IMMUNOLÓGIAI ÉS BIOTECHNOLÓGIAI INTÉZET

13th practice: Autoantibody diagnostics

Basic Immunology

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

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 **immunodefficiencies**.

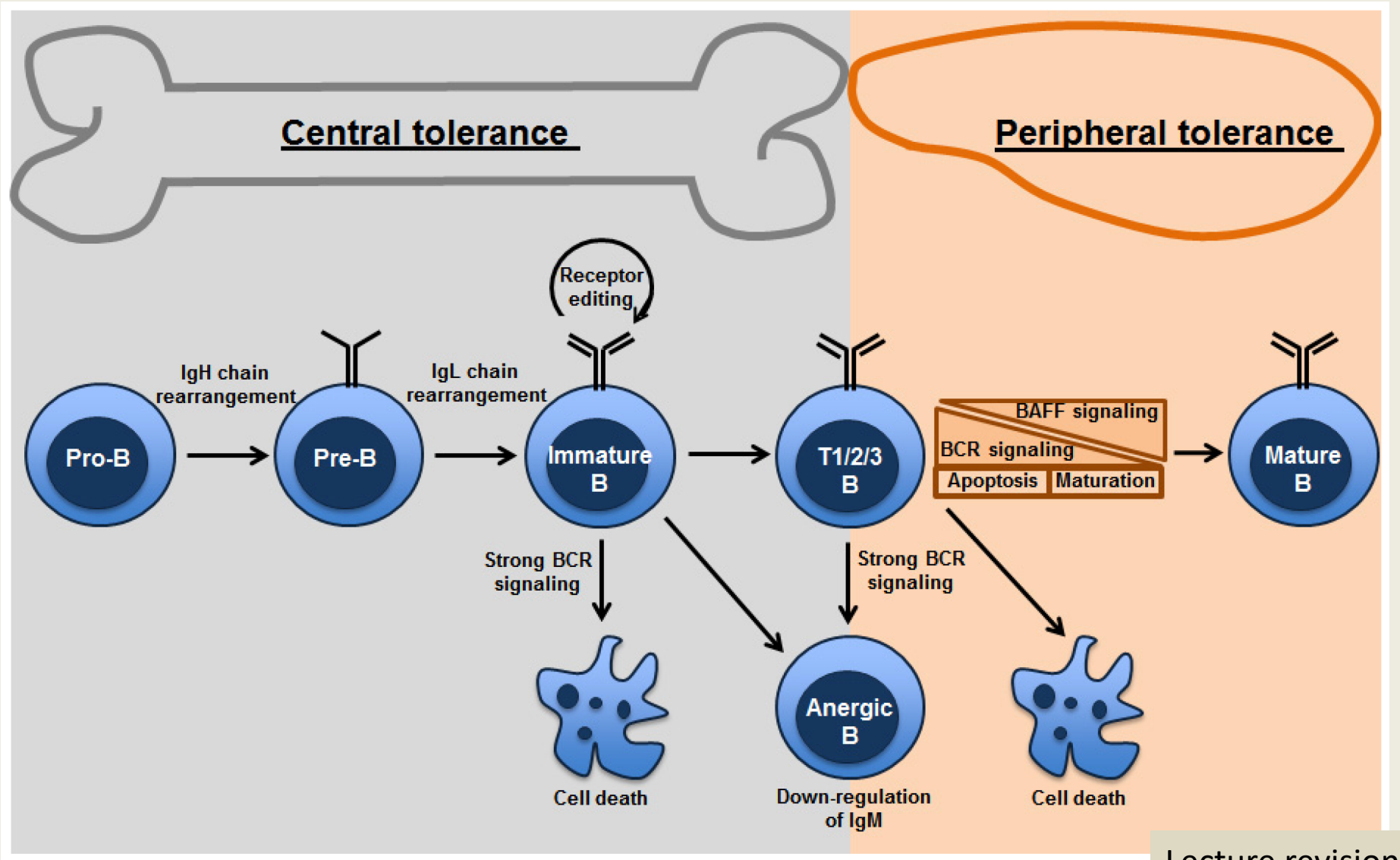
T-cell tolerance

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



Lecture revision

ACTIVE TOLERANCE

Anti-idiotype network

- Anti-idiotype 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
- γ/δ T, iγ/δT, 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

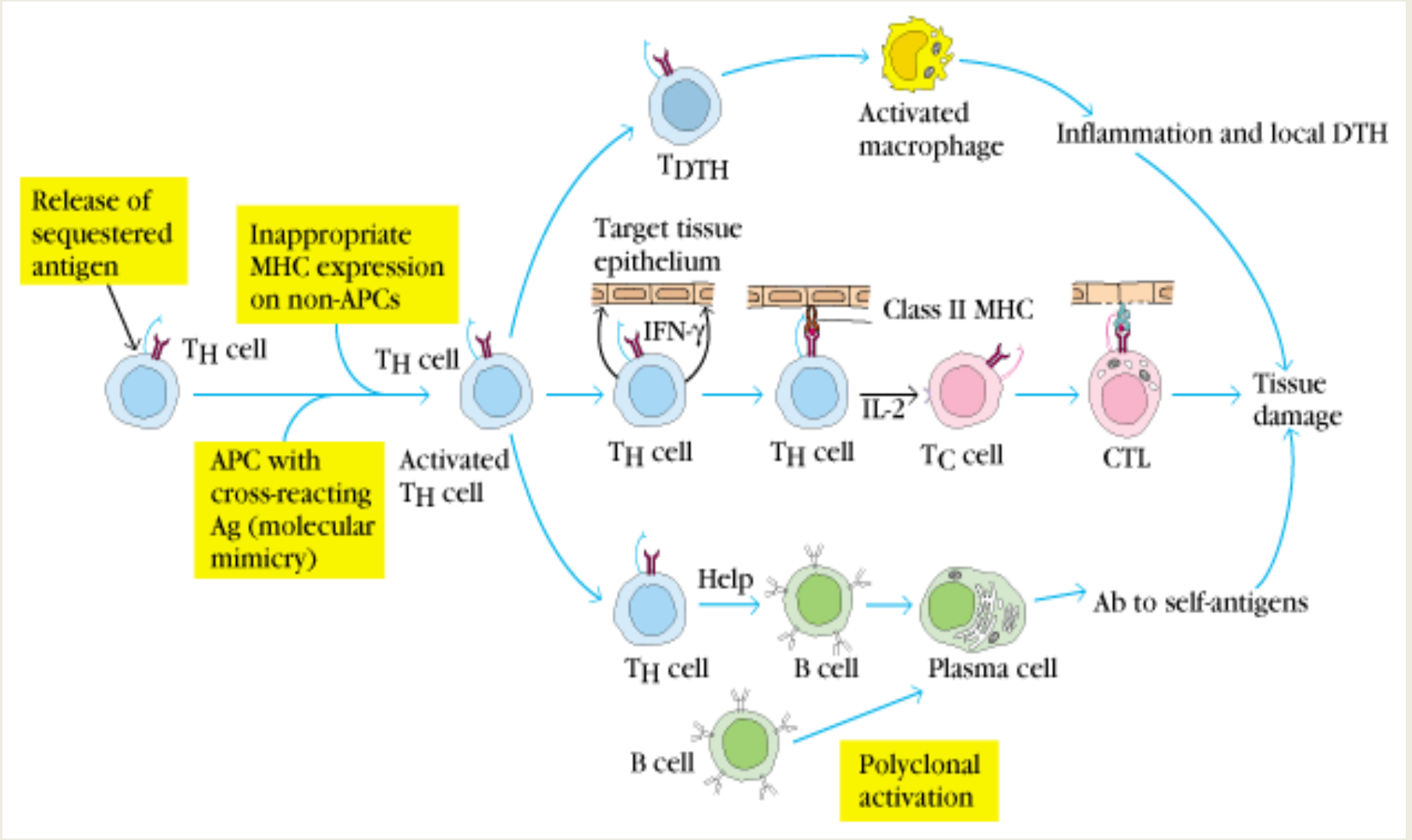
Pathomechanism of autoimmunity

- **Multifactor 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)



Lecture revision

Autoimmunity

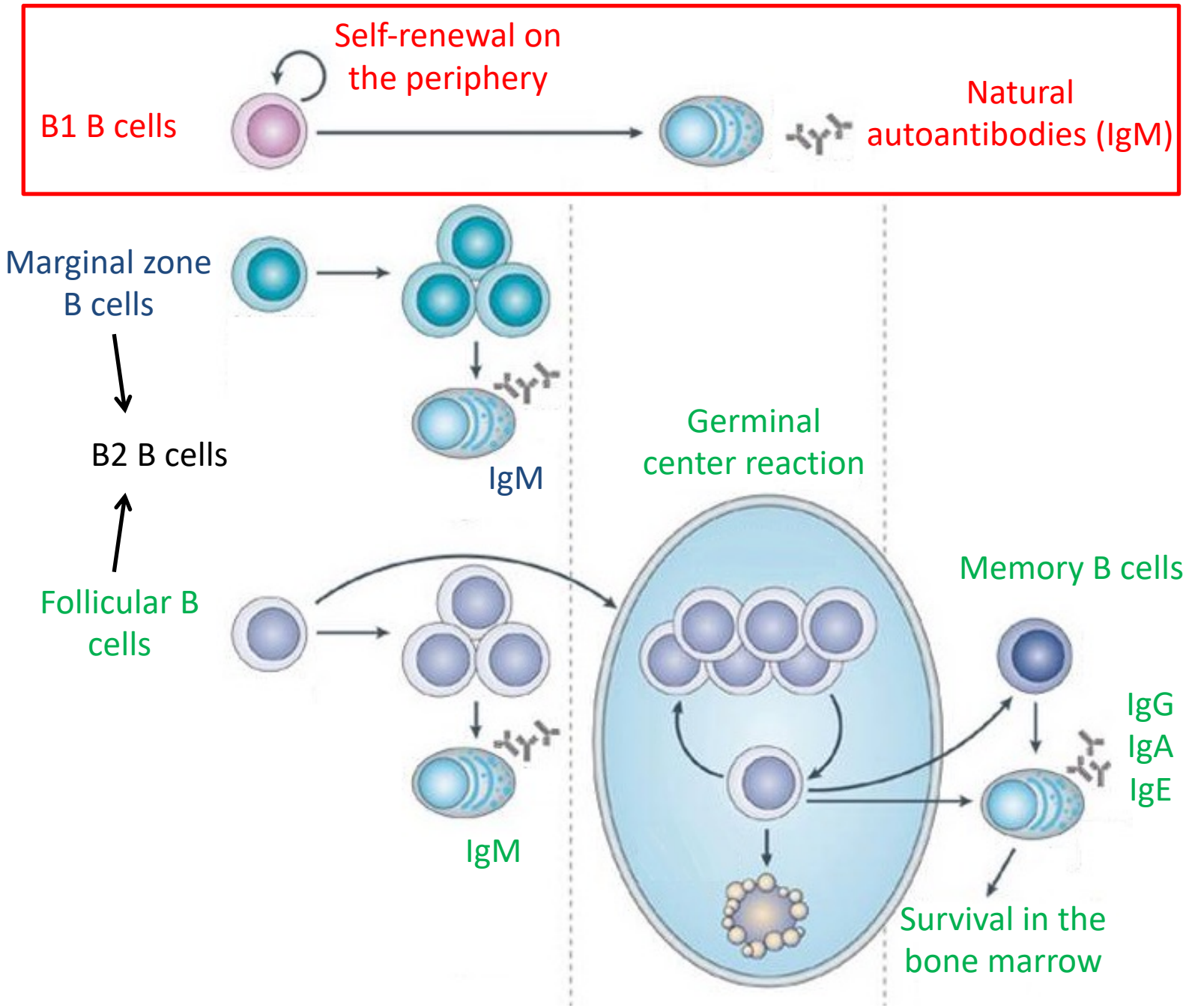
- Autoimmunity: Recognition and immune response to **self-antigens**.

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**

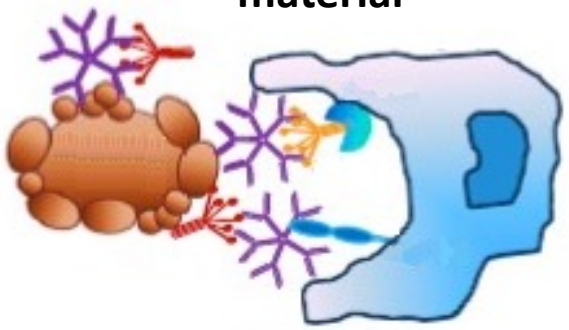

B1 B cells
Self-renewal on the periphery
Natural autoantibodies (IgM)
Marginal zone B cells
B2 B cells
Follicular B cells
IgM
Germinal center reaction
Memory B cells
IgM
IgG
IgA
IgE
Survival in the bone marrow

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**.

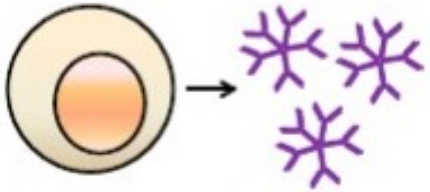
Immunological homunculus or **Immunculus**

Removal of apoptotic material



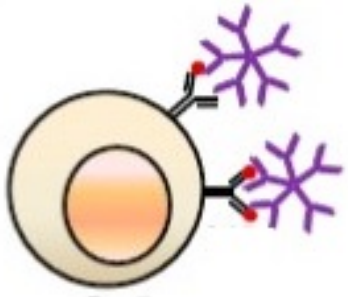
Prevention of autoimmunity (Elimination of DAMPs)

B1 B cells



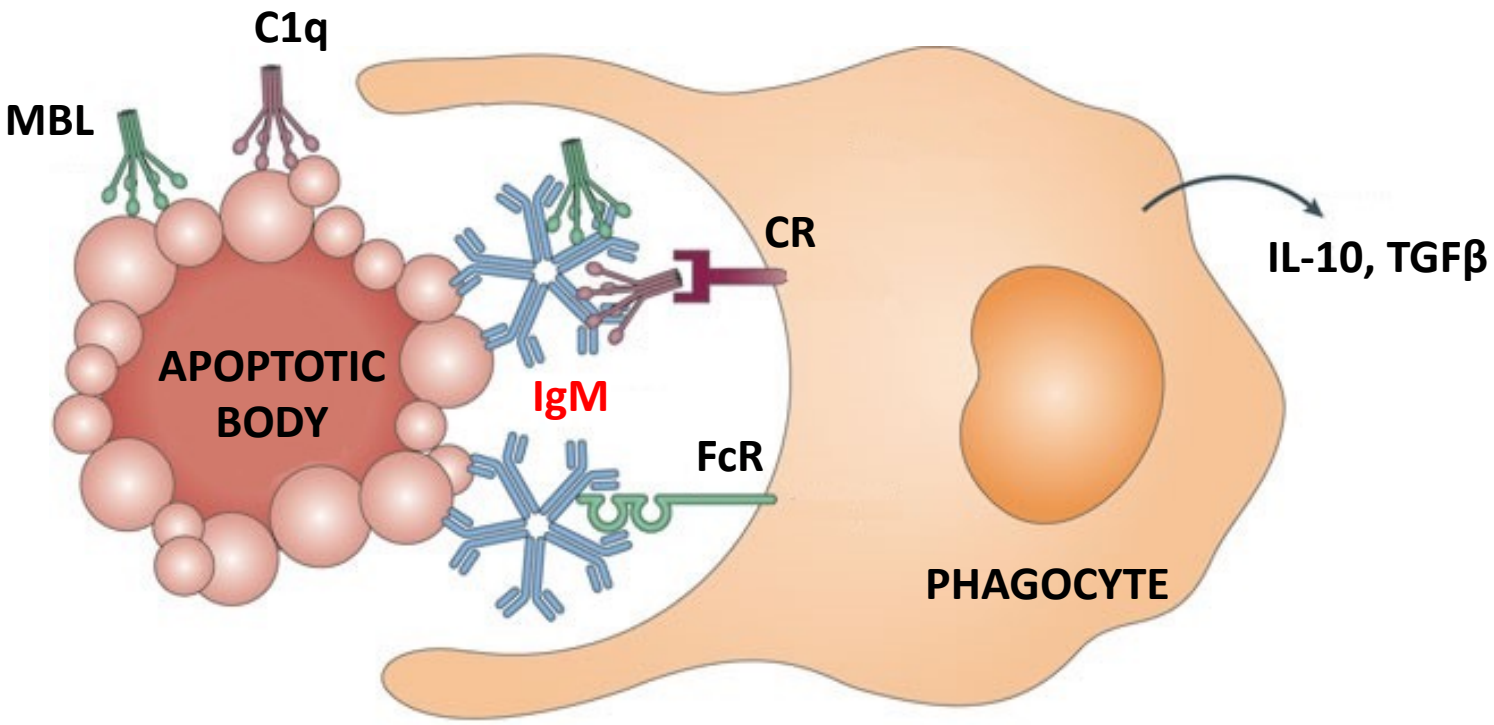
Natural autoantibodies

Immune regulation and homeostasis



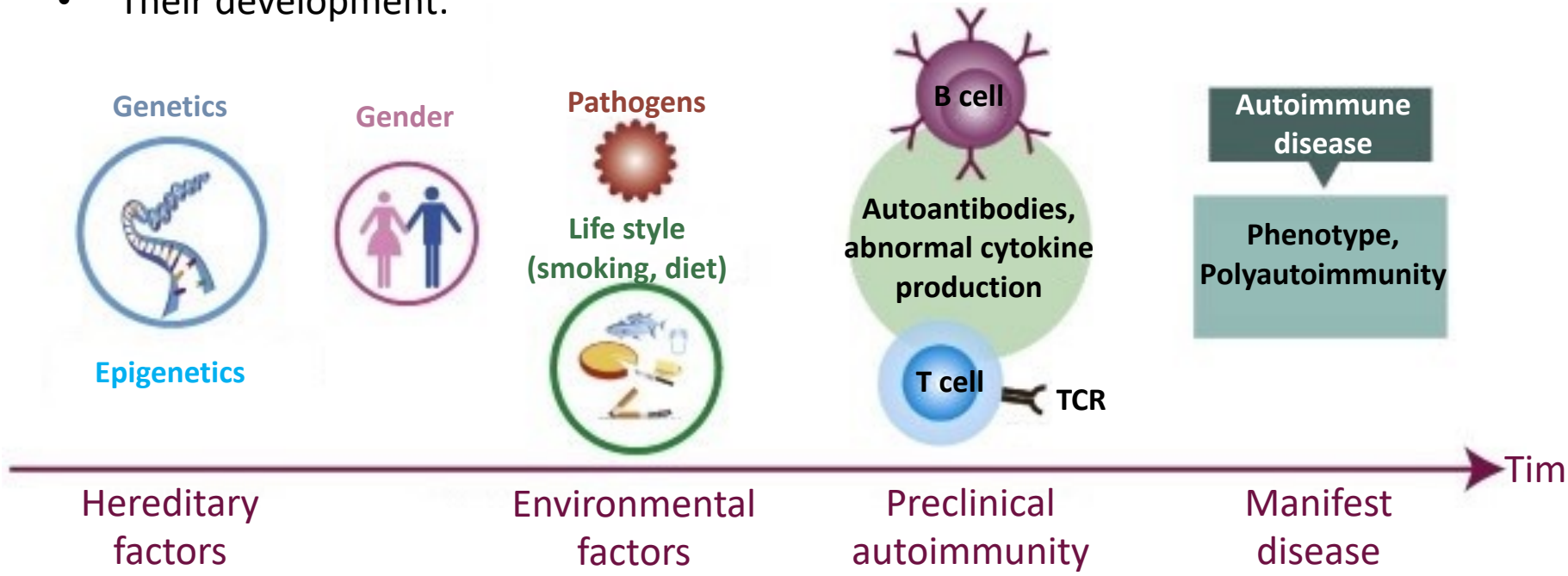
Removal of apoptotic bodies

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



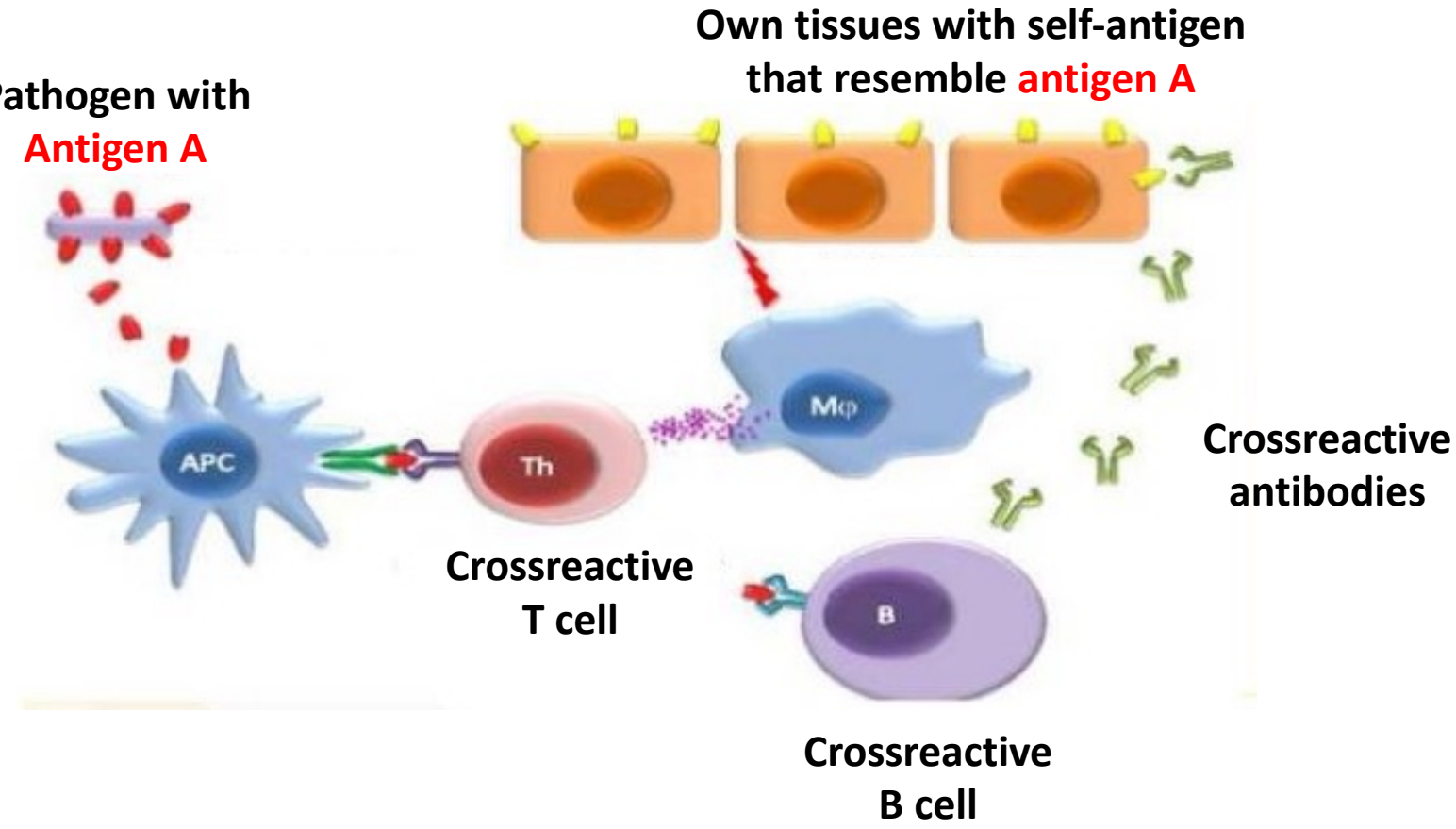
Autoimmune disorders

- 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:

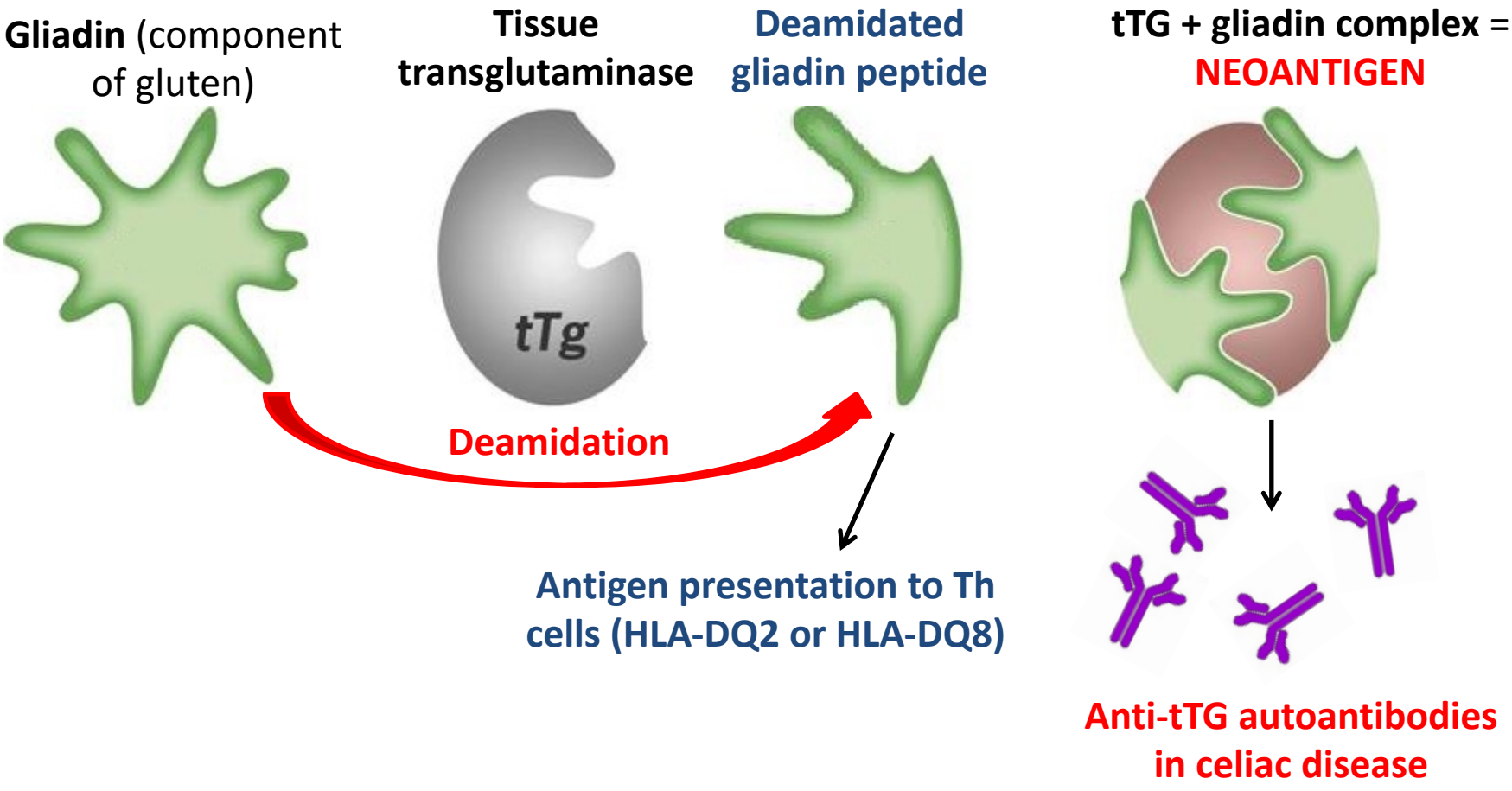


Molecular mimicry

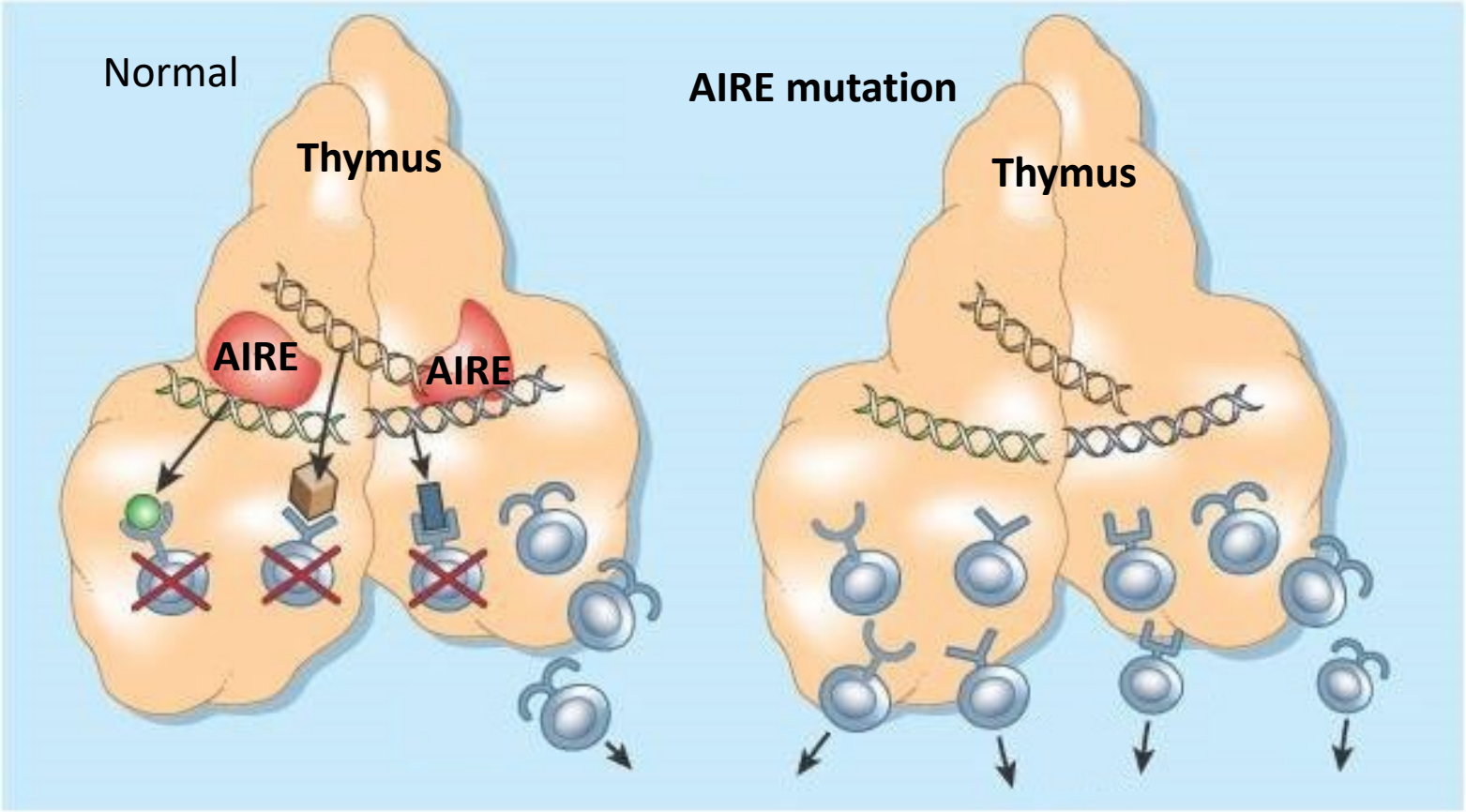
The pathogen's antigen shares a high degree of **structural similarity** with self-antigens. → Can lead to **cross-reaction and autoimmunity** in genetically susceptible people. (e.g. rheumatic fever after S*treptococcus pyogenes* infections)



Formation of neoantigens



Loss of central tolerance



Negative selection

Autoreactive T cells released to the periphery

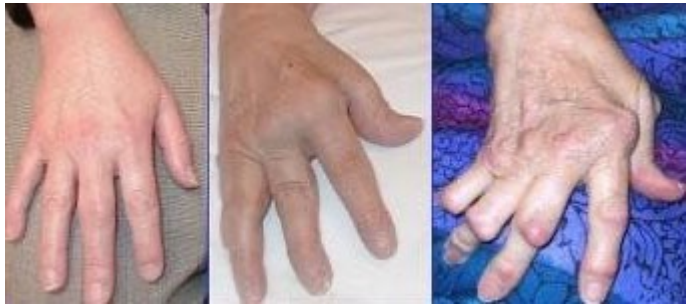
APECED (**A**utoimmune **p**oly**e**ndocrinopathy-**c**andidiasis - **e**ctodermal **d**ystrophy)

Classification of autoimmune diseases

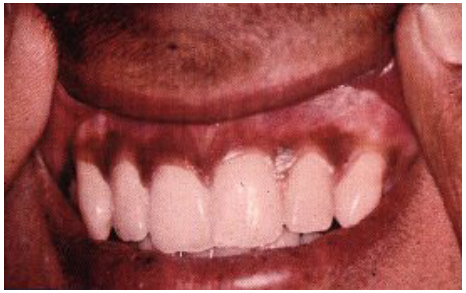
Autoimmune disorders
(see in the clinical phase)

Systemic diseases
(Affect the entire body)

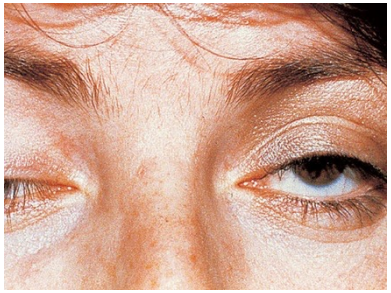
Organ-specific diseases
(Affect a specific organ)



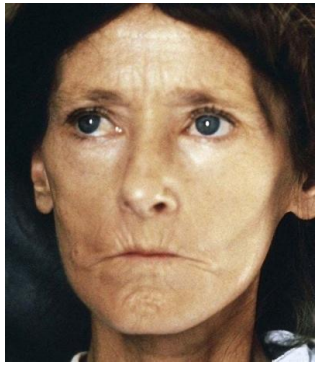
Rheumatoid arthritis



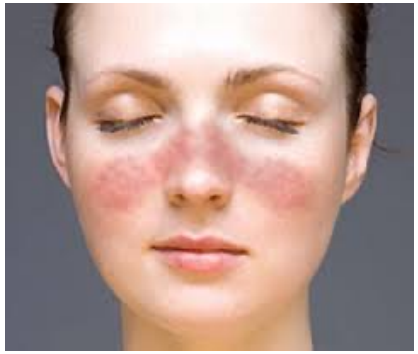
Addison's disease



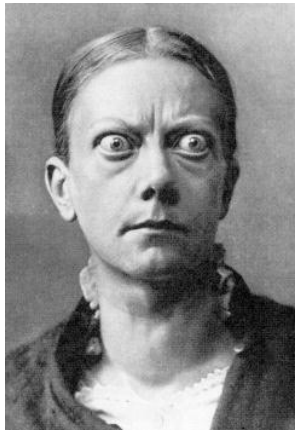
Myasthenia gravis



Scleroderma
(Systemic sclerosis)



SLE (Systemic lupus erythematosus)

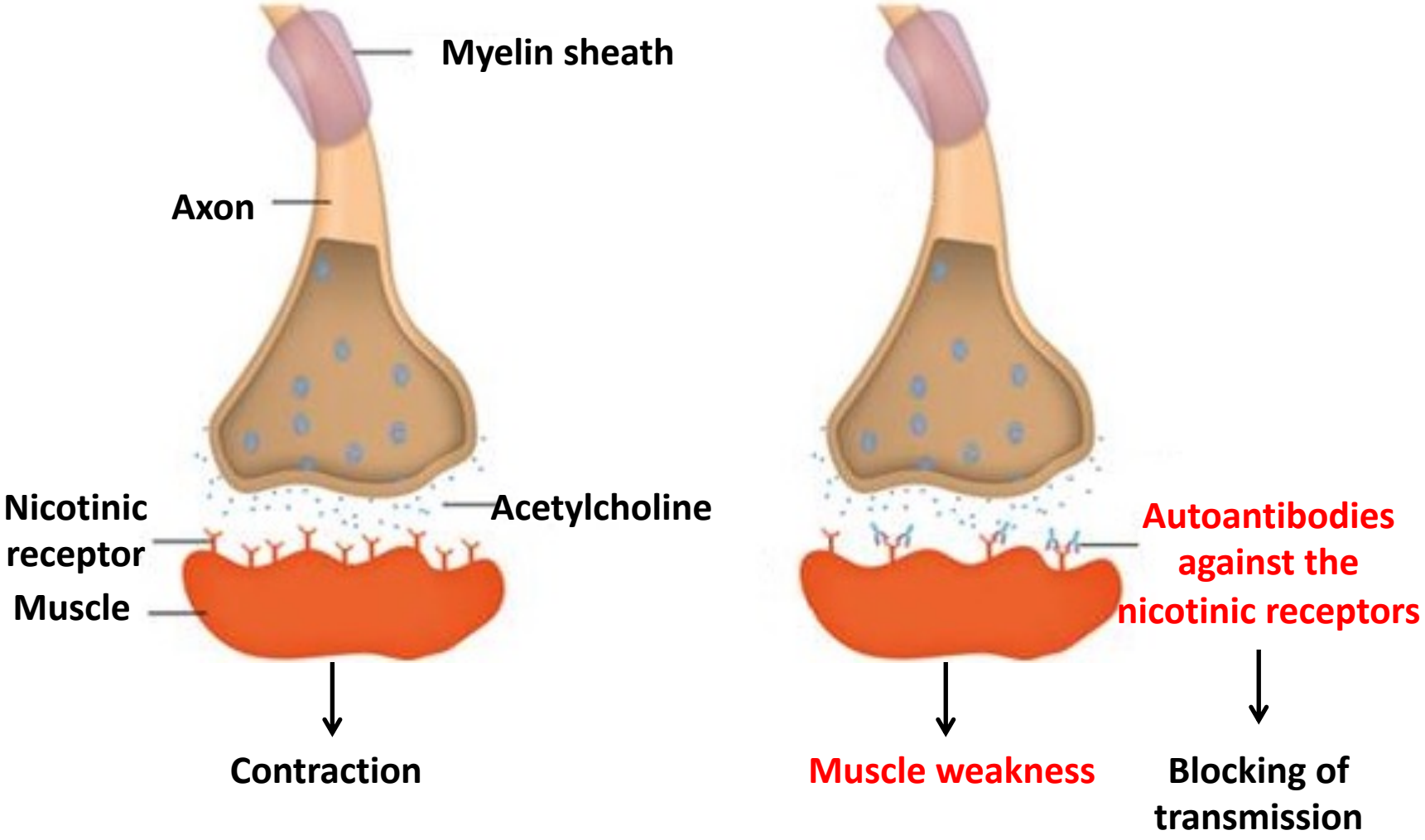


Graves-Basedow disease

Organ-specific autoantibodies

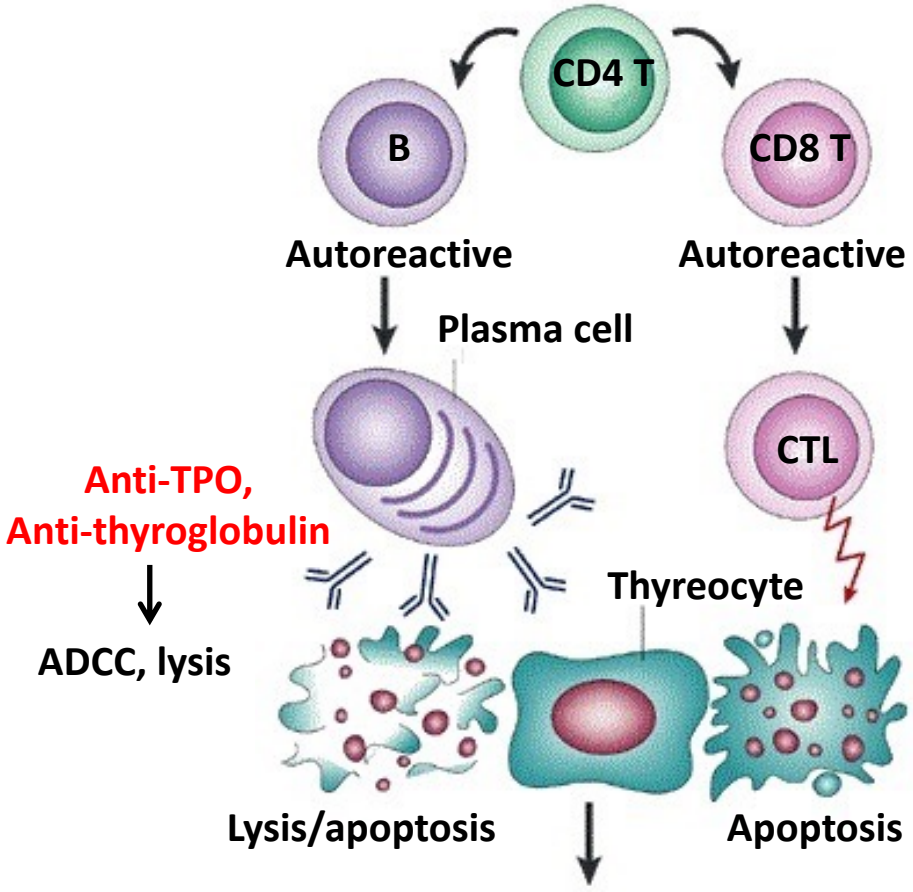
- Occur in organ-specific autoimmune diseases.
- Some examples of the targets of organ-specific autoantibodies:
  - Type I diabetes mellitus (IDDM): glutamic acid decarboxylase (GAD), tyrosine phosphatase-like protein (IA-2)
  - **Autoimmune thyroid diseases**: **thyroperoxidase** (TPO), **thyroglobulin**
  - 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



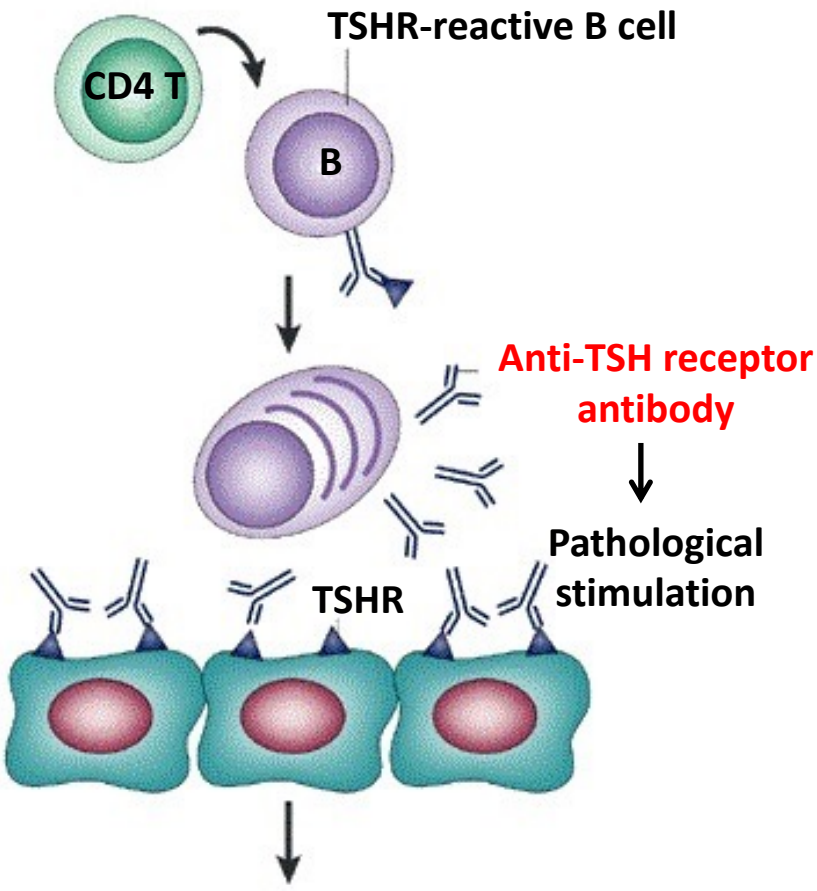
Autoimmune thyroid diseases

Hashimoto's thyroiditis:



Hypothyroidism
(T3/T4↓, TSH↑)

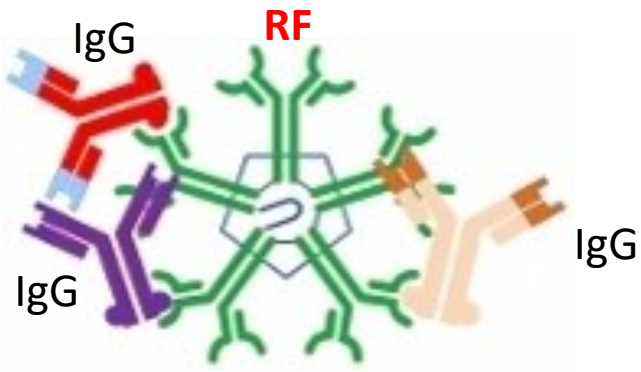
Graves-Basedow disease:



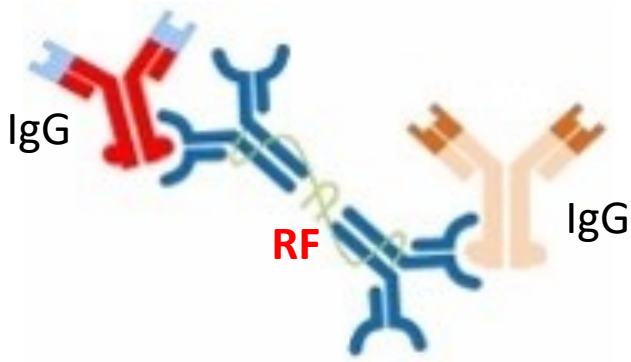
Hyperthyroidism
(T3/T4↑, TSH↓)

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-β2 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

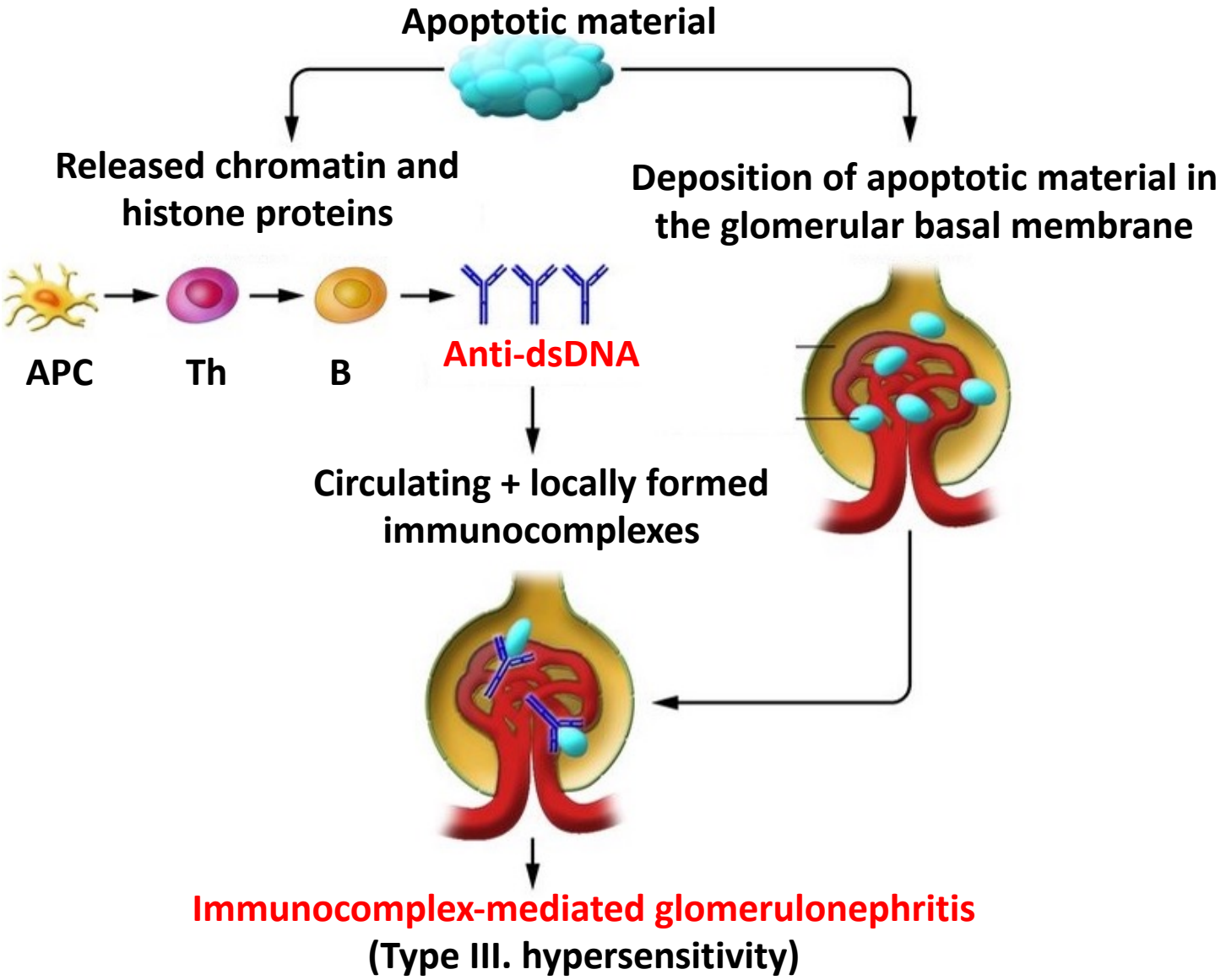


Rheumatoid factor of IgA isotype

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

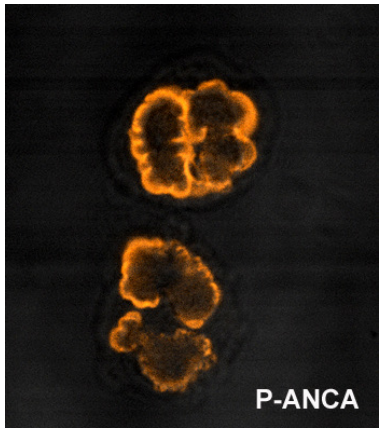
Lupus nephritis



Anti-neutrophil cytoplasmic antibodies (ANCA)

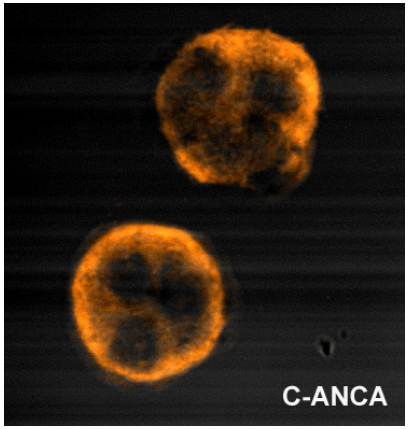
Have two main types:

Perinuclear pattern (p-ANCA)



Indirect immunofluorescence microscopy

Cytoplasmic pattern (c-ANCA)



- 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 polyangitis)

Detection of autoantibodies

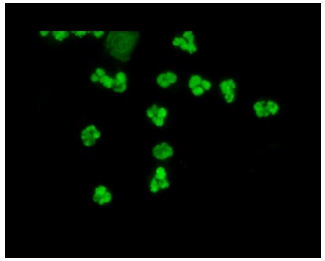
- **Screening**:
  - **Direct immunofluorescence**: Performed on tissue samples taken from the patient. (e.g. dermatological or nephrological diseases, see in the clinical phase)
  - **Indirect immunofluorescence**: The patient's serum is tested in different cell cultures or tissue types for autoantibodies.
  - 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?

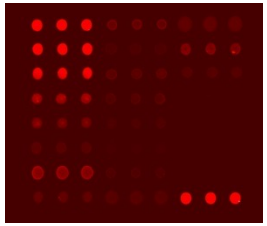


ELISA: antigen-specific, can measure individual autoantibodies, can be automated, can identify new autoantigens

Time ?

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

Quick and specific

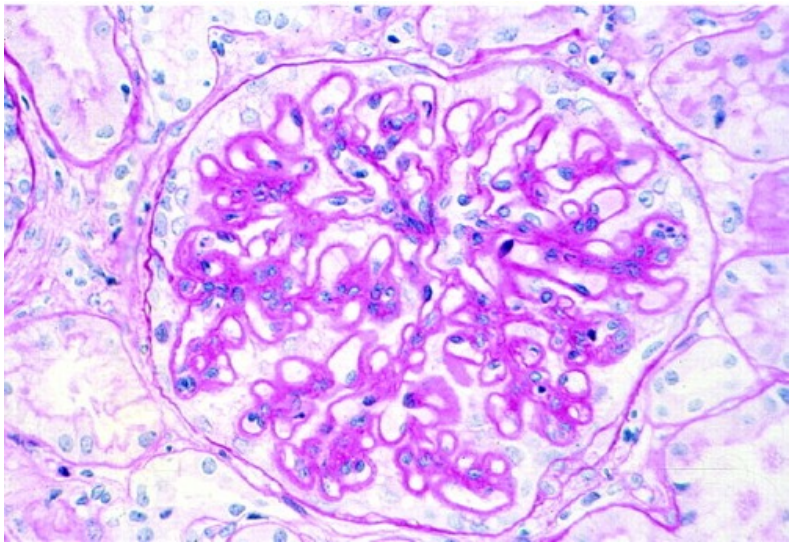


Immunofluorescence I.

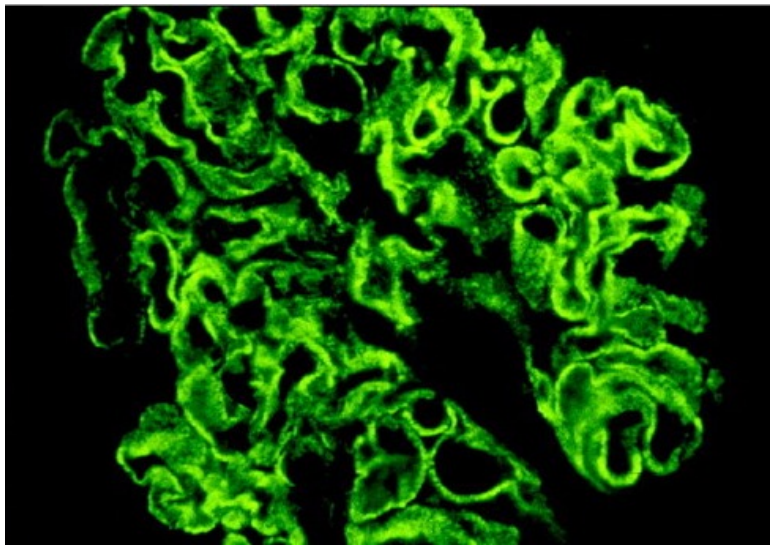
Direct immunofluorescence test from the renal manifestation of SLE (lupus nephritis):

- Membranous glomerulonephritis (see from pathology and nephrology)

1. Biopsy from the kidney of the patient

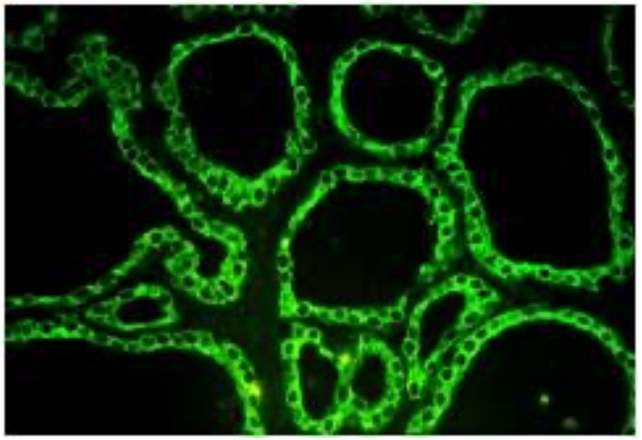


2. Glomerulus with PAS reaction: thickened basal membrane

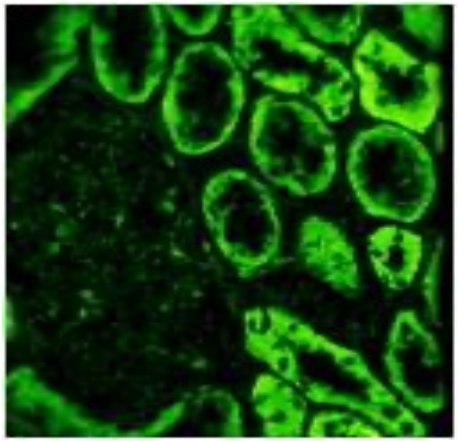


3. Direct IF microscopic image of a glomerulus: Deposition of **IgG immunocomplexes** in the GBM (Type III. hypersensitivity)

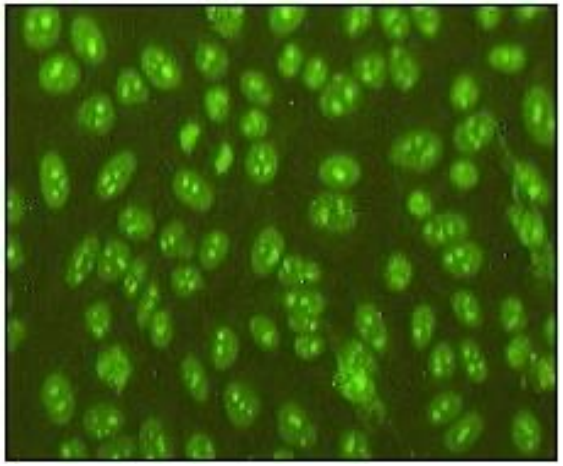
Immunofluorescence II.



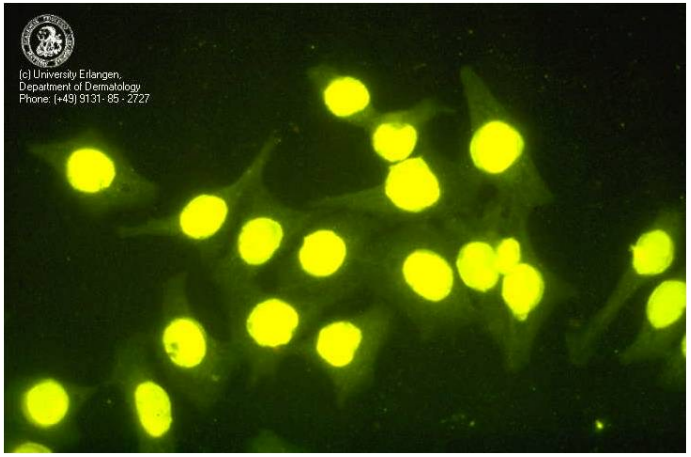
Anti-TPO autoantibodies in a thyroid tissue section



Mitochondrial staining pattern in a kidney tissue



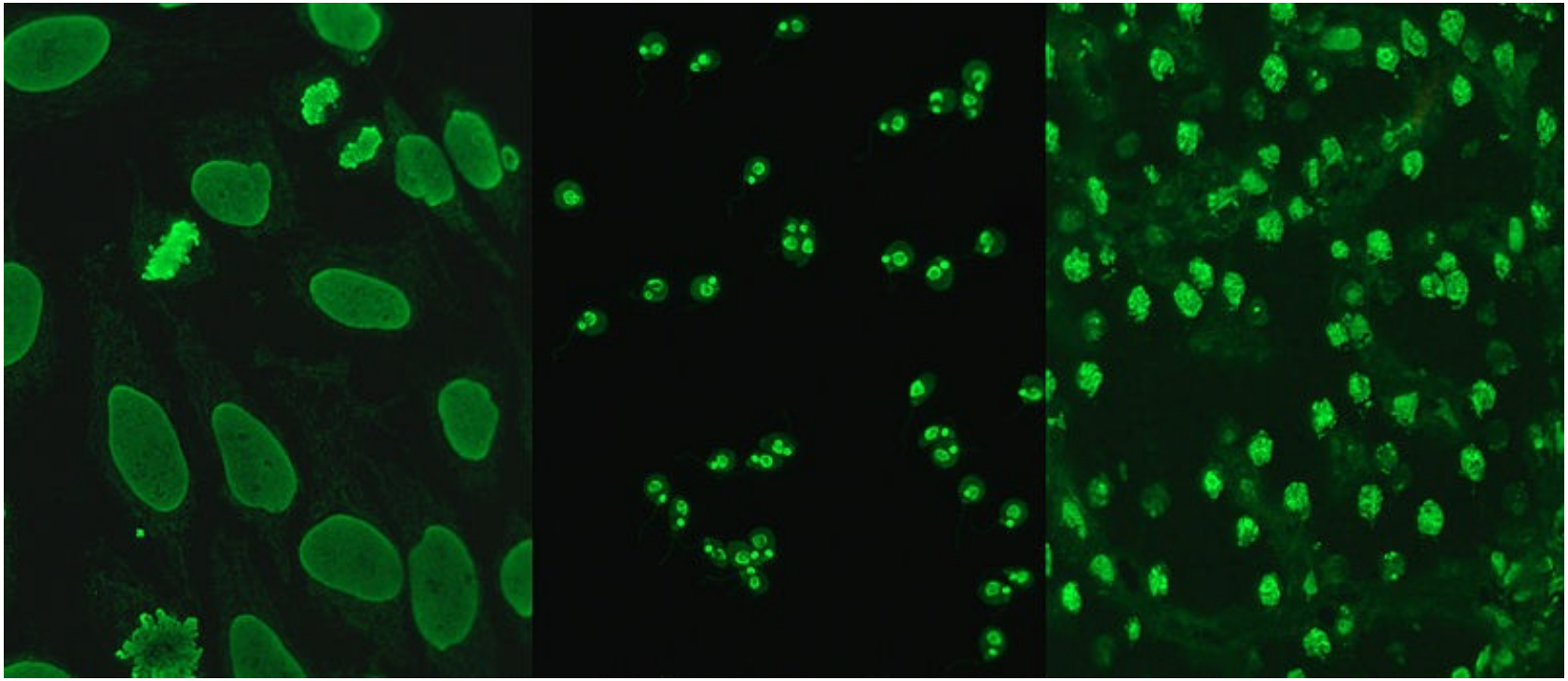
Nucleolar pattern in Hep2 cells (anti-SSA)



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)

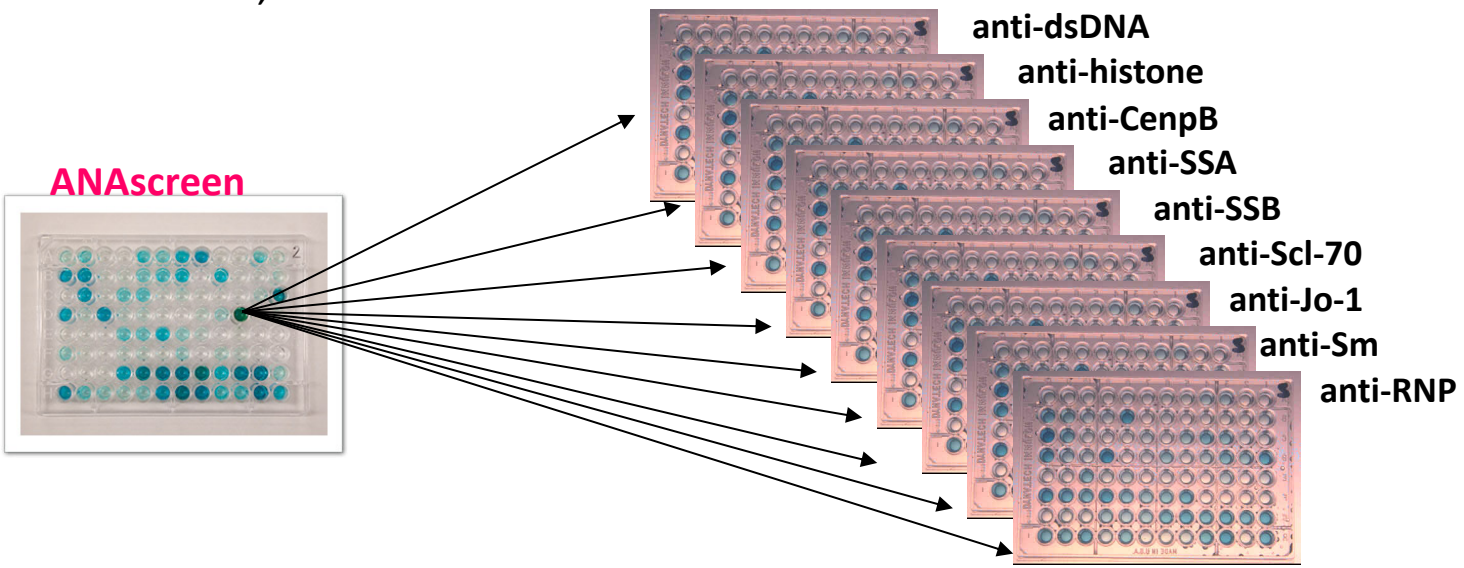
Rat liver tissue

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**.



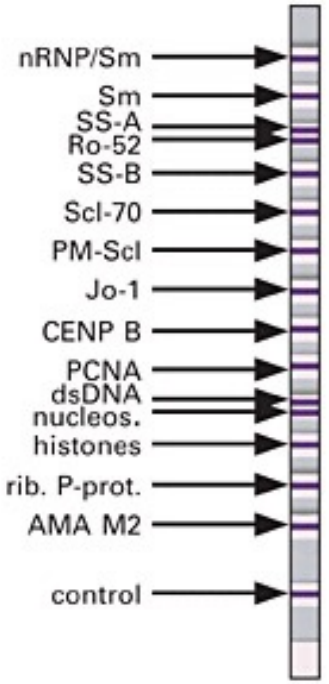
Multiplex immunszerological methods

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

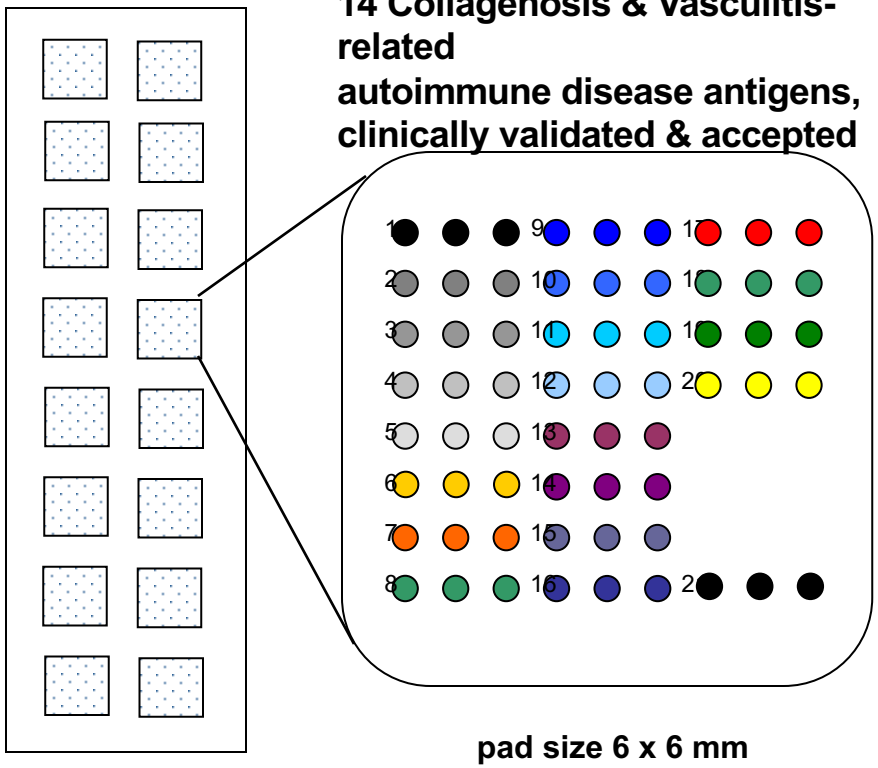
ANA-Profil Immunoblot



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



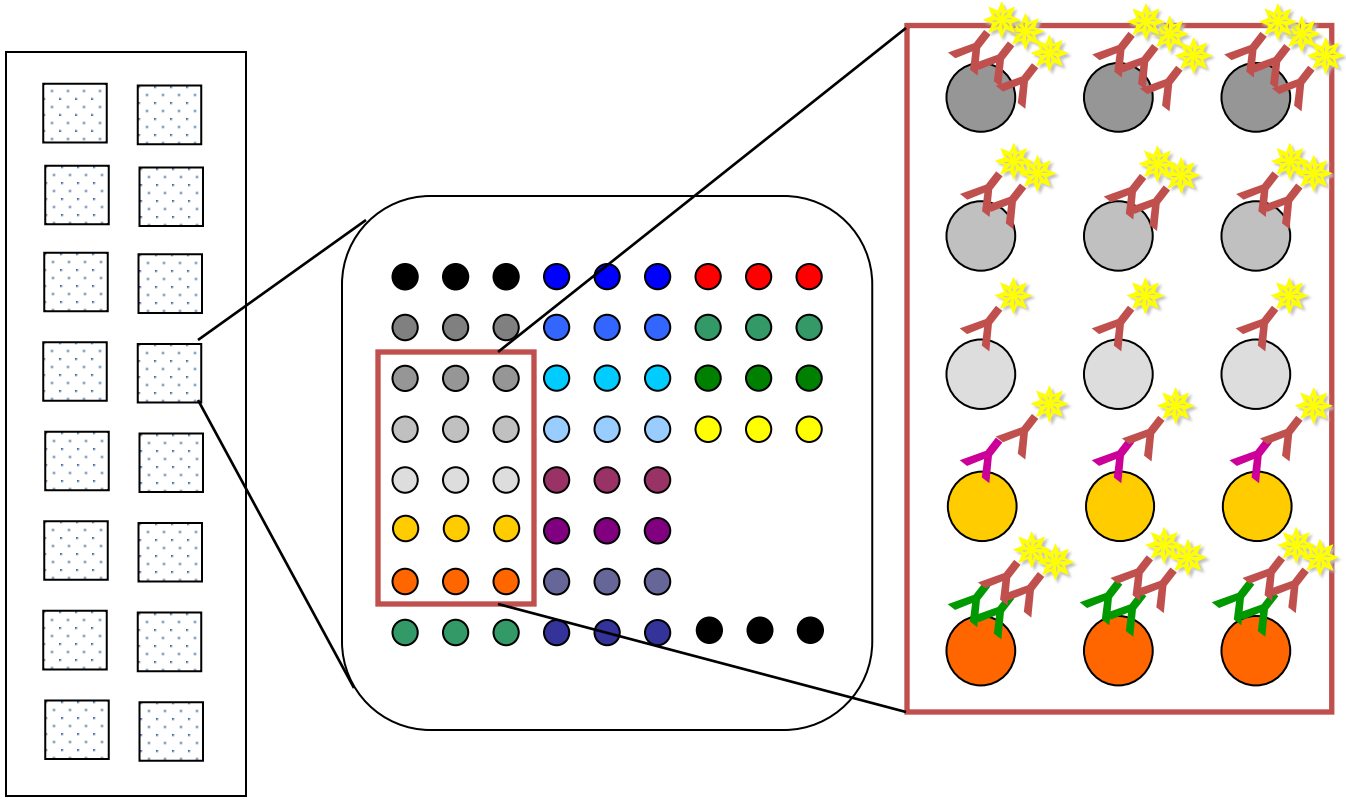
14 Collagenosis & Vasculitis-related autoimmune disease antigens, clinically validated & accepted

pad size 6 x 6 mm

1-5 = calibrator*
6 = CENP-B
7 = U1-70k
8 = Sm
9 = SSA/Ro52
10 = SSA/Ro60
11 = SSB/La
12 = Mi-2
13 = PM/Scl-75
14 = PM/Scl-100
15 = Jo-1
16 = Scl-70
17 = Pr3
18 = MPO
19 = dsDNA
20 = buffer
21 = “Landing Lights” (= positive assay control)

* patent pending

CombiChip Autoimmune 1.0 Layout



References (not in order)

- Poletaev AB[1], et al.: **Immunophysiology versus immunopathology: Natural autoimmunity in human health and disease.** *Pathophysiology.* 2012 Jun;19(3):221-31. doi: 10.1016/j.pathophys.2012.07.003. Epub 2012 Aug 9.
- Nguyen TT[1], Elsner RA[2], Baumgarth N[3]: **Natural IgM prevents autoimmunity by enforcing B cell central tolerance induction.** *J Immunol.* 2015 Feb 15;194(4):1489-502. doi: 10.4049/jimmunol.1401880. Epub 2015 Jan 16.
- Binder CJ[1]: **Naturally occurring IgM antibodies to oxidation-specific epitopes.** *Adv Exp Med Biol.* 2012;750:2-13. doi: 10.1007/978-1-4614-3461-0_1.
- Lutz HU[1]: **Homeostatic roles of naturally occurring antibodies: an overview.** *J Autoimmun.* 2007 Dec;29(4):287-94. Epub 2007 Sep 12.
- Lutz HU[1], Binder CJ, Kaveri S: **Naturally occurring auto-antibodies in homeostasis and disease.** *Trends Immunol.* 2009 Jan;30(1):43-51. doi: 10.1016/j.it.2008.10.002. Epub 2008 Dec 4.
- Aziz M[1], et al.: **The role of B-1 cells in inflammation.** *Immunol Res.* 2015 Dec;63(1-3):153-66. doi: 10.1007/s12026-015-8708-3.
- Savage HP[1,2], Baumgarth N[1,2,3]: **Characteristics of natural antibody-secreting cells.** *Ann N Y Acad Sci.* 2015 Jun 23. doi: 10.1111/nyas.12799.
- Grönwall C[1], Vas J, Silverman GJ: **Protective Roles of Natural IgM Antibodies.** *Front Immunol.* 2012 Apr 4;3:66. doi: 10.3389/fimmu.2012.00066. eCollection 2012.
- Elkon K[1], Casali P: **Nature and functions of autoantibodies.** *Nat Clin Pract Rheumatol.* 2008 Sep;4(9):491-8. doi: 10.1038/ncprheum0895.
- Grönwall C[1], Silverman GJ: **Natural IgM: beneficial autoantibodies for the control of inflammatory and autoimmune disease.** *J Clin Immunol.* 2014 Jul;34 Suppl 1:S12-21. doi: 10.1007/s10875-014-0025-4. Epub 2014 Apr 2.
- Cohen IR[1]: **Biomarkers, self-antigens and the immunological homunculus.** *J Autoimmun.* 2007 Dec;29(4):246-9. Epub 2007 Sep 20.

References

- Vas J[1], Grönwall C, Silverman GJ: **Fundamental roles of the innate-like repertoire of natural antibodies in immune homeostasis.** *Front Immunol.* 2013 Feb 5;4:4. doi: 10.3389/fimmu.2013.00004. eCollection 2013.
- Xu X[1], et al.: **Human-derived natural antibodies: biomarkers and potential therapeutics.** *Future Neurol.* 2015;10(1):25-39.
- Casali P[1], Schettino EW: **Structure and function of natural antibodies.** *Curr Top Microbiol Immunol.* 1996;210:167-79.
- Panda S[1], Ding JL[2]: **Natural antibodies bridge innate and adaptive immunity.** *J Immunol.* 2015 Jan 1;194(1):13-20. doi: 10.4049/jimmunol.1400844.
- Mannoor K[1], Xu Y, Chen C: **Natural autoantibodies and associated B cells in immunity and autoimmunity.** *Autoimmunity.* 2013 Mar;46(2):138-47. doi: 10.3109/08916934.2012.748753. Epub 2013 Jan 10.
- Madi A[1], et al.: **The natural autoantibody repertoire in newborns and adults: a current overview.** *Adv Exp Med Biol.* 2012;750:198-212. doi: 10.1007/978-1-4614-3461-0_15.
- Kaveri SV[1]: **Intravenous immunoglobulin: exploiting the potential of natural antibodies.** *Autoimmun Rev.* 2012 Sep;11(11):792-4. doi: 10.1016/j.autrev.2012.02.006. Epub 2012 Feb 12.
- Choi YS[1], et al.: **B-1 cells in the bone marrow are a significant source of natural IgM.** *Eur J Immunol.* 2012 Jan;42(1):120-9. doi: 10.1002/eji.201141890. Epub 2011 Nov 28.
- Rahyab AS[1], et al.: **Natural antibody - Biochemistry and functions.** *Glob J Biochem.* 2011;2(4):283-288.
- Baumgarth N[1]: **The double life of a B-1 cell: self-reactivity selects for protective effector functions.** *Nat Rev Immunol.* 2011 Jan;11(1):34-46. doi: 10.1038/nri2901. Epub 2010 Dec 10.
- Chan AY[1,2], Anderson MS[1]: **Central tolerance to self revealed by the autoimmune regulator.** *Ann N Y Acad Sci.* 2015 Nov;1356:80-9. doi: 10.1111/nyas.12960.

References

- Anaya JM[1]: **The diagnosis and clinical significance of polyautoimmunity.** *Autoimmun Rev.* 2014 Apr-May;13(4-5):423-6. doi: 10.1016/j.autrev.2014.01.049. Epub 2014 Jan 11.
- Pisetsky DS[1]: **Anti-DNA antibodies - quintessential biomarkers of SLE.** *Nat Rev Rheumatol.* 2015 Nov 19. doi: 10.1038/nrrheum.2015.151.
- Czömpöly T[1], et al.: **Anti-topoisomerase I autoantibodies in systemic sclerosis.** *Autoimmun Rev.* 2009 Jul;8(8):692-6. doi: 10.1016/j.autrev.2009.02.018. Epub 2009 Feb 13.
- Nihtyanova SI[1], Denton CP: **Autoantibodies as predictive tools in systemic sclerosis.** *Nat Rev Rheumatol.* 2010 Feb;6(2):112-6. doi: 10.1038/nrrheum.2009.238.
- Liberal R[1], et al.: **Diagnostic and clinical significance of anti-centromere antibodies in primary biliary cirrhosis.** *Clin Res Hepatol Gastroenterol.* 2013 Dec;37(6):572-85. doi: 10.1016/j.clinre.2013.04.005. Epub 2013 Jul 19.
- Zampieri S[1], et al.: **Anti-Jo-1 antibodies.** *Autoimmunity.* 2005 Feb;38(1):73-8.
- Aggarwal A[1]: **Role of autoantibody testing.** *Best Pract Res Clin Rheumatol.* 2014 Dec;28(6):907-20. doi: 10.1016/j.berh.2015.04.010. Epub 2015 May 23.
- Csernok E[1], Moosig F[1]: **Current and emerging techniques for ANCA detection in vasculitis.** *Nat Rev Rheumatol.* 2014 Aug;10(8):494-501. doi: 10.1038/nrrheum.2014.78. Epub 2014 Jun 3.
- Poletaev AB[1], Stepanyuk VL, Gershwin ME: **Integrating immunity: the immunculus and self-reactivity.** *J Autoimmun.* 2008 Feb-Mar;30(1-2):68-73. doi: 10.1016/j.jaut.2007.11.012.
- Poletaev AB[1]: **The immunological homunculus (immunculus) in normal state and pathology.** *Biochemistry (Mosc).* 2002 May;67(5):600-8.