



IMMUNOLÓGIAI ÉS
BIOTECHNOLÓGIAI
INTÉZET



2nd practice: Production of the cells of the immune system: hemopoiesis (myeloid vs lymphoid differentiation). Types and functions of myeloid cells.

Basic Immunology

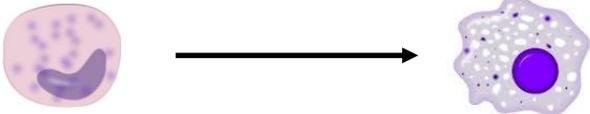
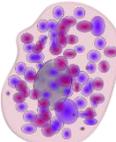
University of Pécs, Clinical Center

Department of Immunology and Biotechnology

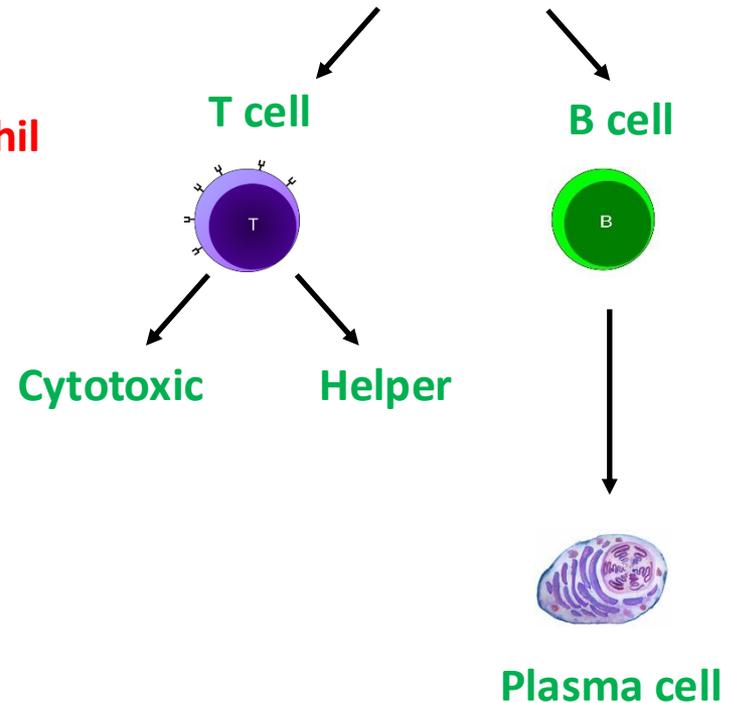
Pécs

Cells of the innate and adaptive immune system

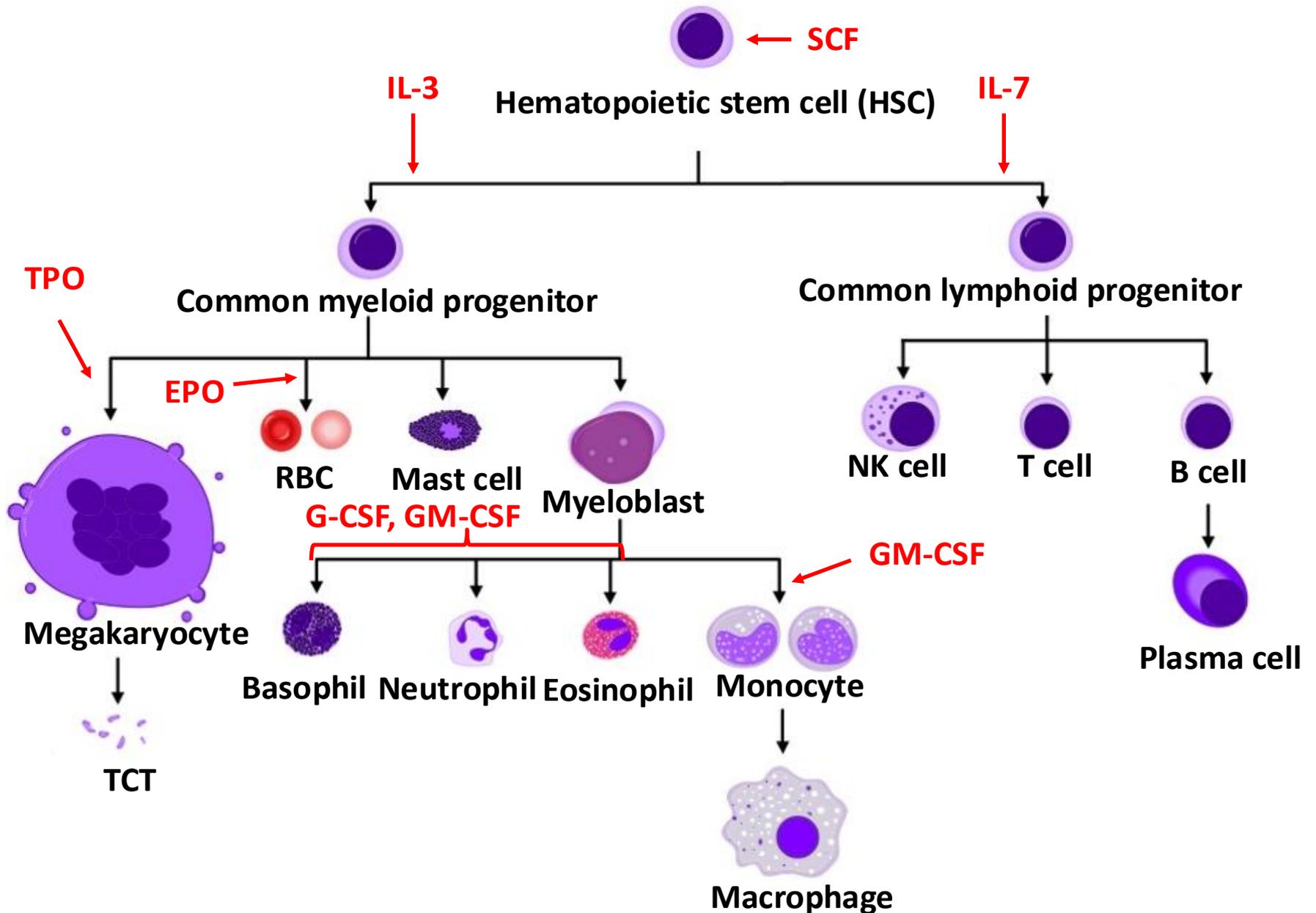
Innate:

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

- 3. Dendritic cell (DC)** 
- 4. Mast cell** 
- 5. NK cell (natural killer)** 

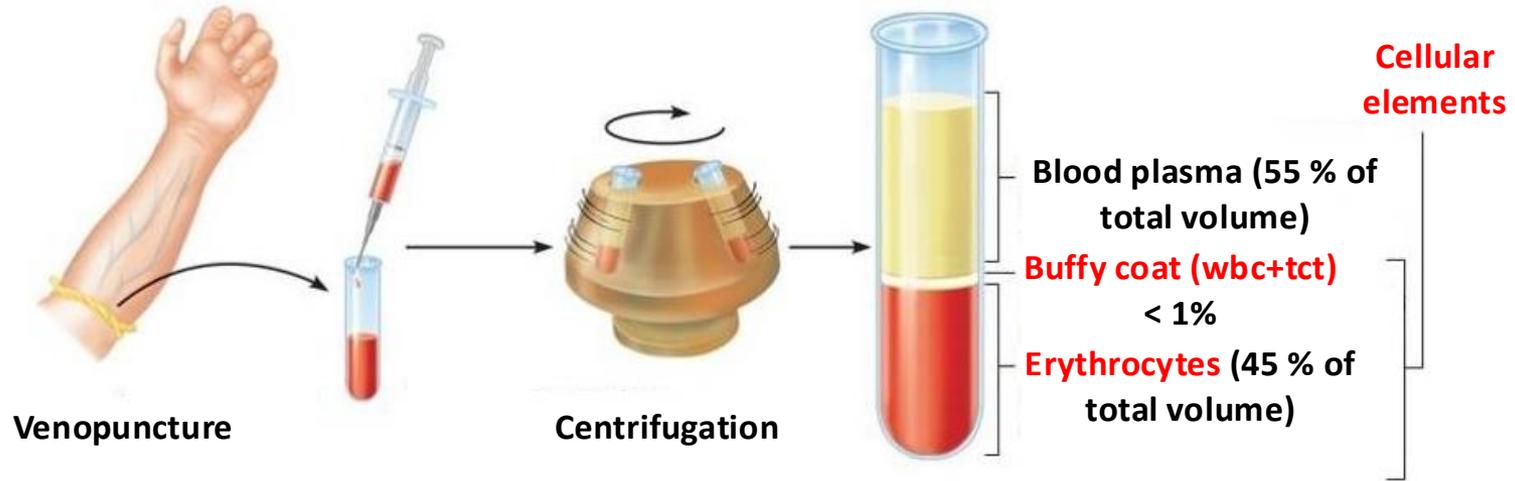
Adaptive:



Hematopoiesis



Components of the peripheral blood

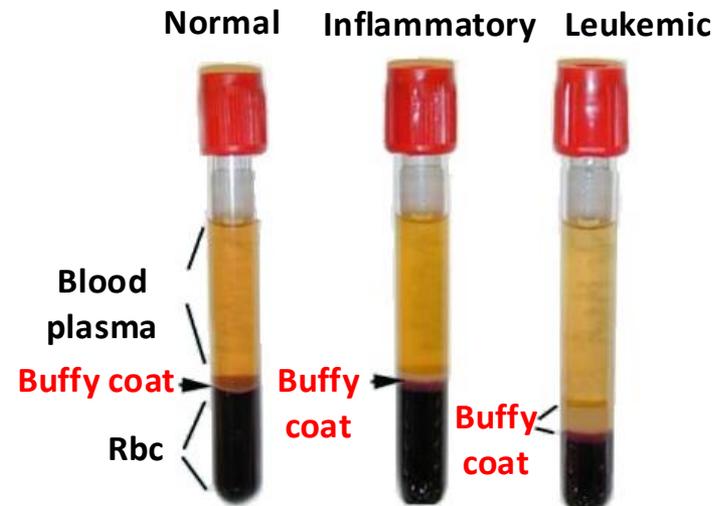


Blood plasma: supernatant of anticoagulated blood

Blood serum: supernatant of coagulated blood



They are suitable for different laboratory tests, e.g. coagulation tests cannot be performed from blood serum.

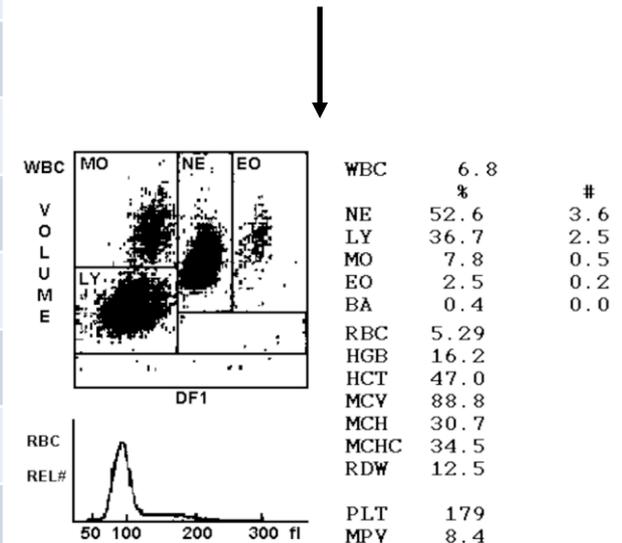


Cellular components of blood

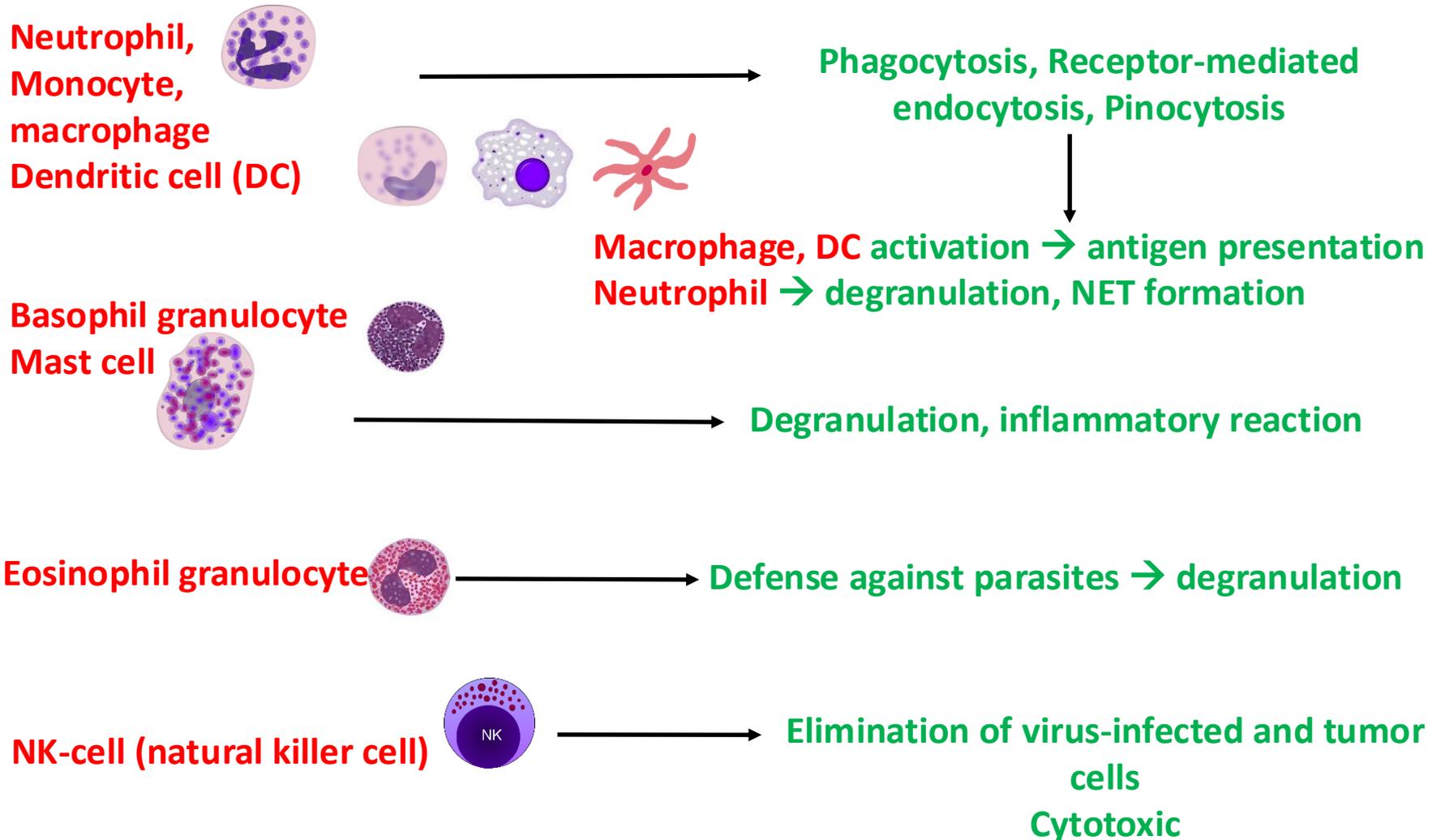
The laboratory blood count measurements are done by automated hematology analyzers based on the principles of **flow cytometry**. (see later)

Qualitative blood count

Cell type	Absolute number (cells/ μ l)	Ratio (%)
White blood cells	4500-10.000	
Neutrophil	2200-6300	55-70
Band form	120-450	3-5
Segment form	2000-6300	50-70
Eosinophil	80-360	2-4
Basophil	< 90	0-1
Monocyte	80-540	2-6
Lymphocyte	1000-3600	25-40

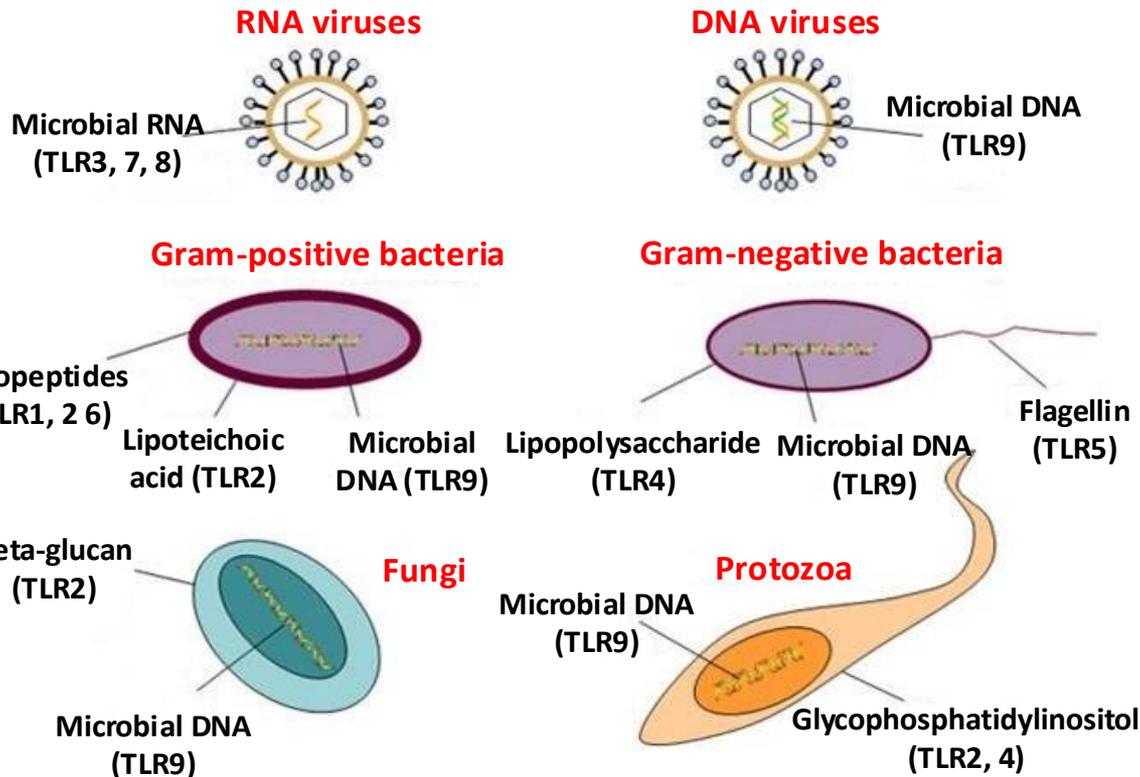


Functions of the cells of the innate immune system



Pattern-recognition

- **PAMP** (pathogen-associated molecular pattern): Molecules frequently found on pathogens which can be recognized by cells of the innate immune system.



Examples of PAMPs:

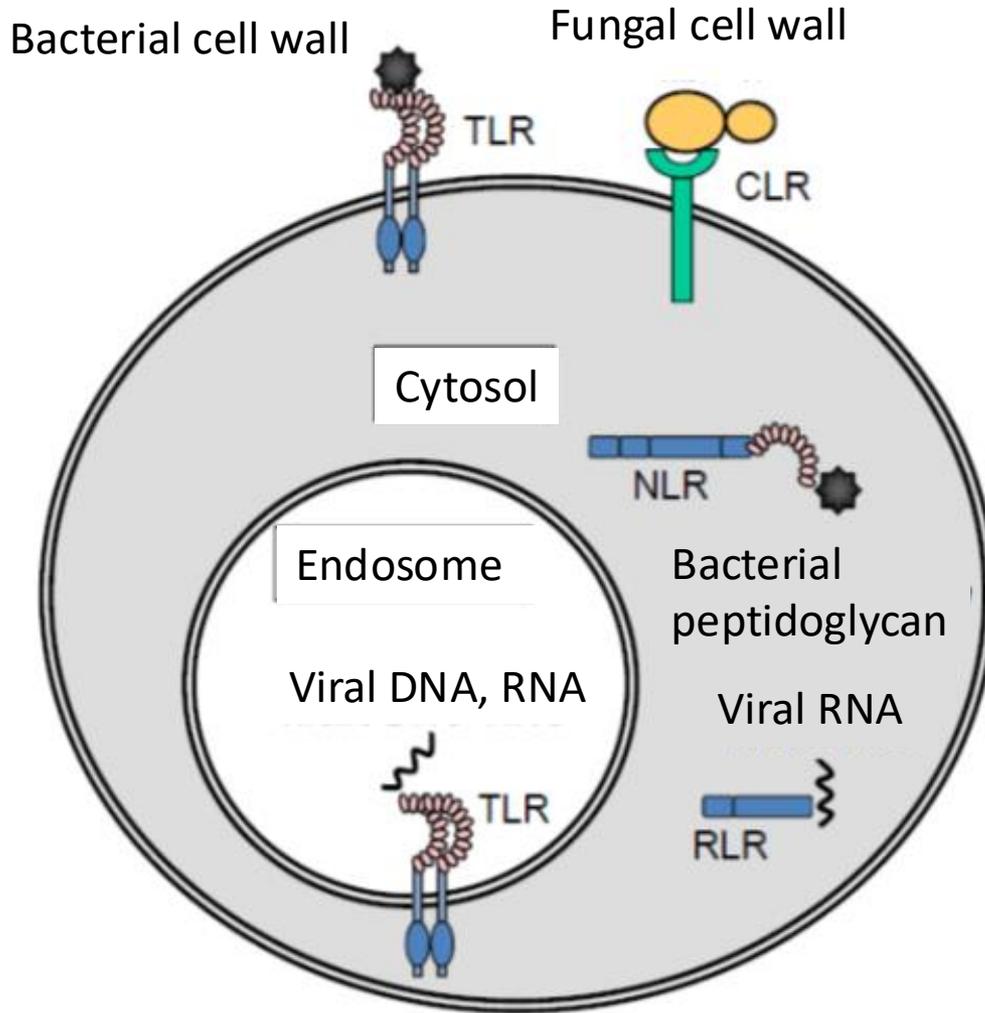
- **LPS (lipopolysaccharide)**
- Beta-glucan
- dsRNA (double stranded RNA)
- CpG-DNA (microbial DNA)
- Flagellin

Examples of PRRs:

- **LPS-receptor (CD14)**
 - **Mannose-receptor**
 - **Glucan-receptor**
 - **Toll-like receptors (TLR)**
 - **Scavenger receptors**
- } C-lectin-R

- **PRR** (Pattern-recognition receptor): Their genes do not rearrange; all our innate immune cells express the very same PRRs throughout our entire life.
- Some of the receptors are found on the **cell surface**, others are **intracellular**.

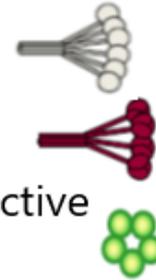
Pattern recognition molecules



Extracellular space

Plasma proteins

- Complement system
- Collectins
- Pentraxins (eg. C-reactive protein)



PRR

- No clonal distribution
- Less than 100 type
- Same on every celltype

Examples of PRRs:

- **LPS-receptor (CD14)**
 - **Mannose-receptor**
 - **Glucan-receptor**
 - **Toll-like receptors (TLR)**
 - **Scavenger receptors**
- } C-type lectin-R

TLR: Toll-like receptor

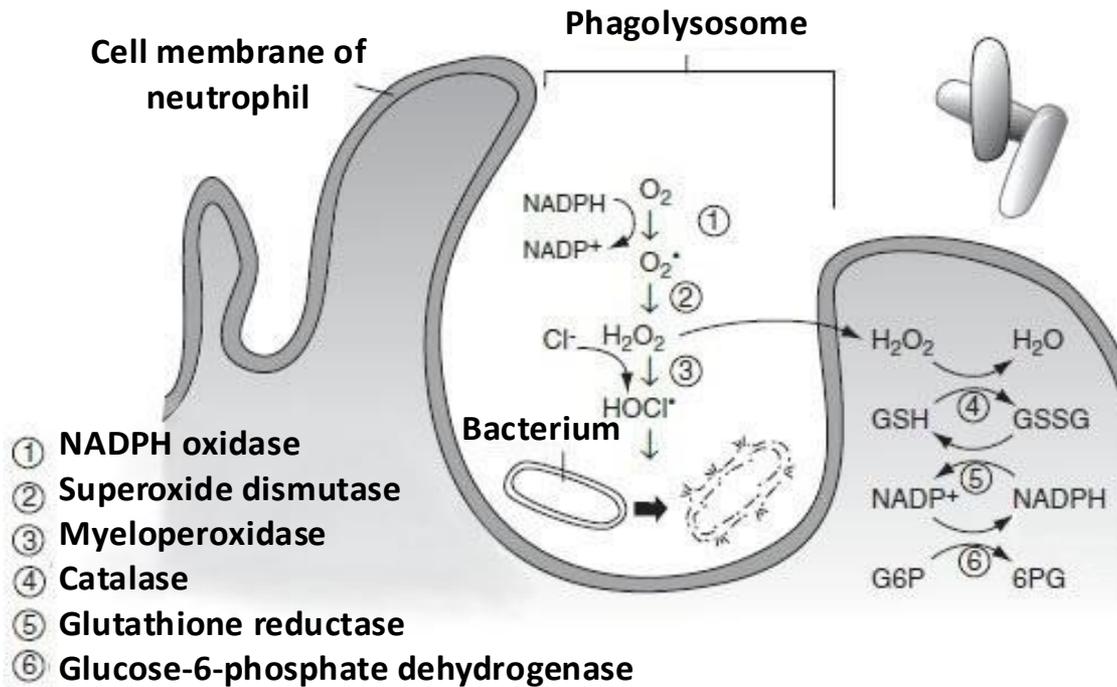
NLR: NOD-like receptor

CLR: C-type lectin receptor

RLR: RIG-like receptor

Based on A. Abbas

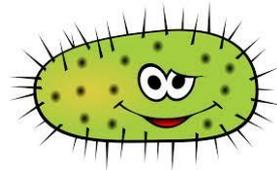
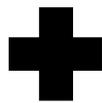
Respiratory burst



1. Phagocytosis (phagosome)
 - ↓
2. Phagosome + lysosome containing enzymes and reactive oxygen species → **phagolysosome**
 - ↓
3. The enzymes and the reactive oxygen species kill the pathogen



Neutrophil



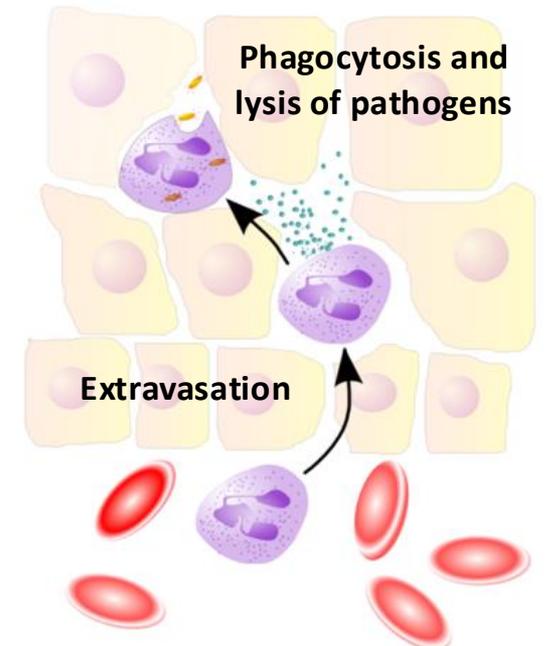
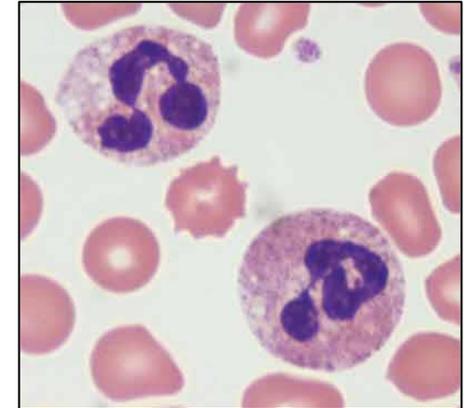
Pathogen
bacterium



Neutrophil granulocyte

Leukocyte %	55-70
Main function:	Elimination of pathogens, removal of tissue debris
Recognition:	PRR, Fc receptor, Complement receptor
Content of granules:	Digesting enzymes
Elimination of pathogens:	Phagocytosis, respiratory burst, degranulation
Produced mediators:	Inflammatory cytokines
Fc receptor:	FcγR (binds IgG)
Role in diseases:	Inflammatory reactions

Red: Only possible after the activation of the adaptive immunity



Degranulation

Neutrophil granulocytes are able to release the content of three granule types in their environment. Enzymes found in these have antimicrobial characteristics.

Granule type

Protein

azurophilic (or primary)

[Myeloperoxidase](#) (MPO),
[bactericid/permeability increasing protein](#)
(BPI), [defensins](#), protein degrading
[neutrophil elastase](#) and [cathepsin G](#)

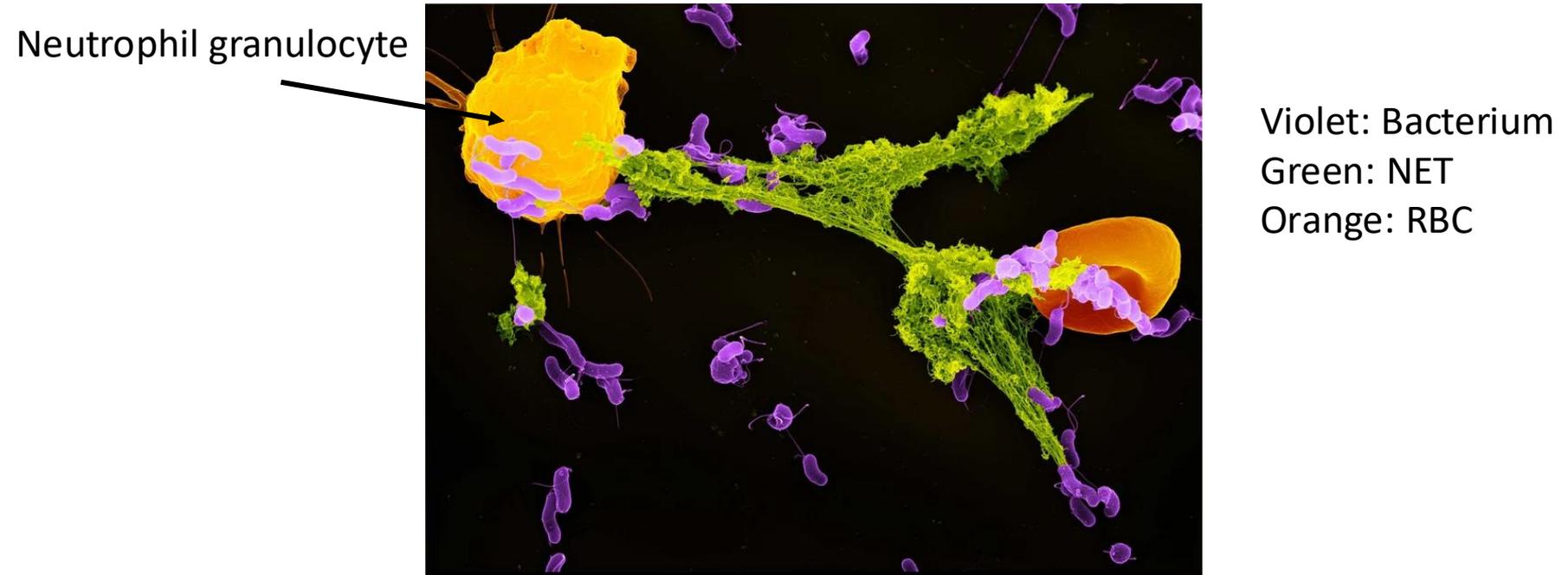
specific (or secondary)

[Alkaline phosphatase](#), [lysozyme](#), [NADPH-](#)
[oxidase](#), [collagenase](#), [lactoferrin](#),
[histaminase](#), [cathepsin](#)

tertiary

[cathepsin](#), [gelatinase](#), [collagenase](#)

NET: Neutrophil Extracellular Trap

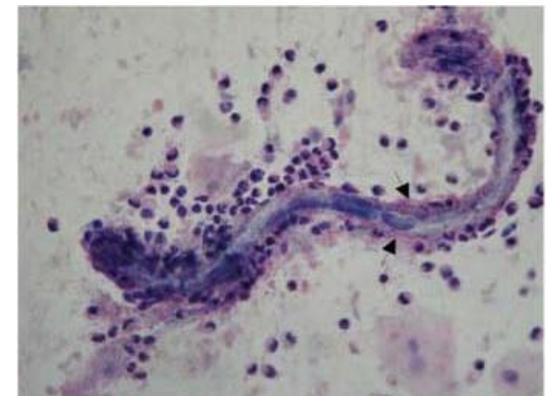
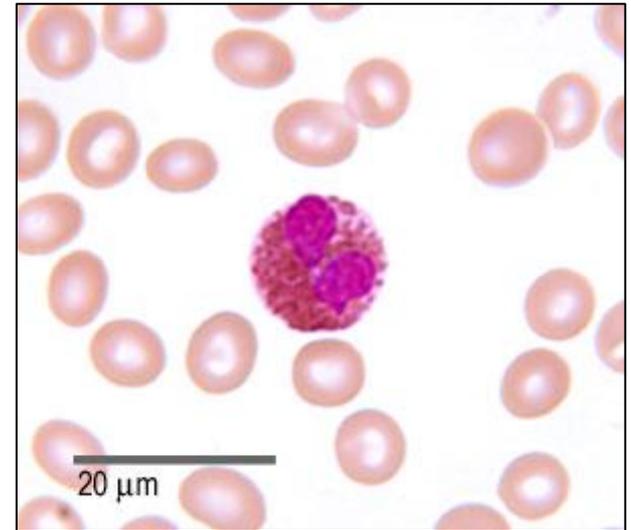


In 2004 it was discovered that the activated neutrophils release network-like DNA structures against bacteria. These so called **extracellular traps (neutrophil extracellular trap, NET)** are composed from materials of chromosomes, chromatin and [serin-proteases](#). These fibrils are able to destroy bacteria and can inhibit the physical spreading of the bacteria.

In systemic sepsis NETs can be formed in the circulation, and can help blood clotting.

Eosinophil granulocyte

Leukocyte %	2-4
Main function:	Defense against multicellular parasites
Recognition:	PRR, Fc receptor
Content of granules:	Toxic proteins, enzymes
Elimination of pathogens:	Degranulation
Produced mediators:	Prostaglandins, Leukotrienes, Inflammatory cytokines
Fc receptor:	FcεR (binds IgE)
Role in diseases:	Allergic reactions

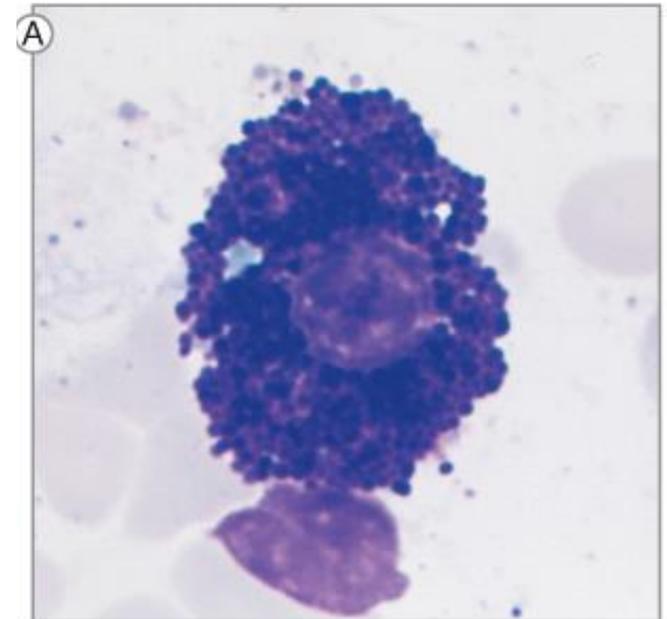
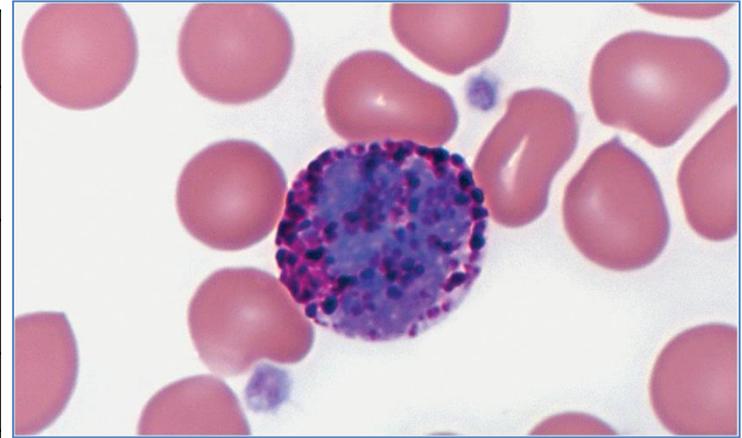


Red: Only possible after the activation of the adaptive immunity

Eosinophils surrounding a *Strongyloides stercoralis* larva. (sputum from a parasitic pneumonia case)

Basophil granulocyte

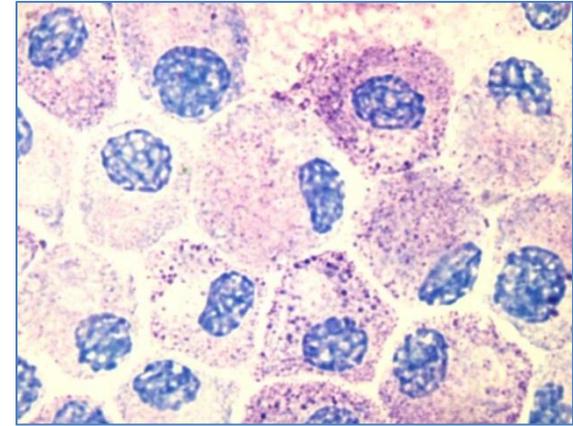
Leukocyte %	0-1
Main function:	Defense against multicellular parasites
Recognition:	PRR, Fc receptor
Content of granules:	Histamine, heparin
Elimination of pathogens:	Degranulation
Produced mediators:	Cytokines (e.g. IL-4), Leukotrienes
Fc receptor:	FcεR (binds IgE)
Role in diseases:	Allergic reactions



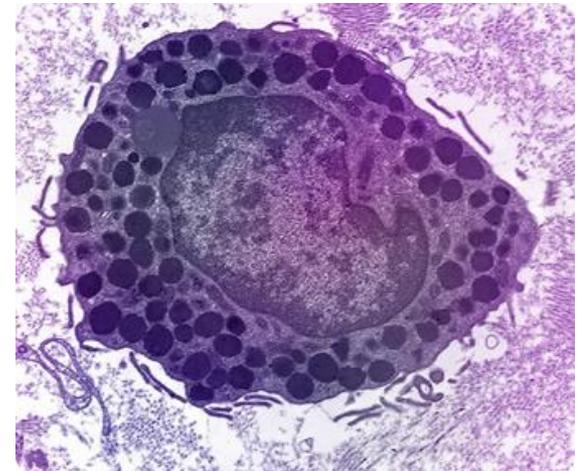
Red: Only possible after the activation of the adaptive immunity

Mast cell (mastocyte)

Found in:	Tissues
Main function:	Defense against multicellular parasites
Recognition:	PRR, Fc receptor
Content of granules:	Histamine, heparin, enzymes
Elimination of pathogens:	Degranulation
Produced mediators:	Cytokines, Leukotrienes
Fc receptor:	FcεR (binds IgE)
Role in diseases:	Allergic reactions



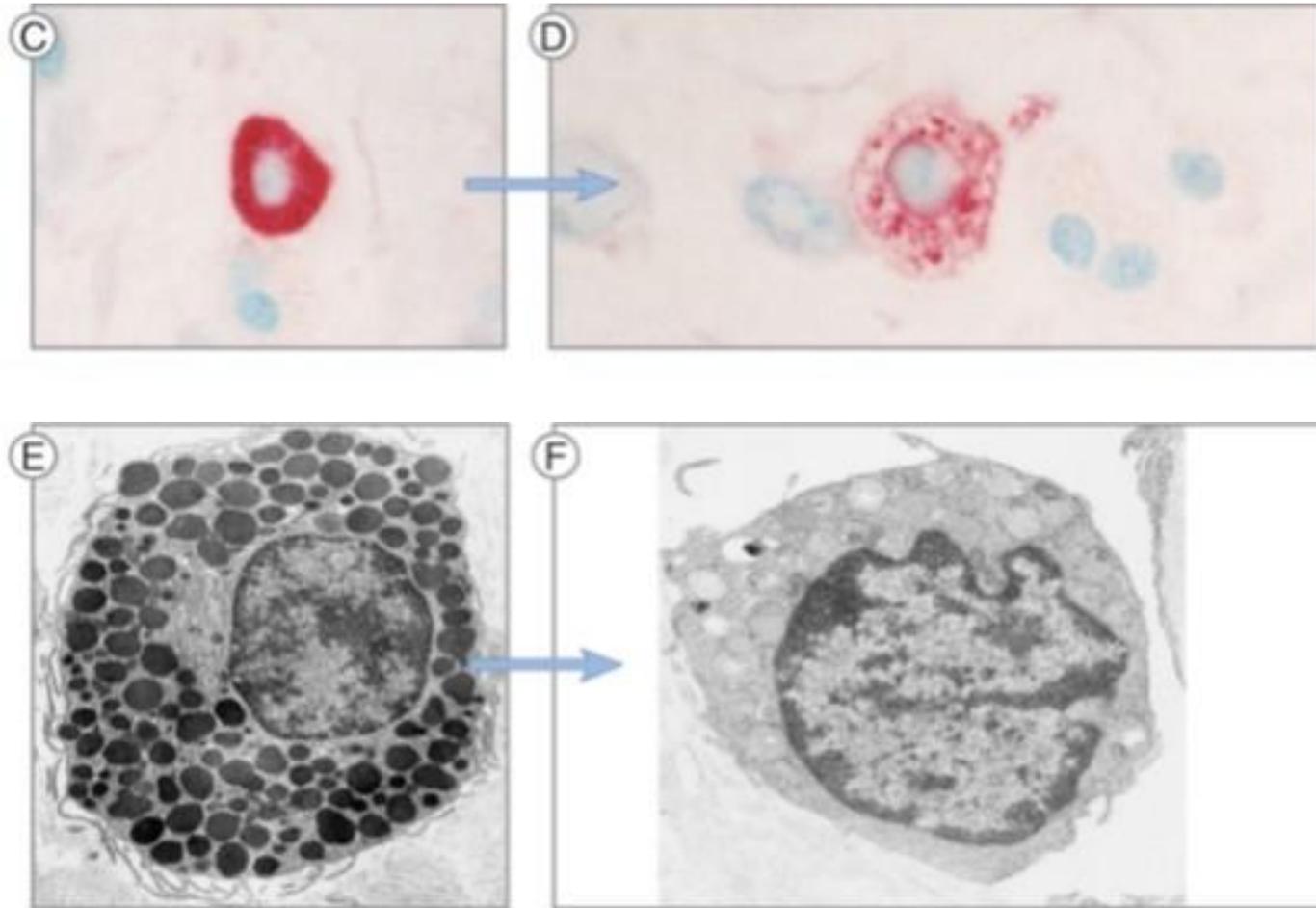
Cultured mast cells
(Toluidine blue staining)



Mast cell (electron microscopy image)

Red: Only possible after the activation of the adaptive immunity

Quick degranulation of a mast cell



Monocyte, macrophage

Leukocyte %:	2-8
Main function:	Phagocytosis, Antigen presentation, Cytokine production,
Site of antigen presentation:	Locally, in the tissues
Recognition:	PRR, Fc receptor, Complement receptor
Elimination of pathogens:	Phagocytosis, Respiratory burst
Produced mediators:	Cytokines
Fc receptor:	FcγR (binds IgG)
Role in diseases:	Type IV. hypersensitivity



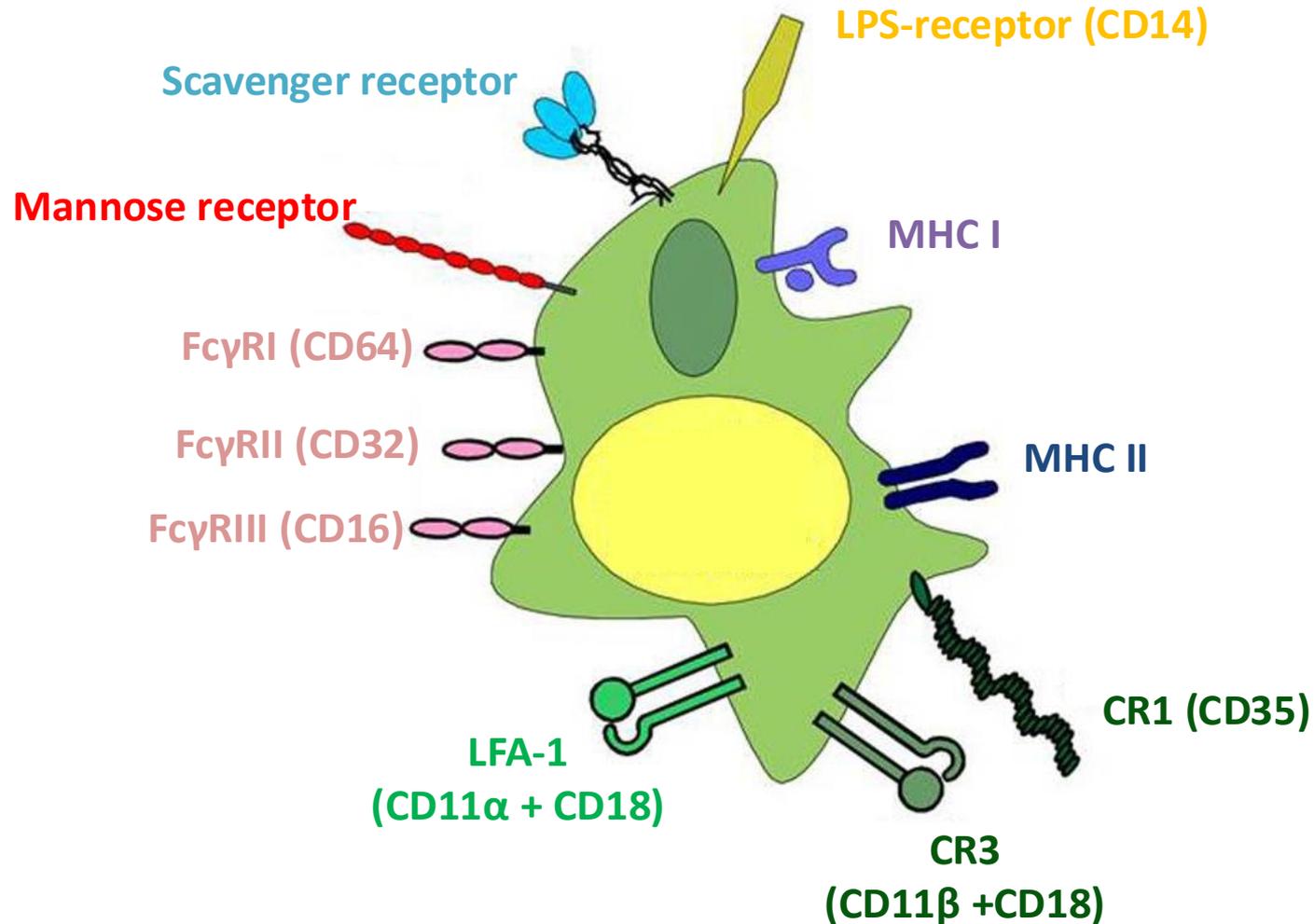
A macrophage ingesting (phagocytosing) bacteria (SEM image)



A monocyte in a blood smear

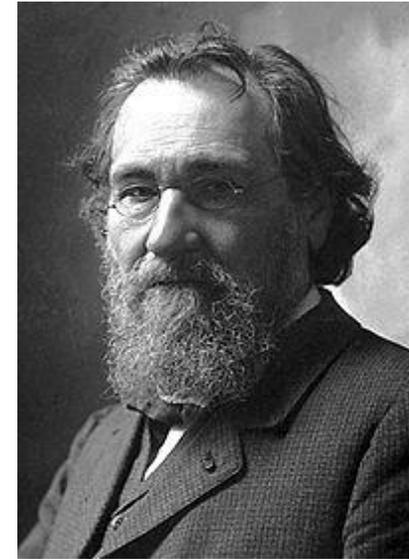
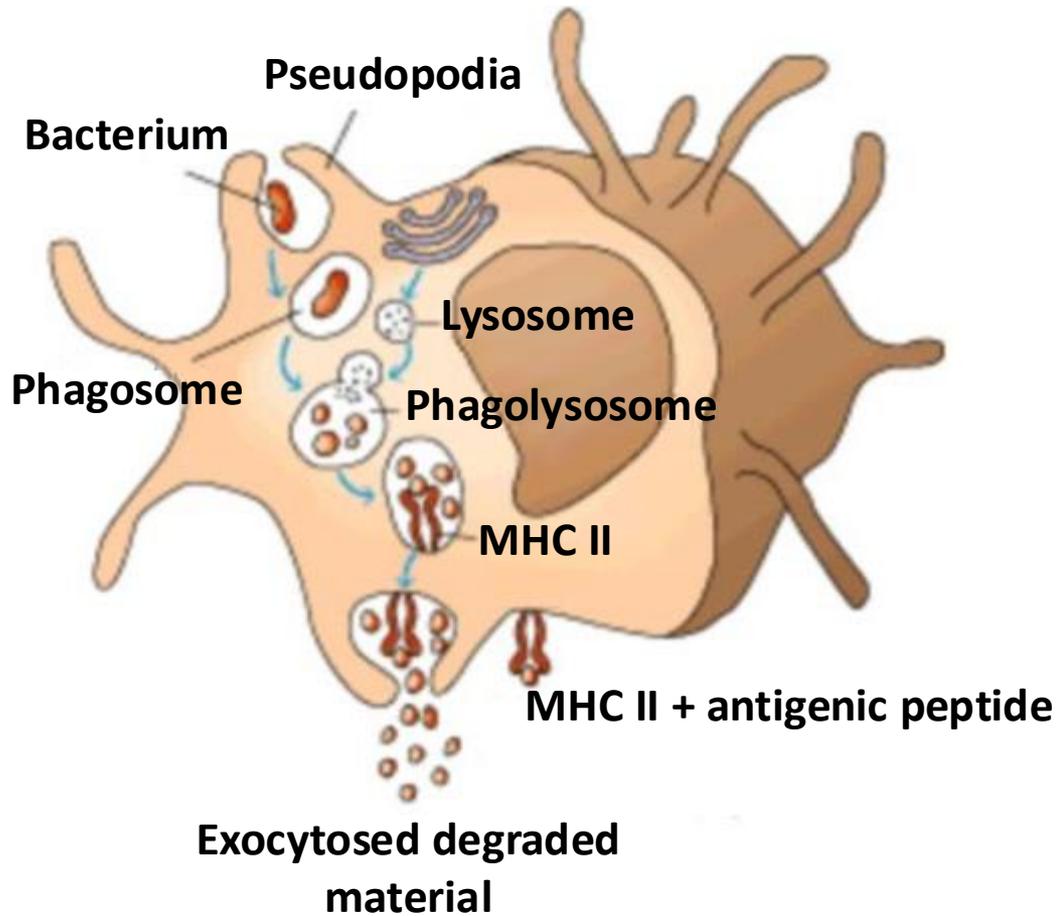
Red: Only possible after the activation of the adaptive immunity

Surface molecules of macrophages



Phagocytosis

Phagocytosis and antigen presentation of macrophages:

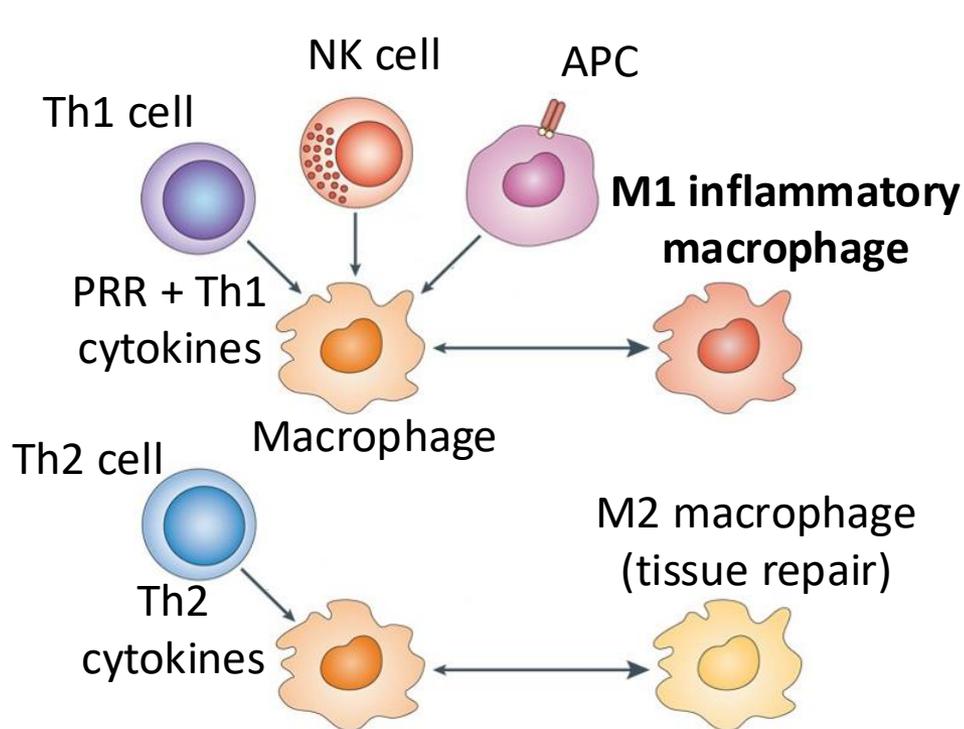


Ilya Ilyich Mechnikov who discovered macrophages and the phenomenon of phagocytosis.

Was awarded the 1908 Nobel Prize in Physiology or Medicine jointly with Paul Ehrlich „in recognition of their work on immunity”.



Activation of macrophages

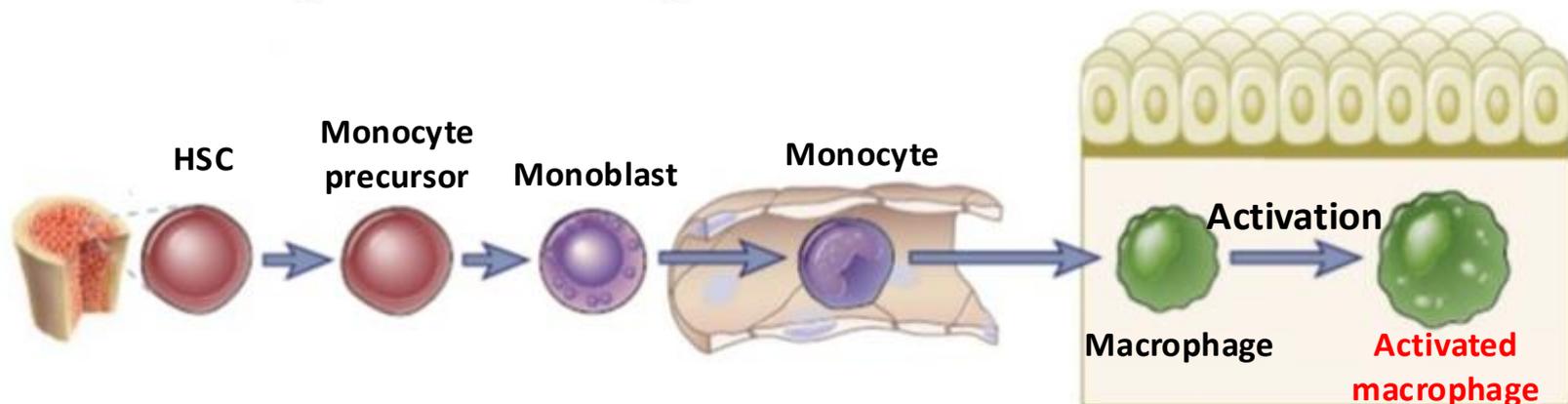


EFFECTS OF CLASSIC ACTIVATION^[10.]:

- **Increased phagocytosis**
- Production of **inflammatory cytokines** (see later)
- Production of **chemokines**
- **Increased expression of MHC II**
- **They express costimulatory molecules** on their surface (e.g. CD40, CD80, CD86)

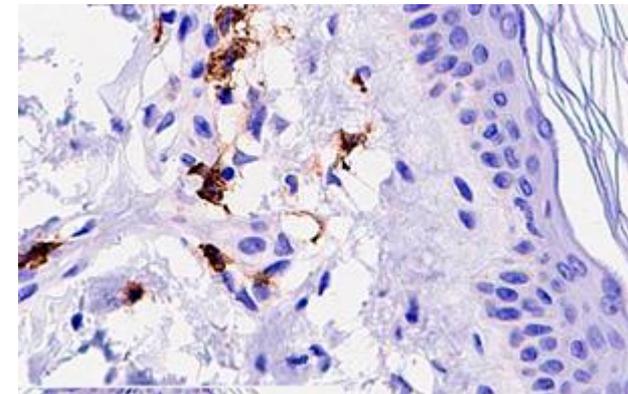
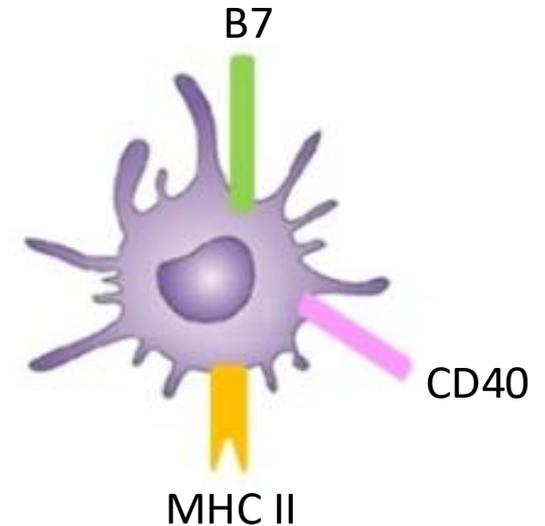
HYPERACTIVATED M1 MACROPHAGE:

- **Has anti-tumor activity**



Dendritic cell (DC)

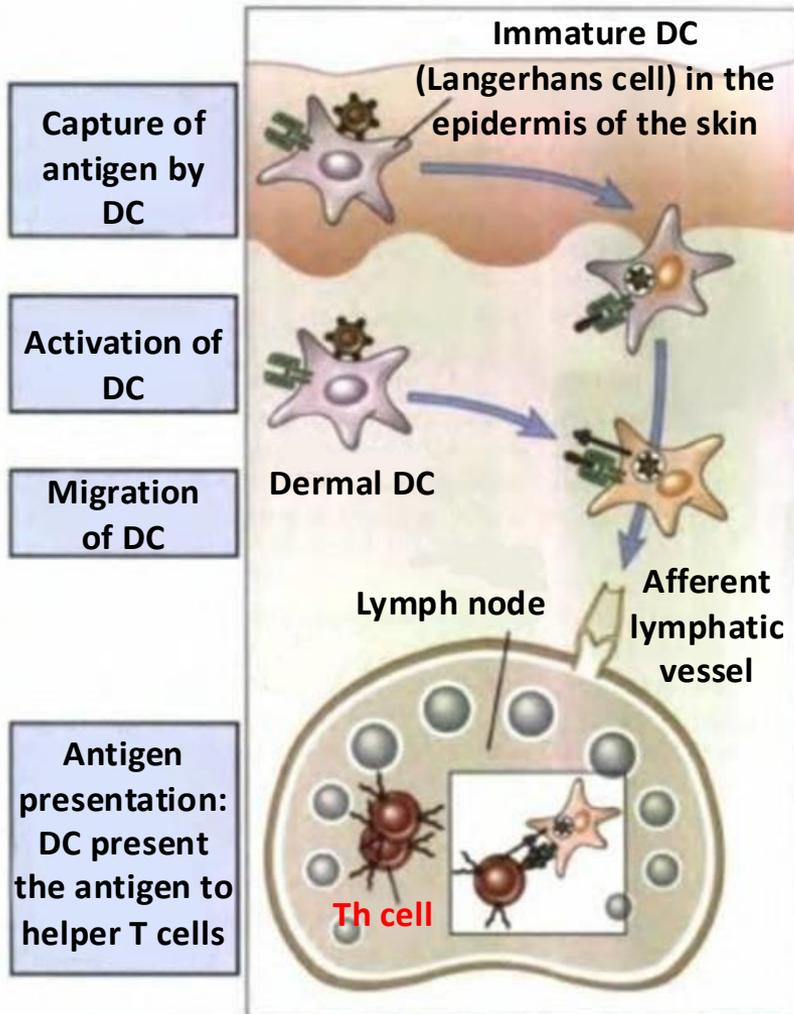
Found in:	Tissues
Main types:	Myeloid (cDC) and plasmacytoid (pDC)
Main function:	Antigen presentation (cDC) Type I IFN production (pDC)
Site of antigen presentation:	In the secondary lymphoid organs
Recognition:	PRR, Fc receptor
Produced mediators:	Cytokines
Fc receptor:	FcγR (binds IgG)
Role in diseases:	Autoimmunity, HIV infection



Dendritic cells in the skin
(DC-SIGN immunoperoxidase staining)

Red: Only possible after the activation of the adaptive immunity

Function of dendritic cells



1. They **capture antigens** in the tissues with **PRRs**
2. They transport the captured antigen to the draining **lymph node**
3. They present the antigen on **MHC II** molecules to **helper T cells**

Antigen presentation of resting, „immature“ DCs:
MHC II^{low}/CD80^{low}/CD86^{low} (no costimulation)

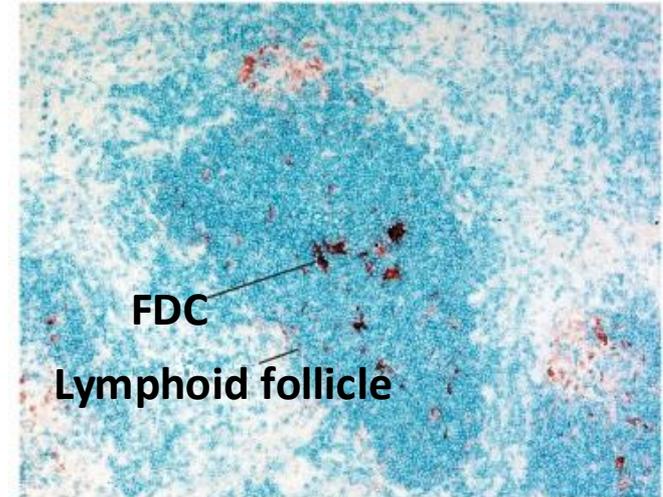
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TOLERANCE

Antigen presentation of DCs activated by PRRs:
MHC II^{high}/CD80^{high}/CD86^{high}/CD40⁺

↓
IMMUNE RESPONSE

Follicular dendritic cell (FDC)

Found in:	Lymphoid follicles
Origin:	Non-hemopoetic (connective tissue stroma)
Main function:	Formation of follicles, Keeping the antigen in the follicle for B cells
Recognition:	Fc receptor, Complement receptor
Produced mediators:	Cytokines
Fc receptor:	FcγR (binds IgG)



Red: Only possible after the activation of the adaptive immunity

Iccosome:

- Antigen
- Antibody + Fc receptor
- Complement + Complement receptor

