



IMMUNOLÓGIAI ÉS  
BIOTECHNOLÓGIAI  
INTÉZET



# 13th practice: Immunological aspects of organ transplantation.

## HLA typing

### Basic Immunology

University of Pécs, Clinical Center

Department of Immunology and Biotechnology

Pécs, 2026.

# Basic terms

- **autolog, allogeneic, xenogeneic graft**
- **auto-, allo-, xeno-transplantation**

**Cornea**

From cadaver  
 Immunosuppression not required  
 40,000 transplants per year

**Skin**

Mostly autologous (burn victims)  
 Temporary grafts of nonviable tissue  
 Allogeneic grafts rare, require immunosuppression

**Lung**

From brain-dead donor  
 Procedure recently developed;  
 little data available  
 845 transplants in 1998  
 Often heart/lung transplant (45 in 1998)

**Blood**

Transfused from living donor  
 ABO and Rh matching required  
 Complications extremely rare  
 An estimated 14 million units used each year

**Heart**

From brain-dead donor  
 HLA matching useful but often impossible  
 Risk of coronary artery damage, perhaps mediated by host antibody  
 2,340 transplants in 1998

**Pancreas**

From cadaver  
 Islet cells from organ sufficient  
 253 transplants in 1998  
 Increasingly, pancreas/kidney transplant for advanced diabetes (965 in 1998)

**Liver**

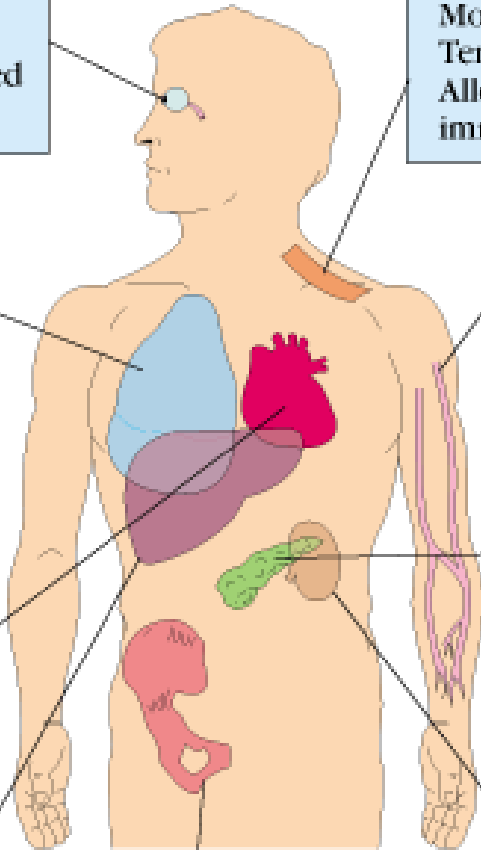
From cadaver  
 Surgical implantation complex  
 Resistant to hyperacute rejection  
 Risk of GVHD  
 4,450 transplants in 1998

**Kidney**

From live donor or cadaver  
 ABO and HLA matching useful  
 Immunosuppression usually required  
 Risk of GVHD very low  
 11,900 transplants in 1998

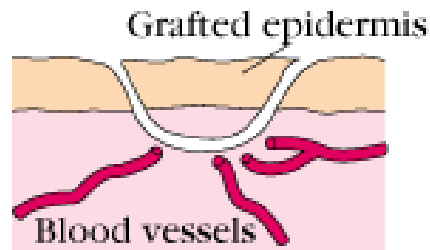
**Bone marrow**

Needle aspiration from living donor  
 Implanted by IV injection  
 ABO and HLA matching required  
 Rejection rare but GVHD a risk

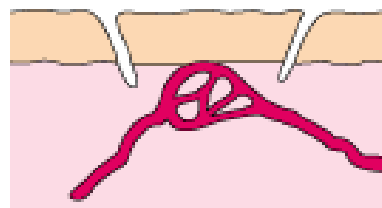


# Graft acceptance and rejection

(a) Autograft acceptance



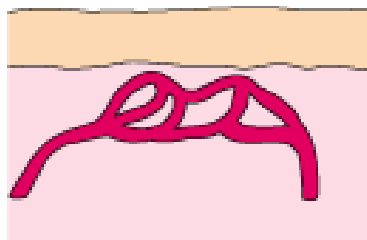
Days 3-7: Revascularization



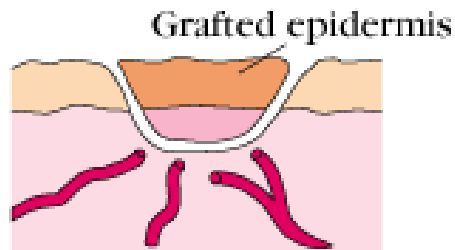
Days 7-10: Healing



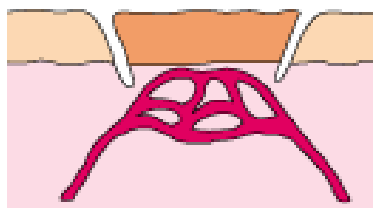
Days 12-14: Resolution



(b) First-set rejection



Days 3-7: Revascularization



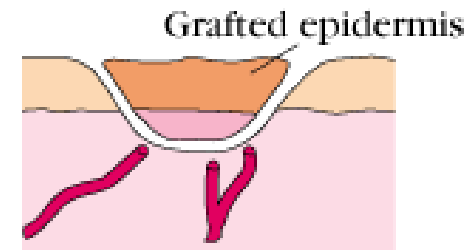
Days 7-10: Cellular infiltration



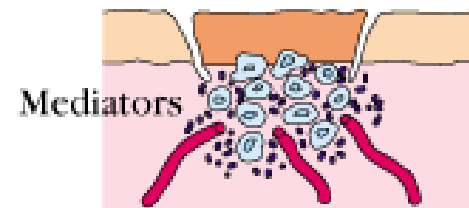
Days 10-14: Thrombosis and necrosis



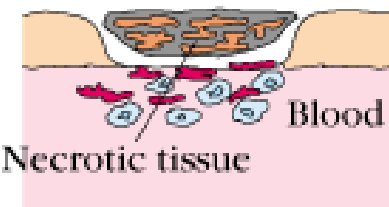
(c) Second-set rejection



Days 3-4: Cellular infiltration



Days 5-6: Thrombosis and necrosis



Necrotic tissue

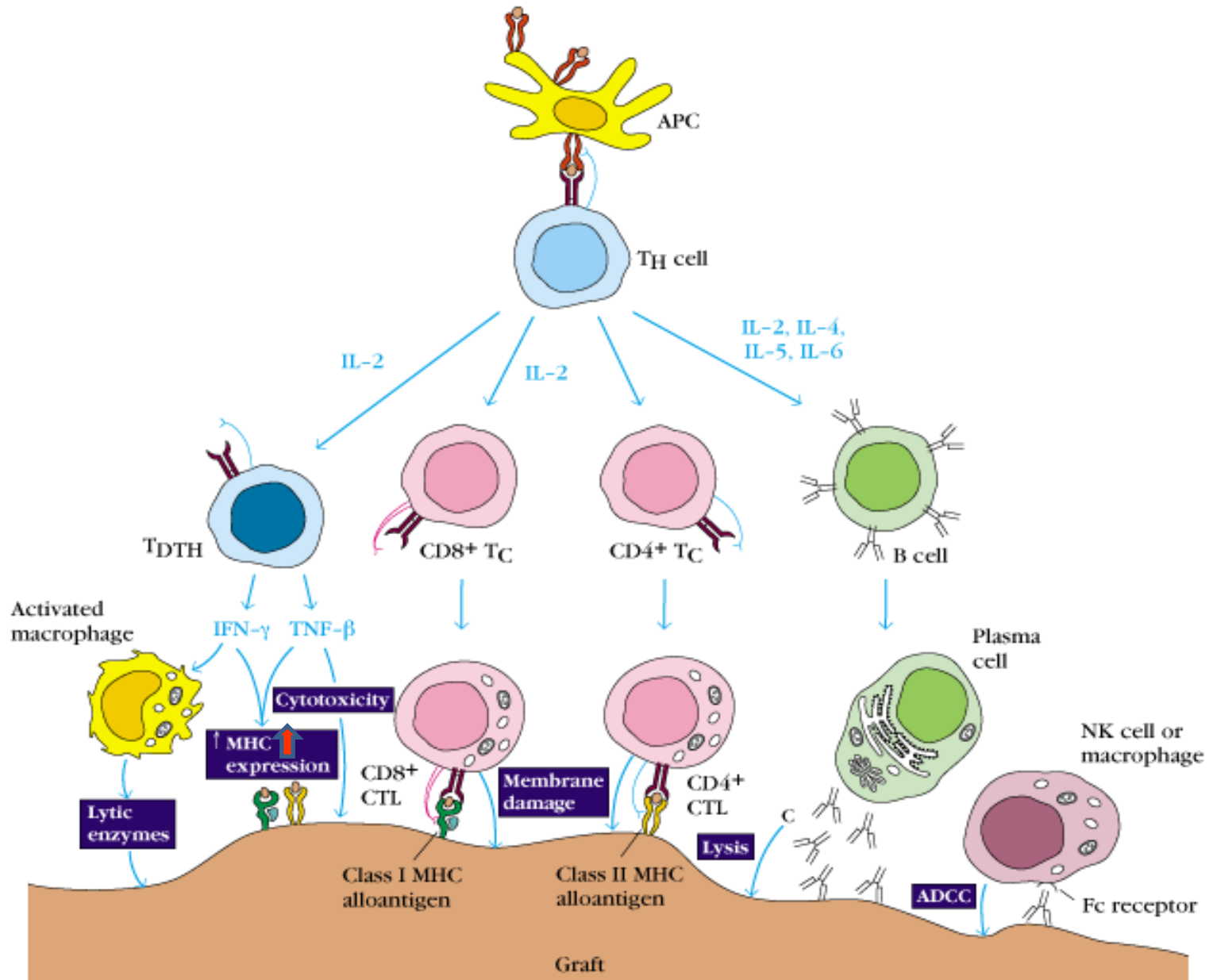
Blood clots

Damaged blood vessels

# Host versus graft reaction

- hyperacute rejection caused by pre-existing antibodies
- acute rejection managed by T cells, ADCC and DTH
- chronic rejection induced by permanent endothelial injuries and complement activation

# Mechanisms of host versus graft reactions



# Graft versus host reaction – bone marrow or hemopoietic stem cell transplantation

- acute GVHD (acute tissue necrosis of the targeted organs)



- chronic GVHD (autoimmune-like phenomenon)

# Bone marrow transplantation

<b>Advantage</b>	<b>Disadvantage</b>
<b>Autologous</b>	<b>Allogeneic</b>
no GVH no rejection no matching needed	GVH rejection need matching tumour in donor cells
<b>Allogeneic</b>	<b>Autologous</b>
no tumour transfer graft vs. tumour myelosuppression avoided	grafting tumour cells (myelosuppression possible)

# Discovery of HLA system

- **George Snell** performed experiments on mice.  
Vaccinated different individuals with mouse tumours.  
The rejection or engraftment was determined by the genetical difference or identity of the cells.  
Genetic system: MHC (Major Histocompatibility Complex)

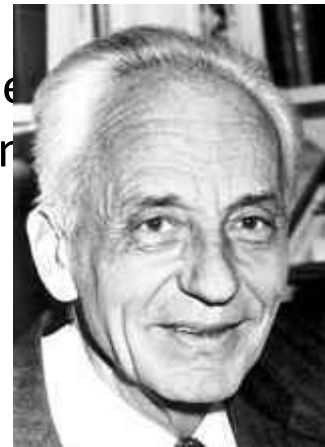
- **Jean Dausset** the same system in humans (1950s):  
HLA system: Human Leukocyte Antigen

- **Baruj Benacerraf**: the structure of the genes determining histoincompatibility between mice and man.

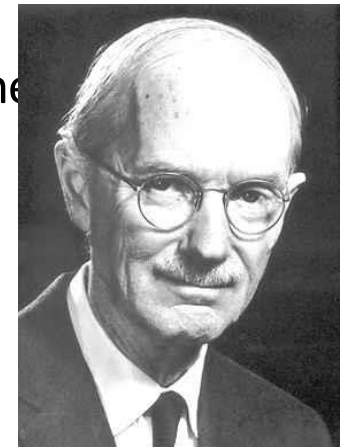
- **Nobel prize (1980)**



Baruj Benacerraf  
(1920 - 2011)



Jean Dausset  
(1916 - 2009)



George D. Snell  
(1903 - 1996)

# Clinical significance of HLA typing

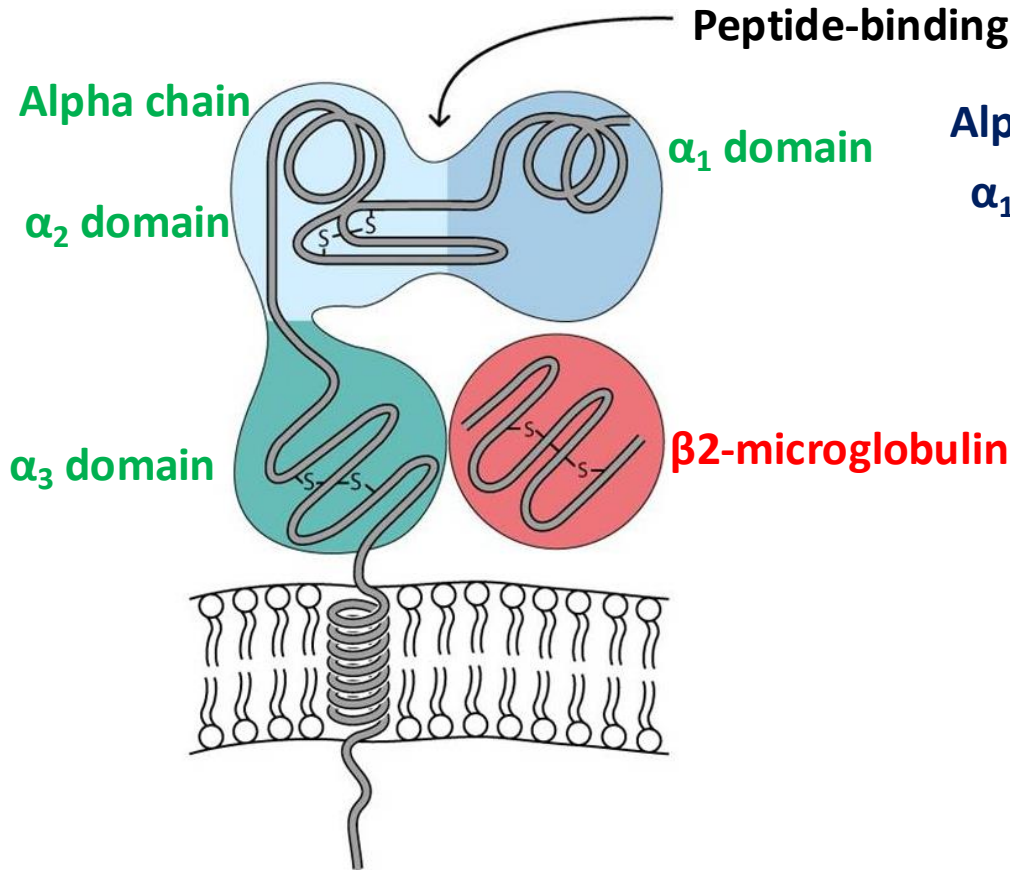
- Checking the immunological compatibility of the donor and the recipient before **transplantations** to prevent rejections.
- Further diagnostic confirmation of some **autoimmune disorders** as certain HLA types are more frequent in autoimmune conditions than others, e.g.:
  - **HLA-B27**: Bechterew's disease, Inflammatory bowel diseases (IBD), Psoriasis
  - HLA-DR1: Rheumatoid arthritis, Ulcerative colitis
  - HLA-DR3: Type I diabetes mellitus, Myasthenia gravis, Hashimoto's thyroiditis
  - HLA-DR4: Rheumatoid arthritis, SLE
  - **HLA-DQ2**: Celiac disease, Type I diabetes mellitus
  - **HLA-DQ8**: Celiac disease, Type I diabetes mellitus

# Basics of HLA typing I.

HLA (Human leukocyte antigen)

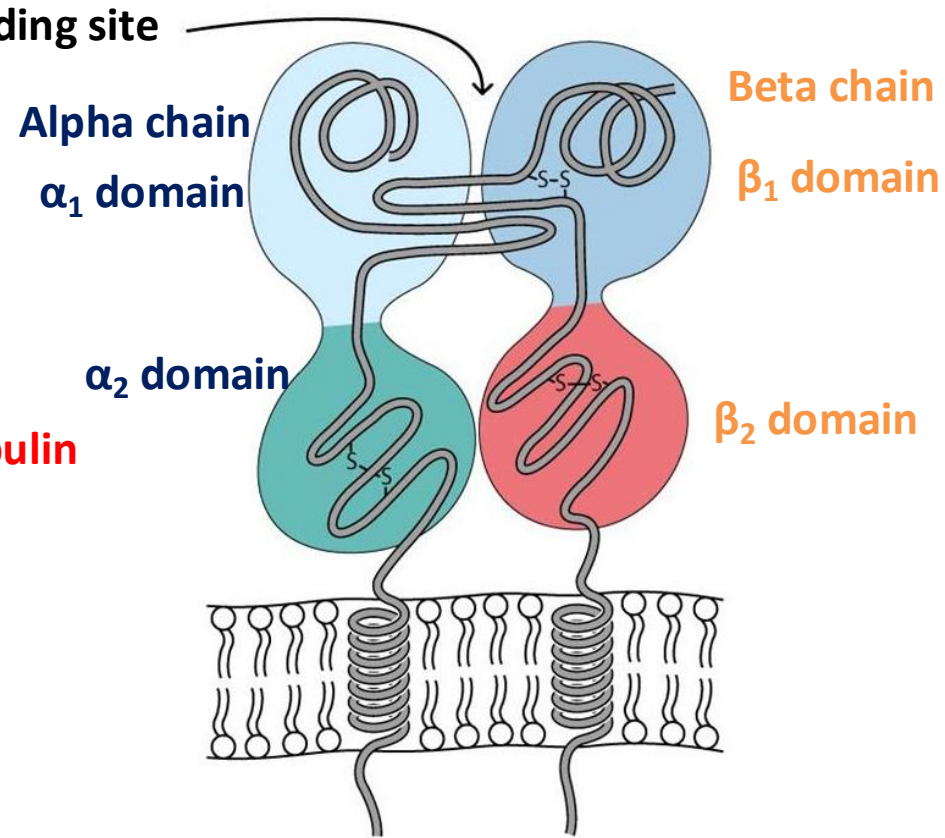
MHC (Major histocompatibility complex)

MHC I



On all nucleated cells and thrombocytes!

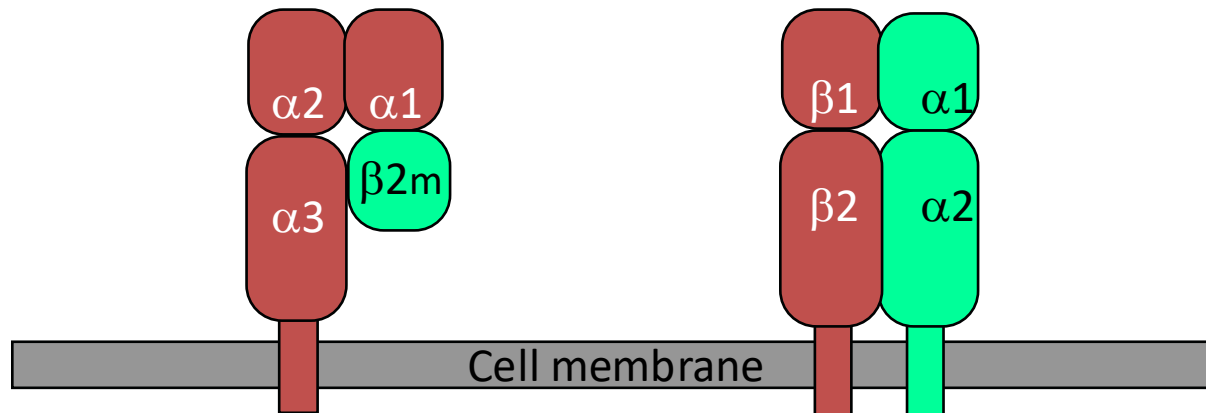
MHC II



On antigen presenting cells!  
(e.g. macrophage, dendritic cell, B cell)

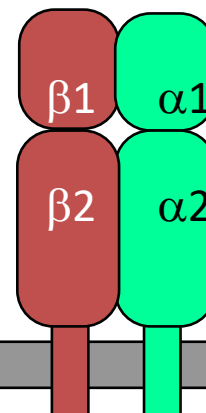
# HLA class I.

- HLA-A, B, C genes
- Present in all nucleated cells and platelets.
- Different level of expression:
  - high on the cells of the immune system;
  - low eg. on nerve cells, on myocytes
- Consists of alpha chain and  $\beta 2$  microglobulin.



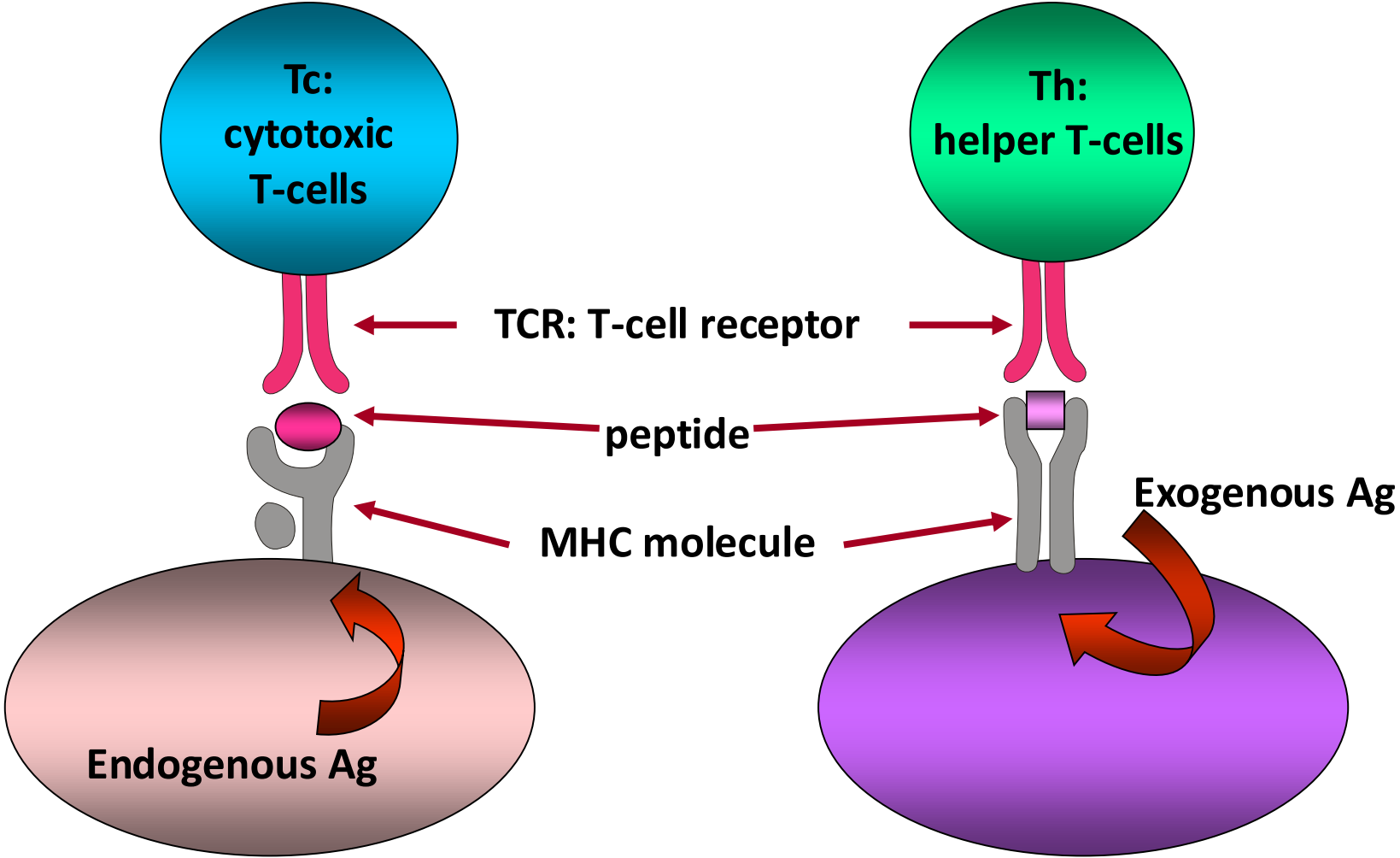
# HLA class II.

- HLA-DR, DQ, DP genes
- Present on the surface of the cells of the immune system (professional antigen presenting cells):
  - B cells
  - dendritic cells
  - macrophages
- Can be induced on endothel.
- Consists of alpha and beta chains.



# HLA class I.

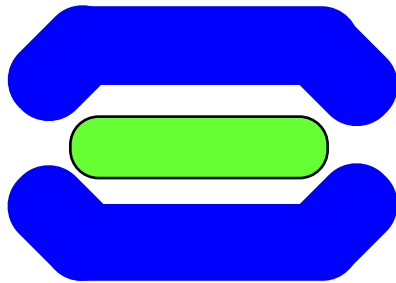
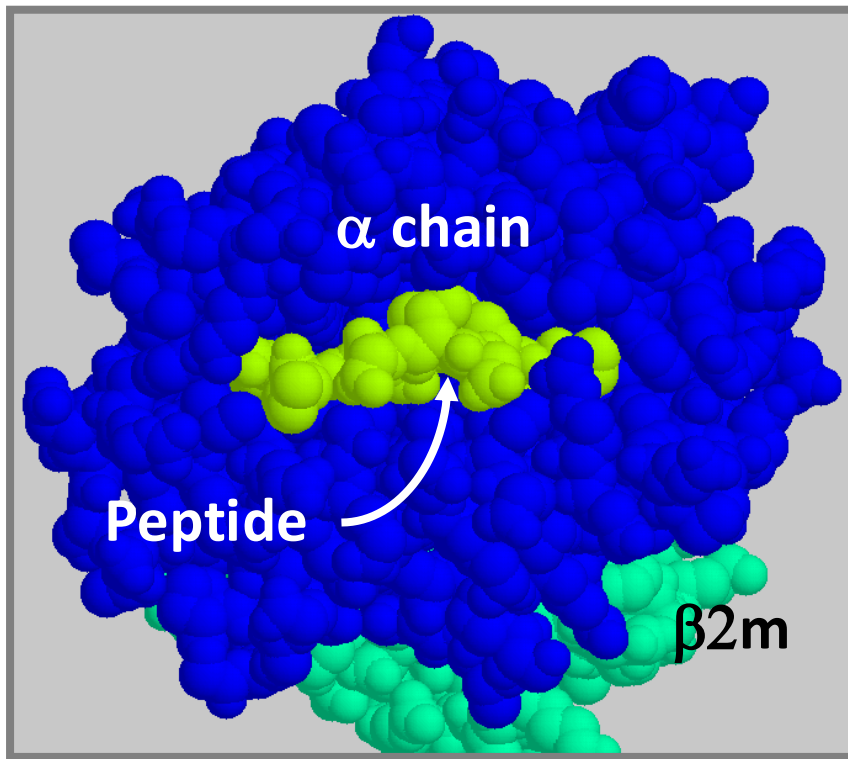
# HLA class II.



Peptides from endogenous proteins  
(own, virus, intracellular bacteria, tumour)

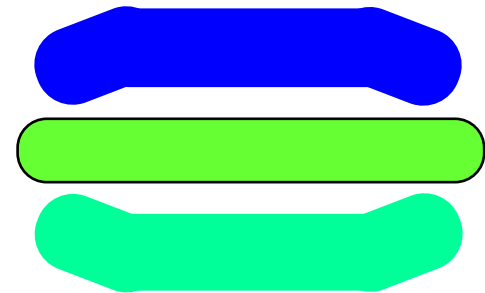
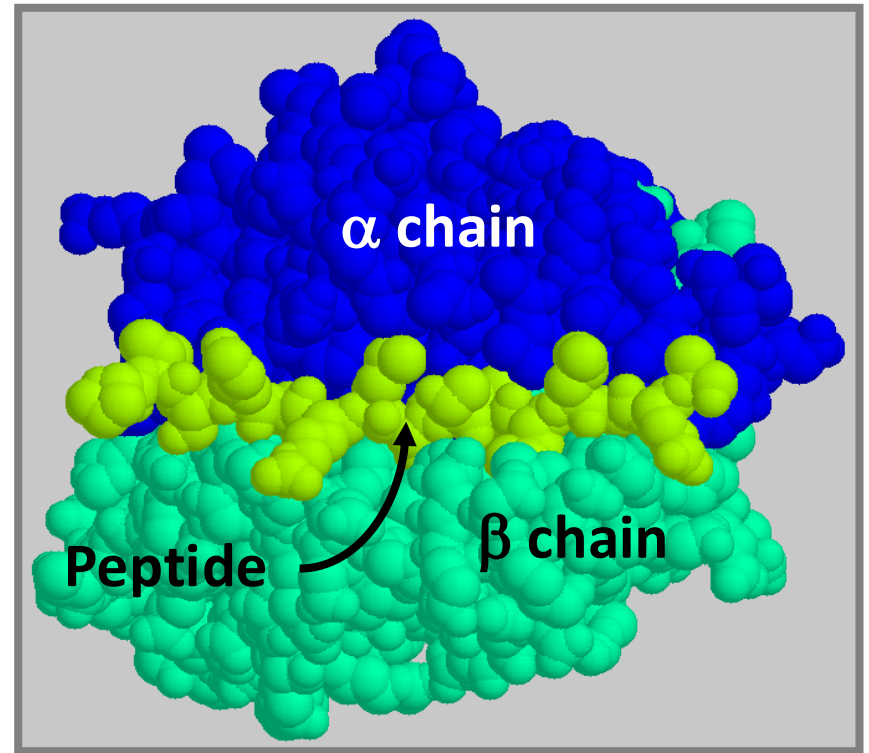
Peptides from exogenous proteins  
(extracellular pathogen, allergen)

# HLA class I.



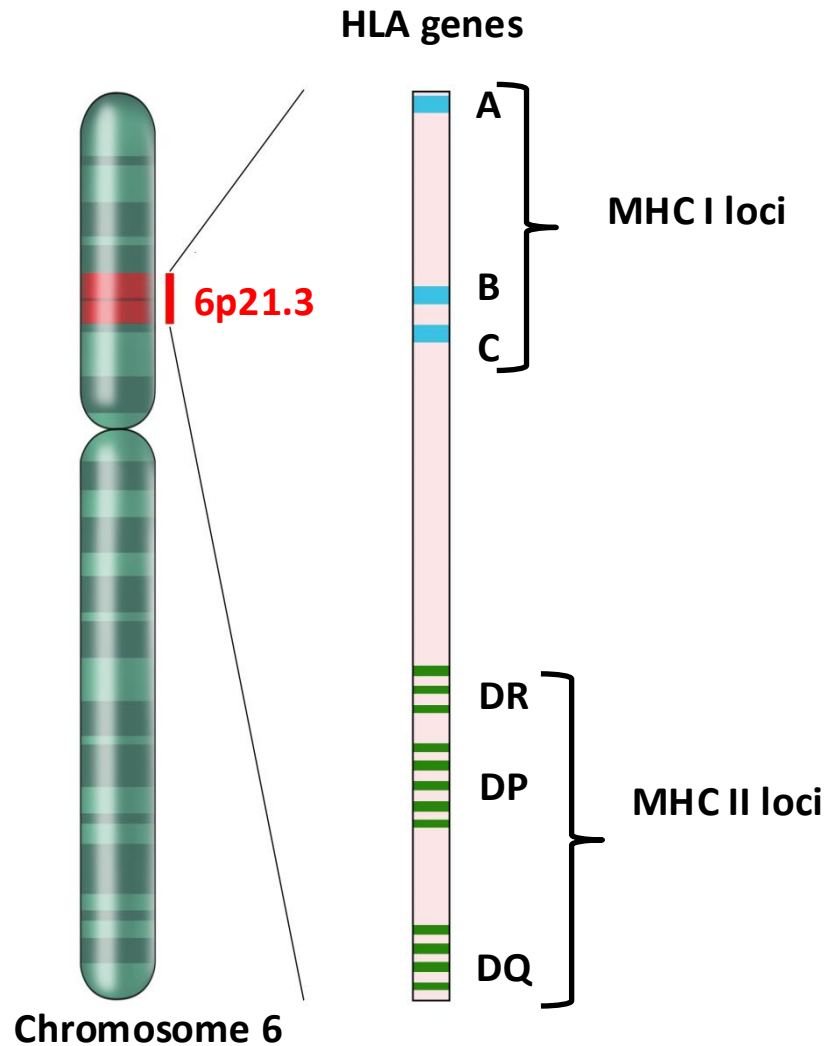
Binds peptides of 8-10 amino acids

# HLA class II.



Binds peptides of >13 amino acids

# Inheritance of HLAs: MHC loci



In humans both HLA-A, B, C, DQ, DR, DP and DP are expressed simultaneously both from the maternal and paternal chromosomes. → **Many types of MHC are present on the cells.**

# Basics of HLA nomenclature

- **MHC I** has only **1 chain**, e.g.: **HLA-B\*27:01** → B type MHC I of the 27 serological group
- The **MHC II** is a **heterodimer** with two chains.

**HLA-DQA1:05:01**

1. What type of MHC does it encode?

E.g.: A, B, C, DQ, DR, DP

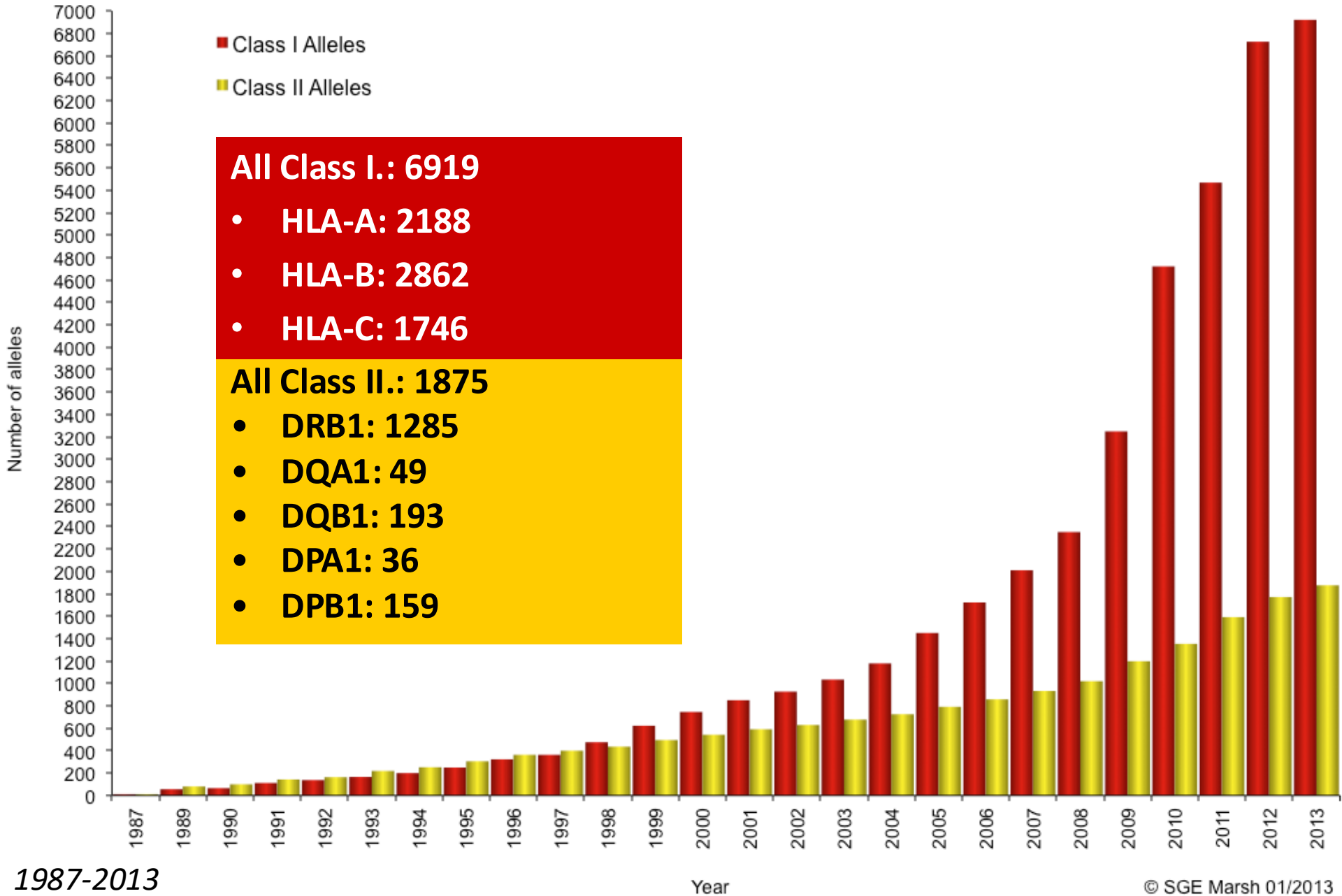
2. Locus (A1 = encodes alpha chain,  
B1= encodes beta chain)

3. Into which serological group does the chain belong? (05 = alleles resulting a  $\alpha^5$  chain)

4. The specific allele in the group

**Attention!** Slide is solely for illustration, we will not ask HLA nomenclature.

# Number of identified HLA alleles



1987-2013

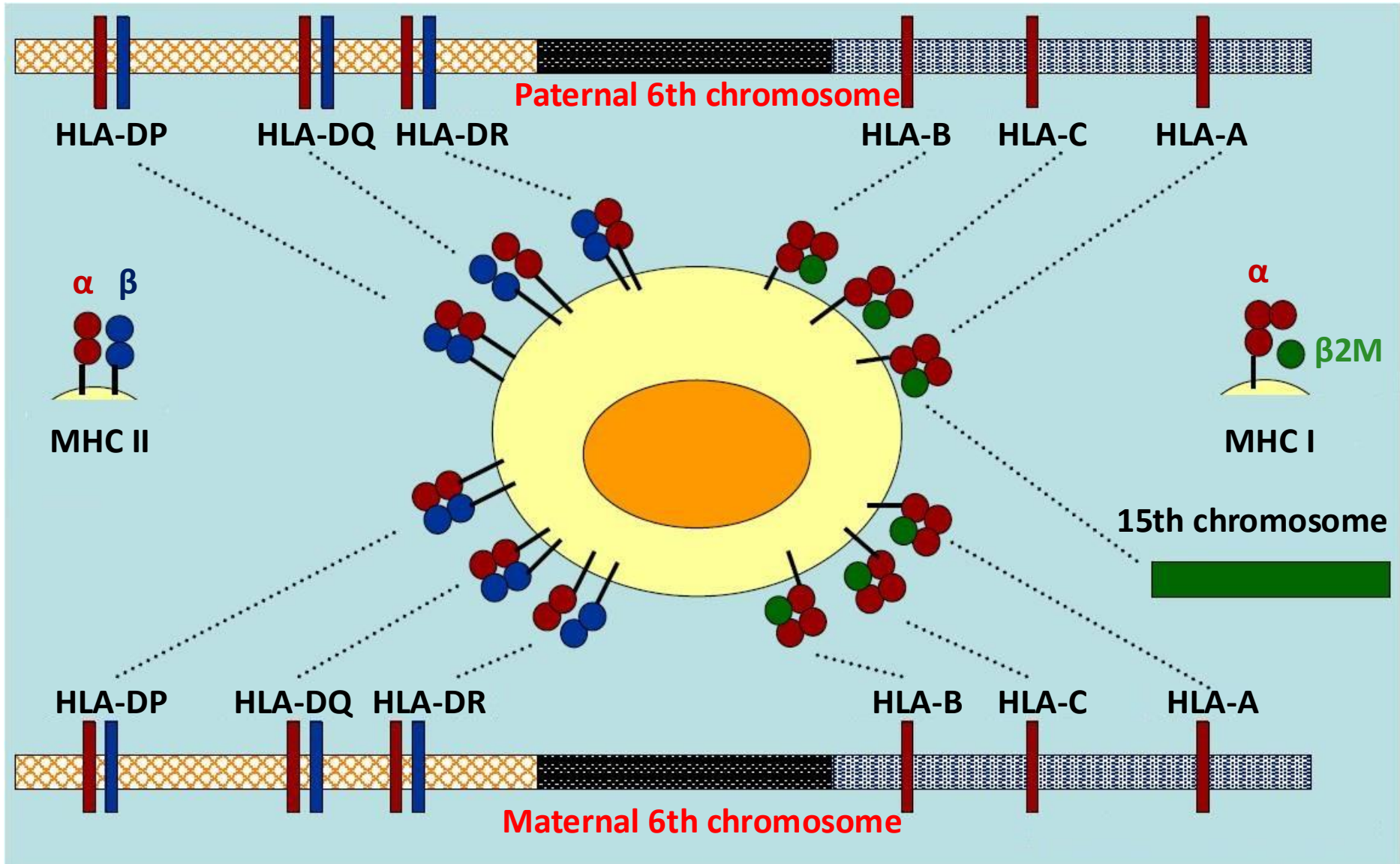
# Inheritance of HLAs

- **Polygenic:** Several genes encode MHC I and MHC II molecules. (e.g. HLA-A, B and C type I MHCs and DP, DQ and DR type II MHCs)
- **Polymorphic:** There are many different alleles of each gene in the population, therefore it is highly variable.
- **Codominant:** Both the maternal and the paternal alleles are expressed in an individual.



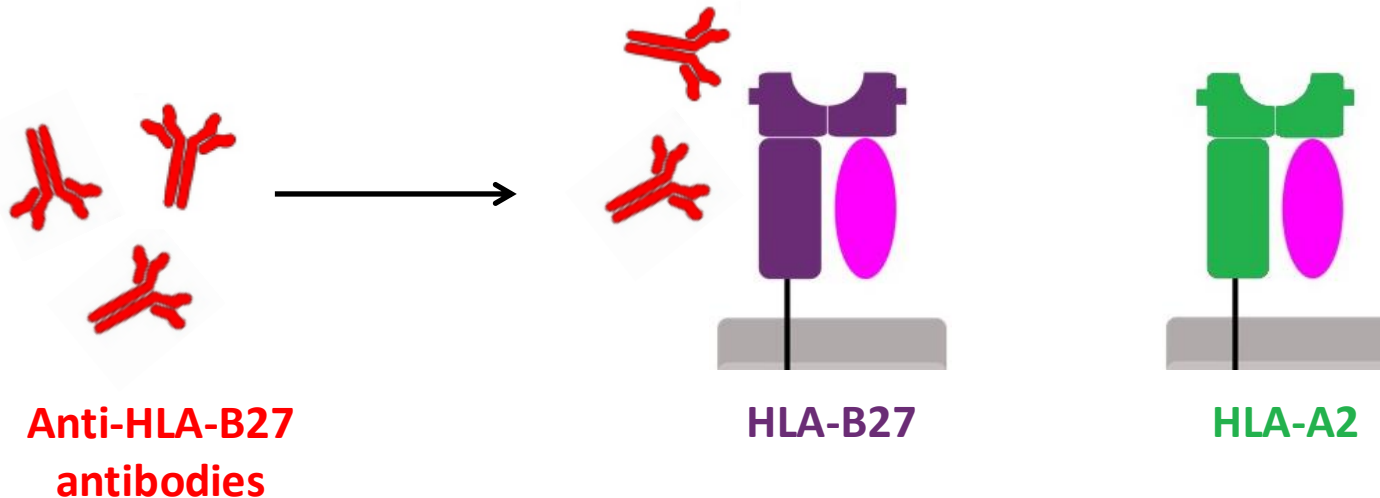
Each person has a characteristic **MHC pool** with different peptide-binding capabilities.

# Codominant expression



# HLA serotype vs genotype

- HLA **serotype**: MHC molecules are distinguished by their different antigenicity. E.g.:



- HLA **genotype**: MHC molecules are distinguished by identifying the **encoding HLA alleles**. A different genotype will not necessarily lead to a different serotype, **there are more alleles than HLA serotypes**. (6959 HLA alleles were known in 2010 but it is increasing each year. → Polymorphism)

# Methods of HLA typing

- **Serological methods:**
  - **Microcytotoxicity assay (MCA)**
  - Mixed lymphocyte culture (MLC)
- **Molecular biological methods:** (→ see from molecular cell biology)
  - Restriction fragment length polymorphism (RFLP)
  - Sequence-specific oligonucleotide probes (SSOP) → DNA hybridization
  - **Sequence-specific primers → SSP-PCR**
  - **DNA sequencing**

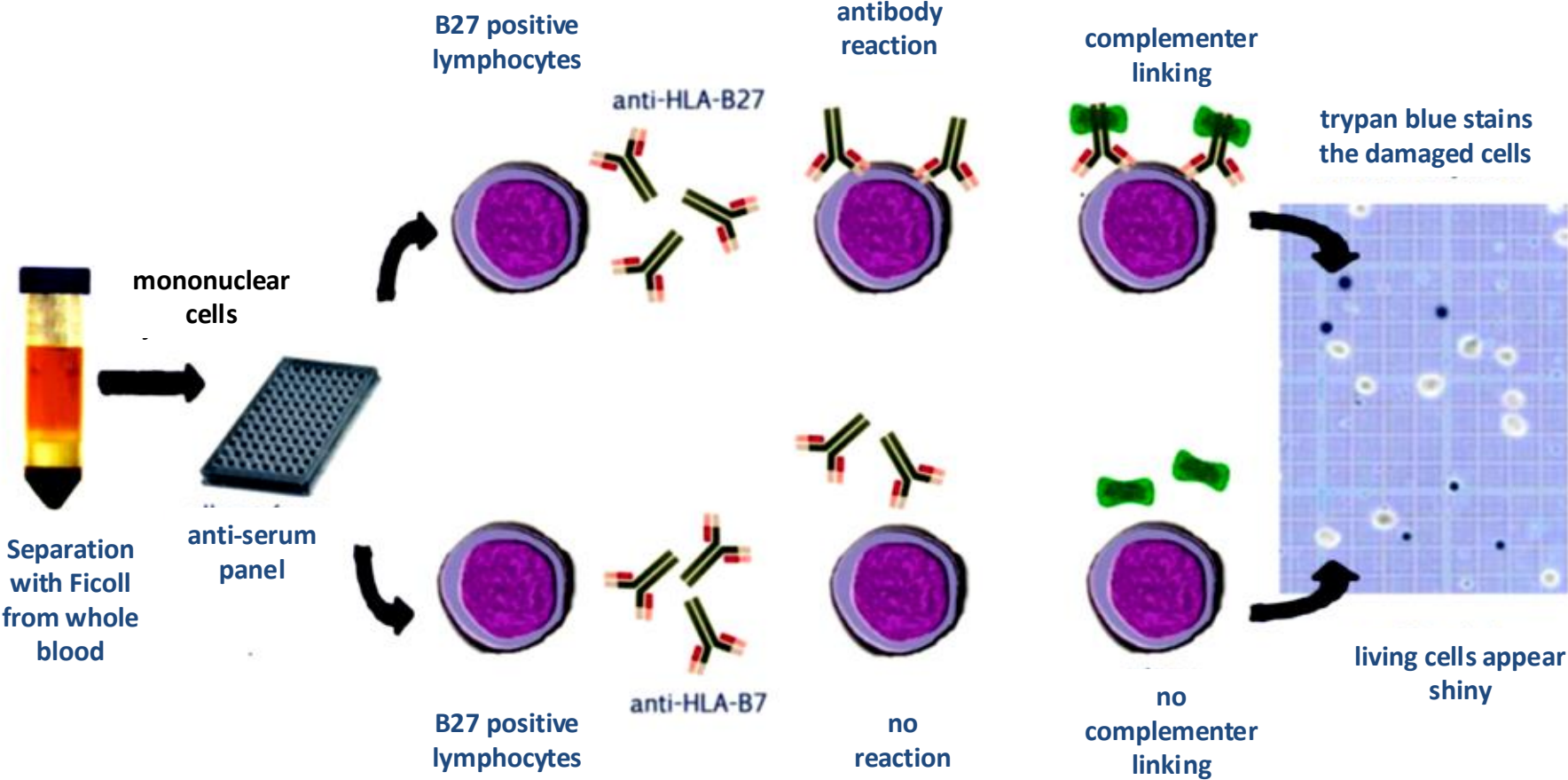
The **molecular biological methods are the preferred ones** because they are:

- **More specific** (well-defined probes and primers are used)
- **More flexible** (new oligonucleotide probes or primer can be designed as soon as a new allele described)
- **More reliable** (doesn't require a specific cell type and is less dependent on the condition of the patients)

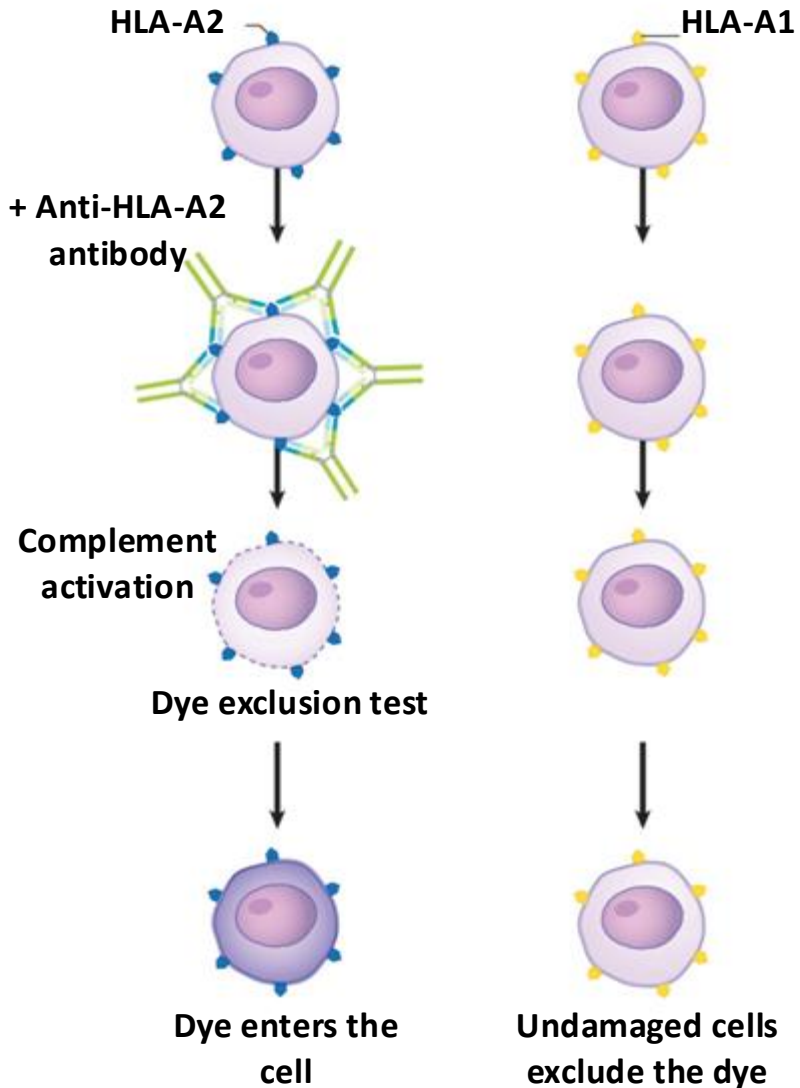
# HLA serology

- 1. Typing** – investigation of polymorphism of HLA antigens : lymphocytes with unknown antigenicity added to known specificity antibodies.
- 2. Antibody screening - PRA** (panel reactive antibody) test – detection of antibodies against HLA antigens: the sera that need to be tested is added to known antigenicity cell panel.
- 3. Cross match:** in vitro model of antigen-antibody reaction that might occur during transplantation, might result in graft rejection in vivo.

# Microcytotoxicity assay (Terasaki)



# Microcytotoxicity assay (MCA) – Cross match Terasaki plate

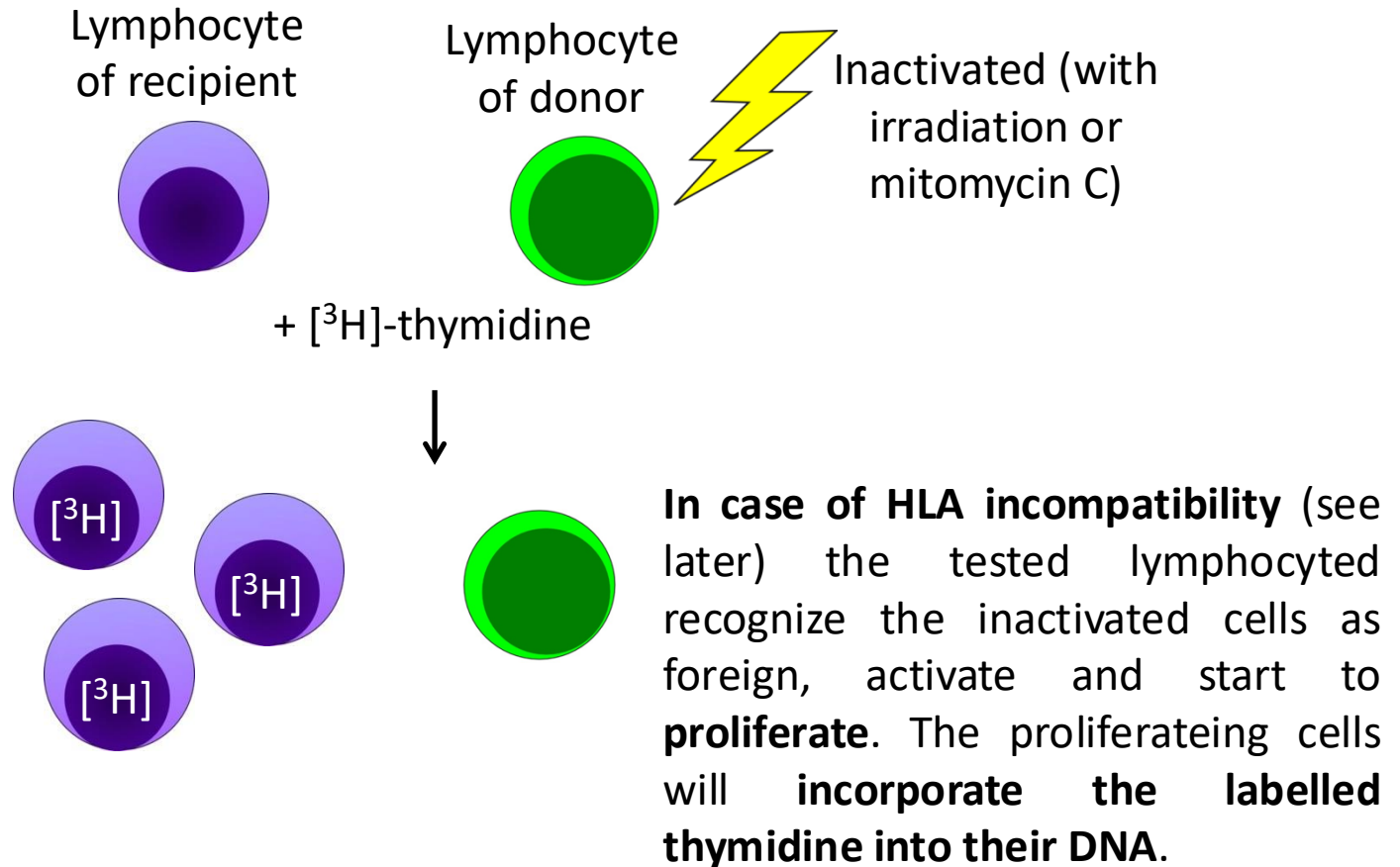


Used to check the **immunological incompatibility** of the donor and the recipient before transplantations. E.g.:

	1	2	3	4	5	6	7	8	9
Donor	●	○	○	○	○	○	●	○	○
Recipient 1	●	○	○	○	○	○	●	○	○
Recipient 2	○	●	●	○	○	○	○	○	○

The donor and recipient 1 matches based on the serological test

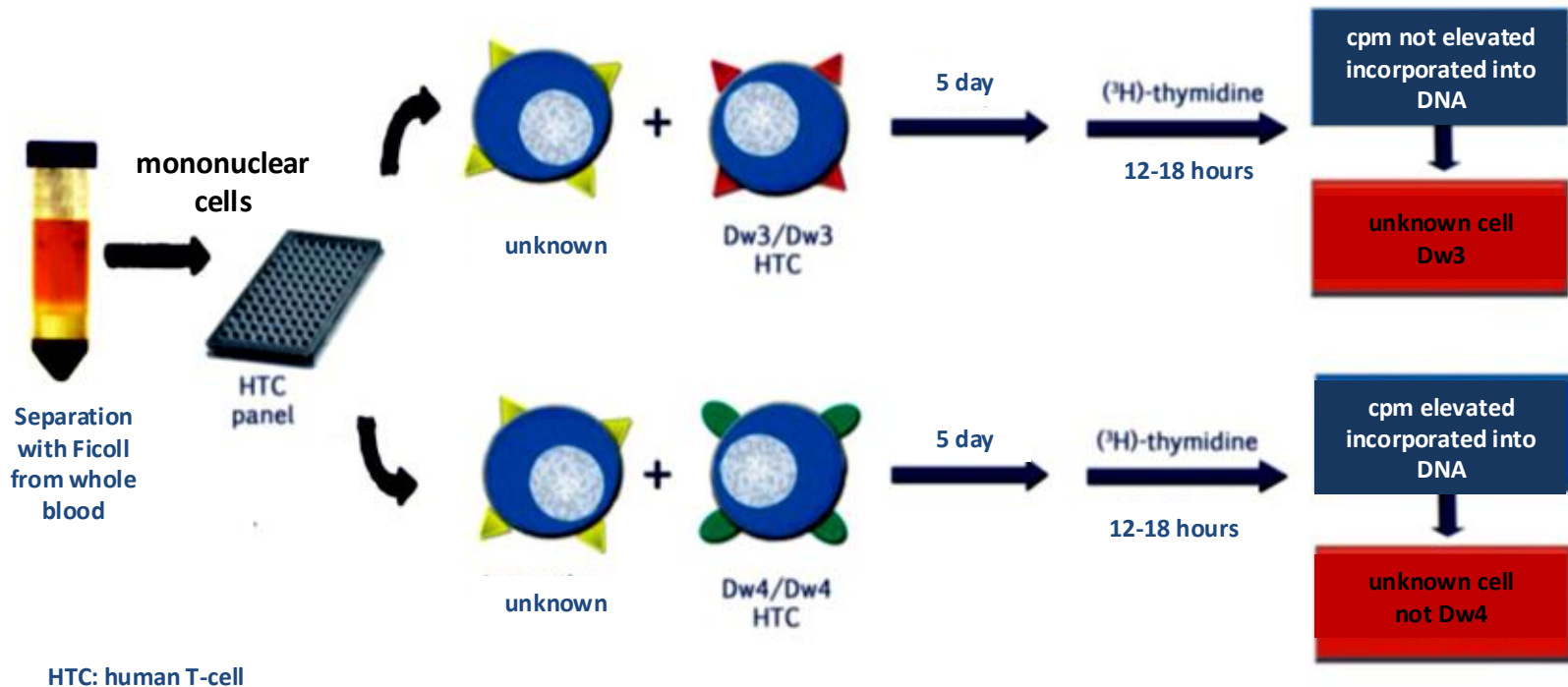
# Mixed lymphocyte culture



Application:

To check the **immunological incompatibility** of the donor and the recipient before transplantations.

# Mixed lymphocyte culture (MLC)

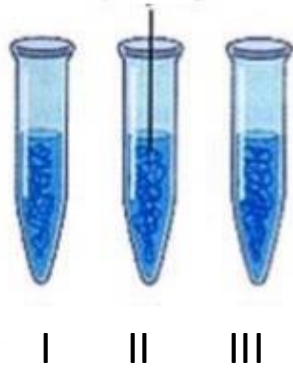


Mixed Lymphocyte Culture (MLC): The lymphocytes of two individuals (eg. a patient's unknown lymphocyte and a laboratory sustained known lymphocyte expressing HLA-Dw ) are mixed in a Petri dish and incubated in cell culture for days. If the unknown (patient's) lymphocyte doesn't carry HLA-Dw, which can be found on the test lymphocyte, then the lymphocyte becomes stimulated and proliferates, which can be measured by the thymidine incorporation. *Used solely to determine MHCII*, it has clinical significance in case of transplantation. Requires tissue culture laboratories that sustain homogeneous cell population. Isotope test.

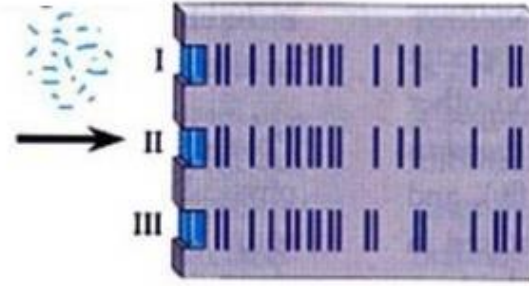
# RFLP

(Restriction fragment length polymorphism)

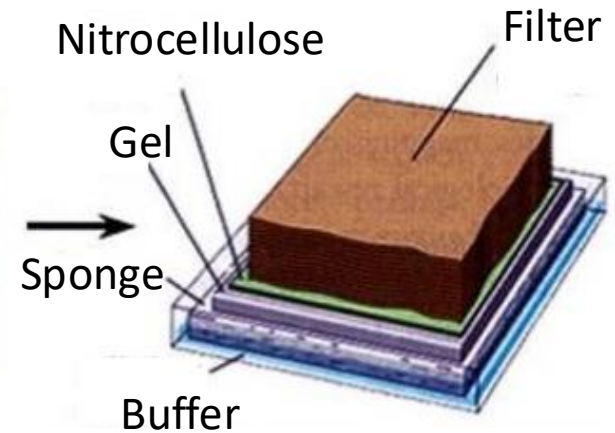
DNA + Restriction  
endonuclease



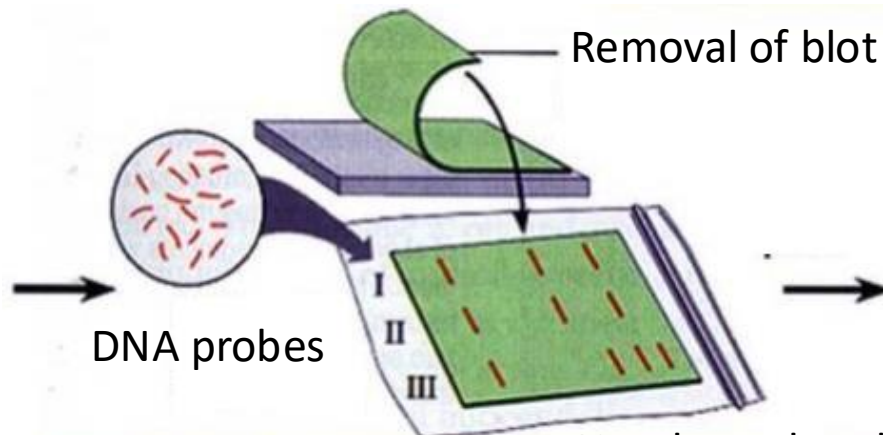
Electrophoresis



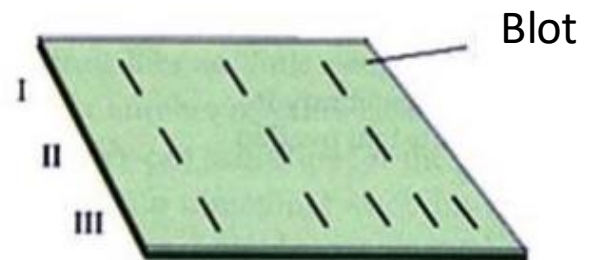
Blotting



Hybridization

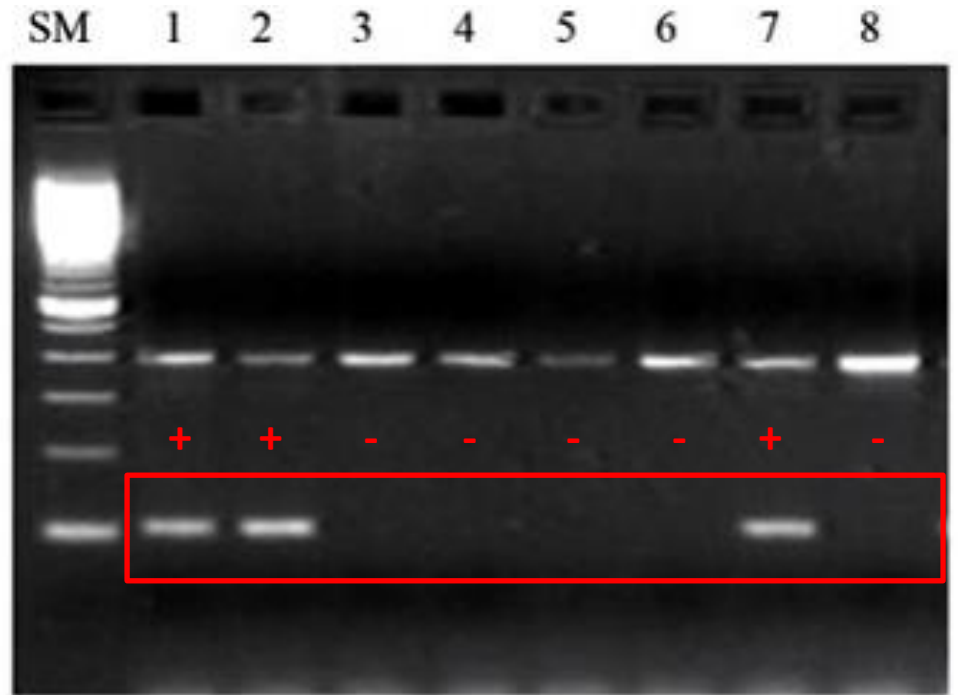
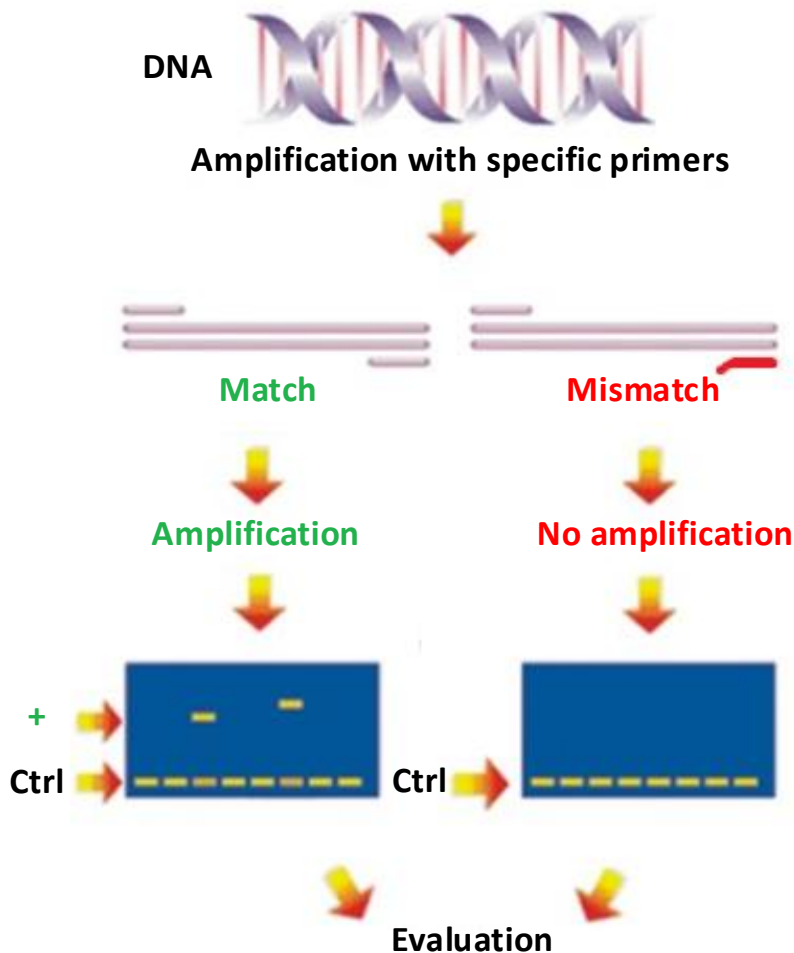


Detection



Non-bound probes removed by washing

# PCR with sequence-specific primers



HLA-A\*01 genotyping: The visible bands in samples 1, 2 and 7 mark HLA-A\*01 alleles.

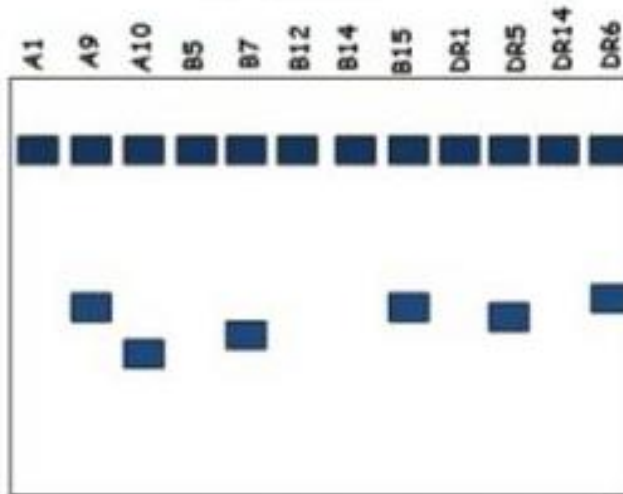
# Steps of organ transplantation alert

- The blood sample of the donor arrives
- Selection of possible recipients from the registry
- DNA isolation, SSP PCR for HLA-A, B and DRB1 genes, gel electrophoresis, evaluation
- Cross match → testing of donor's cells with the sera of the possible recipients in the presence of complement. Checking the recipient's sensitization

# HLA matching before kidney transplantations

The survival of the graft is mainly determined by the degree of matching of the **HLA-A**, **HLA-B** and **HLA-DR** alleles, these are the one investigated before transplantations.

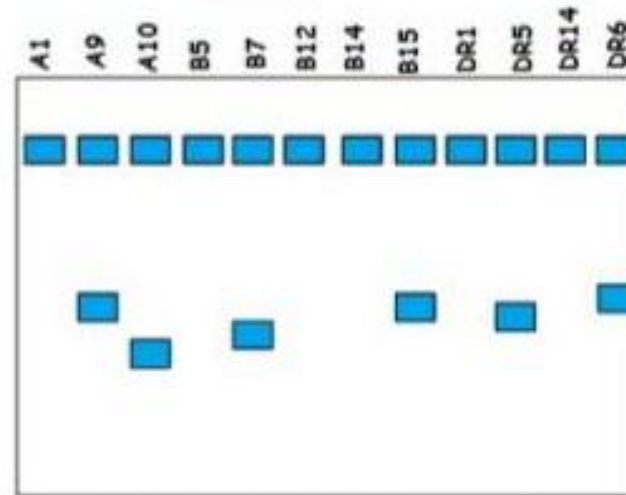
**Recipient**



HLA genotype:

- A9, A10
- B7, B15
- DR5, DR6

**Donor**



HLA genotype:

- A9, A10
- B7, B15
- DR5, DR6

12/12 match ✓

# Graft versus host disease 1. (GVHD)

- May develop after **allogeneic hematopoietic stem cell transplantations** (HSCT).
- The donor-derived immune cells attack and damage the host tissues.
- Main risk factor: **HLA mismatch** between the donor and the recipient.
- Therapy: Steroids (immunosuppression), lethality is roughly 15%, but steroid resistant acute GVHD has a lethality of 90%.

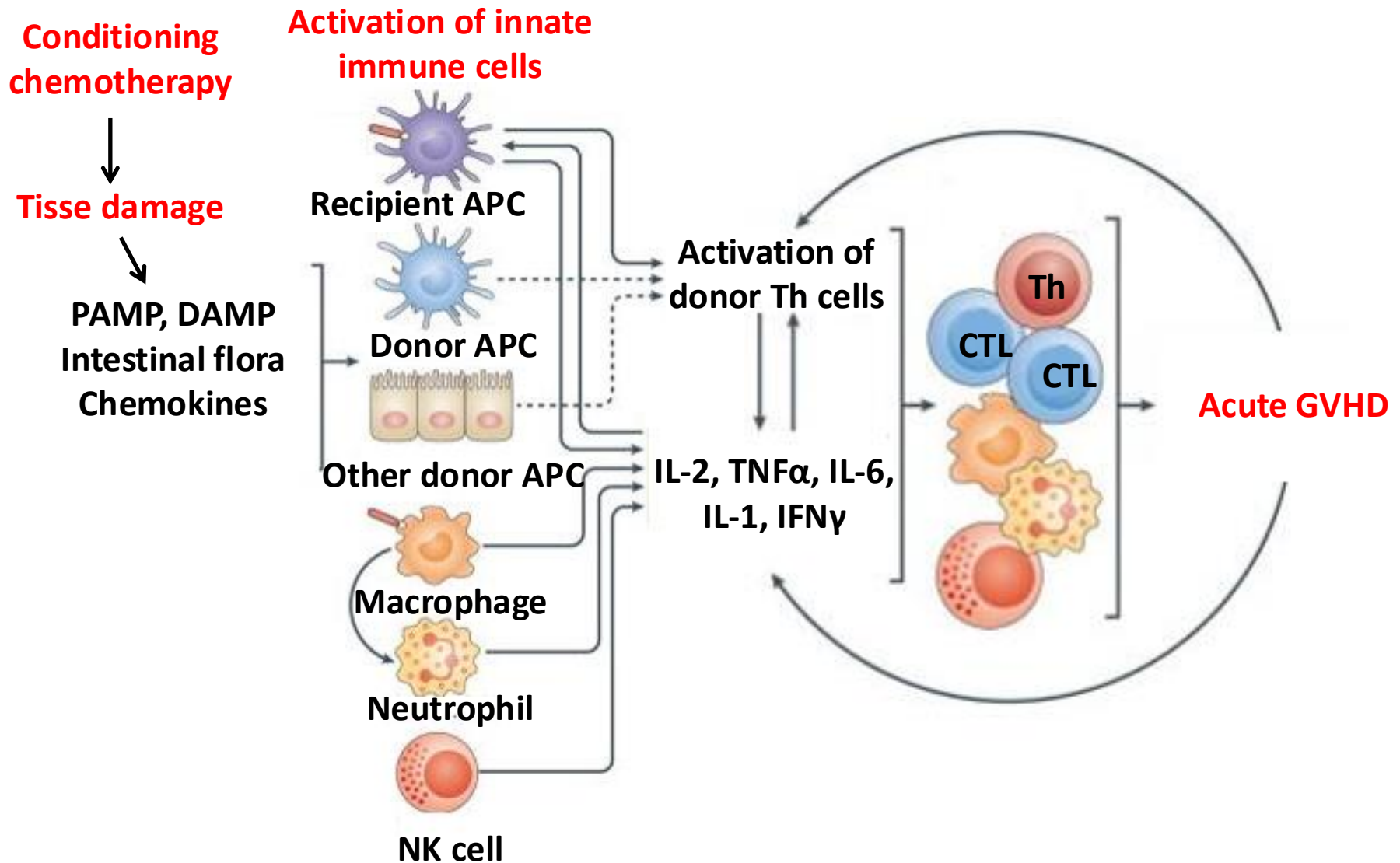


Severe skin GVHD



Acute intestinal GVHD  
(endoscopic image)

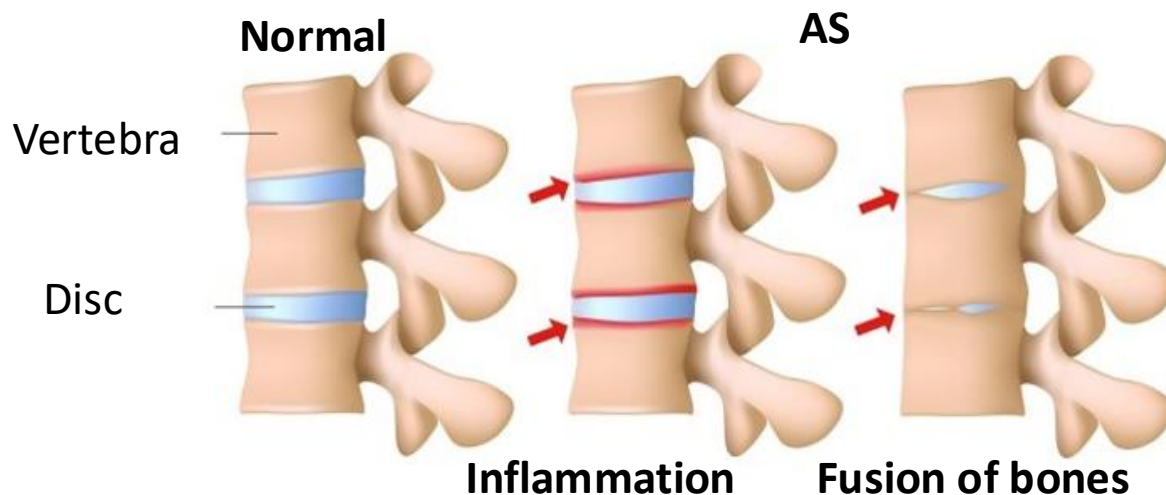
# Graft versus host disease 2. (GVHD)



# HLA and disease associations 1.

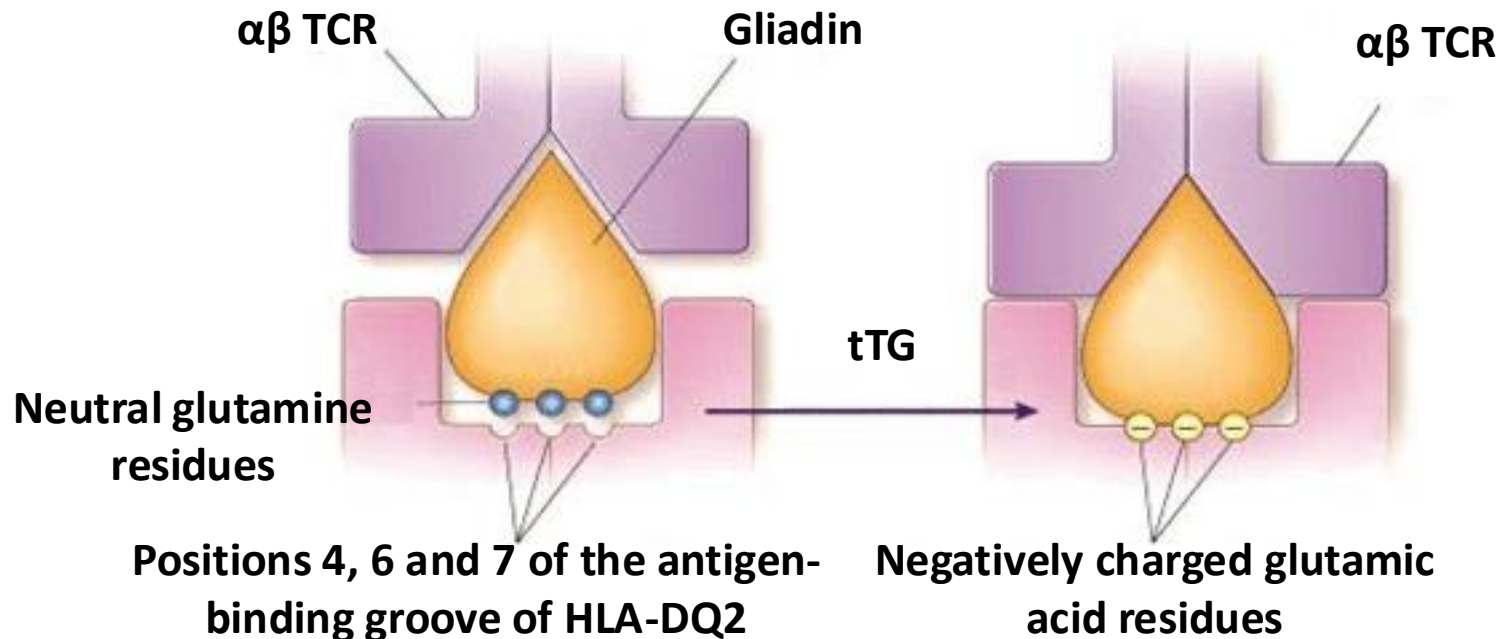
- **Bechterew's disease** (Ankylosing spondylitis, AS): **HLA-B27**
- Approx. **90%** of AS patient are HLA-B27 positives.
- Prevalence of HLA-B27 in the Caucasian race is 8%, in Scandinavia it reaches 24%.
- Approx. 1,8% of HLA-B27 positive individuals develop manifest AS.

HLA-B27 positivity **only increases the risk of the disease**, it is **not enough on its own** for developing the disease! (This is also true for all the HLA associations.)



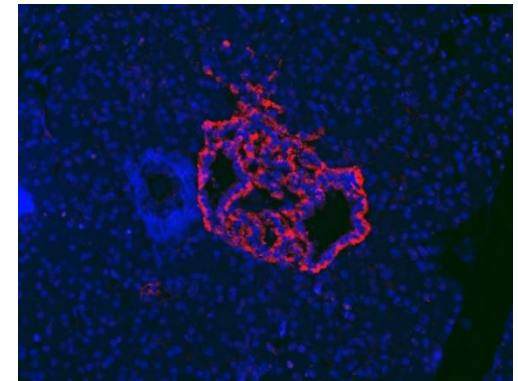
# HLA and disease associations 2.

- **Celiac disease** (gluten-sensitive enteropathy): **HLA-DQ2** and **HLA-DQ8**
- At least one of them is present in roughly **98%** of the patients. (Strongest known HLA association and the most well-understood role in the pathogenesis)
- Prevalence of HLA-DQ2 in the Caucasian race is 30%, but the prevalence of the disease is only 1%. → Positivity is not enough for developing the disease.
- These show a **higher affinity towards gliadin** than other MHC types, especially when binding the deamidated forms.



# HLA and disease associations 3.

- **Type I diabetes mellitus (IDDM): HLA-DR3, HLA-DR4**
- HLA-DR3-DQ2 → 3X risk
- HLA-DR4-DQ8 → 10X risk
- **HLA-DR3-DR4 heterozygotes → 25X risk**
- HLA-DQ6.2 → 0,1X risk (protective role)



**Direct IF: Human Langerhans islet (patient with IDDM)**

**Green: Insulin (lacking)**

**Red: glucagon**

**Blue: cell nuclei**

# Student Research

- Main fields of research at the department:
  - **Differentiation of lymphoid tissues and recirculation of immune cells**
  - **Role of T cells in the murine model of rheumatoid arthritis**
  - **Signaling and role of regulatory T cells in autoimmune disease**
  - **Effects of glucocorticoids on T cells**
  - **Evolution of the immune system in invertebrate animal models**
  - **Efficacy monitoring of vaccines by immunoserological techniques**

The exact topics can be found on the link below, for further info please contact the supervisors. Own topics are also welcomed if they are related to immunology.

[http://aok.pte.hu/en/egyseg/tdk\\_temak/120](http://aok.pte.hu/en/egyseg/tdk_temak/120)



**I WANT YOU**  
**FOR STUDENT RESEARCH!**

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