Immunology of allogeneic organ transplantation and allogeneic hematopoietic stem cell transplantation (HSCT)

By Viviana Marin-Esteban

Objectives

Explain the **allogeneic immune response**.

Understand the **immunological mechanisms underlying rejection reactions** in solid organ transplantation:

- **Hyperacute rejection**,
- **acute rejection**
- **chronic rejection**

Identify the main **cytotoxicity mechanisms** involved in the rejection of an allogenic organ:

- direct cell cytotoxicity by T CD8+ lymphocytes
- antibody-mediated cell cytotoxicity ADCC
- antibody mediated complement-dependent cytotoxicity).

Identify molecular **targets of immunosuppressive therapies**.

Understand the immunopathology underlying the **graft vs. host reaction (GVHD)** in hematopoietic stem cell transplantation (HSCT).

Program

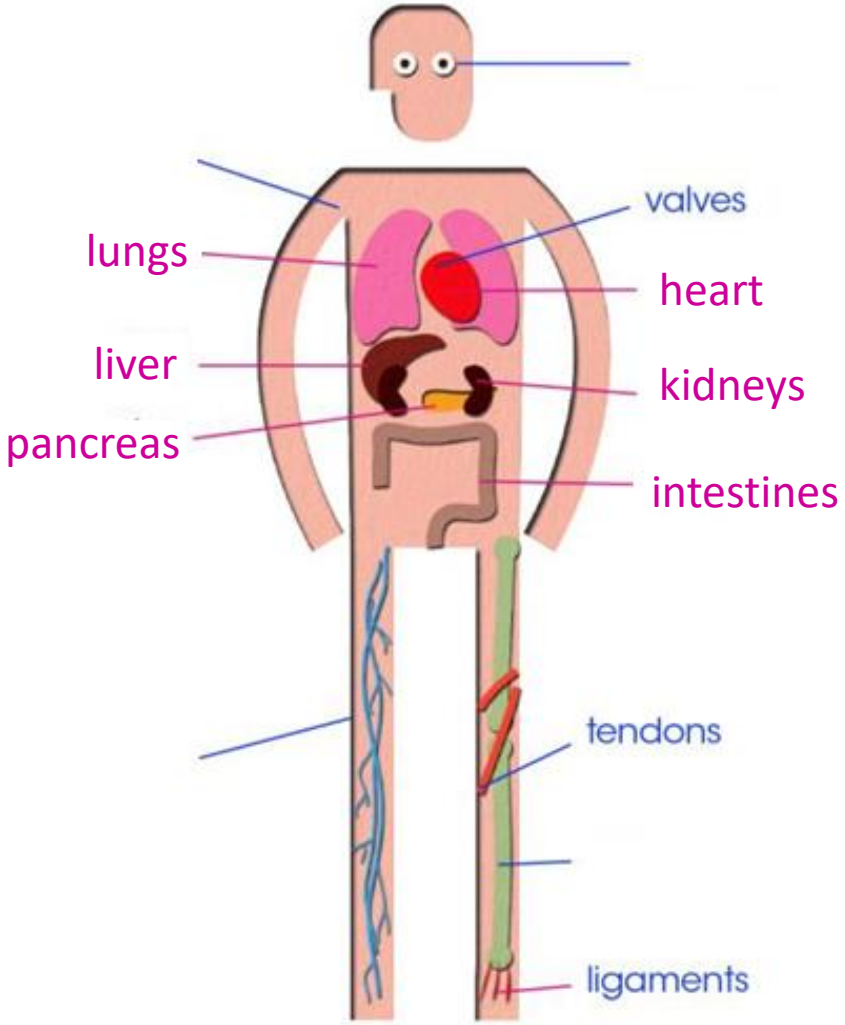
1. Agence de la biomédecine (French Agency of Biomedicine)
2. Definitions
3. History
4. Alloantigens : definition, recognition (direct, indirect, semi-direct), and allogeniec reaction
5. Organ transplantation:
   - Interest
   - Immunological explorations pre-transplantation
   - Donors
   - Donor – recipient selection
   - Complications: immunological mechanisms of rejection
6. Hematopoietic stem cell transplantation (HSCT):
   - Complications : infections and graft vs. host disease (GVHD)

Agence de la Biomédecine

French agency in charge of medical, scientific and ethical aspects related with human body elements :

- **Mange national registries** for organ and HSC donors and transplantations
- Fix rules for transplant priorities
- Promoting organ, tissue, bone donation
- Regulate medically assisted procreation
- Prenatal diagnosis
- Authorizing embryonic and stem cell research
- Biosafety for human body derived elements (except blood)

Organ transplantation activity in France in 2023



Heart	384
Heart-lungs	9
Liver	1342 (18)
Intestines	1
Pancreas	74
Lung	289
Kidney	3525 (557)
Total	**5633 (575)**

*in brackets living donors

Definitions

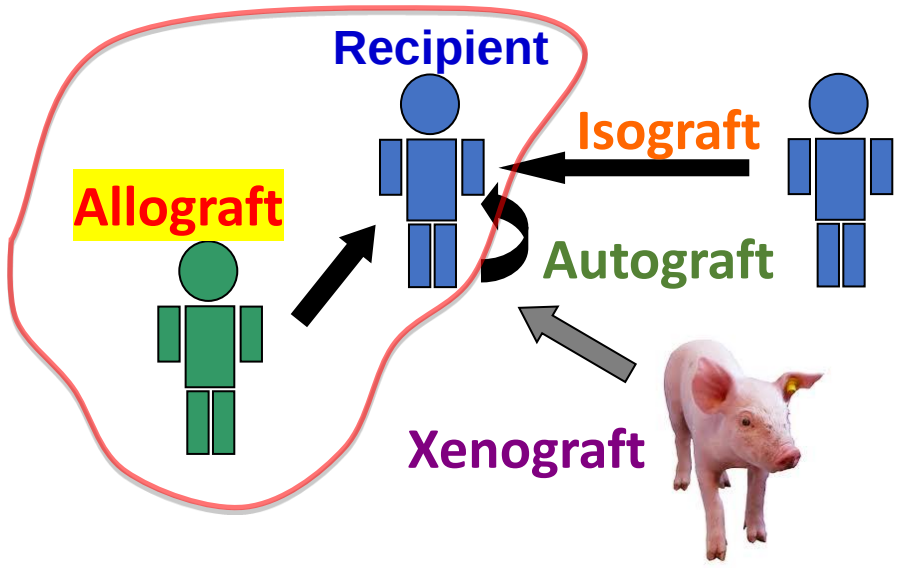
Transplantation is the act of transferring cells, tissues, or organs from one site to another, typically between different individuals.

Autograft**:** tissue transferred from one site to another site on an individual.

Isografts or syngeneic graft: grafting of tissue between two genetically identical individuals such that there are no antigenic differences.

Allograft**:** transplantation of tissues from genetically different individuals.

Xenograft**:** tissue transplanted between individuals of different species, not in clinical use.



History

WW II Skin allograft test for burn victims

Allograft always fail. Second allograft fails faster = memory

1947-1951 Medawar and Gibson works on immune tolerance in mice

Tolerance through high-dose total body irradiation (**TBI**)of the recipient + bone marrow transplantation (**BMT**) from the donor

1952. First allogeneic renal transplant - Paris

Donor: mother. Recipient: her son

Graft/patient **survival: 3 weeks**.

1954. First syngeneic renal transplant - USA

Donor and recipient: Identical twins

Graft/patient **survival: 9 years**.

1956. First syngeneic bone marrow transplant - USA

1958. First successful allogeneic renal transplant - USA

Recipient pre-treatment TBI +/- donor BMT. Out of 12 patients: 11 died within the 1st month, **1 survived 20 years** (TBI, no BMT)

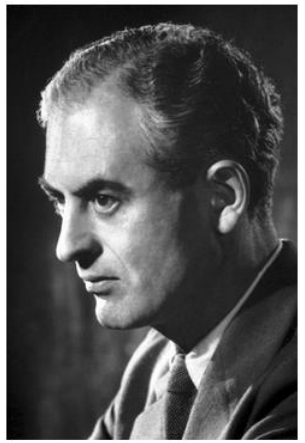
→ Conclusion: clinically, immunosuppression alone is sufficient.

1958-1965 – Discovery of MHC / HLA proteins.

1963. First hepatic transplant - USA

1969. First lung transplant - USA.

1977. First HLA-matched BM transplant from an un-related donor•••



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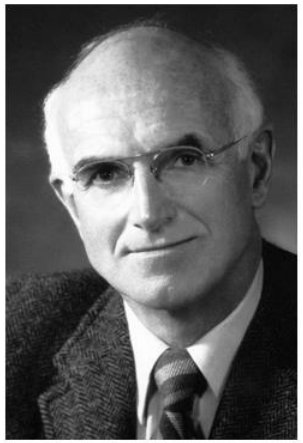
Peter Brian Medawar



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Joseph E. Murray

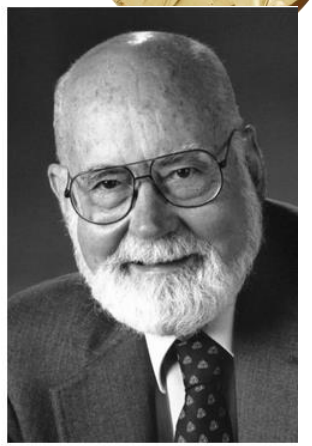
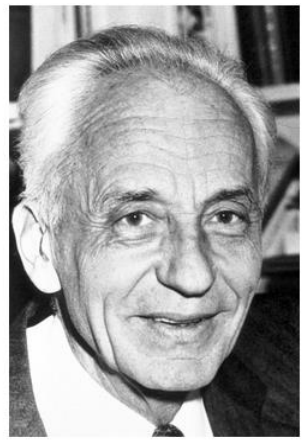


Photo from the Nobel Foundation archive.
E. Donnall Thomas

1960 1990

Photo from the Nobel Foundation archive.
Baruj Benacerraf



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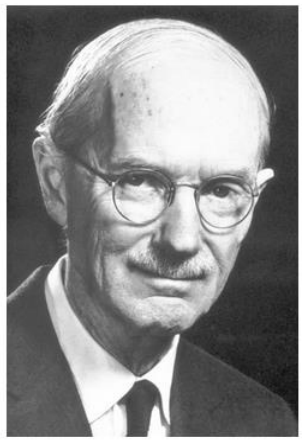
Jean Dausset



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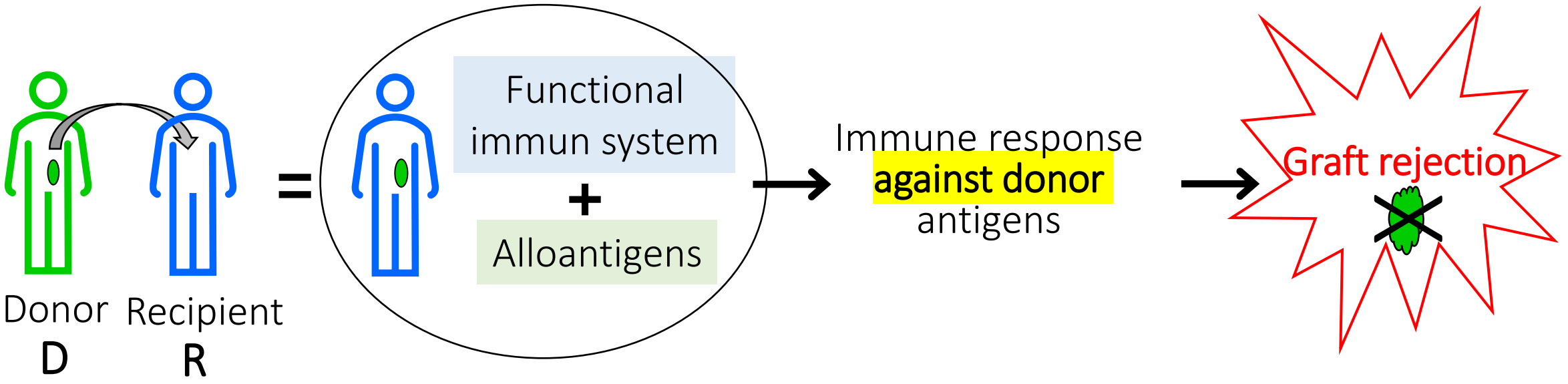
George D. Snell

1980

Allogeneic transplants are recognized as foreign by the recipient's immune system

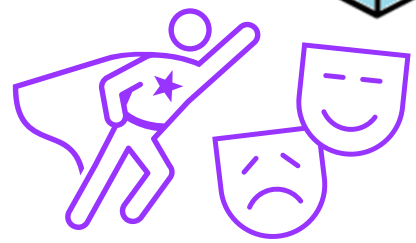
Renal allogeneic transplant

Donor and Recipient genetically different



Immune response against non-Self antigens is the **normal** expected function of the immune system

Allogeneic transplants are accepted with the help of an immunosuppressive treatment



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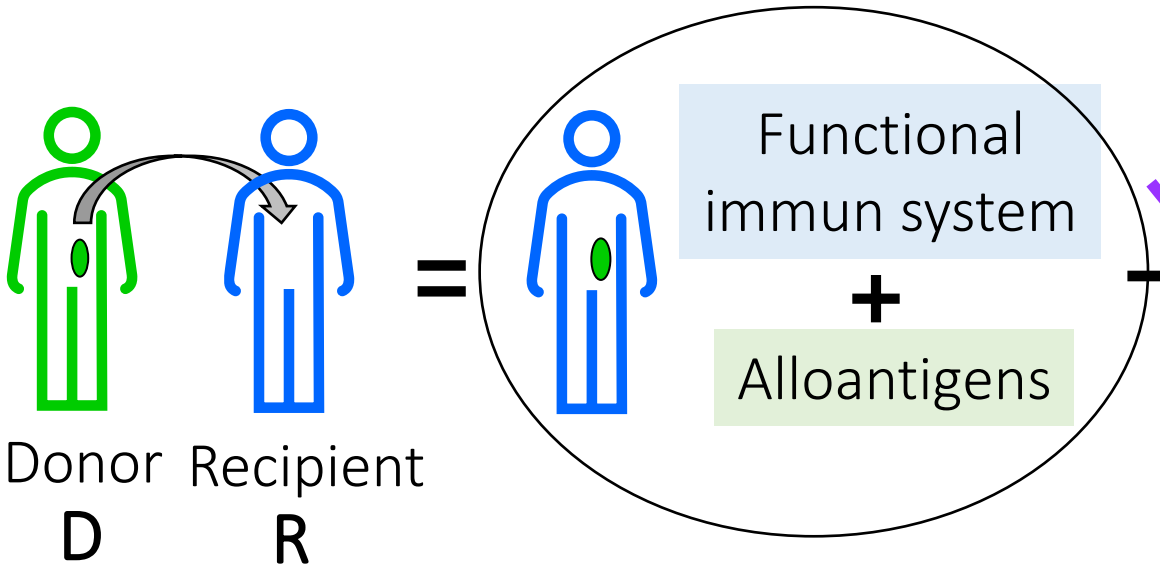


Immunosuppressors

… but they are associated with some adverse effects

Immune response against donor antigens → Graft rejection

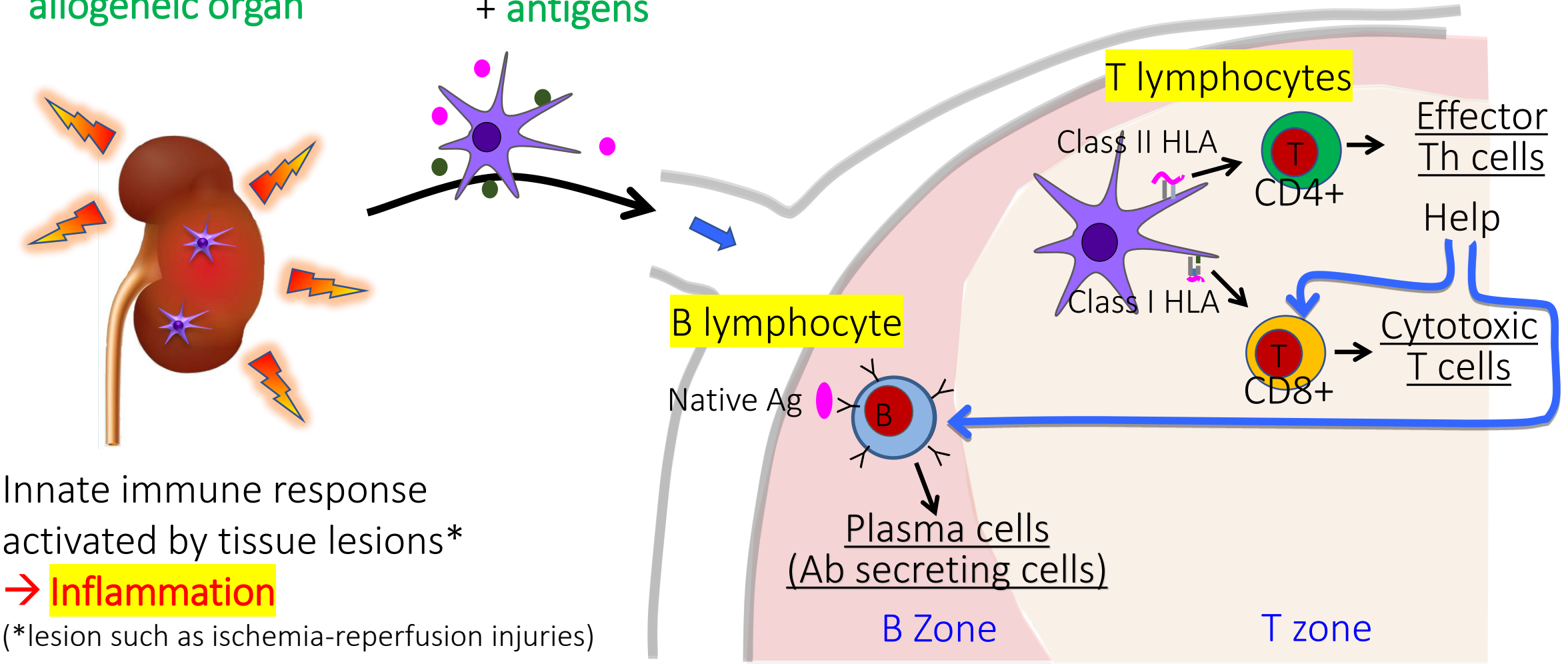
Immunosuppressors block the immune response preventing the aggression against the allogeneic organ

Activation of the allogeneic immune response

Transplanted allogeneic organ

Activated dendritic cell + antigens

Secondary lymphoid organs
Activation of **Ag-specific** T and B lymphocytes



Innate immune response activated by tissue lesions*

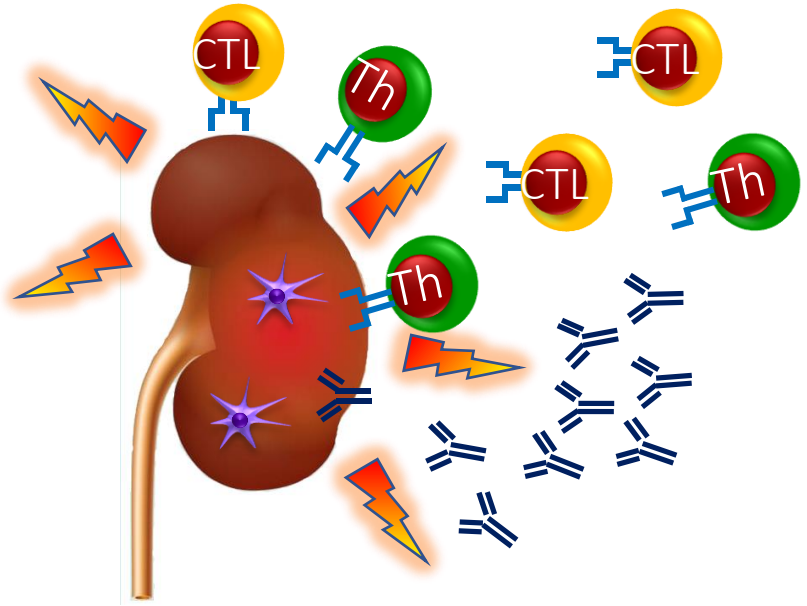
→ **Inflammation**

(*lesion such as ischemia-reperfusion injuries)

Allogeneic immune response in action

To learn

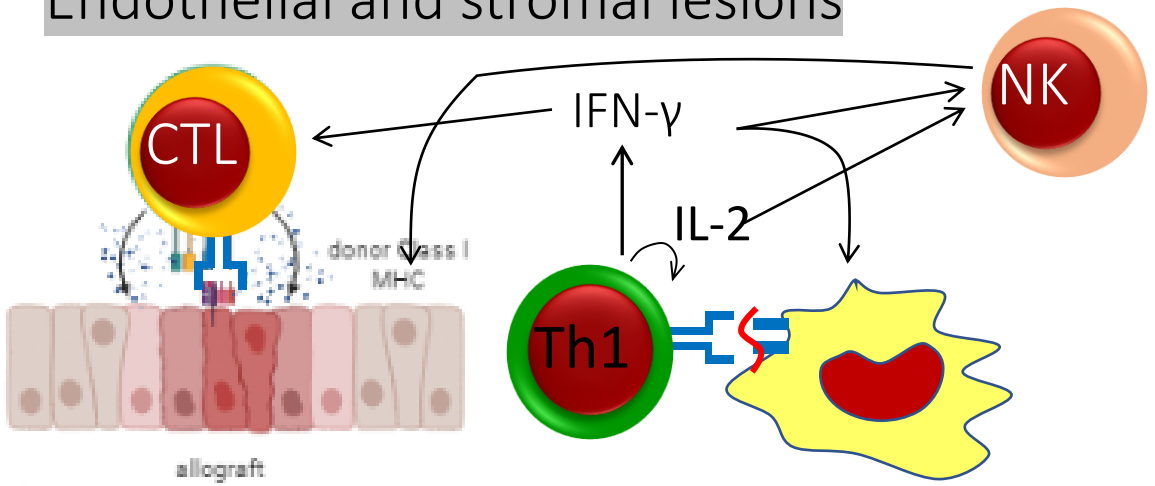
Effector T lymphocytes and allo-antibodies attack the transplanted organ



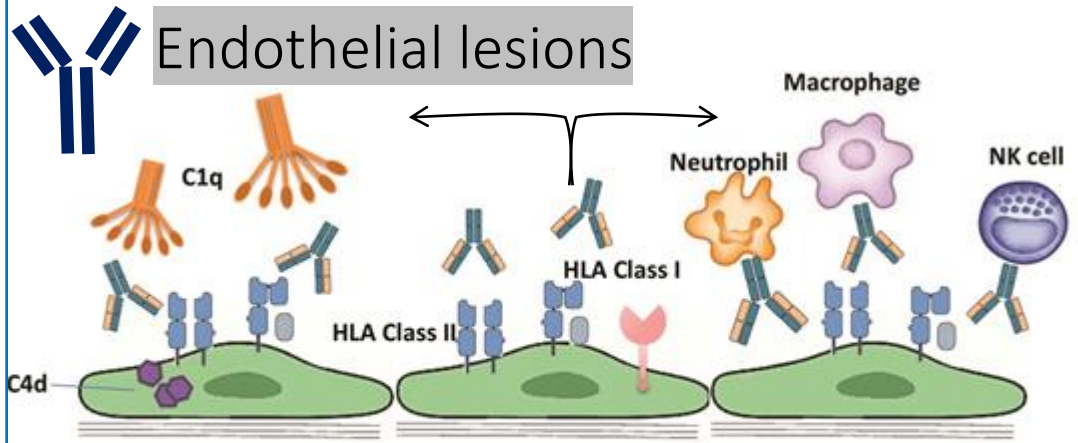
Inflammation

+ tissue lesions promoted by the adaptive immune response

Endothelial and stromal lesions



Endothelial lesions



Three groups of alloantigens - Immunological incompatibility between donor and recipient in transplants

To learn

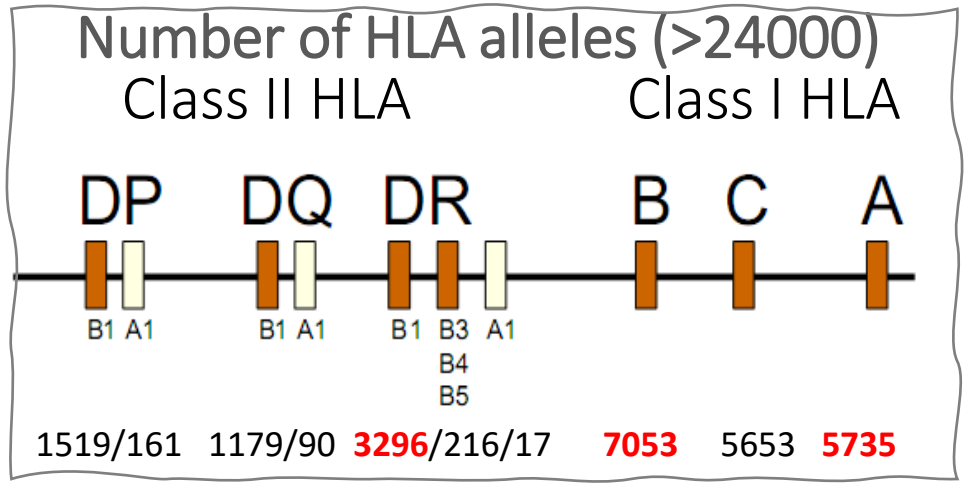
ABO blood antigens

Expressed by different cell types (red blood cells, vascular endothelium, …)

Natural Ab anti-A or anti-B (IgM) are produced in individuals not expressing these antigens

HLA molecules (MHC)

HLA incompatibilities are at the origin of very strong immune responses.



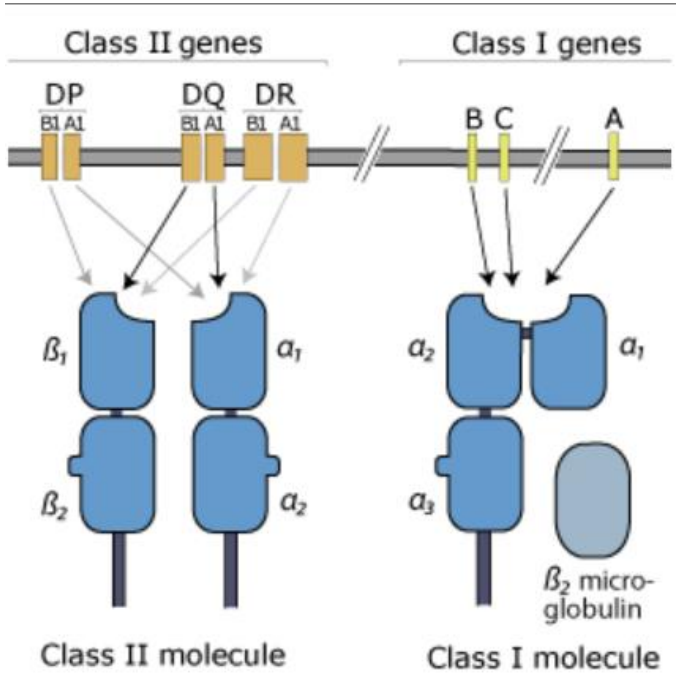
Minor Histocompatibility antigens (mHAgs)

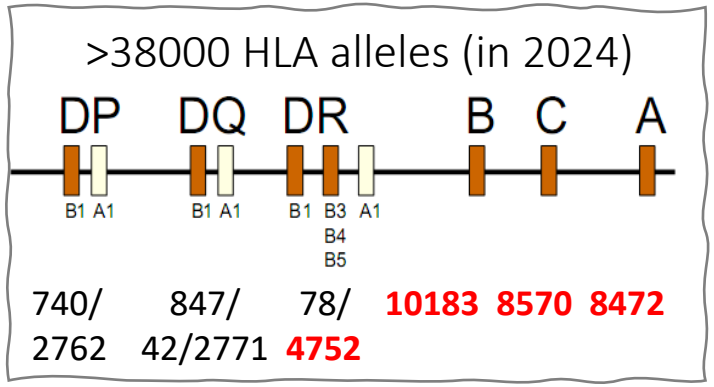
HLA: human Major Histocompatibility (MHC) genes and molecules

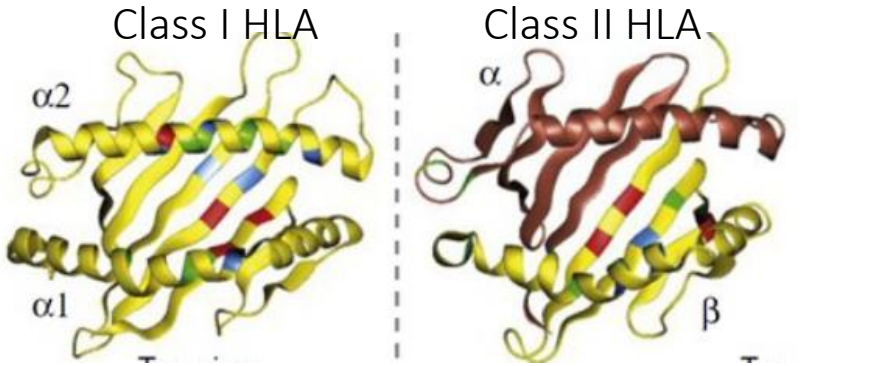
HLA Function: Presentation of peptides to TCR molecules (Ag receptor of T lymphocytes).

Polygenic, polymorphic and codominant system

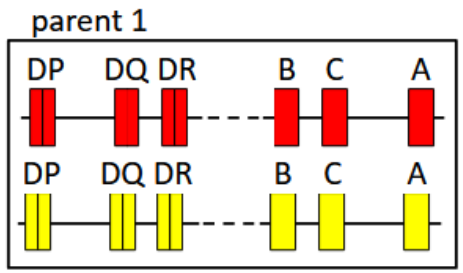
- 3 loci for classic HLA class I molecules. 6 or 7 loci for classic HLA class II molecules (heterodimers)
- Usually inherited by "haplotypes".
- > 38000 HLA alleles (2024). Polymorphic residues clustered in peptide binding region and in surface of interaction with TCR. Large polymorphism = rare to find a 100 % HLA identical unrelated donor.

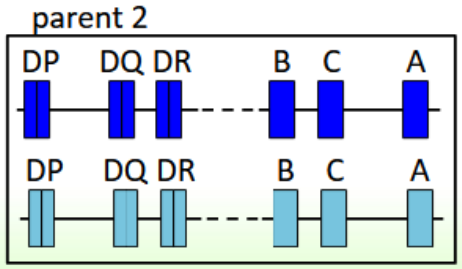




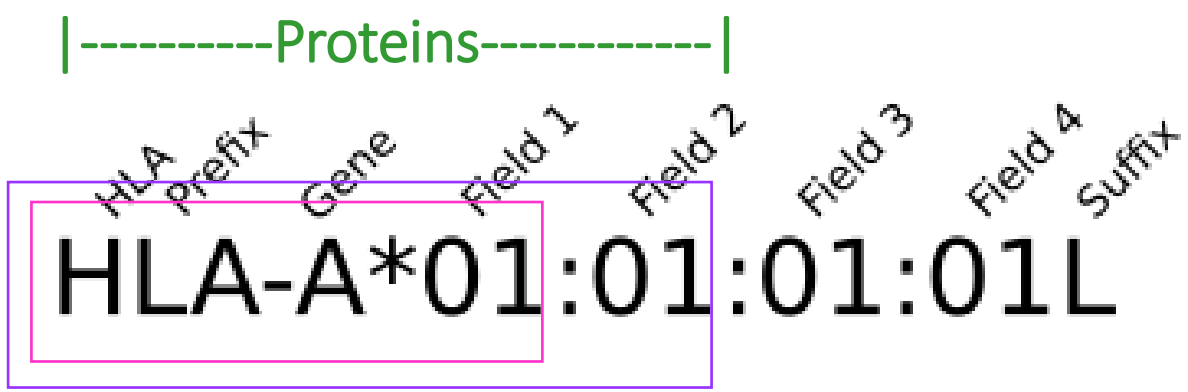


Top view. Polymorphic residues: red blue green





Nomenclature of HLA alleles (2010)



Field 1: allele group (Low resolution typing)
Field 2: specific HLA protein (High or allelle resolution typing)
Field 3: synonymous DNA substitution in coding region
Field 4: difference in non-coding region
Suffix: difference in expression

- A: aberrant
- C: cytoplasm
- L: low expression
- N: null, no expression
- Q: questionable
- S: secreted

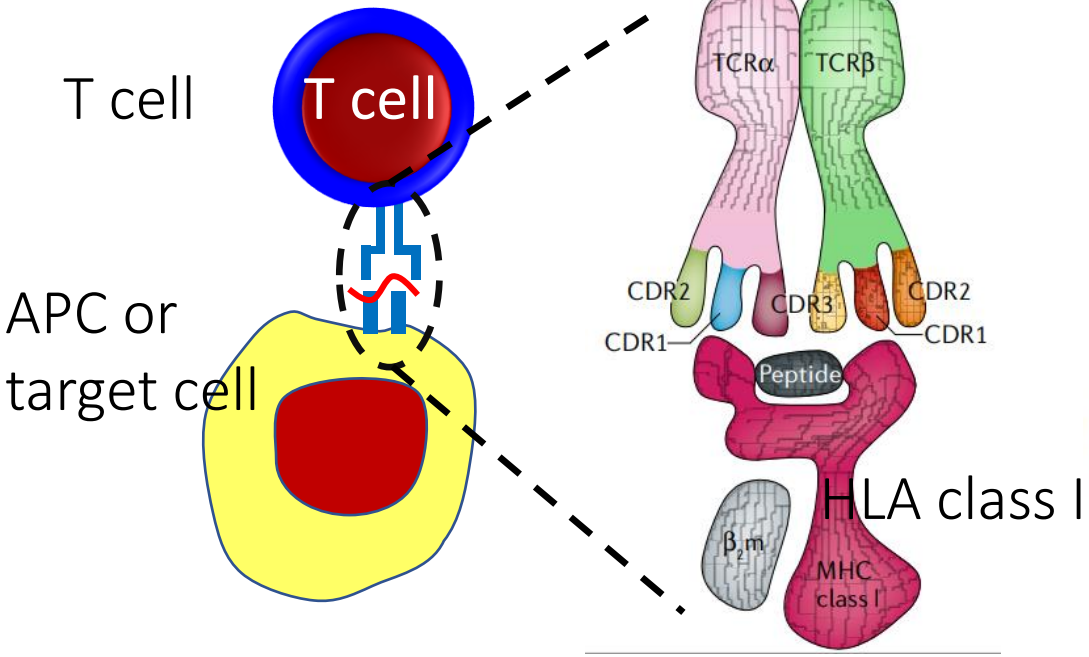
*Typed by molecular biology techniques

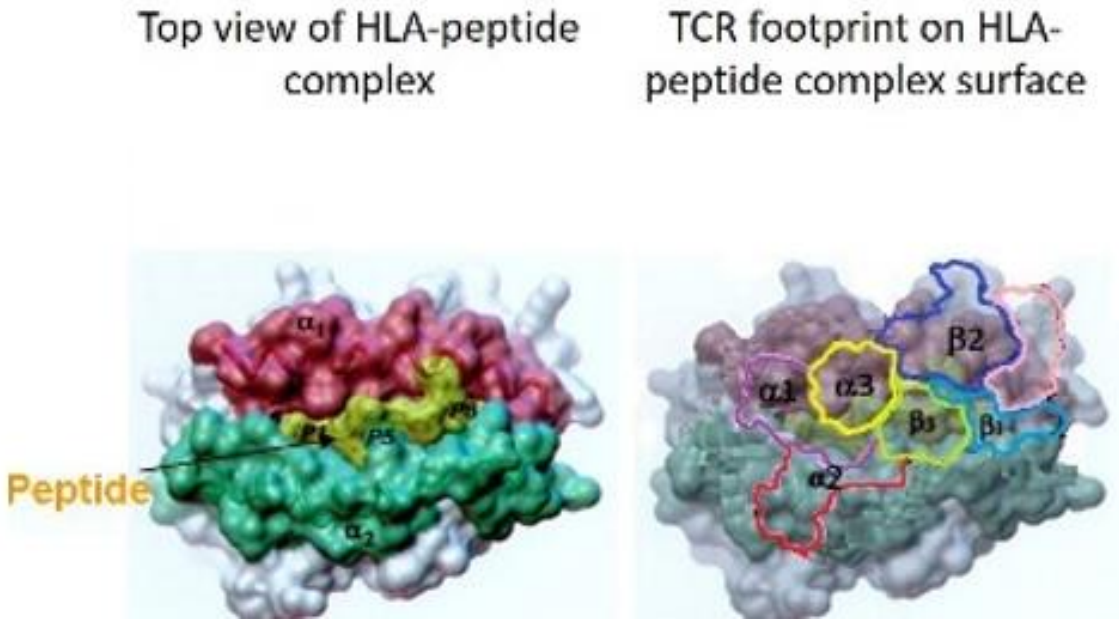
https://www.anticorps-enligne.fr/areas/human-leukocyte-antigen/

Allogeneic HLA molecules (donor) can directly activate recipient's TCRs

When scanning cell surfaces, **TCR interacts with** both the **HLA and the peptide**.

The TCR repertoire of an individual is selected (thymus) to have a weak interaction with **self HLA / self peptide complexes** (central tolerance). In every day immune responses TCR affinity is only affected by variations related to the presented peptides (non Self-Ag), as self HLA molecules don't change.

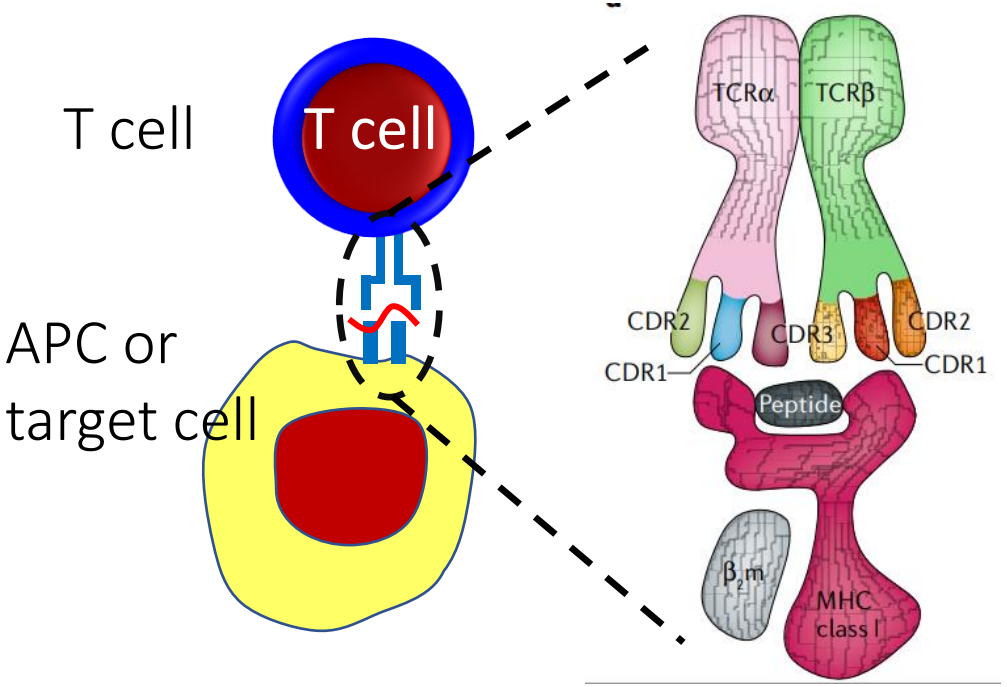


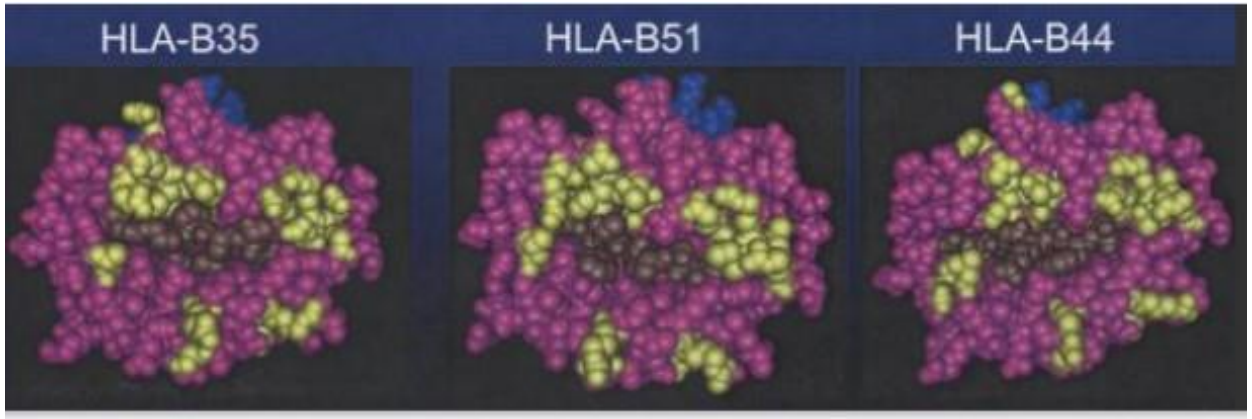


HLA molecules from donor can directly activate TCRs from recipient

Face to **allogeneic HLA molecules**, certain TCRs (hypervariable CDR1, CDR2 regions) can interact with high affinity with polymorphic HLA residues, resulting in the activation of the T lymphocytes bearing these TCRs.

Allogeneic (donor) HLA molecule can directly activate some T cells form recipient (up to 2% recipient naive T cells!). In comparison, the estimated frequency of naïve T cells specific to a conventional antigen (a non-self peptide) is only 0.0001%.





Top view of three HLA-B molecule models. Polymorphic residues are in yellow, peptide in brown

Three groups of alloantigens - Immunological incompatibility between donor and recipient in transplants

ABO blood antigens

Expressed by different cell types (red blood cells, vascular endothelium, …)

Natural Ab anti-A or anti-B (IgM) are produced in individuals not expressing these antigens

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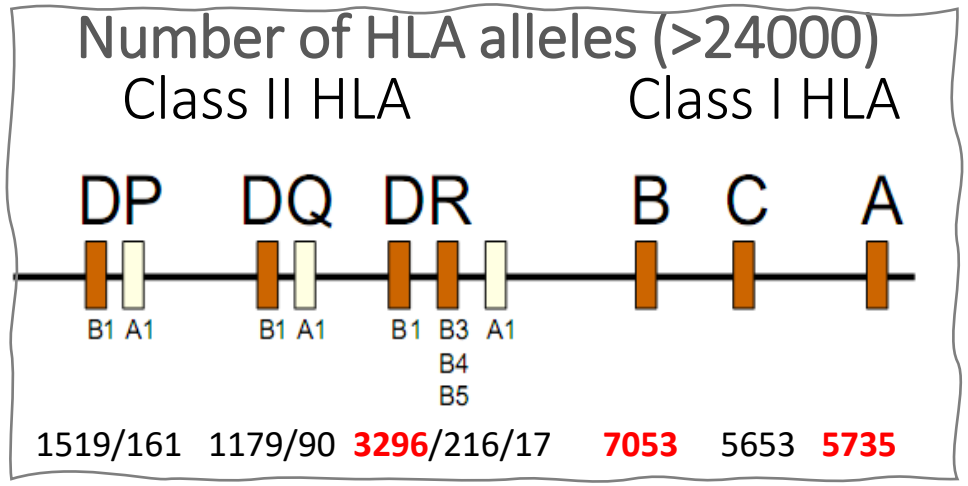
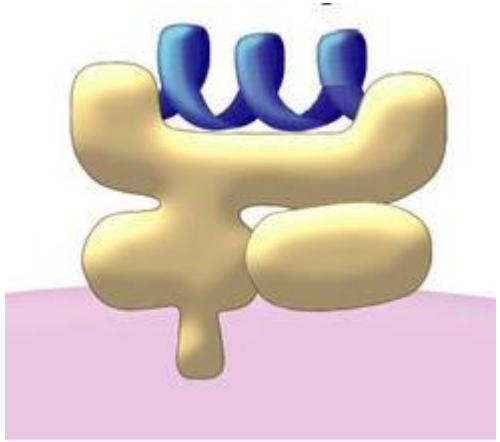
Minor Histocompatibility antigens (mHAgs)



Minor Histocompatibility antigens (mHAgs)

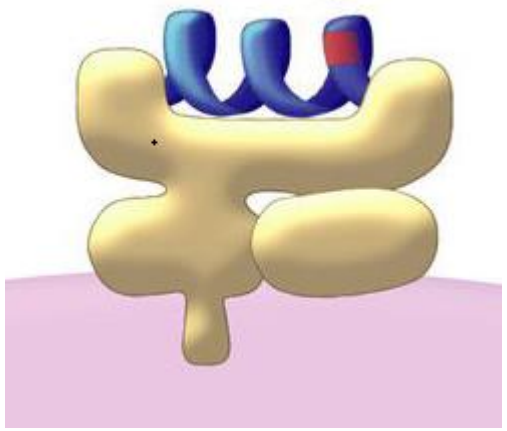
Antigens (Ag) derived from any donor protein, other than HLA proteins, that display sequence differences from recipient's proteins. The recipient has no developed immune tolerance to these "foreign Ags". These differences exist because **normal protein polymorphism** and **natural genetic difference such the presence of Y chromosome** in men.

Self peptide in patient



Patient immune system is tolerant to this peptide

One aa polymorphisme in donor

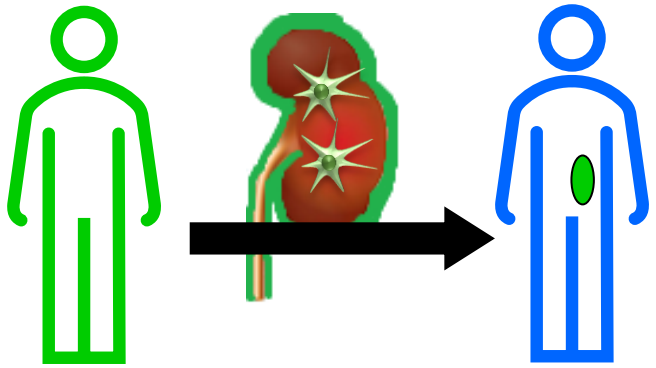


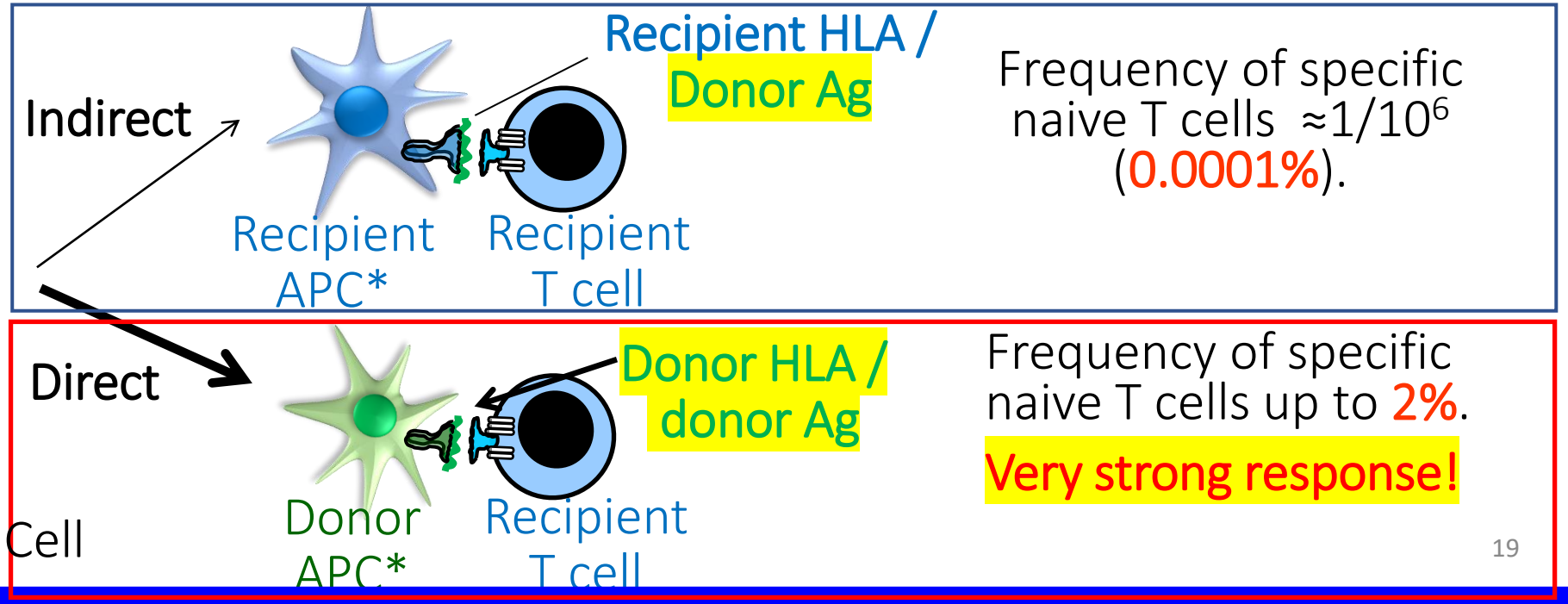
The transplanted organ produces this protein variant. This peptide could be recognized with high affinity by certain recipient TCRs, triggering an aggressive T cell response against the transplanted organ.

Pathways of alloantigen recognition

1. **Indirect allorecognition**: "**Classic mode**" of recognition of non-self Ag. The recipient APCs process the allogeneic antigens and present them to the recipient T cells.
2. **Direct allorecognition:** recipient T cells recognize intact allogeneic HLA/peptide complexes expressed on donor's APCs
3. **Semidirect allorecognition:** donor MHC proteins are transferred intact* to recipient APCs enabling them to present allogeneic MHC–peptide complexes directly to recipient T cells.
   (*tr**ogocytosi**s: acquiring part of the plasma membrane and cytoplasm of another cell through direct contact)

Renal allograft





*APC = Antigen Presenting Cell

Activation of T allogeneic response

The allogeneic immune response against transplants is a normal immune response initiated by the recipient to eliminate non-self antigens. However, it is unwanted.

The expected issue in transplant medicine is ensuring the acceptance of transplants:

- Preventing immune rejection (achieved through immunosuppressants).
- Ideally, inducing tolerance toward the transplanted organ (a goal not yet achieved and a major area of research).

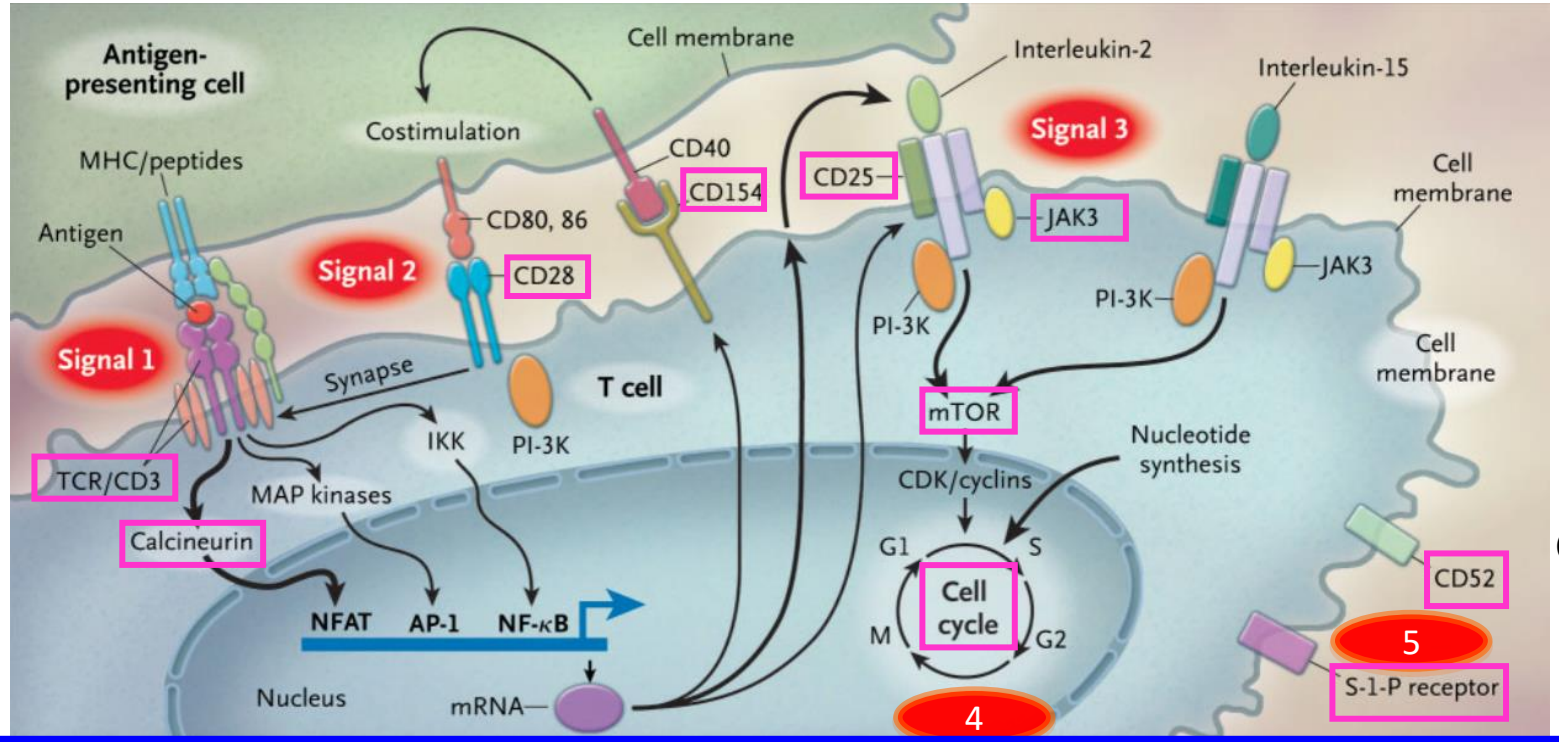
Activation of T allogeneic response

Classic steps of activation of **naive T lymphocytes** (in part valid for effector and memory T cells):

1. **TCR activation** by specific **HLA / Ag complex**
2. **Costimulation**
3. **Cytokines**: IL-2 production and CD25 synthesis
4. Clonal expansion and differentiation

} Secondary lymphoid organs / tissues

5. Migration to the inflammatory focus and direct or indirect cytotoxic action



Chemokine receptors and cell adhesion molecules

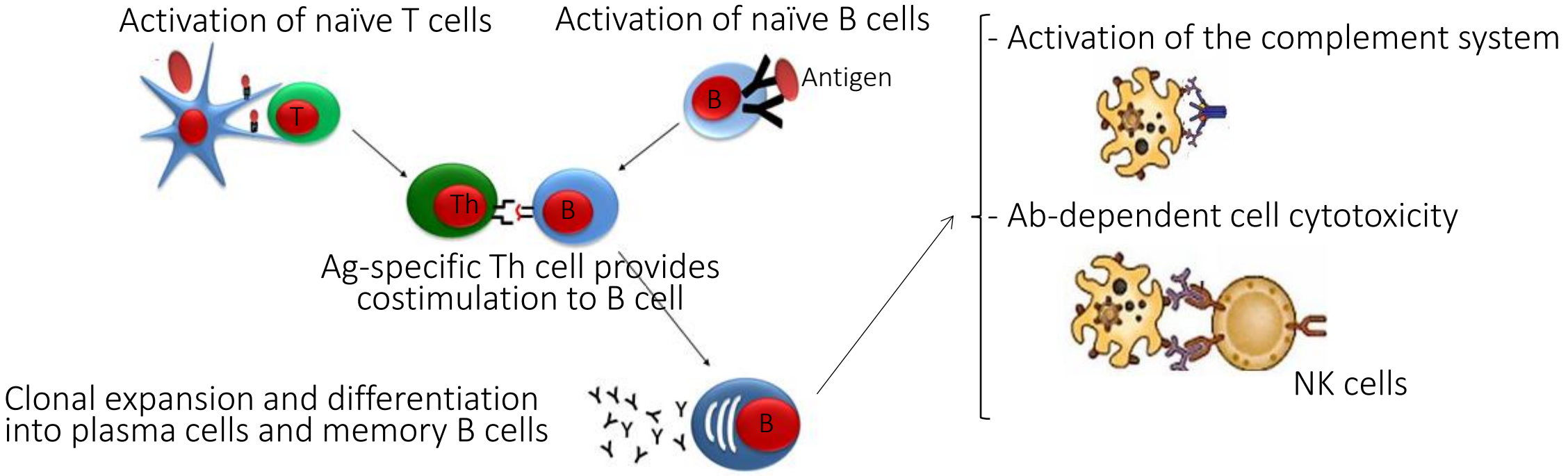
Halloran, New Engl J Med 2004

Activation of B allogeneic response

Classic steps of activation of **naive B lymphocytes** (T-dependent response):

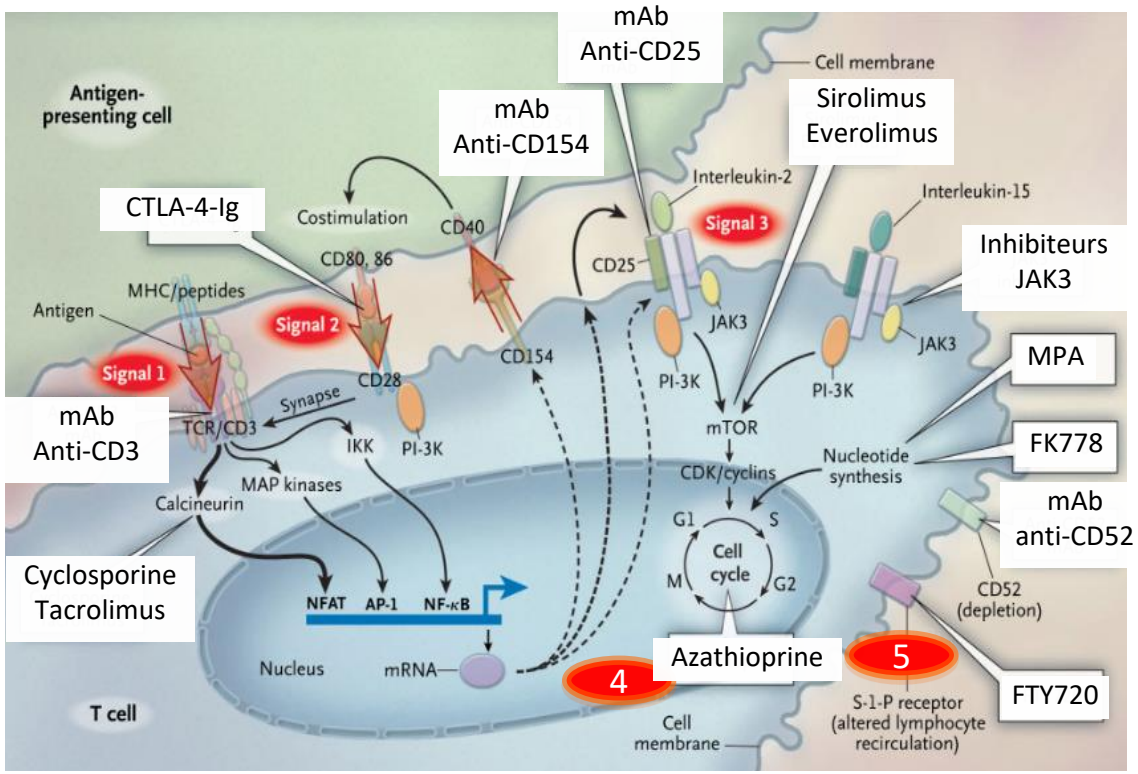
1. **BCR activation** by specific Ag
2. **Costimulation** provided by Ag-specific effector T helper cells
3. **Cytokines** provided by effector T helper cell
4. Clonal expansion and differentiation
5. Migration of plasma cells to the bone marrow. Production of Ag-specific IgG

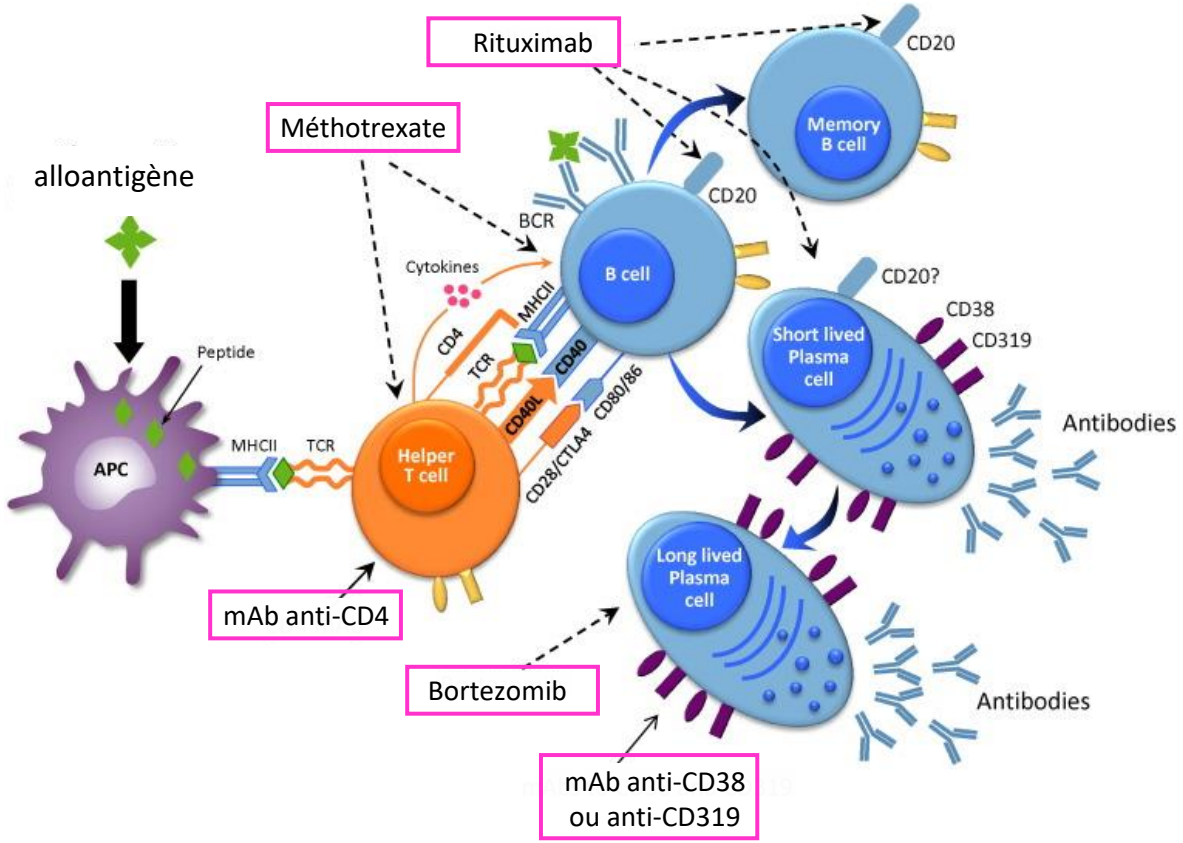
} Secondary lymphoid organs / tissues



Multiple targets for immunosuppressor treatments

→ Course by Aurélie Tran-Barrail





Halloran, New Engl J Med 2004

Immunological aspects of solid organ transplantation
- Rejection responses -

Clinical benefits of ortgan transplantation

Vital transplants (heart, lungs, liver): only solution for a patient with terminal organ disfunction to continue livng (no substitute methods).

Non-vital transplants (kidney, intestine, pancreas - beta cells) : improve the quality and quantity of life for patients.

Donors:

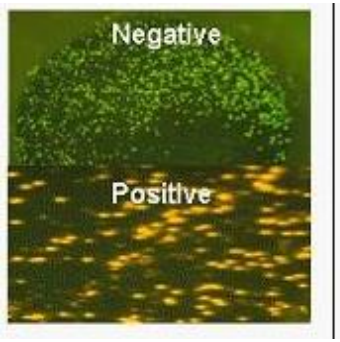
- Donor in brain-death state: multi-organ and multi-tissue donor
- Heart-stopped donor: kidneys, (potentially liver)
- Living donor: a related adult or a adult who has lived with the patient for at least two years*. Can donate **one kidney**, **a lobe of the liver**, a portion of pancreas, or a lobe of a lung.

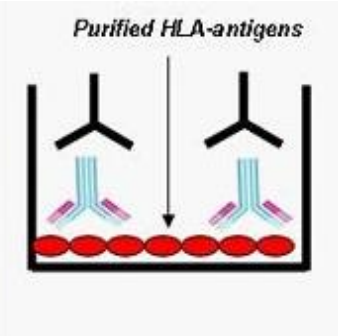
Pre-transplant immunological evaluation

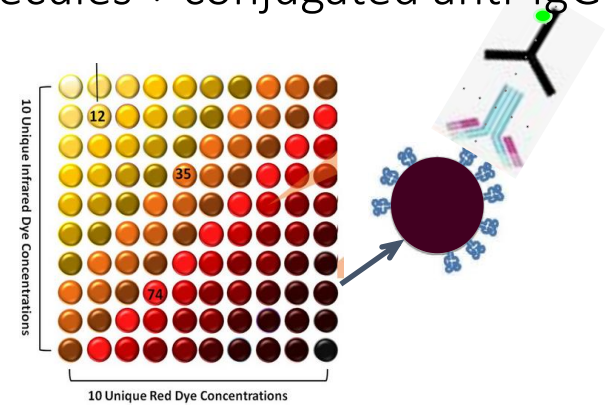
Recipient: pre-transplant testing and waitlist enrolment (for non-vital transplant).

Exploration to minimize immune complications:

1. **HLA typing** (by PCR, 8 to 10 loci: the two loci of HLA-A, B, C, DRB and DQB).
2. **Detection of anti-HLA antibodies in recipient.** Immunization circumstances are previous transfusion, pregnancies or prior allotransplants.
   - Microlymphocytotoxicity test (no longer done): serum + cell lines expressing known HLAs + complement + vital dye.
   - LUMINEX / ELISA: serum + solid support coated with known HLA molecules + conjugated anti-IgG Ab.







To anticipate infectious complications: identification of seropositivity for VIH, VHB, VHC,…

Other medical check-ups

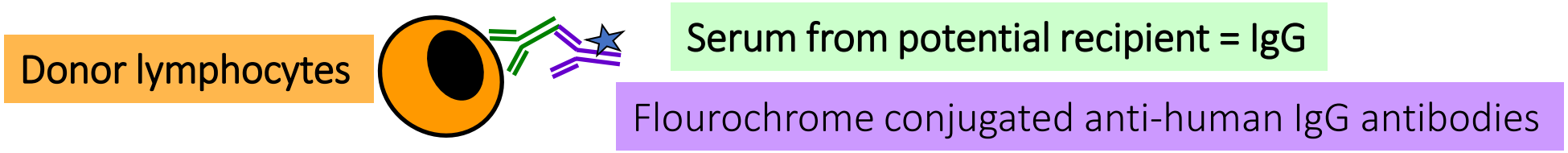
Final cross-match test

When a potential donor is identified →

ABO compatibility + **best possible HLA compatibility** based on the urgency of the situation (Renal Tx: best possible compatibility. Heart Tx: is an urgency)

Number of HLA incompatibilities or **mismatches**: refers to the number of HLA alleles presents in donor but not in recipient*.

3. **Cross-match test**: Just before transplantation to determine if recipient have Anti-donor Abs



Detection of Abs by indirect immunofluorescence, analysed by cell cytometry.

This result must be negative to proceed with the transplant.

*Next slide

Example of HLA incompatibilities or mismatches

typing of 3 loci = 6 alleles (low resolution typing for HLA-A, -B, -C, and -DRB1)

Recipient

HLA-A 01, HLA-A 24, HLA-B 39, HLA-B 44, HLA-DRb1 01, HLA-DRb1 02

Donor

HLA-A 01, HLA-B 09, HLA-B 44, HLA-DRb1 01, HLA-DRb1 11

- Low resolution typing
- Donor is homozygous for HLA-A1, heterozygous for the other HLA loci here presented.
- Two HLA mismatches exist between donor and recipient from the recipient point of view

https://ascopost.com/issues/october-25-2016/donor-selection-for-hla-matched-hematopoietic-cell-transplantation-1/

Post-transplant follow-up

1. Resumption of transplant function

2. Immunosuppression:

Preventive and curative treatment of graft rejection.

Patient under immunosuppressors throughout whole life.

3. Complications:

Immunological complications: rejection episodes

Infectious complications*: viral reactivation (HCV, HBV, CMV), pathogens transmitted by the donor, opportunistic and nosocomial infections (Pneumocystis, Candida)

Toxicity of immunosuppressors*

Lymphoproliferative syndromes and cancer*

Patient's underlying disease

*Unwanted effects of immunosuppressive treatments

Different physiopathologies of graft rejection

1. Hyperacute rejection (immediate < 24h… to 5 days)
2. Acute cellular or antibody-mediated rejection (after 5 days to >1 year)
3. Chronic rejection and chronic allograft dysfunction (> 90 days to several years later)

→ Different underlying pathological mechanisms

1. Hyperacute rejection: immediate

Causes

Presence of Ab against "donor" before transplantation (alloimmunized patient) or shortly after procedure.

- Anti-HLA Abs
- Anti-ABO Abs (ABO expressed by red blood cells, epithelial and endothelial cells)
- Abs against other alloantigens e.g. anti-endothelium

Pathologic Mechanism

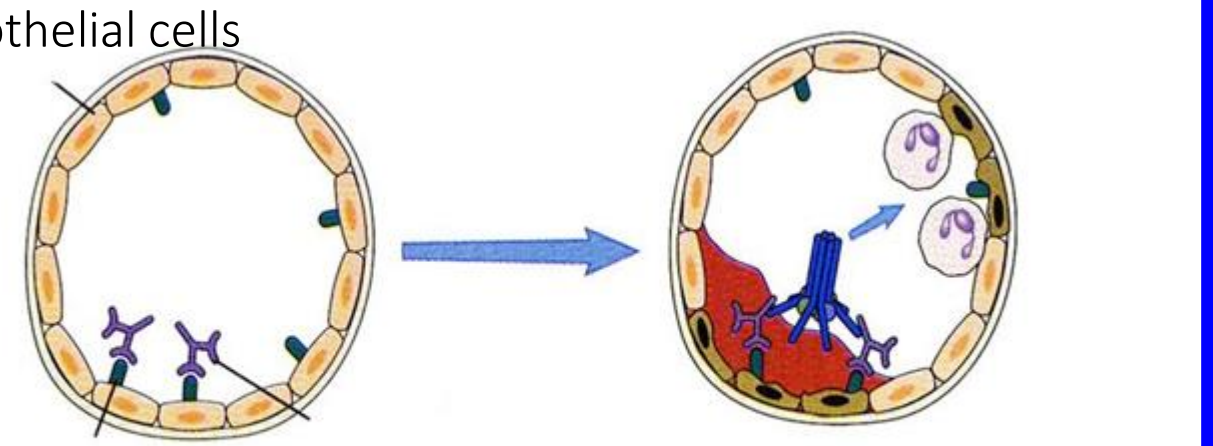
Fixation of Ab on endothelial allo-Ag in the transplanted organ

→ complement activation,

→ ADCC

→coagulation

→ thrombosis, ischemic necrosis

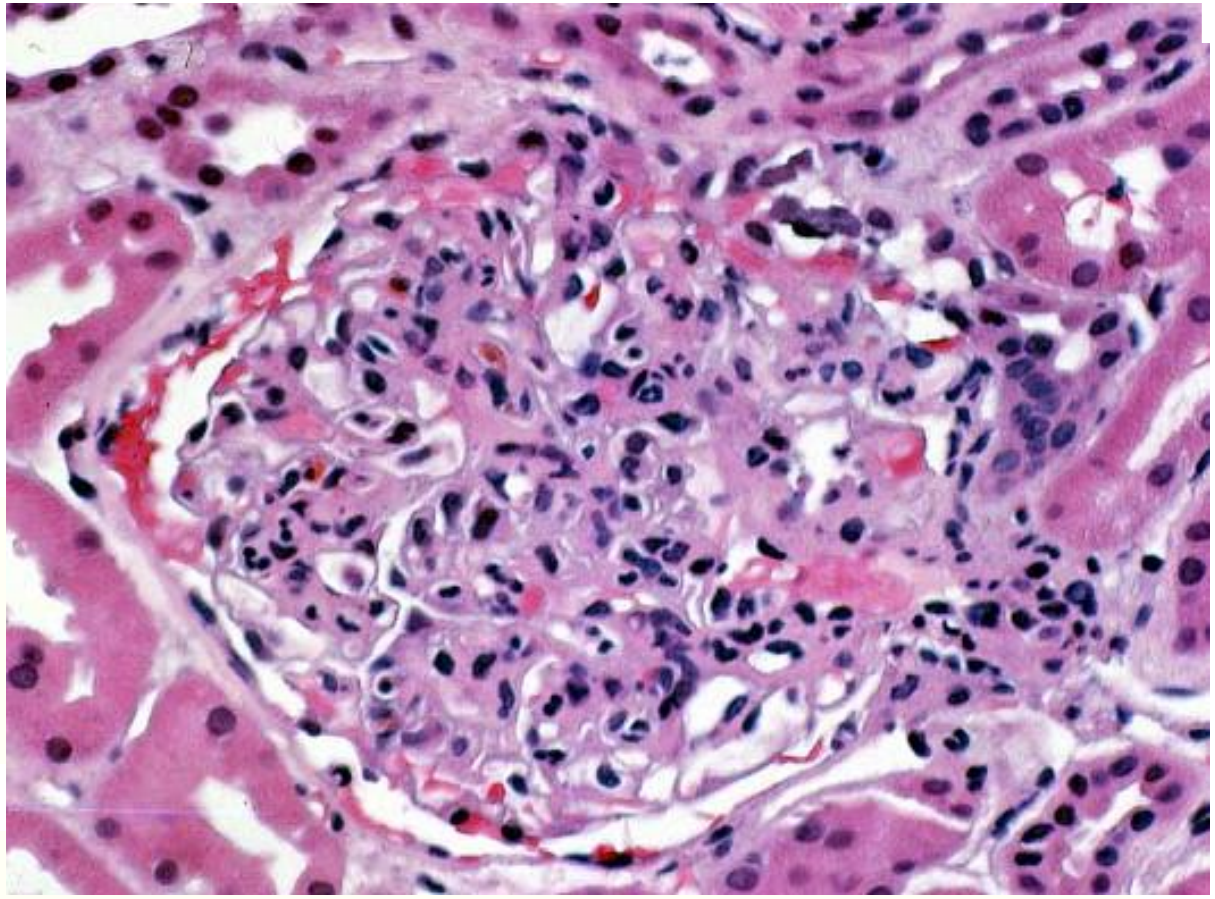


1. Hyperacute rejection: immediate

To learn

Histological features

Abundant accumulation of Abs, complement cleavage products (e.g. C4d), neutrophils, platelets and thrombi in the transplant.



Incidence

Very rare, <1% if cross-match test and ABO compatibility are respected.

Treatment

Only preventive measures are implemented prior transplant (HLA matching, cross-match, matching with absence of anti-HLA antibodies).

No therapeutic strategy to block the hyperacute rejection once it has started.

2. Acute cellular rejection

Clinical features

Fever, functional failure of the transplanted organ

Cause (see activation steps of T lymphocytes)

Activation of anti-donor T lymphocytes. (biopsy)

Mechanism

Infiltration of the transplant by anti-donor effector T lymphocytes.

Direct cytotoxicity (perforin, Grz, FasL) and **indirect effect** by recruitment and activation of monocytes and NK cells and by production of cytotoxic cytokines (TNF, IFNγ)

→ Parenchymal damage, interstitial inflammation

2. Acute cellular rejection

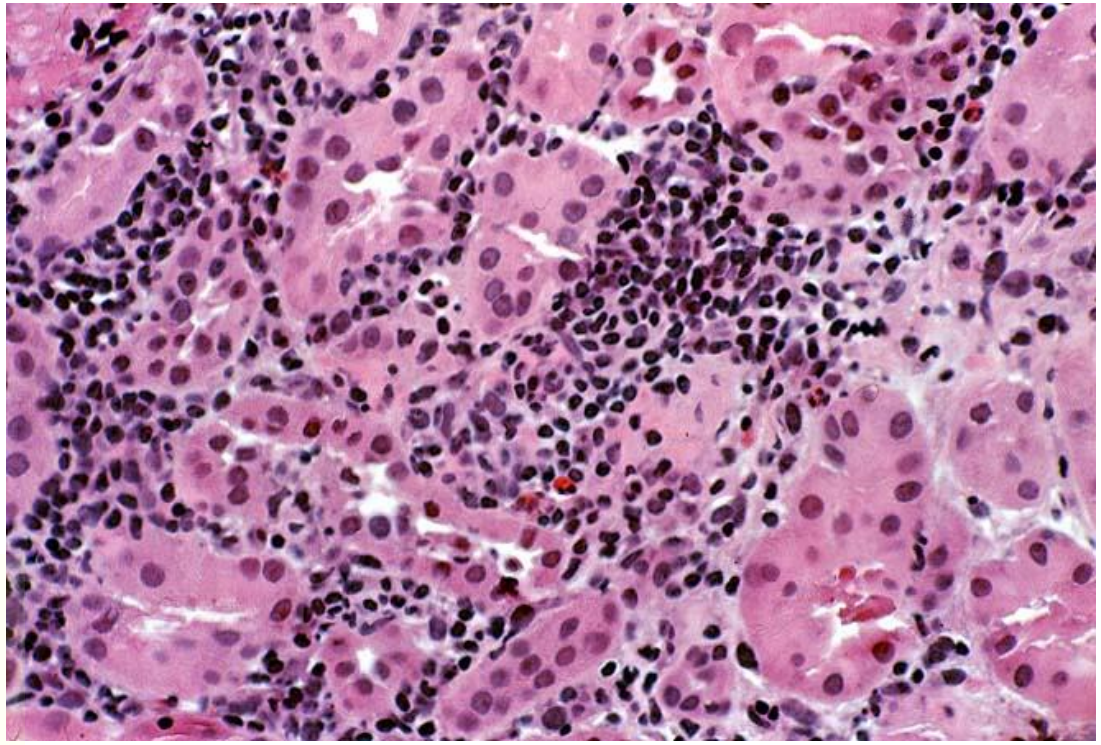
To learn

Histology: Mononuclear leukocyte infiltrate (T lymphocytes and monocytes).
→ Necrosis of parenchyma cells

Incidence: in 40 to 50% of patients.
Loss of the transplant in <10% of patients.

Treatment :
Immunosuppressors targeting T lymphocytes.
(Lecture from Aurélie Tran-Barrail)

Prevention:
Limit HLA mismatches.
Watch out for minor histocompatibility Ag.



The definitive diagnosis of a rejection episode is based on biopsy analysis

3. Antibody-mediated humoral rejection

To learn

Clinical features

Fever, functional failure of the transplanted organ

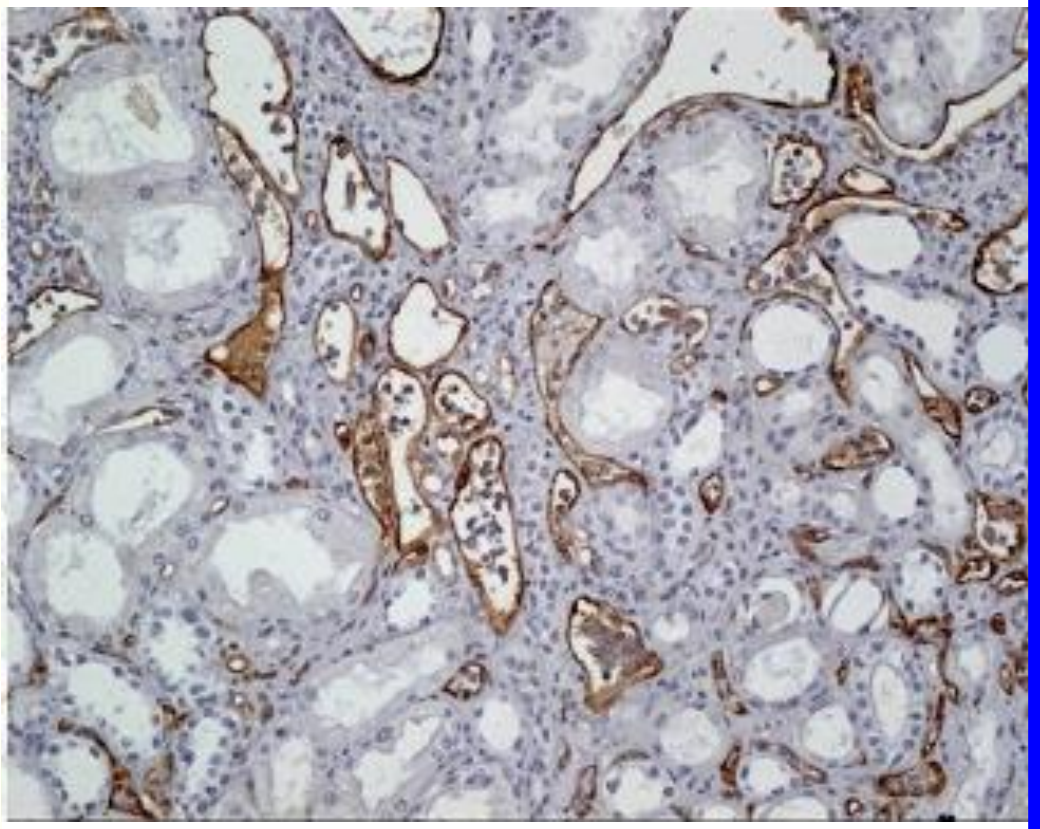
Cause (see activation steps of B lymphocytes)

Production of Ab anti-donor (mainly anti HLA).

Histology: Accumulation of inflammatory elements (C4d, neutrophils, ...) in the capillaries and small vessels of the transplant.

→ Vascular lesions

Treatment: Immunosuppressors targeting B and T lymphocytes. (Course by Aurélie Tran-Barrail)



Biopsy of kidney graft. Staining of C4d complement fragment.

4. Chronic graft rejection and dysfunction

Progressive and irreversible degradation of the graft function.

Causes: (multiple)

1. **Immunologic factor: Subclinical, chronic (persistent) alloimmune reaction.**
2. Non-immunological factors (hypertension, toxicity of immunosuppressors, …).

Immunologic mechanism:

Chronic alloreactive aggression (subclinical + clinical rejection episodes) are at the origin of local lesions persistently triggering tissue repair mechanisms. Treg lymphocytes are involved, producing **TGFβ**.

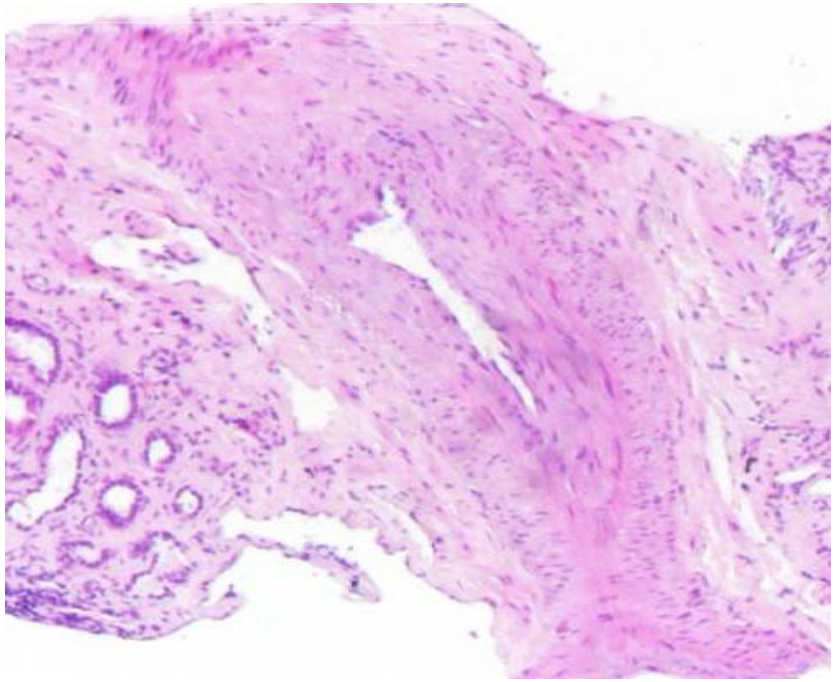
This regulatory but also **profibrotic cytokine** stimulates the proliferation of fibroblasts, endothelial cells and smooth muscle cells within the intima.

4. Chronic graft rejection and dysfunction

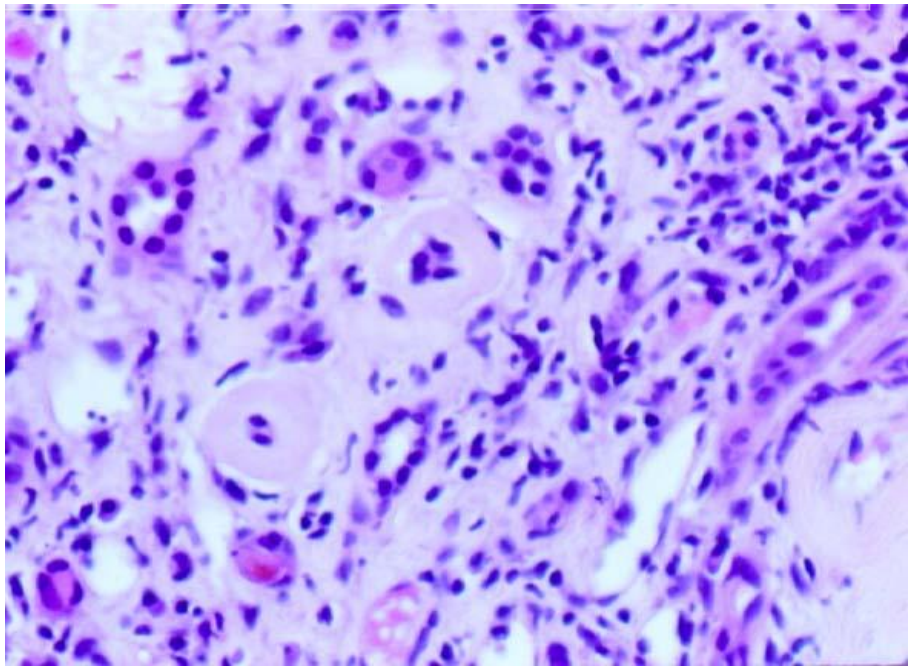
Histology:

Chronic **fibroproliferative vasculopathy** specific to the transplant indicating a chronic endothelial aggression and interstitial fibrosis.

Occlusive arteriosclerosis



Tubular atrophy



Treatment : At this stage, the fibrosis can not be reversed with immunosuppressive therapy. Discovering treatments thar induce fibrosis regression is an active research field

Average lifespan of a transplanted organ

Kidney: 10 -15 years.
Cases over 40 years.

Liver: ~ 10 years.

Heart: 10 to 13 years.

Lung: 5.7 years.

Intestine: 3 years.

Pancreas: 8 years.

Summary
Allogeneic immune response

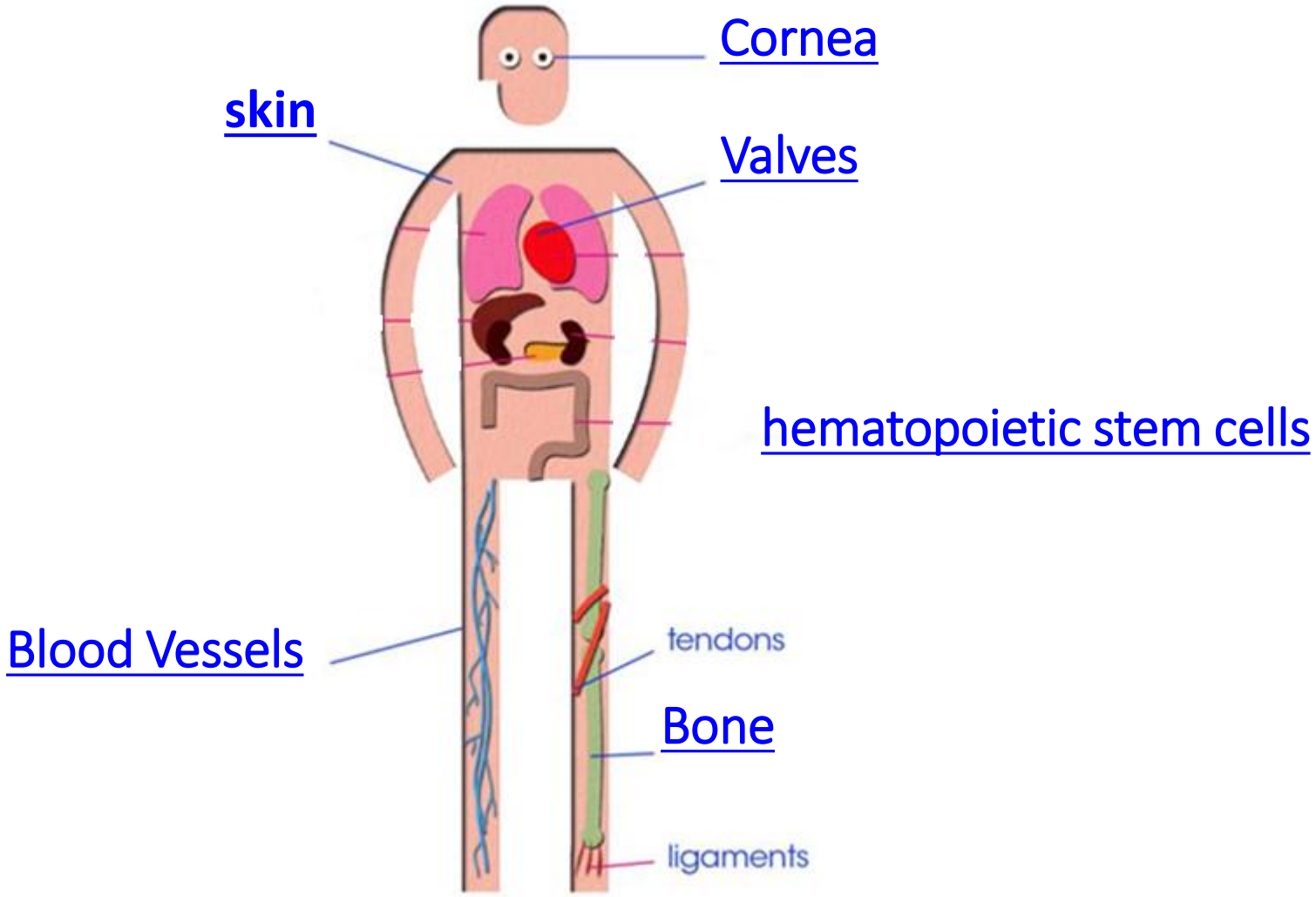
- Circumstance **created by medicine** with transfusions of blood products and transplants.
- Graft rejection is a normal immune response to eliminate the "no-Self“. But in this context of transplant, it is an undesirable reaction.
- Can be reduced by choosing a good donor – recipient pair with the fewest genetic differences possible: blood type, HLA antigens and mHAg.
- Acute rejection episodes are generally well controlled by immunosuppressors.
- Chronic rejection can be slowed by effectively managing acute rejection episodes, but there is currently no treatment to reverse fibrosis.

Immune tolerance

Not discussed.
This is a highly active area of research focused on identifying key elements and control mechanisms to induce true immune tolerance to transplants.

Tissue and cell transplantation

Tissue and hematopoietic stem cell transplantation



Allogeneic tissue and cell transplants

Tissues :

Cornea (immune privileged tissue), reduced risk of rejection.
Only tissue with waiting list for transplantation.

Skin: Short term therapeutic purposes. Pretreated to minimize immune risks.
Frequently used to aid healing after severe burning.

Composite tissues (e.g. a hand): High risk of immune-rejection.

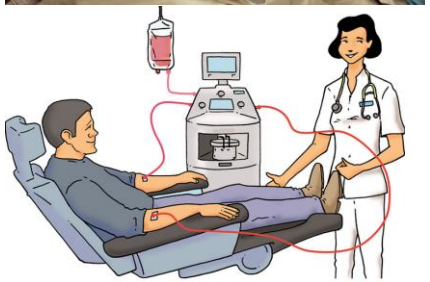
Cells:

Hematopoietic stem cells (HSC): used to reconstitute an hematopoietic system that is either abnormal or intentionally destroyed .

→ replace genetic deficiencies or restore a hematopoietic system affected by malignant diseases.

Sources of HSC

1. Bone marrow
   ~50 samples, > 1 L of marrow, under general anesthesia.
   Then sorting of CD34+ cells
2. **Peripheral stem cells** mobilized by G-CSF
   Apheresis, then sorting of CD34+ cells
3. **Umbilical cord blood**. Whole PBMC could be used



Different sources = Different qualities
– Hematopoietic capacities (reconstitution speed)
– Immunological qualities (memory T cells)

CD34+ cell-dose to transplant ≥ 3×10^6 / kg

www.mathec.com
www.britanica.com
www.lifebankusa.com

Steps for Managing a HSCT

Pre-transplant

Clinical assessments for the patient and donor.

Remission of the underlying malignant disease

HLA and blood group typing for donor and recipient

- HLA typing: high allelic resolution (HLA-A, -B, -C, -DRB, -DQB)
- ABO compatibility is not required

Assessment of organs function

Search for infectious foci and viral serology (CMV, EBV, HBV, HCV, HIV, HTLV)

Conditioning

Steps for Managing a HSCT

Pre-transplant
4 weeks

Clinical assessments for the patient and donor.
Conditioning

Transplant
6 weeks

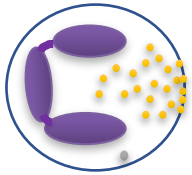
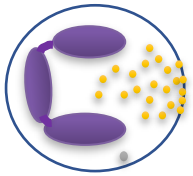
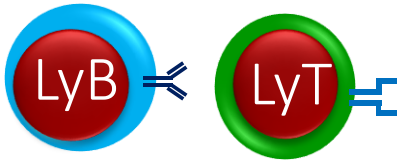
Graft transfusion
3 weeks of isolation in a **sterile room**
Period for acute toxicities emergence
Recovery from aplasia, gradual immune system reconstitution

Types of donors based on HLA and general genetic compatibility levels

1. Genetically identical (siblings)
2. **HLA genetically identical** and **related** (same family)
3. **Phenotypically identical** and **unrelated** (HLA 10/10 match, not from the same family)
4. **Haploidentical** and related (HLA 5/10 match, brotherhood)
5. Unrelated cord blood (at least 5/6 HLA match required)

Cases 3 and 4 with high risk of allogeneic immune reactions

Hematopoietic-Immune Reconstitution after HSCT

5 to 10 days before	**day 0**	day 12-15	day 20	weeks months	Months-years
Conditioning	 HSC infusion	 Reconstitution neutrophils (if autograft)	 Reconstitution neutrophiles (allograft)	 Reconstitution NK cells	 Reconstitution B and T lymphocyts

Recovery from aplasia: WBC > 500 / µL

3 consecutive days

concept of chimerism after allograft

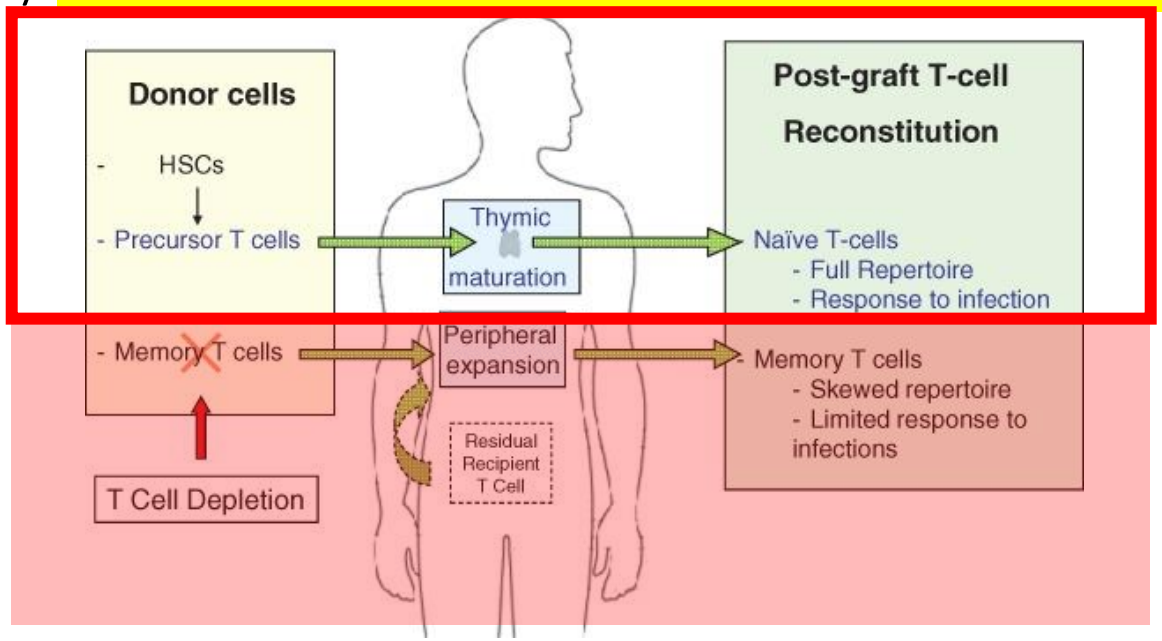
Immunodeficincy state

Lehman, OpenPeditrics 2017; Toubert et al, Tissue Antigens 2012

Immune Reconstitution after HSCT

To learn

Is accompanied by **the establishment of immune tolerance to the graft**



→ Allows gradual cessation of immunosuppressors (cyclosporine)

~ 1 year post transplant

The vaccination program must be resumed: Tetanus, Polio, Pneumococcus, Influeza vaccines, …

Steps for Managing a HSCT

Pre-transplant 4 weeks	Clinical assessments for the patient and donor. **Conditioning**
Transplant 6 weeks	**Graft transfusion** 3 weeks of isolation in a **sterile room** Period for acute toxicities emergence **Recovery from aplasia, gradual immune system reconstitution**
Post-transplant 1st year	Weekly/Monthly Follow-up Patients on **immunosuppressants, antibiotics**, etc.. Gradual reconstitution of immune system **Risk of GVHD**
Post-transplant Long term	Annual Follow-up (1 or 2 times per year) Vaccinations, discontinuation of immunosuppressants **Risk of GVHD** Long-term effects on growth, puberty, fertility, etc.

Potential complications after HSCT

Infectious complications:

Due to **aplasia*, incomplete reconstitution of the immune system** and immunosuppressors.

→ Opportunistic infections with atypical, severe symptoms and rapid dissemination.

Prevention involves patient isolation, extensive antimicrobial profilaxis and regular monitoring to promptly identify infections.

Immunological complications in allogeneic HSCT:

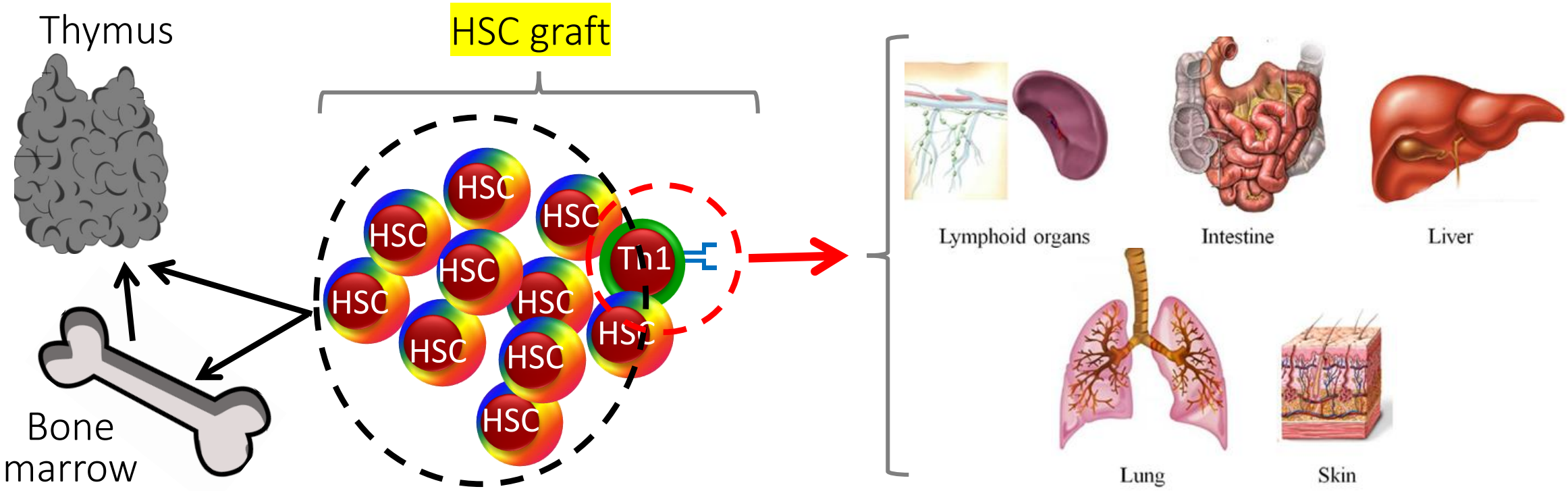
- Reject (rare): recipient's immune system rejects the graft.
- Graft versus host disease (GvHD): **Immunocompetent T lymphocytes** transferred with the graft attack the host's tissues, primarily affecting different epithelia.

*Bone marrow aplasia: Not enough production of blood cells

Graft vs. host réactions

- The allogeneic GVH immune response can have a **beneficial** effect controlling hematologic malignancies (GvL effect) and infectious risk (anti-infectious response)

- The **negative** consequence of these cells' presence is the development of a **graft vs. host disease** (GVHD).



Castor et al, Front Pharmacol 2012

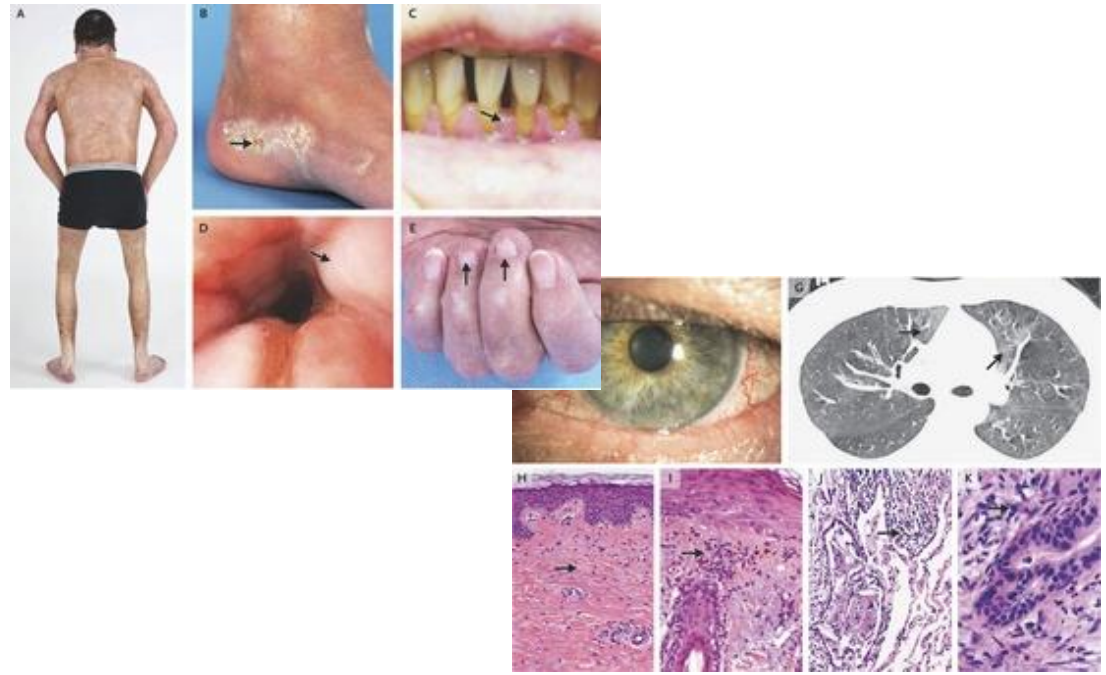
Graft vs. host disease (GVHD)

- Acute GvHD: <3 months. Skin manifestations (erythroderma, pruritic & painful rash), digestive (diarrhea, pain, …), hepatic (jaundice). The thymic epithelium is also targeted.

- Chronic GvHD (> 3 months): Similar to scleroderma (autoimmune manifestations)
  → Lichenoid lesions, fibrosis and vascular obliteration in the skin and other organs.

Prophylaxis : Cyclosporine from day -1
- Other I.S.: Methotrexate, Cellcept, Tacrolimus
- Depletion of T lymphocytes in the graft.

Treatment: corticosteroids



Seizer and Blazar, NEJM 2017

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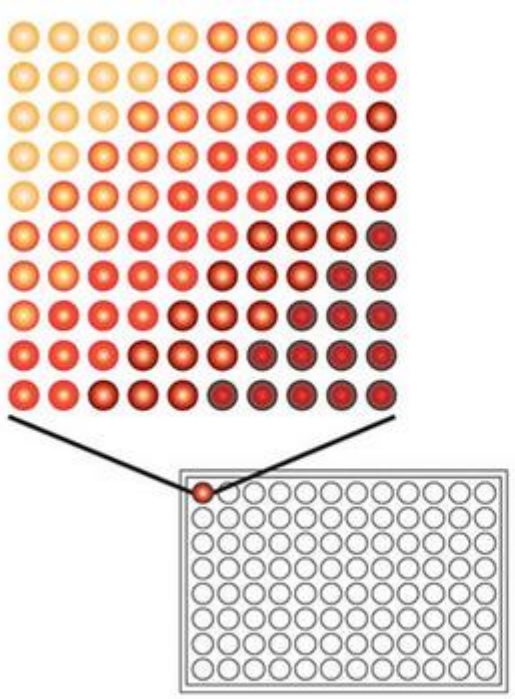
- Can be reduced by choosing a good donor – recipient pair with the fewest genetic differences possible: blood type, HLA antigens and mHAg.
- Acute rejection episodes are generally well controlled by immunosuppressors.
- Chronic rejection can be slowed by effectively managing acute rejection episodes, but there is currently no treatment to reverse fibrosis.
- The immunological risks in HSCT: rejection and GvHD, are managed through both preventive and active control using immunosuppressive drugs.

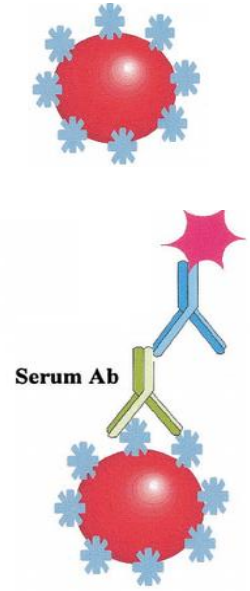
Thank you for your attention

Multiplex methods for detection of anti-HLA antibodies

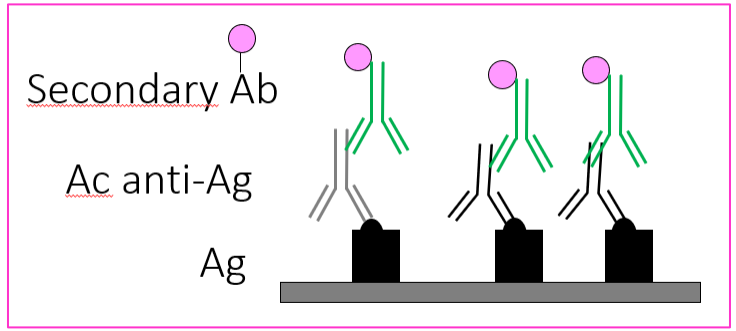
Multiple anti-hla specific antibodies are detected (quantified) in a single test, in a small sample (e.g. 50 µL). Polystyrene micro-beads are used as solid phase to immobilize HLA molecules.
Each bead population has its own fluorescence 'signature' and is coated with a single identity of recombinant HLA molecules.
Different bead populations are pooled together and used for exploring a biological sample.



For **Ab** detection
→ Ag-coated beads

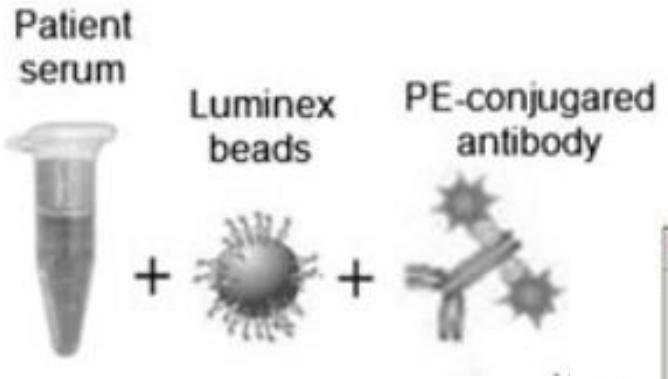


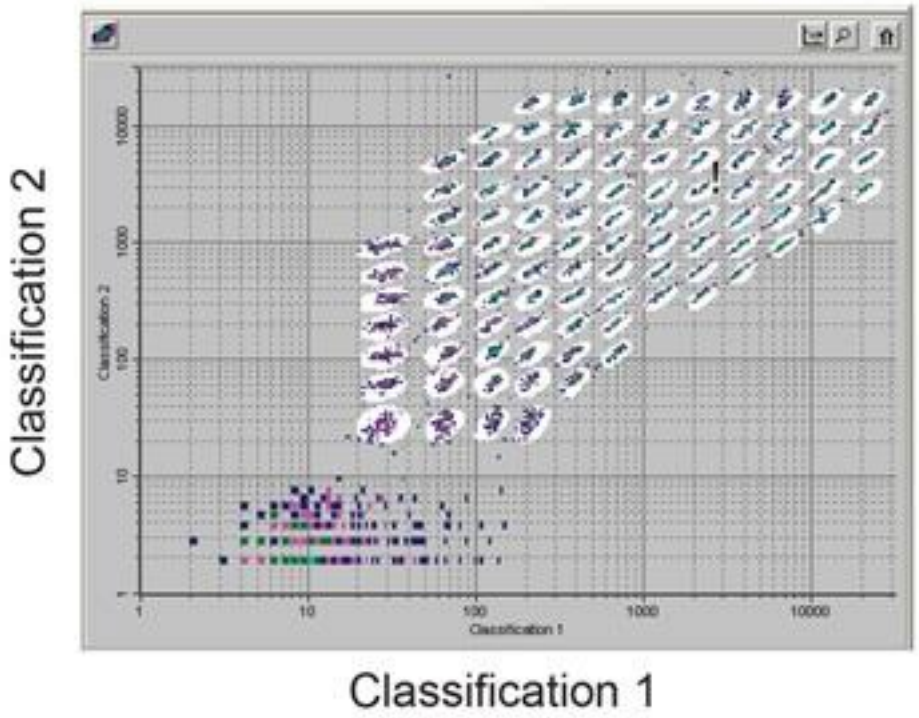
Indirect reaction

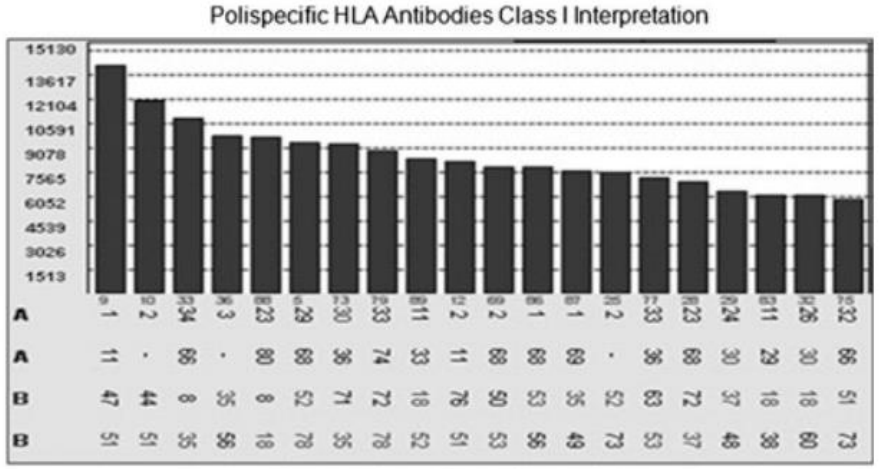


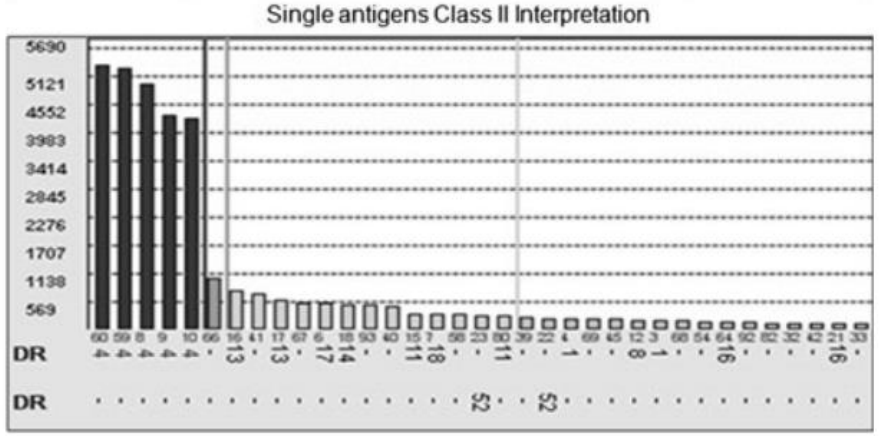
Multiplex methods for detection of anti-HLA antibodies

Results are read in cell cytometer and analysed







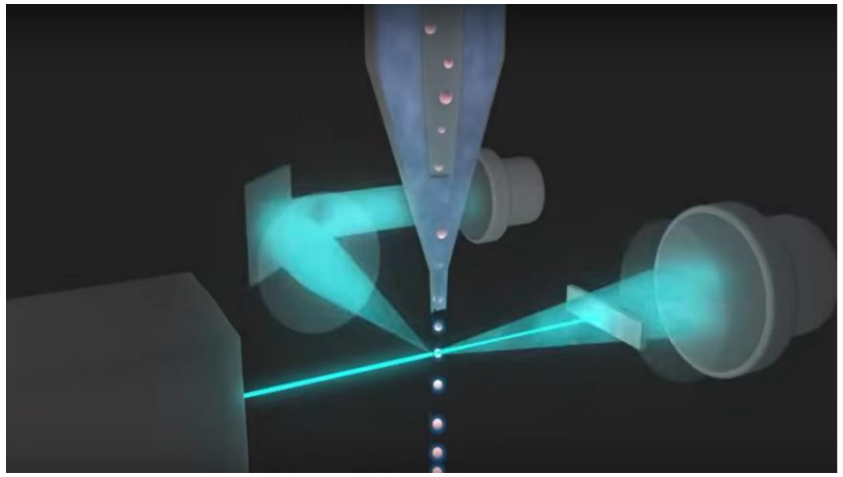
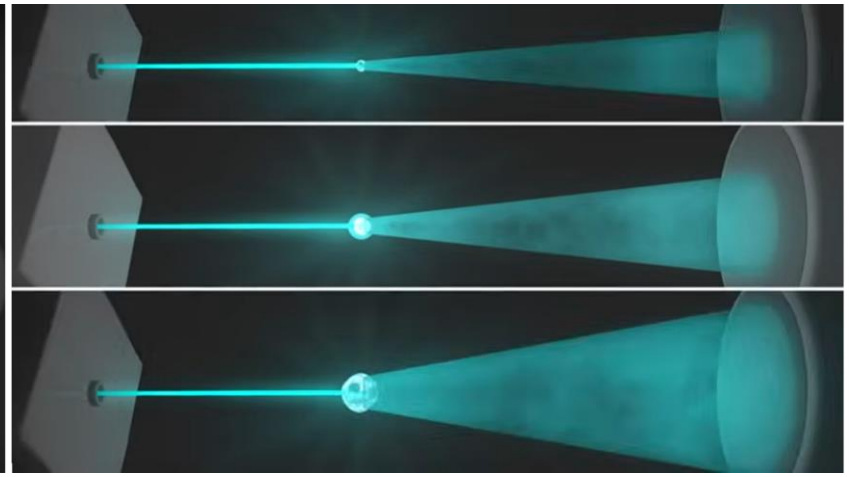
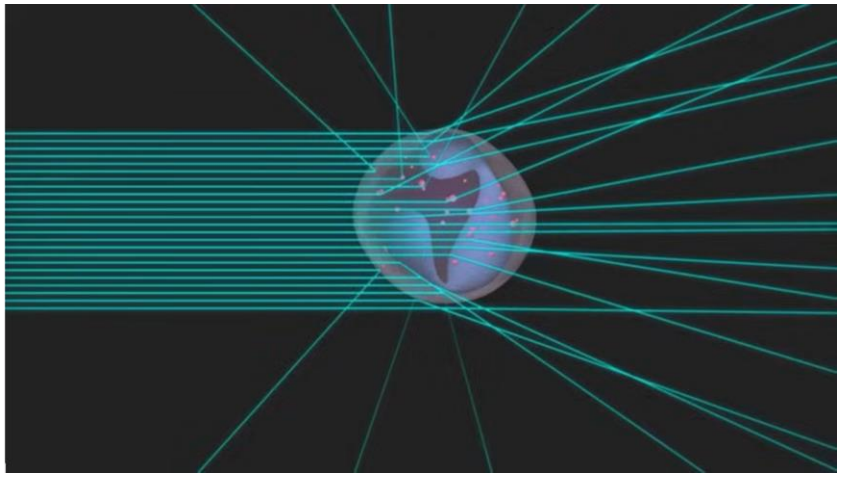


Cell cytometry 1

Enables the counting and characterization of single-cell suspensions based on two optical properties.

The cell suspension is drawn into the machine and directed through a narrow channel, where a strong buffer flow aligns the cells in single file. In the analysis chamber, cells are illuminated by a laser beam (lambda 488 nm). First optical property:

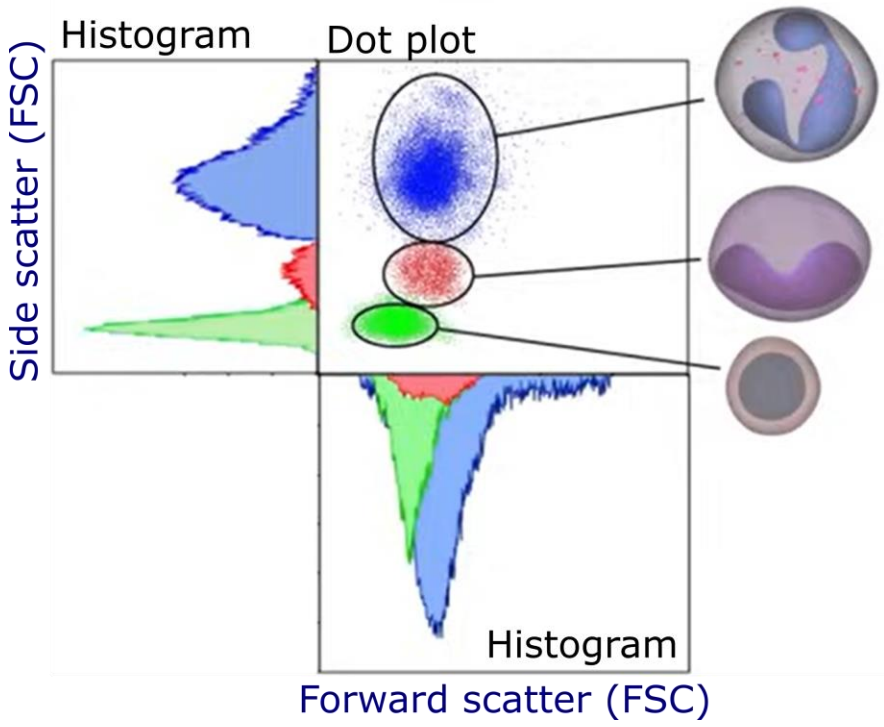
1. Light scattering. Diffusion of laser light is measured in a straight line (forward scatter, FCS) and at 90° (side scatter, SSC), providing **information about cell morphology**: cell size and internal complexity, correspondingly.

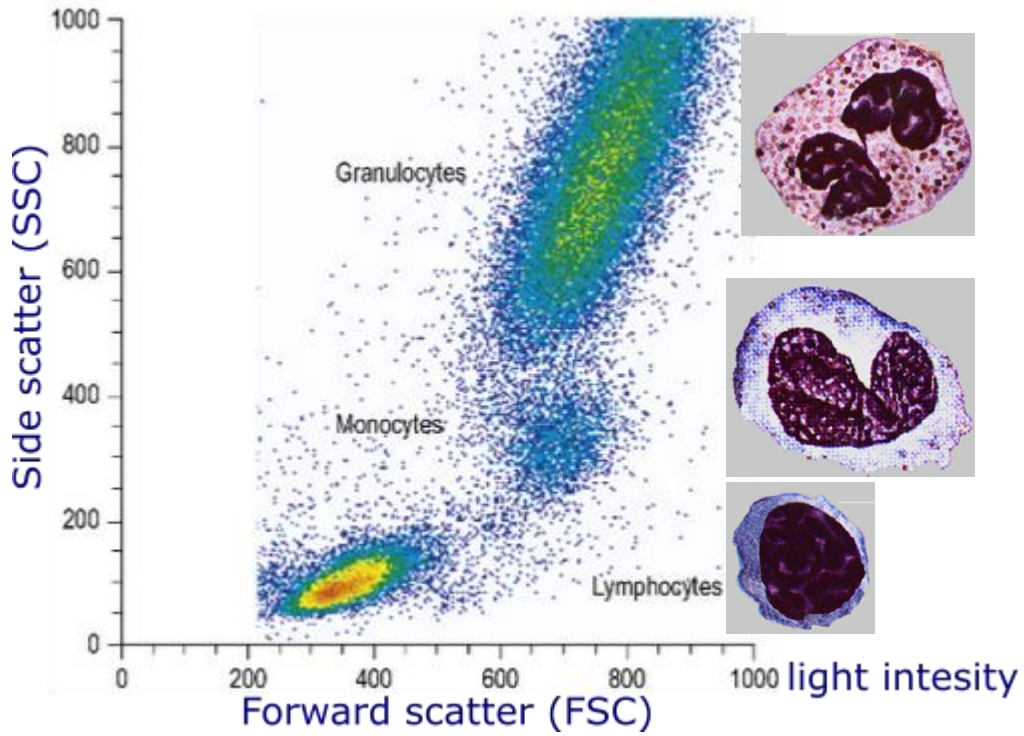




Cell cytometry 2

Enables the counting and characterization of single-cell suspensions based on two optical properties.

1. <u>Light scattering</u>. In a heterogeneous cell suspension like white blood cells, SSC vs. FCS analysis distinguishes different cell populations: lymphocytes, monocytes, polymorphonuclear neutrophils.

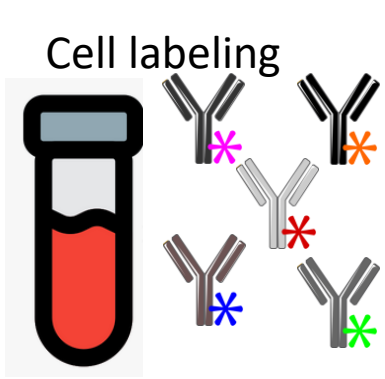


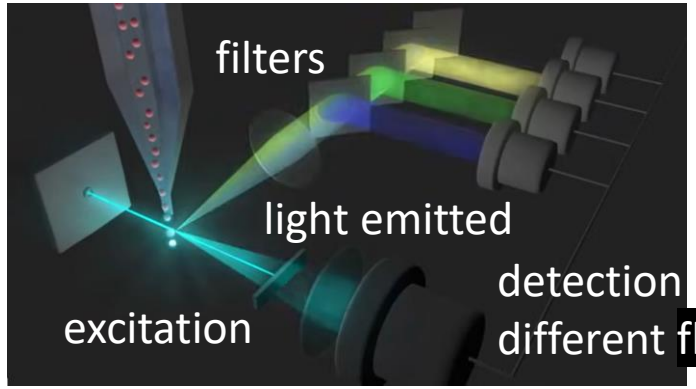


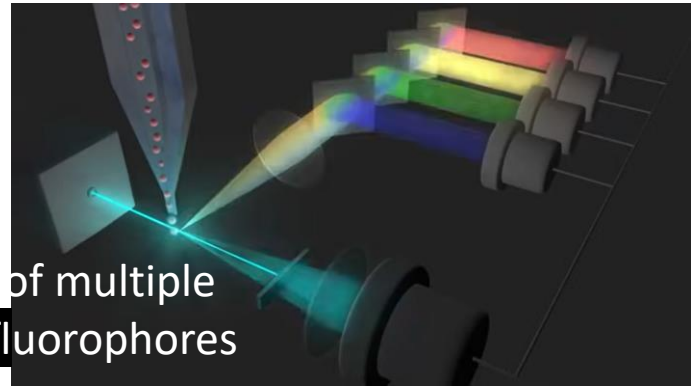
Cell cytometry 3

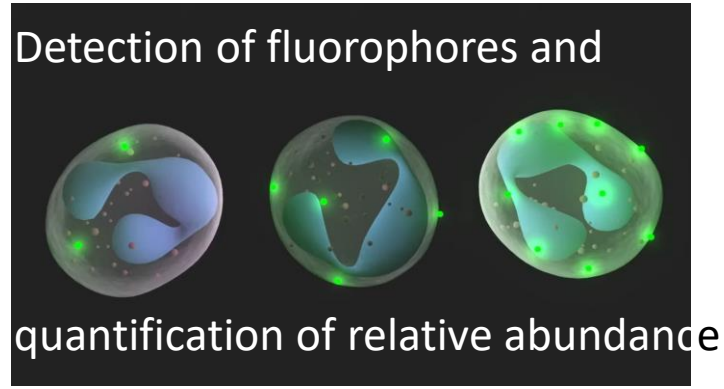
Enables the counting and characterization of single-cell suspensions based on two optical properties:

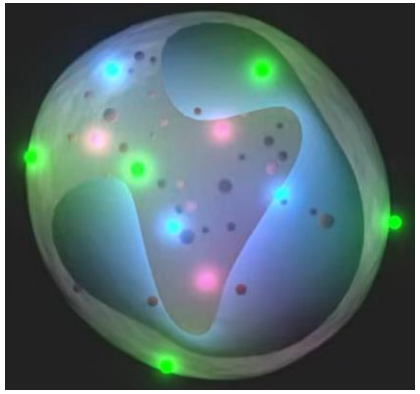
1. <u>Light scattering</u>. Provides information about cell morphology
2. <u>Fluorescence Detection</u>. Excited fluorochromes emit ‘color’ signals that provide information about phenotype, functional status of cells. For example, fluorochrome conjugated Abs enable the identification of cells based on the expression of proteins recognized by the Abs.

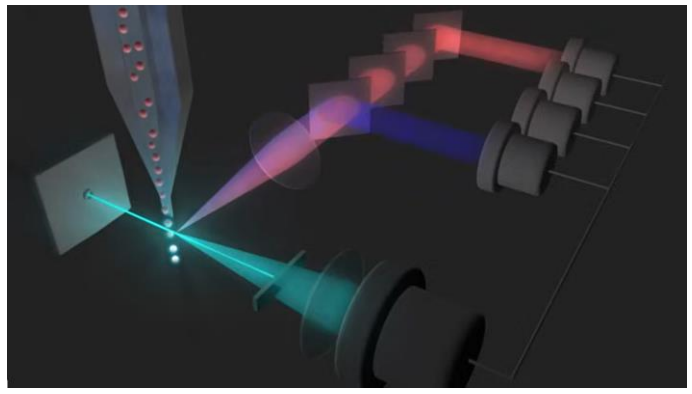


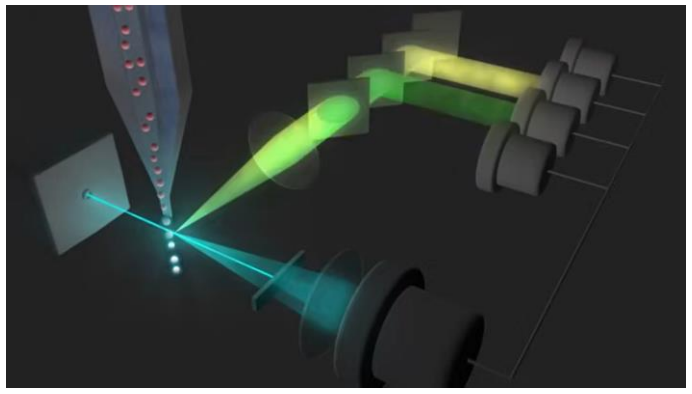


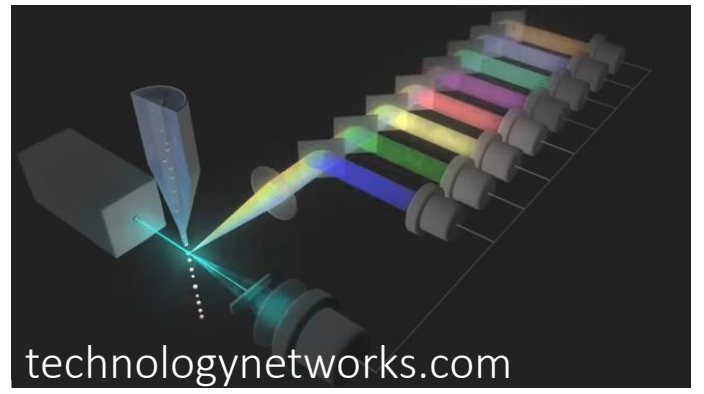








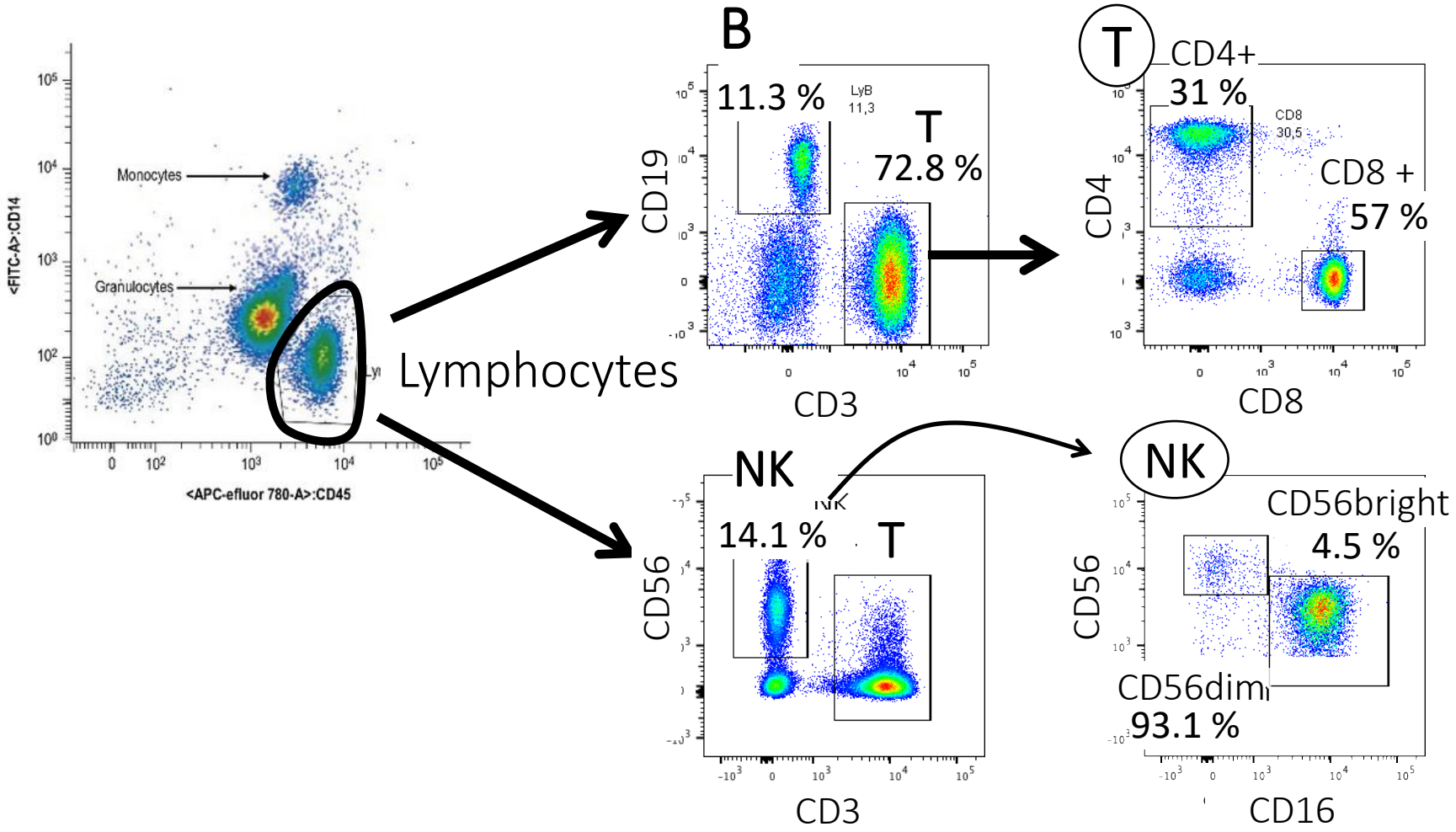




Blood Lymphocyte Immunophenotyping - Gating Concept

Total leukocytes

Analysis restrained to lymphocyte population = gate on lymphocytes



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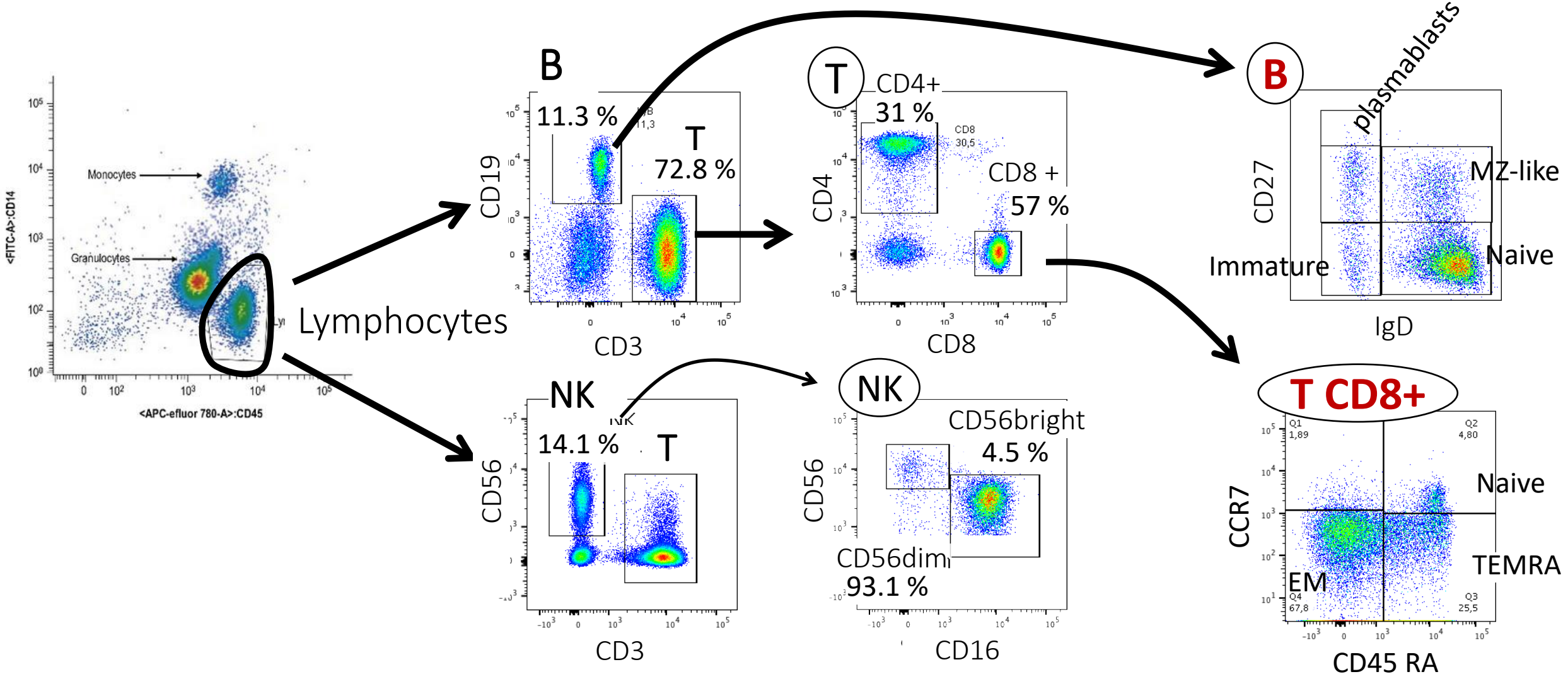

Routine applications of flow cytometry

include:

- Identifying and counting **CD4 T-cell in HIV-infected patients**
- Diagnosing and monitoring **lymphoproliferative disorders**,
- **Characterizing immune cells in immunodeficiencies** and other immune-mediated diseases.

Blood cells are assessed by **direct immunofluorescence labelling of cell surface and intracellular molecules** with fluorochrome-conjugated antibodies.

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