IMMUNOLÓGIAI ÉS BIOTECHNOLÓGIAI INTÉZET

14th practice: HLA typing

Basic Immunology

University of Pécs, Clinical Center
Department of Immunology and Biotechnology
Pécs, 2024.

Tumor Specific Antigen

- **TSA – mutations of somatic cells induced by chemical carcinogenesis, viruses or x-rays**

- **Each carcinogenic factor induces a unique and specific class of antigens. NO GENERAL TUMOR SPECIFIC ANTIGEN EGSISTS!**

- **TSA is recognized** (according to the individual MHC haplotype) **by the immune system and induces targeting type immune response or tolerance**

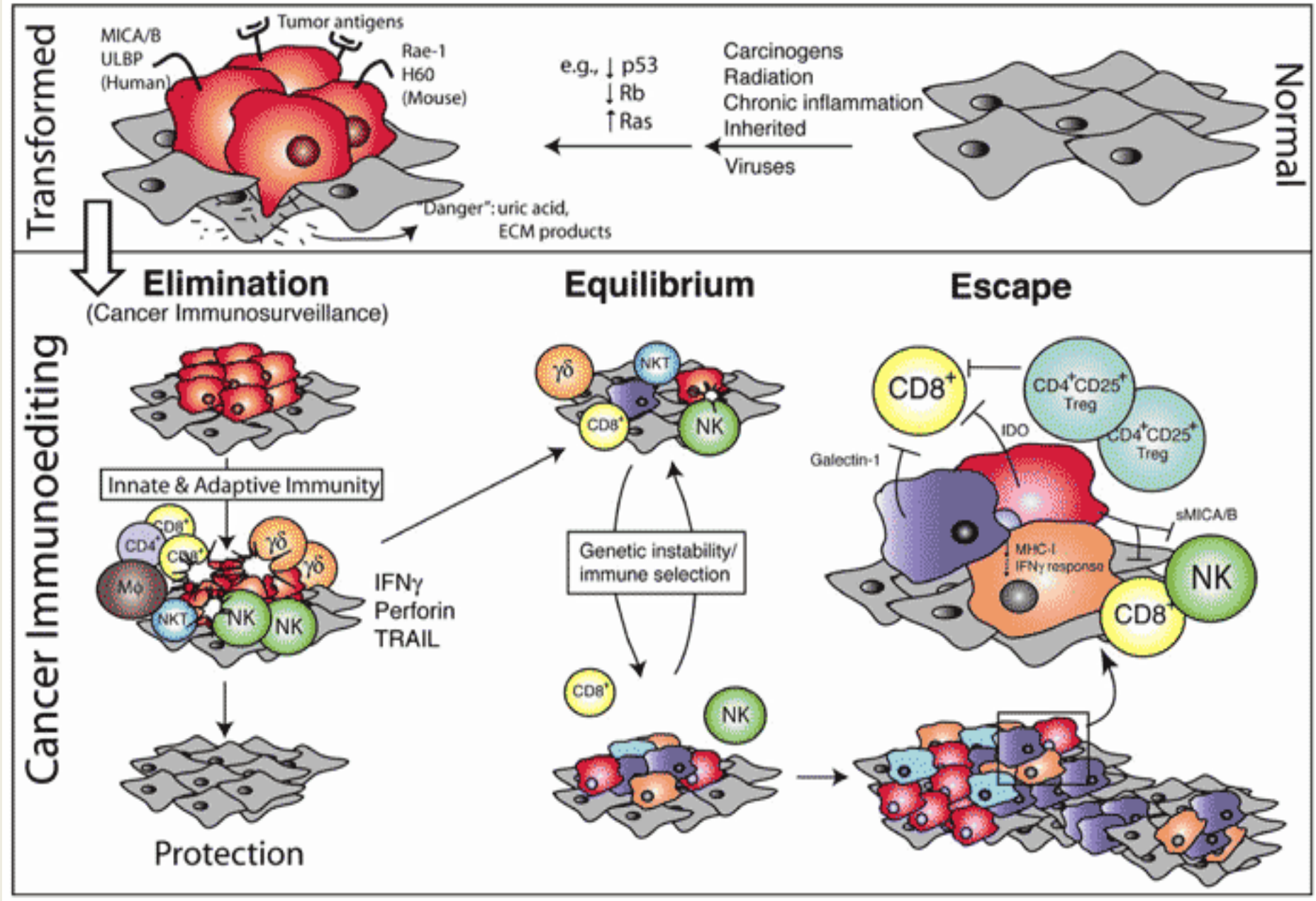
Tumor Associated Antigen

Products (e.g. hormones, growth factors, cell surface receptors, differentiation molecules etc.) of both normal and altered cells during their differentiation.

Production of TAAs is not related with tumorous transformation exclusively, however, expression profile of TAAs could be characteristic in some tumors, and useful as „tumor markers" in differential diagnosis or in the monitoring of therapeutic efficiency.

Tumor markers

Tumor markers	Abbreviation	Oncological application
Alfa-foetoprotein	AFP	Liver and germ cell tumors
Cancer antigen 125	CA 125	ovarian tumors
Cancer antigen 15,3	CA 15,3	Breast cancer
Cancer antigen 72,4	CA 72,4	Gastric cancer
Cancer antigen 19,9	CA 19,9	Pancreatic cancer
Carcinoembrional antigen	CEA	Gastrointestinal cancers
Neuronspecific enolase	NSE	Small cell lung cancer
Prostate specific antigen	PSA	Prostate cancer
Squamous cell carcinoma antigen	SCC	Planocellular cancers
Tissue polypeptide antigen	TPA	Urinary bladder and lung cancer
Tissue polypeptide-specific antigen	TPS	Metastatic breast cancer



Lecture revision

Cancer immunotherapy

Complementary therapeutic tools after the surgical, chemotherapeutic and/or irradiation treatments:

- **Therapeutic monoclonal antibodies**
- **Checkpoint inhibitors (PD-1/PDL-1)**
- **Immuno-modulation**
- **Cancer vaccines**
- **Oncolytic viruses**

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

Bone marrow transplantation

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

Discovery of HLA system

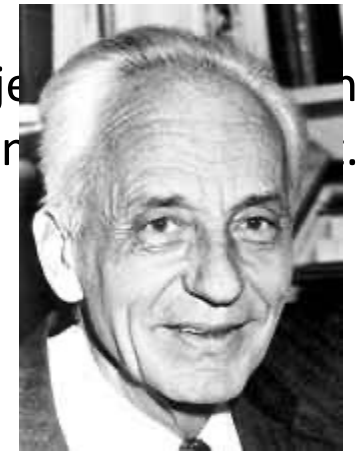
- **George Snell** performed experiments on mice.
  Vaccined different individuals with mouse tumours.
  The rejection or engraftment was determined by the genetical difference or identity of the cells.
  Genetic system: MHC (Major Histocompatibility Complex)

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

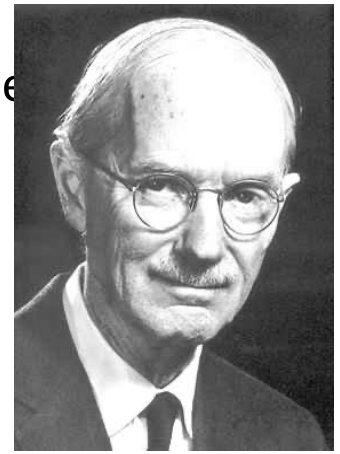
- **Baruj Benacerraf**: the str eje nine
  histoincompatibility betw ar .

- **Nobel prize (1980)**

Baruj Benacerraf
(1920 - 2011)



Jean Dausset
(1916 - 2009)



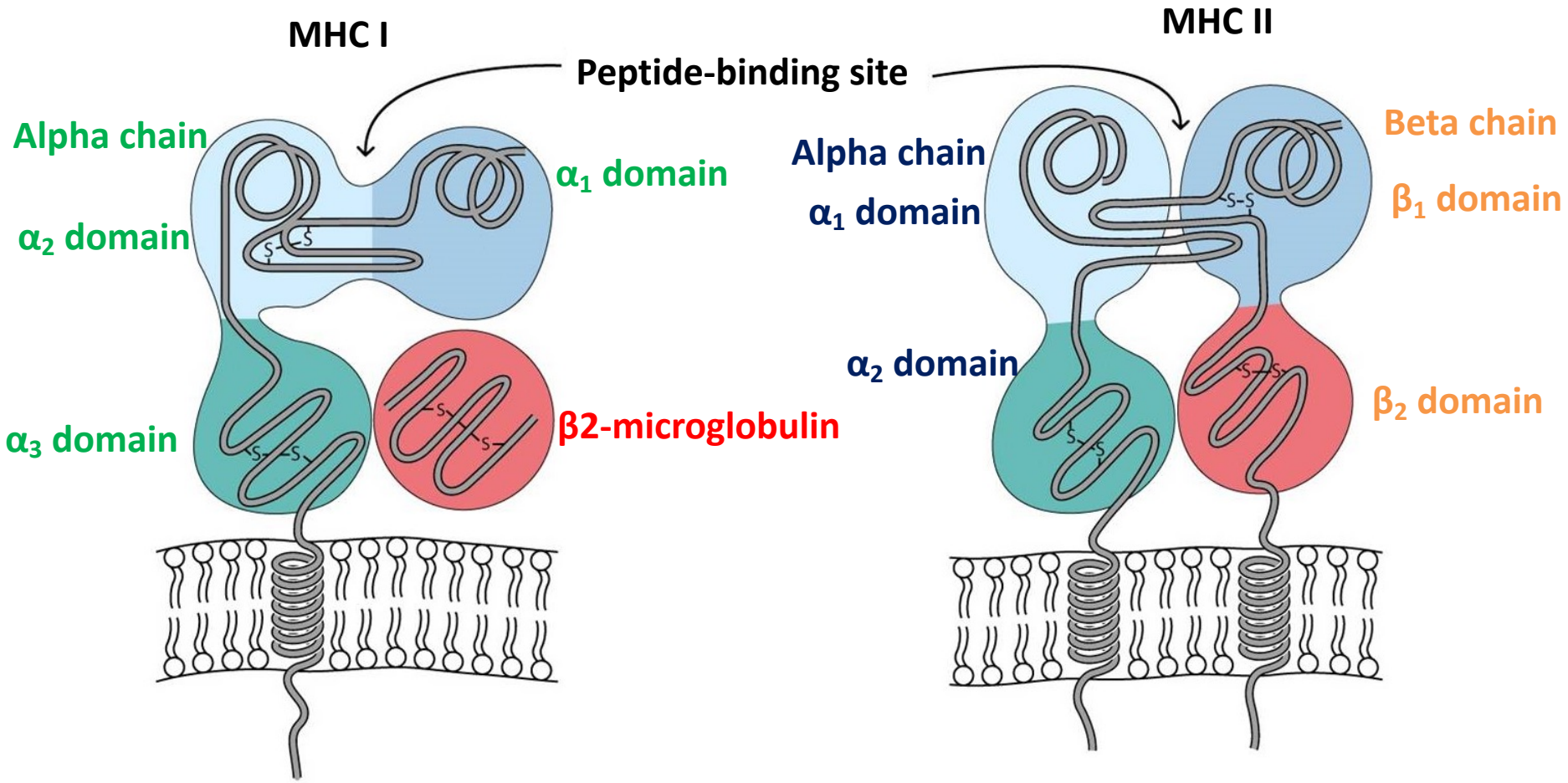
George D. Snell
(1903 - 1996)

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**: Becheterew'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)



On all nucleated cells and thrombocytes!

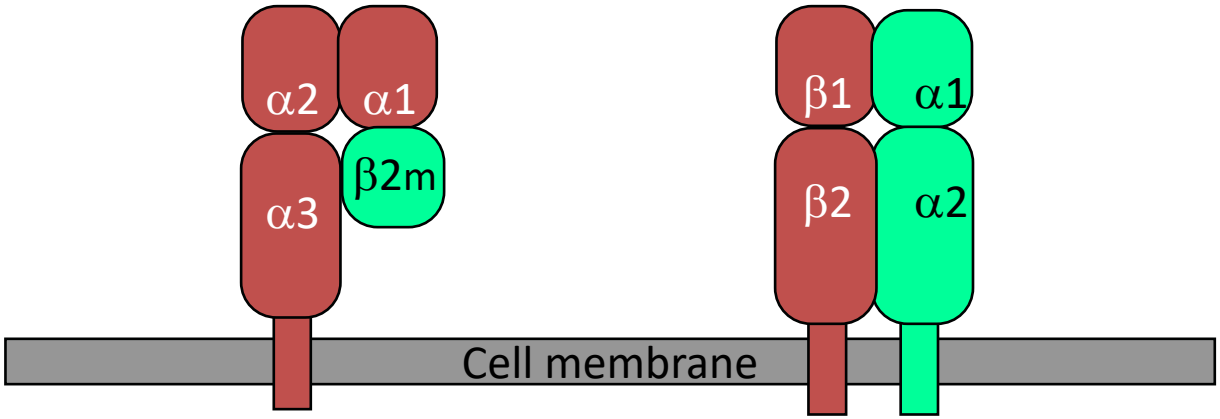
On antigen presenting cells!
(e.g. macrophage, dendritic cell, B cell)

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 β2 microglobulin.

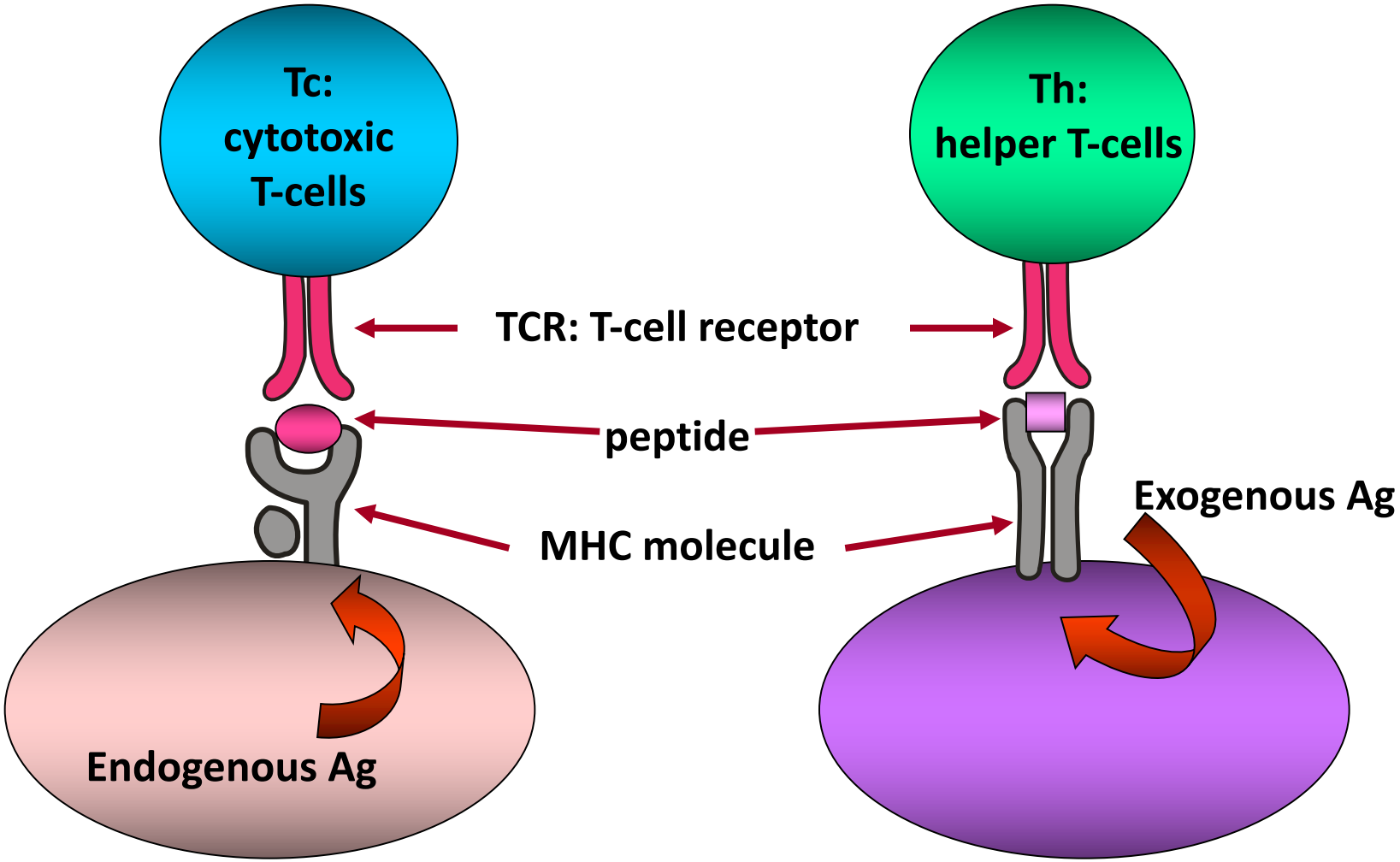
HLA class II.

- 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.

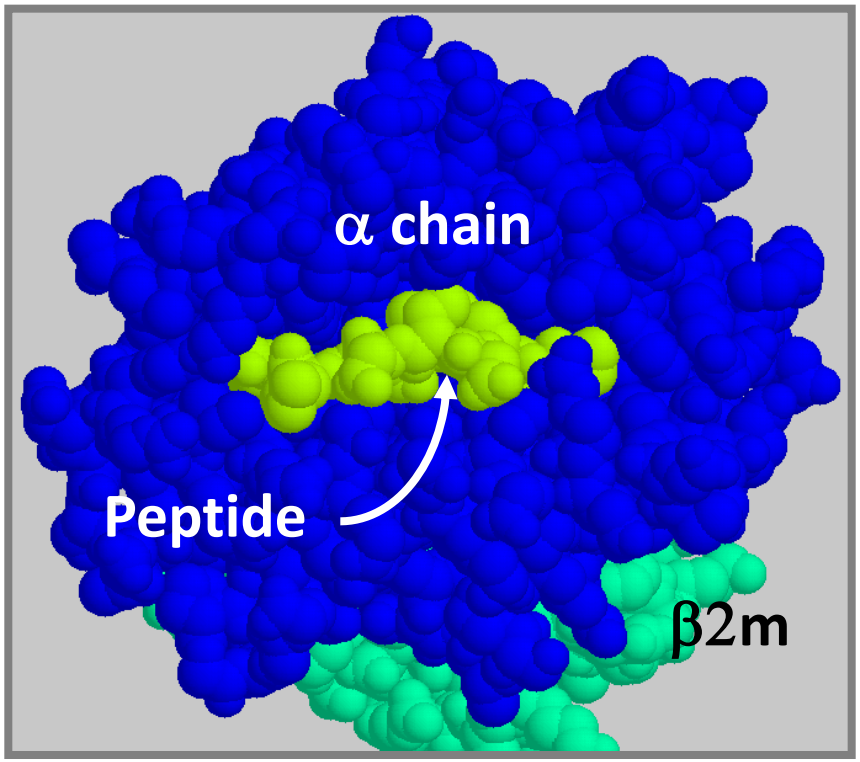
HLA class II.

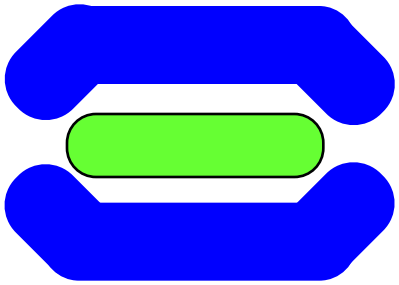


Peptides from endogenous proteins
(own, virus, intracellular bacteria, tumour)

Peptides from exogenous proteins
(extracellular pathogen, allergen)

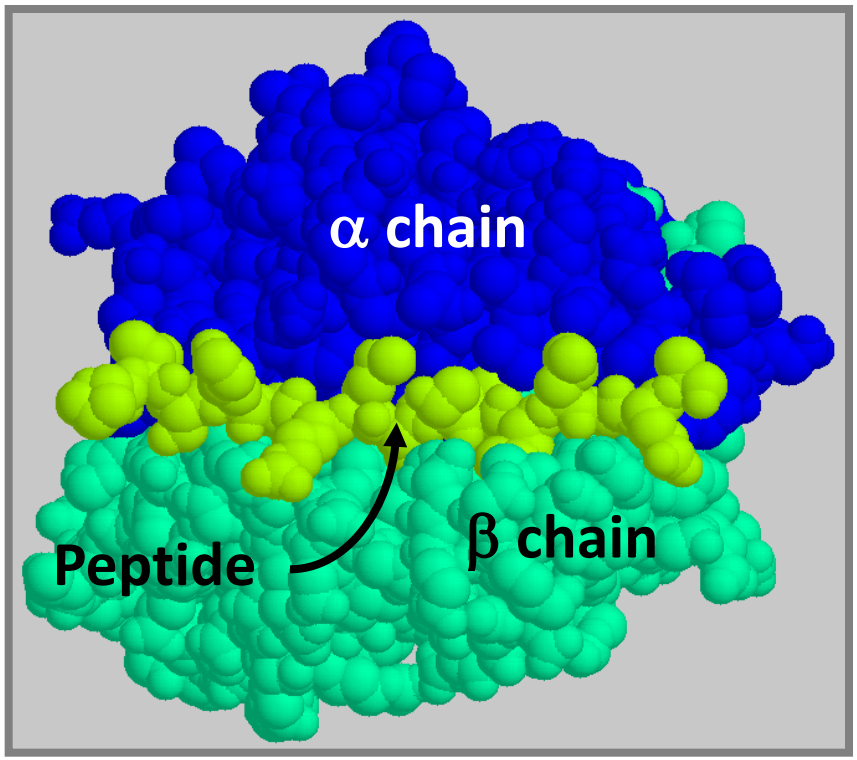
HLA class I.

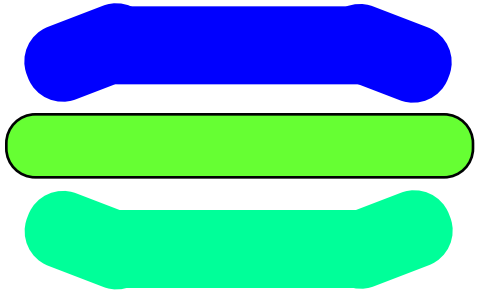




Binds peptides of 8-10 amino acids

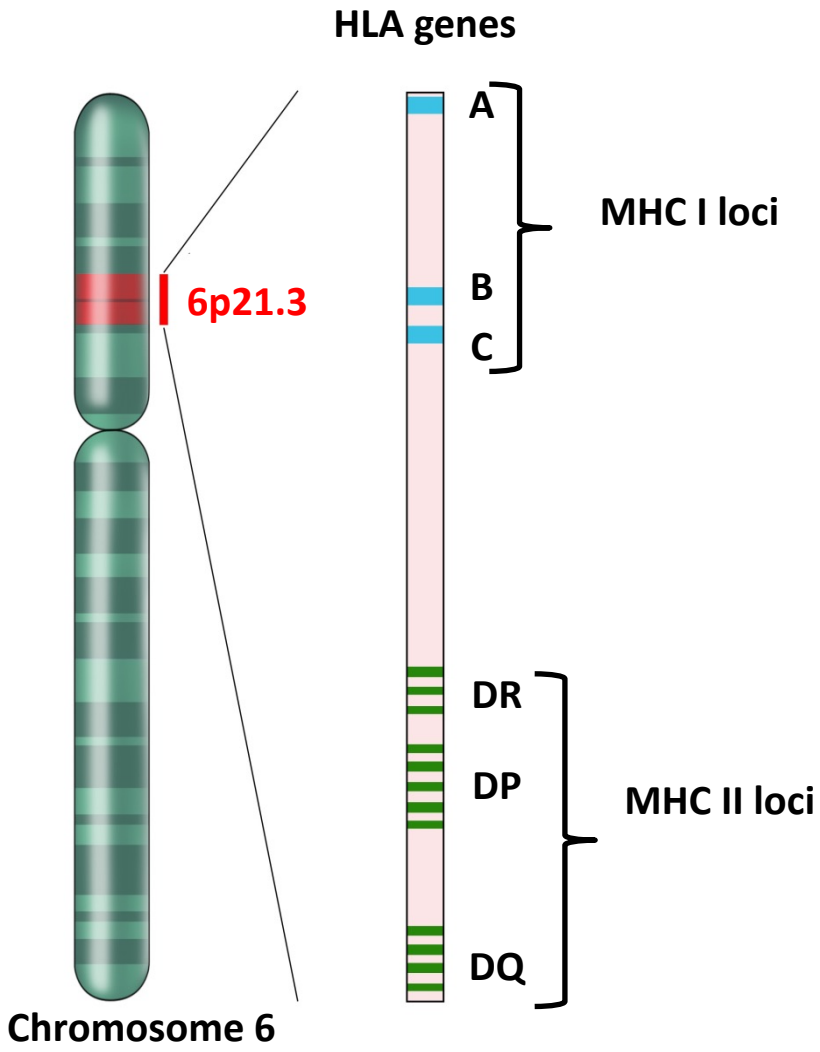
HLA class II.





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

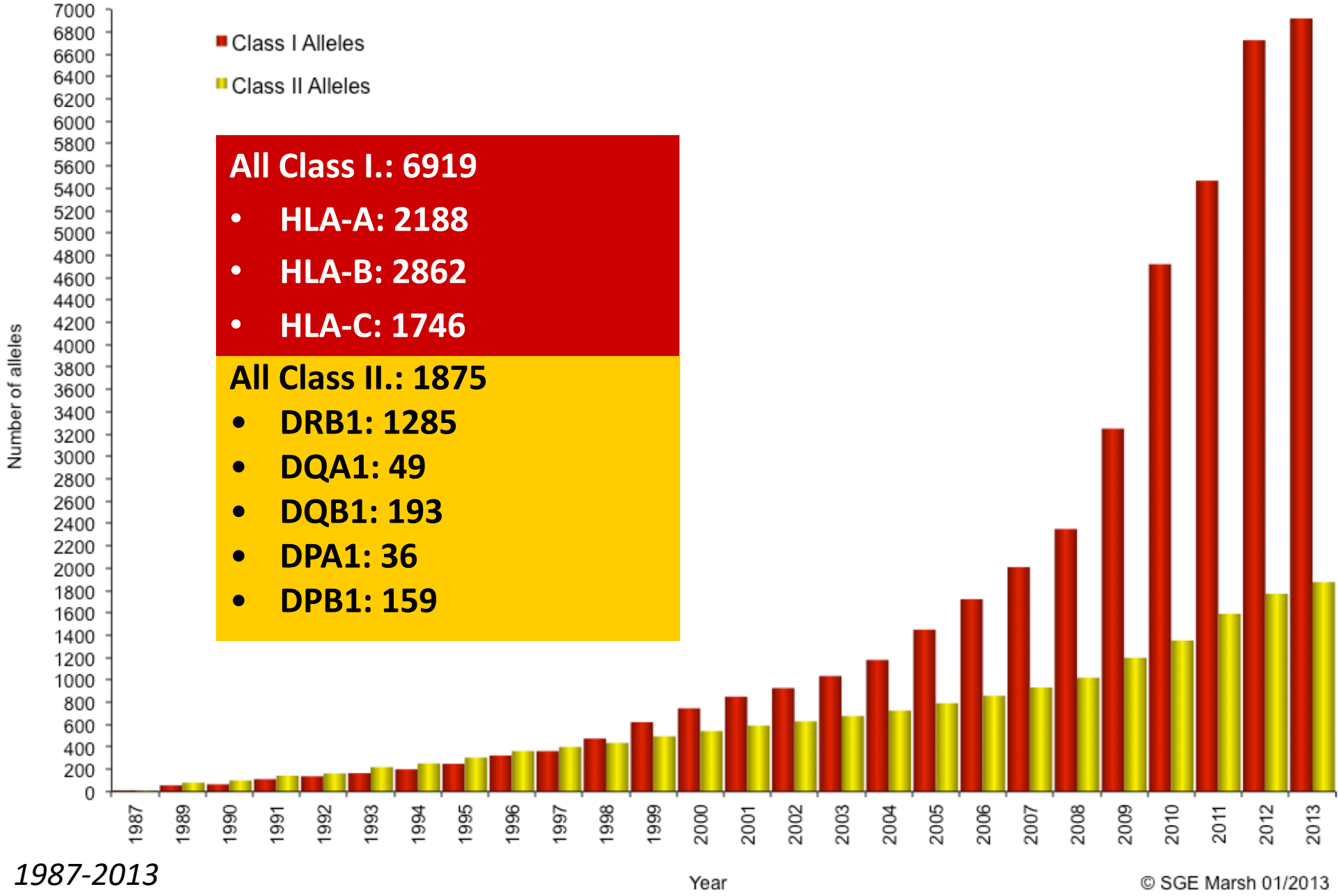
- **MHC I** has only **1 chain**, e.g.: **HLA-B*27:01** → B type MHC I of the 27 serological group
- The **MHC II** is a **heterodimer** with two chains.

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 α^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

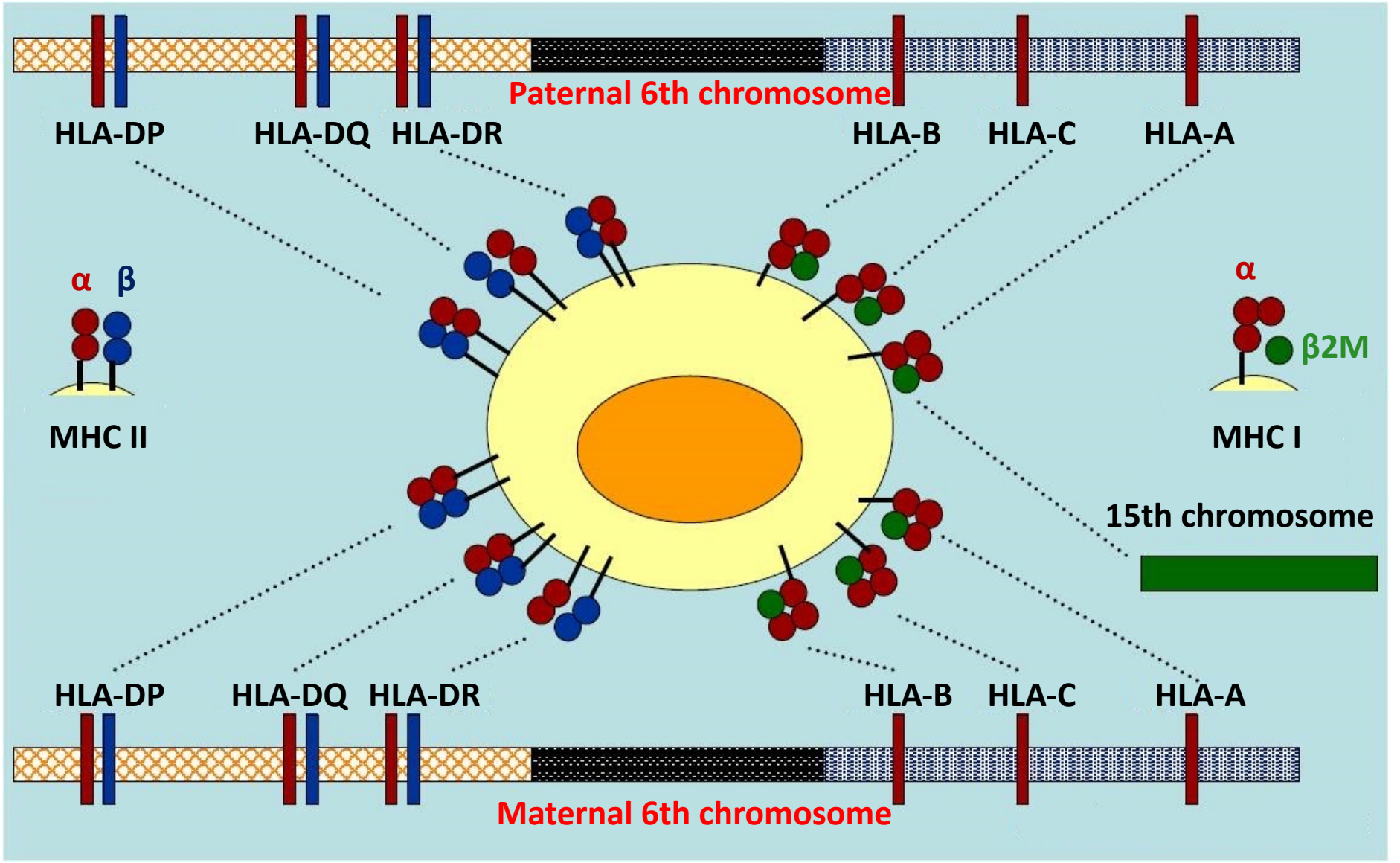
Inheritance of HLAs

- **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.

↓

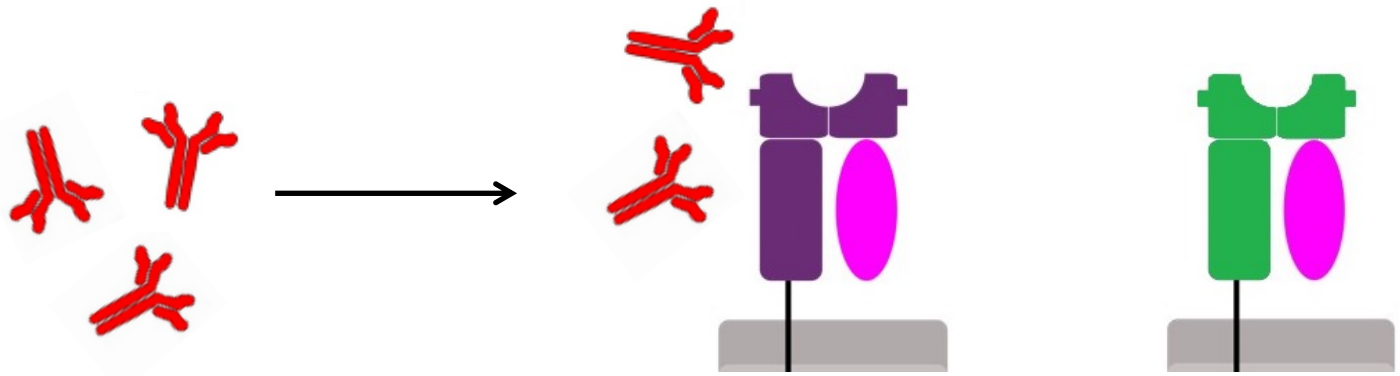
Each person has a characteristic **MHC pool** with different peptide-binding capabilities.

Codominant expression



HLA serotype vs genotype

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



Anti-HLA-B27 antibodies | **HLA-B27** | **HLA-A2**

- 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)

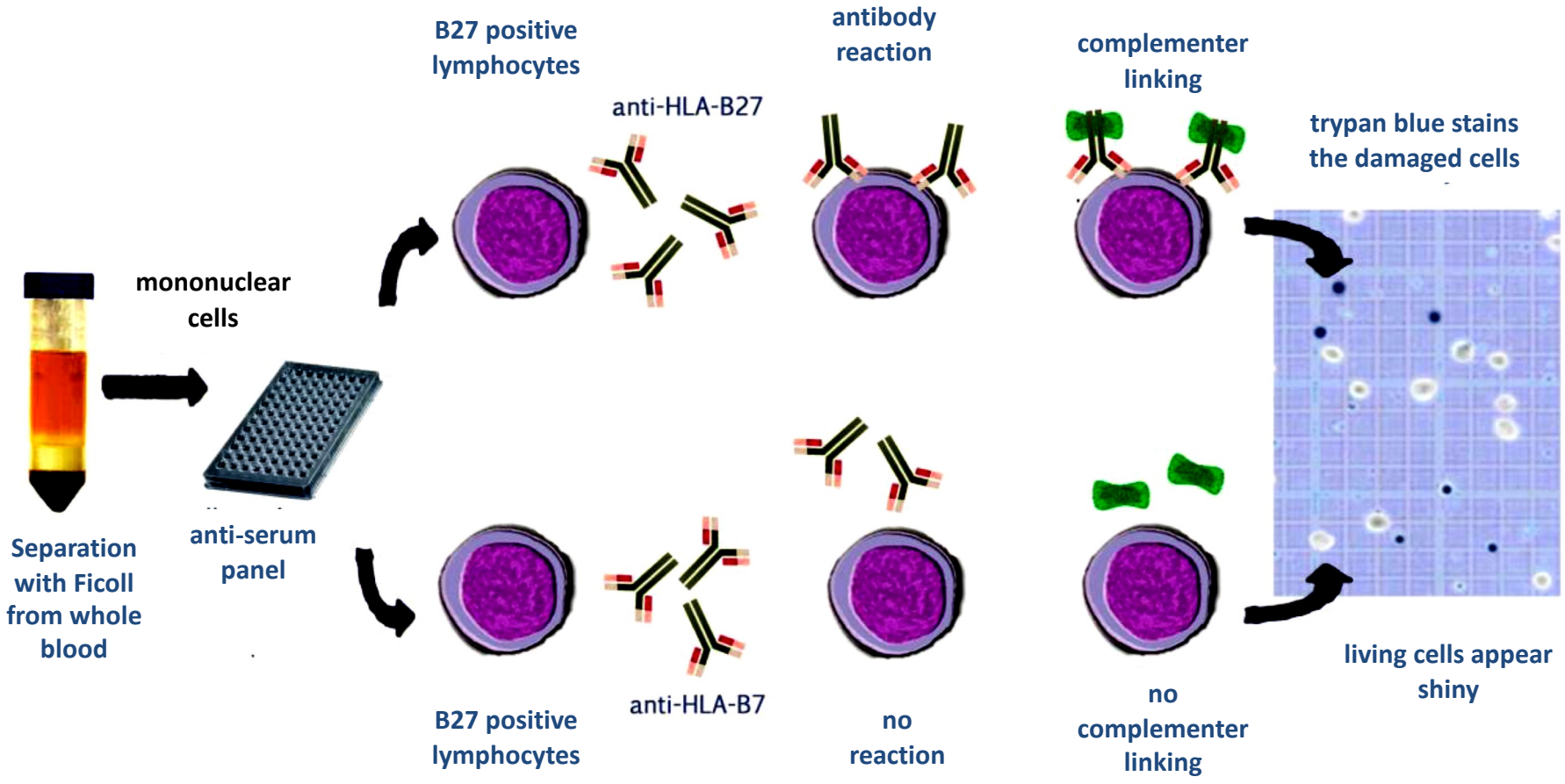
HLA serology

1. **Typing** – investigation of polymorphism of HLA antigens : lymphocytes with unknown antigenicity added to known specificity antibodies.

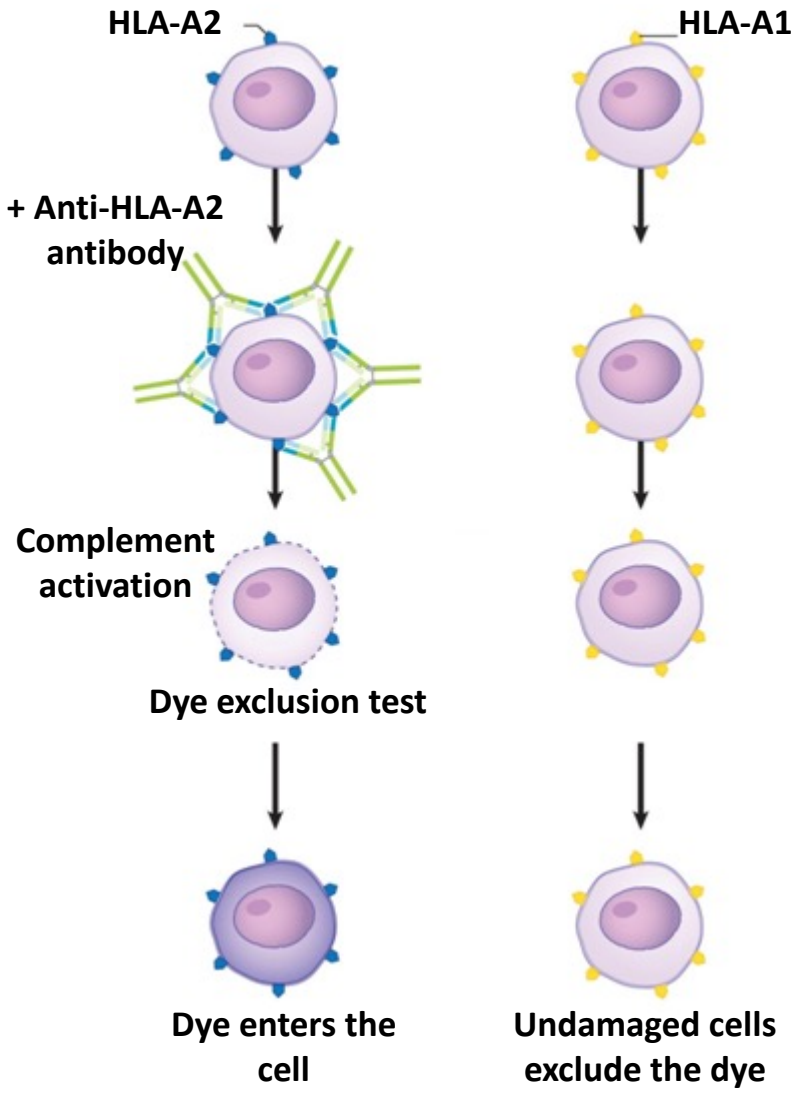
2. **Antibody screening - PRA** (panel reactive antibody) test – detection of antibodies against HLA antigens: the sera that need to be tested is added to known antigenicity cell panel.

3. **Cross match**: in vitro model of antigen-antibody reaction that might occur during transplantation, might result in graft rejection in vivo.

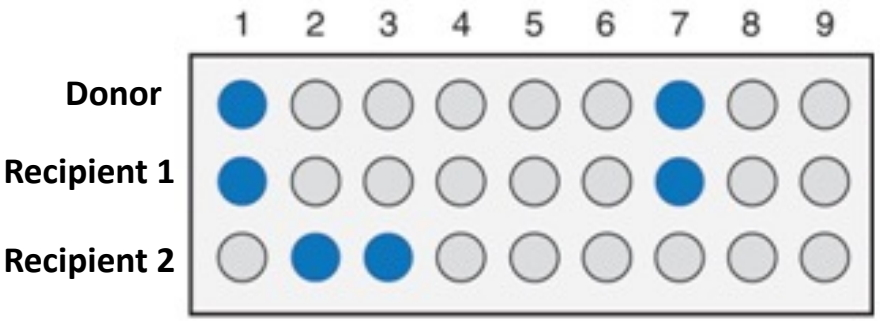
Microcytotoxicity assay (Terasaki)



Microcytotoxicity assay (MCA) – Cross match Terasaki plate

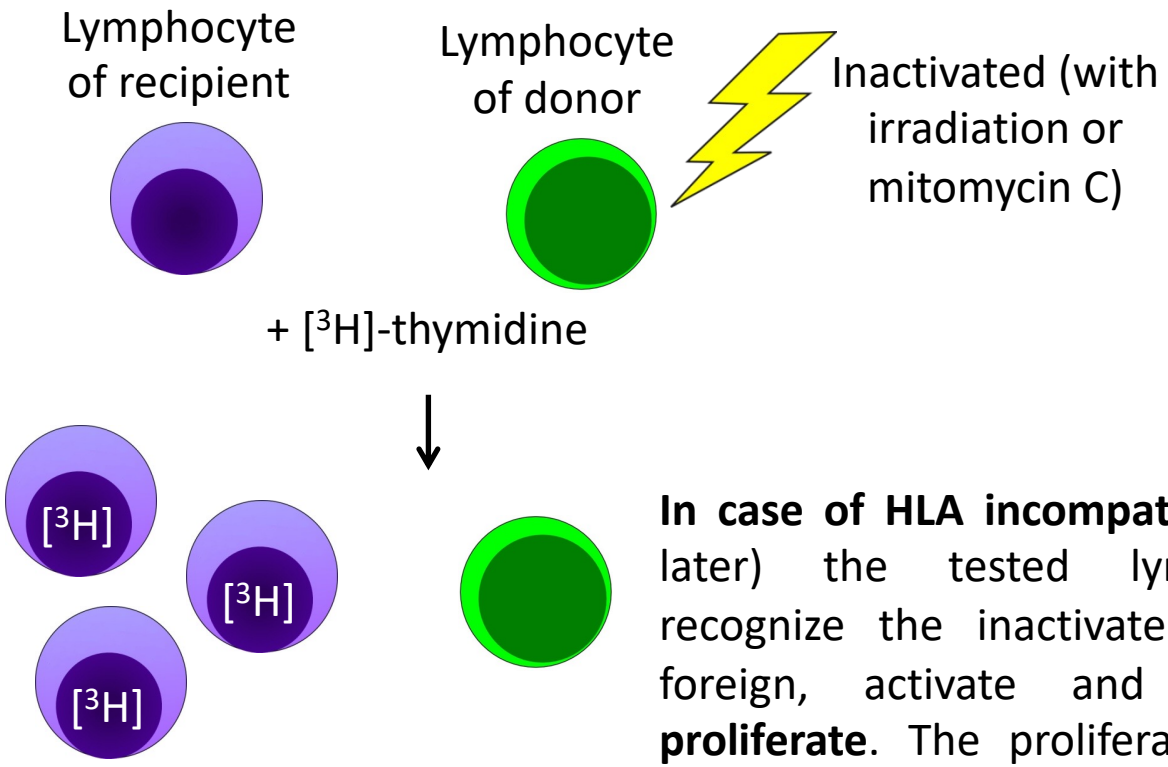


Used to check the **immunological incompatibility** of the donor and the recipient before transplantations. E.g.:



The donor and recipient 1 matches based on the serological test

Mixed lymphocyte culture

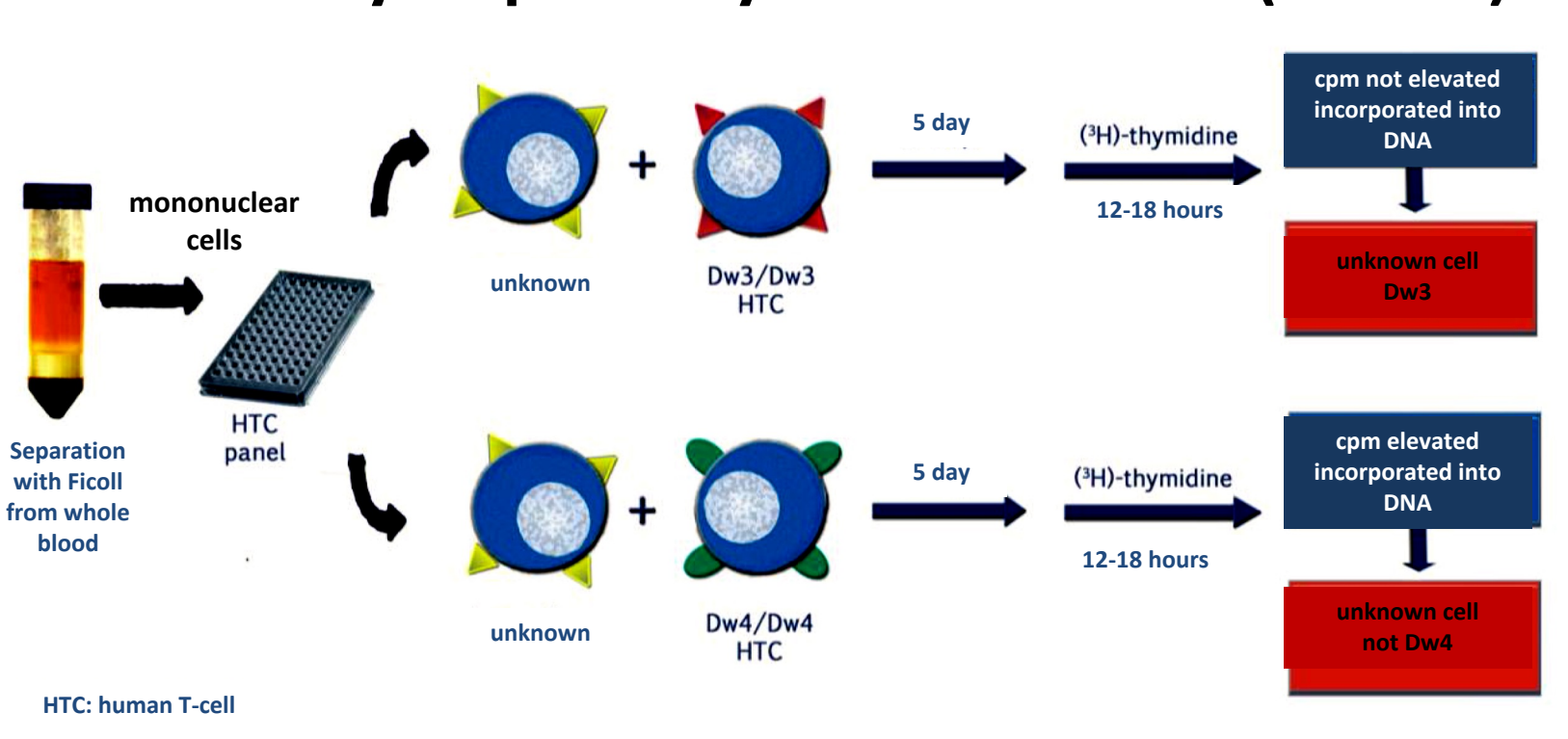


In case of HLA incompatibility (see later) the tested lymphocyted recognize the inactivated cells as foreign, activate and start to **proliferate**. The proliferateing cells will **incorporate the labelled thymidine into their DNA**.

Application:

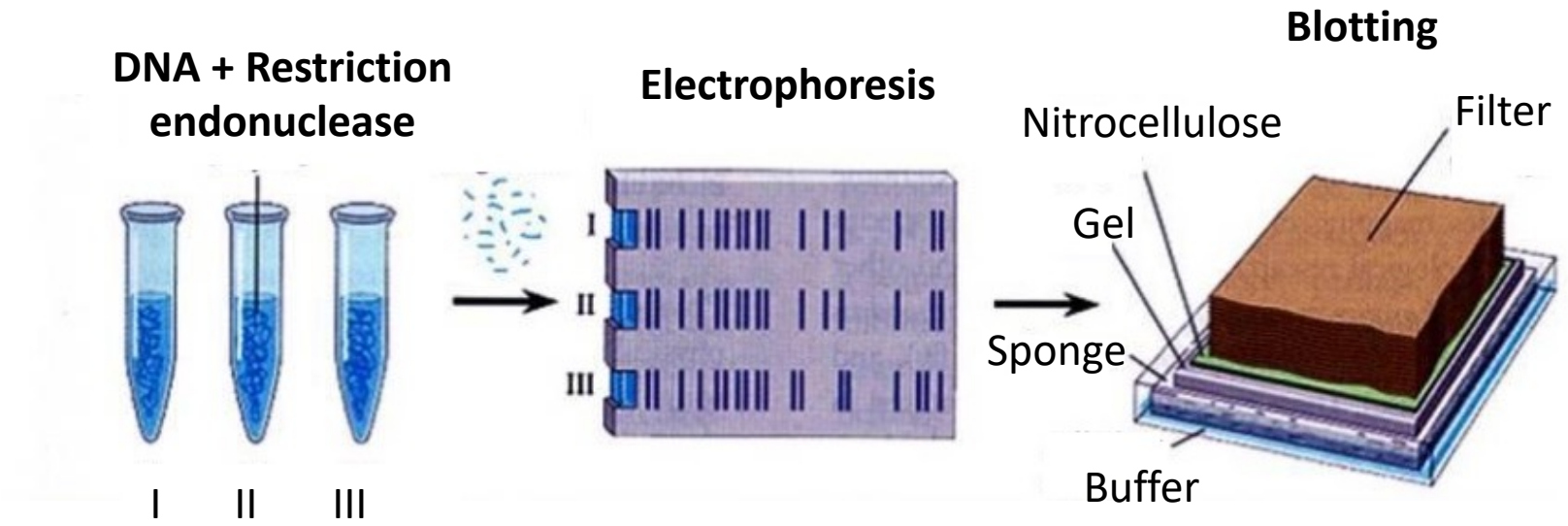
To check the **immunological incompatibility** of the donor and the recipient before transplantations.

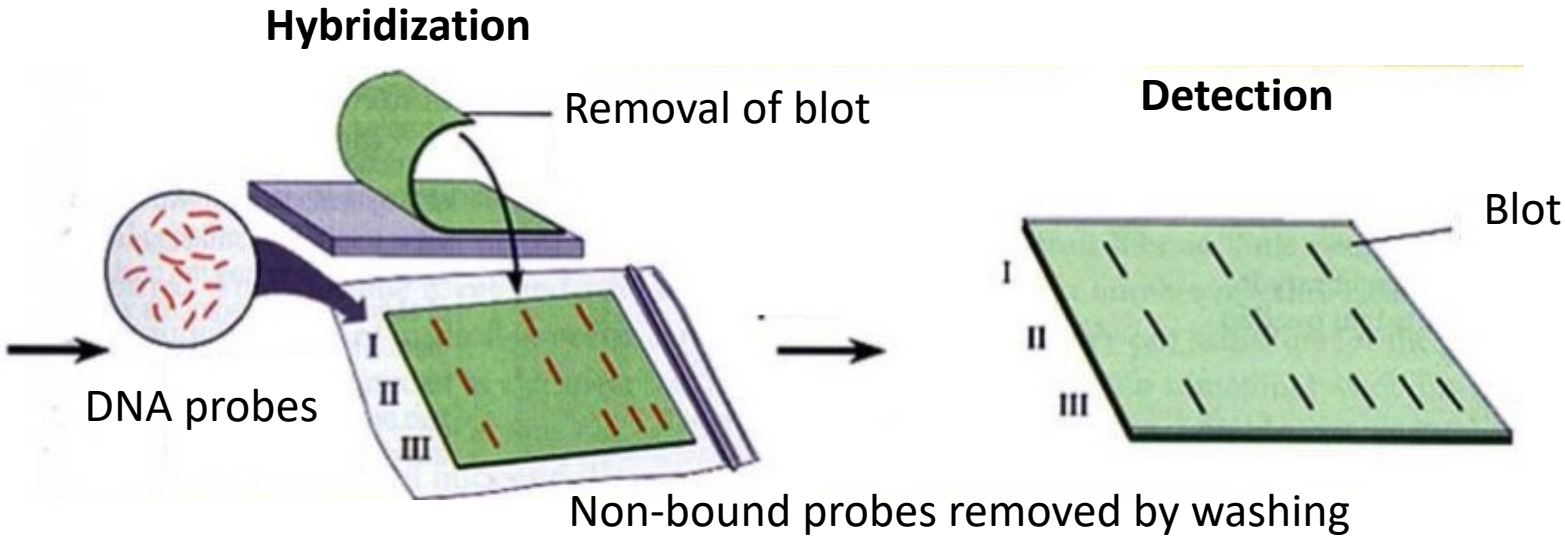
Mixed lymphocyte culture (MLC)



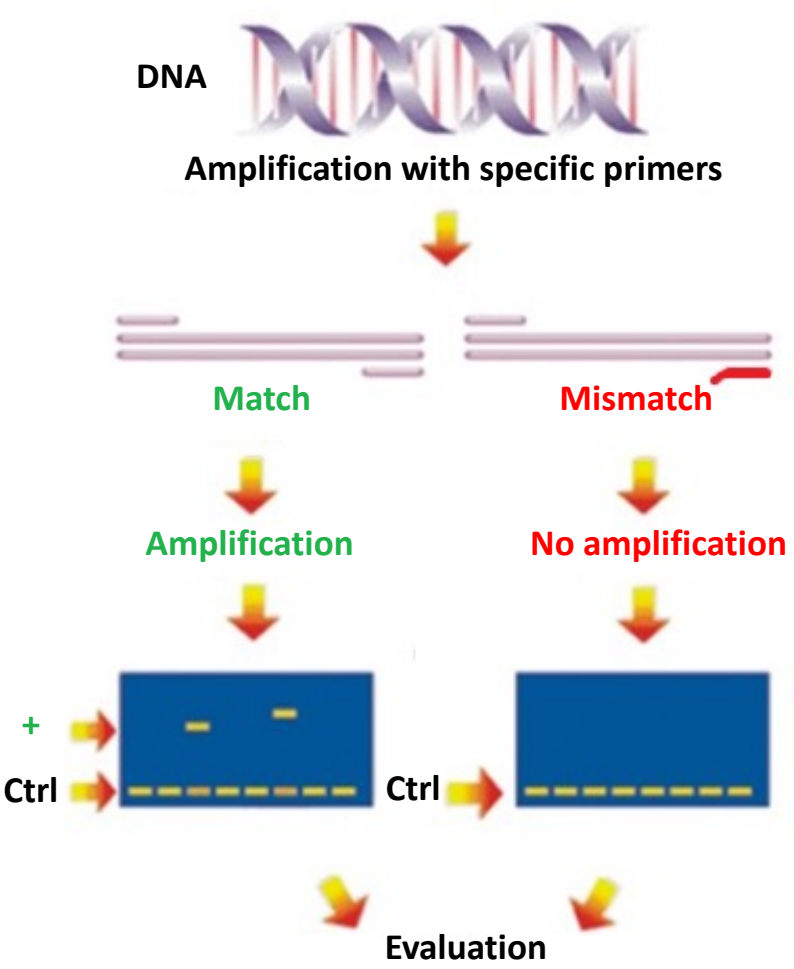
Mixed Lymphocyte Culture (MLC): The lymphocytes of two individuals (eg. a patient's unknown lymphocyte and a laboratory sustained known lymphocyte expressing HLA-Dw) are mixed in a Petri dish and incubated in cell culture for days. If the unknown (patient's) lymphocyte doesn't carry HLA-Dw, which can 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.

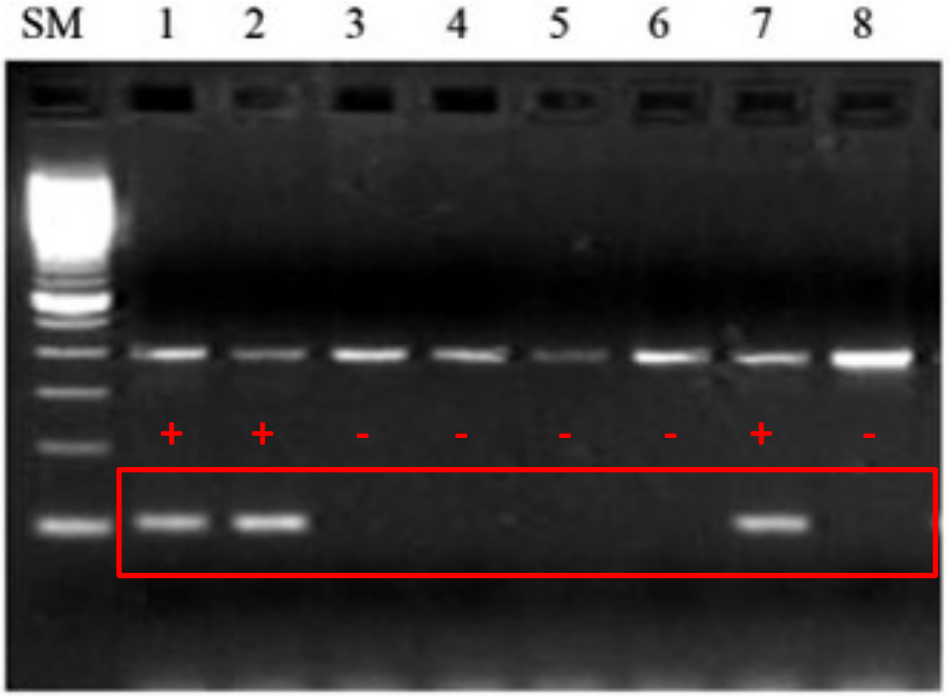
RFLP
(Restriction fragment length polymorphism)





PCR with sequence-specific primers





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

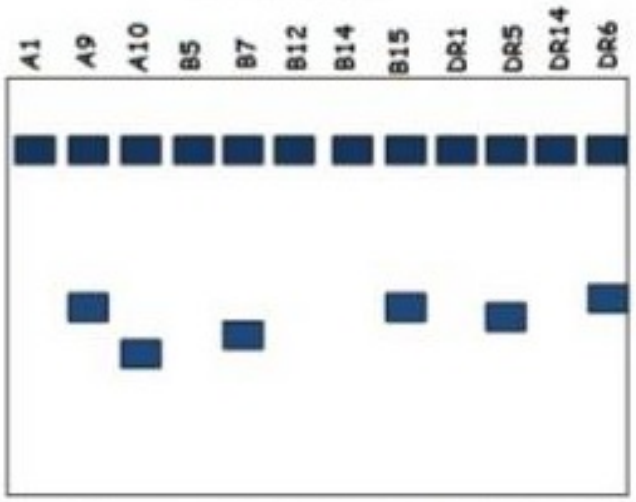
Steps of organ transplantation alert

- 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.

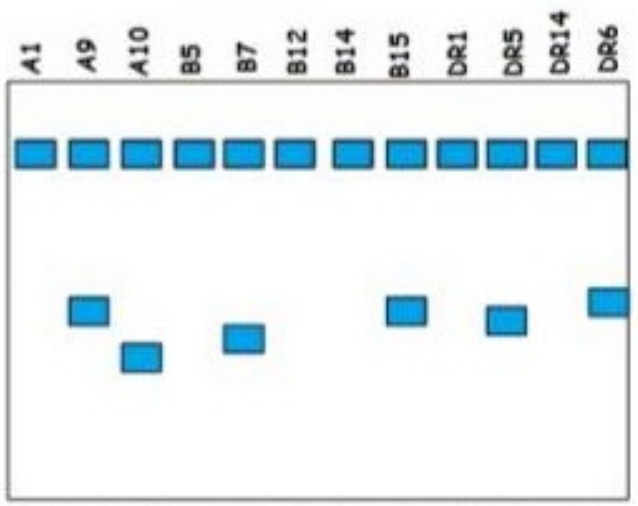
Recipient



HLA genotype:

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

Donor



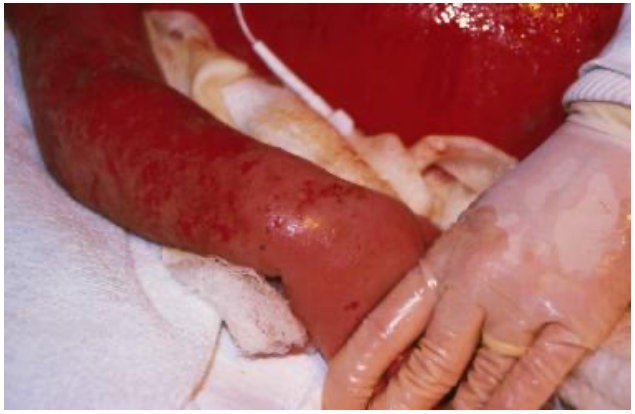
HLA genotype:

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

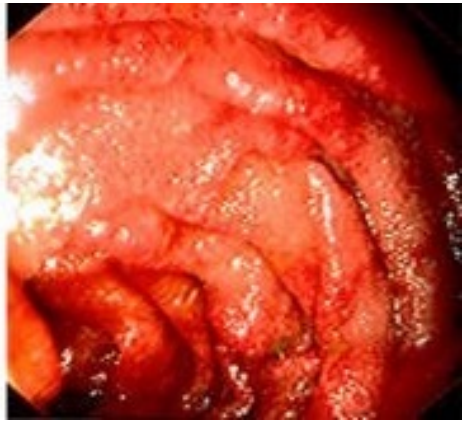
12/12 match ✓

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%.

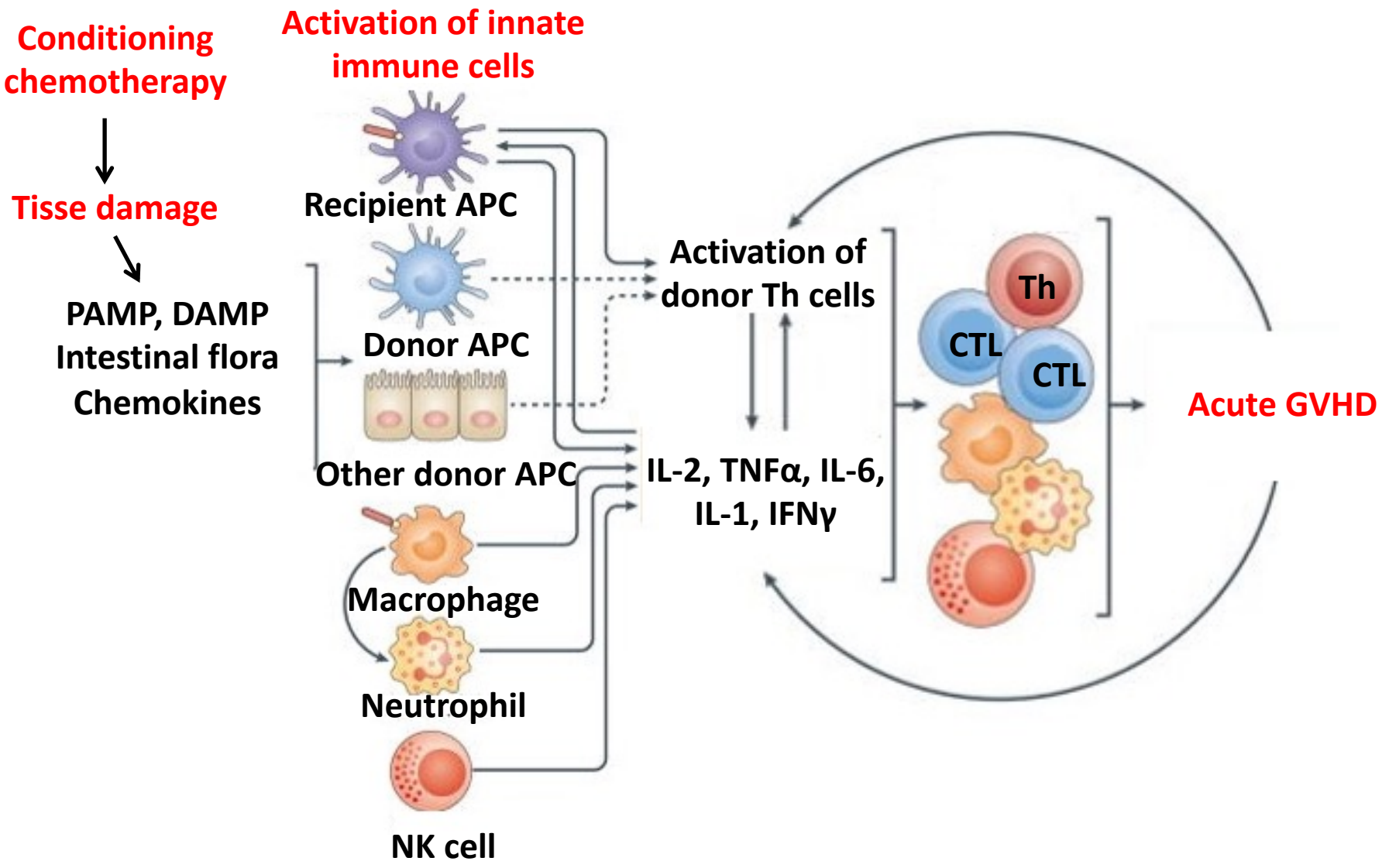


Severe skin GVHD



Acute intestinal GVHD
(endoscopic image)

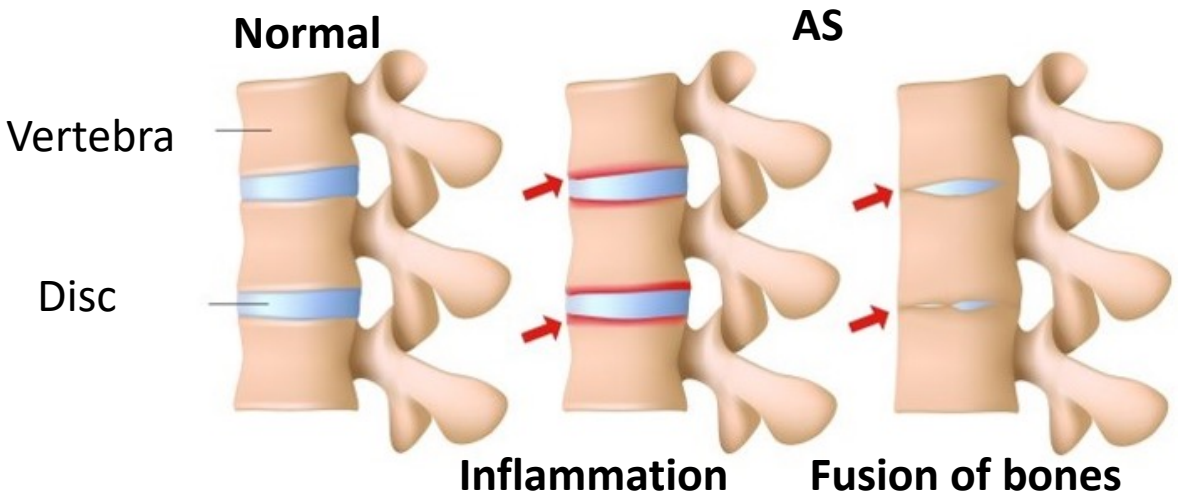
Graft versus host disease 2. (GVHD)

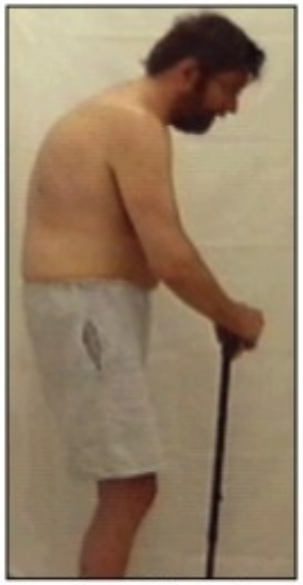


HLA and disease associations 1.

- **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.

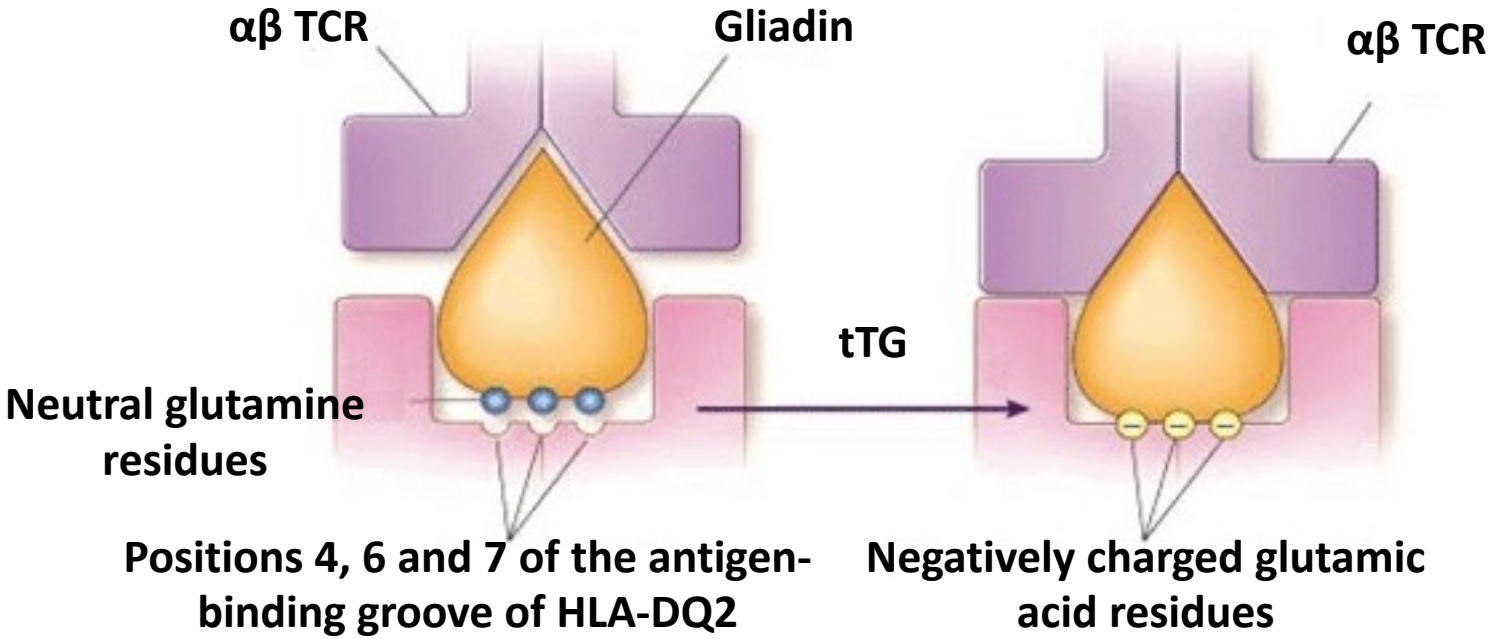
HLA-B27 positivity **only increases the risk of the disease**, it is **not enough on its own** for developing the disease! (This is also true for all the HLA associations.)





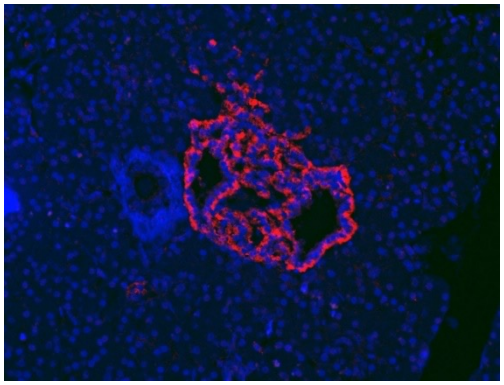
HLA and disease associations 2.

- **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 (mainly SSc)
  - 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

I WANT YOU

FOR STUDENT RESEARCH!

References (not in order)

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