



IMMUNOLÓGIAI ÉS  
BIOTECHNOLÓGIAI  
INTÉZET



# 14th practice: Autoantibody diagnostics

Basic Immunology

University of Pécs, Clinical Center

Department of Immunology and Biotechnology

Pécs, 2025.

# TOLERANCE & AUTOIMMUNITY

- Upon encountering an antigen, the immune system can either develop an immune response or enter a state of unresponsiveness called tolerance.
- Immunological tolerance is thus the lack of ability to mount an immune response to epitopes to which an individual has the potential to respond.
- Targeting type and tolerating type immune responses composed by the same cellular and molecular components, the differences are in the effector phase only.
- Targeting type immune response or tolerance needs to be carefully regulated since an inappropriate response – whether it be autoimmune reaction to self-antigens or tolerance to a potential pathogen – can have serious and possibly life-threatening immunodeficiencies.

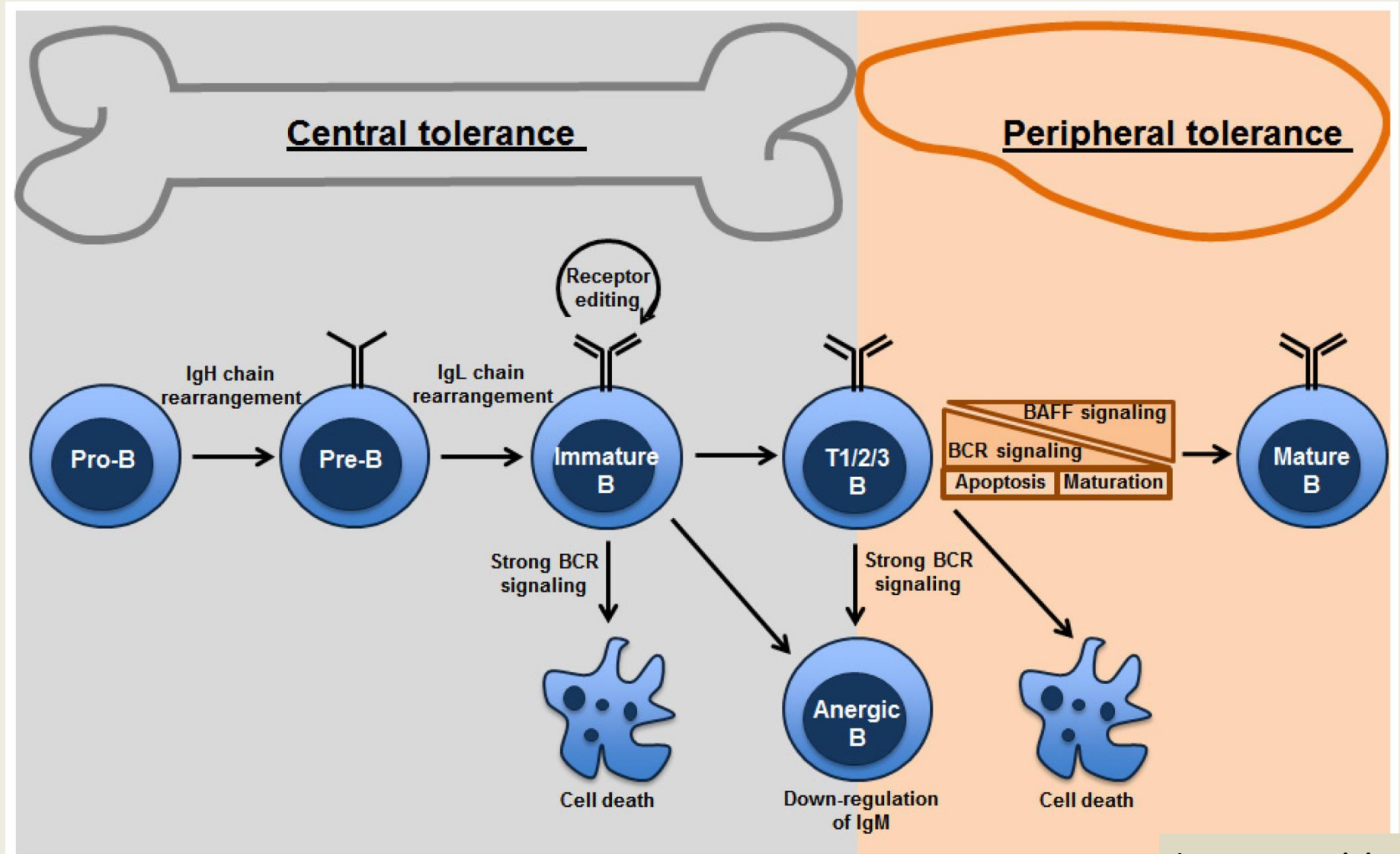
# **T-cell tolerance**

**Central Tolerance (selection in the Thymus)**

**Peripheral Tolerance**

- **Lack of co-stimulation**
- **Receipt of death signal (high dose of antigen)**
- **Control by regulatory T cells**

# Mechanisms of B-cell tolerance in bone marrow and periphery



# ACTIVE TOLERANCE

## Anti-idiotypic network

- Anti-idiotypic antibodies against T cell and B cell receptors and immunoglobulins
- Antigen-specific inhibition and induction of memory

## Natural immune system (*“Immunological homunculus”*)

- Low affinity IgM, IgG or IgA natural autoantibodies produced by CD5+ B1B cells
- $\gamma/\delta$  T,  $i\gamma/\delta$ T, ILCs1,2,3, MAIT, IEL, iNKT cells

# AUTOIMMUNITY

- **Physiological autoimmunity:** part of the normal immunological regulation
- **Pathological autoimmunity:** diseases caused by self reacting inflammatory immune responses with permanent tissue/organ injury

# Pathomechanism of autoimmunity

- Multifactorial mechanism

(general catastrophe of bio-regulation caused by external and internal factors)

- **Autoimmune “*steady state*”** (failure of dynamic balance on self tolerance and autoimmunity)
- **Role of infections** (molecular mimicry or inefficient natural antibody network)





# Autoimmunity

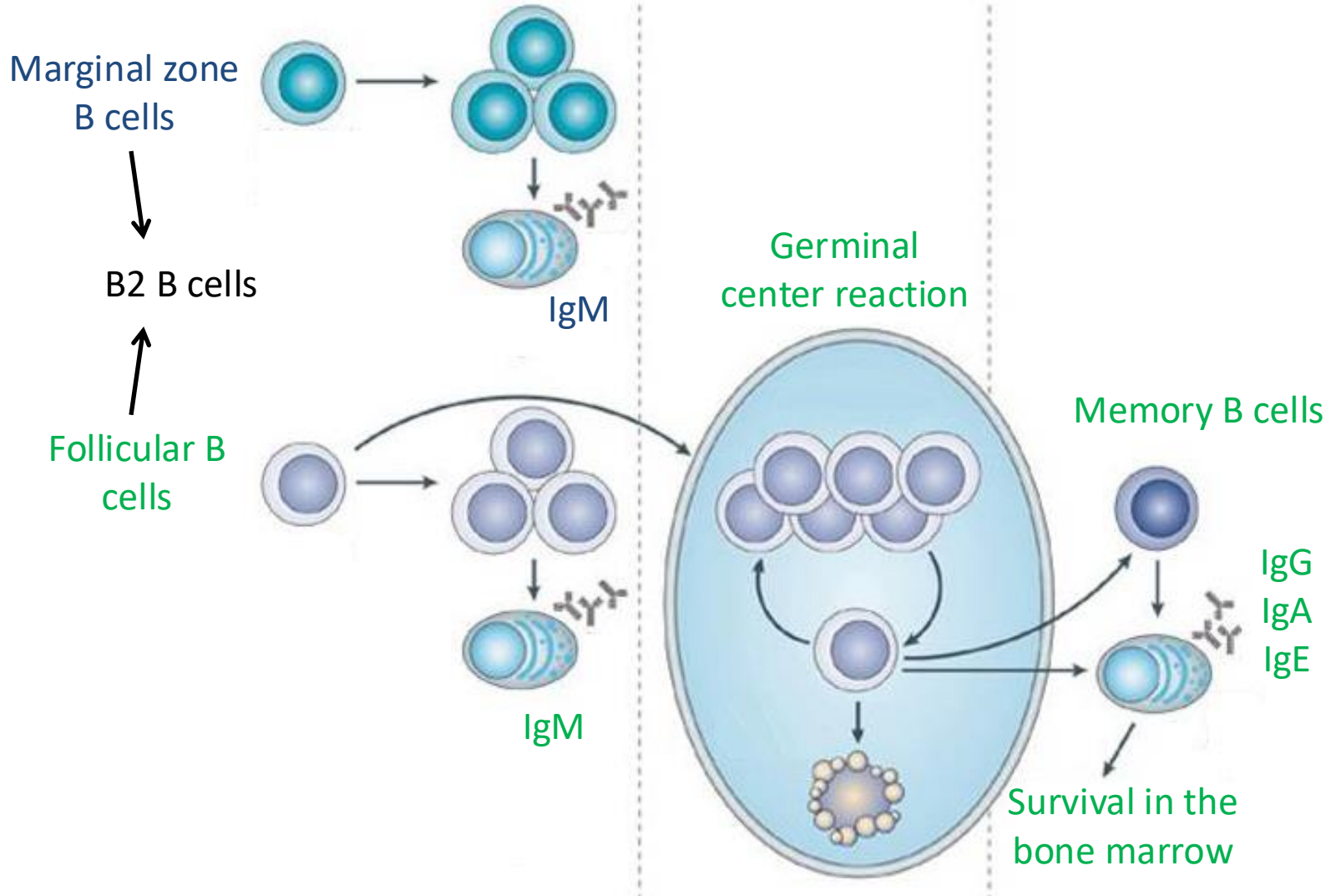
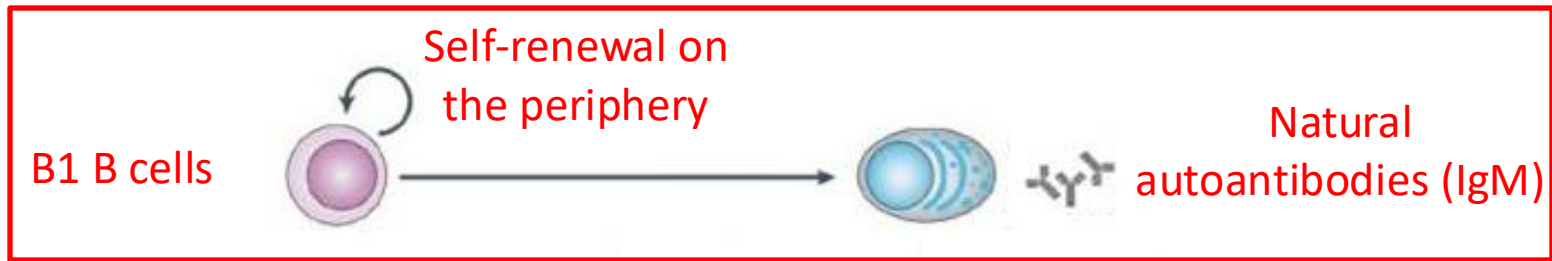
- Autoimmunity: Recognition and immune response to **self-antigens**.

## Natural autoimmunity

- Has immunoregulatory role
- Antibody production without external stimuli
- **Natural autoantibodies:**
  - **Low affinity**
  - **Polyspecific**
  - Mainly **IgM**
  - Low serum concentrations
  - Produced by **B1 B cells**

## Pathological autoimmunity

- Causes or is associated with pathological conditions
- **Pathological autoantibodies:**
  - **High affinity**
  - **Monospecific**
  - Mainly **IgG**
  - High serum concentrations
  - Produced by **B2 B cells**

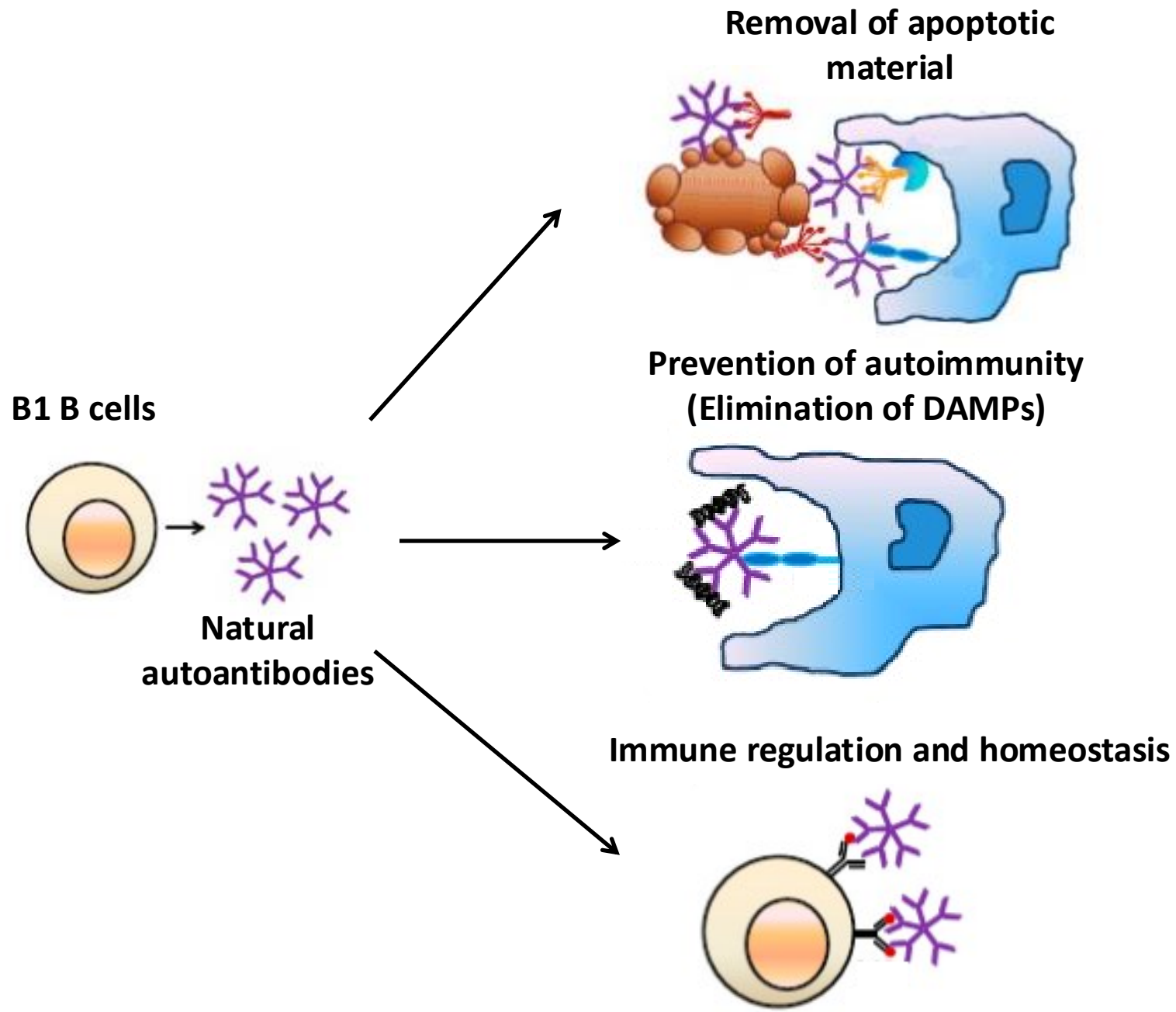


# Targets of natural autoantibodies

- **Evolutionary conserved** structures that are usually found in all cells, e.g.:
  - Heat shock proteins
  - Proteins of the cytoskeleton
  - Metabolic enzymes
  - Nuclear structures
- Hypothesized role: **Maintaining immunological tolerance**, removal of self-antigens (e.g. DAMP: Damage-associated molecular pattern), prevention of pathological autoimmunity
- Natural autoantibodies are constitutively present and their repertoires are constant, independent of age or gender and **characteristic for each individual**.

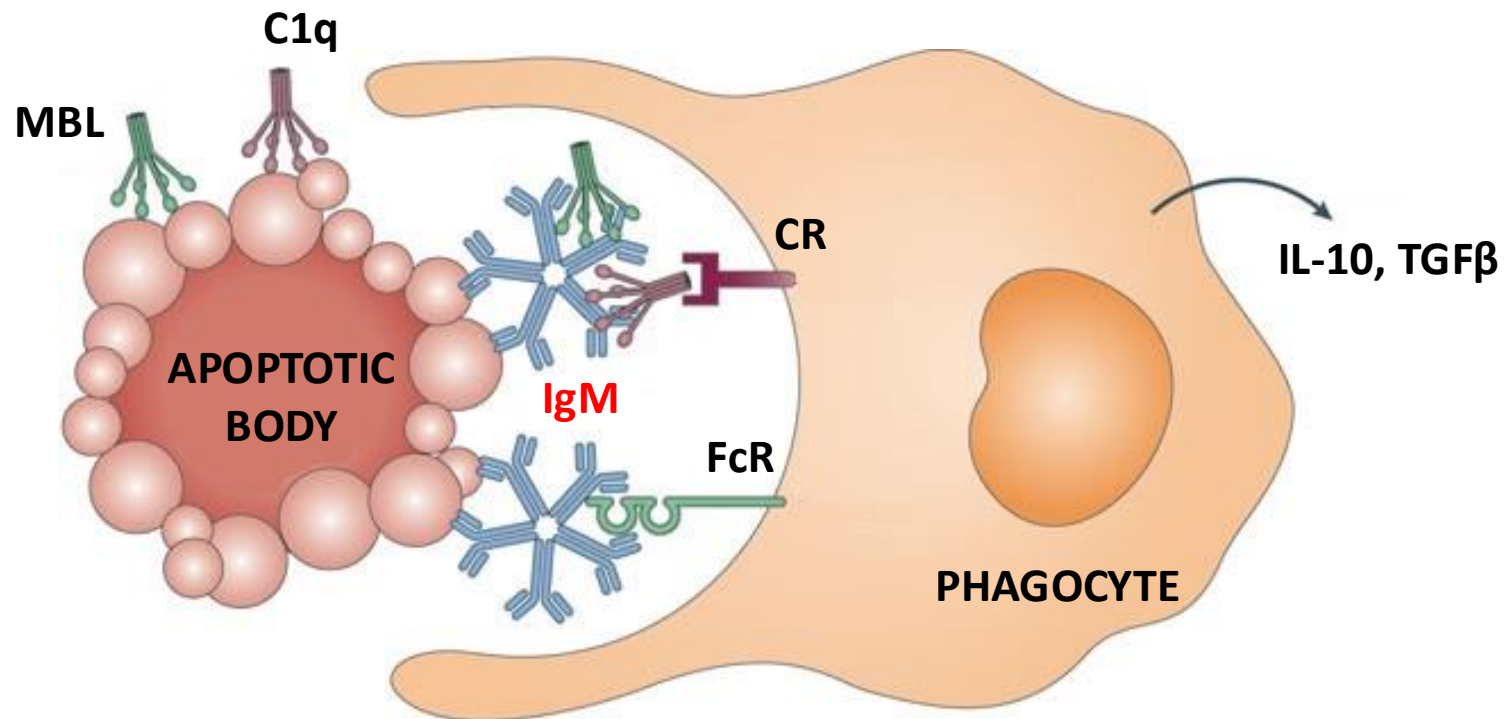


**Immunological homunculus or Immunculus**



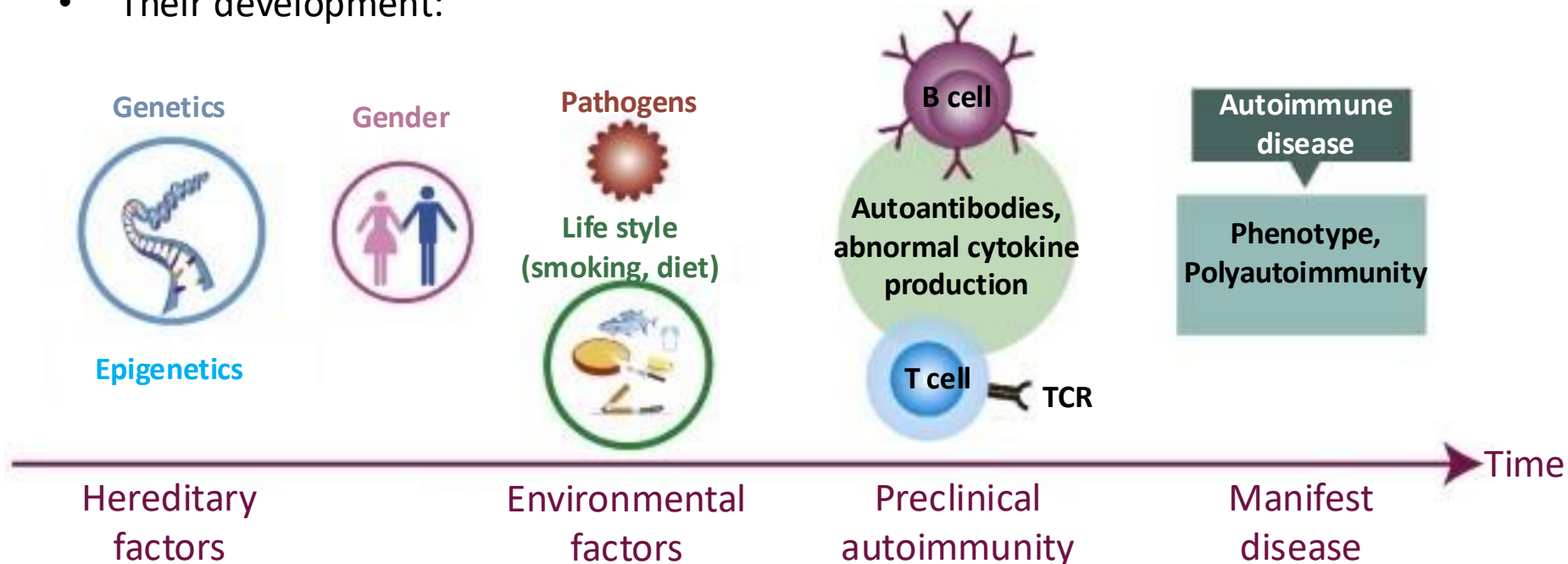
# Removal of apoptotic bodies

- Opsonization of **apoptotic bodies**, phagocytosis via **Fc** and **complement receptors**.



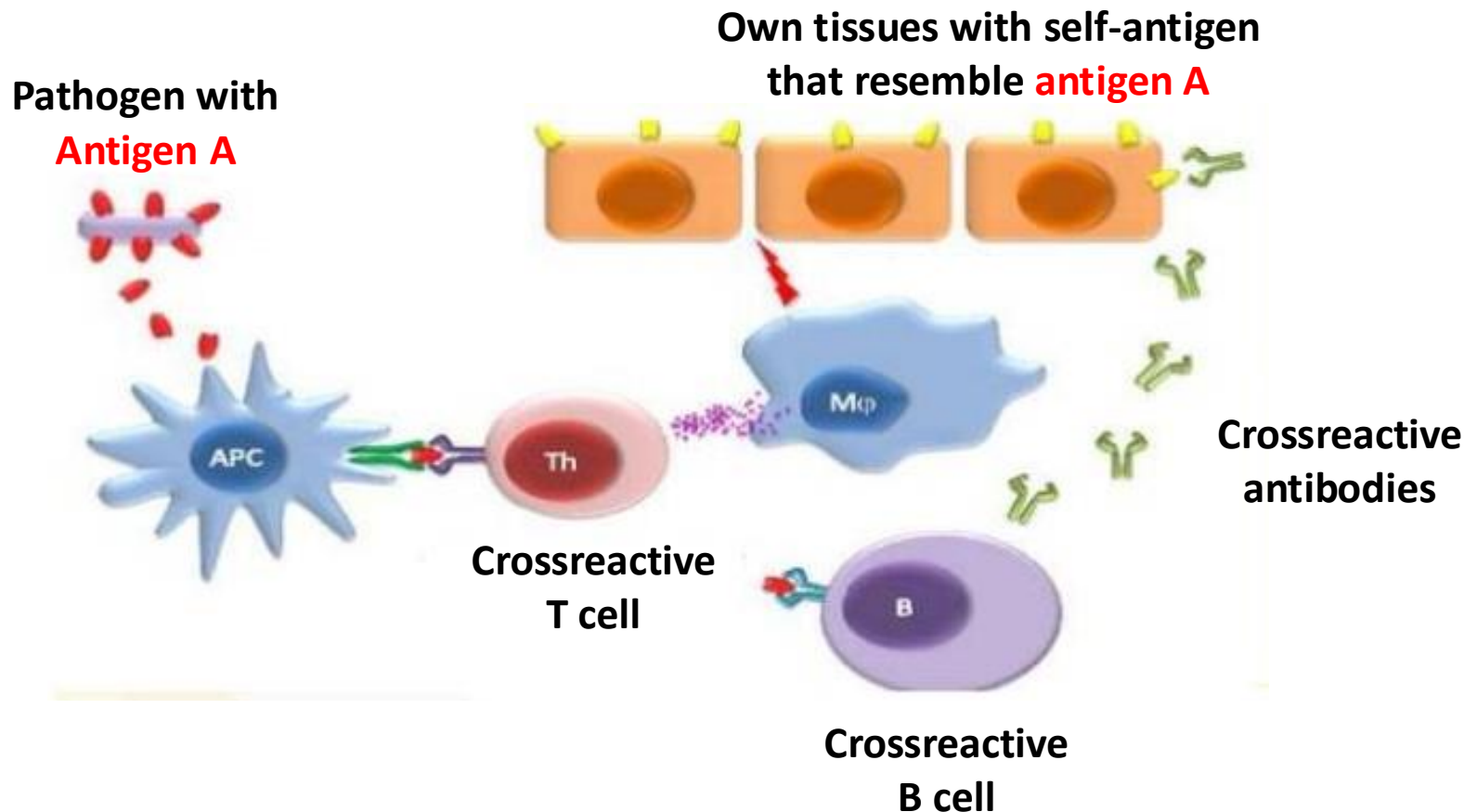
# Autoimmune disorders

- They affect 7-8% of the population.
- Have a strong **female dominance**. (e.g. the male:female ratio in SLE is 1:9)
- Many affect **young adults**.
- Can occur jointly in the same patient. → **Polyautoimmunity** (e.g. a second autoimmune disease occurs in 41% of all patients with SLE)
- **They are chronic illnesses!**
- Their development:

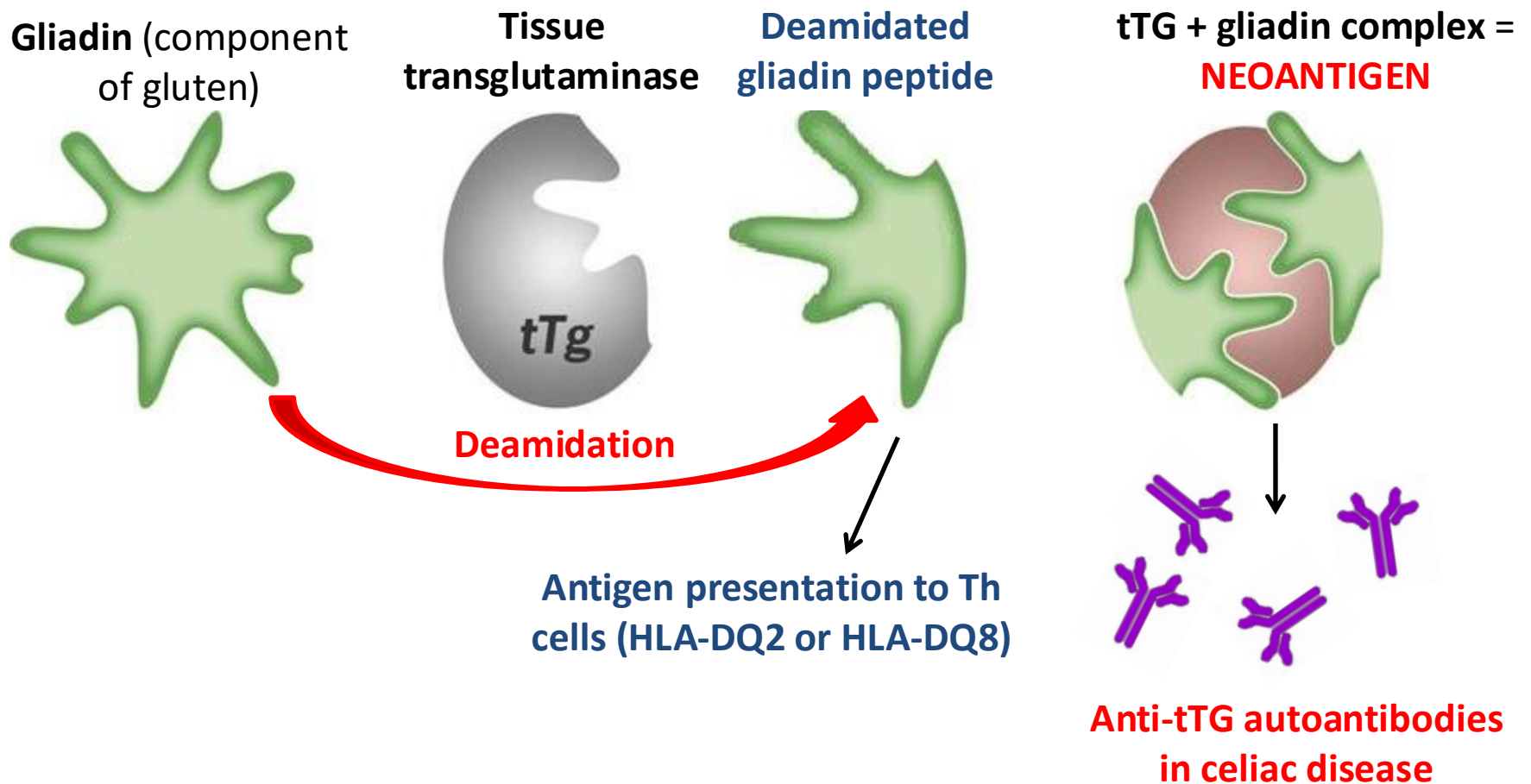


# Molecular mimicry

The pathogen's antigen shares a high degree of **structural similarity** with self-antigens. → Can lead to **cross-reaction and autoimmunity** in genetically susceptible people. (e.g. rheumatic fever after *Streptococcus pyogenes* infections)

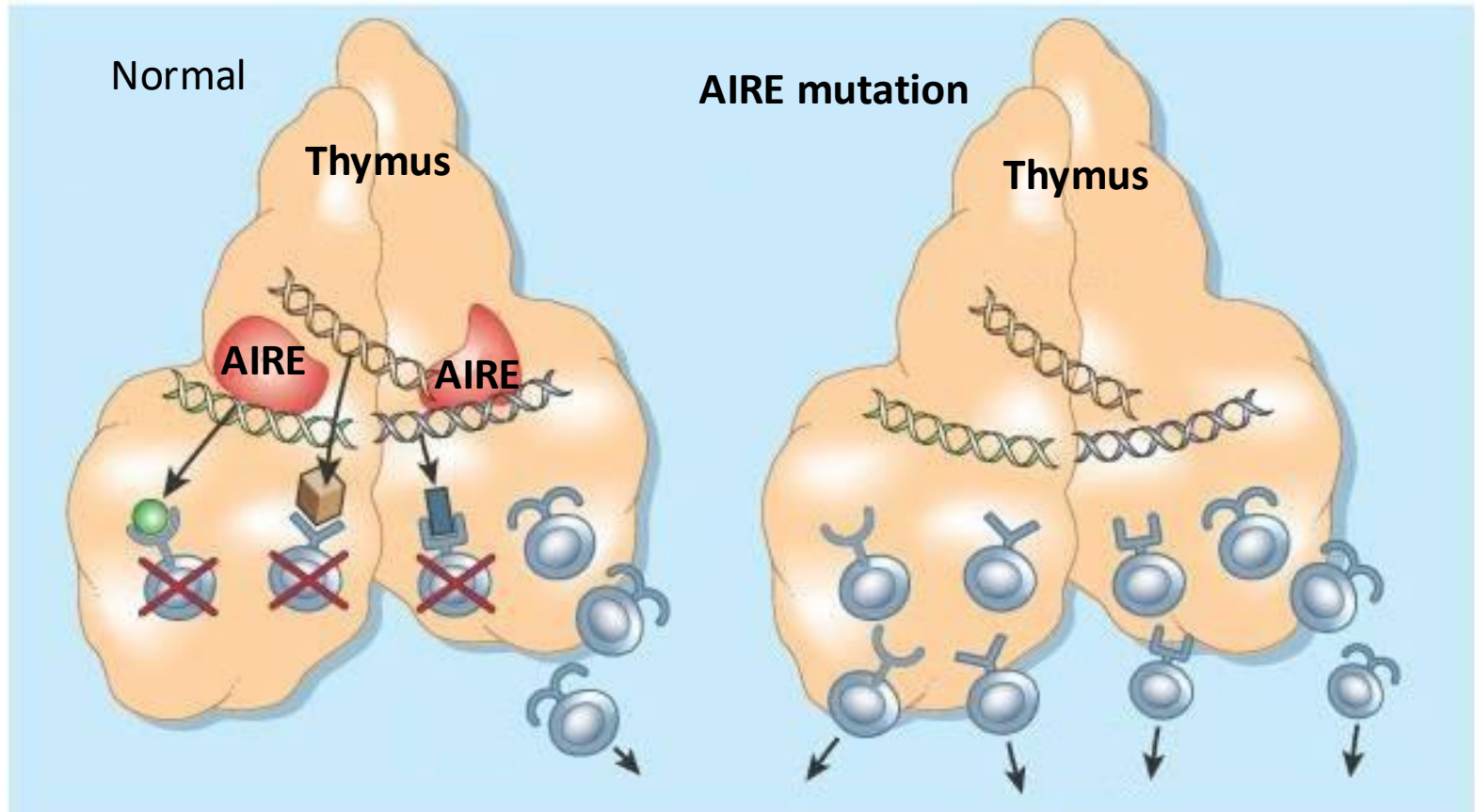


# Formation of neoantigens





# Loss of central tolerance



**Negative selection**

Autoreactive T cells  
released to the periphery

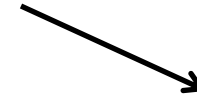
↙  
**APECED** (Autoimmune polyendocrinopathy-candidiasis - ectodermal dystrophy)

# Classification of autoimmune diseases

**Autoimmune disorders**  
(see in the clinical phase)



**Systemic diseases**  
(Affect the entire body)



**Organ-specific diseases**  
(Affect a specific organ)



Rheumatoid arthritis



Addison's disease



Myasthenia gravis



Scleroderma  
(Systemic sclerosis)



SLE (Systemic lupus  
erythematosus)



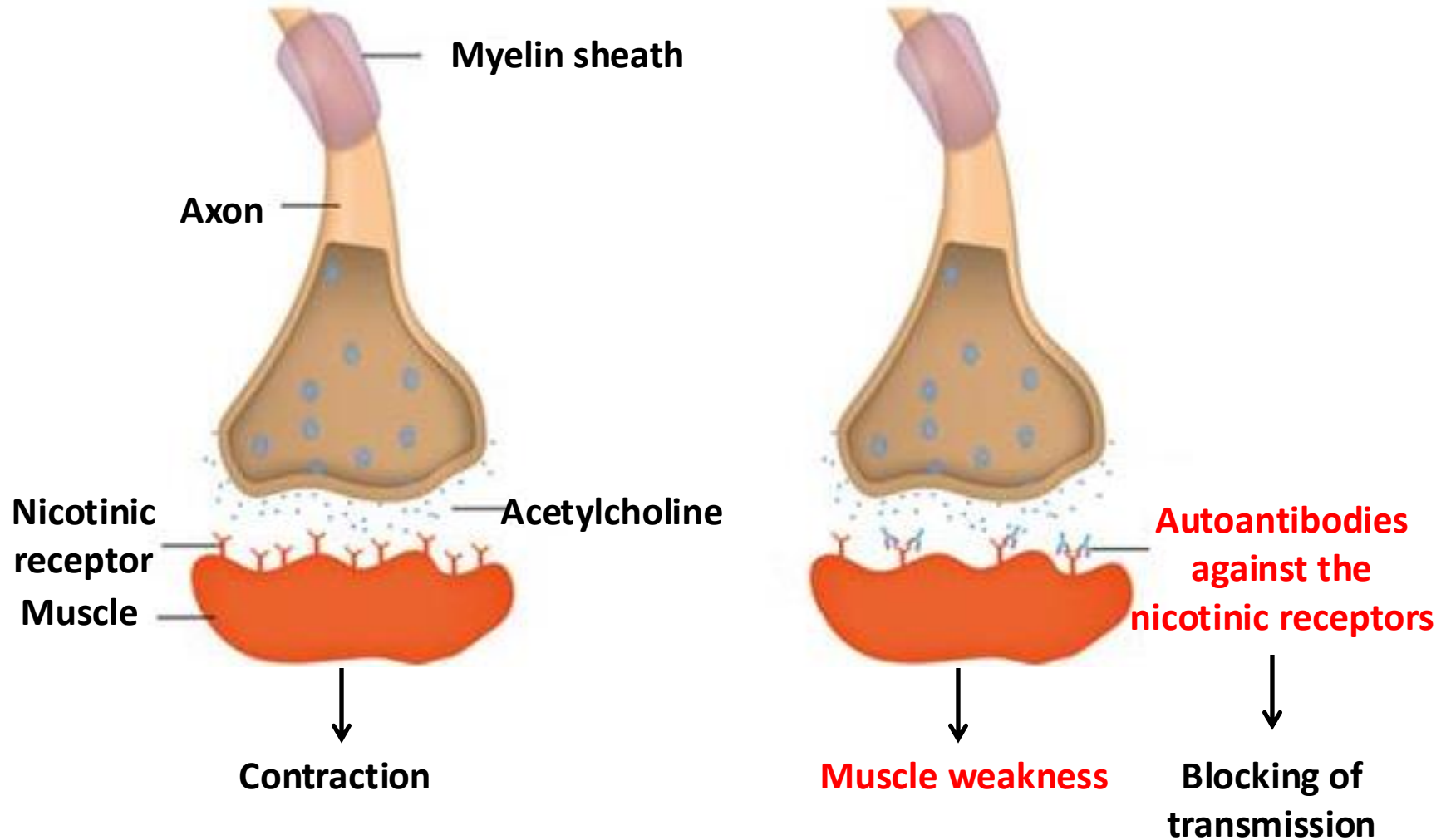
Graves-Basedow disease



# Organ-specific autoantibodies

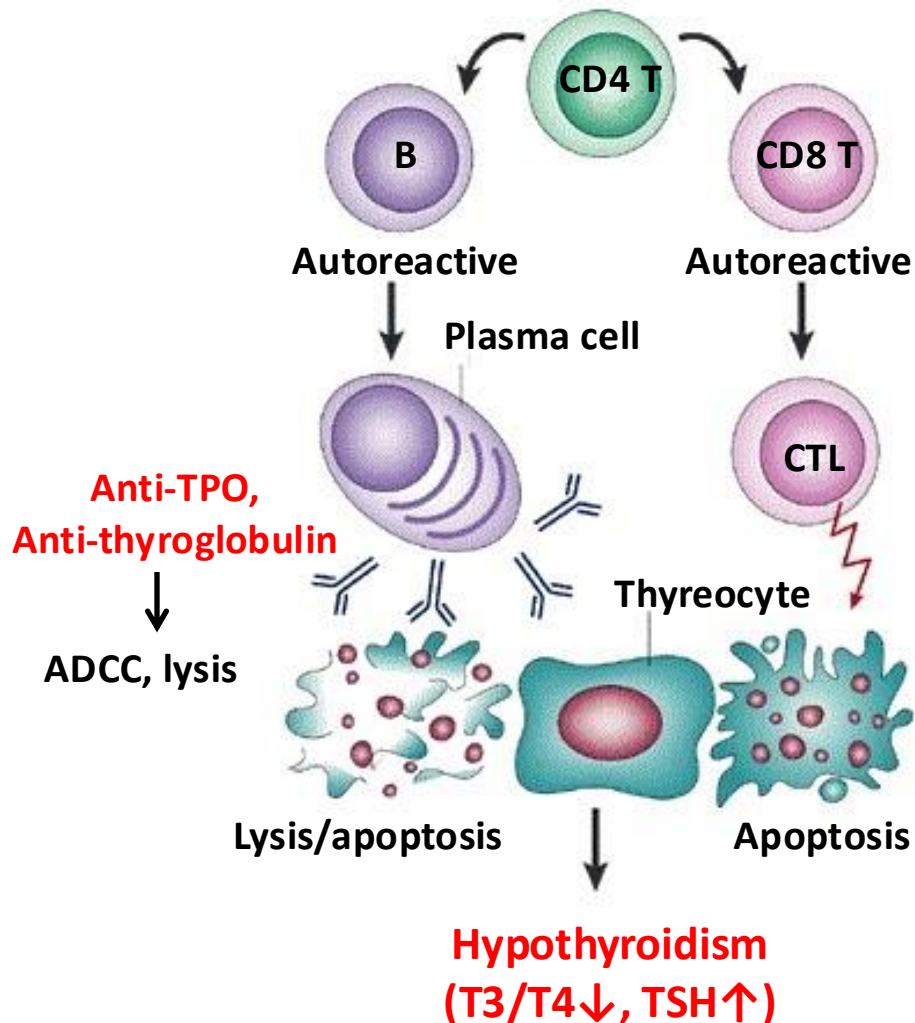
- Occur in organ-specific autoimmune diseases.
- Some examples of the targets of organ-specific autoantibodies:
  - Type I diabetes mellitus (IDDM): glutamic acid decarboxylase (GAD), tyrosine phosphatase-like protein (IA-2)
  - **Autoimmune thyroid diseases: thyroperoxidase (TPO), thyroglobulin**
  - Goodpasture syndrome: type IV collagen (basal membrane in the glomeruli and the lung)
  - **Myasthenia gravis:** postsynaptic nicotinic **acetylcholine receptors** (neuromuscular junction)
  - **Celiac disease: tissue transglutaminase (tTG)**, endomysium, gliadin (the latter is a component of gluten found in cereals and therefore is not an autoantigen!)
  - Primary biliary cirrhosis: several mitochondrial antigens
- Detection of such autoantibodies can be **diagnostic** and also have **prognostic significance**. Their levels can also be measured to **check the efficacy of treatment**.

# Myasthenia gravis

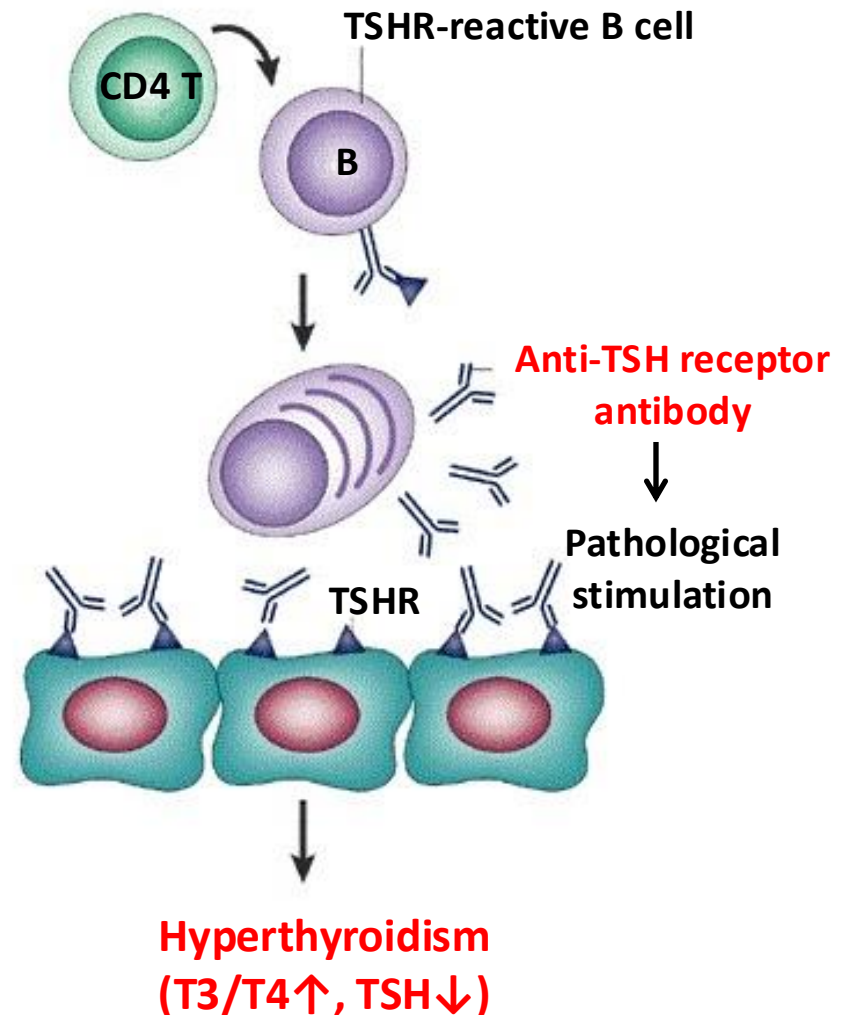


# Autoimmune thyroid diseases

Hashimoto's thyroiditis:



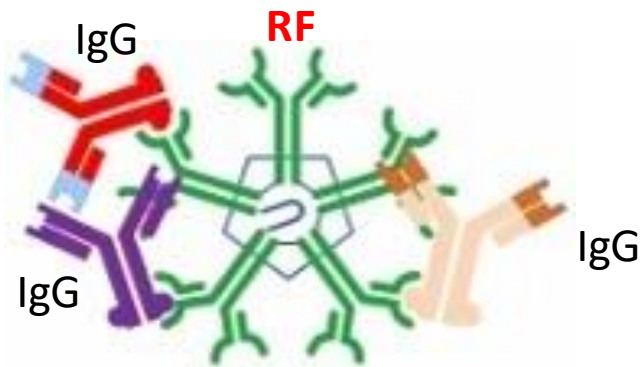
Graves-Basedow disease:



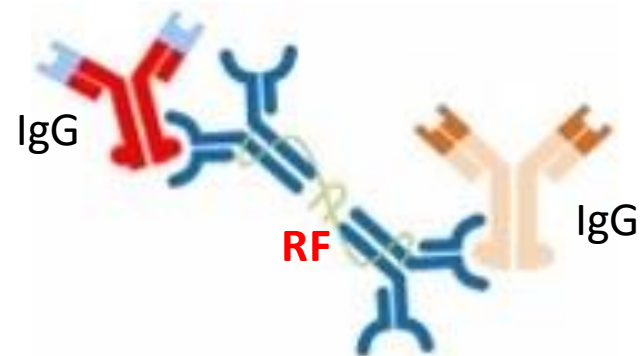


# Autoantibodies in systemic diseases

- Examples:
  - **Anti-nuclear antibodies (ANA)**
  - Anti-citrullinated protein antibodies (ACPA)
  - **Anti-neutrophil cytoplasmic antibodies (ANCA)**
  - **Rheumatoid factor (RF, anti-IgG antibodies, usually of IgM isotype but can be IgG or IgA)**
  - Antiphospholipid autoantibodies (pl. anti-cardiolipin, anti- $\beta$ 2 glycoprotein I)
- Detection of such autoantibodies can be **diagnostic** and also have **prognostic significance**. Their levels can also be measured to **check the efficacy of treatment**.



Rheumatoid factor of IgM isotype

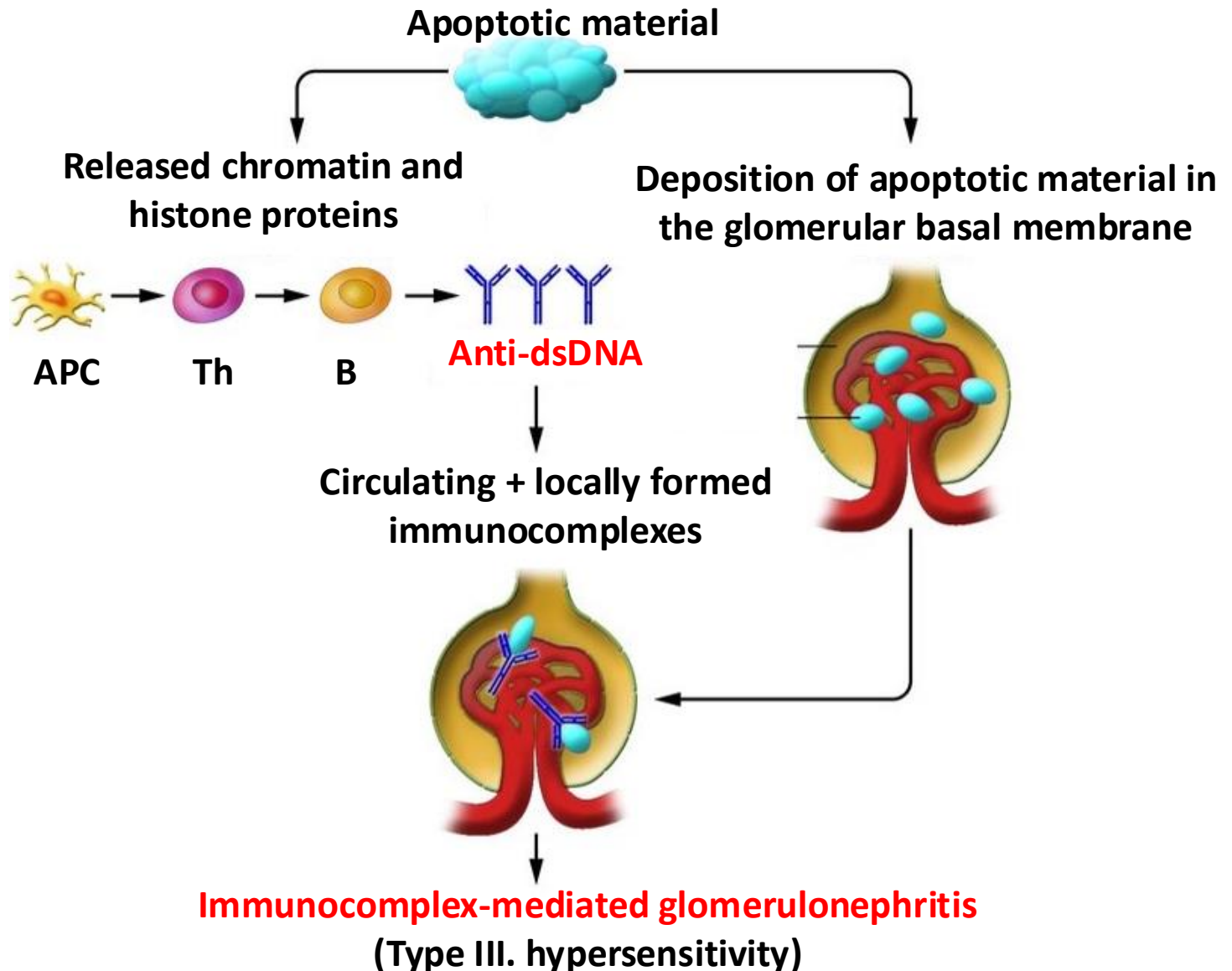


Rheumatoid factor of IgA isotype

# Anti-nuclear antibodies (ANA)

- They recognize different **nuclear structures**, such as:
  - **Anti-double-stranded DNA antibodies** (anti-dsDNA) → Mainly in **SLE**
  - Anti-Smith (anti-SM), against ribonucleoproteins → **SLE**
  - **Anti-Scl-70**, against topoisomerase I → **Scleroderma**
  - **Anti-centromere** antibodies → **Scleroderma**, primary biliary cirrhosis
  - Anti-Ro (anti-SSA) and anti-La (anti-SSB) → Sjögren's syndrome, SLE
  - **Anti-Jo-1** against histidyl-tRNA synthetase → **Polymyositis, dermatomyositis**
  - Anti-histone antibodies → Drug-induced SLE

# Lupus nephritis

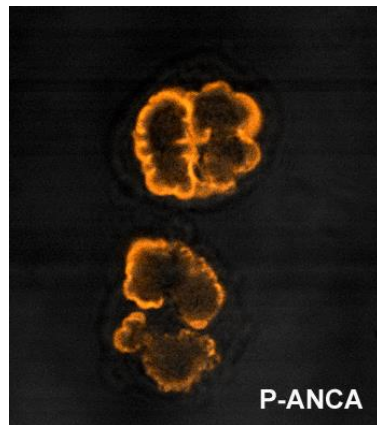




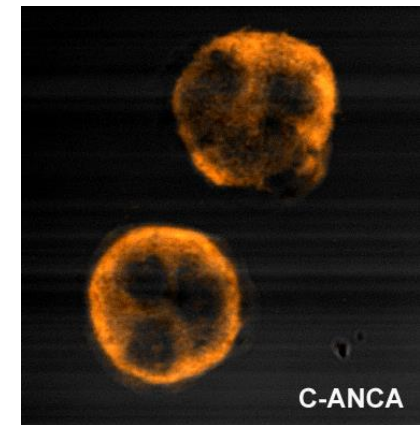
# Anti-neutrophil cytoplasmic antibodies (ANCA)

Have two main types:

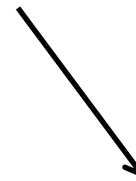
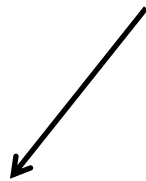
**Perinuclear pattern (p-ANCA)**



**Cytoplasmic pattern (c-ANCA)**



Indirect immunofluorescence  
microscopy

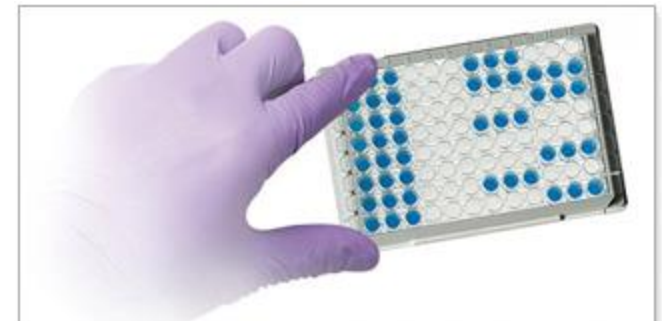


- Antigen: mainly **myeloperoxidase** (MPO)
- Examples:
  - Ulcerative colitis
  - Churg-Strauss syndrome
  - Microscopic polyangiitis
  - Primary sclerosing cholangitis
- Antigen: mainly **proteinase 3** (PR3)
- Examples:
  - Wegener's granulomatosis (GPA: Granulomatosis with polyangiitis)

# Detection of autoantibodies

- **Screening:**
  - **Direct immunofluorescence:** Performed on tissue samples taken from the patient. (e.g. dermatological or nephrological diseases, see in the clinical phase)
  - **Indirect immunofluorescence:** The patient's serum is tested in different cell cultures or tissue types for autoantibodies.
  - Different autoantibodies produce specific patterns with certain tissues or cells (e.g. homogenous nuclear, nucleolar, centromeric, mitochondrial, cytoplasmatic, etc.)
- **Antigen-specific serological methods:**
  - Done **to confirm the diagnosis** after a positive screening test or after the evaluation of the clinical signs and symptoms of the disease.
  - Methods:
    - **ELISA**
    - **Western blot**
    - **Radial immunodiffusion**

} 6-8th practices

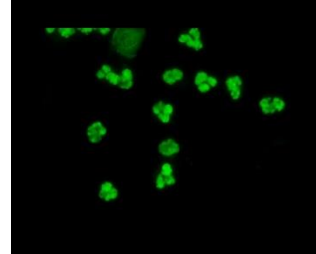


# Characteristics of tests

**Immunofluorescence:** not specific, shows patterns, multiplex image, manual, many autoantibodies cannot be detected



What is this?



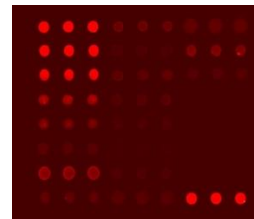
**ELISA:** antigen-specific, can measure individual autoantibodies, can be automated, can identify new autoantigens



Time ?

**Multiplex microarray technology:** antigen-specific, complex autoantibody patterns, can be automated

Quick and specific

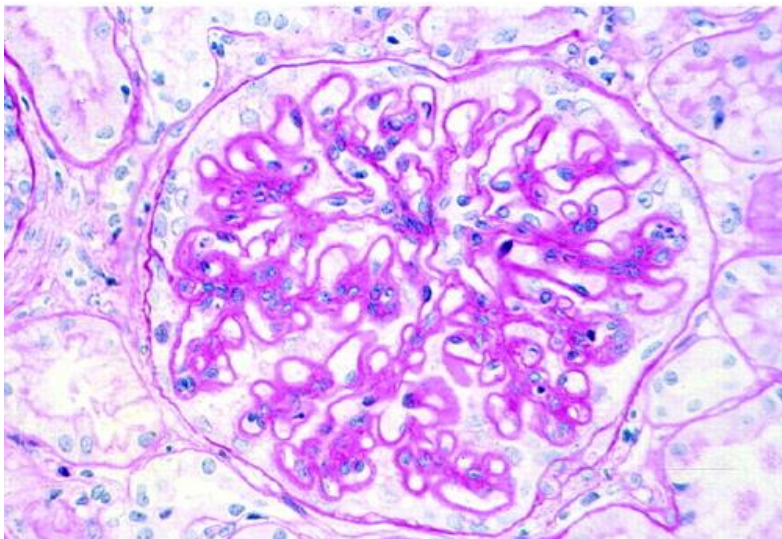
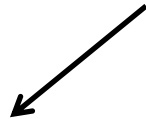


# Immunofluorescence I.

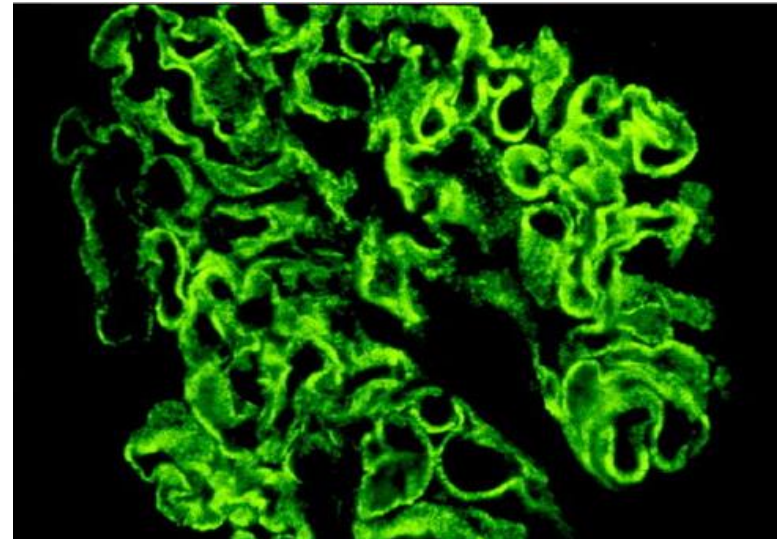
**Direct immunofluorescence** test from the renal manifestation of SLE (lupus nephritis):

- Membranous glomerulonephritis (see from pathology and nephrology)

1. Biopsy from the kidney of the patient

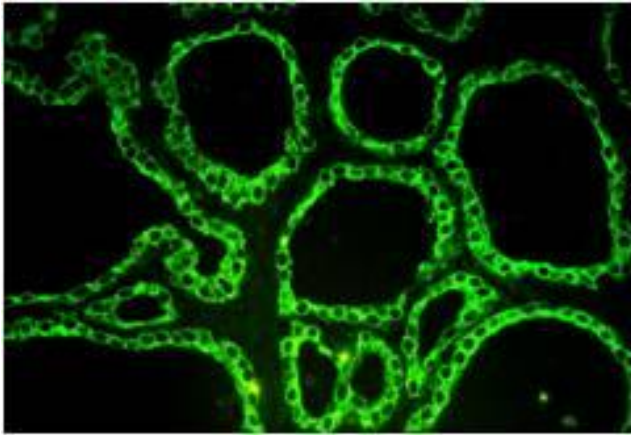


2. Glomerulus with PAS reaction:  
thickened basal membrane

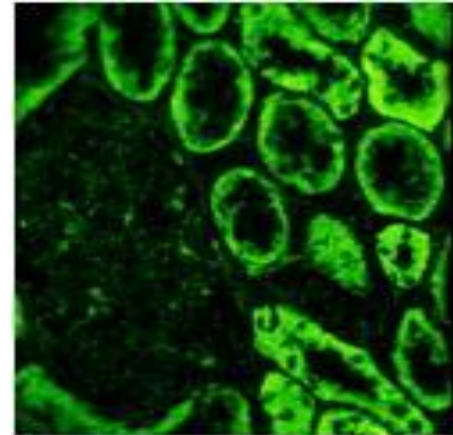


3. **Direct IF** microscopic image of a glomerulus:  
Deposition of **IgG immunocomplexes** in the GBM  
(Type III. hypersensitivity)

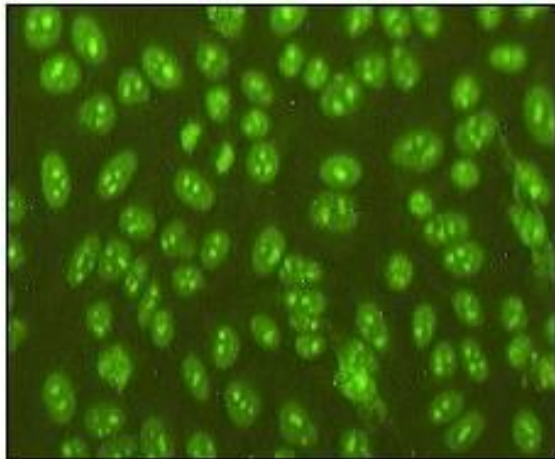
# Immunofluorescence II.



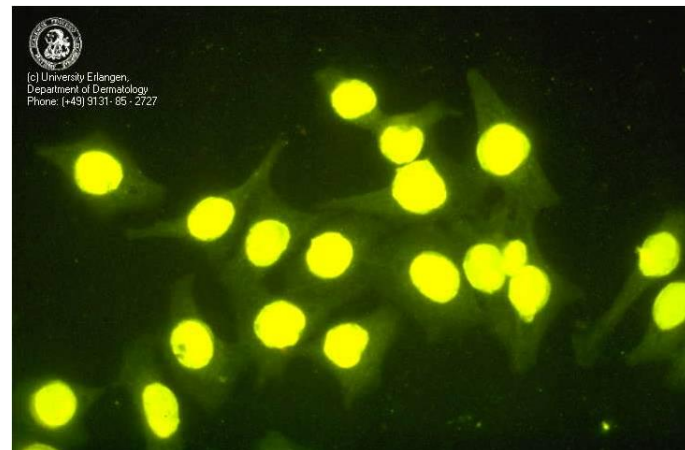
Anti-TPO autoantibodies in a thyroid tissue section



Mitochondrial staining pattern in a kidney tissue



Nucleolar pattern in Hep2 cells (anti-SSA)

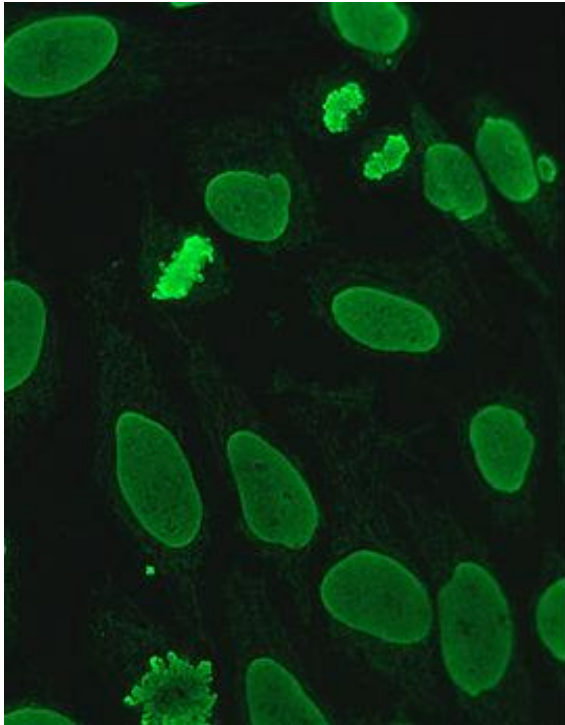


Homogenous nuclear staining in Hep2 cells (anti-dsDNA)

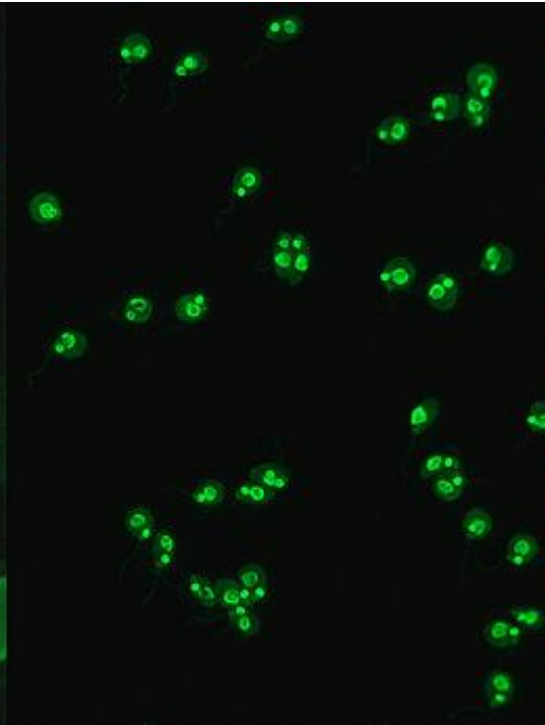


# Immunofluorescence III.

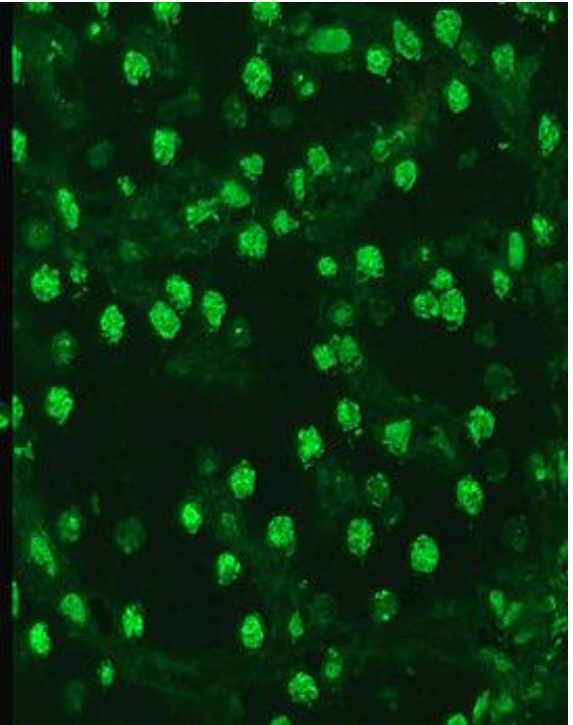
Detection of **Anti-dsDNA** from the serum of the patient with **indirect immunofluorescence microscopy** in different cell types:



HEp-20-10 cell culture  
(human epithelial tumor)



*Crithidia luciliae* cells  
(protist parasite)



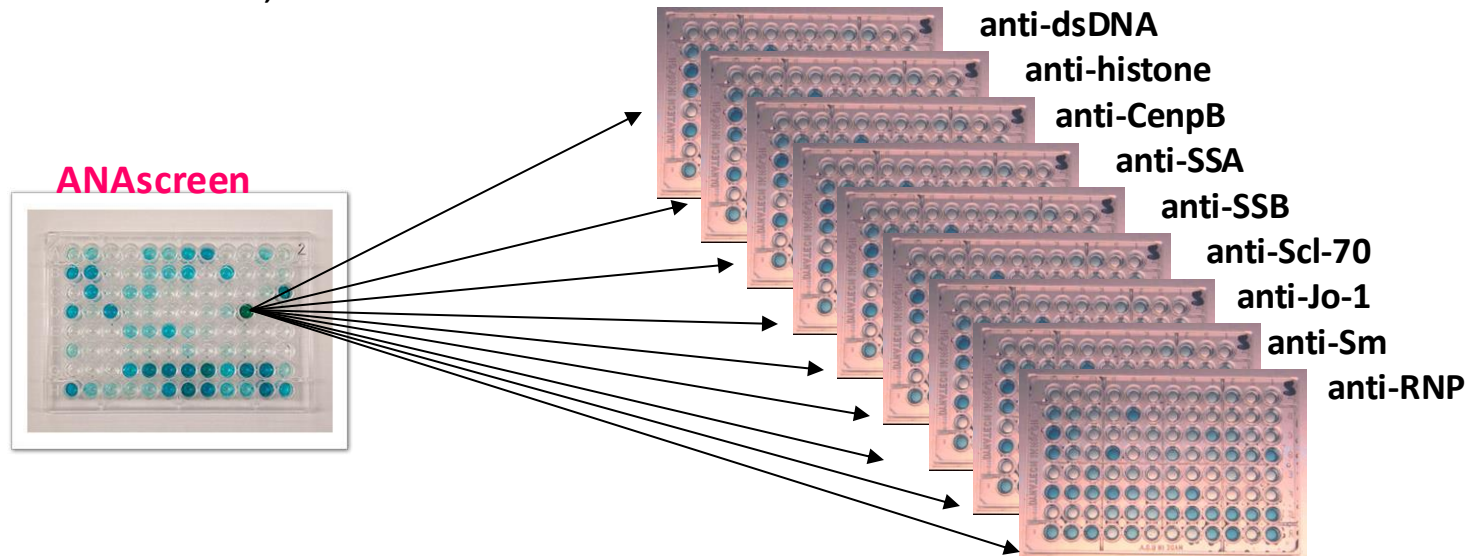
Rat liver tissue

# ANAscreen - microplate ELISA

The ELISA plate is sensitized with a **mixture of antigens** derived from the nuclei of Hep2 cells:

- dsDNA
- histone
- centromere
- SSA/Ro, SSB/La
- Sm, Sm/RNP
- Scl-70, Jo-1

→ If **positive**, than the test is repeated with ELISA plates **each sensitized with only one specific antigen.**

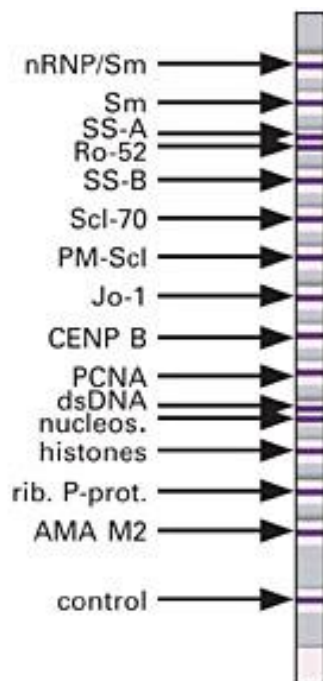


# Multiplex immunszerological methods

1. **Immunoblot:** the antigen is in a denatured conformation, high number of false positive/negative results, not used
2. **Protein chip methods: „microarray“:** - chip reader
  - glass
  - plastic
  - silicon
  - nano-well
3. **Microbead-based methods:** - Flow cytometer
  - Becton Dickinson
  - Bender MedSystem
  - Luminex platform



# ANA-Profil Immunoblot



Előnye: - 1 beteg mintája is vizsgálható

- gyors

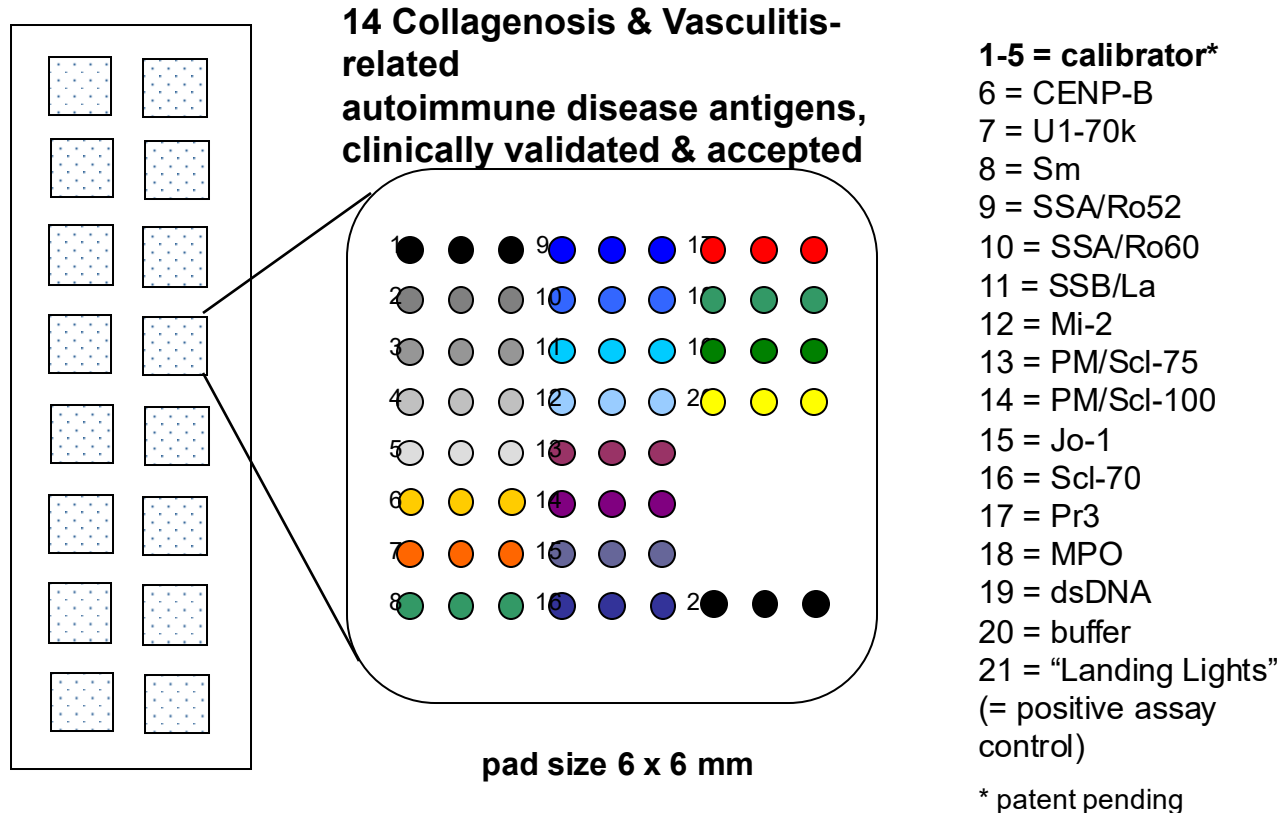
Hátrány: - antigén denaturált, nem megfelelő konformációban

- ál-negatív reakciók

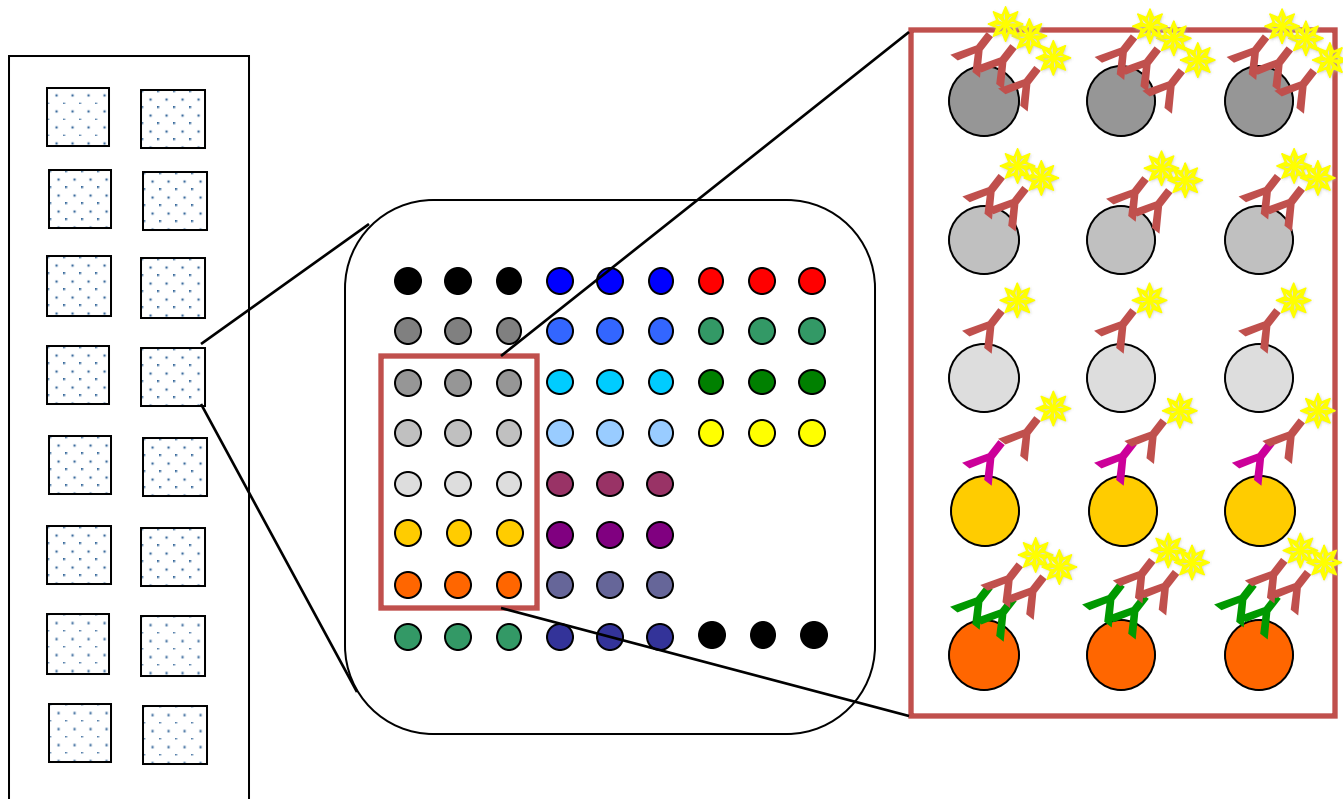
- sok antigén nem

vizsgálható

# CombiChip Autoimmune 1.0 Layout



# CombiChip Autoimmune 1.0 Layout



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