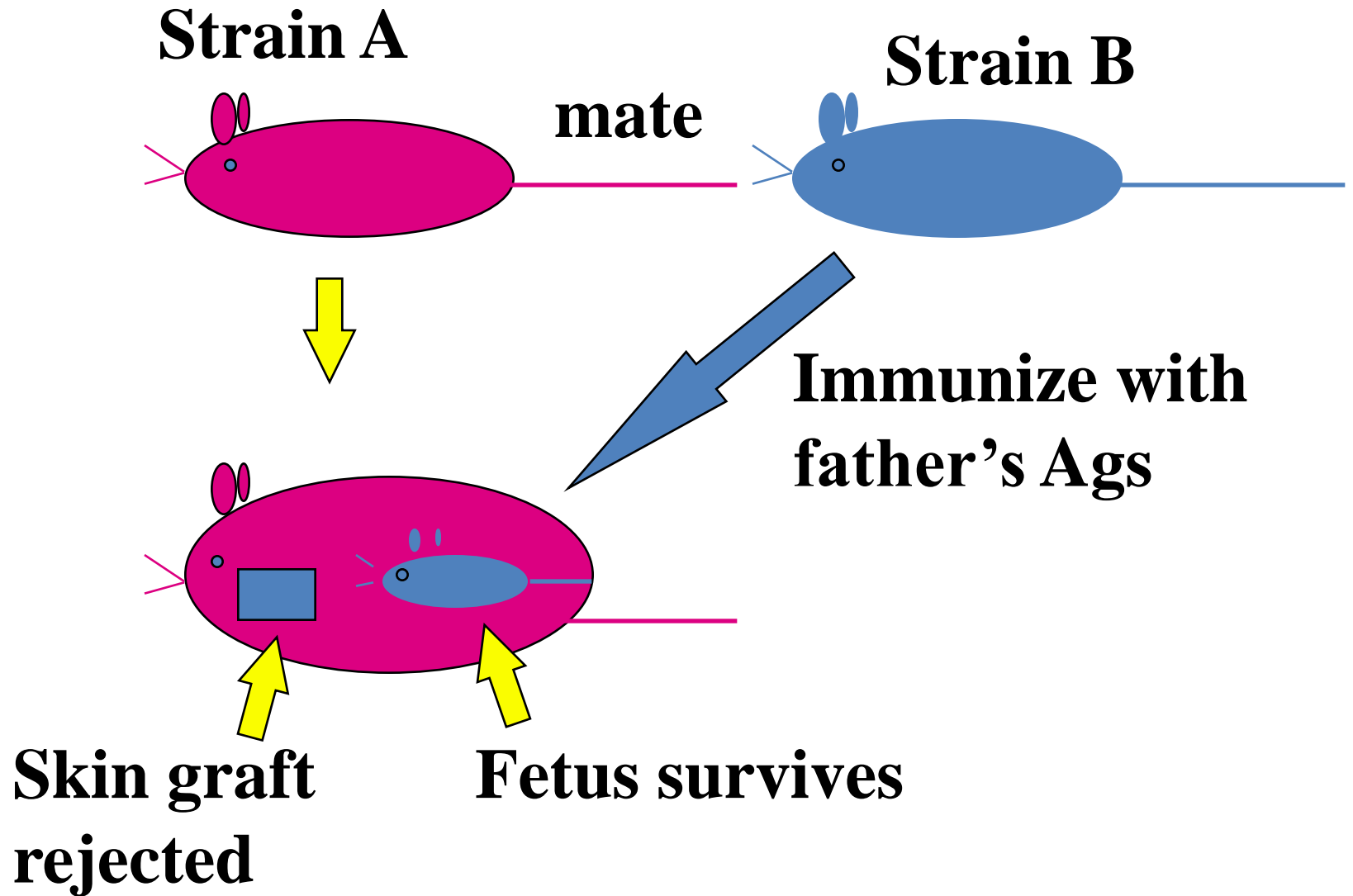


C/D



A/C, A/D, B/C, B/D

# Fetus as an allograft



# Why is the fetus not rejected ?

## Local immunosuppression

- Outer layer of placenta; **does not express MHC** and Placenta expresses FasL and secretes a substance that depletes tryptophan and therefore T cell stimulation
- **Progesterone** itself acts as immunosuppressive
- Fetal blood rich in **Alpha feto protein** is also immunosuppressive
- Mucopolysaccharide barrier rich in **Sialic acid** surrounds Trophoblasts
- Uterine epithelium and trophoblast secrete cytokines that suppresses TH1
- Tolerance of paternal MHC antigens
- Spontaneous abortions are some times triggered by maternal immune response against fetus.

# Immunologically privileged sites

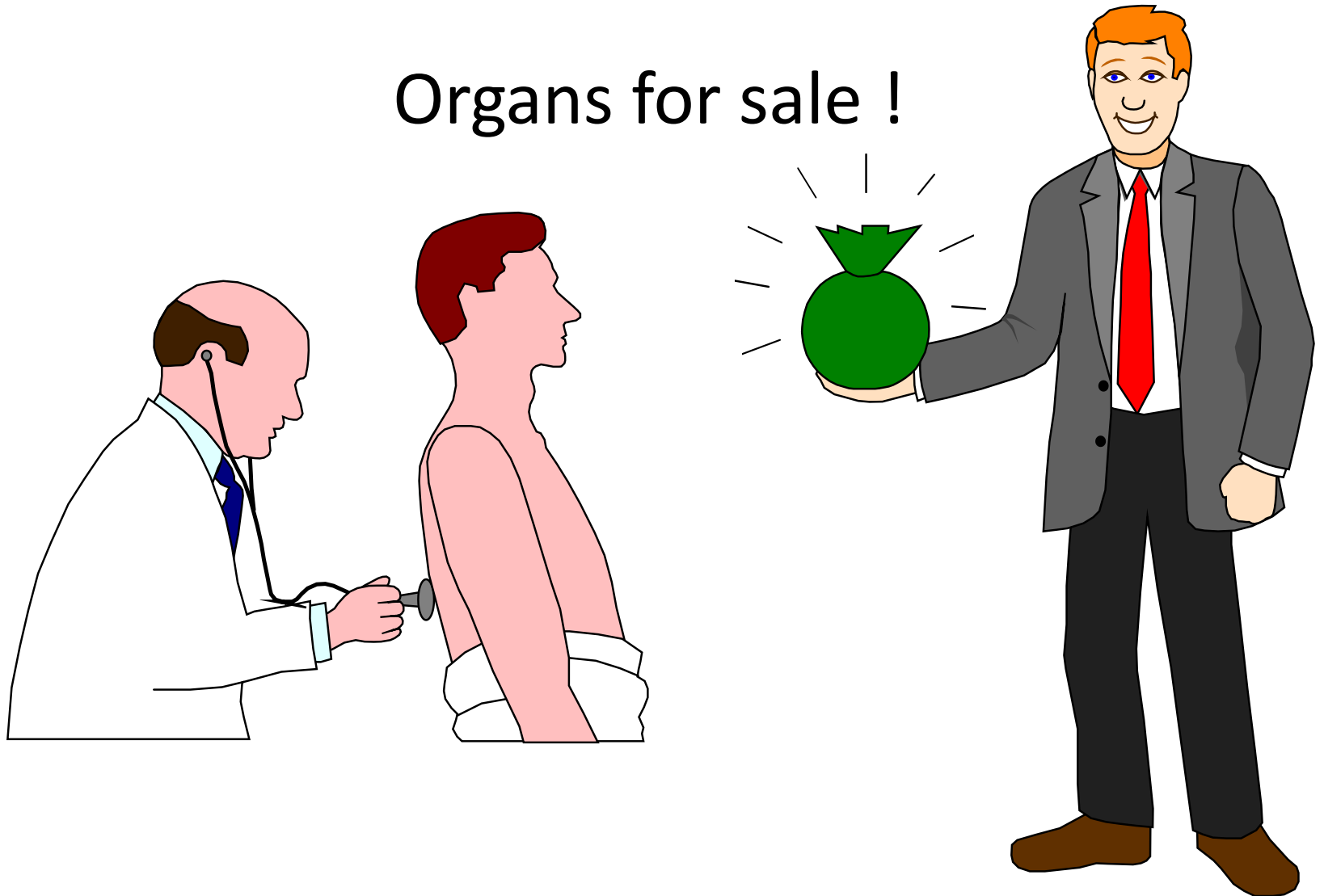
Some areas not infiltrated by immune cells

- cornea, brain, uterus, testes
- thymus?



# Ethical aspects

Organs for sale !



# Ethical aspects:

- Thanks to Allah ---  
MHC is  
polymorphic.



# *Contents*

*Part I Introduction*

*Part II Immunologic mechanism of allogeneic transplantation rejection*

*Part III Classification and effector mechanisms of allograft rejection*

*Part IV Prevention and treatment of allograft rejection*

*Part V Xenotransplantation*

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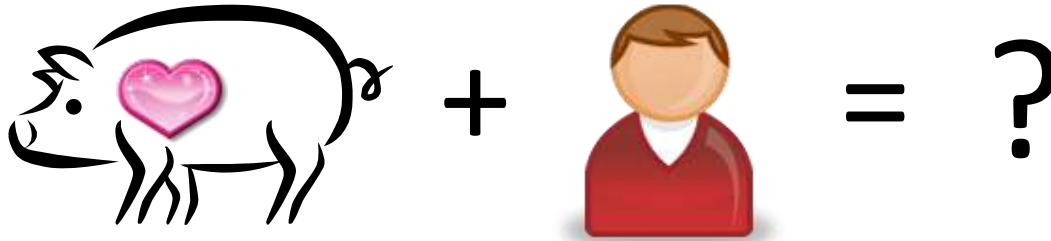
*Part IV Prevention and treatment of allograft rejection*

*Part V Xenotransplantation*

## *Part V Xenotransplantation*

- Potential advantage due to larger accessibility of animal organs
- Monkeys are apparently the most suitable donors, but dangerous because of potential risk of retrovirus transfer within graft

# Correct functioning



- Pigs are now considered because of similar sizes of organs and erythrocytes to human ones
- The major obstacle – presence in man (1%) of natural antibodies vs. Gal (galactose- $\alpha$ -1,3-galactose) causing hyperacute rejection

## Pigs as donors

- Multiple offspring per litter.
- Reach sexual maturity rapidly
- Risk of transmission of viruses low
- Genetically engineered
- Herds similar in size to humans developed
- Insulin used to treat diabetes

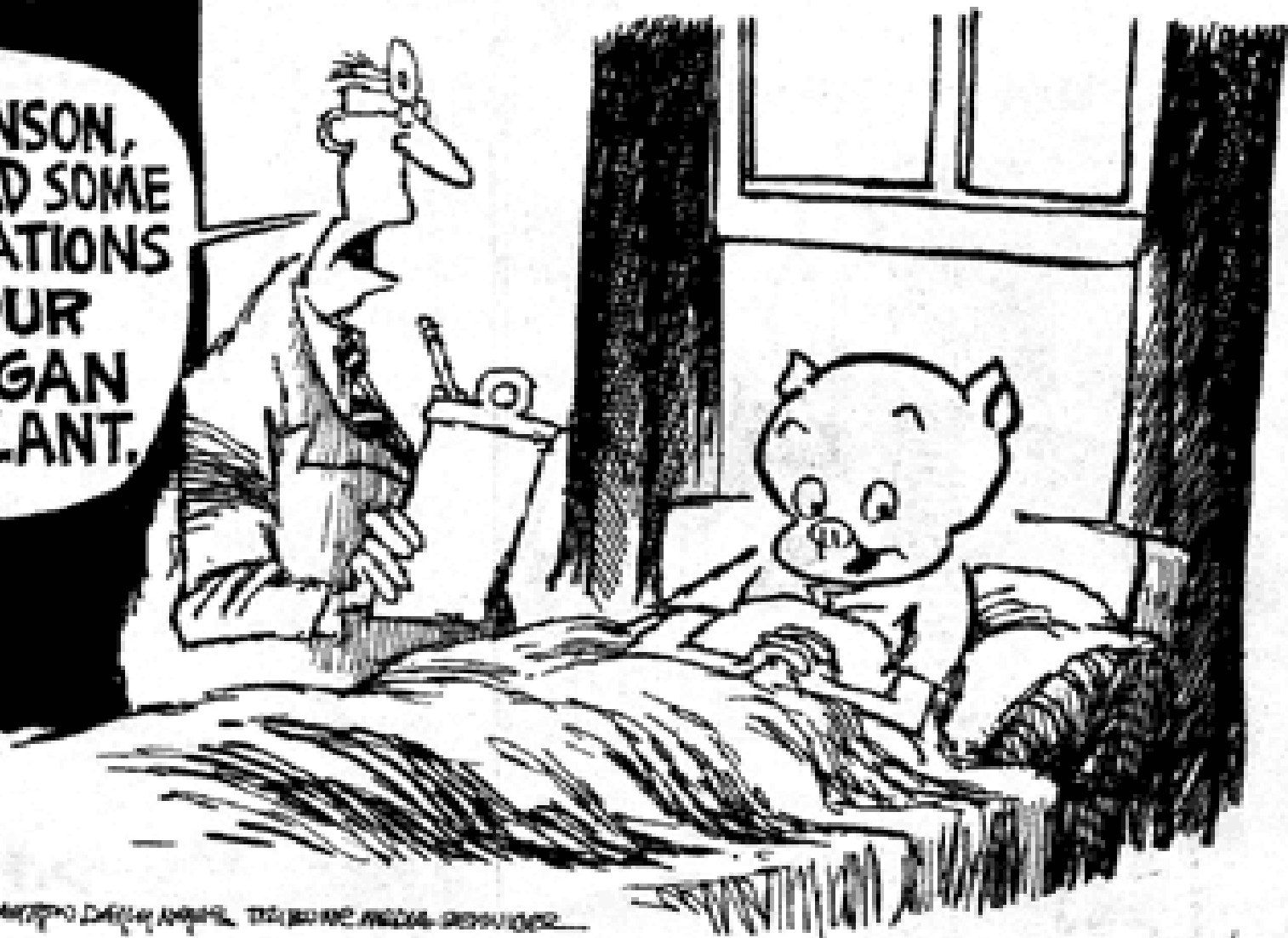




- Hyperacute xenograft rejection (HXR)
  - Human anti-pig nature Abs reactive with Gal $\alpha$ 1,3Gal
  - Construct transgenic pigs expressing human proteins that inhibit complement activation
- Delayed xenograft rejection (DXR)
  - Acute vascular rejection
  - Incompletely understood
- T cell-mediated xenograft rejection



MR. JOHNSON,  
WE'VE HAD SOME  
COMPLICATIONS  
WITH YOUR  
PIG ORGAN  
TRANSPLANT.



TIM  
PETERS

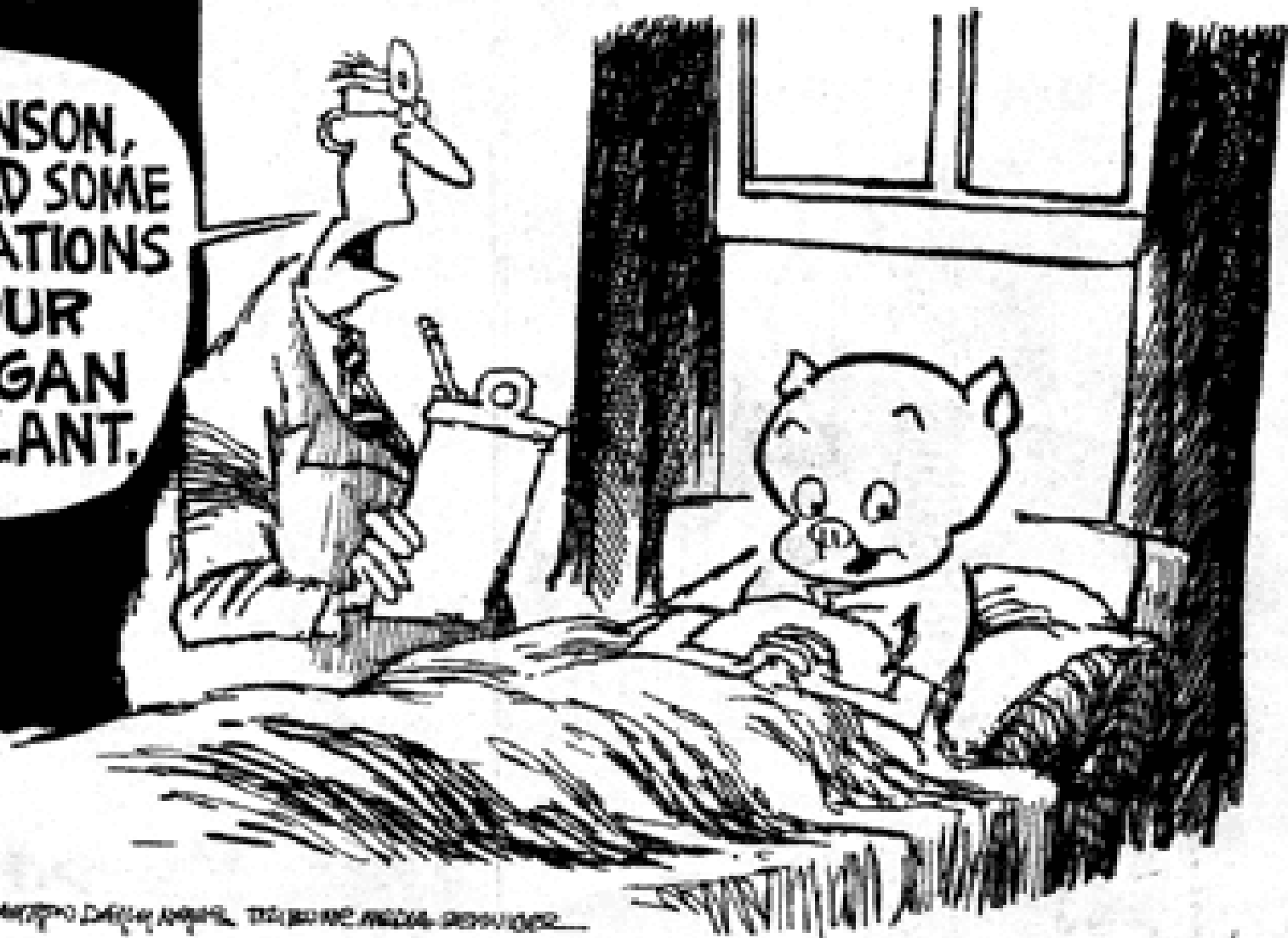
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Thank You

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