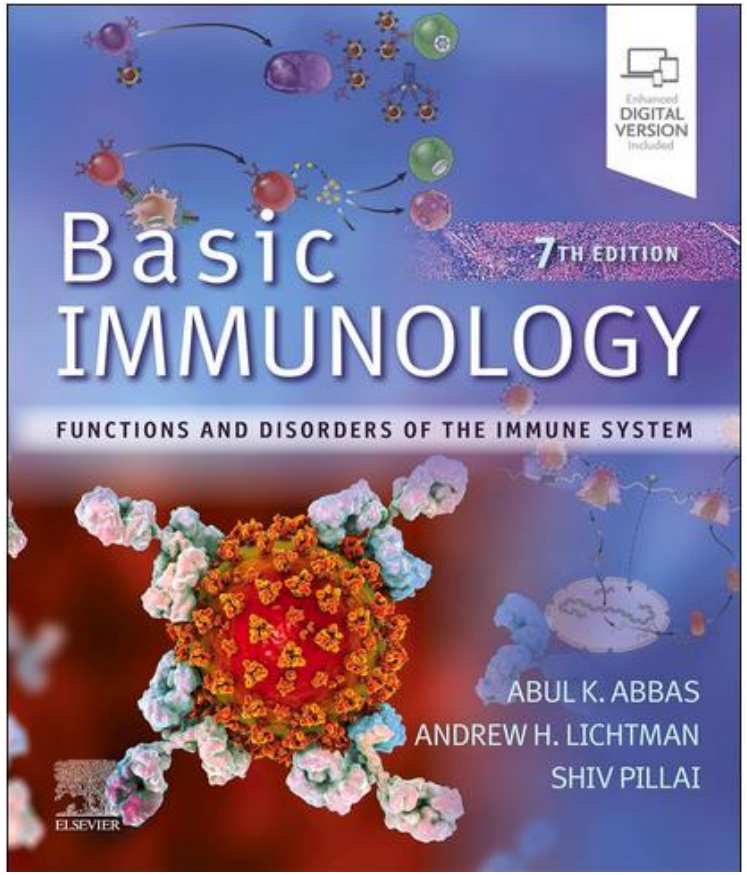
Adaptive immunity generalities



By Viviana Marin-Esteban

Immune response : two response modes

Immunity: state or quality of being resistant to a particular infectious disease or pathogen … and being protected against cancer.

Innate immunity (born with) : Recognition of "predefined" common molecular signatures of potential pathogens (pattern recognition receptors).

Effector functions (defence against pathogens) deployed without prior exposure to a pathogen.

Adaptive immunity (shaped with the exposure to non-Self) : Recognition of "non predefined" specific regions of any macromolecule (antigen, non-Self). Receptors : BCR/TCR.

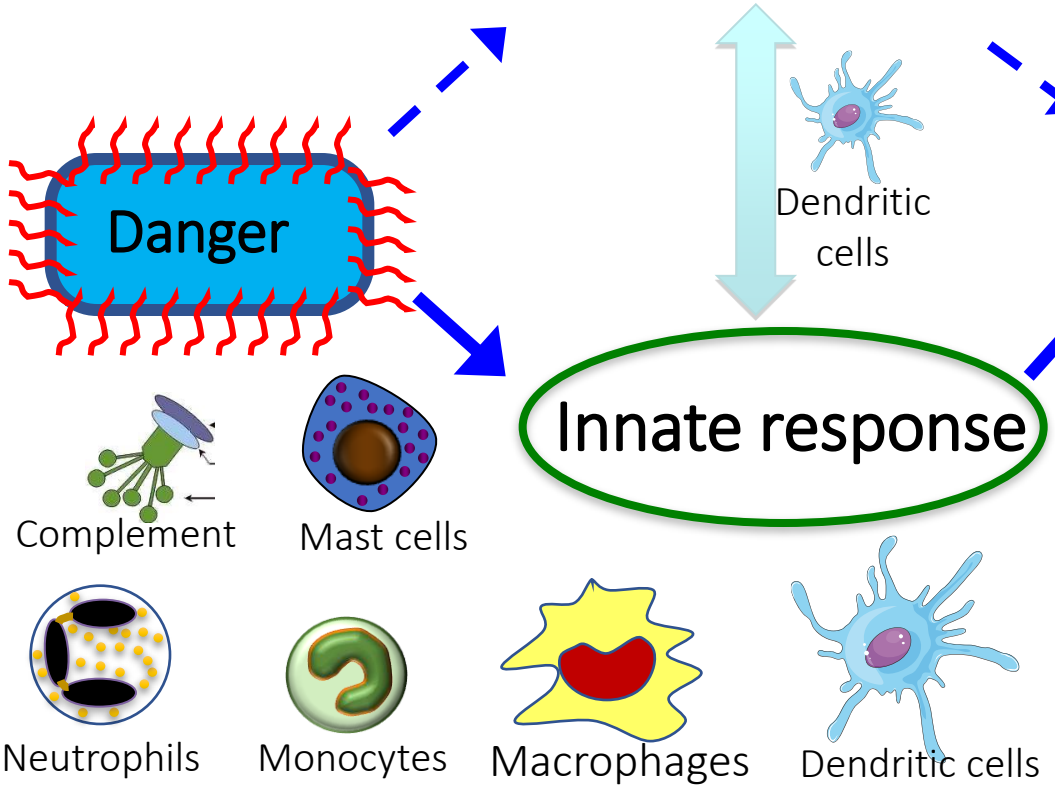
Effector functions require a prior exposure to a non-Self (pathogen/antigen) : naïve cells → effector and memory cells.

Most immune responses include participation of both modes of response.

Action / function of both of them involves elaboration of soluble products acting locally and systemically (humoral effectors) or requiring direct cell-to-cell contact (cell mediated responses).

BCR : B cell receptor; TCR: T cell receptor

Adaptive response



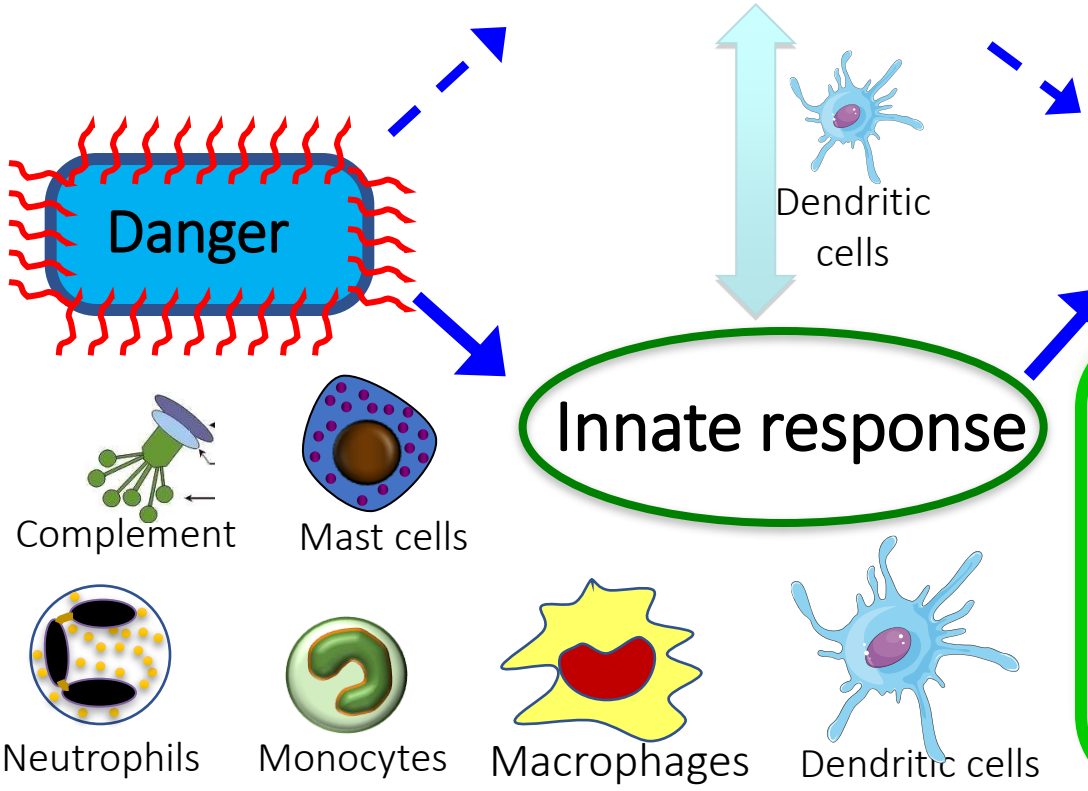
Elimination of the danger

Regulation (homeostatic functions)

- Myeloid cells
- Short half-life
- Capacity to phagocyte (large cytoplasm)
- Released to blood with effector functional capacity
- A single cell = different receptor identities
- Receptors with limited number of specificities (coded by the genome)

To learn

Adaptive response



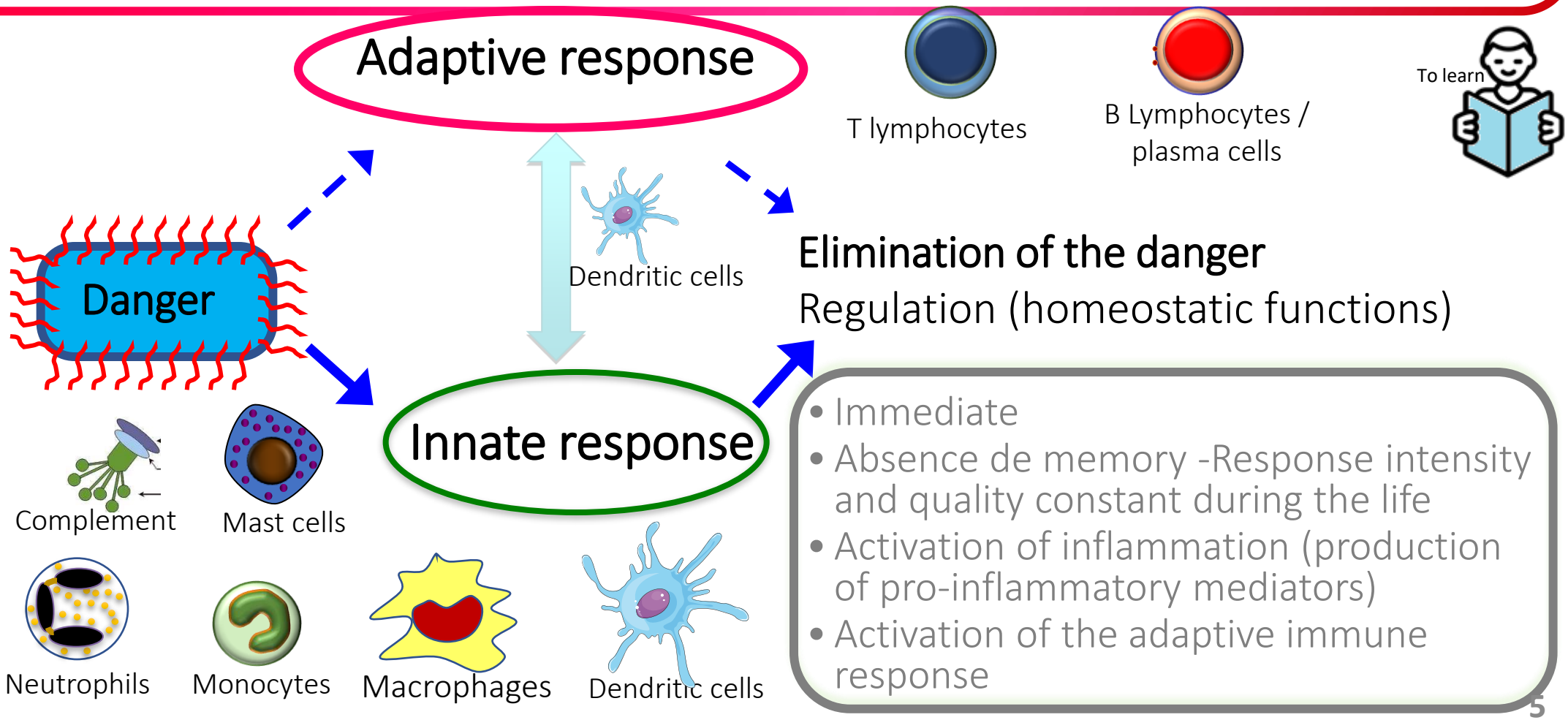
Elimination of the danger

Regulation (homeostatic functions)

- Immediate
- Absence de memory: Response intensity and quality constant during the life
- Activation of inflammation (production of pro-inflammatory mediators)
- Activation of the adaptive immune response

- A single naïf lymphocyte expresses only one antigen receptor identity (= 1 sequence = 1 specificity)
- Specificity of BCR/TCR is generated aleatorily (tremendous diversity, genes ~lego kit)
- Newly generated BCR/TCR undergo selection (central tolerance) to guaranty a functional and non auto-reactive BCR/TCR repertoire
- Effector response not immediate (delayed) (naïve to effector, clonal expansion ~8 to 10 days)
- Memory : reduced response delay (~4 days), larger, improved Ab/BCR quality, long lived cells (years)

Ab: antibody



Cardinal features of adaptive immune responses

Specificity: Response to an antigen (Ag) is targeted to that Ag. Differences in a single a.a. between two peptides can be critical to loss recognition by BCR or TCR.

👉 Remember: one lymphocyte = only one Ag receptor identity.

Diversity: Enables the immune system to respond to a large variety of Ag. Theoretical number of different BCR able to be generated 10^{11}, and 10^{16} different TCR.

👉 Number of potential genes in human genome is $3x10^4$.

Nonreactivity to self: Different mechanisms are active to warrant elimination or control of Self-reactive lymphocytes and maintain Self-tolerance (central and peripheral mechanisms).

Clonal expansion: Increases the number of Ag-specific lymphocytes to keep pace with microbes.

Specialization: Responses are adapted (optimized) to the particular type of invading pathogen.

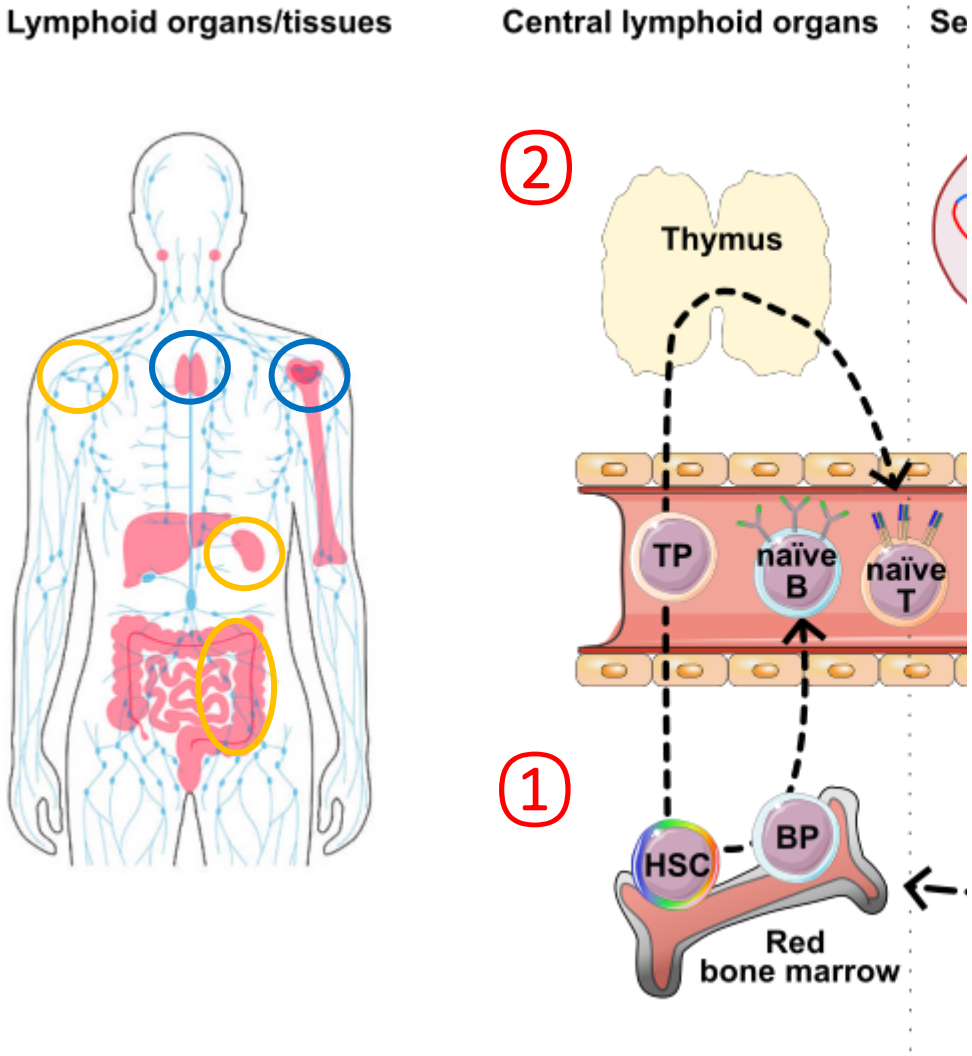
Contraction and homeostasis: When Ag is eliminated the effector lymphocytes is reduced, eliminated. Only a small pool of Ag-specific memory lymphocytes is maintained.

Memory: Increases the ability to combat repeat infections by the same microbe.

Abbas et al. Basic Immunology: Functions and Disorders of the Immune System 2023 (Recommended book)

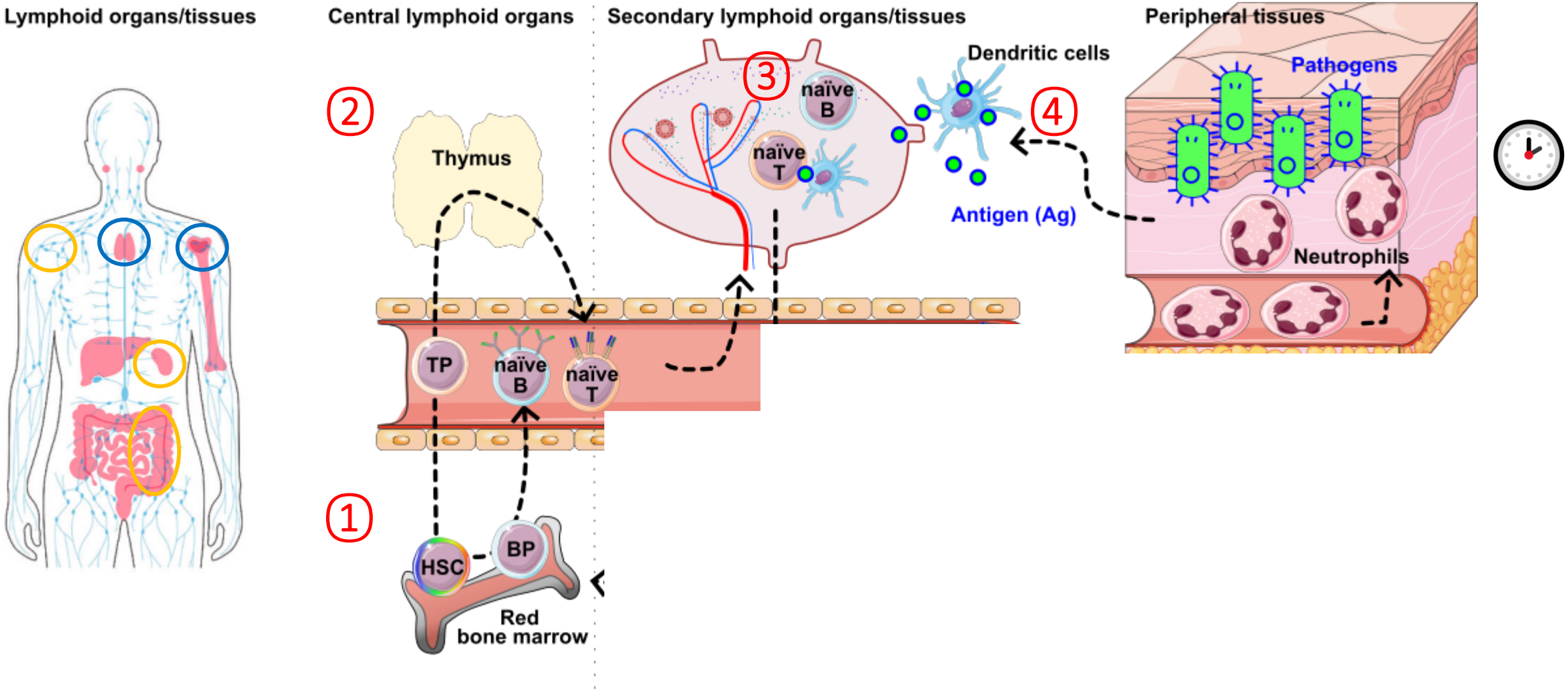
Organisation of the immune system

1 & 2: Lymphopoiesis de central lymphoid organs



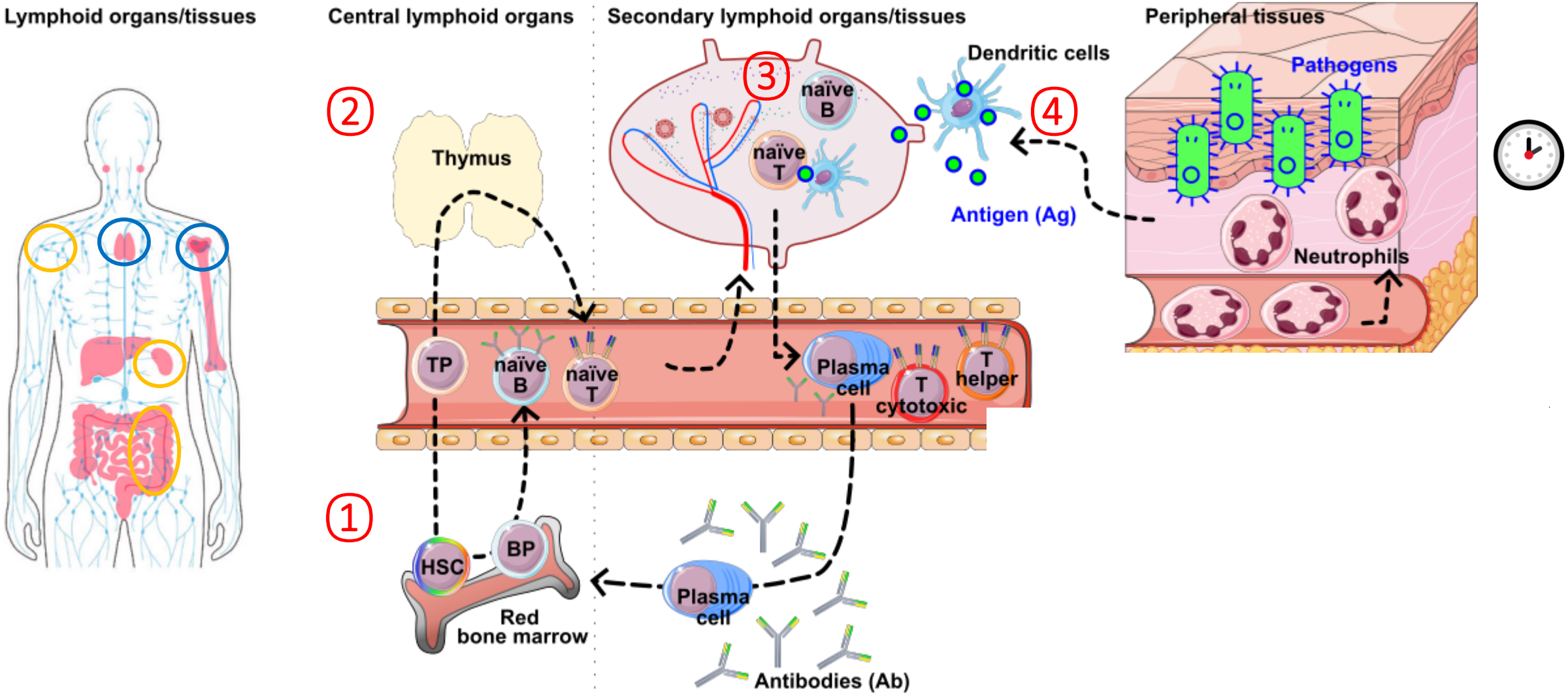
Organisation of the immune system

1 & 2: Lymphopoiesis; 3 & 4: Ag-dependent activation of naïve lymphocytes to become effector and memory lymphocytes in secondary lymphoid organs;



Organisation of the immune system

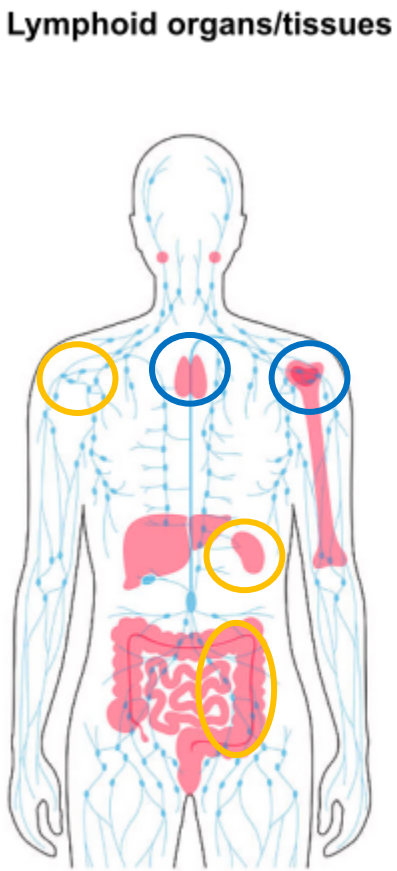
1 & 2: Lymphopoiesis; 3 & 4: Ag-dependent activation of naïve lymphocytes to become effector and memory lymphocytes in secondary lymphoid organs;

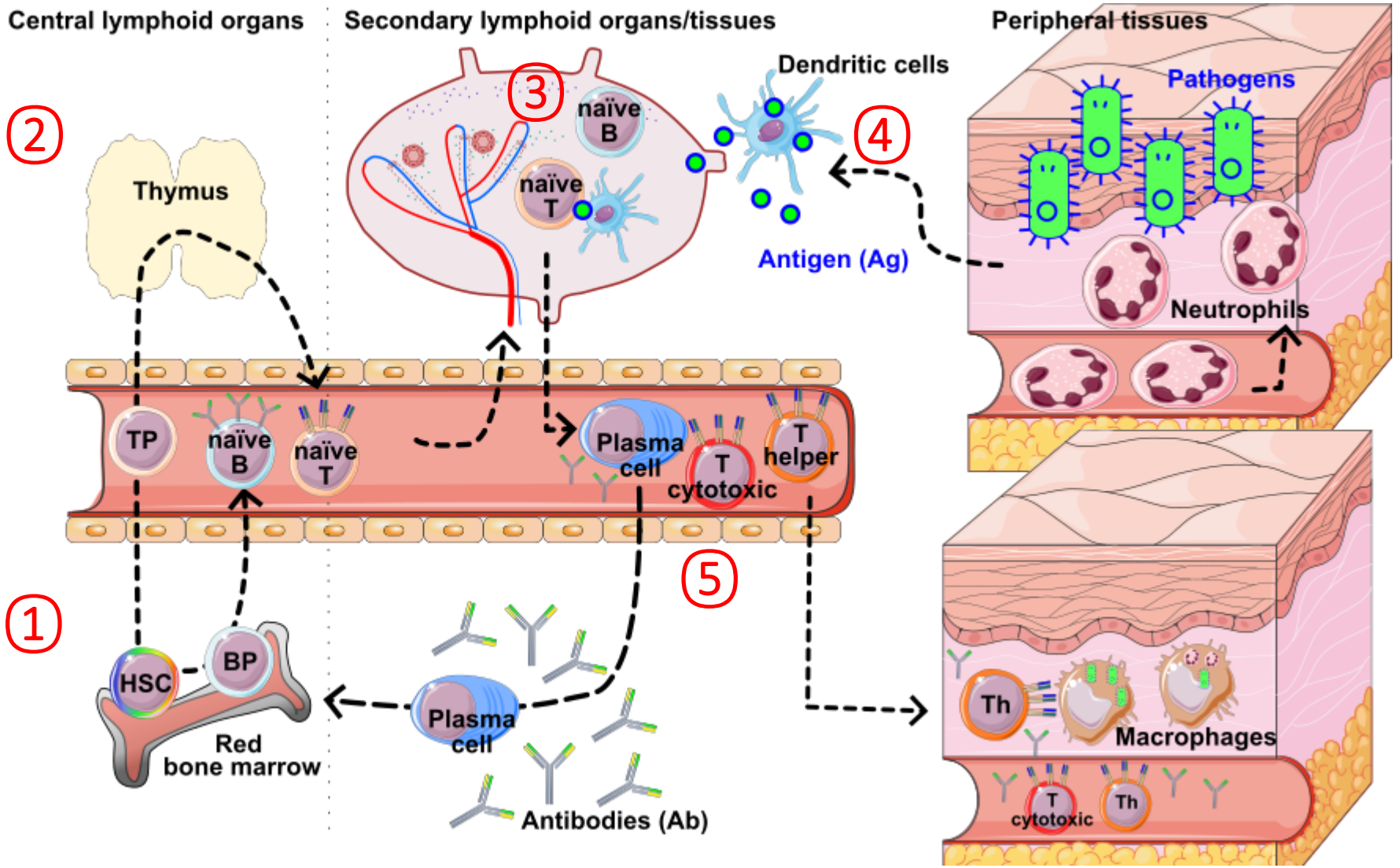


Organisation of the immune system

To learn

1 & 2: Lymphopoiesis ; 3 & 4: Ag-dependent activation of naïve lymphocytes to become effector and memory lymphocytes ; 5: effector function of B and T lymphocytes participate in the elimination of pathogens





Summary: Compartmentalization of the stages of the adaptive immune response

Primary lymphoid organs (bone marrow and thymus):

→ Place of lymphopoiesis (production and maturation of lymphocytes)

→ **Lymphopoiesis** allows

- Acquisition of a functional BCR or TCR; only one per cell (diversity, specificity)
- Elimination of cells with non-functional or with Self-reactive TCR and BCR (central tolerance : non Self reactivity)

Secondary lymphoid organs (lymph nodes, spleen, MALT):

→ Place of activation of naïve lymphocytes (activation, proliferation, differentiation in effector and memory cells)

→ Activated Dendritic cells (DC) migrate to these organs to present Ag to T lymphocytes

→ Effector T lymphocytes migrate to infected tissues to mount an Ag-specific immune response

→ Effector B lymphocytes (plasma cells) migrate to bone marrow and release Ab that reach the blood

Distribution of immune cells is regulated by expression of tissue/cell chemokines (homeostatic, inflammatory) and of chemokine receptors

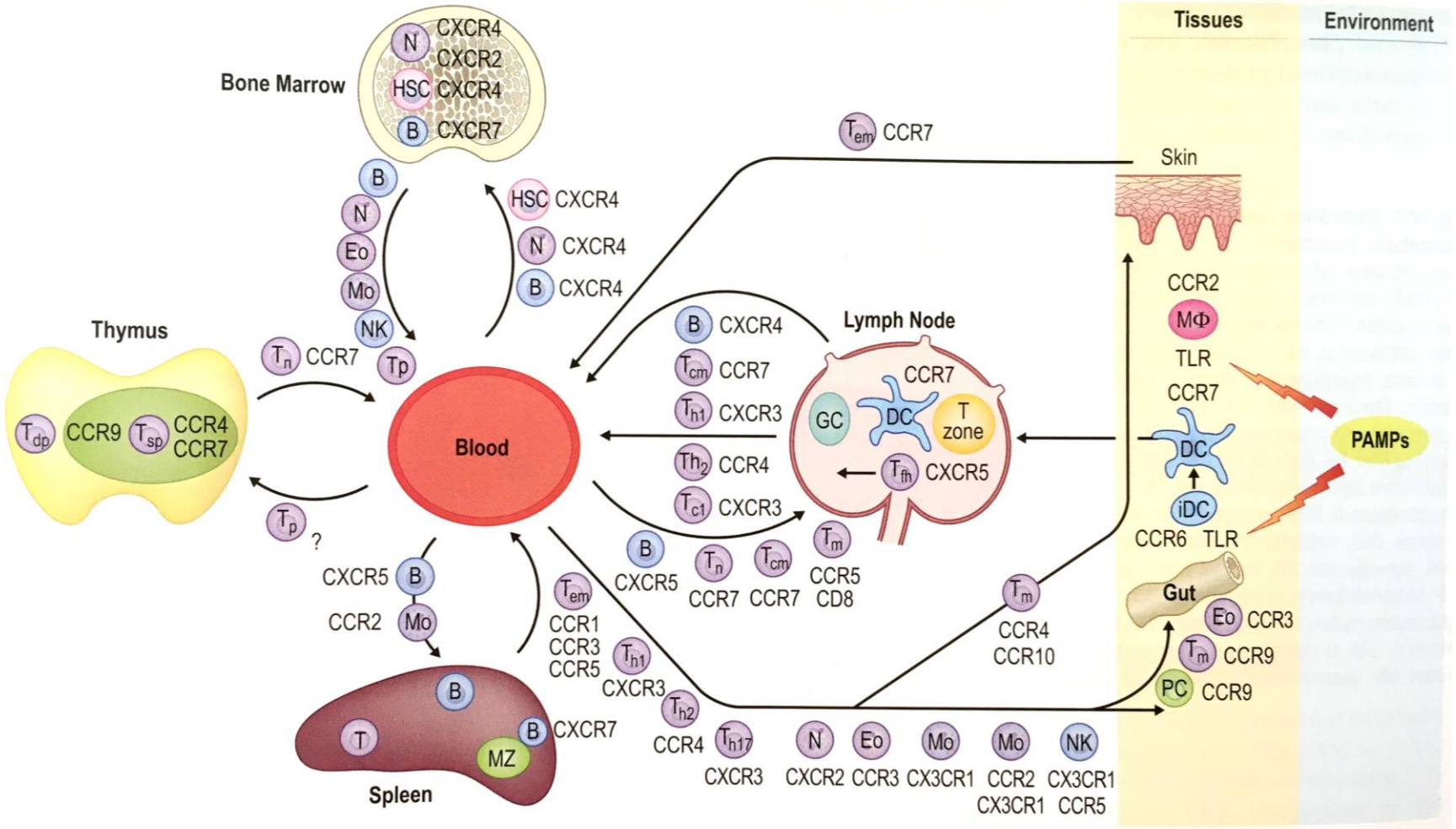
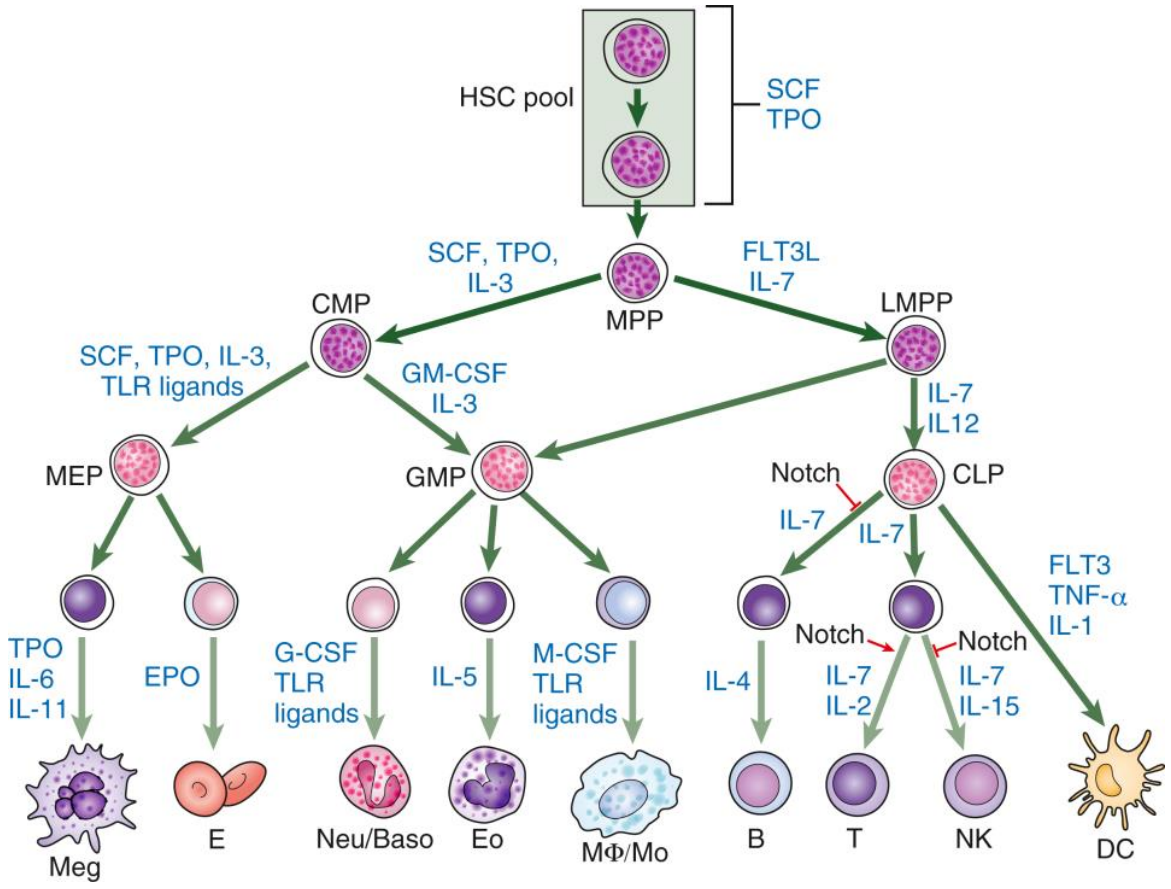


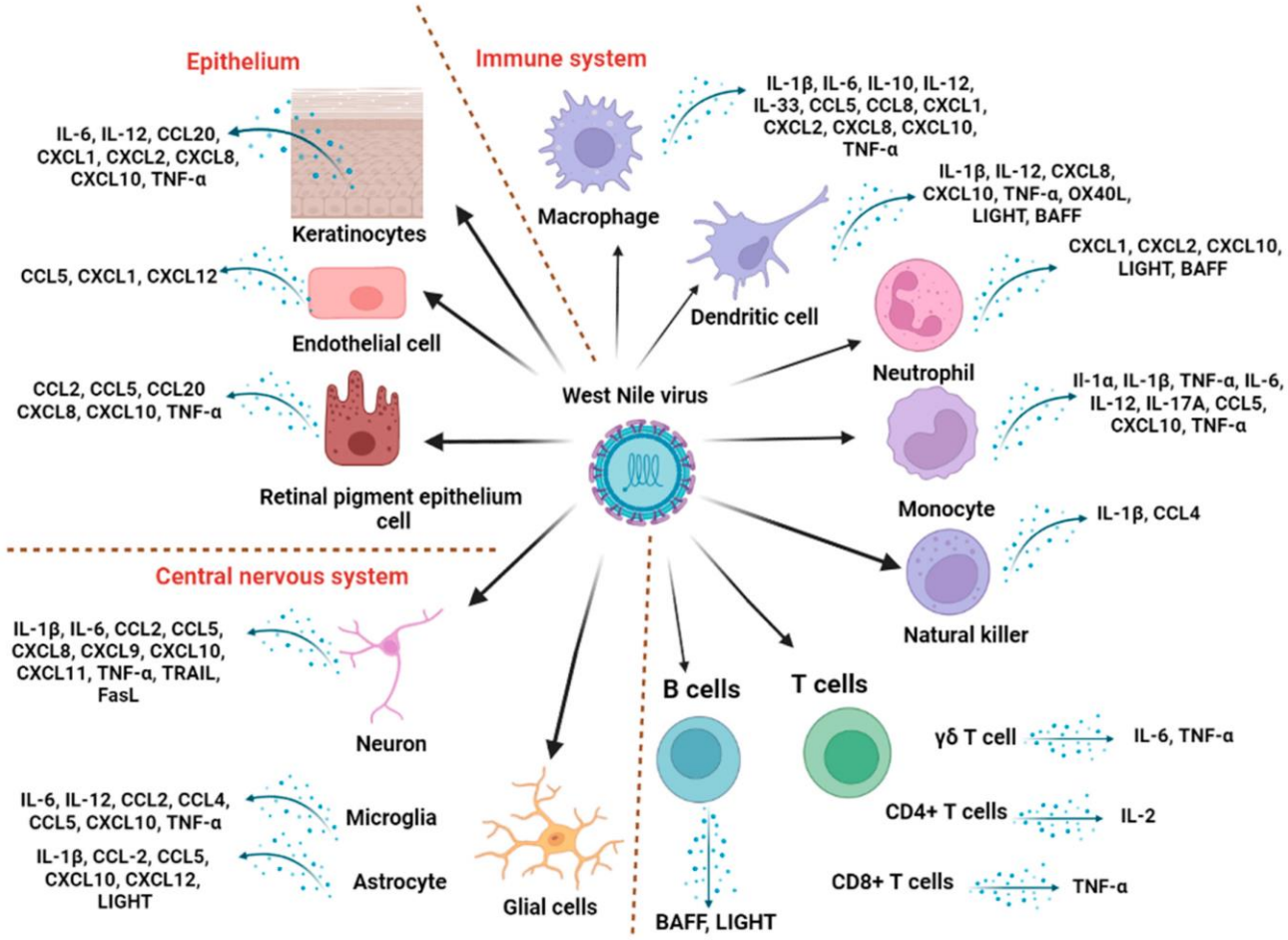
Fig. 10.4 Chemokine receptor control of leukocyte trafficking. Shown are routes among primary and secondary immune organs and the periphery, leukocyte subtypes trafficking on those routes and some of the chemokine receptors that appear to be most important in regulating each route. Tn, naïve T cells; Tp, precursor T cells; Tm, memory T cells; T_{EM}, effector memory T cells; T_{CM}, central memory T cells; T_{FH}, follicular help T cells; iDC, immature dendritic cells; N, neutrophil; Eo, eosinophil; MΦ, macrophage; Mo, monocyte; NK, natural killer cell; PC, plasma cell; HSC, hematopoietic stem cell; GC, germinal center. The model is based primarily on studies of mice where the relevant gene has been inactivated by gene targeting.

Cytokine (and their receptors) also participate in the differentiation, growth, and activation of immune cells (growth factors, chemokines, interleukins, TNF, IFN and TGF family)

Hematopoietic cytokines



Proinflammatory cytokines



Proportions of immune cell populations on BM and in blood

Hematopoietic cells in **bone marrow**:

Stem and precursor cells (CD34+)	1%
Megakaryocytes	1%
Red blood cell precursors	2%
Immature and mature red blood cells	10-20%
Myeloid precursors	4%
Granulocytes(neutrophils)	50-70%
Monocytes	2%
Dendritic cells (DC)	2%
Lymphocytes	15%
Plasma cells	1%

White blood cells in **peripheral blood**:

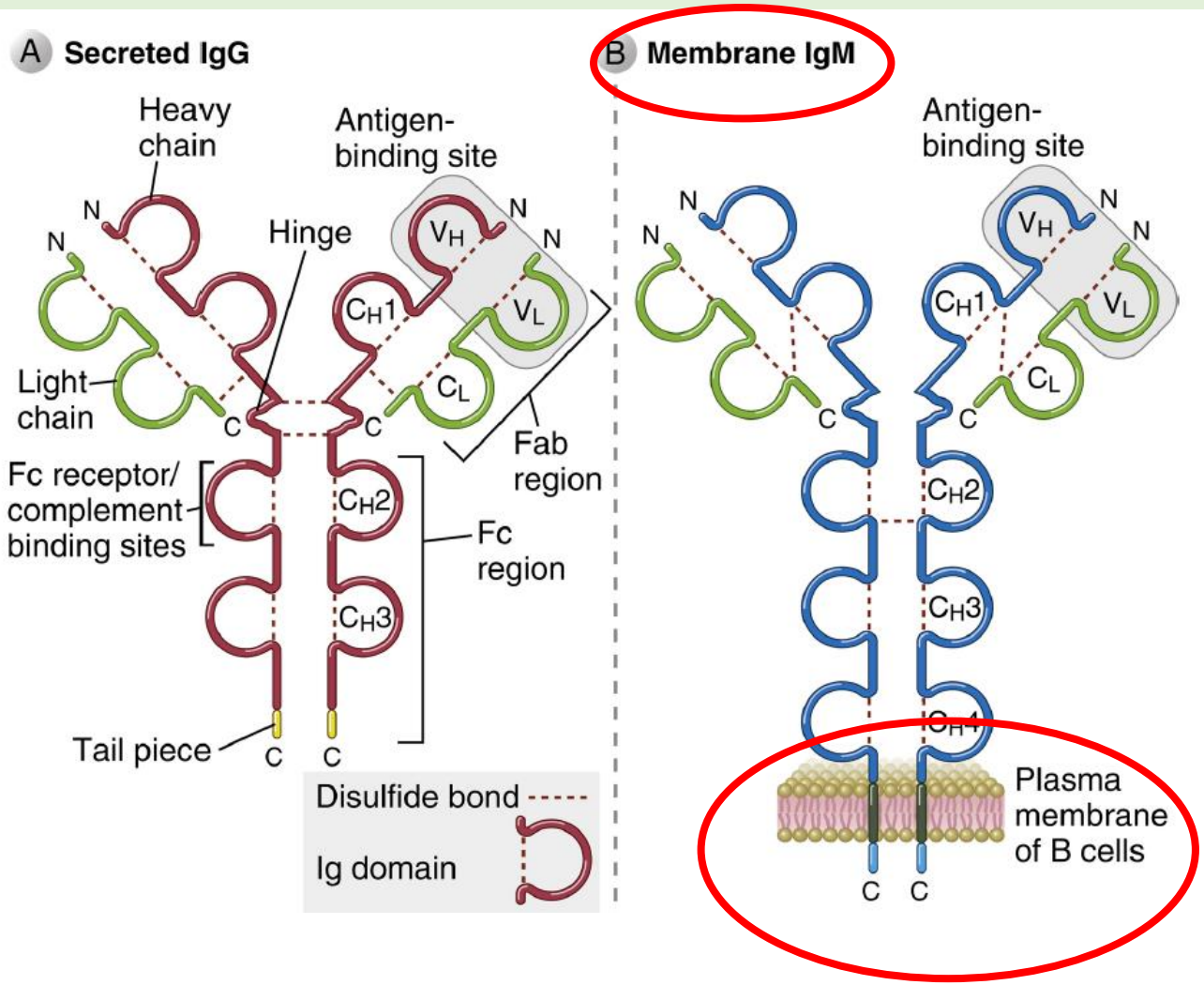
Granulocytes (neutrophils)	35-73%
Lymphocytes	15-52%
Monocytes	4-13%
Dendritic cells	< 1%

% of lymphocytes

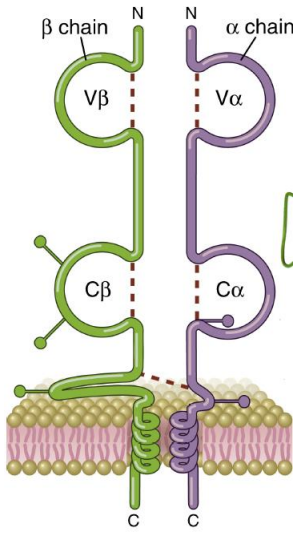
T cells (~2/3 are CD4+, 1/3 CD8+)	75-85%
B cells	5-15%
NK cells	5-15%

Structure of BCR and TCR

Structure of BCR, immunoglobulins (Igs) or antibodies (Abs)



Structure of TCR



Abbas et al. 2012

Antibody amino acid sequence alignment (light chain N-term domain)

→ Variable domain with 3 hypervariable regions

= Complementarity-determining regions (CDR)

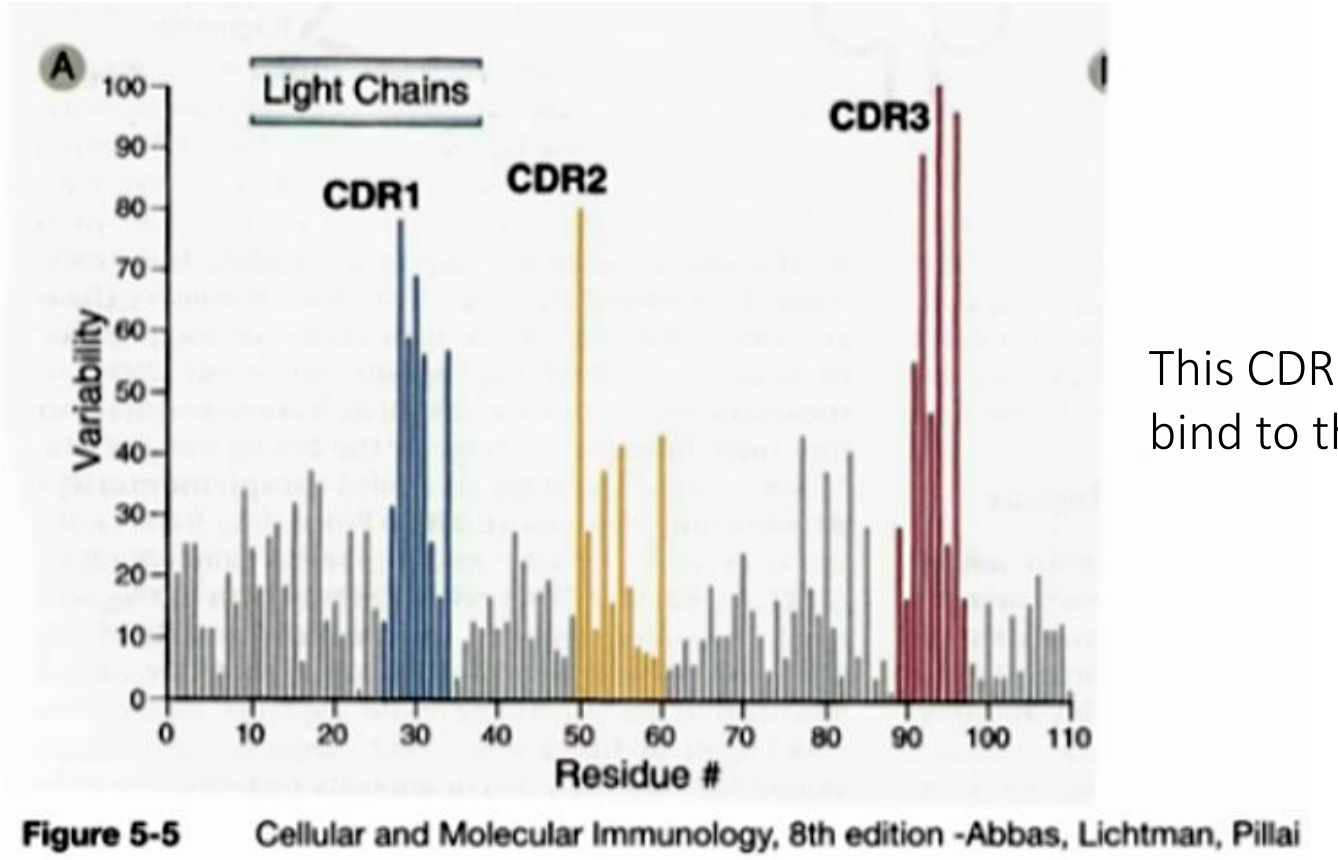
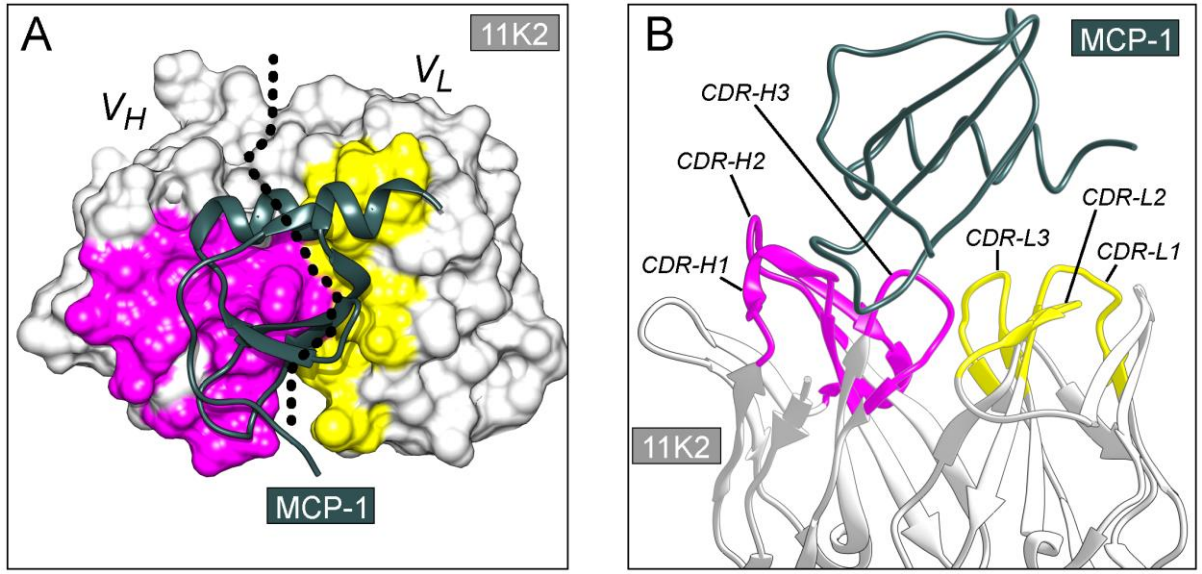


Figure 5-5 Cellular and Molecular Immunology, 8th edition -Abbas, Lichtman, Pillai

This CDR are the actual regions (loops) that bind to the antigen (Ag)

Binding of BCR and TCR to the Ag – 6 CDR loops

Immunoglobulins directly bind to the Ag via 6 CDR loops

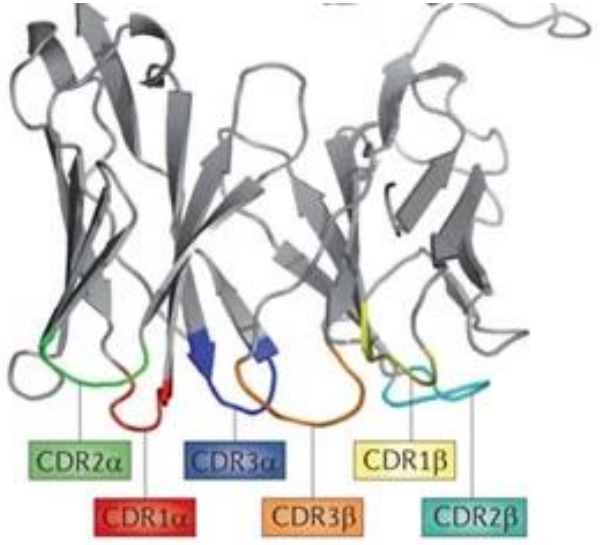


Crystal structure model of Fab domain of 11K2 Ab in complex with its antigen MCP-1

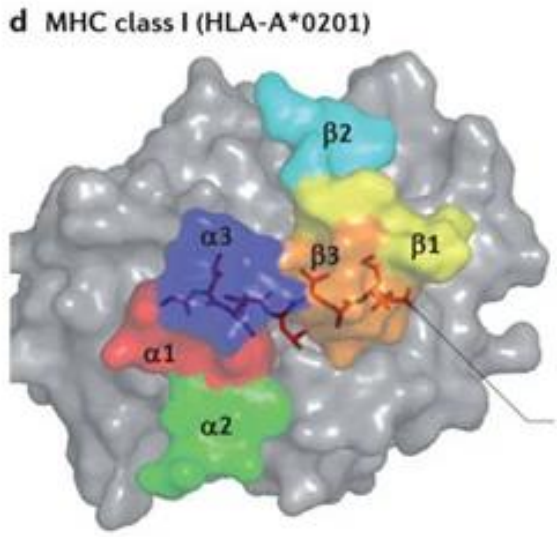
Antigens recognized by Ab = proteins, small chemical molecules, carbohydrates, DNA, lipids;

Kiyoshi et al, Plos One 2014

TCR binds to the Ag / MHC complex via 6 CDR loops



Cristal structure model of TCR Va Vb domains – the 6 CDR loops are indicated



HMC / Antigen complex structure model indicating the surfaces where TCR CDRs bind to.

Antigens recognized by TCR = **peptides presented by self MHC molecules.** MHC restriction of Ag recognition / presentation

Sewell, Nat Rev Immunol 2012

Antibody amino acid sequence alignment (light chain N-term domain)

→ Variable domain with 3 hypervariable regions

= Complementarity-determining regions (CDR)

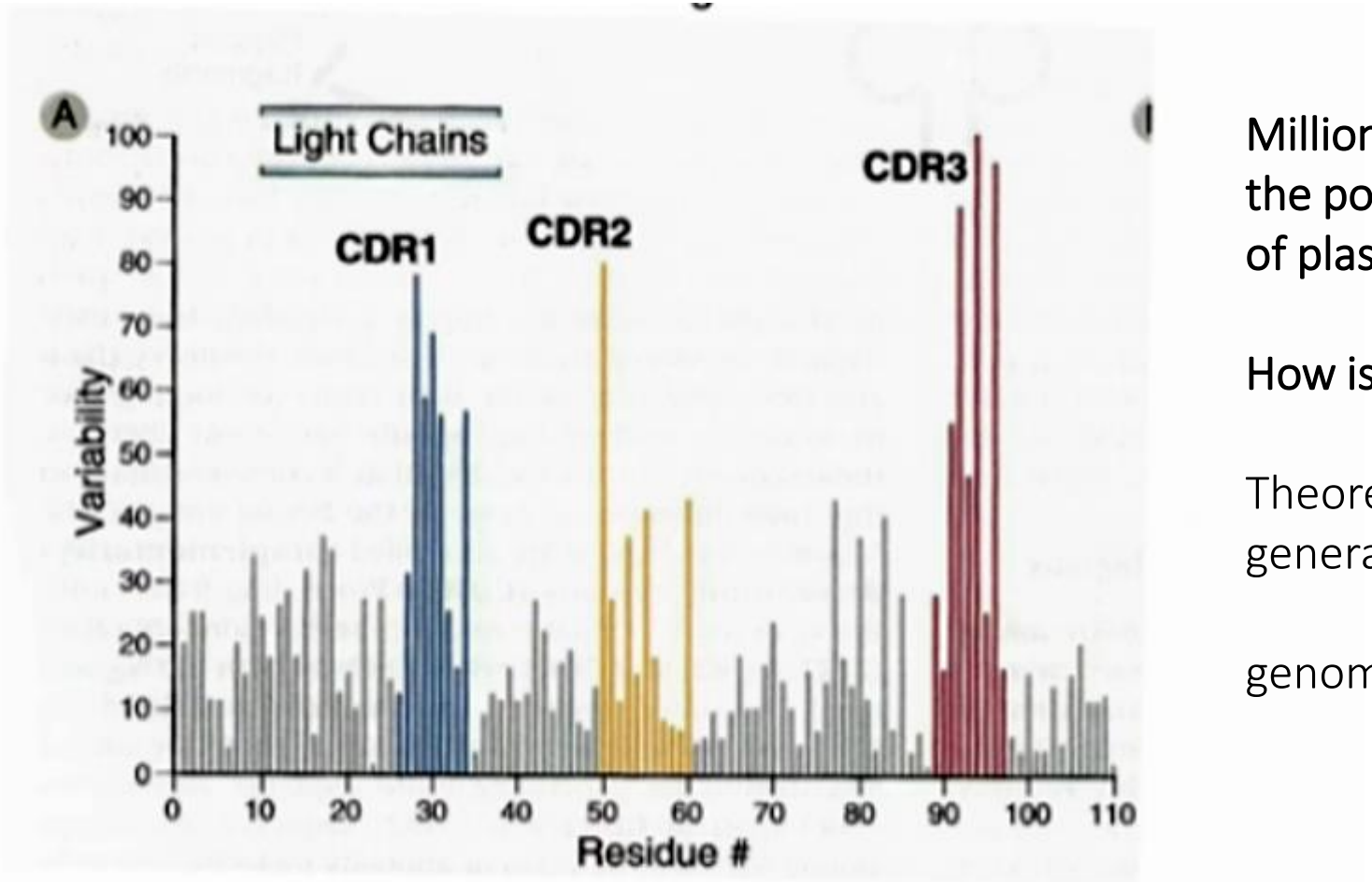


Figure 5-5 Cellular and Molecular Immunology, 8th edition -Abbas, Lichtman, Pillai

Millions of different Ig identities/sequences in the pool of naïve B lymphocytes and in the pool of plasmatic immunoglobulins.

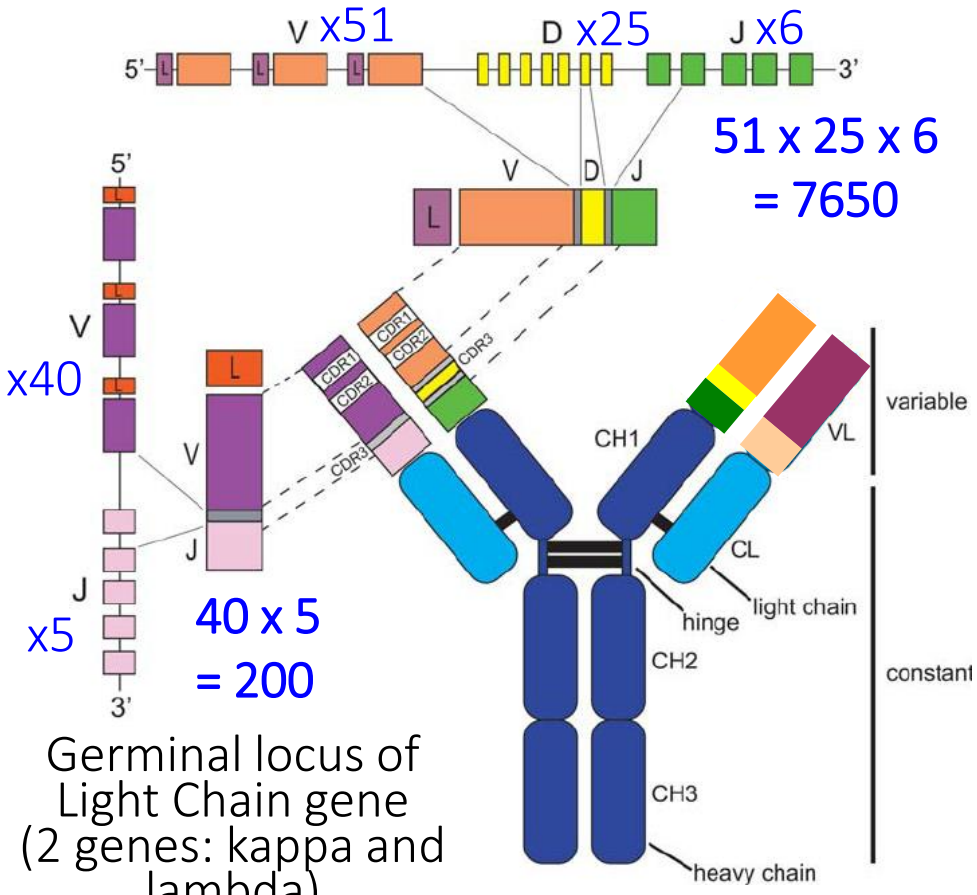
How is this enormous diversity achieved ?

Theoretical number of different BCR able to be generated 10^{11}, and 10^{16} different TCR.

☛Number of potential genes in human genome is $3x10^4$.

One naive B lymphocyte = a single identity of BCR generated randomly by DNA recombination of gene segments

Germinal locus of Heavy Chain gene



Germinal locus of Light Chain gene (2 genes: kappa and lambda)

Model of a immunoglobulin and of the germinal gene loci coding for the heavy and the light chains.

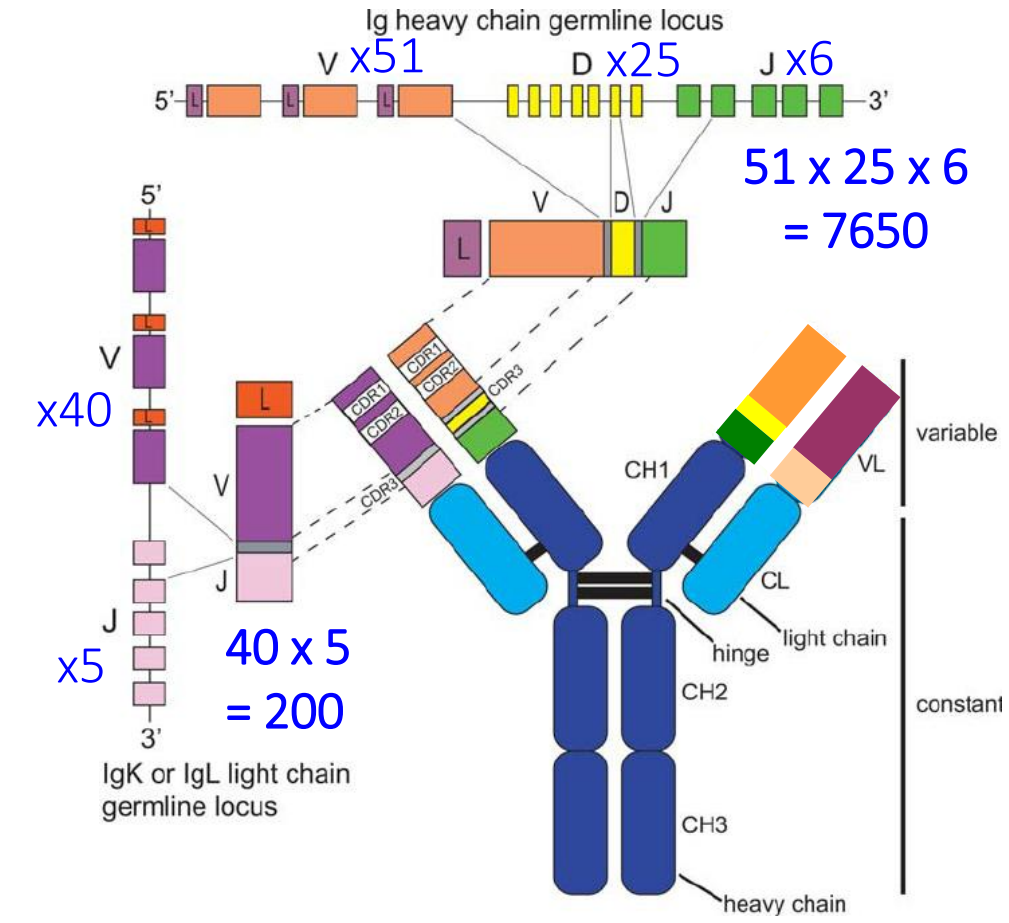
→ **Somatic recombination** generates the diversity of BCR molecules.

The **germinal immunoglobulin genes** coding for heavy and light chains, **are not functional** (rather a kind of a Lego kit).

They contains multiple V, D and J segments. Each segment has a unique DNA sequence.

One of each will be **randomly "chosen"** an brought together to form a functional heavy chain gene and a functional light chain gene.

One naive B lymphocyte = a single identity of BCR generated randomly by DNA recombination of gene segments



To learn

① Somatic recombination of gene fragments :

Combinatorial diversity Recombination-activating genes (RAG1/2)

1- Random assembly of 1 D segment with 1 J segment, then 1 V segment (heavy chains)

2- Random assembly of 1 V segment with 1 J (light chains)

Junctional imprecision Terminal Deoxynucleotidyl Transferase (TdT)

Insertion and deletion of nucleotides in the junction between gene segments

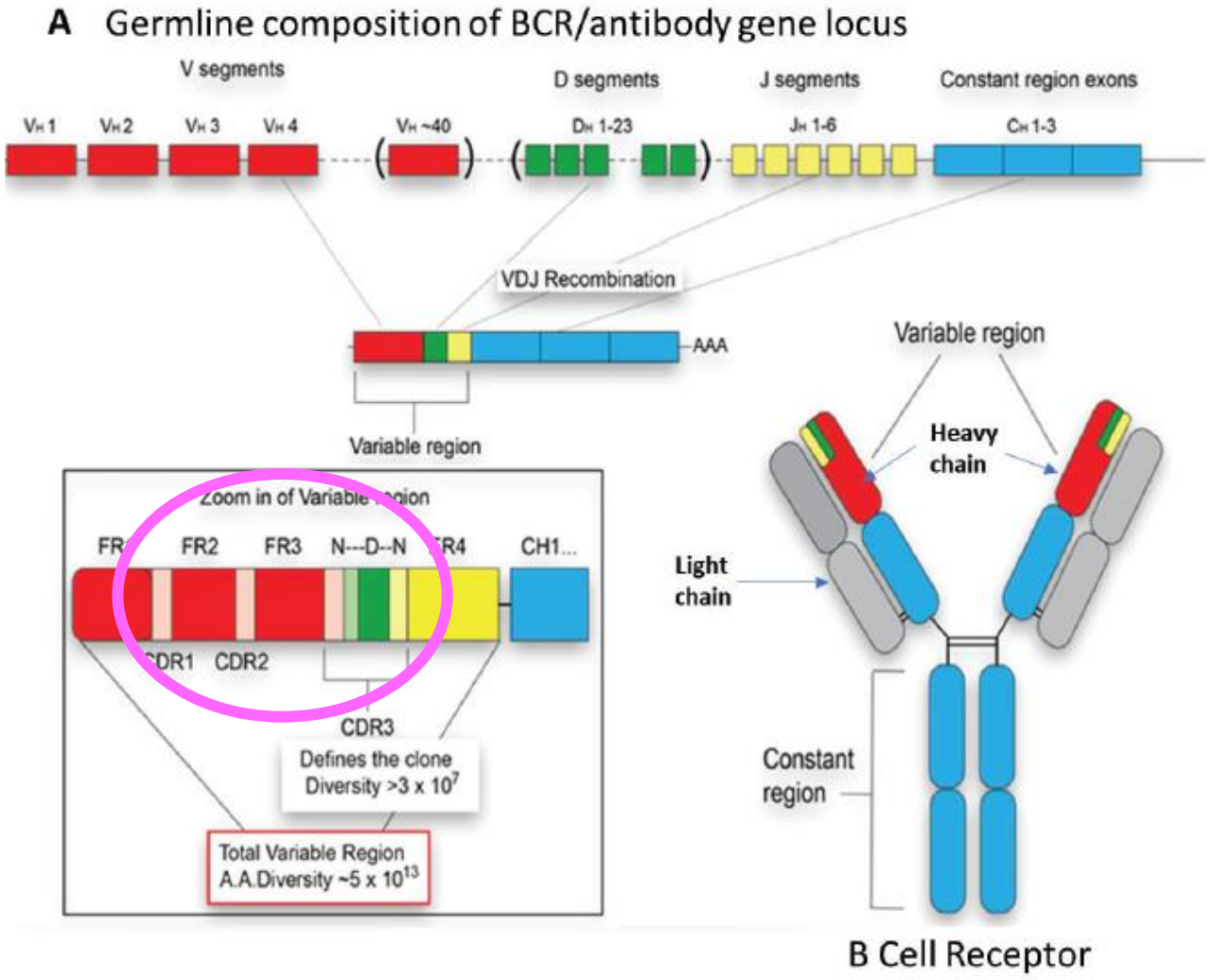
→ **Allelic exclusion**: only 1 HC gene and 1 LC gene expressed per cell = a single identity of BCR

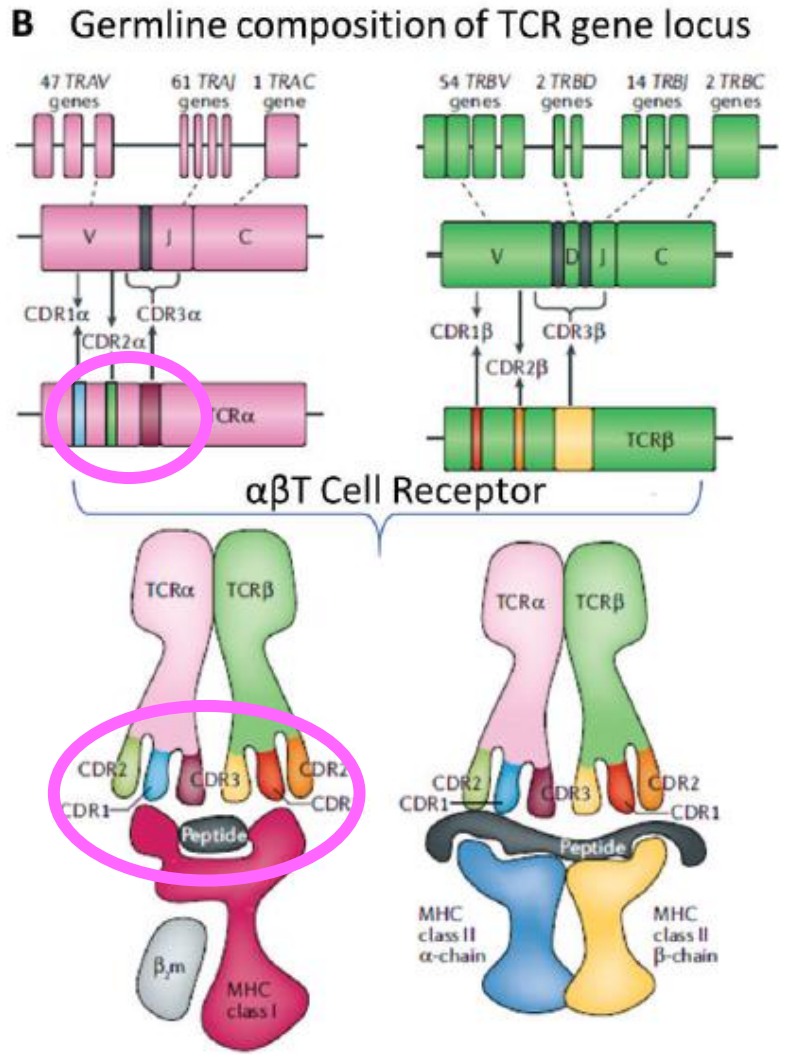
→ **Isotypic exclusion:** only 1 LC gene expressed per cells, either Kappa (2/3 of naïve cells) or lambda (1/3)

② Association diversity

1 HC + + 1 LC, both randomly generated

TCR identity and diversity are as well generated by a random DNA recombination of gene segments in the germline α, β TCR loci





https://bioinfoworld.wordpress.com/2019/11/08/diversity-based-immunodiagnostics/

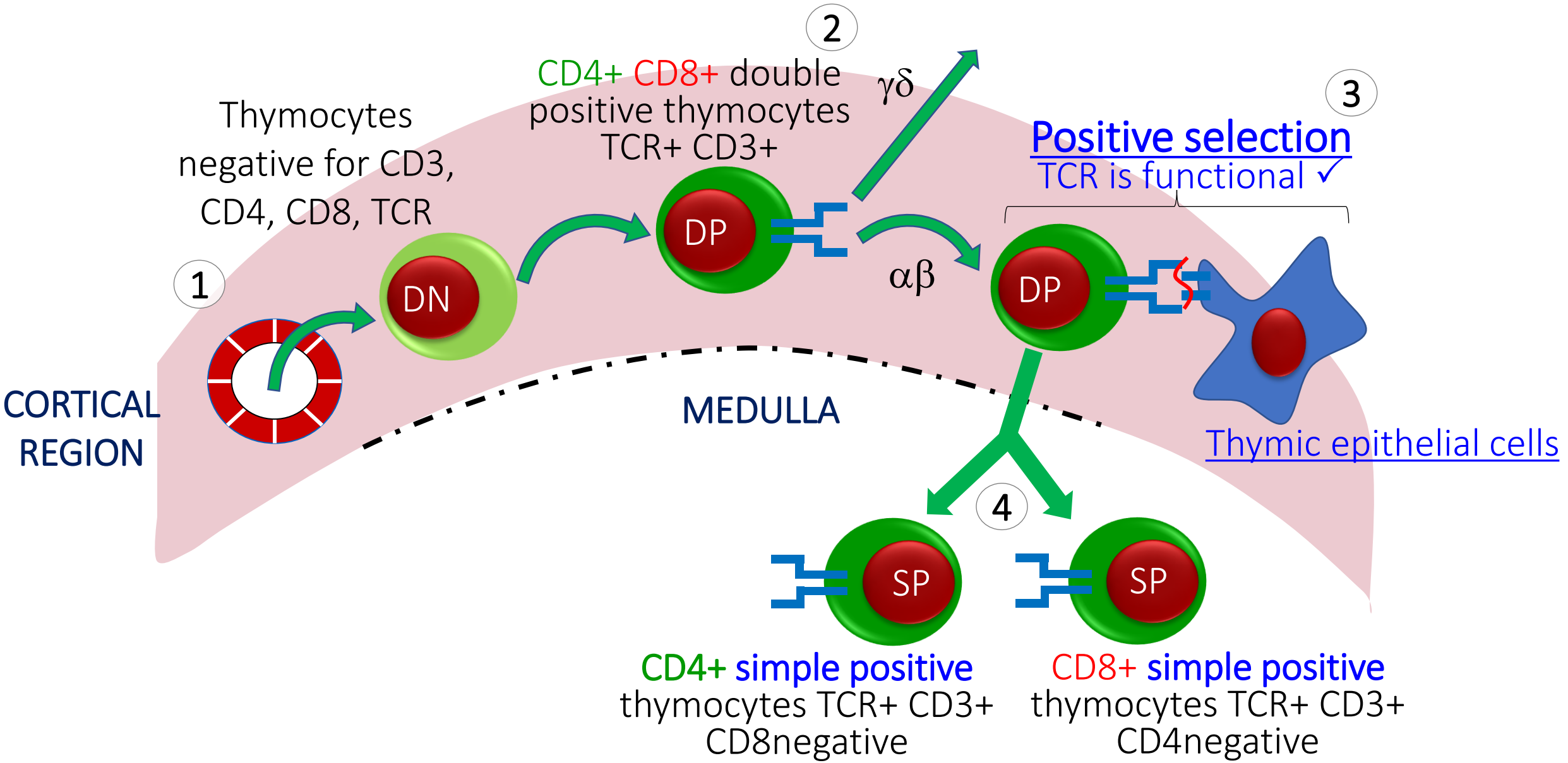
The specificity of antigen recognition receptors (BCR and TCR) is generated randomly

How lymphocyte precursors learn to
differentiate between Self and non-Self? (answers)

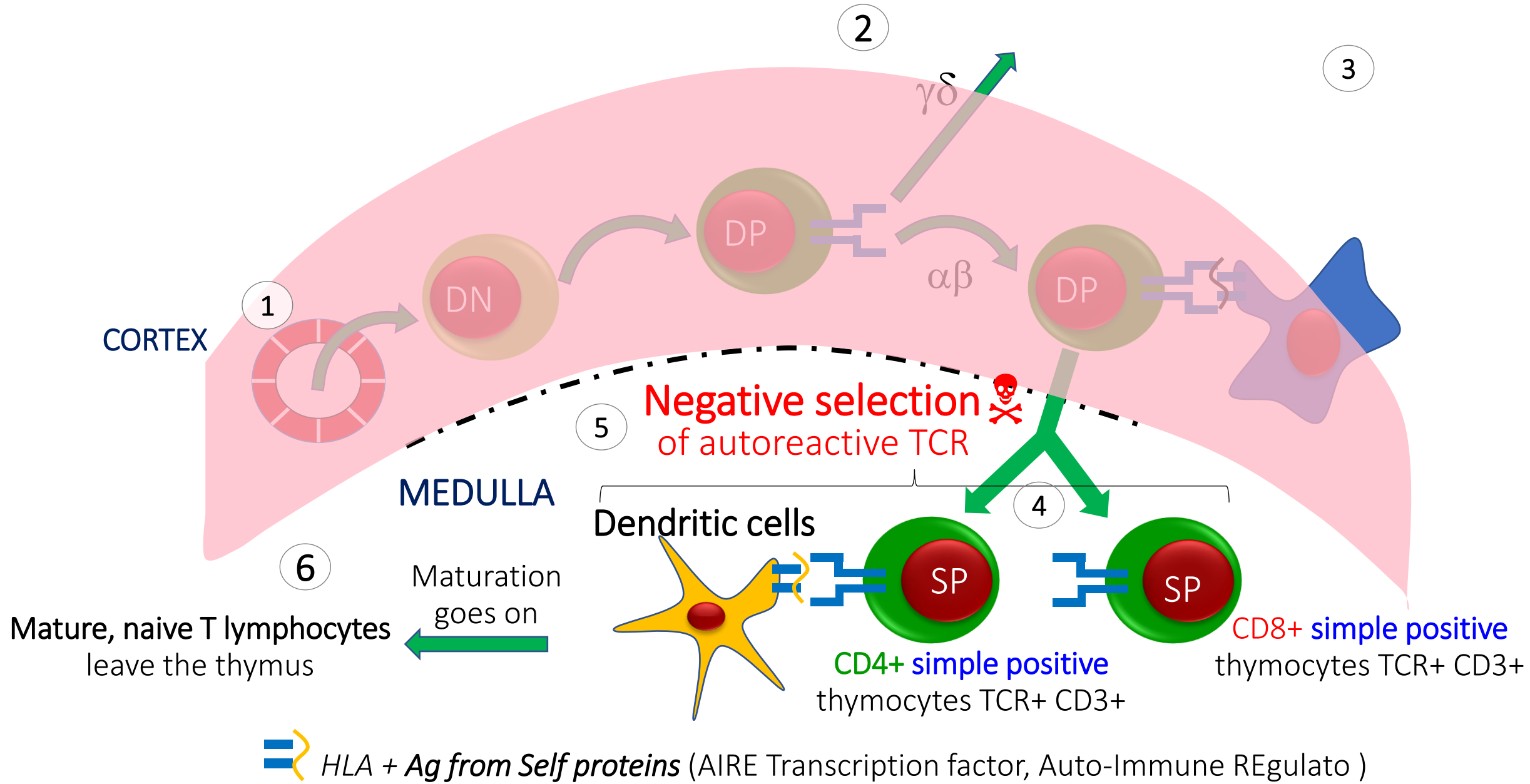
Central tolerance

Example of T lymphocytes

T lymphopoiesis and thymic selection – Central tolerance



Thymic selection of T cells, central tolerance



*HLA + **Ag from Self proteins*** (AIRE Transcription factor, Auto-Immune REgulato)

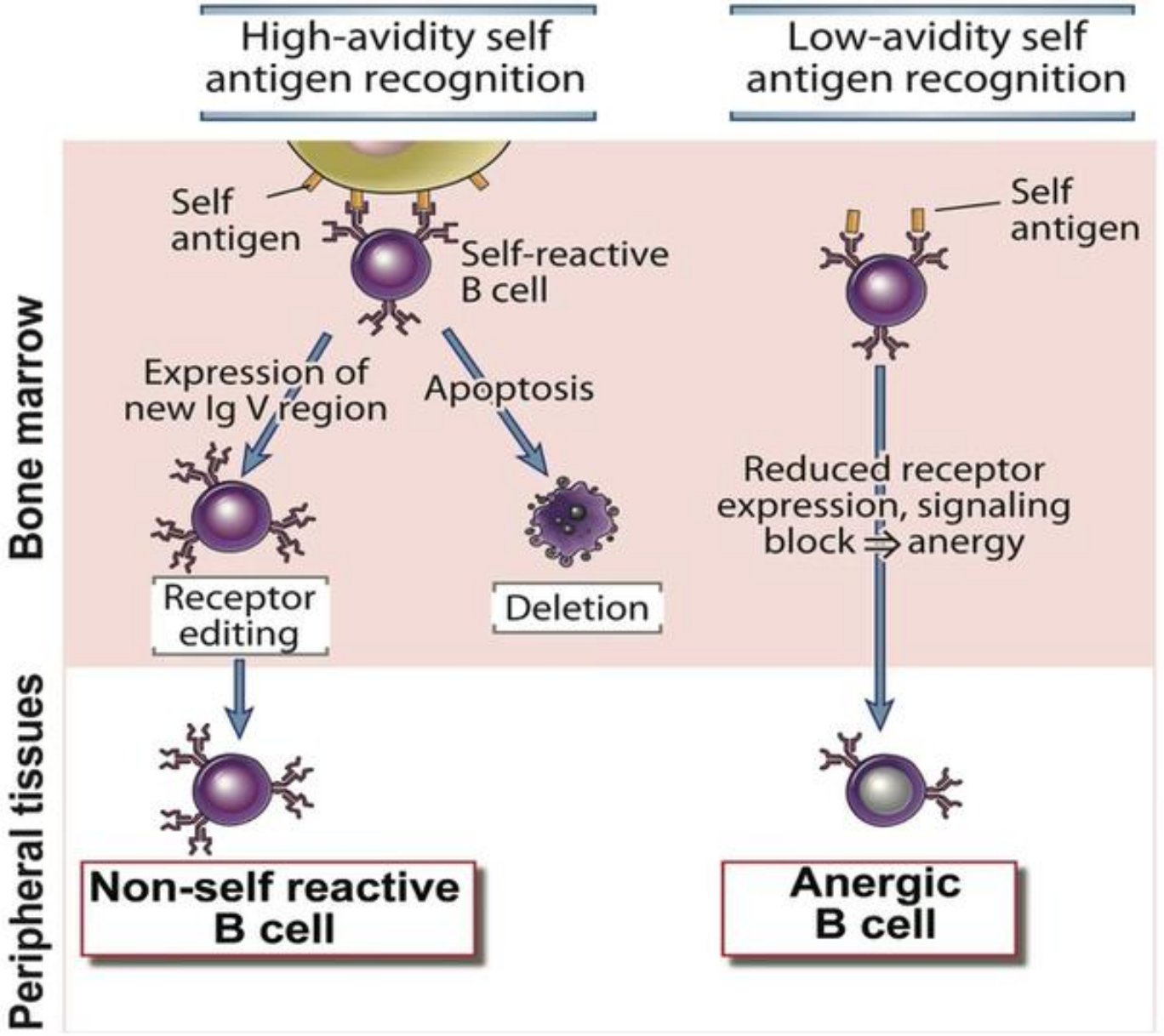
Summary: T lymphopoiesis and thymic selection – Central tolerance

Steps in T lymphopoiesis in view to produce mature, naïve T lymphocytes

- Expression of TCR
- Positive selection: restriction to CMH
- Engagement into the CD4+ or the CD8+ lineage
- Negative selection : establishing central tolerance

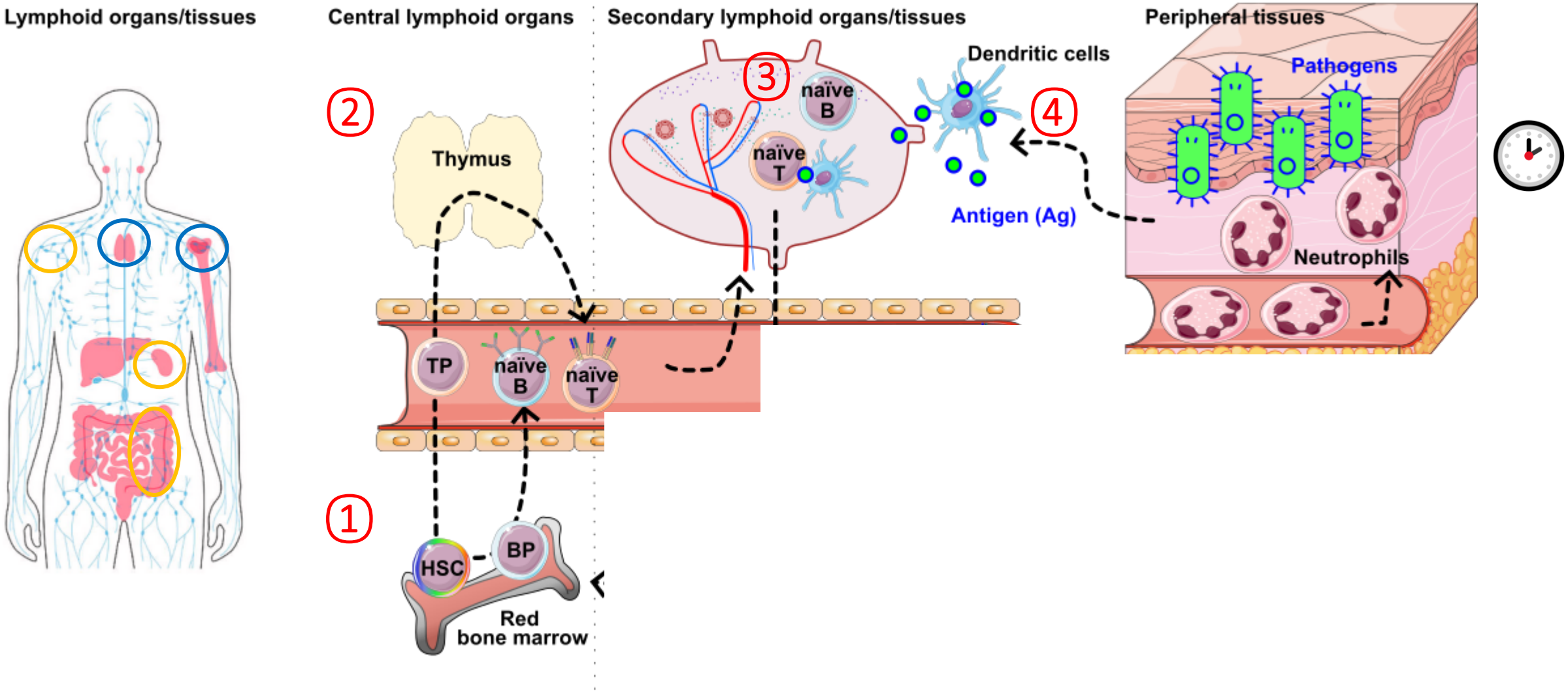
The thymocytes that have not succeeded the selections die by apoptosis

B cell tolerance



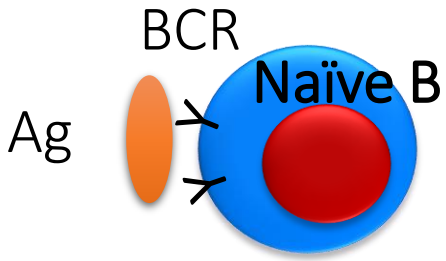
Organisation of the immune system

1 & 2: Lymphopoiesis; **3 & 4:** Ag-dependent activation of naïve lymphocytes (**primary response**) to become effector and memory lymphocytes in secondary lymphoid organs;



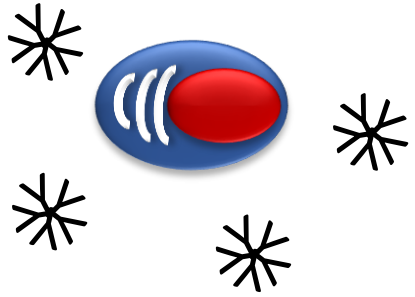
Activation of naïve B lymphocytes

To learn



Thymo-independent response

→ Carbohydrate antigens



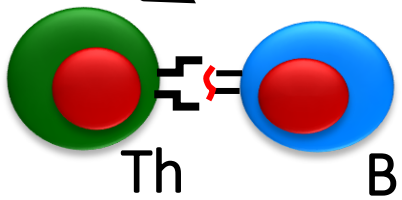
Rapid differentiation into **IgM** producer plasmablast.

→ **Short lived** effector cells

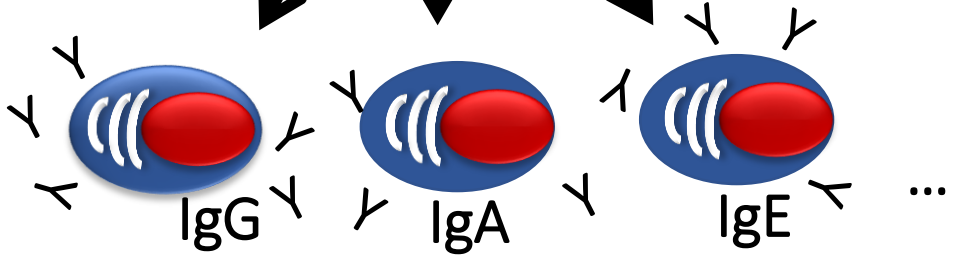
Thymo-dependent response

→ Protein Ag

Internalisation, processing and presentation of the Ag to T effector cells = T B cooperation



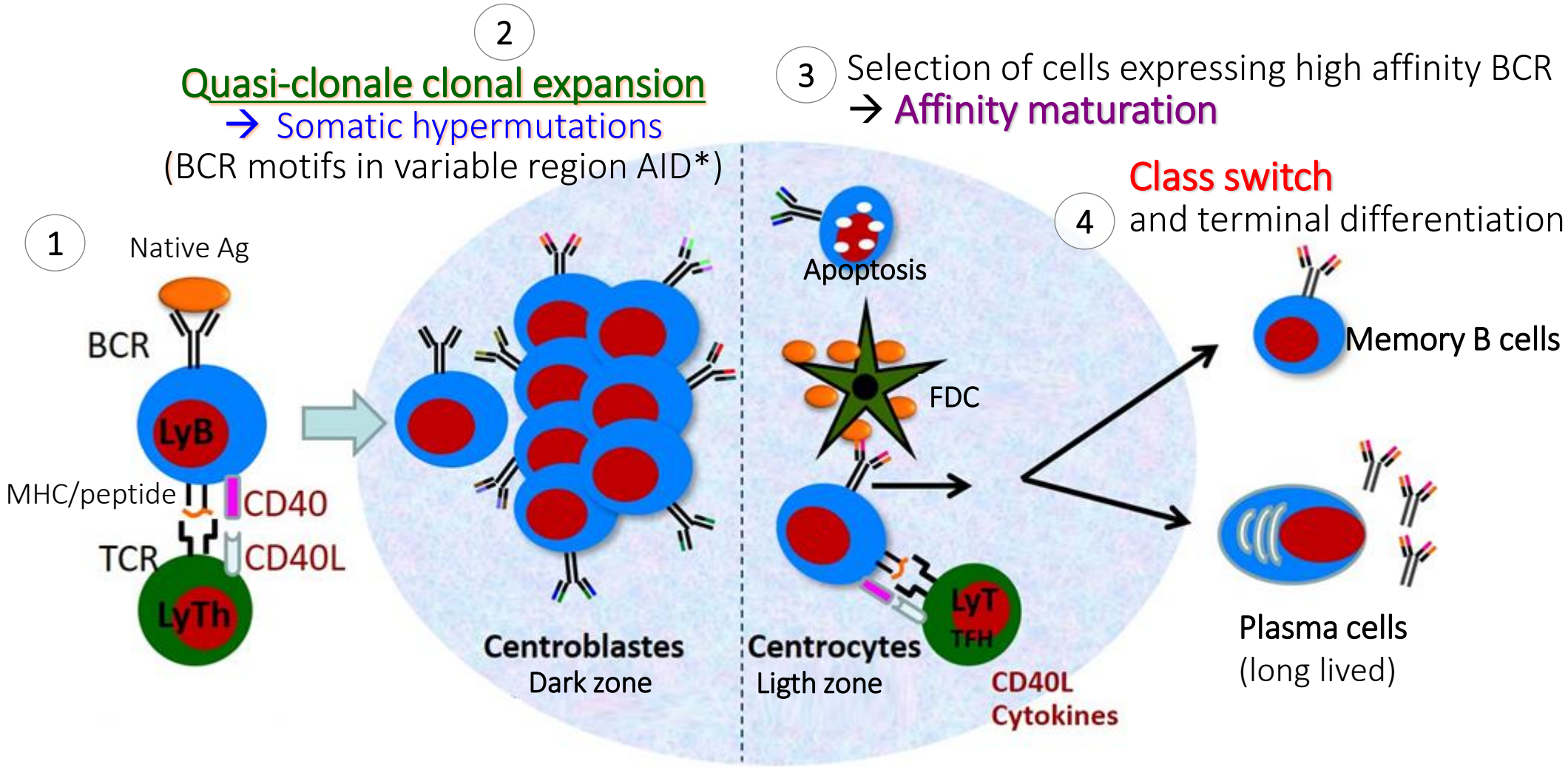
Germinal center



Long lived plasma cells producers of IgG, IgA, IgE,

Igs with improved affinity and generation of **memory B cells**

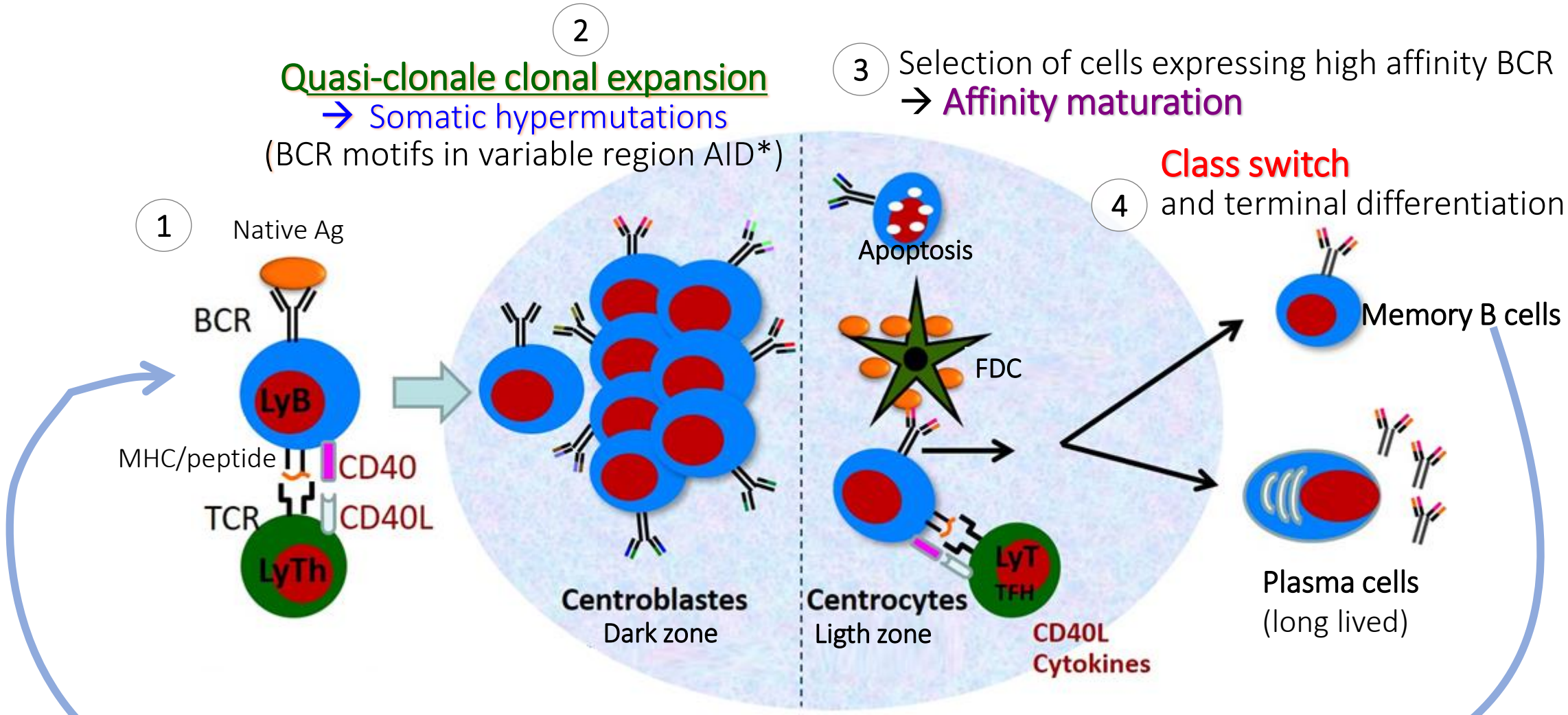
T-dependent B cell response - Germinal center reaction



*AID: activation-induced cytidine deaminase, converts Cytosines to Uracil residues at specific tetranucleotide (AGCT) hotspots in V regions. Us change to Ts during DNA replication, or are excised and repaired by an error-prone DNA repair process.

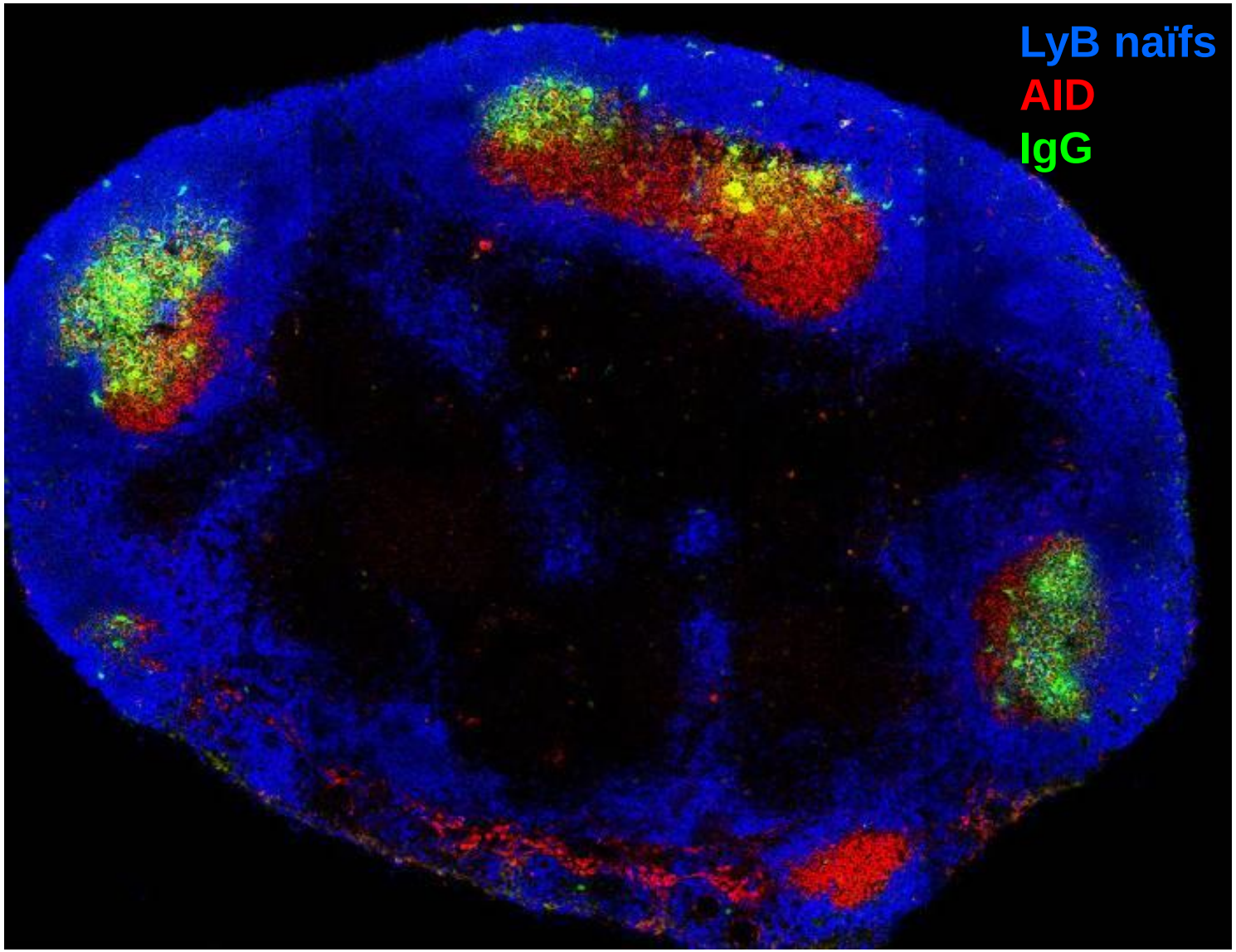
FDC= follicular dendritic cell, stromal non hematopoietic cells

T-dependent B cell response - Germinal center reaction



An Ag-specific B cell response can improvs the whole life by BCR hypermutation and affinity maturation selection.
Instead, TCR is never modified in periphery.

Germinal centers (dark zone / light zone) in a **lymph node**



Cortical zone

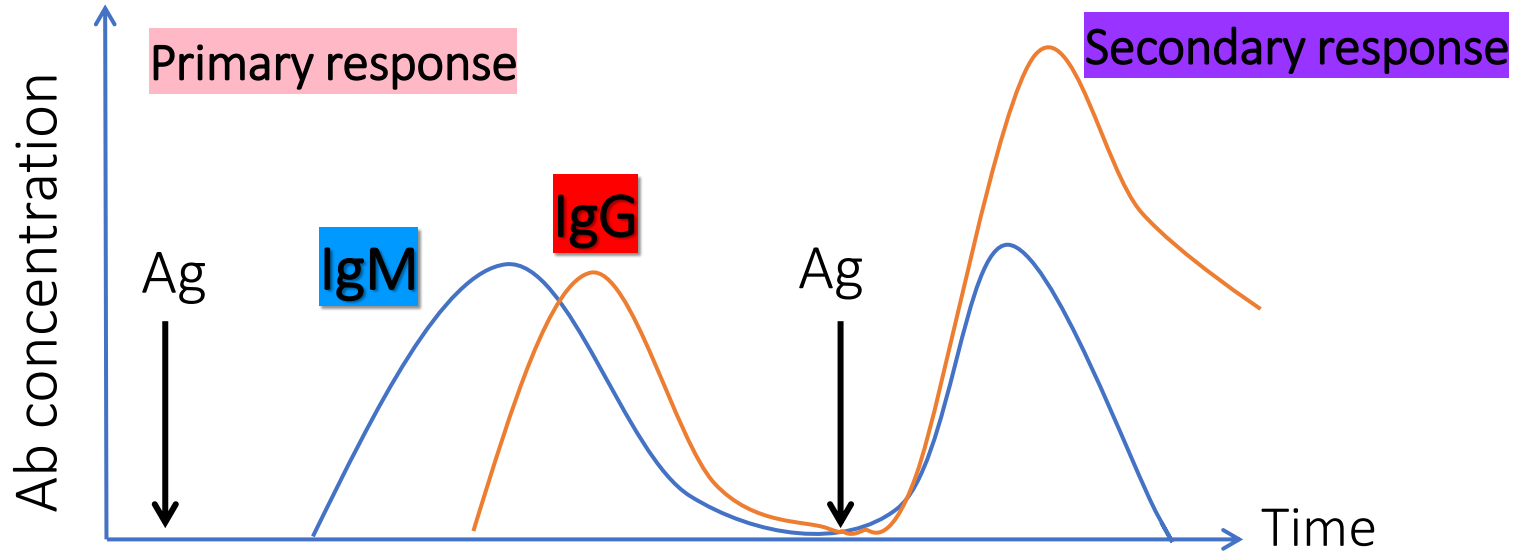
Follicles

Dark zone: High cell density (clonal expansion)

Light zone: Low cell density (apoptosis of cells no recognizing the Ag)

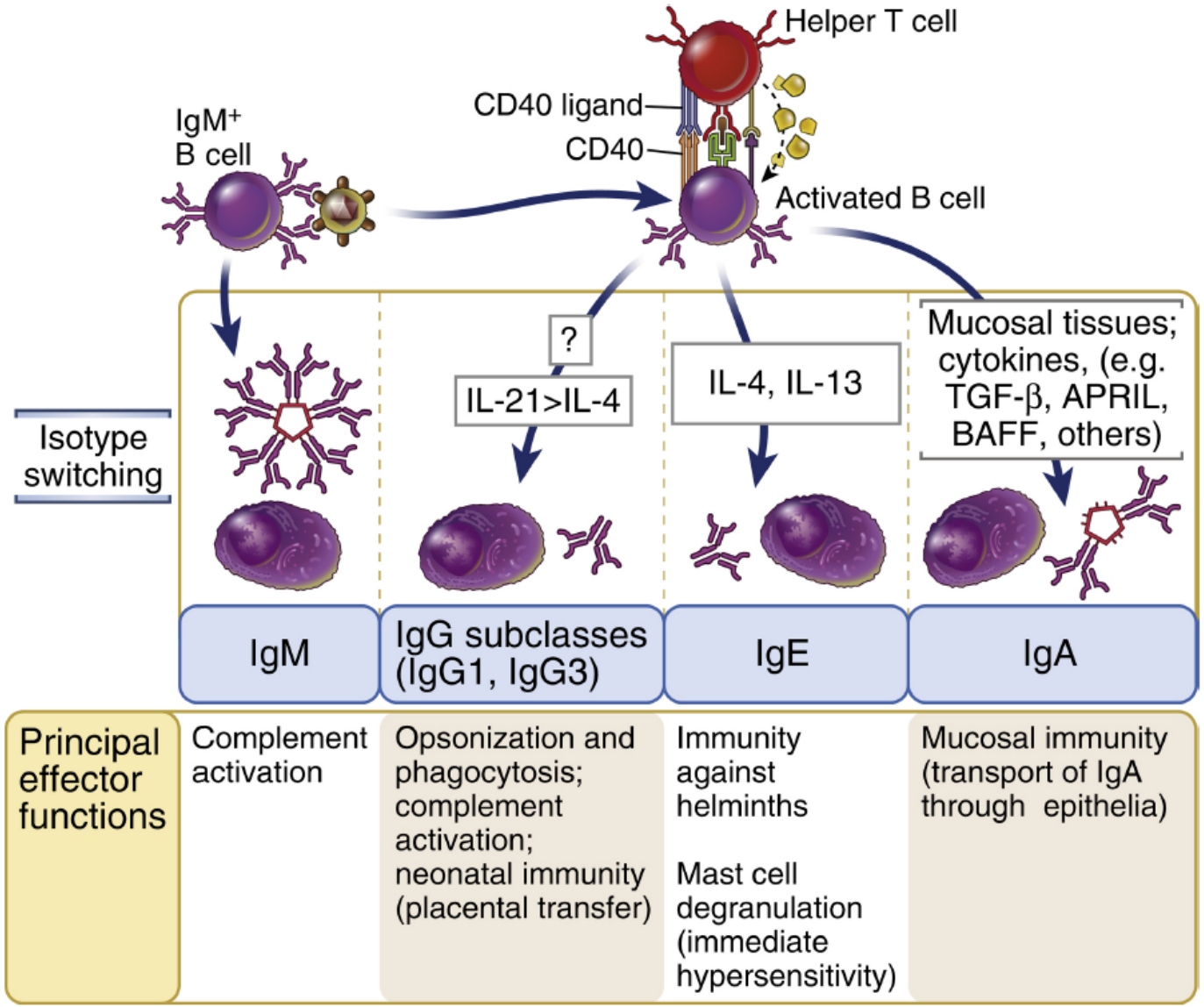
Ab production in primary vs. secondary B lymphocyte response

To learn



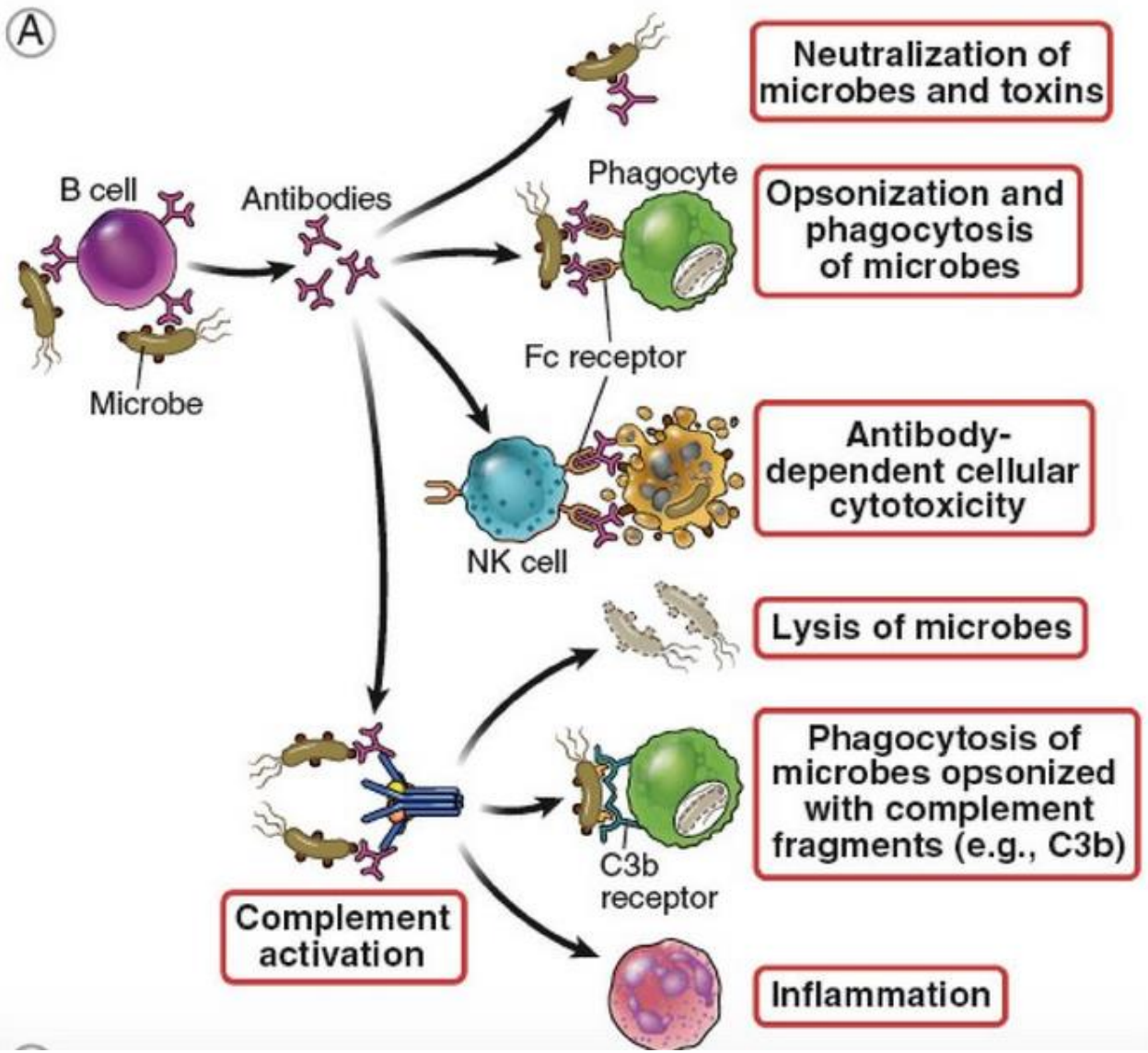
	Primary response	Secondary response
When	1st contact with the Ag (naïve cells)	Activation of memory B lymphocytes
IgM response	Relatively fast (3-4 days), short, Abs with relatively low affinity (kd 10-7M)	No modification : relatively fast, short, no memory
IgG Response delay	> 7-10 days	**Shorter delay** (4 days)
Intensity / duration IgG response	Relatively low and short	**Larger and longer Ab production**
Affinity des IgG	**Improved Ab affinity**	**Even higher affinity** (kd 10-11M)

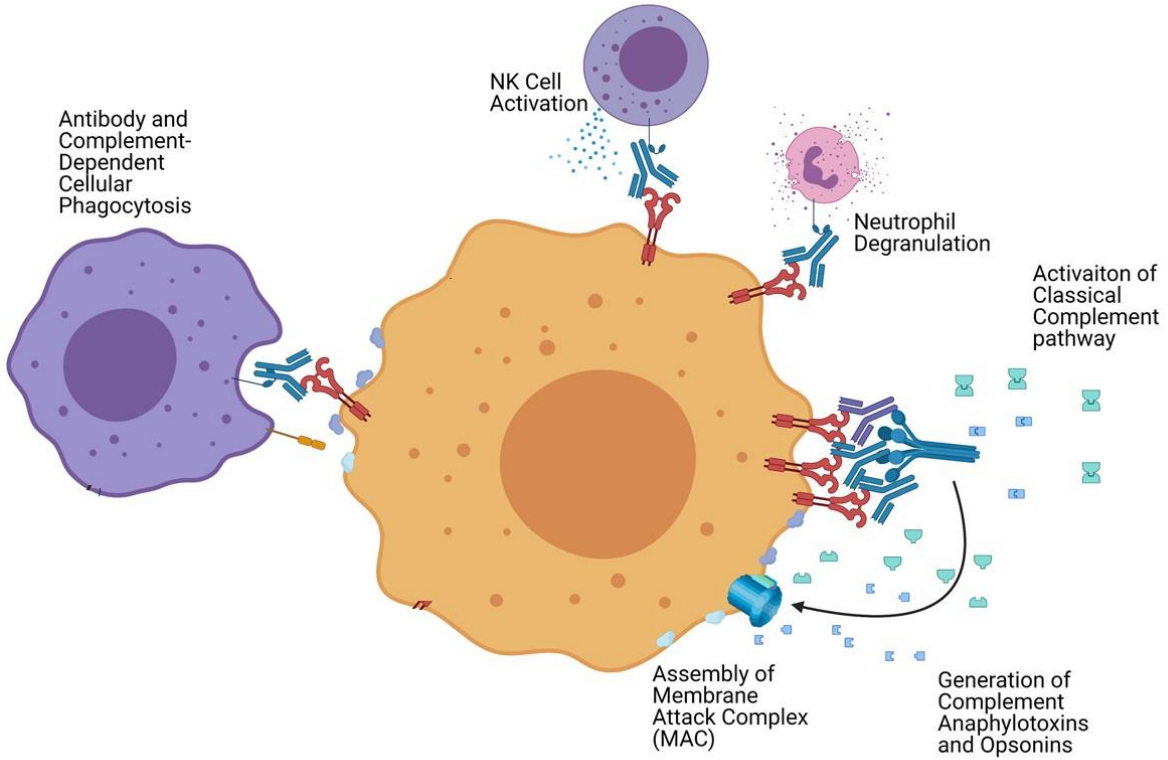
Immunoglobulins heavy chain isotype - function



Effector function of antibodies

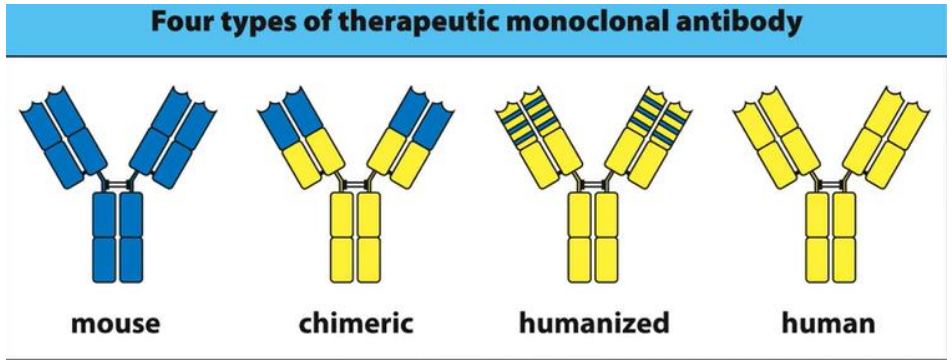
To learn





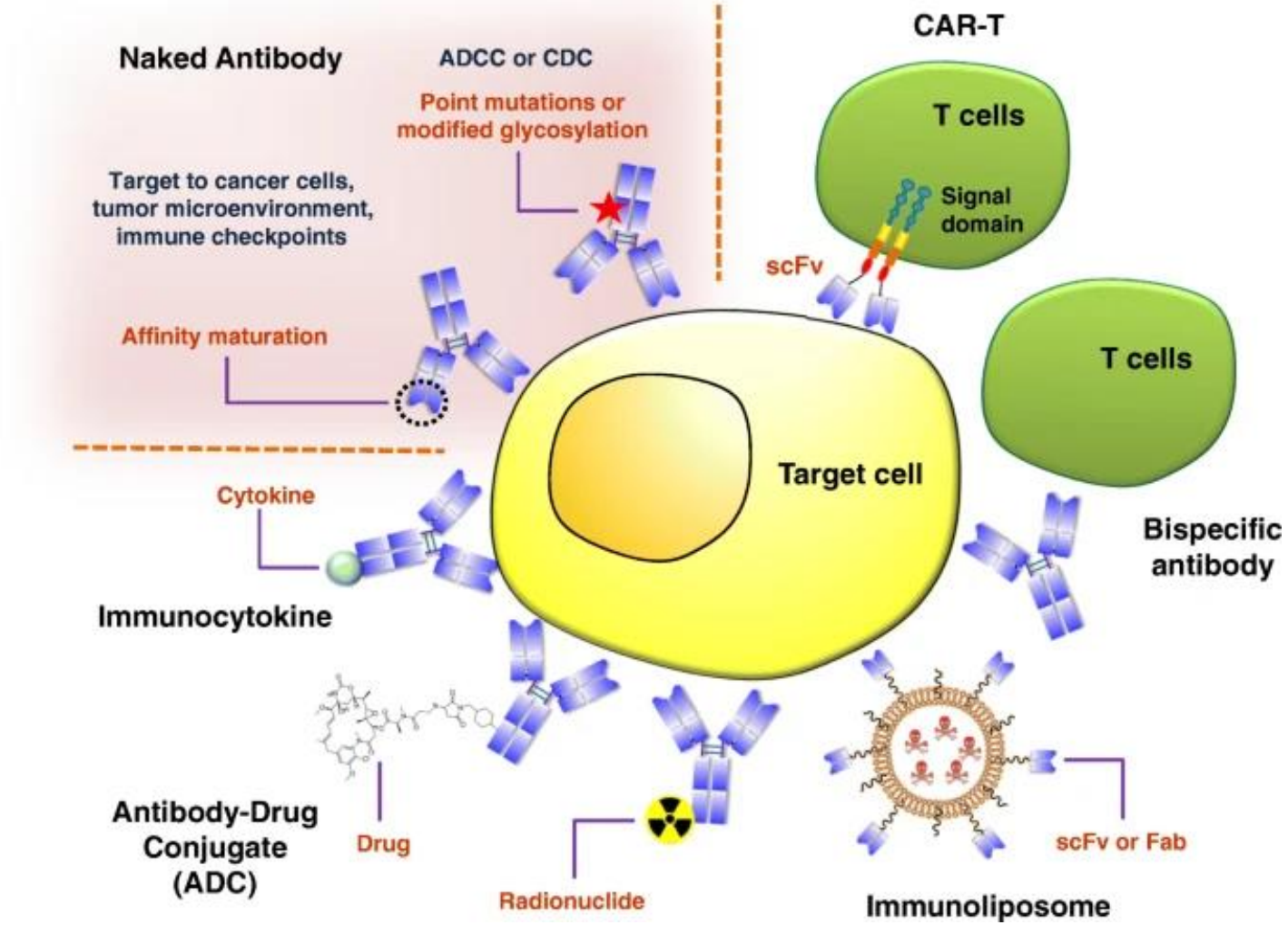
Tsao et al, Cancer Res 2021

Therapeutic application of monoclonal antibodies



"Naked" Abs can activate (**agonist**) or block activation **(antagonist**) of given receptors, **elicit** cell death by different mechanisms, including **ADCC/CDC,** or target immune checkpoints.

Ab-drug conjugates require additional engineering to enhance their therapeutic efficacy



Lu et al, J Biomedical Sci 2020

Summary: B lymphocyte activation

It takes place in secondary lymphoid organs

- The BCR of a naïve B lymphocyte is **activated** upon binding a **specific antigen** (Ag).
- Without further stimuli, activated B cells proliferate and differentiate into **short-lived IgM**-producing **plasmablasts** (T-independent response).
- If the Ag is a protein, it is processed and presented to T-helper lymphocytes, leading to a **T-dependent response** and initiation of a **germinal center reaction**, which includes:
  - Clonal expansion with somatic hypermutations in Ig variable regions.
  - **Affinity maturation** / selection of modified Igs, is driven by competition for the Ag.
  - Class switching and production of **long-lived plasma cells (IgG, IgA, or IgE) and memory B cells**.
- Memory B cells enable a faster, more effective response with high-affinity antibodies (mainly IgG) upon re-exposure to the antigen (immune memory).

Antibodies cannot penetrate cells to detect pathogens or non-self antigens inside.

T lymphocytes (CD8+) perform this function through a coupled mechanism of antigen presentation by all nucleated cells in the body.

Antigens (peptides) are displayed on the cells surface, loaded onto Major Histocompatibility Molecules (MHC), known as HLA in humans).

→There are **two classes of classic HLA molecules** that load peptides from two distinct cell compartments and interact with different T cell subsets : CD4+ or CD8+.

HLA: Human Major Histocompatibility molecules

Functions (pleiotropic): •Presentation of Self and non-Self peptides to **TCR** (Ag receptor of T lymphocytes).

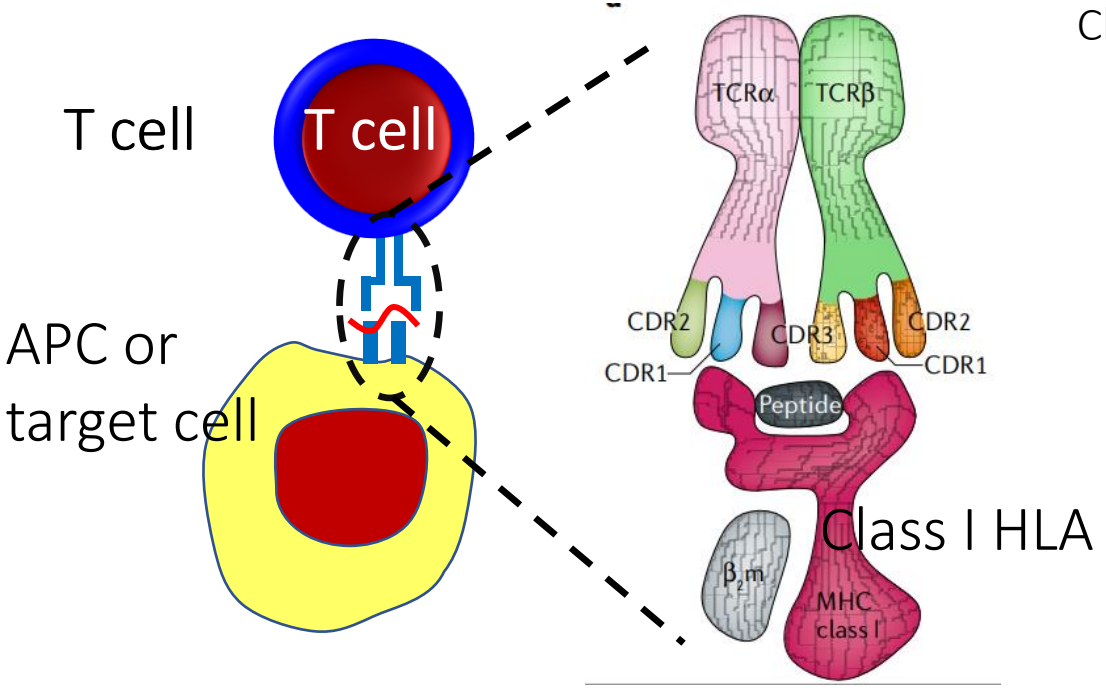
→ Education and selection of TCR repertoire in thymus (homeostasis)

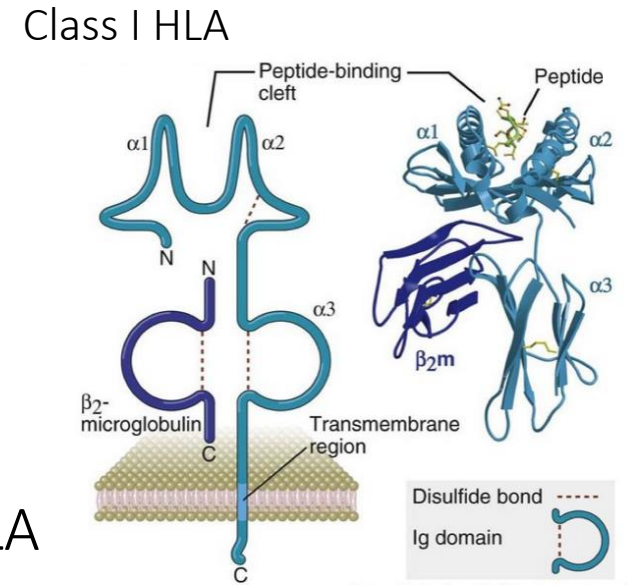
→ Maintain of peripheral T cell pool (homeostasis)

→ Activation of T lymphocyte response by presenting non-Self antigens

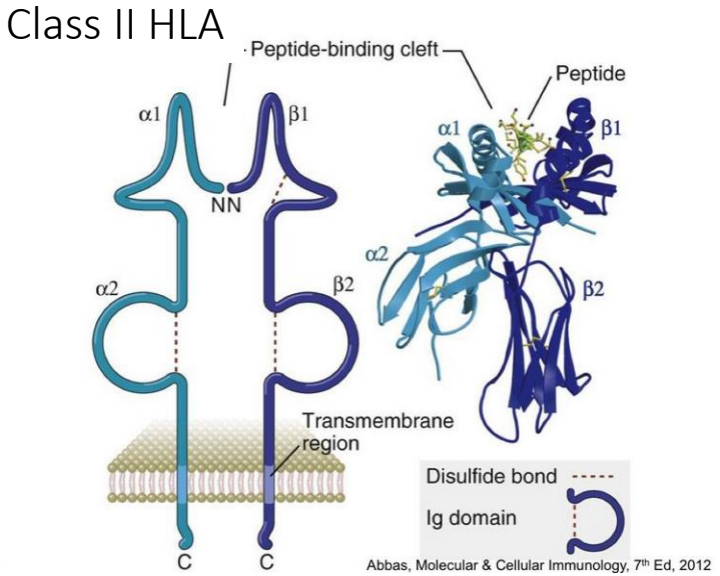
•Class I HLA molecules are inhibitory ligands of **NK KIR receptors** ("Self-signal")

Two classes of HLA classic molecules: Class I (α chain) and Class II (α β chains), 3 isotypes for each Class.





HLA-A, HLA-B, HLA-C

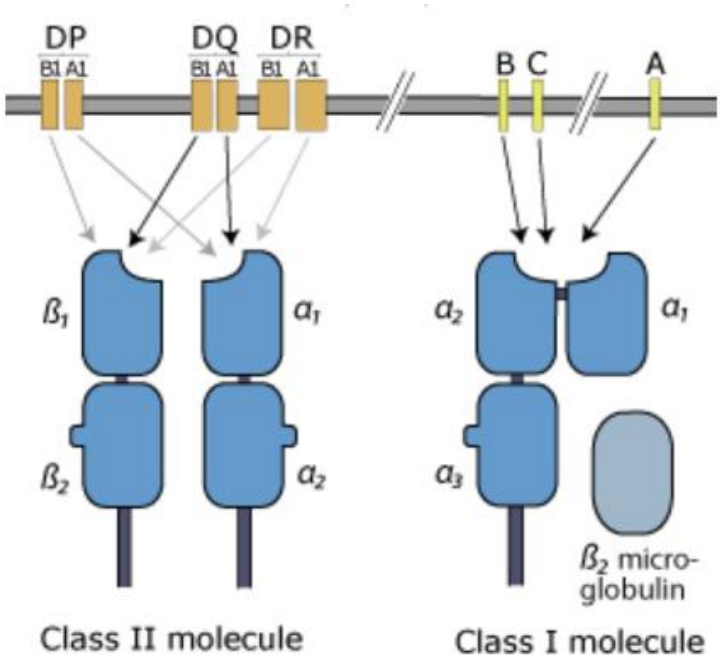


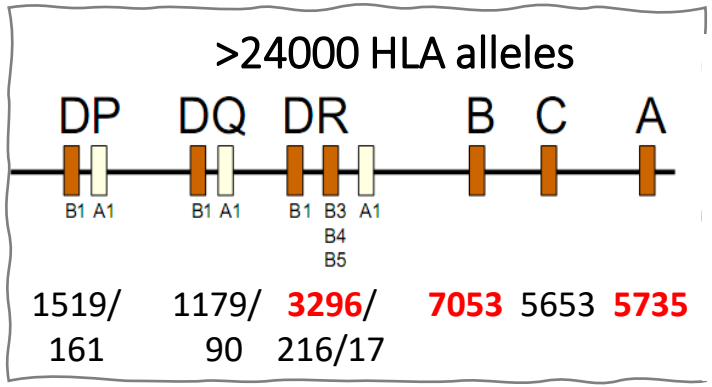
HLA-DP, HLA-DQ, HLA-DR

Genes encoding for classic HLA molecules

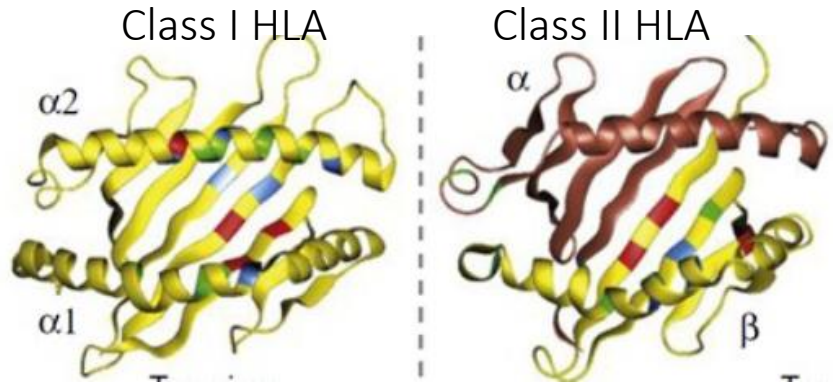
Polygenic, polymorphic and codominant system

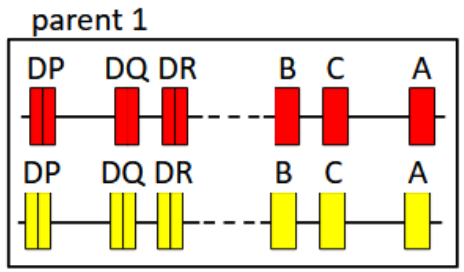
- 3 loci for classic HLA class I molecules, 6 or 7 to 8 loci for classic HLA class II molecules (heterodimers)
- > 24000 HLA alleles in human population. Polymorphic residues clustered in peptide binding region and in the surface of interaction with TCR.

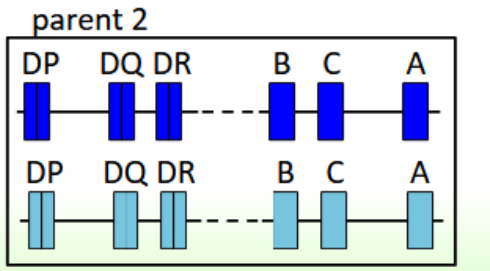




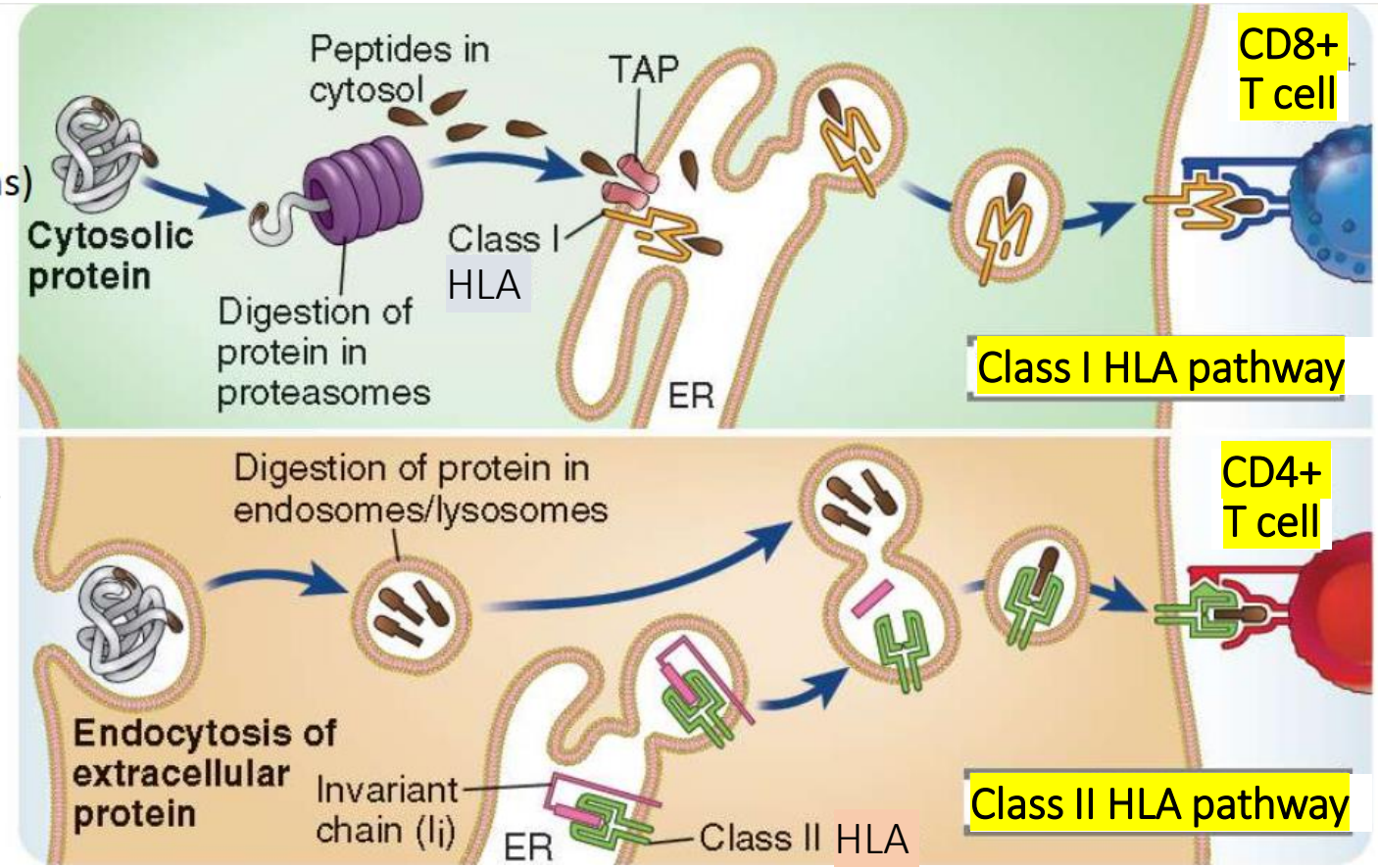
Top view. Polymorphic residues: red blue green







Peptides associated to HLA molecules : Processing and presentation pathways



To learn

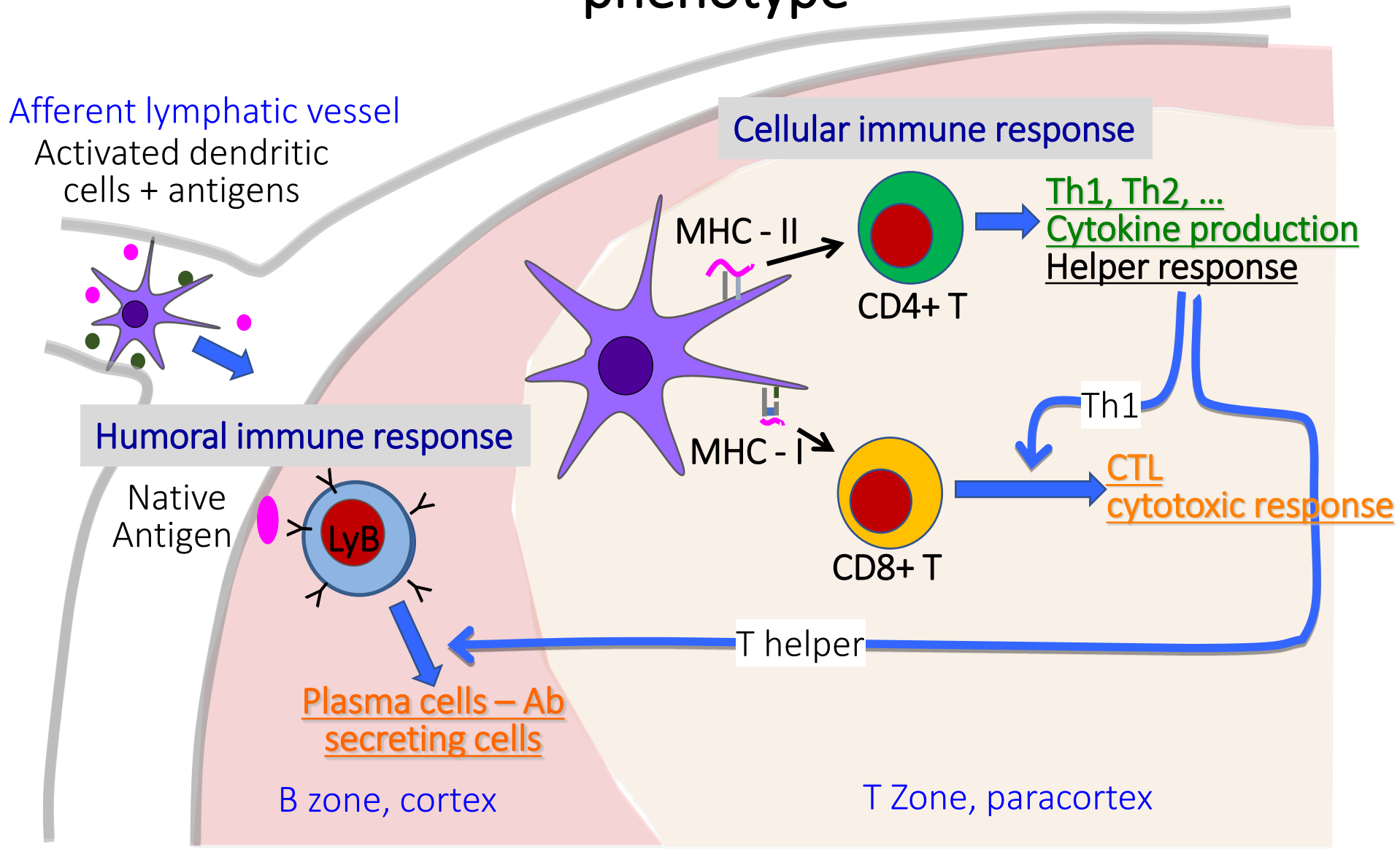
Class I HLA molecules :

- Expressed by **all nucleated cells**
- Present peptides mostly derived form endogenous / cytoplasmic proteins
- Peptides are presented to CD8+ T lymphocytes → cytotoxic response

Class II HLA molecules

- Expressed by professional antigen-presenting cells (DCs, B cells, mono/macrophages)
- Present peptides mostly derived form exogenous proteins.
- Peptides are presented to CD4+ T lymphocytes → cytokine response

Primary adaptive immune response activation in secondary lymphoid tissues triggers **clonal expansion** and acquisition of an **effector phenotype**



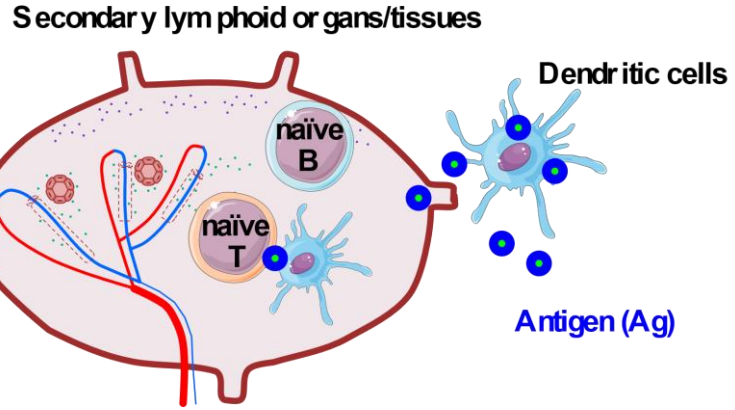
Activation of T lymphocytes

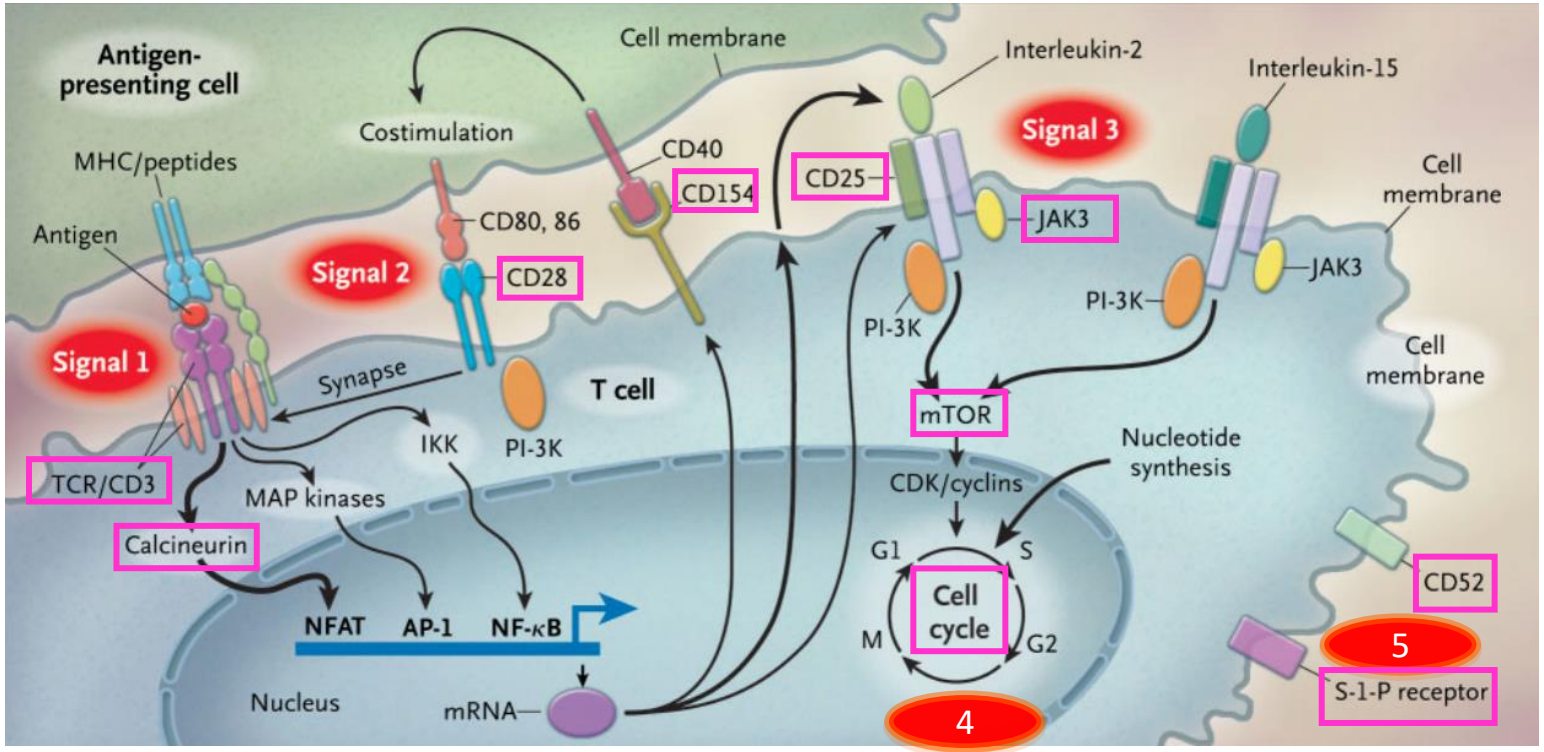
Classic steps of activation of **naive T lymphocytes** (in part valid for effector and memory T cells).

Signal 1. **TCR activation** by specific **HLA / Ag complex**
Signal 2. Costimulation
Signal 3. **Cytokines**: IL-12 (Th1), IL-4 (Th2), … IL-2

} Secondary lymphoid organs / tissues

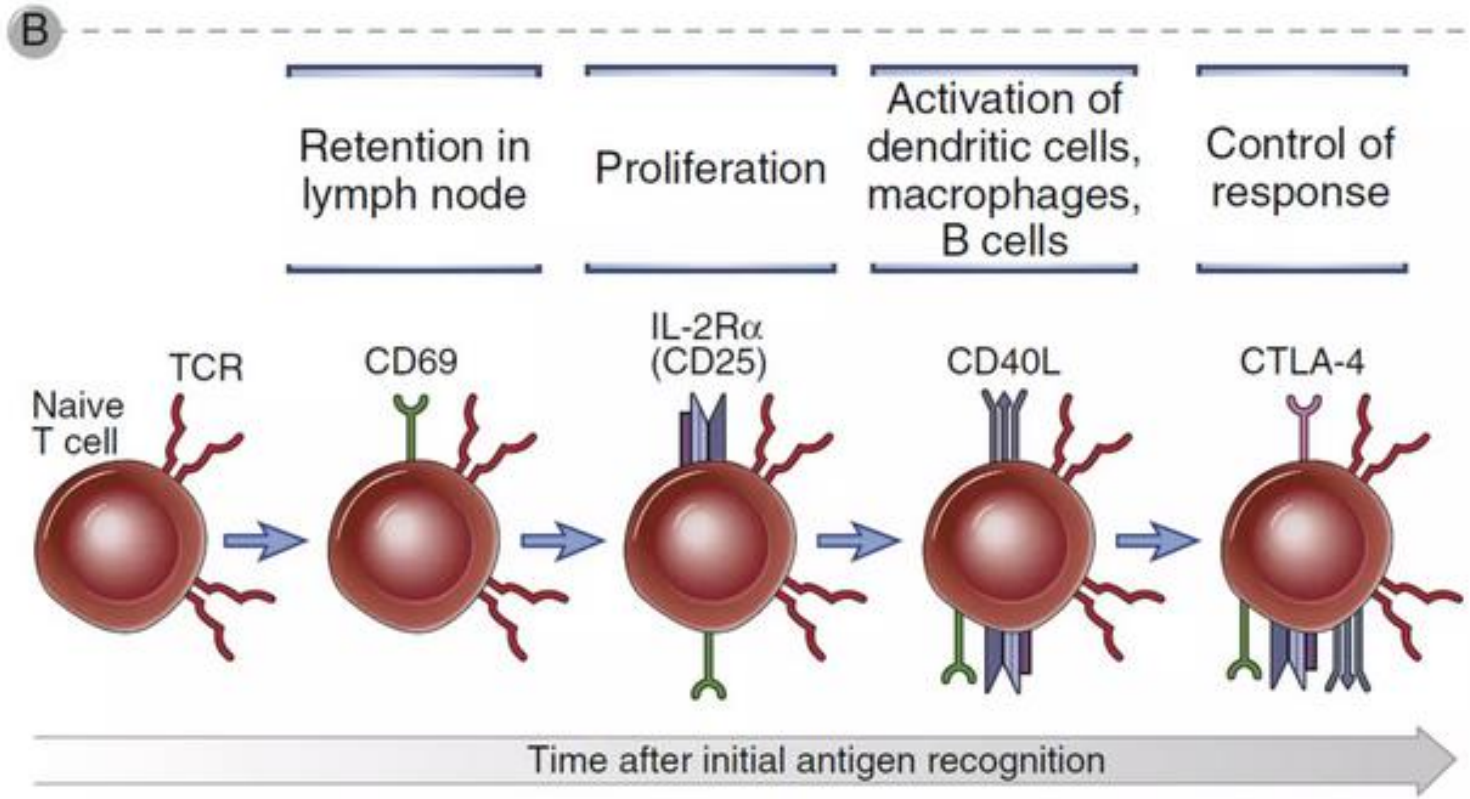
4. Clonal expansion and differentiation
5. Migration to the inflammatory site and direct or indirect cytotoxic action





Halloran, New Engl J Med 2004

Changes in T cell surface markers along activation



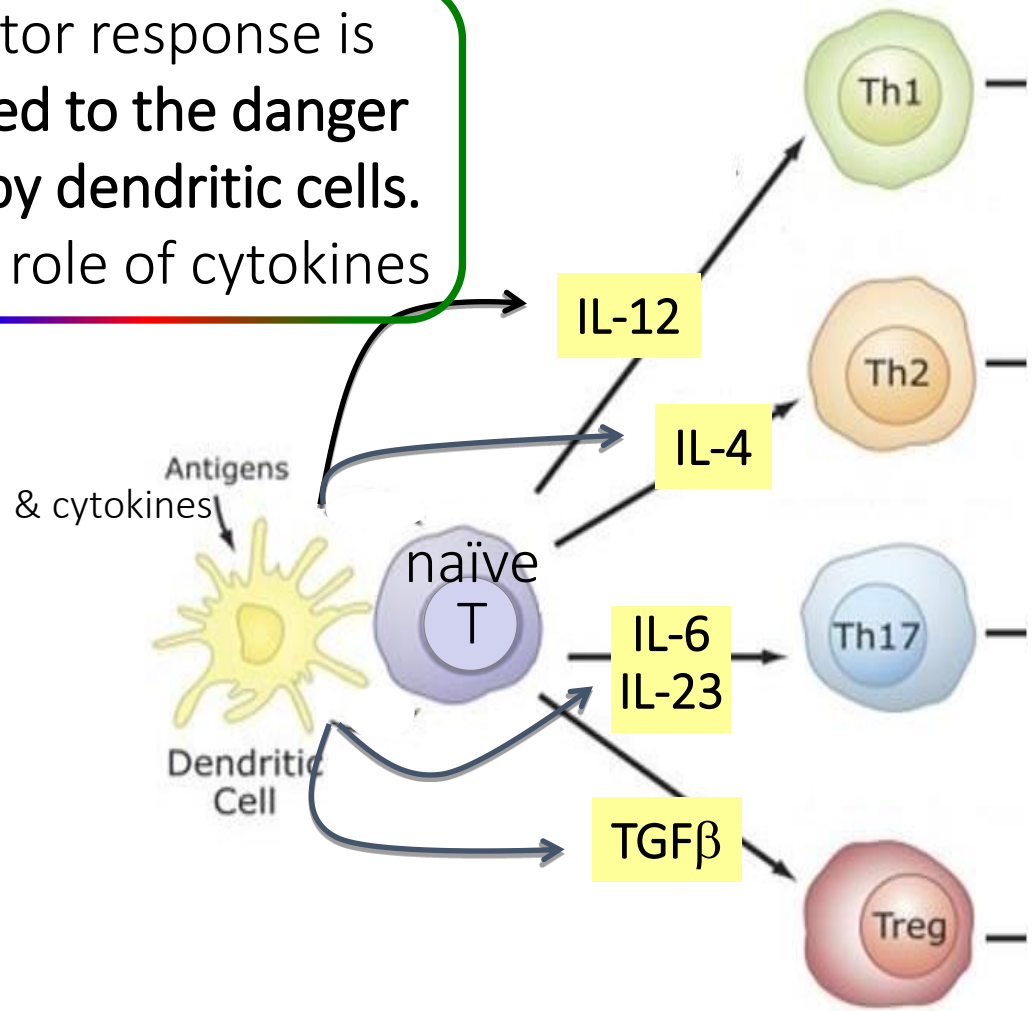
- **CD69 :** retain T cells in lymphoid organ
- **IL-2Rα (CD25):** response to IL-2 (autocrine growth factor)
- **CD40L:** help activate APCs
- **CTLA-4:** inhibit T cell activation

Differentiation of naïve CD4+ T lymphocytes upon Ag-specific activation → Helper T phenotypes

To learn

Effector response is **adapted to the danger faced by dendritic cells.** Crucial role of cytokines

Secondary lymph organs



Peripheral tissues

Effector and memory pool

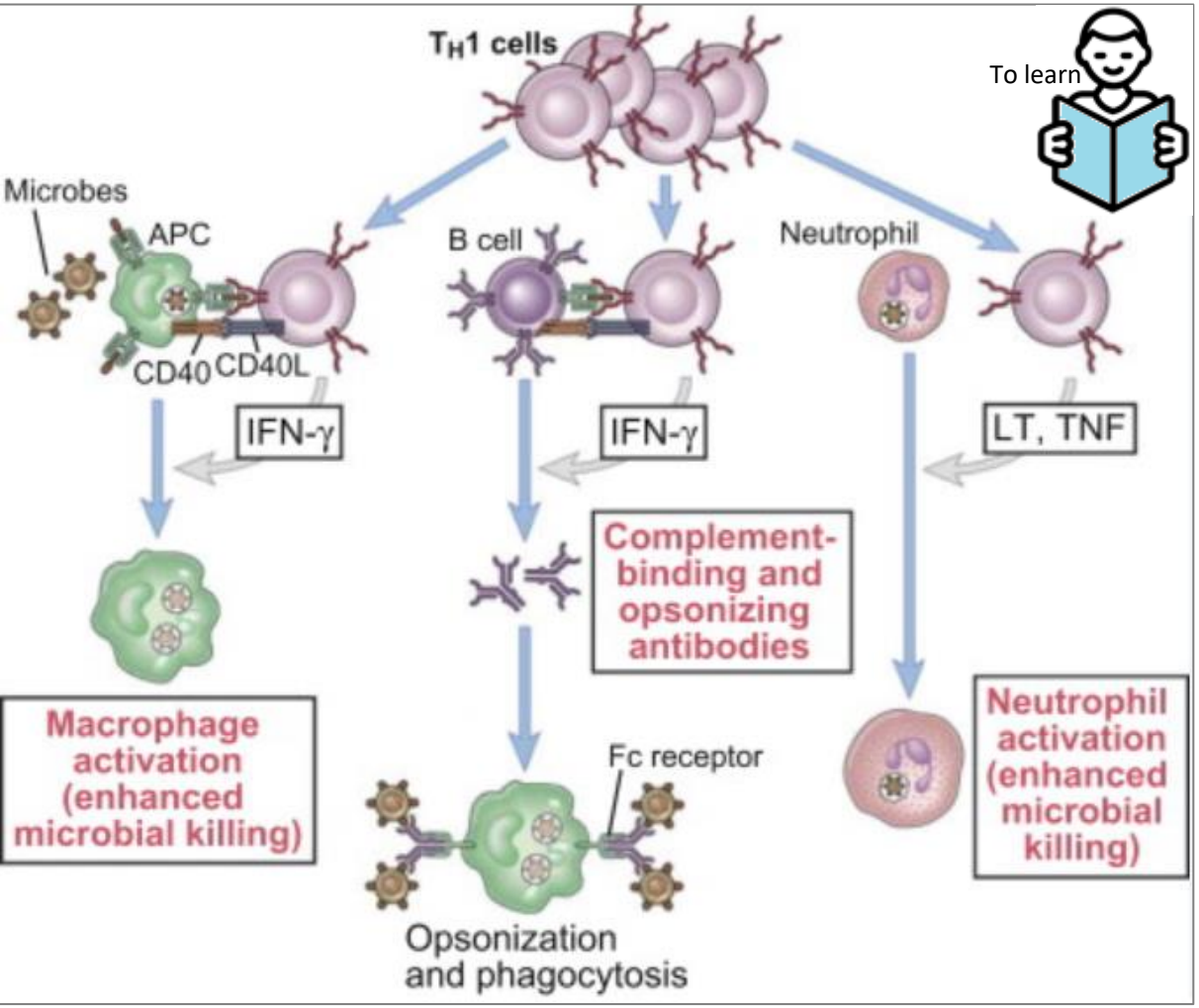
↓

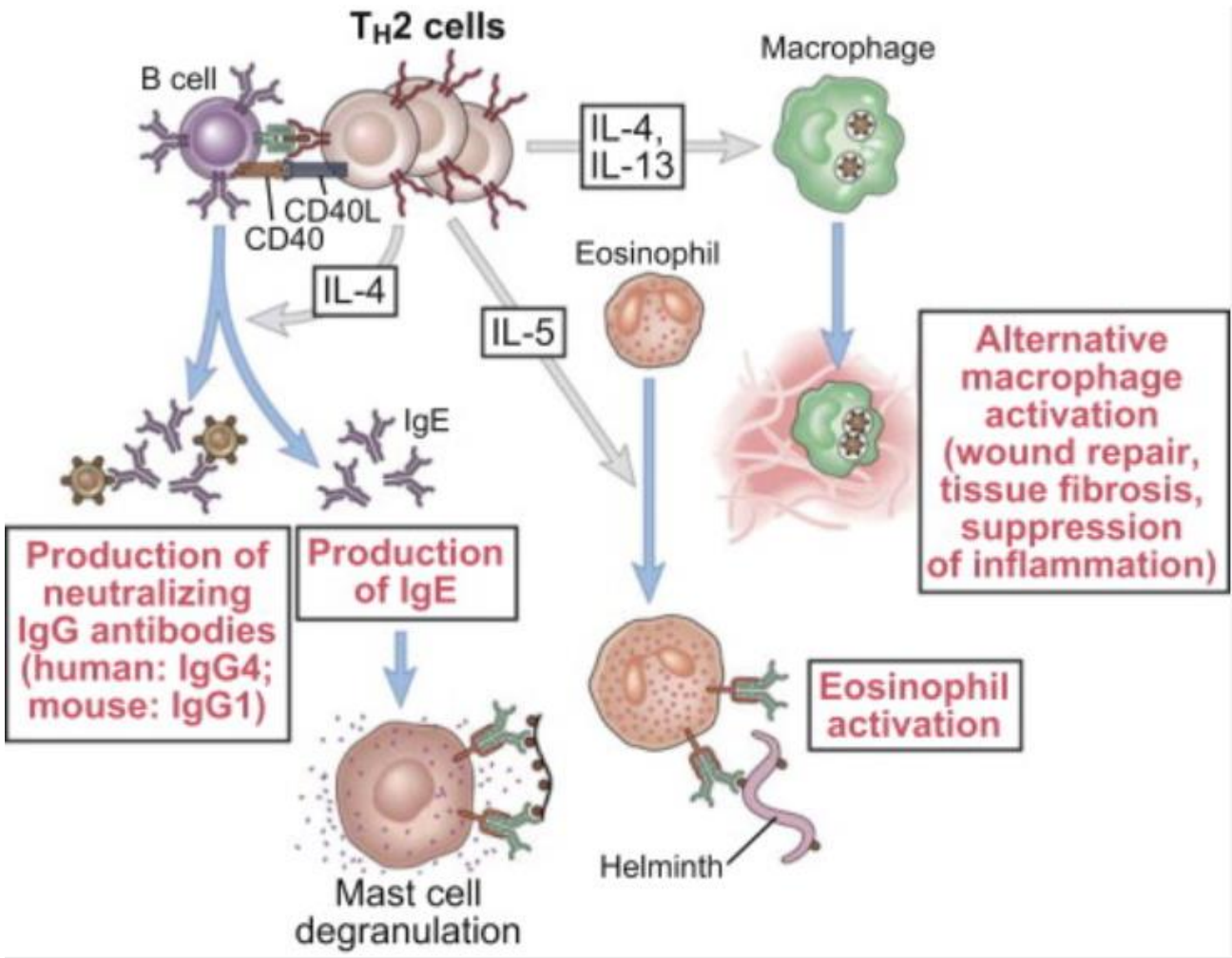
Inflammed tissues

Effector/memory Th cells must be **<u>activated by the specific Ag</u>** to accomplish their effector function

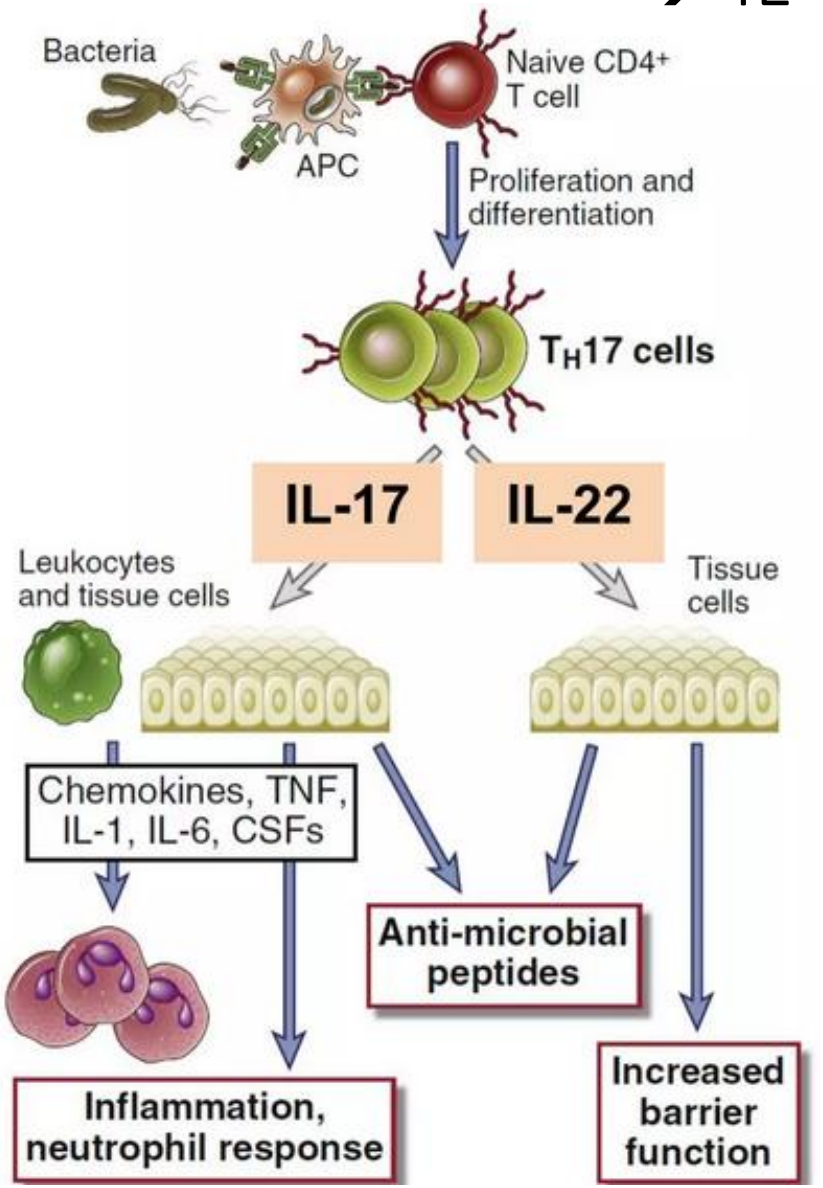
→ A major Th cell function is to **<u>produce particular cytokines</u>** that help to enhance function other immune cells

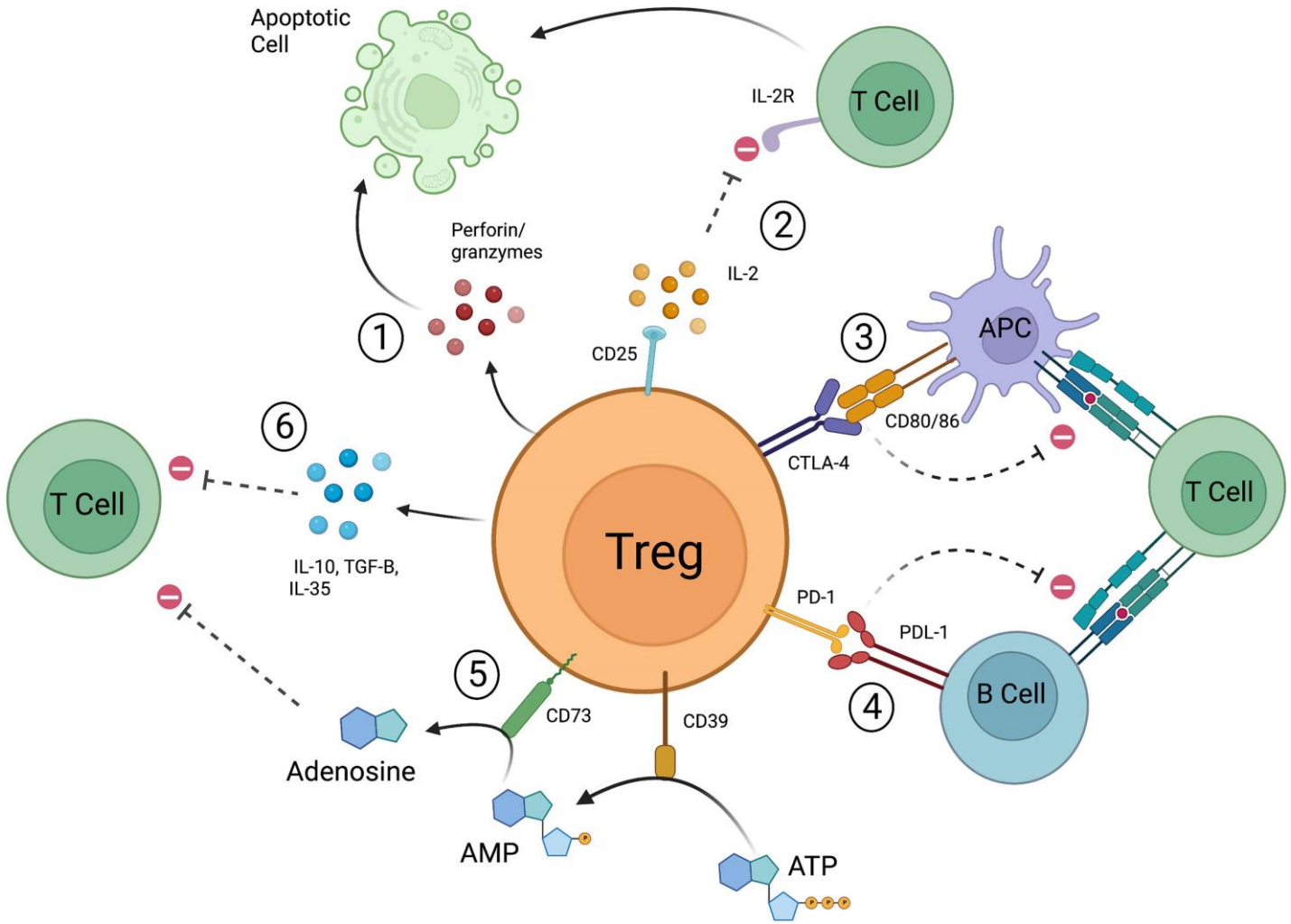
Effector function of TH1 and Th2 lymphocytes
→ IFN-γ, IL-4, IL-5, IL-13





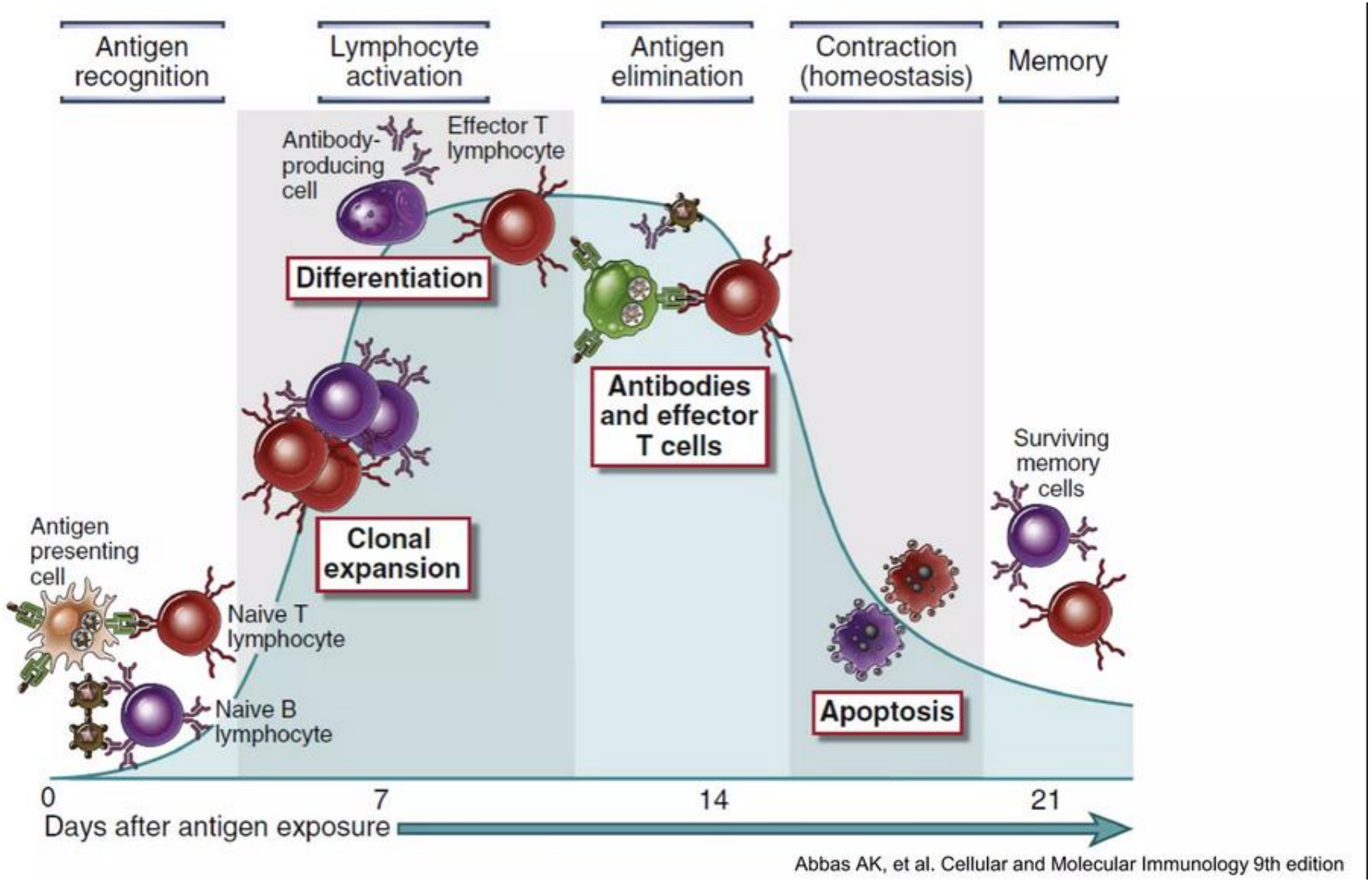
Effector function of TH17 and Treg lymphocytes
→ IL-17, IL-22, IL-10, TGF-β



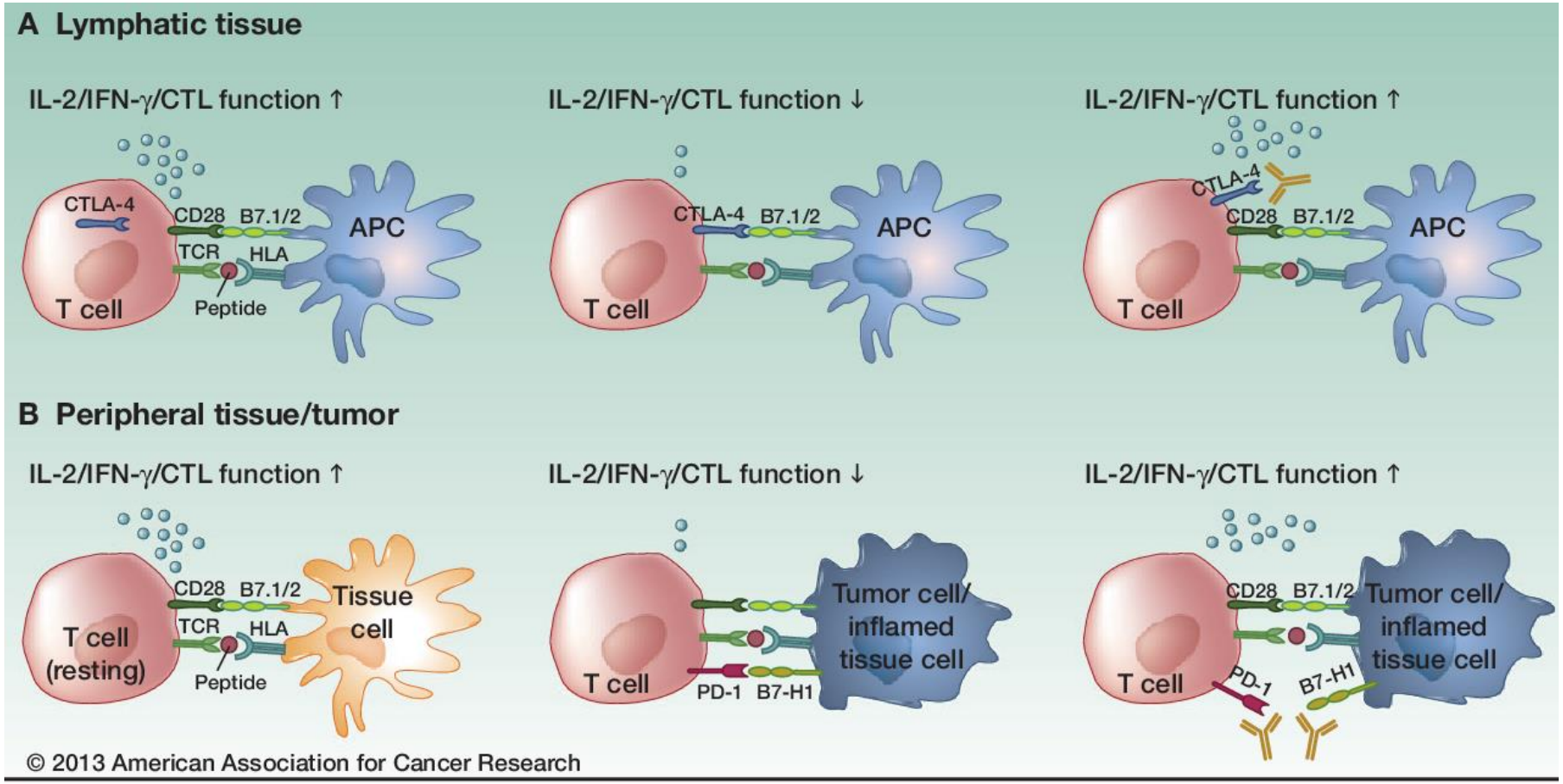


Sanders et al, Front Immunol 2021

Clonal contraction = after Ag elimination the pool of effector lymphocytes are eliminated, memory lymphocytes are preserved

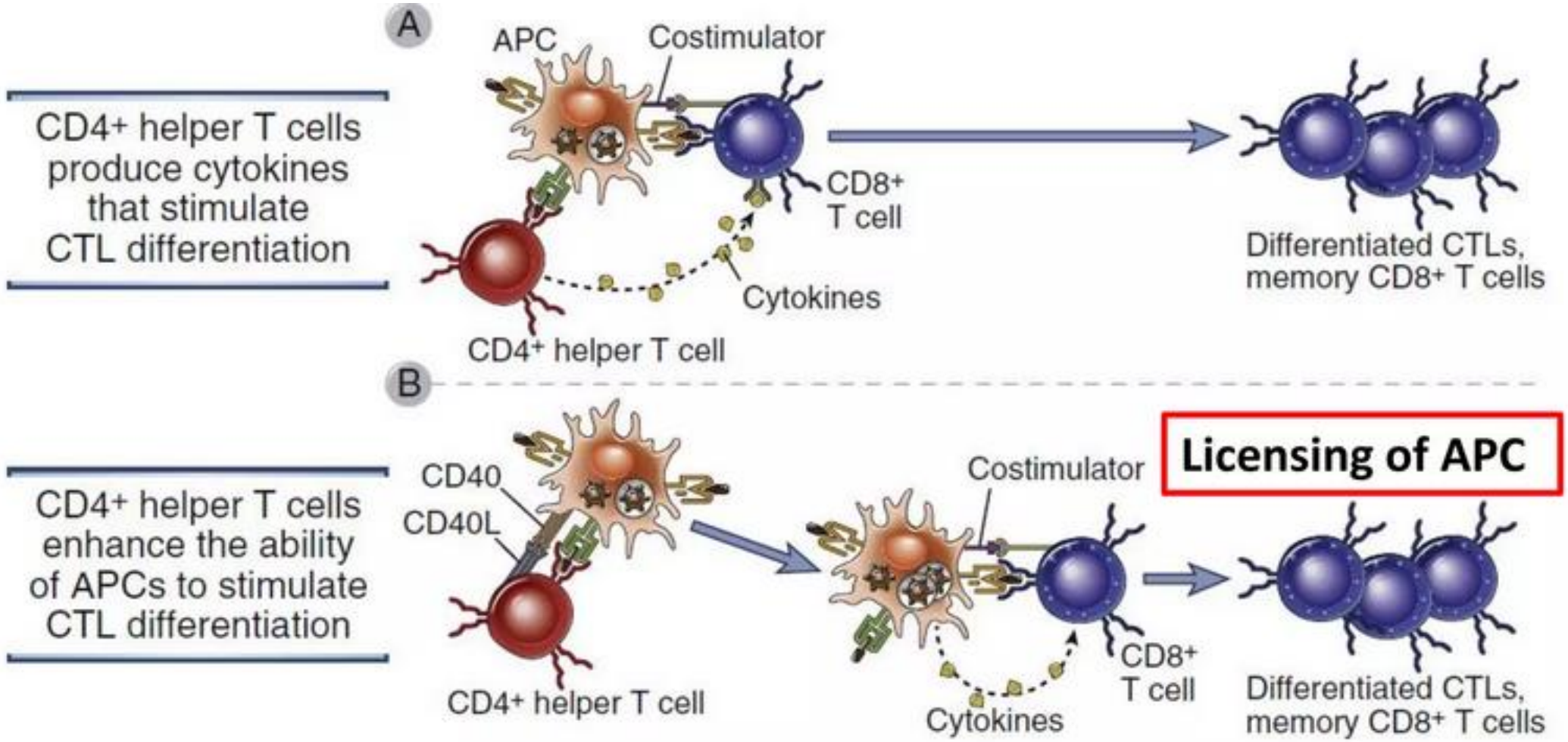


Negative regulators of the T cell activation: CTLA-4, PD1

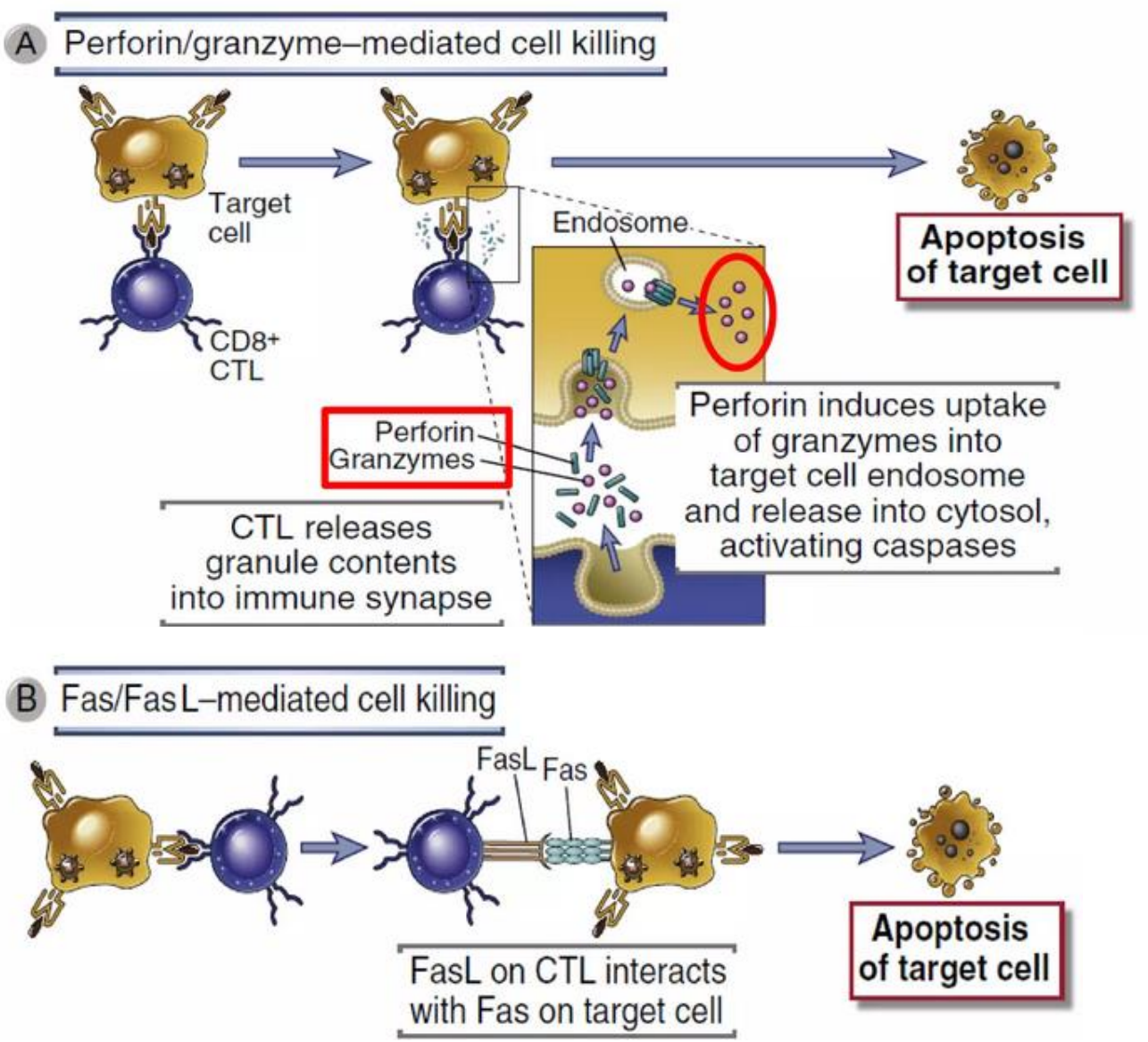


CTLA-4 and PD-1 ensures protection of tissue from collateral damage during an inflammatory response. **CTLA-4 is upregulated after Ag-specific activation of a naïve or memory T cell in lymphatic tissue**, leading to decreased effector function. B, **PD-1 is mainly expressed on antigen experienced memory T cells in peripheral tissues cells**. Tumor cells use this regulatory mechanism to evade a tumor-directed T-cell response by upregulating PD-1 ligands.

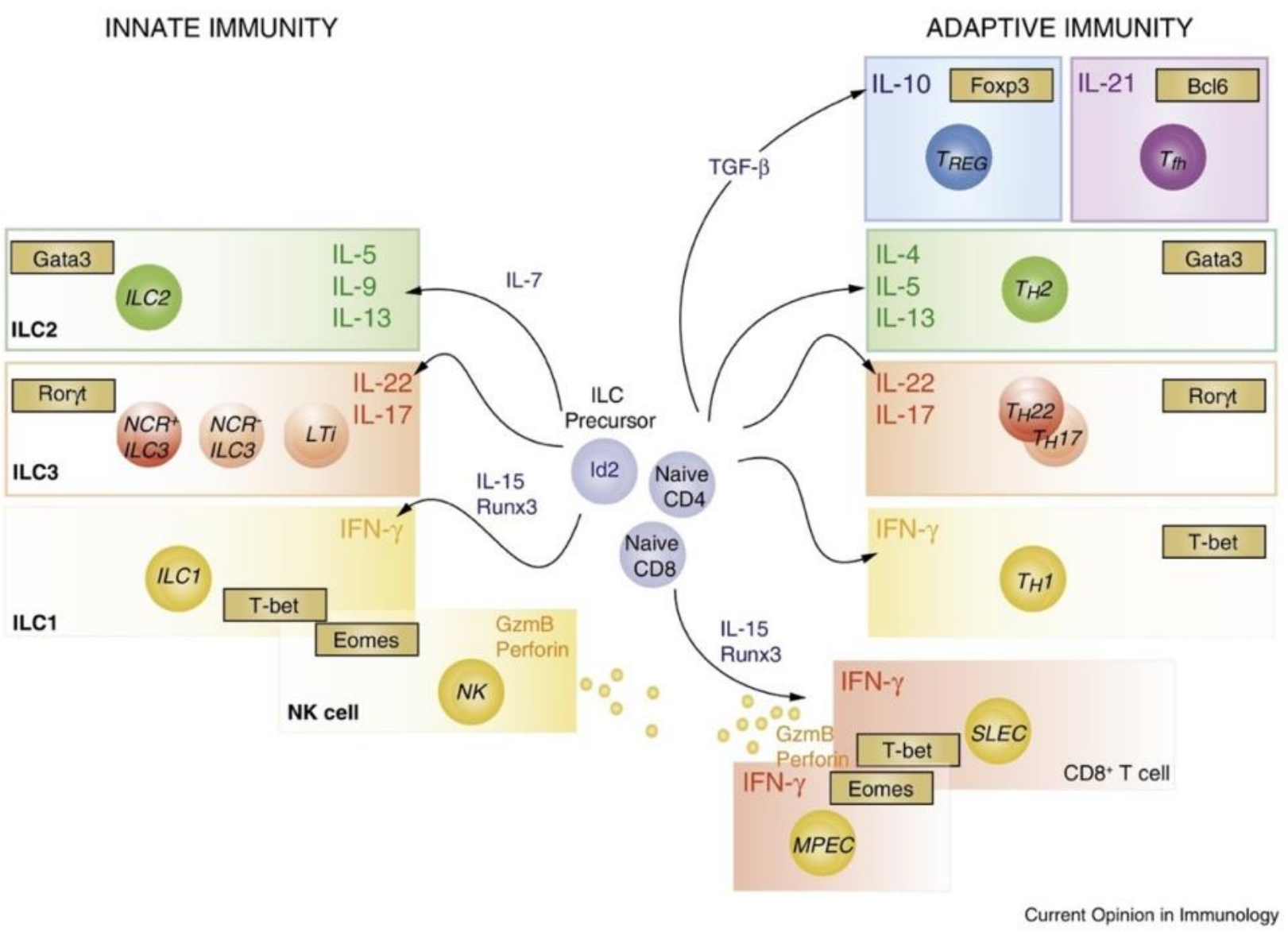
Activation of naïve CD8+ T lymphocytes
Differentiation into CTLs requires CD4+ Th1 help for
Provide IL-2 and APC super-activation



Effector **cytotoxic CD8+ T lymphocytes kill target cells in an Ag-dependent** mode. Perforin/granzyme and Fas/FasL are the major killing mechanisms

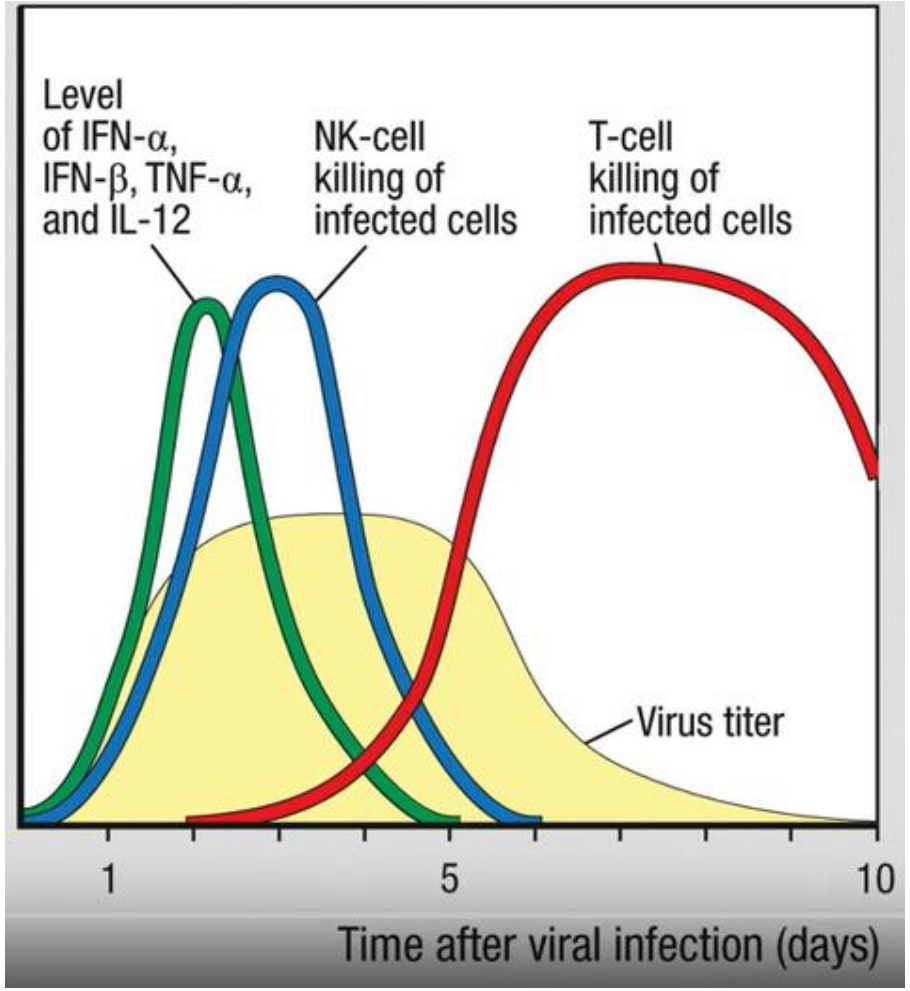


Natural killer (NK) lymphocytes and innate lymphocytes

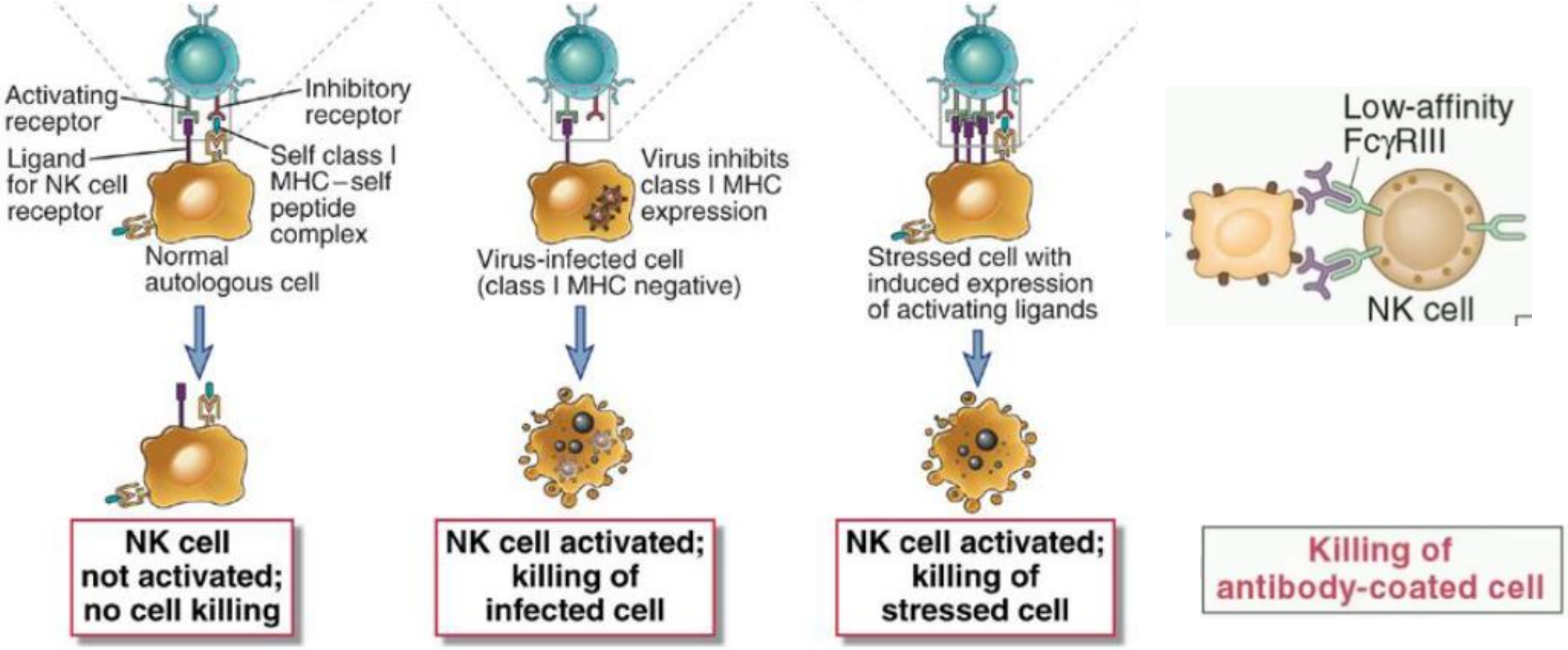


NK lymphocytes are innate-like lymphocytes that resemble CD8+ T lymphocytes but do not express a TCR. They recognize and kill infected cells that evade CD8+ T-cell responses, such as by reducing Class I HLA expression."

NK cells provide early response to viral infection
They belong to the family of innate lymphoid cells



NK cells kill infected cells in an Ag-independent mode. Perforin/granzyme and Fas/FasL are the major killing mechanisms



Cardinal features of adaptive immune responses

Specificity: Response to an antigen (Ag) is targeted to that Ag. Differences in a single a.a. between two peptides can be critical to loss recognition by BCR or TCR.

☞ Remember: one lymphocyte = only one Ag receptor identity.

Diversity: Enables the immune system to respond to a large variety of Ag. Theoretical number of different BCR able to be generated 10^{11}, and 10^{16} different TCR.

☞ Number of potential genes in human genome is $3x10^4$.

Nonreactivity to self: Different mechanisms are active to warrant elimination or control of Self-reactive lymphocytes and maintain Self-tolerance (central and peripheral mechanisms).

Clonal expansion: Increases the number of Ag-specific lymphocytes to keep pace with microbes.

Specialization: Responses are adapted (optimized) to the particular type of invading pathogen.

Contraction and homeostasis: When Ag is eliminated the effector lymphocytes is reduced, eliminated. Only a small pool of Ag-specific memory lymphocytes is maintained.

Memory: Increases the ability to combat repeat infections by the same microbe.

Abbas et al. Basic Immunology: Functions and Disorders of the Immune System 2023 (Recommended book)

Many concepts have not been presented :

- Peripheral tolerance: Immune privileged sites, anergy, ignorance,
- Costimulatory and inhibitory molecules
- Signal transducer molecules !!!! CD3
- …

But with the very fundamental information seen now you can face the world of the immune system response/exploration.

I thank you for your attention