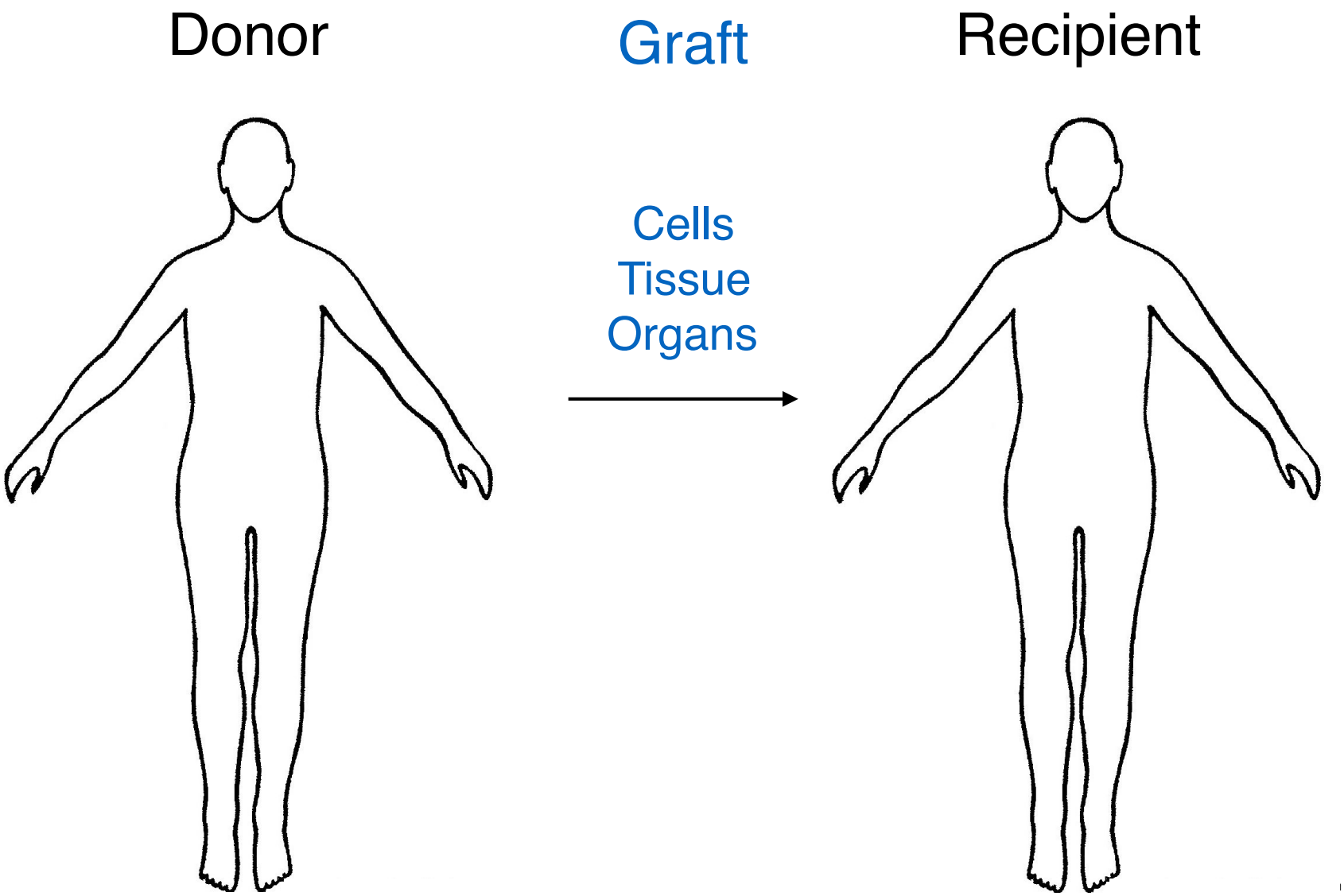
Transplantation Immunology

Transplantation



Definitions

Autograft

Self tissue transferred from one body site to another in the same individual.

E.g.,Skin, blood vessels

Isograft

Tissue transferred between genetically identical individuals.

E.g.,Inbred mice, identical twins

Allograft

Tissue transferred between genetically different individuals of the same species.

E.g. most human transplants

Xenograft

Tissue transferred between different species.

E.g. pig heart valves to humans

Definitions

Autograft — Accepted

Self tissue transferred from one body site to another in the same individual.

E.g.,Skin, blood vessels

Isograft — Accepted

Tissue transferred between genetically identical individuals.

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Allograft — Rejected

Tissue transferred between genetically different individuals of the same species.

E.g. most human transplants

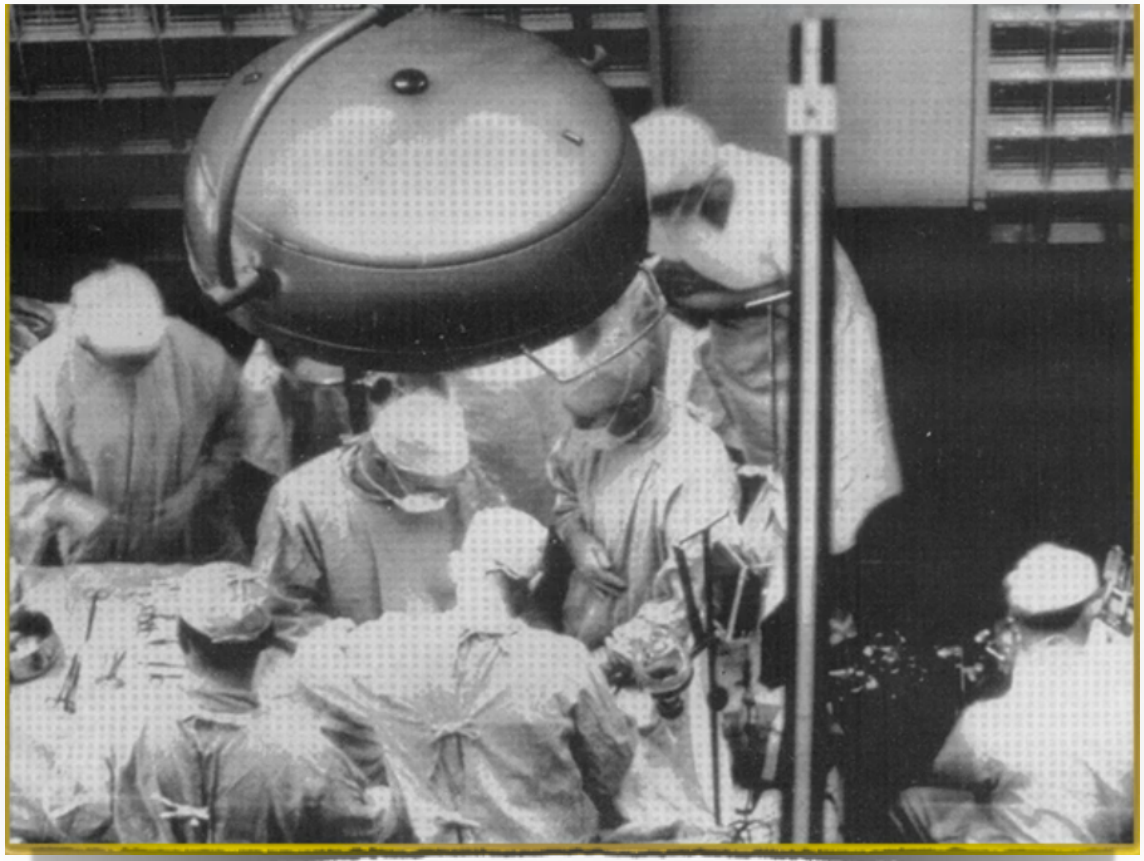
Xenograft — Rejected

Tissue transferred between different species.

E.g. pig heart valves to humans

History of transplantation

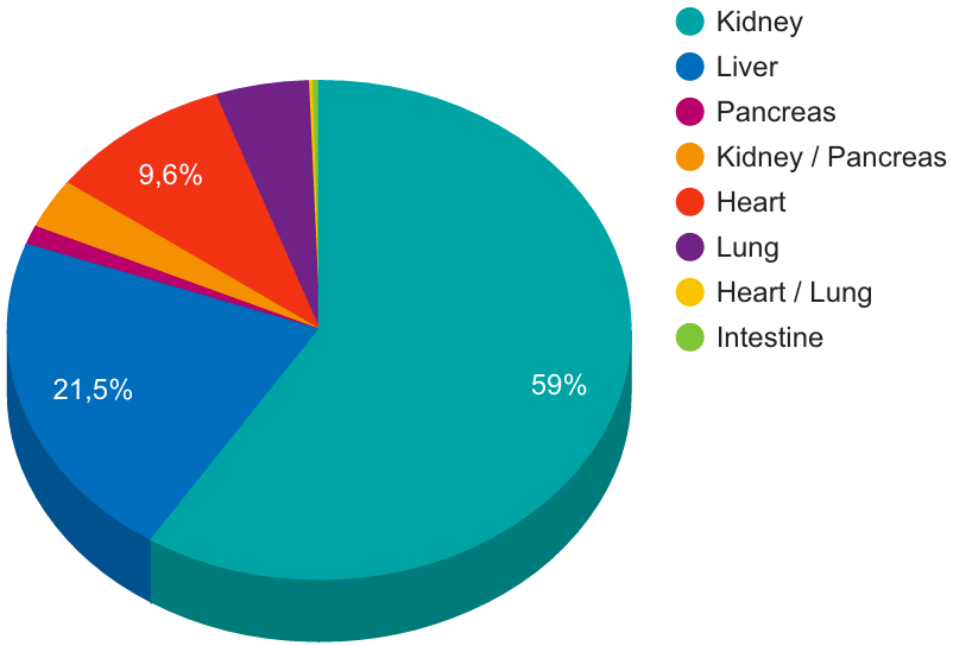
First kidney transplants were performed in 1952 at the Necker Hospital in Paris, although the kidney failed after 3 weeks, and in 1954 in Boston. The donor and recipient were twins and the recipient survived for 8 years.



Transplants By Organ Type January 1, 1988 - September 30, 2016

Based on OPTN data as of October 14, 2016

Organ	Transplants
Kidney	400,348
Liver	145,795
Pancreas	8,291
Kidney / Pancreas	21,922
Heart	64,890
Lung	32,796
Heart / Lung	1,192
Intestine	2,770
Total	**678,004**



Source: unos.org

Improving outcome of transplantation

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IMPROVED GRAFT SURVIVAL AFTER RENAL TRANSPLANTATION IN THE UNITED STATES, 1988 TO 1996

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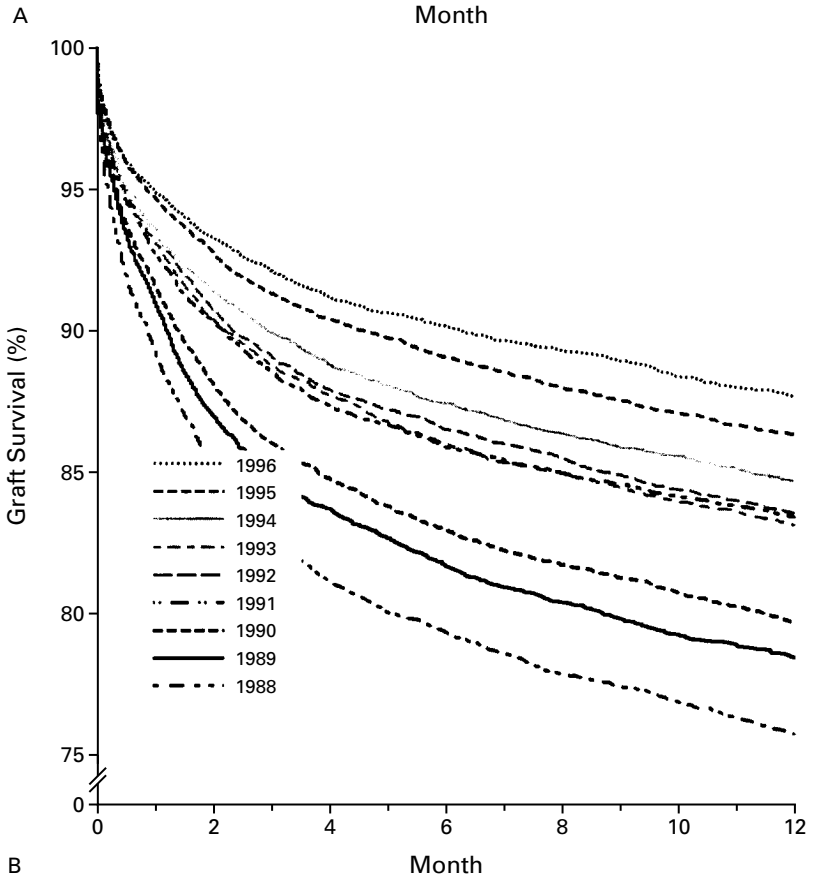
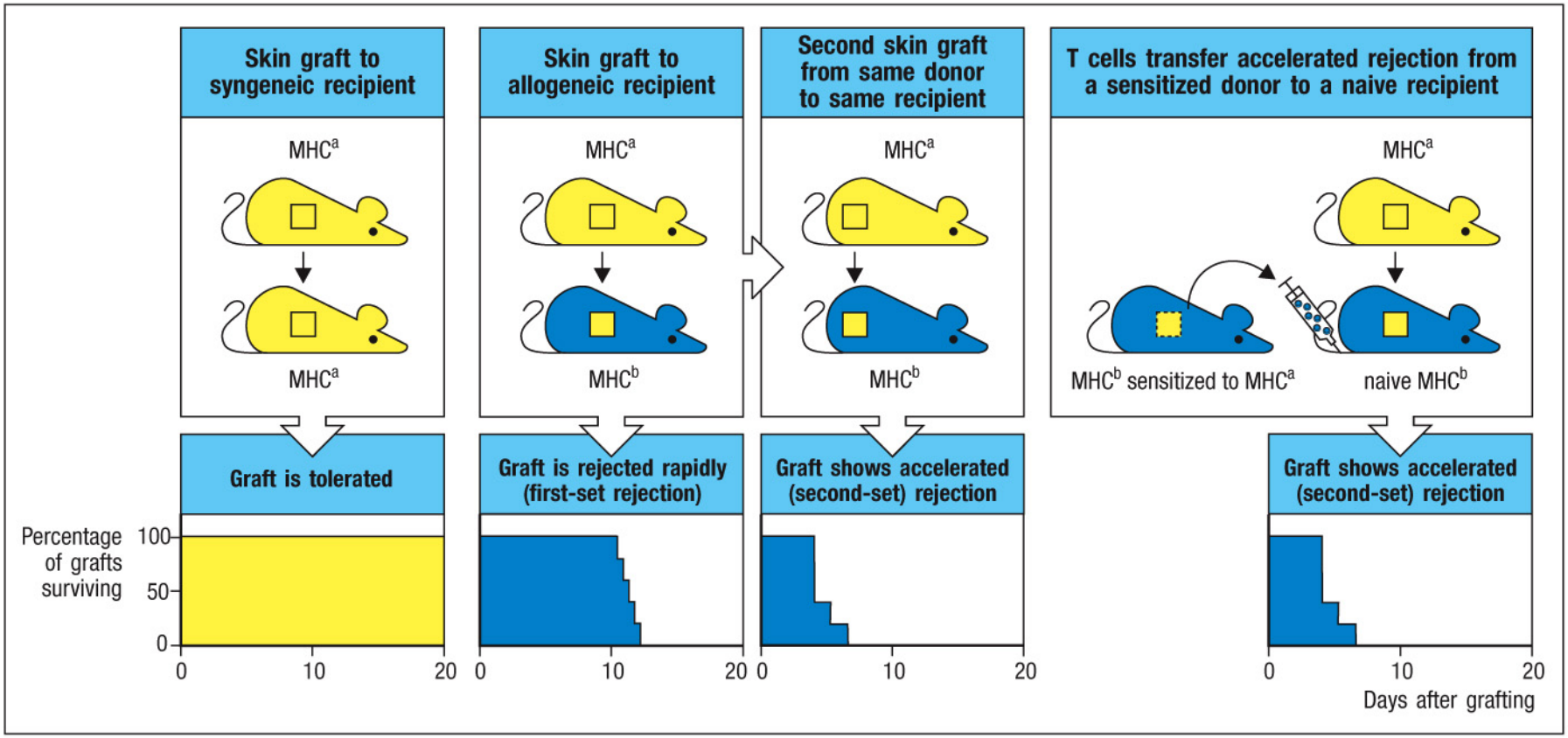


What are the immunological mechanisms of allograft rejection?

How can this knowledge be used to improve the outcome of transplantation?

The role of T cells in allograft rejection

The basic rules of tissue grafting were first elucidated in skin transplantation between inbred strains of mice:



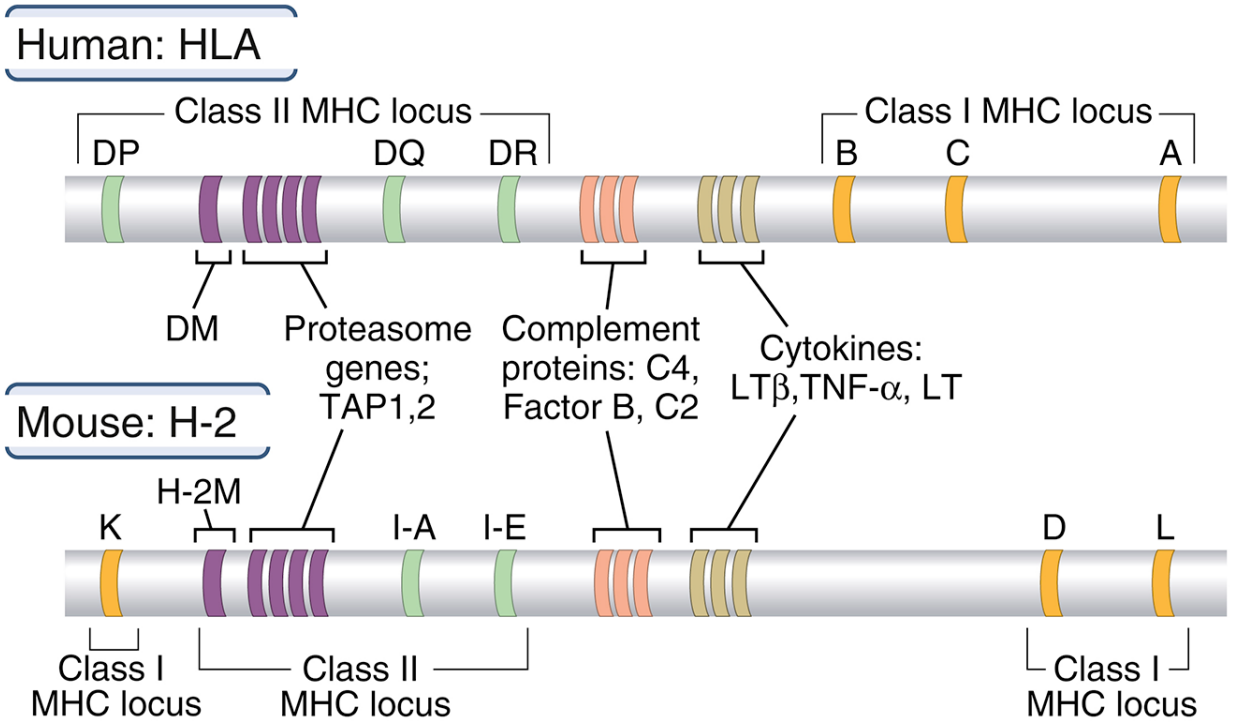
Mechanism of allograft rejection

- Skin graft to different site (**autograft**) or between genetically identical individual (**syngeneic graft**) is tolerated.
- Skin graft between unrelated individual (**allograft**) survives initially, but is then rejected ~ 10/13 days after transplantation (= **first-set rejection**); skin grafts onto nude mice, lacking **T cells**, do not show rejection
- Re-grafting skin to the same recipient is rejected in an accelerated fashion (**second-set rejection**)

Rejection results from inflammatory reactions that damage the transplanted tissues. It is mediated by the **adaptive** branch of the immune system and displays **specificity** and **memory**.

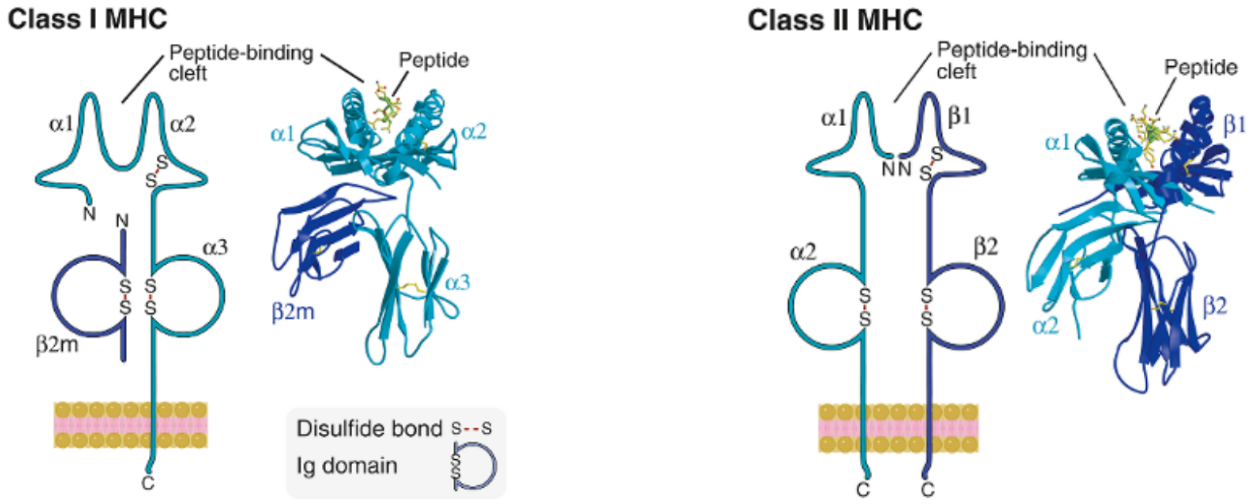
Major histocompatibility complex (MHC)

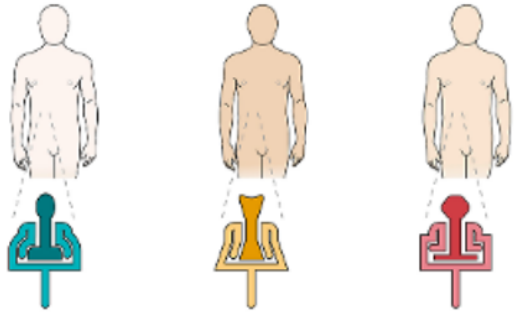
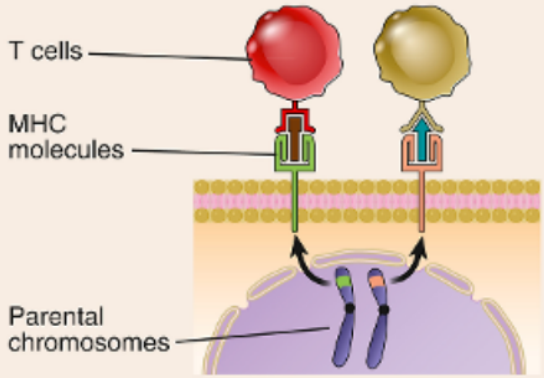
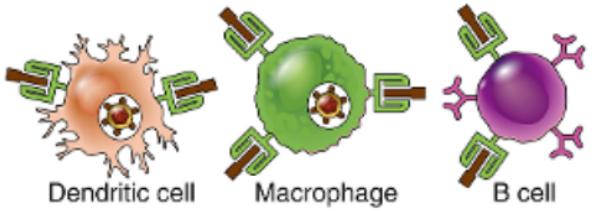
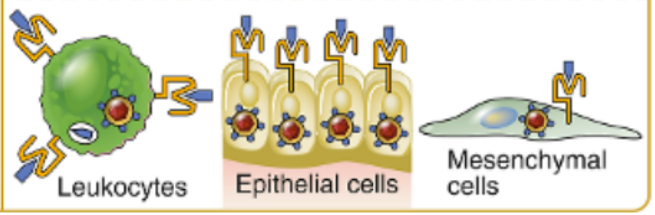
Tissues that are antigenically similar are called „**histocompatible**“. The antigens that determine histocompatibility are various, but the major ones are expressed within the **major histocompatibility couples** (**MHC**).



Major histocompatibility complex (MHC)

MHC locus is found in all mammals.
Human MHC proteins are called **human leukocyte antigens** (**HLA**).
In addition to nonpolymorphic genes (e.g., cytokines) two sets of polymorphic genes are encoded in mammalian MHC locus, namely **MHC class I** and **MHC class II**. MHC molecules are **membrane molecules** that each contains a **peptide-binding cleft** at the N-terminal end.



Feature	Significance	
Polymorphic genes: Many different alleles are present in the population	Different individuals are able to present and respond to different microbial peptides	
Co-dominant expression: Both parental alleles of each MHC gene are expressed	Increases number of different MHC molecules that can present peptides to T cells	
MHC-expressing cell types: Class II: Dendritic cells, macrophages, B cells	$CD4^+$ helper T lymphocytes interact with dendritic cells, macrophages, B lymphocytes	
Class I: All nucleated cells	$CD8^+$ CTLs can kill any type of virus-infected cell	

Major histocompatibility complex (MHC)

MHC class I locus: three gene loci (HLA-A, HLA-B, HLA-C)
MHC class II locus: three gene loci (HLA-DP, HLA-DQ, HLA-DR)

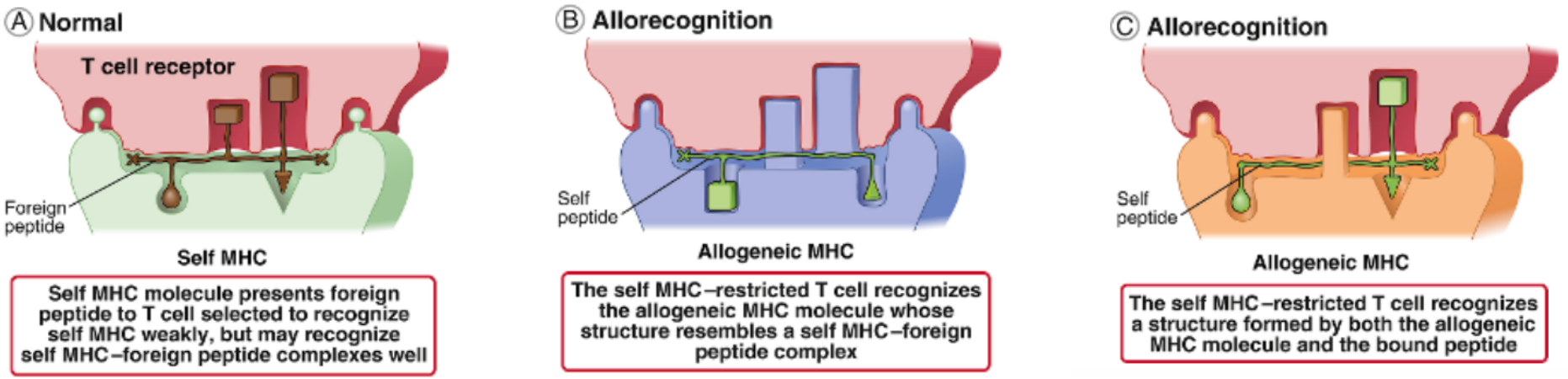
Each person expressed six class I MHC alleles (one from each parent) and usually more than 8 class II MHC alleles (one HLA-DQ/P, one/two HLA-DR, and combinations thereof).

The set of MHC alleles present on one chromosome is called **MHC haplotype**.

MHC alleles are highly polymorphic with > 13.000 different HLA alleles among the human population.

Chance of identical HLA alleles in siblings 1:4.

Recognition of allogeneic MHC molecules

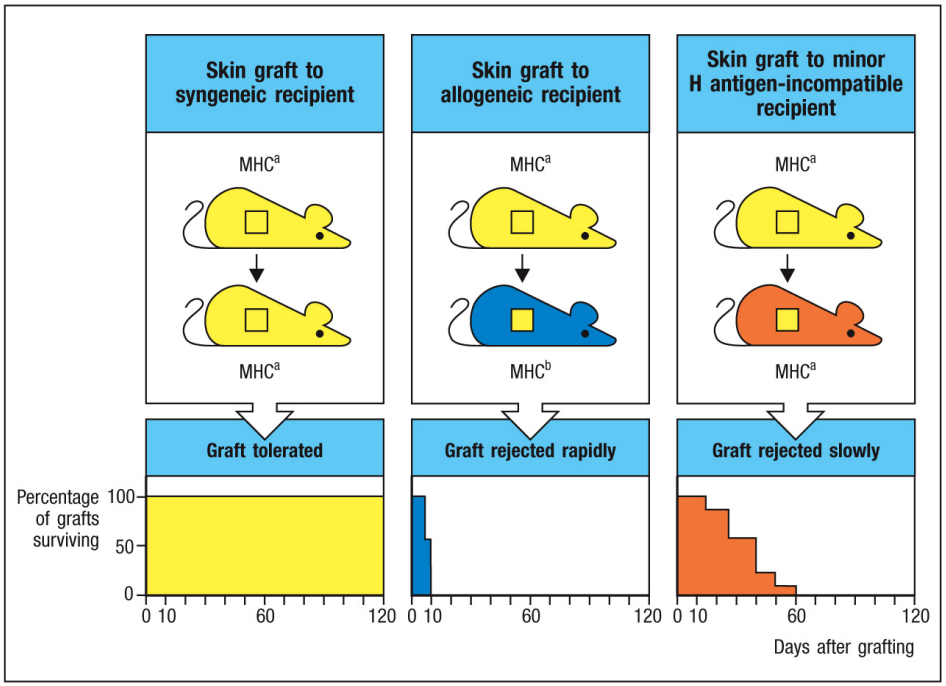


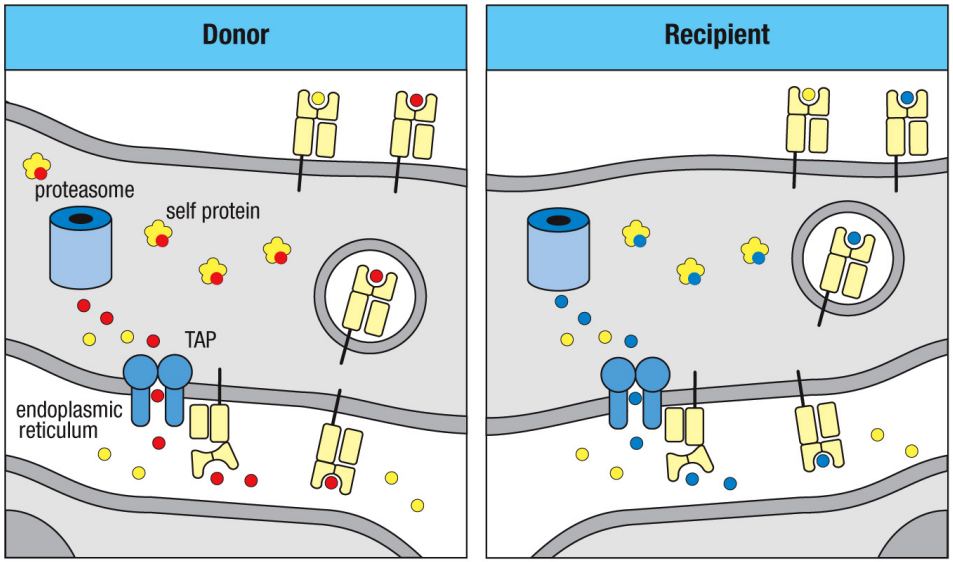
Recognition of alloantigens results in exceptionally strong T cell response:

- Many distinct T cell clone recognize the same allogeneic MHC molecule
- A single graft cell will express many distinct MHC class molecules, which may be recognized.

Minor histocompatibility antigens

Besides MHC other antigens, called minor histocompatibility antigens, may provoke graft rejection, albeit more slowly.





Polymorphic self proteins that differ in amino acid sequence between individuals give rise to minor H antigen differences between donor and recipient

Induction of immune responses against transplants

Graft rejection is caused primarily by a **cell-mediated immune response** to alloantigens (mainly MHC molecules) expressed on graft cells.

The process of graft rejection can be divided into **two stages**:

Sensitization stage → Effector stage

- Recognition of alloantigens
- Proliferation of antigen-specific lymphocytes

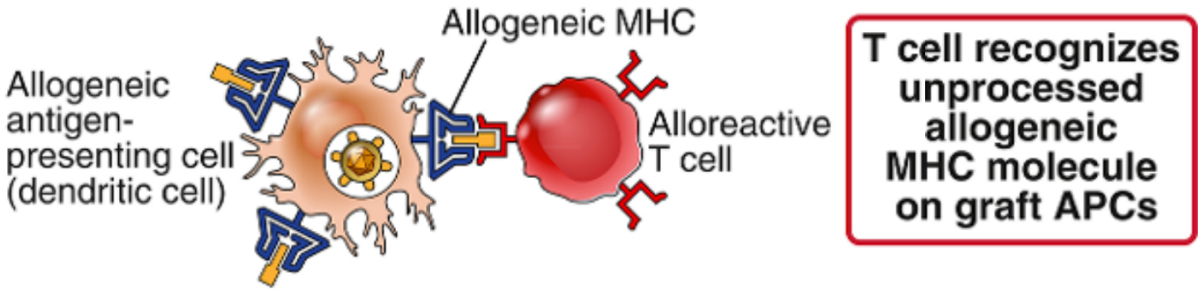
Immune-mediated destruction of the graft

Sensitization (I) - *Direct allorecognition*

T cells may recognize allogeneic MHC molecules through two distinct mechanisms:

1) Direct allorecognition

T cells in the recipient recognize donor allogeneic antigens on graft antigen-presenting cells (e.g., dendritic cells)

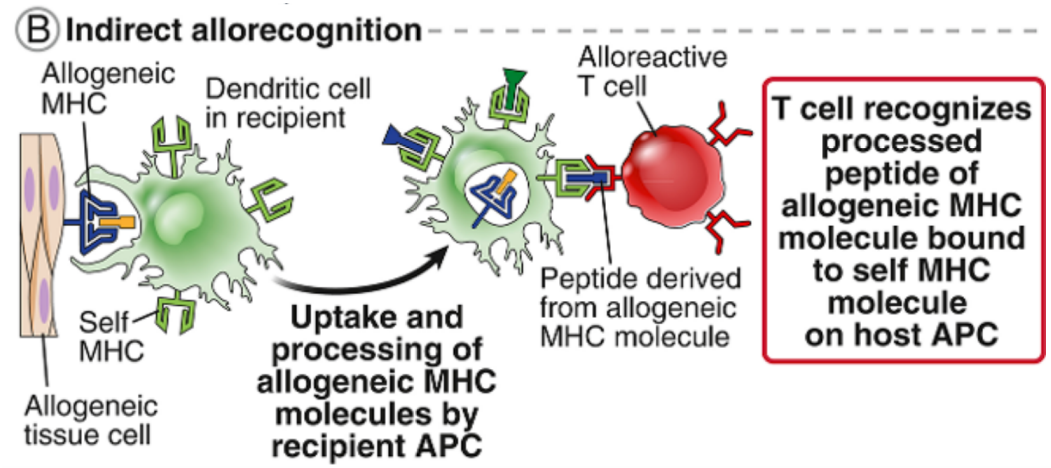


Different populations of cells may serve as APC expressing MHC II: DCs, Langerhans cells (skin), endothelial cells

Sensitization (II) - *Indirect allorecognition*

2) Indirect allorecognition

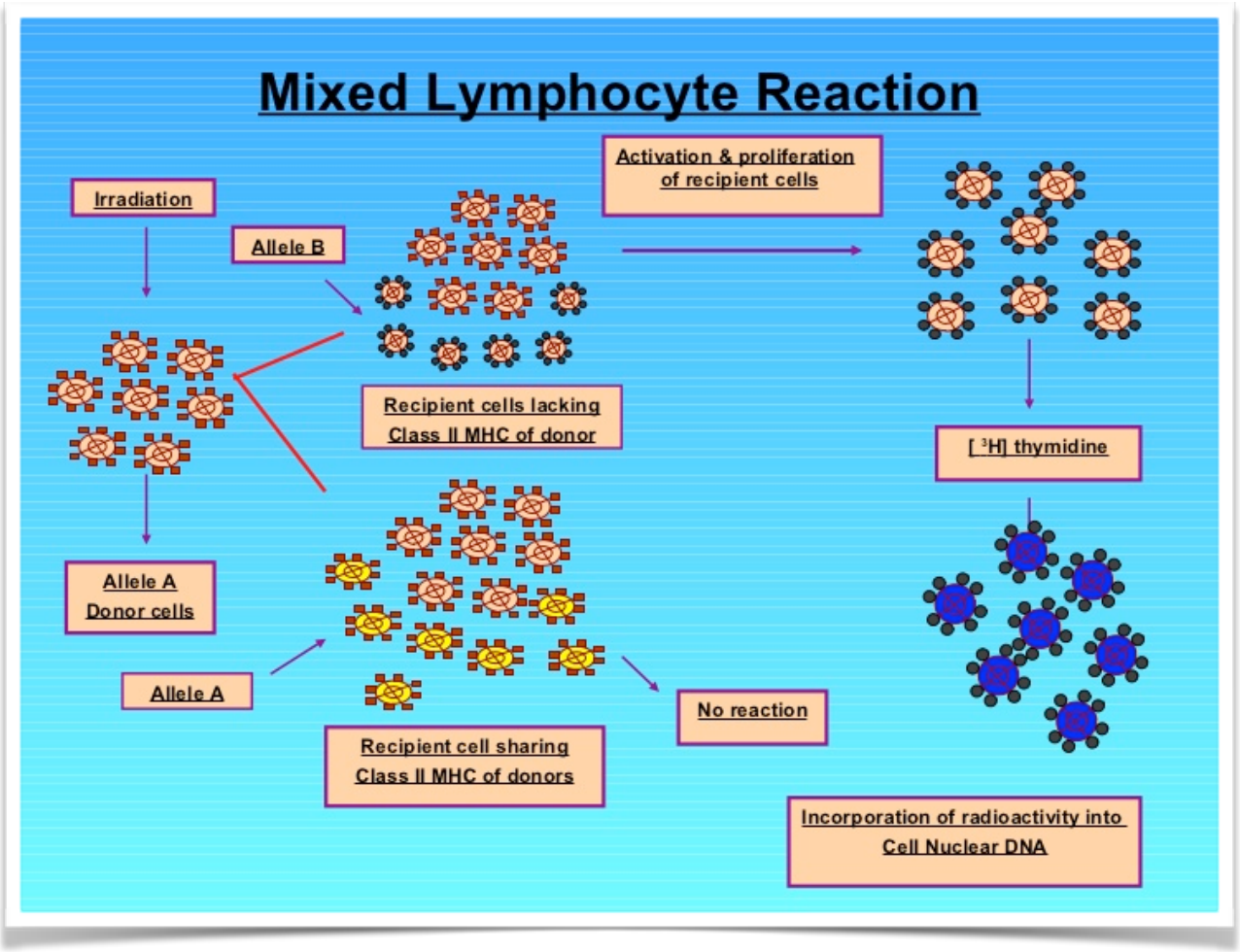
T cells in the recipient recognize donor allogeneic antigens on donor antigen-presenting cells



Cave: CTLs generated through this mechanism are specific for donor alloantigens displayed by recipient MHC molecules, so they cannot recognize and kill cells of the graft (which have alloantigens presented by donors MHC molecules)

Sensitization (III) - Activation of T cells

Recognition of the alloantigens expressed on the cells of the graft induces vigorous T cell proliferation in the host. This can be demonstrated in vitro through as **mixed lymphocyte reaction** (**MLR**):



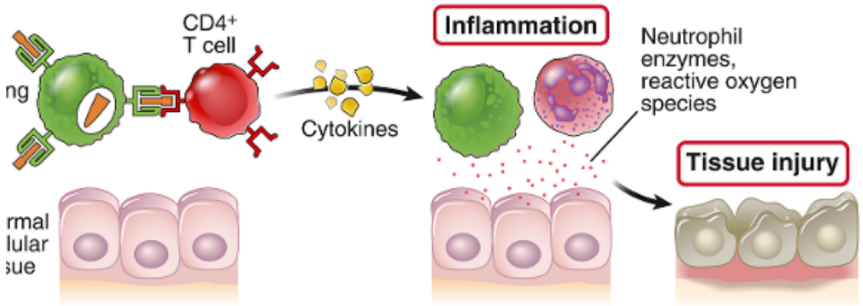
Mixed lymphocyte reaction (MLR)

Lymphocytes from the donor are irradiated to prevent cell division and then added to cells of the recipient.

a) MHC II antigens are same:
   no recognition, no reaction
b) MHC II antigens are different:
   - recognition, proliferation of recipient cells
   - This proliferation can be quantified by measuring proliferation through incorporation of radioactive nucleotides (3H thymidine). The amount of radioactive nucleotide uptake is roughly proportional to the MHC II class differences between the donor and recipient lymphocytes

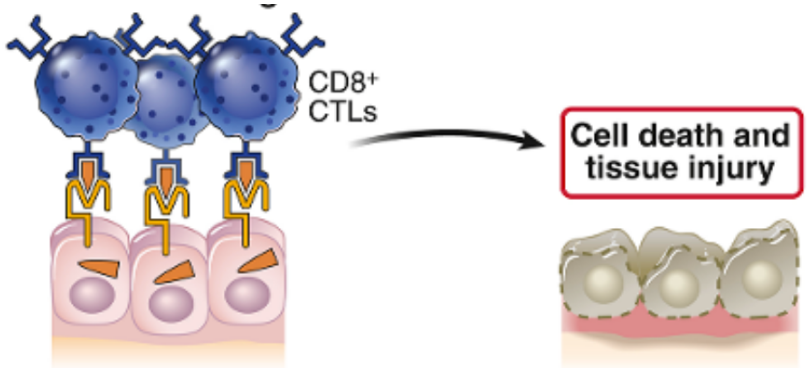
Effector stage (I) - Cell-mediated reactions

Cytokine-mediated inflammatory response



CD4 T cells may react to alloantigens and produce inflammatory cytokines that induce local inflammation and activation of macrophages and/ or neutrophils (*delayed-type hypersensitivity*)

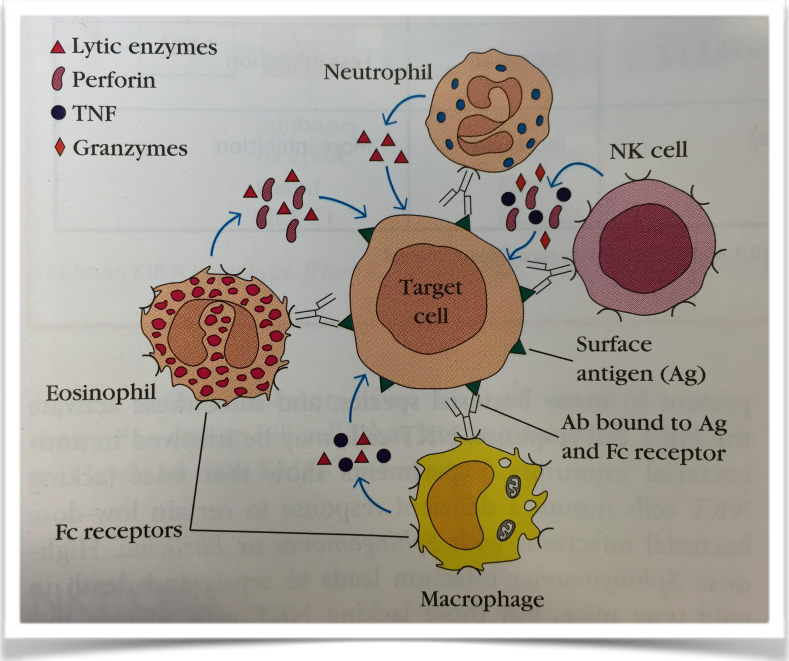
CTL-mediated killing of the graft



Effector stage (I) - Antibody-mediated reactions

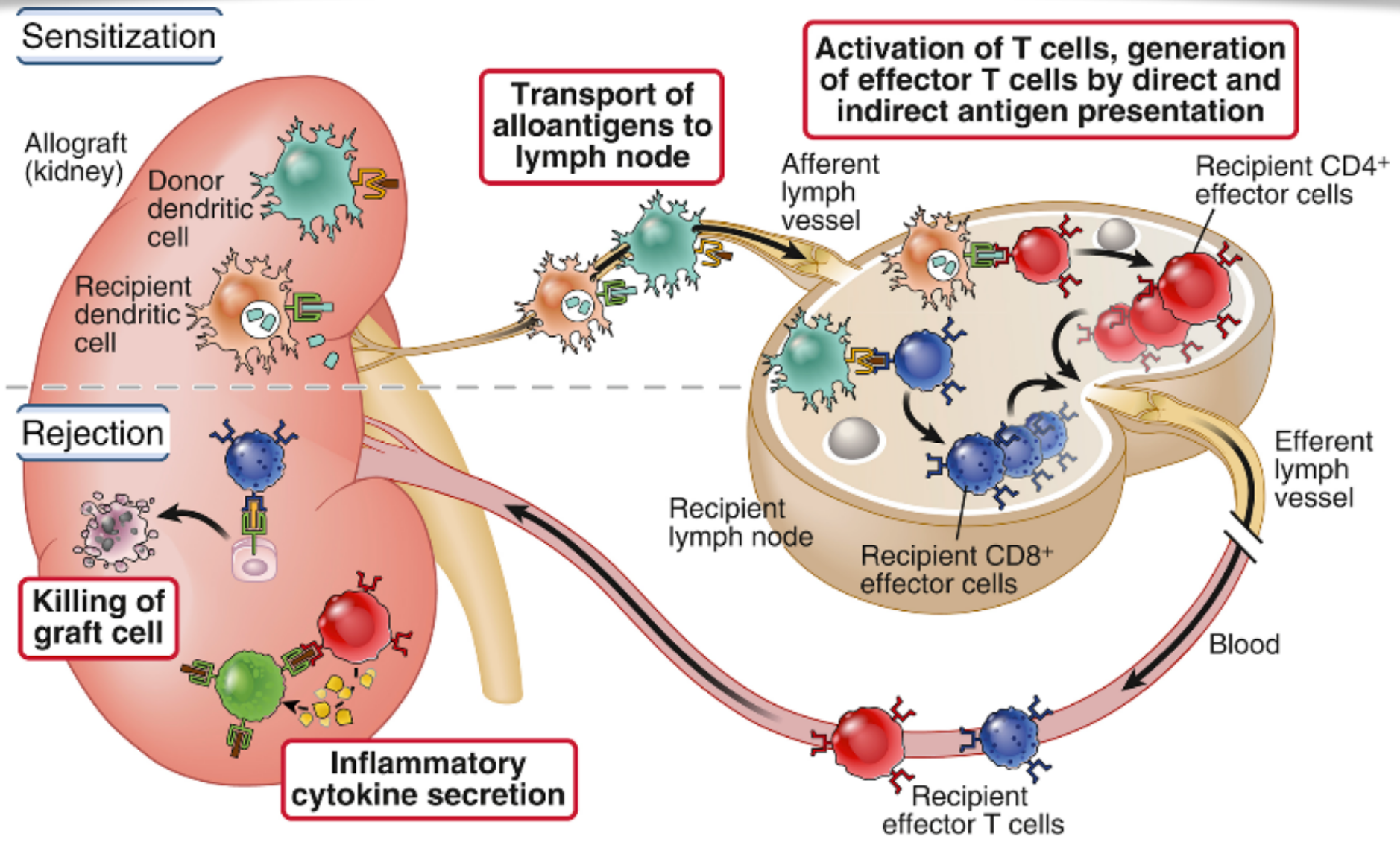
Antibody-dependent cell-mediated cytotoxicity (ADCC)

Some cell types with cytotoxic potential express receptors for the Fc region of the antibody molecule. When an antibody is specifically bound to the target cells, cells bearing the Fc receptor can bind and cause destruction of the target cell.



Antibody-plus-complement lysis (less common)

Induction of immune responses against transplants



Clinical manifestation of graft rejections

Based on clinical and pathological features graft rejection can be classified into the following rejections:

Hyperacute rejection

Begin: first 24 h

Actue rejection

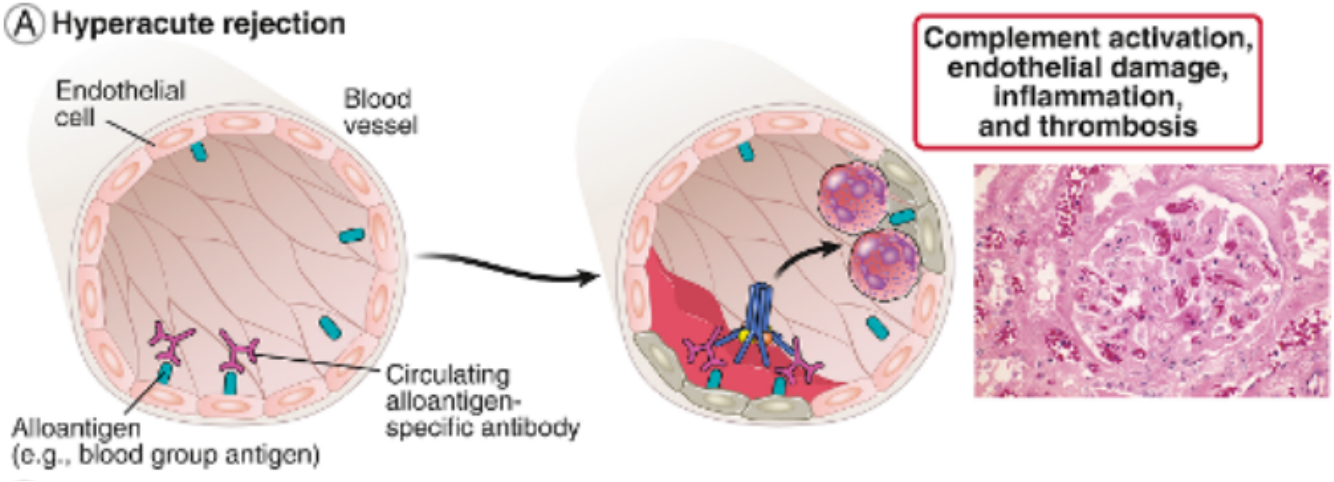
Begin: ~ first week

Chronic rejection

Begin: ~ months to years after transplantation

Hyperacute rejection (I)

Binding of preexisting antibodies to antigens of the graft lead to complement activation, infiltration of neutrophils, inflammatory reaction, endothelial wall destruction, thrombus formation and ischemia.



Transplanted tissue undergoes necrosis due to ischemia

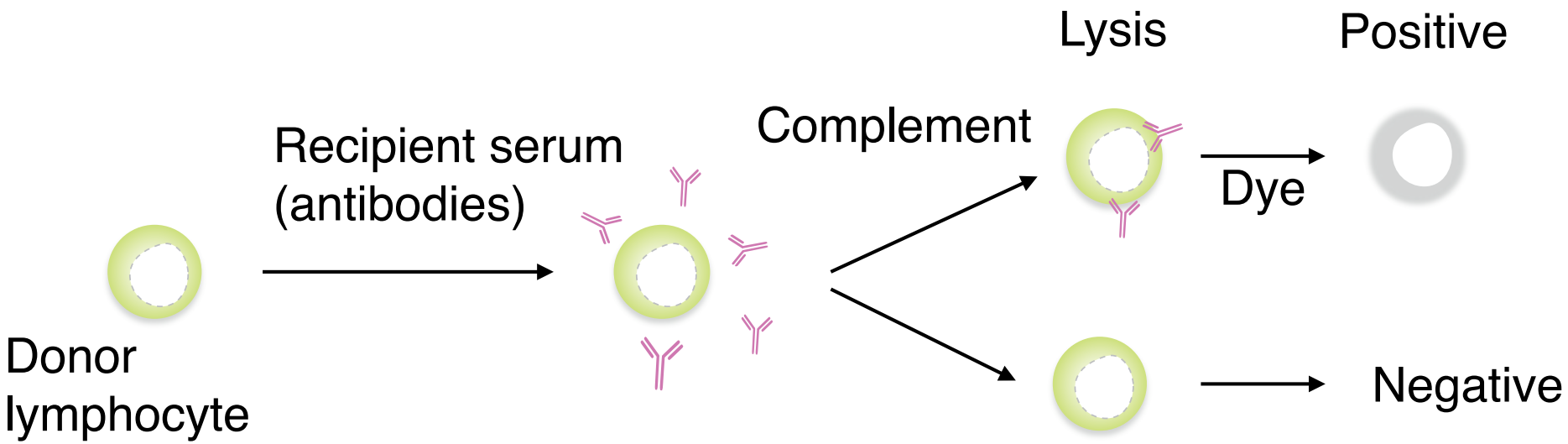
Hyperacute rejection (II)

Mechanisms accounting for presence of specific antibodies:

- Natural antibodies (IgM) specific for blood group antigens, antigens from distinct species (see below)
- Recipients of blood transfusion may develop antibodies against alloantigens expressed on leukocytes present within the transfusion
- After pregnancy, women may develop antibodies against paternal antigens (Rhesus antigen)
- Prior transplantation

Hyperacute reactions are not a common problem in allografts because of rigorous testing for blood-type and presence of antibodies (see below) but are a problem in xenotransplantation. These natural antibodies are thought to result from cross-reactivity of antibodies generated against bacteria. Furthermore, cells from distinct species lack expression of inhibitory molecules.

Hyperacute rejection (III) - cross-match

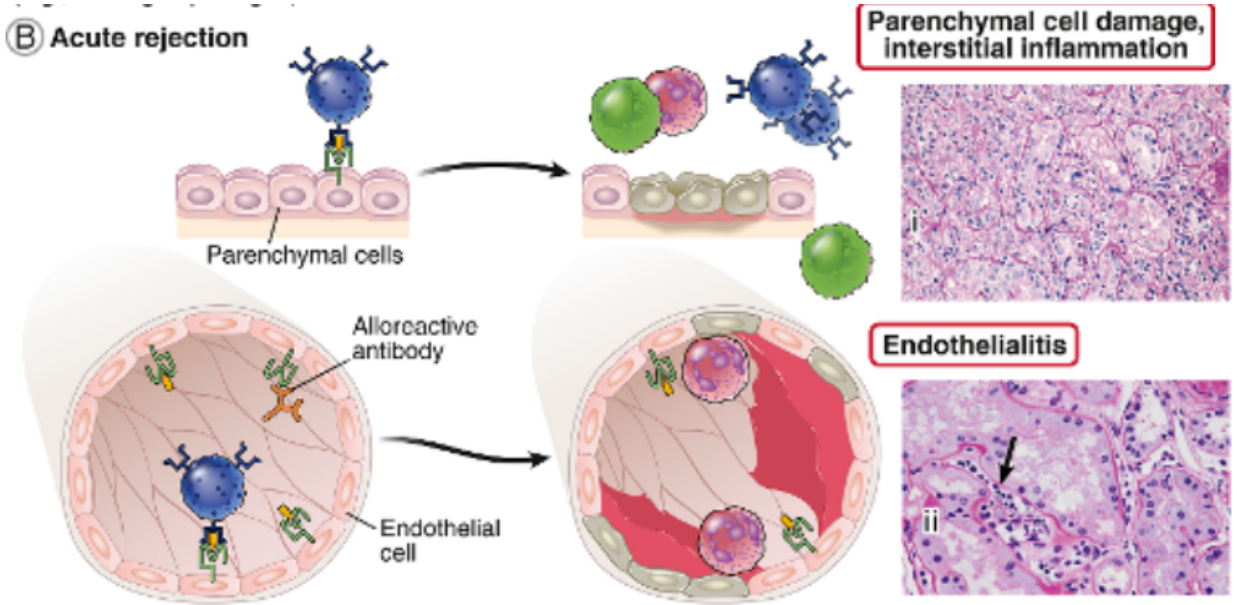


Positive crossmatch:
Recipient`s antibody attack donor`s cell ⇒ not suitable for transplant

Negative crossmatch:
Recipient`s antibody do not attack donor`s cell ⇒ suitable for transplant

Acute rejection

- Acute rejection occurs within days or weeks after transplantation
- Acute rejections are mediated by T cells and antibodies:
  - CD4 and CD8 T cells destroy allograft directly of via cytokines
  - Antibodies attack endothelial cells in the vessels and damage these involving complement

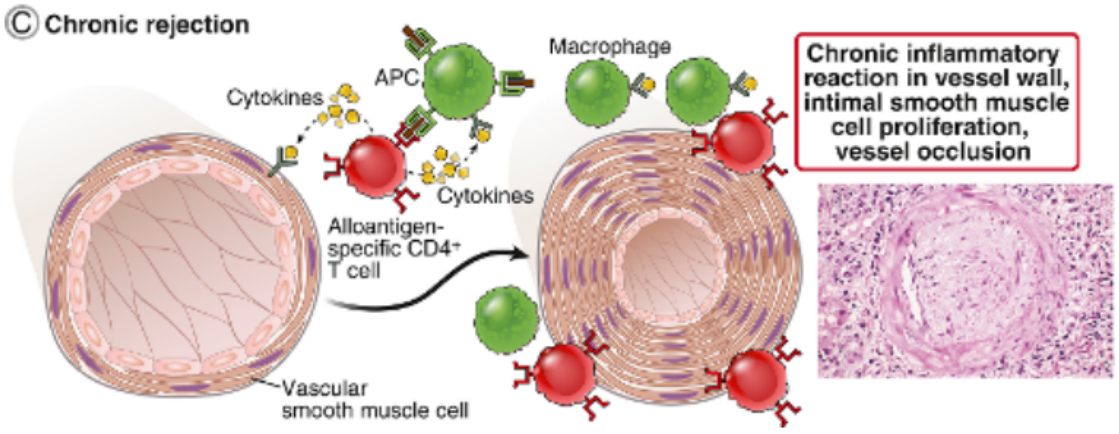


Chronic rejection (I)

- Acute rejection occurs within months or years after transplantation
- Acute rejections are mediated by T cells and antibodies
- Mechanism:

  Cytokines stimulate the proliferation of fibroblasts and vascular smooth muscle cells ⇒ tissue fibrosis and narrowing of blood vessels

  ⇒progressive loss of graft function



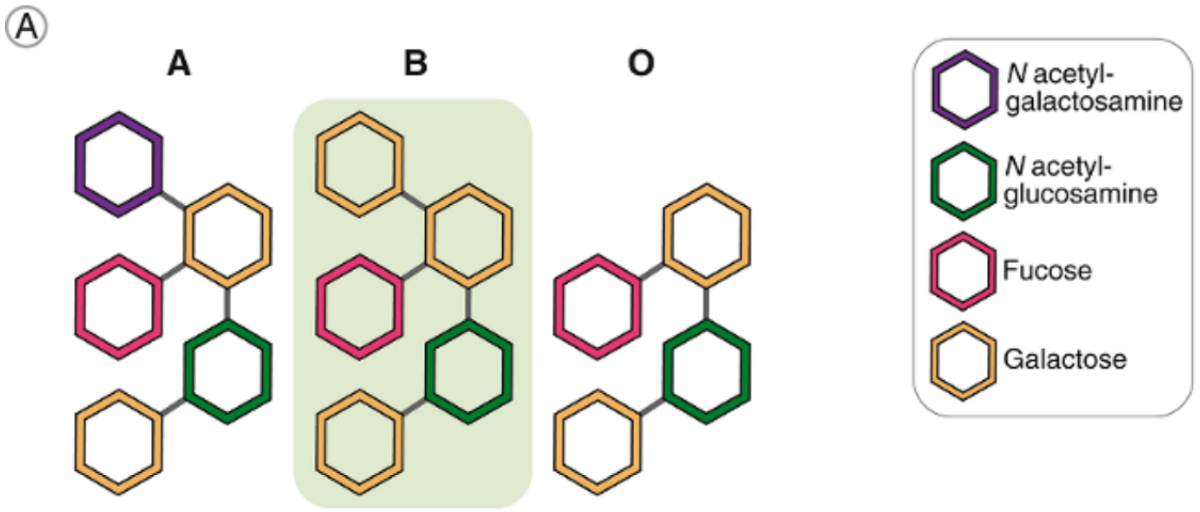
Transfusion reaction

- Transfusion refers to the „transplantation“ of red blood cells
- A major barrier to transfusion is the existence of antigens, which are present of red blood cells

  Special are the AB0 blood group antigens:

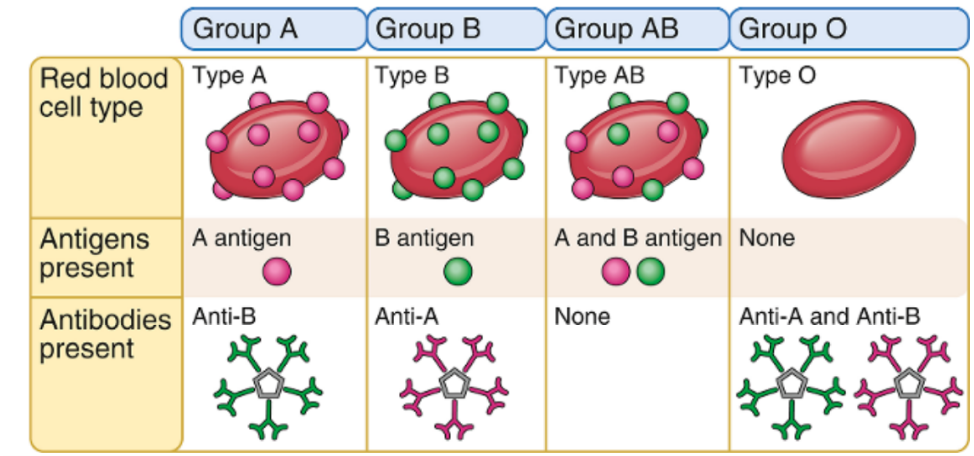
  Carbohydrates present on membrane glycoproteins or glycolipids

  Core glycan may have distinct terminal sugars



Transfusion reaction

- Antibodies against AB0 antigens, called isohemagglutinins are usually of the IgM class
- Isohemagglutinins exist because of similar antigenic determinants on a variety of microorganisms present in the gut



Blood type	Receive blood?	Donate blood?
OO	O only	Any type
AB	Any type	AB only
AA, AO	AA, AO, OO	AA, AO, AB
BB, BO	BB, BO, OO	BB, BO, AB

Transfusion reaction

Mismatch in blood group antigens can lead to **transfusion reactions**:

1) <u>AB0 incompatibility:</u>

- intravascular, complement-mediated lysis of donor red blood cells by IgM isohemagglutinins
- Within hours free hemoglobin is found in plasma, can cause among others severe kidney damage

<u>2) Incompatibility of other blood-group antigens:</u>

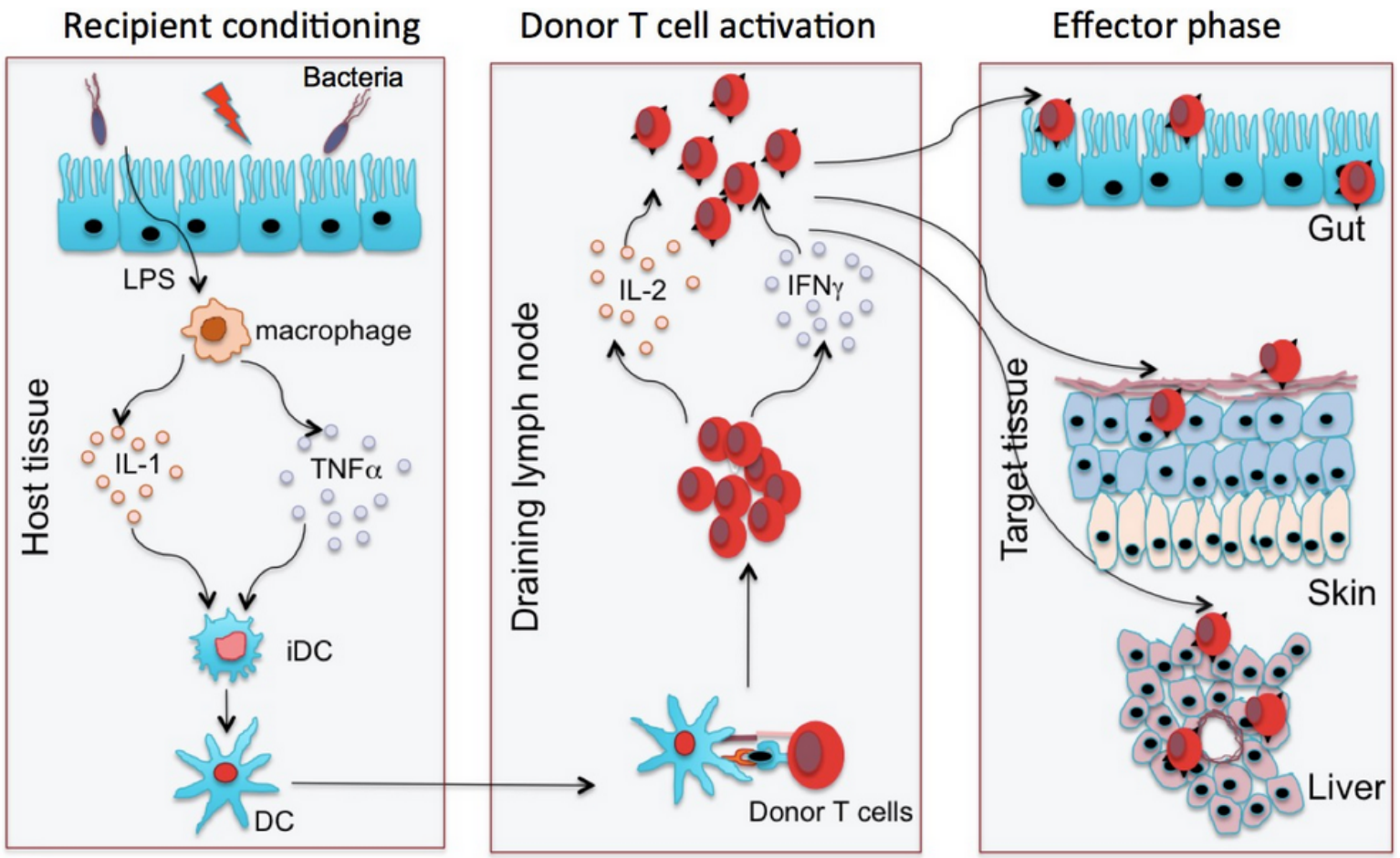
- Delayed reaction (2-6 days) following transfusion
- Repeated transfusion of AB0 compatible blood resulting in IgG against blood-group antigens
- Red blood cells are marked with IgG and are cleared by extravascular phagocytes

Hematopoietic stem cell transplantation

- Enriched hematopoietic stem cells (HSCs) enriched from peripheral blood, bone marrow can be transplanted.
- Indications:
  - Treatment of tumors arising from bone marrow precursor cells (e.g., leukemias, lymphomas)
  - To replace genetically defective stem cells in the context of severe immunodeficiencies

One of the major complications of allogeneic HSC transplantation is **graft-versus-host disease**

Graft-versus-host disease



Therapeutic aspects of GVHD

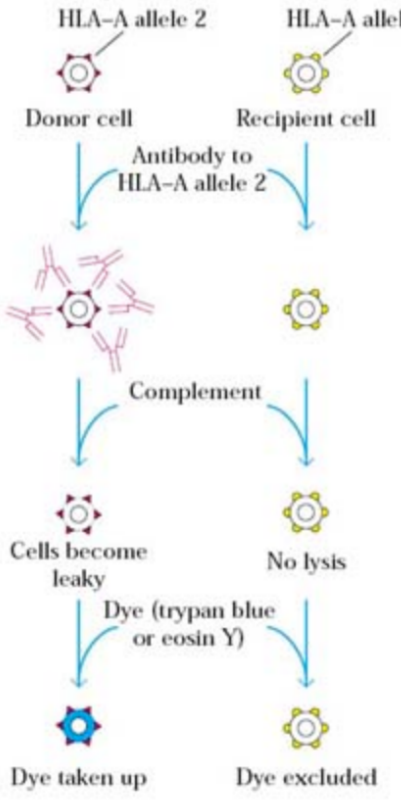
- To prevent GVHD most transplants are undertaken only when donor and recipients are MHC-matched (MLR)
- But, even MHC-matching does not abrogate GVDH, thus immunosuppressive therapy is still essential
- A beneficial aspect of donor T cell-mediated killing of host cells can be the killing of residual tumor cells (**graft-versus-tumor/leukemia effect**)

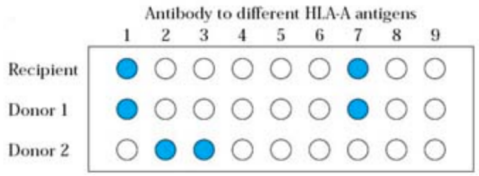
Tissue-typing to prevent graft rejection

- **Screening for AB0 blood-group compatibility**

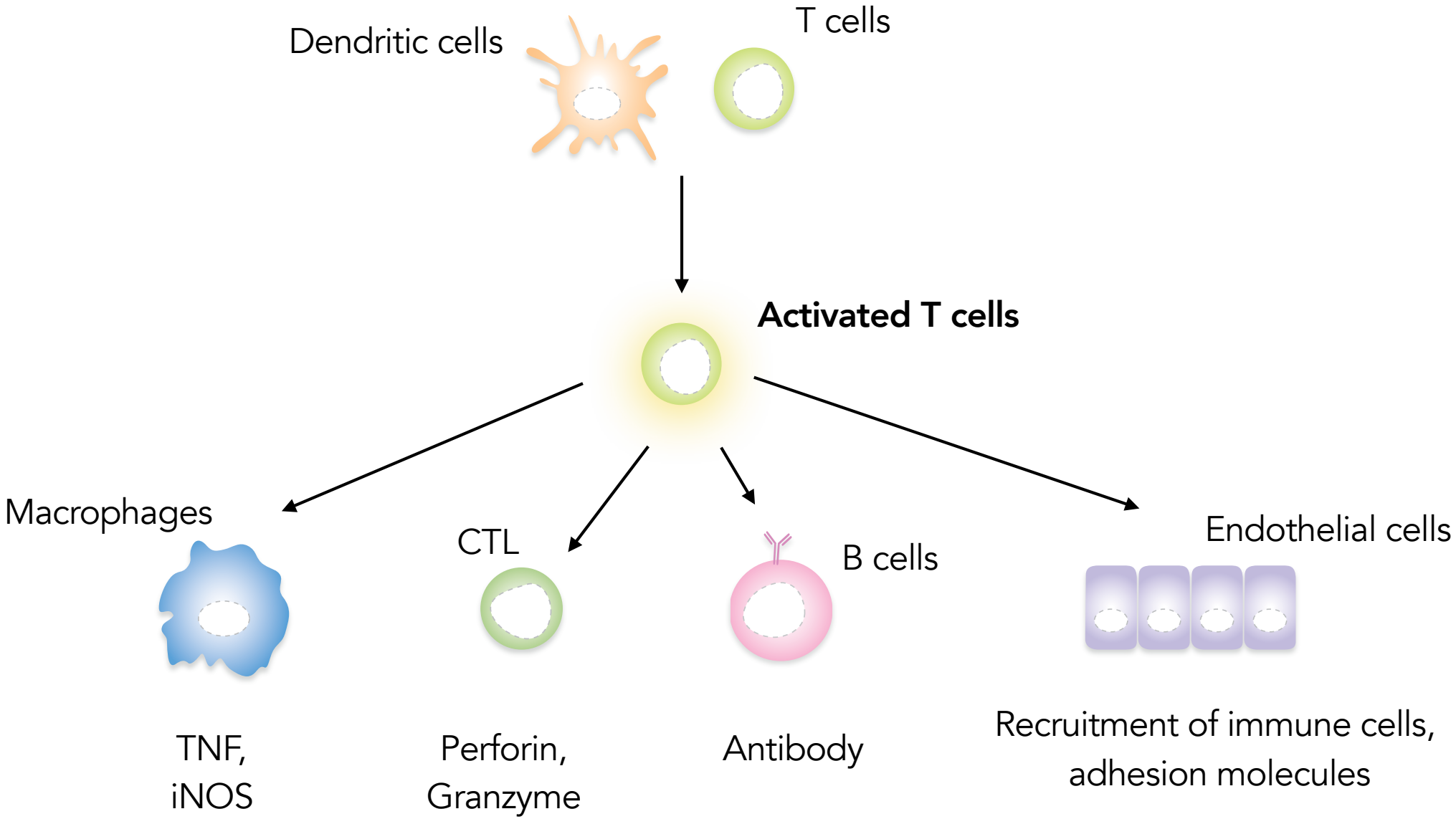
- **HLA-typing: Microcytotoxicity test**

  Check presence of absence of certain HLA antigens on donor and recipient cells





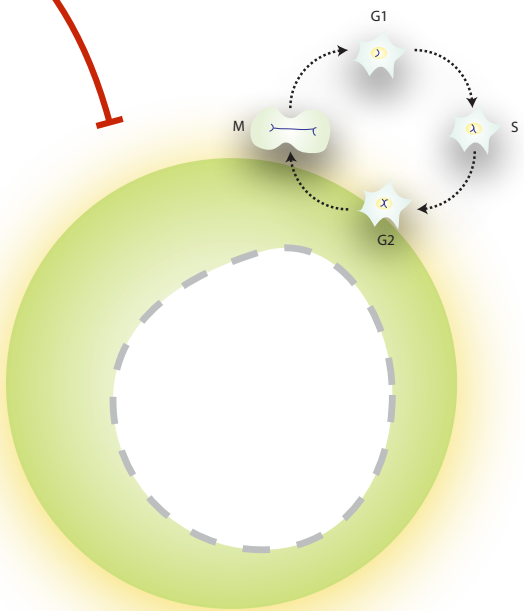
Activated T cells are central in graft rejection



Blockade of T cell activation (I)

Corticosteroids

- Derivatives of glucocorticoid hormones
- Activation of an intracellular receptor results in modulation of gene expression
- Response to corticoids is complex (>20% of leukocyte genes are affected)
- Interferes broadly with T cell activation (proliferation) and inflammatory gene expression (blockade of NF-kB)



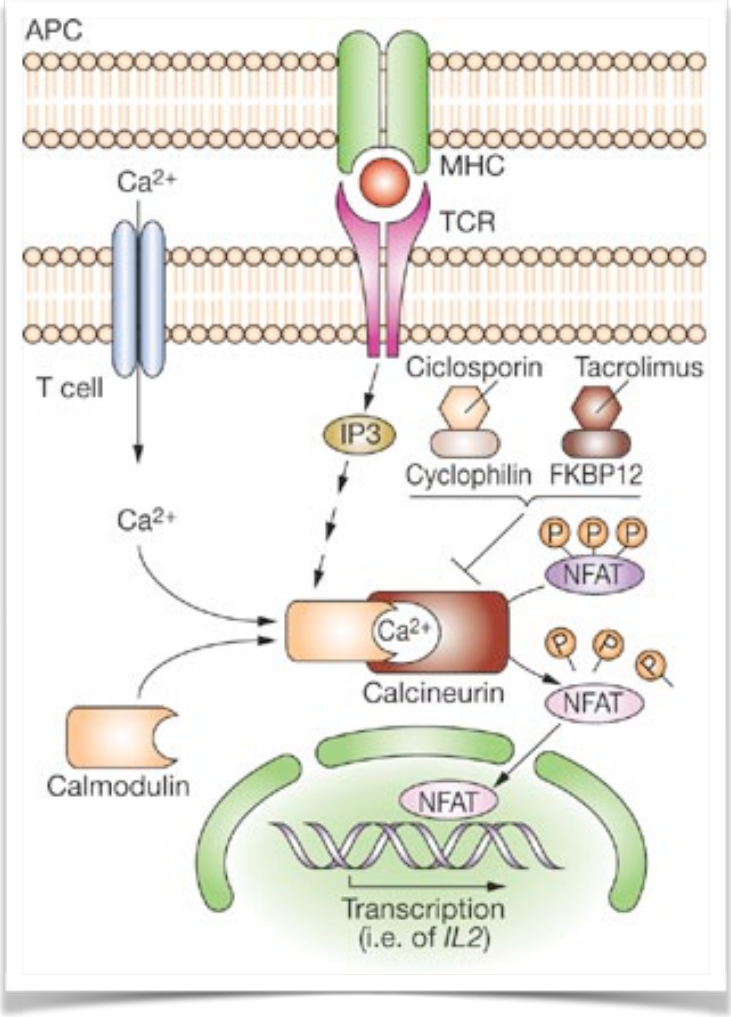
Antimetabolites

- Cyclophosphamide, Azathioprine, Mycophenolate mofetil
- Block DNA synthesis and exert antiproliferative effects of rapidly dividing cell (e.g. T cells)
- Side effects on tissues including skin, bone marrow, gut (anemia, damage of the gut epithelium, hair loss, etc.)

Blockade of T cell signaling

Cyclosporin A, Tacrolimus (FK506)

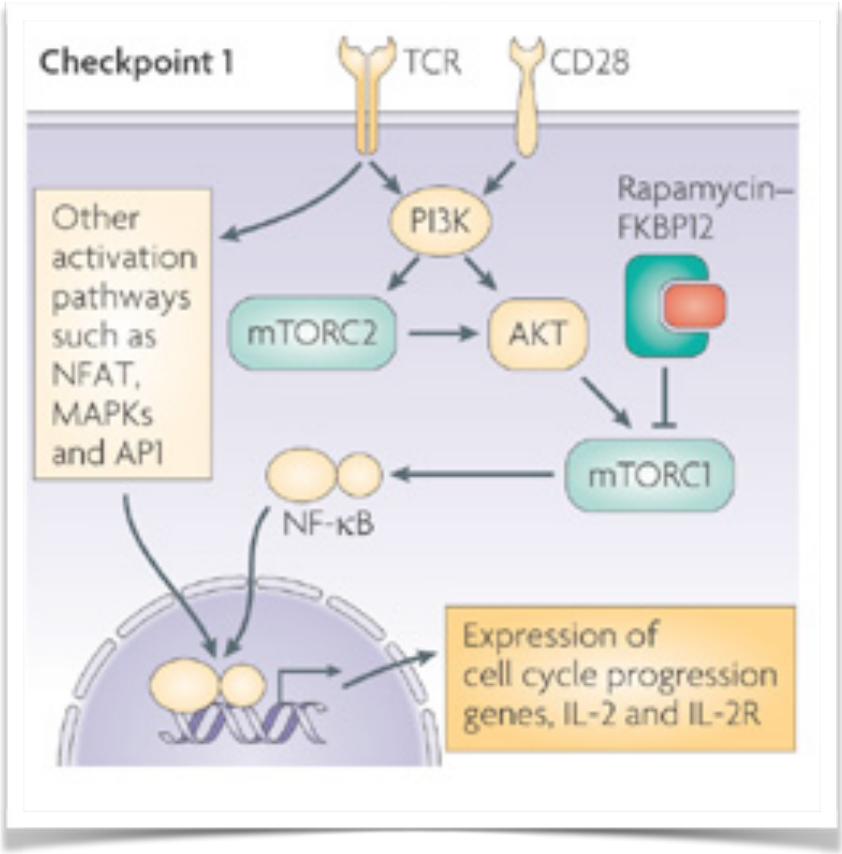
- Blockade of T cell proliferation by inhibiting the phosphatase calcineurin, thus leading to inhibition of NFAT activation
- Reduction of cytokine (IL-2) production
- Mechanism of action: exert their pharmacological activity by binding to immunophilins
- Affect all immune response indiscriminately
- Side effects may affect kidney function



Blockade of T cell signaling

Rapamycin

- Blockade of T cell proliferation by inhibiting the mTOR (*mammlian target of rapamycin*) activation, which is involved in regulating cell growth and proliferation
- Mechanism of action: exert its pharmacological activity by binding to immunophilins (FKBP12)
- Affect all immune response indiscriminately



Antibody-mediated targeting of T cells

Anti-lymphocyte globulin

Preparation of polyclonal immunoglobulins from rabbits immunized with human lymphocytes

In use to treat acute rejection

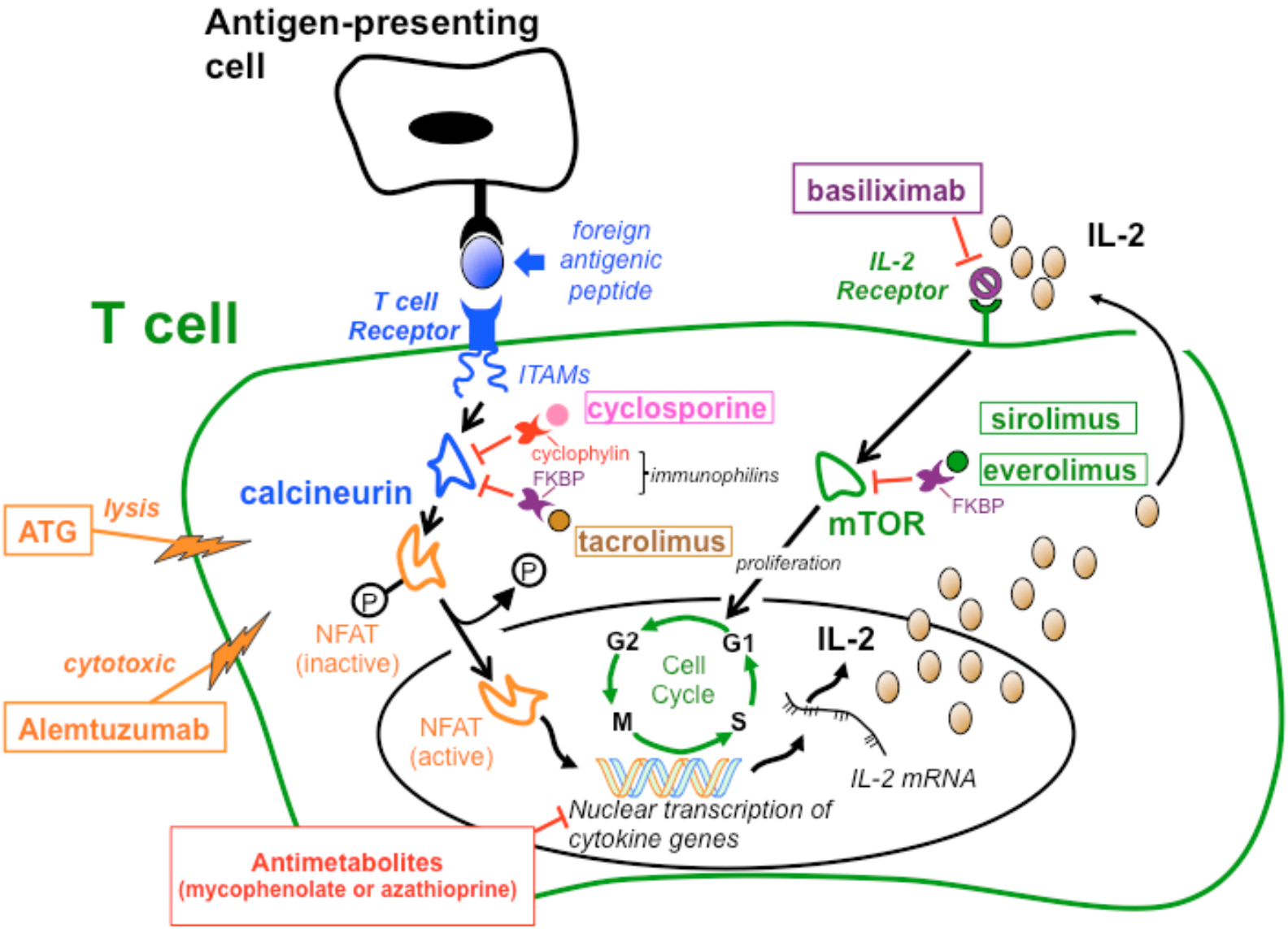
Anti-CD52 (Alemtuzumab)

Depleting antibody targeting CD52 expressed by most lymphocytes

Anti-IL-2 receptor (Basiliximab)

Non-depleting antibody targeting a subunit of the IL-2 receptor, CD25

Summary


Antigen-presenting cell
foreign antigenic peptide
T cell
T cell Receptor
ITAMs
basiliximab
IL-2
IL-2 Receptor
cyclosporine
cyclophylin
FKBP
immunophilins
calcineurin
sirolimus
everolimus
FKBP
mTOR
tacrolimus
ATG
lysis
proliferation
NFAT (inactive)
NFAT (active)
G2
G1
Cell Cycle
M
S
IL-2
IL-2 mRNA
cytotoxic
Alemtuzumab
Nuclear transcription of cytokine genes
Antimetabolites
(mycophenolate or azathioprine)

Exercise and questions

Clinical case (I)

C.M., a computer software salesman, was 48 years old when he came to his primary care physician because of fatigue and shortness of breath. He had not seen a doctor on a regular basis before this visit and felt well until 1 year ago, when he began experiencing difficulty climbing stairs or playing basketball with his children. Over the past 6 months he has had trouble breathing when he lies down in bed. He did not remember ever experiencing significant chest pain and had no family history of heart disease. He did recall that about 18 months ago he had to take 2 days off from work because of a severe flulike illness.

Clinical case (II)

On examination, he had a pulse of 105 beats per minute, a respiratory rate of 32 breaths per minute, a blood pressure of 100/60 mm Hg, and was afebrile. His physician heard rales (evidence of abnormal fluid accumulation) in the bases of both lungs. His feet and ankles were swollen. A chest x-ray showed pulmonary edema and pleural effusions and a significantly enlarged left ventricle. These findings were consistent with right and left ventricular congestive heart failure, which is a reduced capacity of the heart to pump normal volumes of blood, resulting in fluid accumulation in various tissues. C.M. was admitted to the cardiology service of the University Hospital. On the basis of further tests, including coronary angiography and echocardiography, C.M. was given the diagnosis of dilated cardiomyopathy (a progressive and fatal form of heart failure in which the heart chambers become dilated and inefficient at pumping blood). His physicians told him he would benefit from aggressive medical management, including drugs that enhance heart muscle contraction, reduce the workload of the heart, and enhance excretion of accumulated fluid, but if his underlying heart disease continued to progress, the best long-term option would be to receive a heart transplant. Unfortunately, despite optimal medical management, his symptoms of congestive heart failure continued to worsen until he was no longer able to manage even routine activities of daily living, and he was listed for heart transplantation.

Clinical case (III)

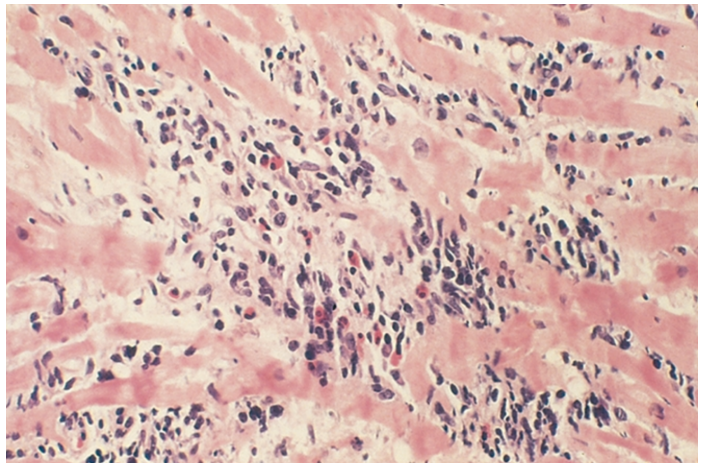
A panel-reactive antibody (PRA) test was performed on C.M.'s serum to determine whether he had been previously sensitized to alloantigens. This test showed the patient had no circulating antibodies against human leukocyte antigens (HLA), and no further immunologic testing was performed. Two weeks later in a nearby city, a donor heart was removed from a victim of a construction site accident. The donor had the same ABO blood group type as C.M. The transplant surgery, performed 4 hours after the donor heart was removed, went well, and the allograft was functioning properly postoperatively.

What problems might arise if the transplant recipient and the donor have different blood types or if the recipient has high levels of anti-HLA antibodies?

Clinical case (IV)

C.M. was placed on immunosuppressive therapy the day after transplantation, which included daily doses of tacrolimus (FK506), mycophenolic acid, and prednisone. Endomyocardial biopsy was performed 1 week after surgery and showed no evidence of myocardial injury or inflammatory cells. He was sent home 10 days after surgery, and within a month he was able to do light exercise without problems. A routinely scheduled endomyocardial biopsy performed within the first 3 months after transplantation was normal, but a biopsy performed 14 weeks after surgery showed the presence of numerous lymphocytes within the myocardium and a few apoptotic muscle fibers. The findings were interpreted as evidence of acute allograft rejection.

What was the patient's immune system responding to, and what were the effector mechanisms in the acute rejection episode?



Clinical case (V)

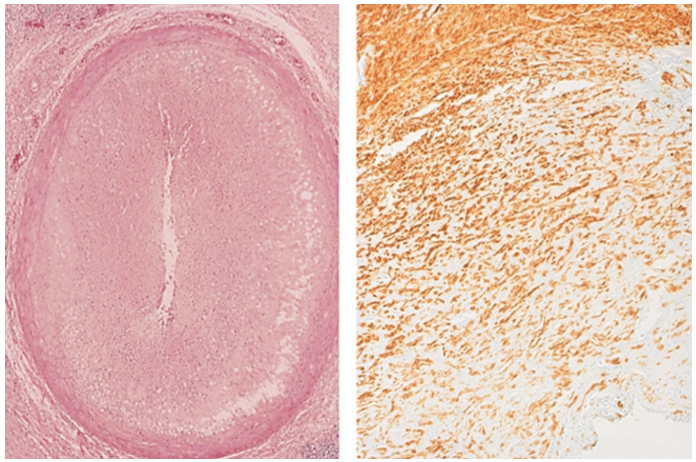
C.M.'s serum creatinine level, an indicator of renal function, was high (2.2 mg/dL; normal, <1.5 mg/dL). His physicians therefore did not want to increase his tacrolimus dose because this drug can be toxic to the kidneys. He was given three additional doses of a steroid drug over 18 hours, and a repeat endomyocardial biopsy 1 week later showed only a few scattered macrophages and a small focus of healing tissue. C.M. went home feeling well, and he was able to live a relatively normal life, taking tacrolimus, mycophenolic acid, and prednisone daily.

What is the goal of the immunosuppressive drug therapy?
Why are three distinct drugs used in combination?
What are the mechanisms of action of each drug?

Clinical case (VI)

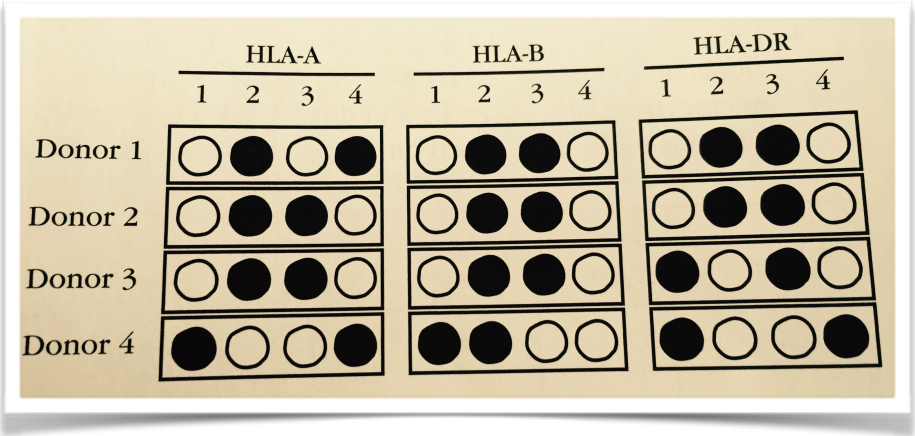
Coronary angiograms performed yearly since the transplant showed a gradual narrowing of the lumens of the coronary arteries. In the sixth year after transplantation, C.M. began experiencing shortness of breath after mild exercise and showed left ventricular dilation on radiographic examination. An intravascular ultrasound examination demonstrated significant thickening of the walls and narrowing of the lumen of the coronary arteries. An endomyocardial biopsy showed areas of microscopic subendocardial infarction, as well as evidence of sublethal ischemia (myocyte vacuolization), C.M. and his physicians are now considering the possibility of a second cardiac transplant.

What process has led to failure of the graft after 6 years?



Exercise 2

You are a surgeon in a major transplantation facility. One of your patients is awaiting a kidney transplant. Several cadaver organs have become available due to a serious accident. Your technician performs a microcytotoxicity test on the potential donors and gets the following results. Your patient was tested previously and was positive for the antibodies used in well 2 and 3 and negative for those used in well 1 and 4 for each HLA determinants.



1) Which donor would be your first choice?
2) If that organ were not usable, which one of the remaining organs would you consider using? Why?
3) What test can you technician perform to confirm that the potential donor is compatible with the recipient?

Exercise 3 (I)

A child who requires kidney transplant has been offered a kidney from both parents and from five siblings.

a) Cells from the potential donors are screened with monoclonal antibodies to the HLA-A, -B and -C antigens in a microcytotoxicity assay. In addition, AB0 blood-group testing is performed. Based on the results in the table below, a kidney graft from which donor(s) is most likely to survive?

	ABO type	HLA-A type	HLA-B type	HLA-C type
Recipient	O	*A1/A2*	*B8/B12*	*Cw3*
Mother	A	*A1/A2*	*B8/B12*	*Cw1/Cw3*
Father	O	*A2*	*B12/B15*	*Cw3*
Sibling A	O	*A1/A2*	*B8/B15*	*Cw3*
Sibling B	O	*A2*	*B12*	*Cw1/Cw3*
Sibling C	O	*A1/A2*	*B8/B12*	*Cw3*
Sibling D	A	*A1/A2*	*B8/B12*	*Cw3*
Sibling E	O	*A1/A2*	*B8/B15*	*Cw3*

Exercise 3 (II)

Now a one-way MLR is performed using various combinations of mitomycin-treated lymphocytes. The results expressed as counts per minute of [3H] thymidine incorporated, are shown in the table below. The stimulation index is indicated also. Based on these results, a graft from which donor(s) is most likely to be accepted?

Respondent cells	Mytomycin C–treated stimulator cells					
	Patient	Sibling A	Sibling B	Sibling C	Sibling D	Sibling E
Patient	1,672 (1.0)	1,800 (1.1)	13,479 (8.1)	5,210 (3.1)	13,927 (8.3)	13,808 (8.3)
Sibling A	1,495 (1.6)	933 (1.0)	11,606 (12.4)	8,443 (9.1)	11,708 (12.6)	13,430 (14.4)
Sibling B	25,418 (9.9)	26,209 (10.2)	2,570 (1.0)	13,170 (5.1)	19,722 (7.7)	4,510 (1.8)
Sibling C	10,722 (6.2)	10,714 (5.9)	13,032 (7.5)	1,731 (1.0)	1,740 (1.0)	14,365 (8.3)
Sibling D	15,988 (5.1)	13,492 (4.2)	18,519 (5.9)	3,300 (1.1)	3,151 (1.0)	18,334 (5.9)
Sibling E	5,777 (6.5)	8,053 (9.1)	2,024 (2.3)	6,895 (7.8)	10,720 (12.1)	888 (1.0)

Exercise 4

Immediately after transplantation, a patient is often given strong doses of anti-rejection drugs and then allowed to taper off as time passes. Describe the effects of the commonly used drugs azathioprine, cyclosporine A, tacrolimus and rapamycin! Why is it possible to decrease the use of some of these drugs at some point after transplantation?

Exercise 5

Why do blood group antigens not lead to T cell responses?

Exercise 6

Indicate whether each of the following statements is true or false. If you think a statement is false explain why.

a) Acute rejection is mediated by preexisting host antibodies specific for antigens on the grafted tissue.
b) Second-set rejection is a manifestation of immunological memory.
c) All allografts between individuals with identical HLA haplotypes will be accepted.
d) Cytokines produced by host T cells activated in response to alloantigens play a major role in graft rejection.