



IMMUNOLÓGIAI ÉS
BIOTECHNOLÓGIAI
INTÉZET



3rd practice: Types and functions of lymphoid cells, CD markers

Basic Immunology

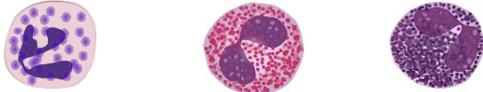
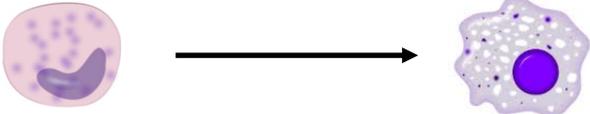
University of Pécs, Clinical Center

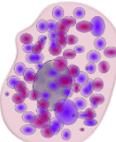
Department of Immunology and Biotechnology

Pécs

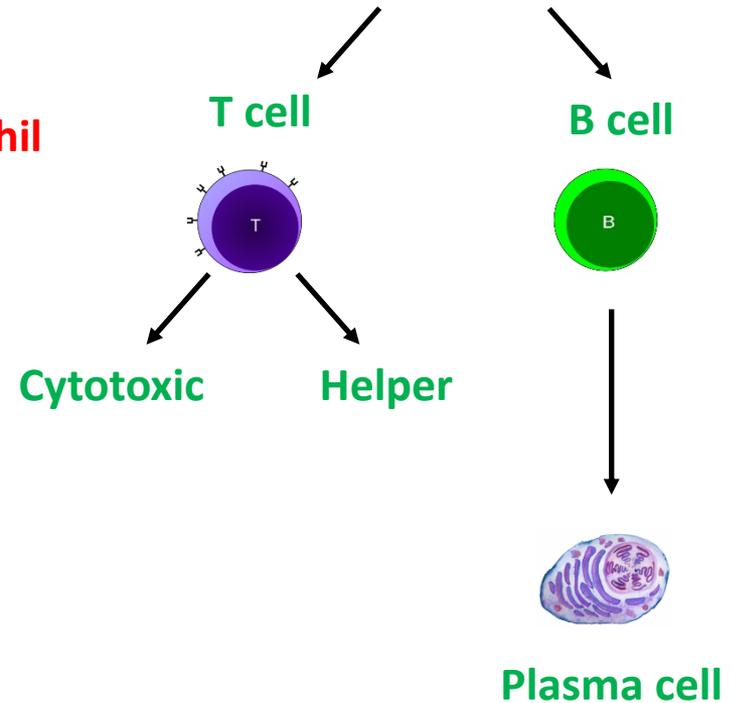
Cells of the innate and adaptive immune system

Innate:

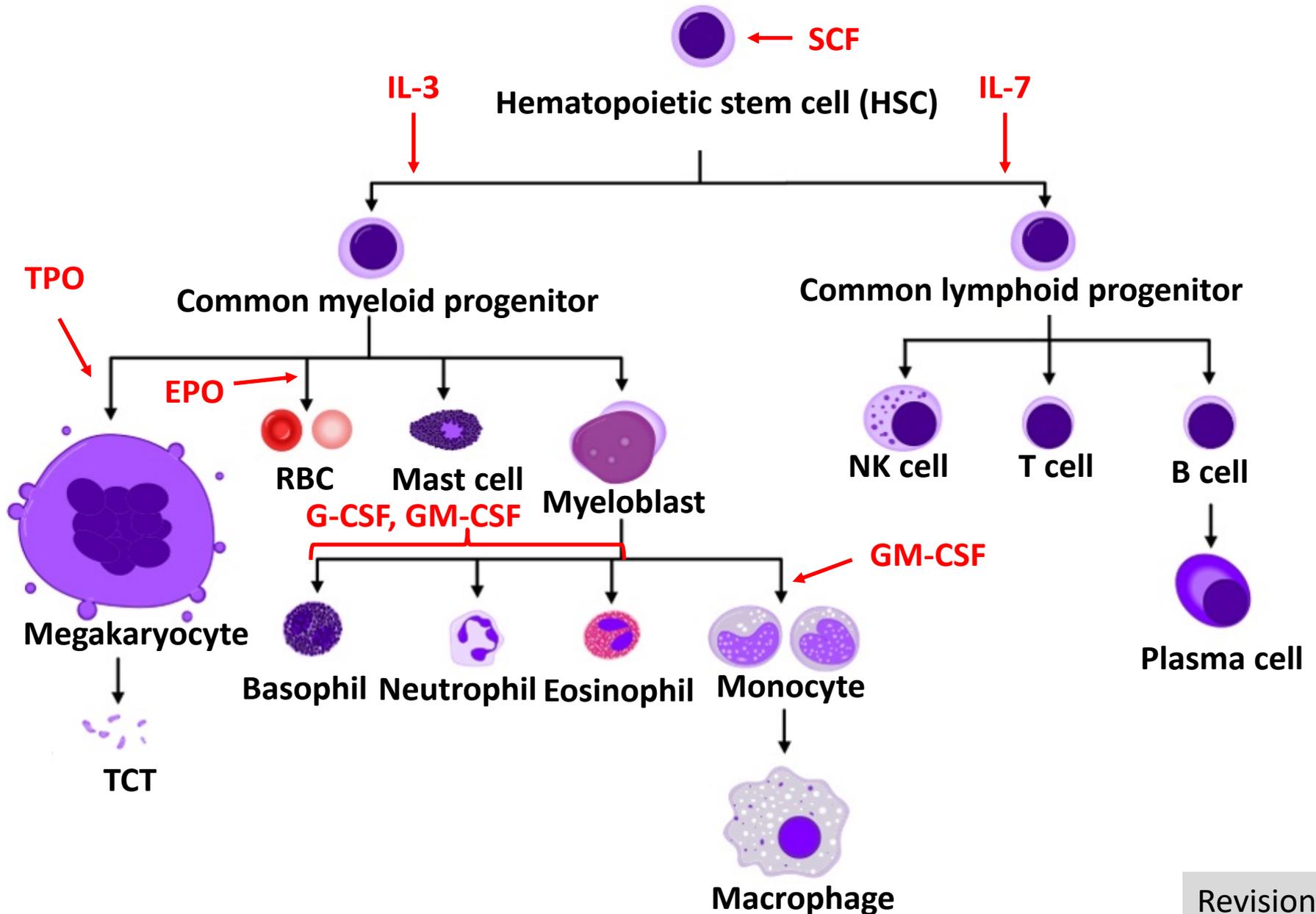
- 1. Granulocytes:** 
neutrophil, eosinophil, basophil
- 2. Monocyte (blood), macrophage (tissues)**

- 3. Dendritic cell (DC), follicular dendritic cell (FDC)**

- 4. Mast cell**

- 5. NK cell (natural killer)**


Adaptive:



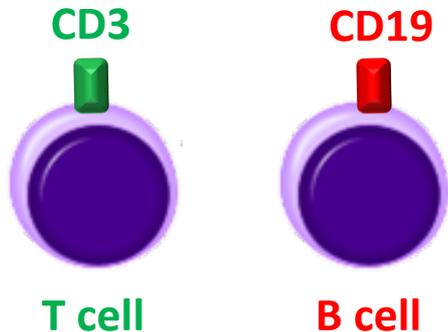
Hematopoiesis



CD markers

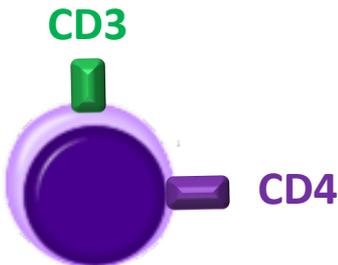


Certain cells (e.g. lymphocytes) cannot always be distinguished based on their morphology.



Different cells can be identified and distinguished by the molecules they express on the cell surface or in the cytoplasm.

IMMUNOPHENOTYPE: The characteristic molecular pattern of a cell type determined with the use of antibodies.



Such SURFACE MOLECULES were given a standardized nomenclature:

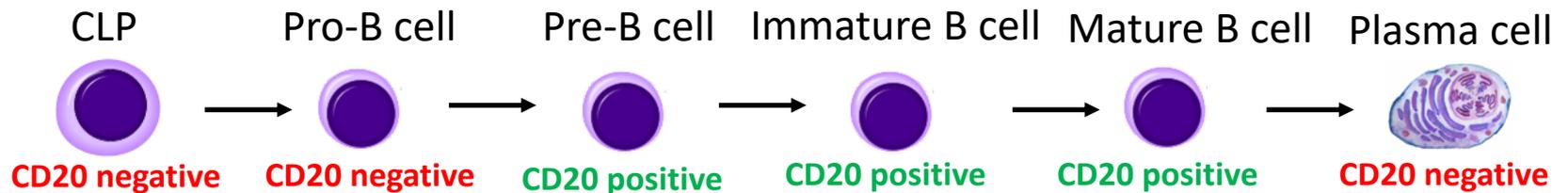
CD = **Cluster of differentiation**, usage: CD+number, e.g.: CD1, CD2, CD3, CD4, etc...

The structure and function of CD marker **varies!**

Example for immunophenotype:
CD3+/CD4+/CD8- → Helper T cell

Types of CD markers

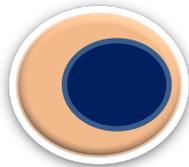
- **Lineage markers:** Molecules expressed exclusively on certain cell lineages.
 - E.g.: CD3 → found on all T cells CD19 → found on all B cells
- **Maturation markers:** The immunophenotype might differ in the phases of cell maturation, certain molecules are only expressed on immature cells, others on mature, fully functioning cells, etc.
 - E.g.: CD20 (It is also a lineage marker of B cells, cannot be found on any other cells)



- **Activation markers:** Molecules expressed by activated cells, whereas resting cells either lack them completely or express them at low levels, e.g.:
 - CD25 (The alpha chain of the interleukin-2 receptor, IL-2R α , see later)
 - CD80 and CD86 (B7-1 and B7-2, so-called costimulatory molecules expressed by activated antigen presenting cells, see later)

Cells of the lymphoid lineage

Innate lymphoid cells (ILC)



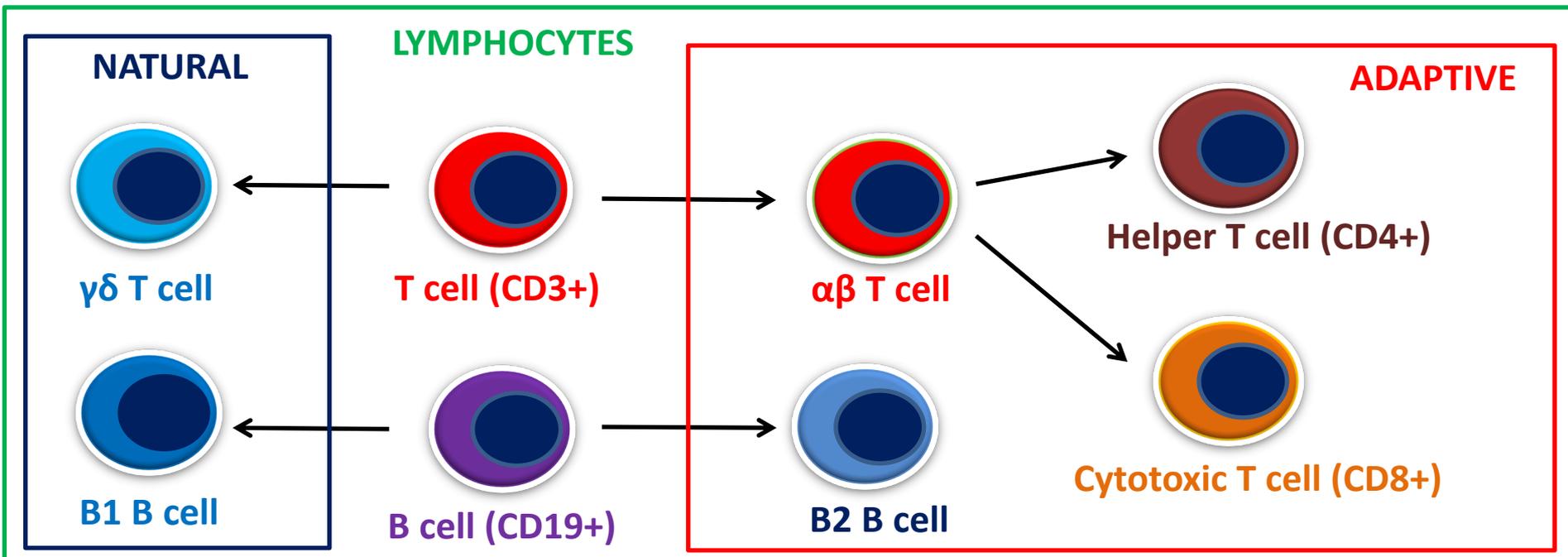
Lymphocyte



There is no difference
in the morphology!

HAVE NO ANTIGEN-RECOGNITION
RECEPTORS

HAVE ANTIGEN-RECOGNITION
RECEPTORS



Innate lymphoid cells (ILC)

- They cannot be distinguished from lymphocytes based on their morphology but unlike adaptive lymphocytes they cannot recognize antigens. → **They have no antigen recognition receptors.**
- They are classified based on the cytokines they produce and the transcription factors that are necessary for their formation. (see in the lectures):

– Group 1 ILCs:

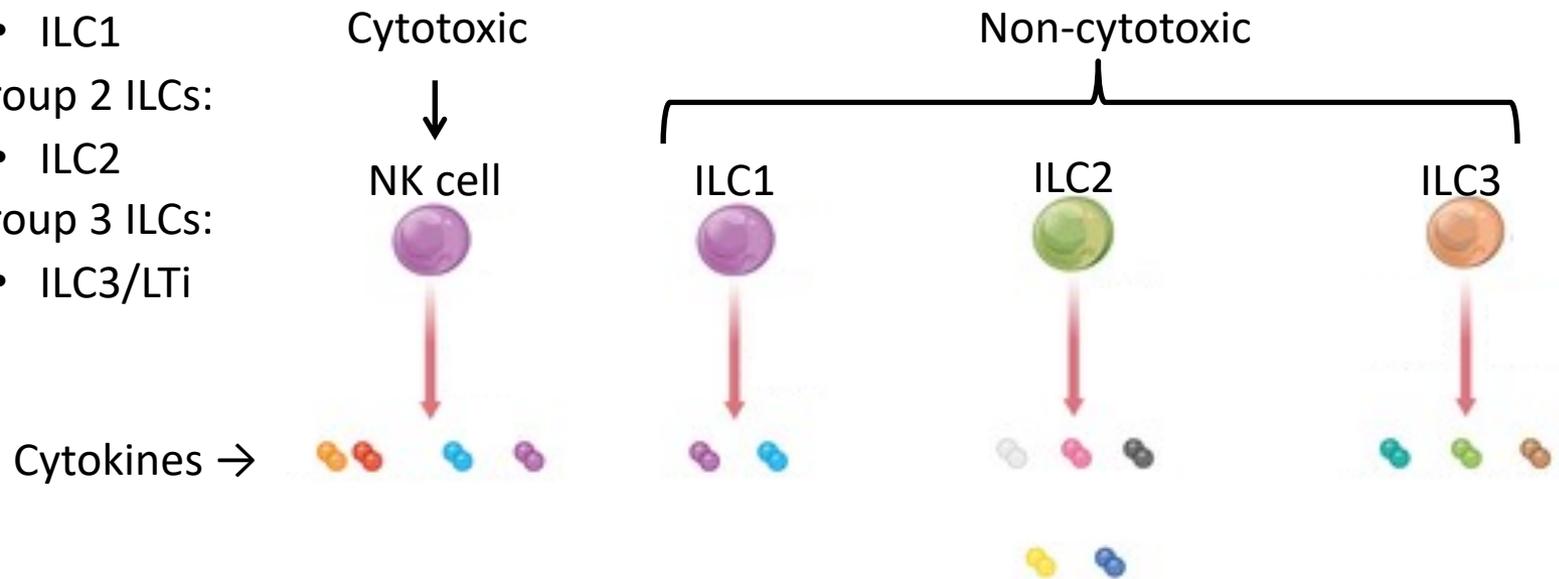
- **NK cells**
- ILC1

– Group 2 ILCs:

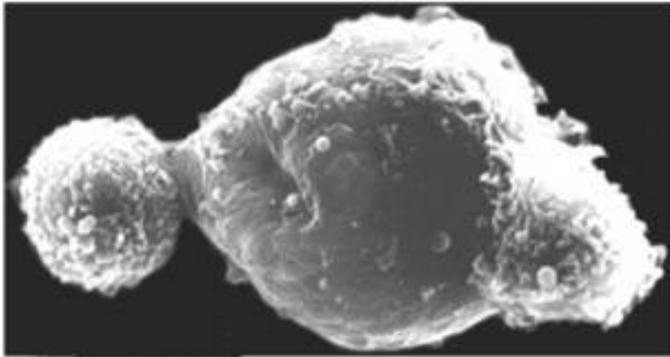
- ILC2

– Group 3 ILCs:

- ILC3/LTi

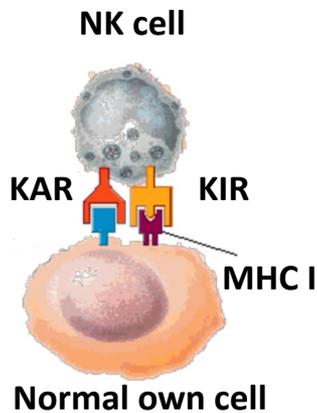


Natural killer cells (NK cells)

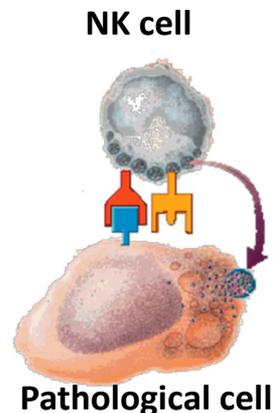


Two NK cells kill a cancerous cell.
(Scanning electron microscopy image)

Blood lymphoid cells %:	≈ 10
Main function:	Killing cells infected with intracellular pathogens, Killing cancer cells
Recognition:	KAR → killing the target KIR → sparing the target Fc receptor, Complement receptor
Cytotoxicity:	Fas-FasL, Perforin, Granzymes
Produced mediators:	Cytokines
Fc receptor:	FcγR (binds IgG)
Characteristic marker:	CD56



CELL IS LEFT ALIVE



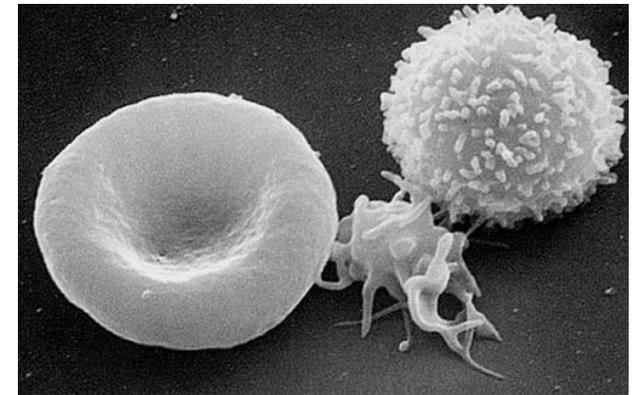
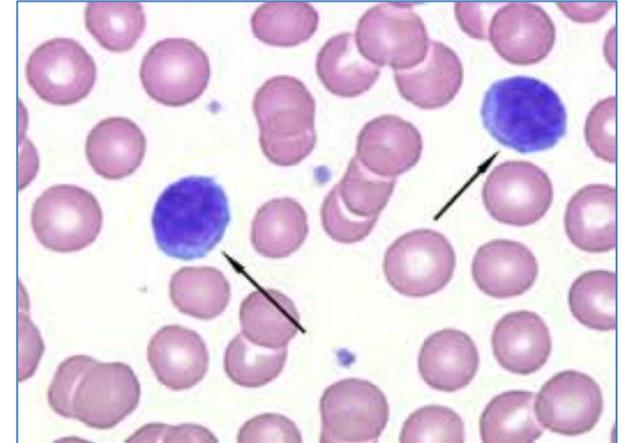
CELL IS KILLED

Red: Only possible after the activation of the adaptive immunity

Lymphocytes

Leukocyte %:	25-40*
Main function:	ADAPTIVE IMMUNITY
Recognition	Antigen-specific receptors (TCR, BCR)

* Including NK cells



A red blood cell, a platelet and a lymphocyte (SEM image)



B cell (CD19+)



Antibody production



Cytotoxic T cell (CD8+)



Direct killing of target cell (infected or cancerous)



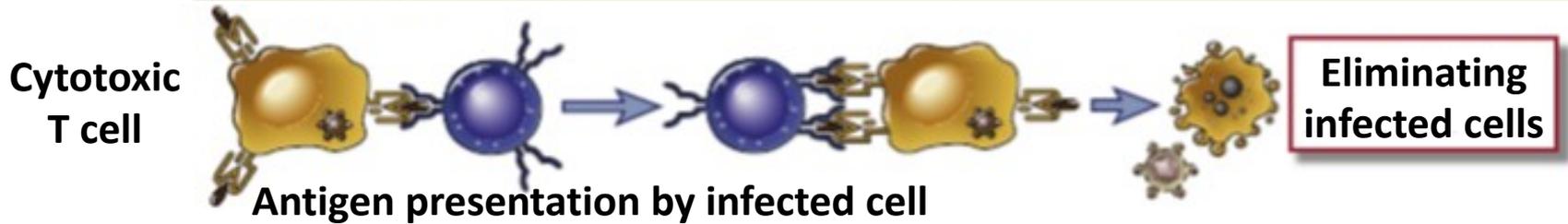
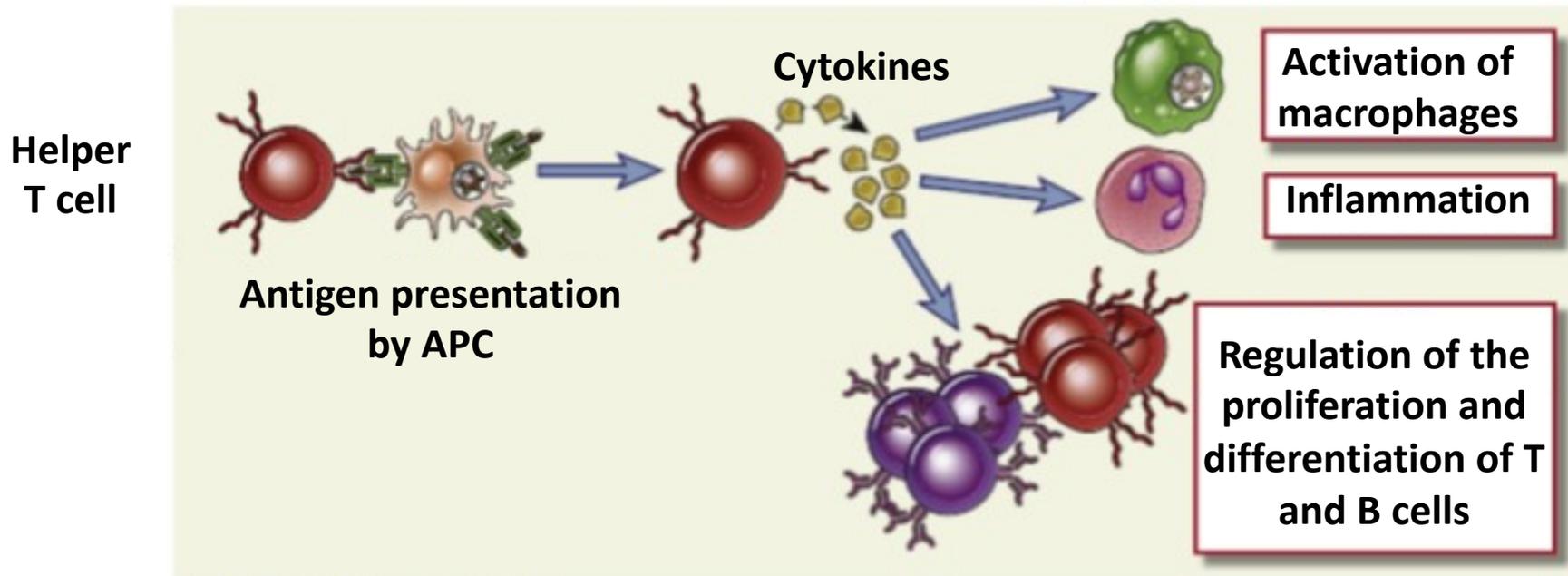
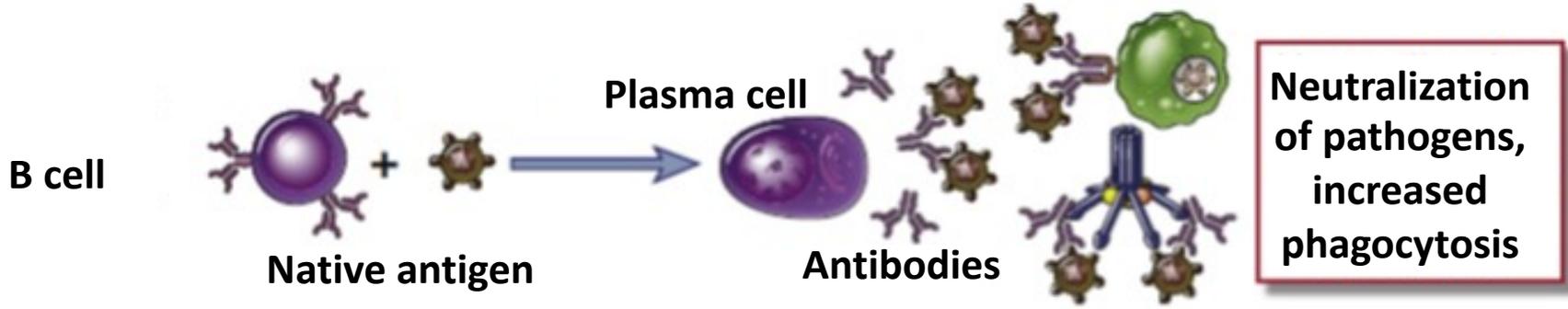
Helper T cell (CD4+)



Regulation of the immune response

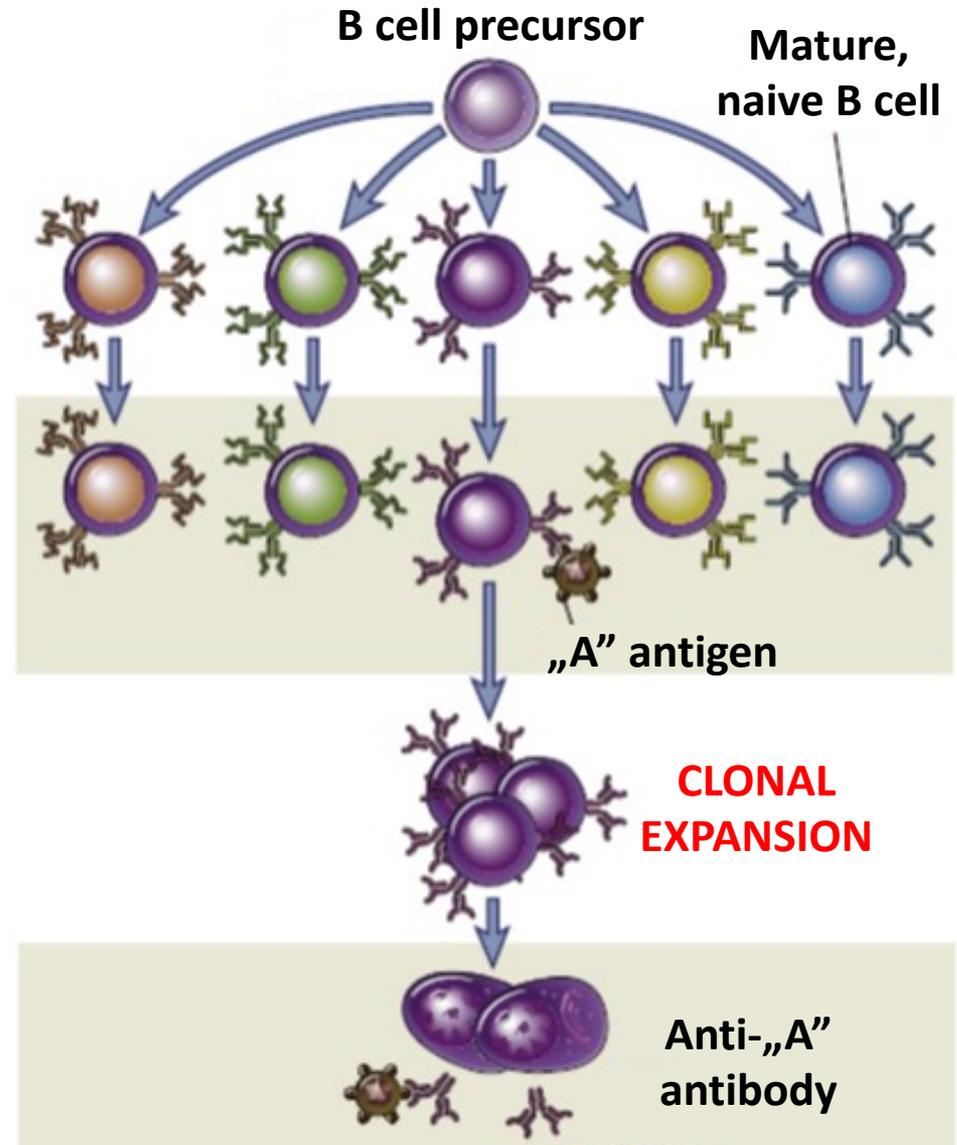
All of the above are done in an ANTIGEN-SPECIFIC manner!

Main groups of lymphocytes



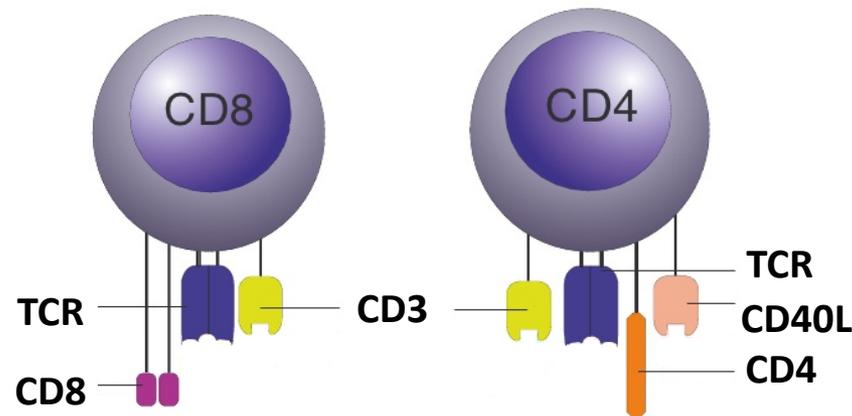
Clonality

1. Each newly produced lymphocyte expresses a **unique antigen-binding receptor**.
2. **Only those lymphocytes will become activated which recognize an antigen.** These selected cells will proliferate and produce **clones** of themselves with each sister cell having the same antigen-recognition receptor.
3. These clones will differentiate into **effector cells** which will participate in the immune response. (e.g. effector plasma cells produce antibodies)



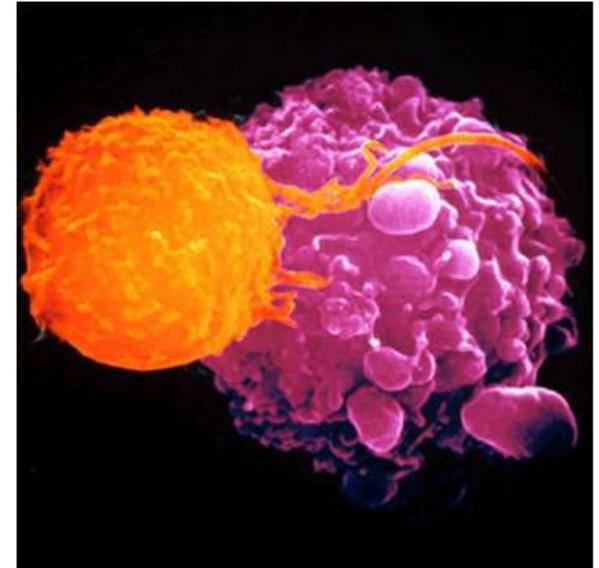
T cells

Main function:	Antigen-specific killing of target cell (CD8+), Regulation of the immune response through cytokines (CD4+)
Recognition:	Through MHC, antigen-specific TCR
Possible type of TCR:	$\alpha\beta$ and $\gamma\delta$
Produced mediators:	Cytokines
Main types of $\alpha\beta$ T cells:	CD4+ Helper CD8+ Cytotoxic
Site of production:	Bone marrow, thymus
Characteristic marker:	CD3 (Makes a complex with the TCR)



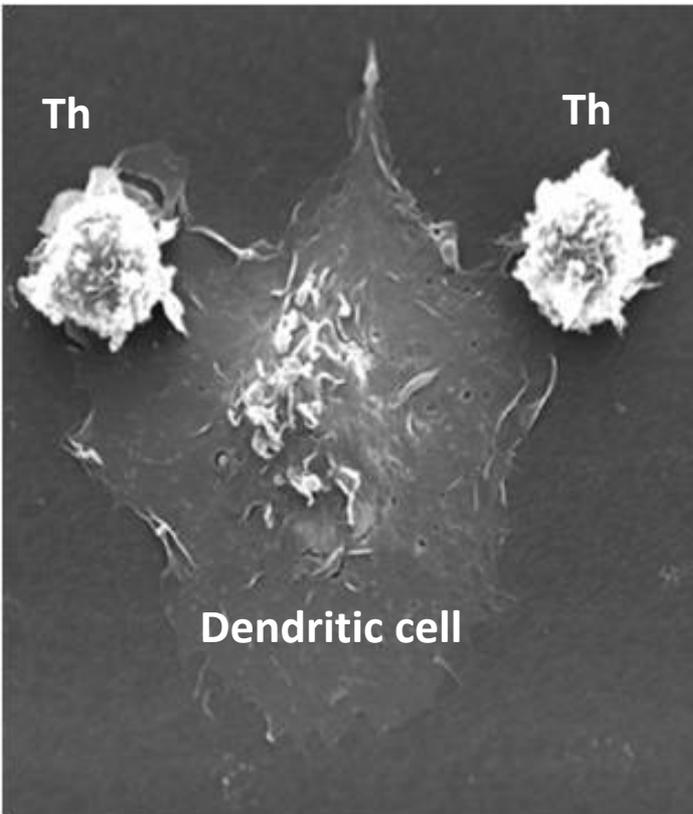
Cytotoxic T cells (Tc or CTL)

Blood T cells:	1/3
Main function:	Effector cell of the cellular immunity
Recognition:	Through MHC I, antigen-specific TCR
Target cells to kill:	Infected with IC pathogens, Cancerous, Foreign (transplantations!)
Recognized antigens:	Endogenous (from the cytoplasm of the target cell)
Cytotoxicity:	Fas-FasL, Perforin, Granzyme
Immunophenotype:	CD3+/CD8+/CD4-



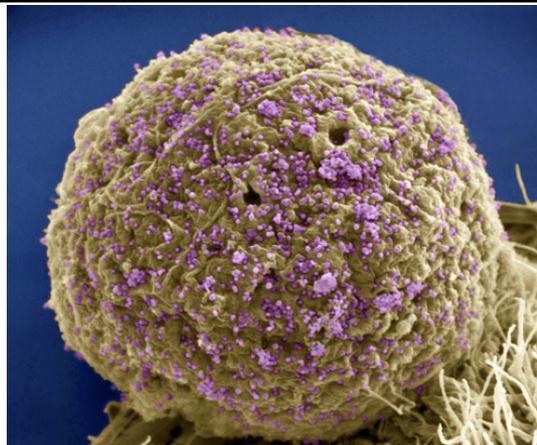
A cytotoxic T cell kills a cancer cell. (SEM image)

Helper T cells (Th)



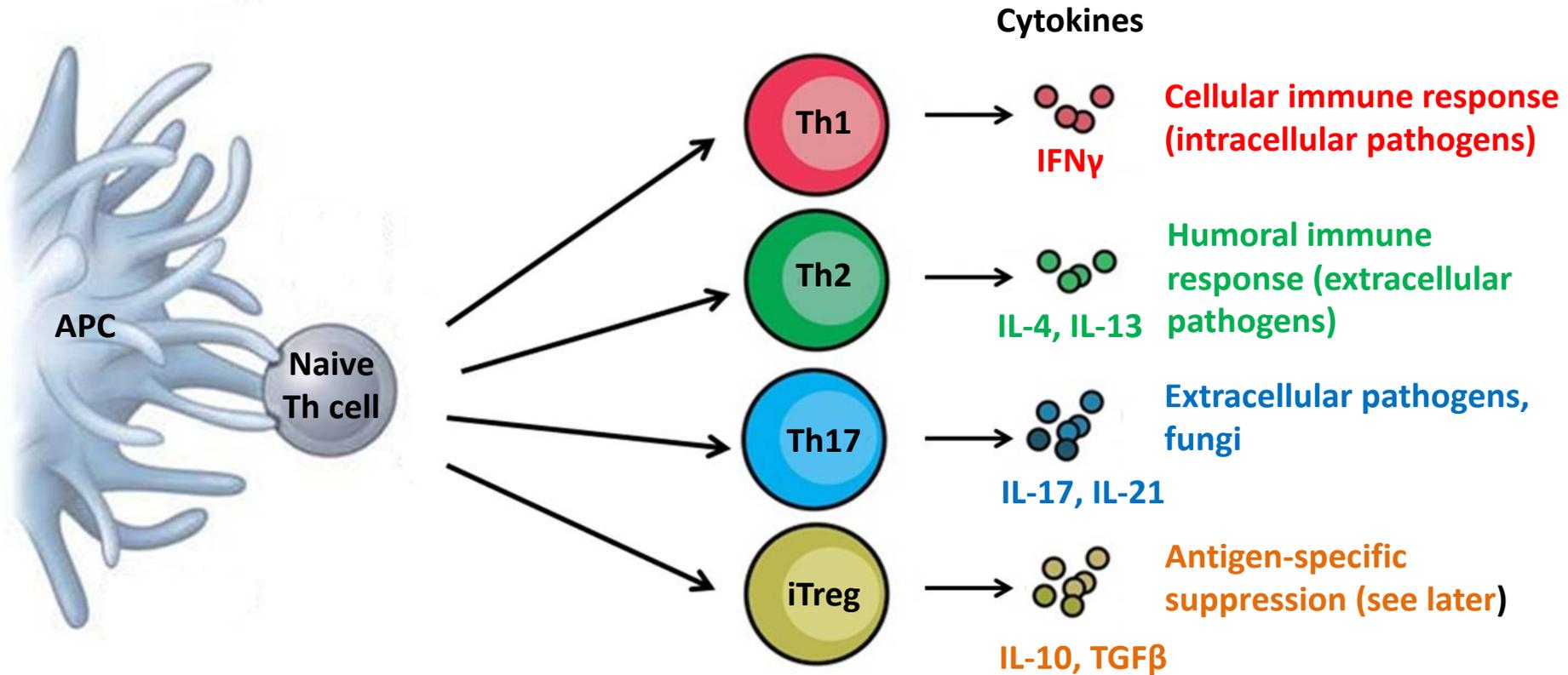
Two helper T cells attached to a dendritic cell. (Scanning electron microscopy image)

Blood T cells:	1/3
Main function:	Regulation of immune response
Recognition:	Through MHC II, antigen-specific TCR
Recognized antigens:	Exogenous (degraded in phagolysosomes)
Immunophenotype:	CD3+/CD4+/CD8-
Role in diseases:	Autoimmunity, HIV infection



Yellowish-brown: Th cell
purple: **HIV** virions
(SEM image)

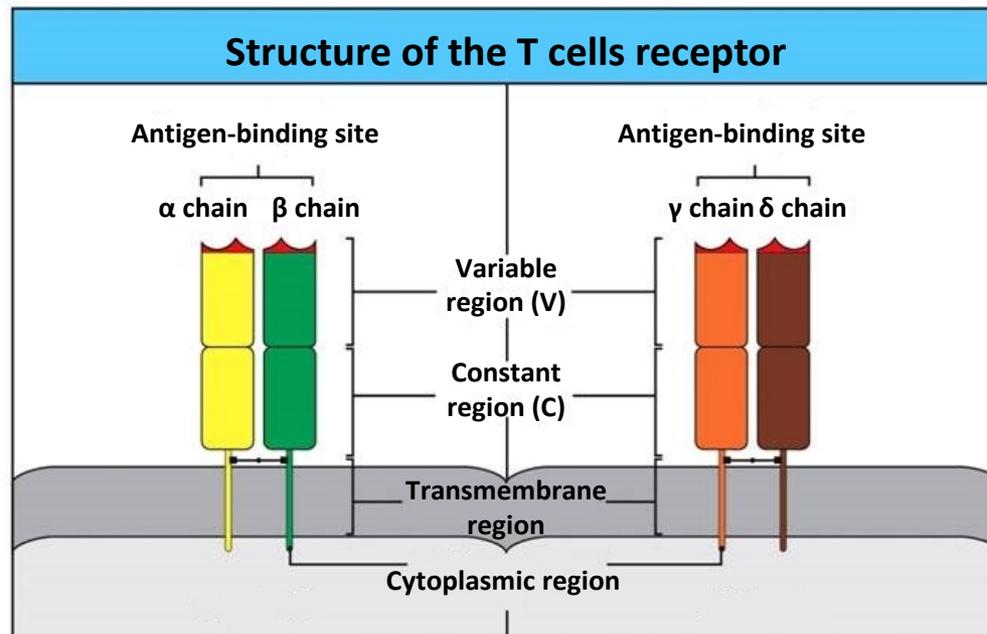
Main subtypes of Th cells



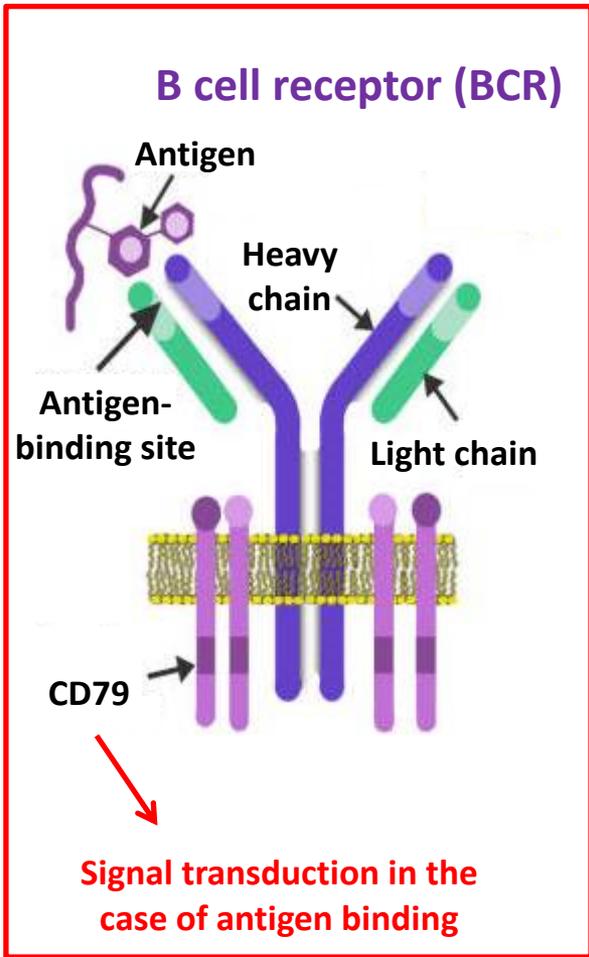
- Th17 cells play an important role in **inflammatory disorders**. (see later)
- **Regulatory T cells** (Treg): They can inhibit other immune cells (**suppression**, see later), their immunophenotype is: **CD4+/CD25+/Foxp3+**

$\gamma\delta$ T cells

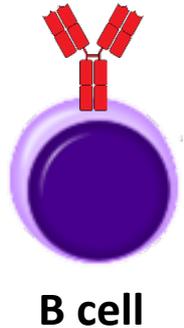
- They express TCRs that consist of γ and δ chains.
- They are **innate-like lymphocytes**, they are not as well-characterized as $\alpha\beta$ T cells.^[17.]
- They are mainly found in the **skin** and the **mucosa**; usually as intraepithelial lymphocytes (IELs). They can be detected in the peripheral blood in low numbers.
- They participate in the early phases of the immune response against invasive pathogens.
- Their antigen-recognition is **MHC-independent**.
- They mainly recognize **lipid antigens**.



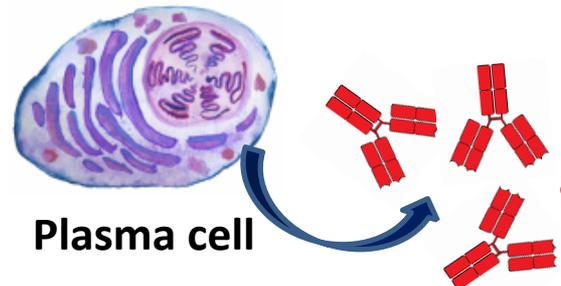
B cells



Blood lymphoid cells %:	10-15
Main functions:	Antibody production, Antigen presentation
Recognition:	Native antigens with antigen-specific BCR
Main types:	B1 and B2
Site of production:	Bone marrow
Characteristic marker:	CD19 (makes a complex with BCR)



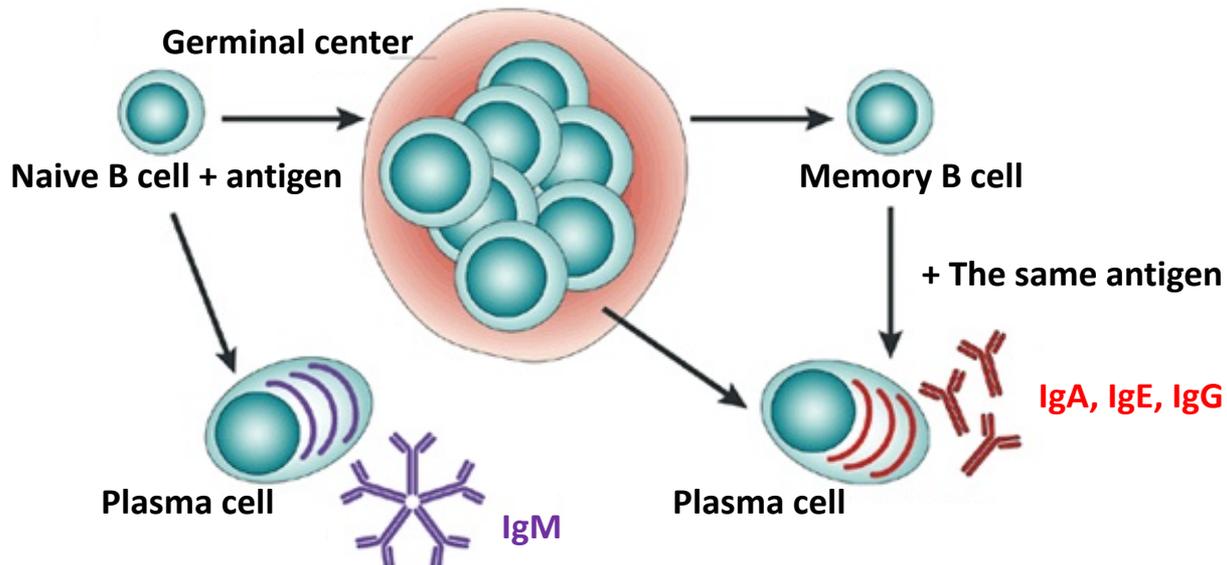
BCR = surface immunoglobulin



Antibody against the same antigen recognized by the BCR (secreted immunoglobulin)

B2 B cells

Found in:	Follicles in secondary lymphoid organs, blood
Main functions:	Antibody production, Antigen presentation
Recognition:	Native antigens with antigen-specific BCR
Site of primary maturation:	Bone marrow
Site of antigen-dependent maturation:	Germinal center
Produced antibodies:	Monospecific, high-affinity, with varying isotype



B1 B cells

- Only few can be found in the peripheral blood.
- **They are innate-like lymphocytes**, most of them reside on serous membranes. (e.g. peritoneum, pleura, pericardium)
- They are first produced in the fetus and later undergo self-renewal in the periphery, not in the bone marrow, as B2 cells do.
- They produce **natural autoantibodies** that can bind that can bind evolutionarily **conserved self-antigens**.
- They were first described as CD5+ B cells in mice.
- The immunophenotype of the human B1 cells is still controversial.

	B1 cells	B2 cells
Spontaneous antibody production	Significant	Minimal
Isotype of produced antibodies	IgM	IgM/IgG/IgA/IgE
Affinity and specificity of antibodies	Polyspecific with low affinity	Monospecific with high affinity
Affinity maturation, memory	No	Yes

References 1.

- Mayo Clinic: **CD20 on B Cells** (<http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/89584>)
- Nauseef WM, et al: **Neutrophils at work**. *Nat Immunol*. 2014 Jul;15(7):602-11. doi: 10.1038/ni.2921.
- Stone KD, et al: **IgE, mast cells, basophils, and eosinophils**. *J Allergy Clin Immunol*. 2010 Feb;125(2 Suppl 2):S73-80. doi: 10.1016/j.jaci.2009.11.017.
- Denzel A, et al: **Basophils enhance immunological memory responses**. *Nat Immunol*. 2008 Jul;9(7):733-42. doi: 10.1038/ni.1621. Epub 2008 May 30.
- Karasuyama et al: **Newly discovered roles for basophils: a neglected minority gains new respect**. *Nat Rev Immunol*. 2009 Jan;9(1):9-13. doi: 10.1038/nri2458.
- Auffray C, et al: **Blood monocytes: development, heterogeneity, and relationship with dendritic cells**. *Annu Rev Immunol*. 2009;27:669-92. doi: 10.1146/annurev.immunol.021908.132557.
- Johnathan CPeiser L, et al: **Scavenger receptors in innate immunity**. *Curr Opin Immunol*. 2002 Feb;14(1):123-8.
- Canton J, et al: **Scavenger receptors in homeostasis and immunity**. *Nat Rev Immunol*. 13, 621–634 (2013) doi:10.1038/nri3515
- Nobelprize.org: **Ilya Mechnikov – Biographical** (http://www.nobelprize.org/nobel_prizes/medicine/laureates/1908/mechnikov-bio.html)
- Mosser DM, et al: **Exploring the full spectrum of macrophage activation**. *Nat Rev Immunol*. 2008 Dec;8(12):958-69. doi: 10.1038/nri2448.

References 2.

- Wu L, et al: **Development of dendritic-cell lineages.** *Immunity.* 2007 Jun;26(6):741-50.
- Liu K, et al: **Origin and development of dendritic cells.** *Immunol Rev.* 2010 Mar;234(1):45-54. doi: 10.1111/j.0105-2896.2009.00879.x.
- Heesters BA, et al: **Follicular dendritic cells: dynamic antigen libraries.** *Nat Rev Immunol.* 2014 Jul;14(7):495-504. doi: 10.1038/nri3689. Epub 2014 Jun 20.
- Juelke K, et al: **Differentiation of human innate lymphoid cells (ILCs).** *Curr Opin Immunol.* 2016 Feb;38:75-85. doi: 10.1016/j.coi.2015.11.005. Epub 2015 Dec 17.
- Vivier E, et al: **Innate or adaptive immunity? The example of natural killer cells.** *Science.* 2011 Jan 7;331(6013):44-9. doi: 10.1126/science.1198687.
- Molecular Biology of the Cell. 4th edition: **Lymphocytes and the Cellular Basis of Adaptive Immunity** (<http://www.ncbi.nlm.nih.gov/books/NBK26921/>)
- Bonneville M, et al: **Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity.** *Nat Rev Immunol.* 2010 Jul;10(7):467-78. doi: 10.1038/nri2781. Epub 2010 Jun 11.
- Rothstein TL, et al: **Human B-1 cells take the stage.** *Ann N Y Acad Sci.* 2013 May;1285:97-114. doi: 10.1111/nyas.12137.
- Covens K, et al: **Characterization of proposed human B-1 cells reveals pre-plasmablast phenotype.** *Blood.* 2013 Jun 27;121(26):5176-83. doi: 10.1182/blood-2012-12-471953. Epub 2013 Apr 23.
- Rothstein TL, et al: **The human counterpart of mouse B-1 cells.** *Ann N Y Acad Sci.* 2015 May 18. doi: 10.1111/nyas.12790.