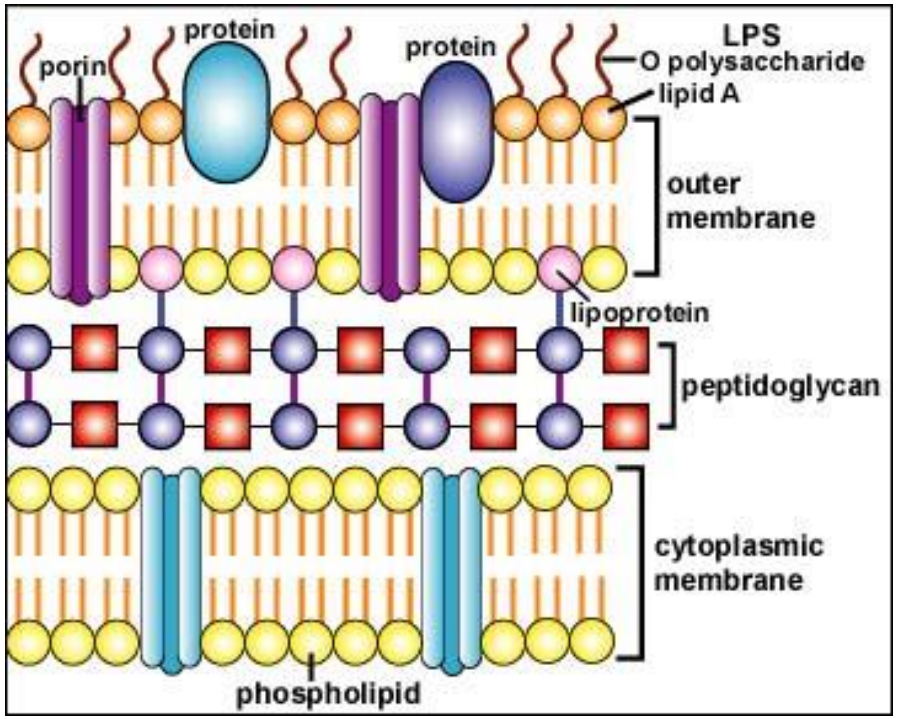
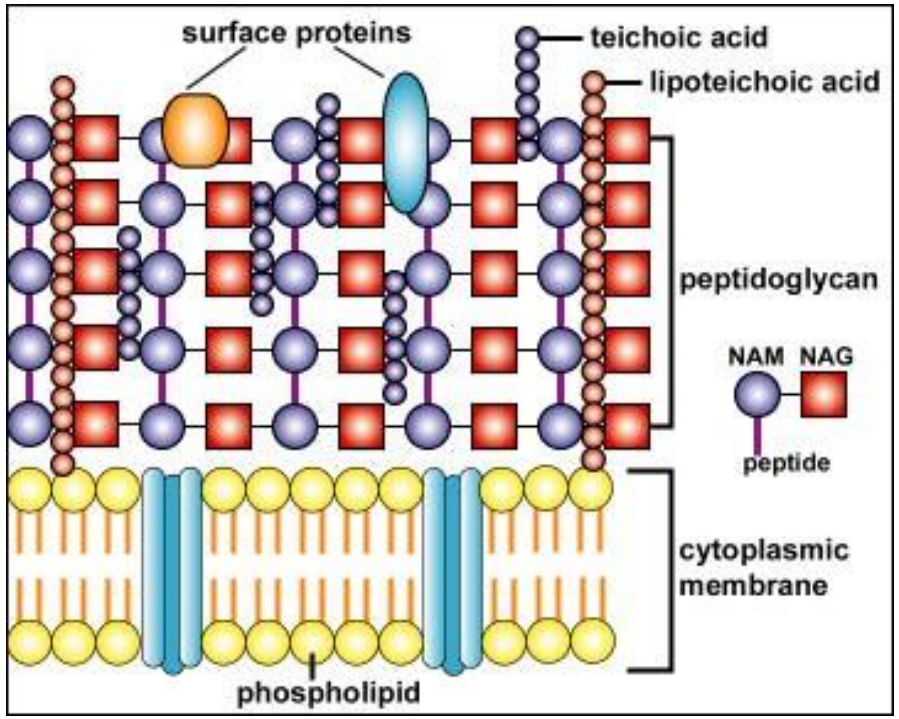
Innate immunity: Non-self

- Pathogen Associated Molecular Patterns (PAMPs)





From Dr. Gary Kaiser (COMMUNITY COLLEGE OF BALTIMORE COUNTY)

Innate immunity: Indirect detection of non-self

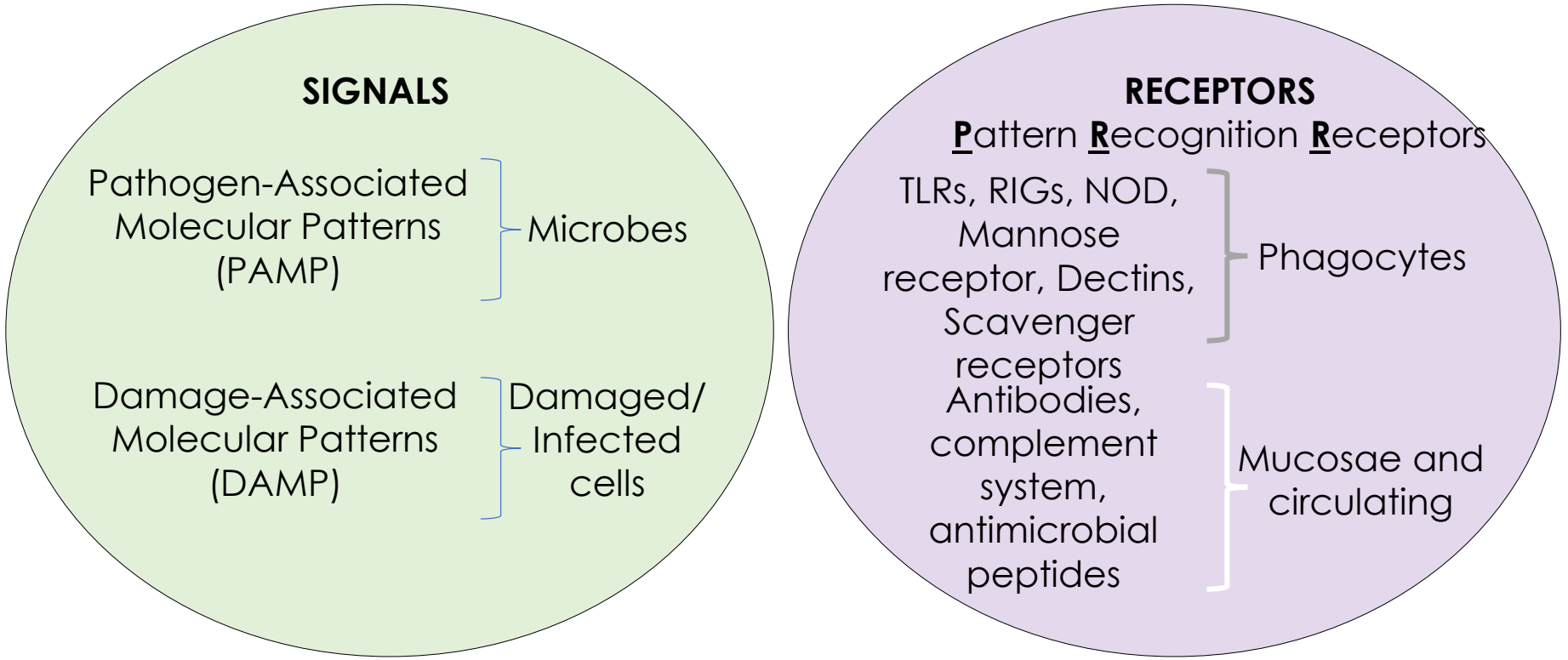
- Danger signals
    - Damage-associated molecular pattern (DAMPs)
    - HMGB1,
    - HSPs
    - mtDNA
    - ATP

Innate immunity: Non-self

- Pathogen Associated Molecular Patterns (PAMPs)

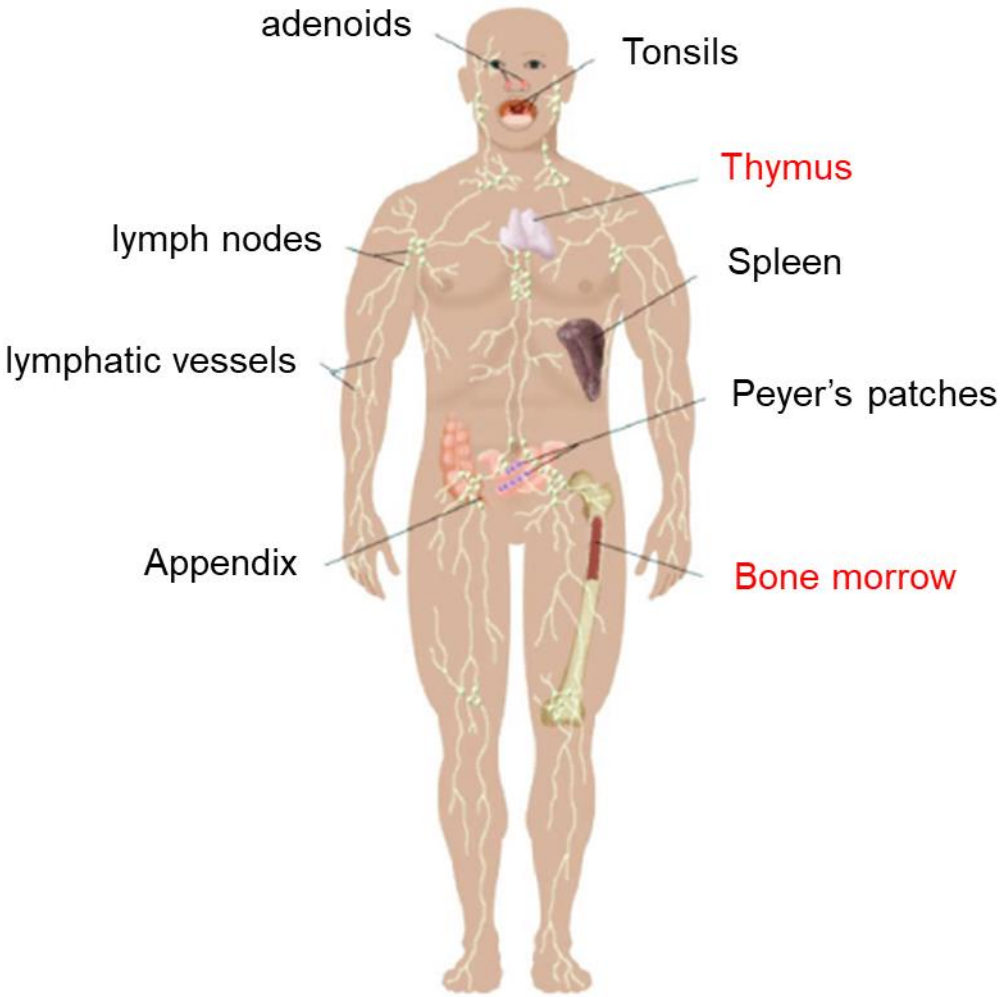
PAMPs		**Microbes**
Nucleic Acids	ssRNA	Viruses
	dsRNA	Viruses
	Non-meth. CpG	Viruses, Bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Wall lipids	LPS	$Gram^-$ bacteria
	LTA	$Gram^+$ bacteria
Carbohydrates	Mannans	Fungi
	Glucans	Fungi, Bacteria
DAMPs		
Stress proteins	HSP	
Crystals	Monosodium urate	
Nuclear proteins	HMGB1	

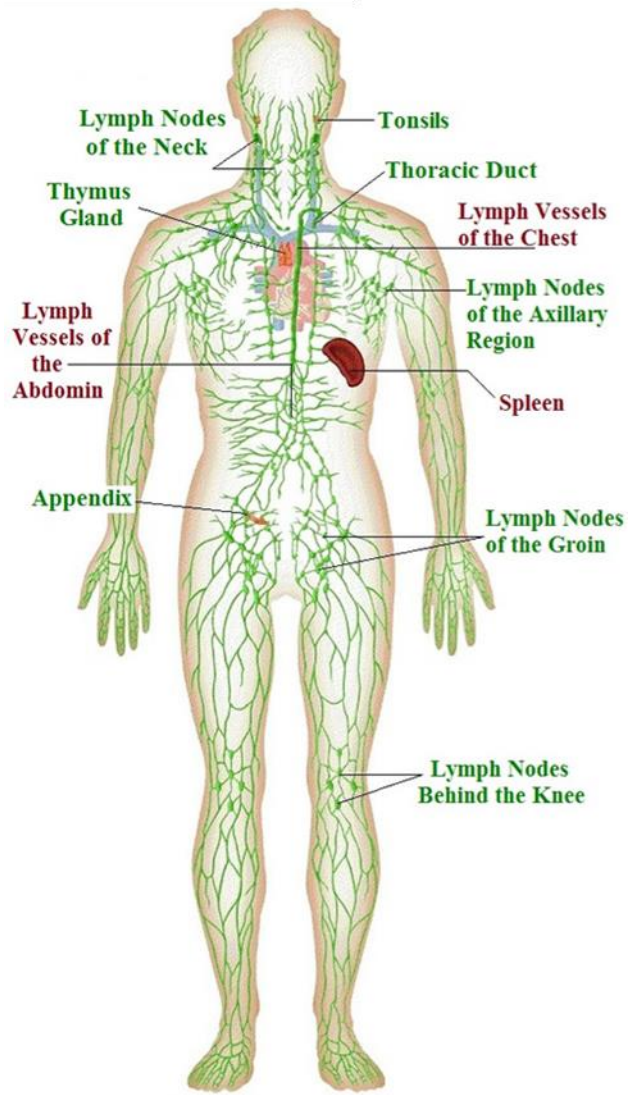
Recognition of microbes and infected cells occurs via



The organs of the immune system

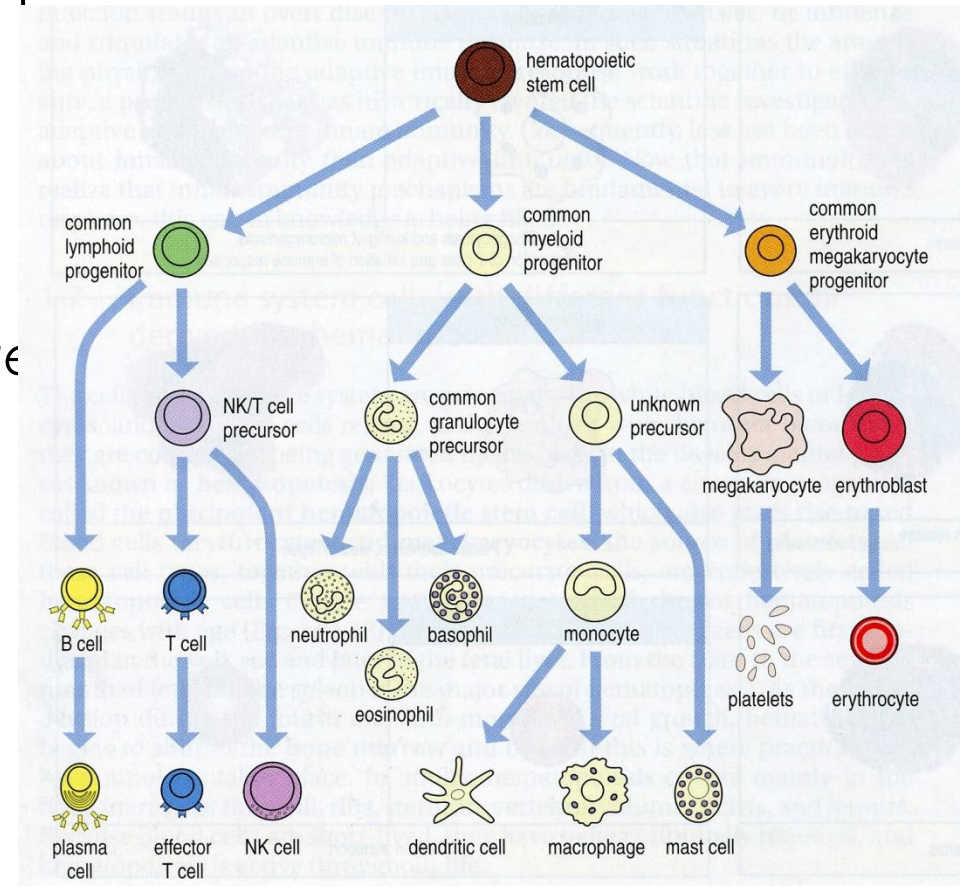
- Primary lymphoid organs
- Secondary lymphoid organs





Cells of the immune system

- Hematopoiesis
  - Pluripotent hematopoietic cells $CD34^+$
    - Lymphoid linage
    - Myeloid linage
    - *erythrocyte linage*

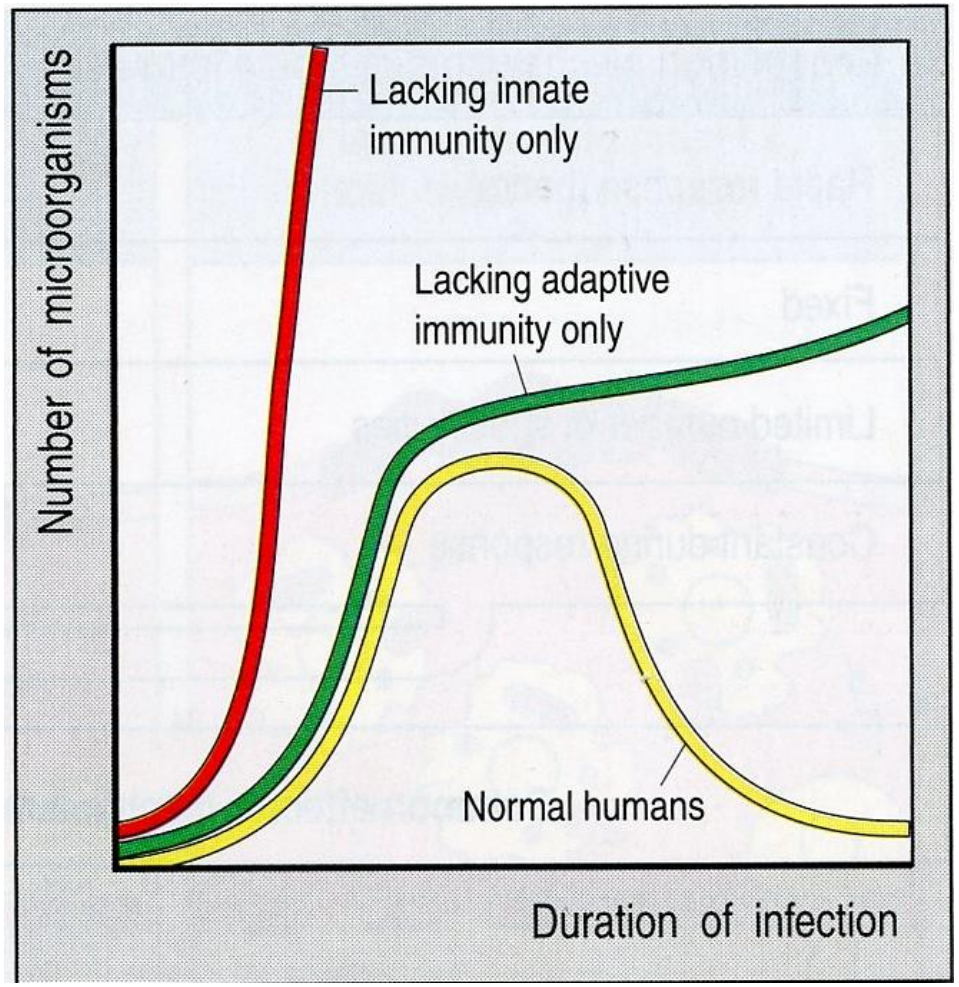


From Parham 4th edition

Two defence mechanisms:

- Innate immunity
- Adaptive immunity

Innate and adaptive immunity are complementary and both necessary for efficient protection



From Parham 4th edition

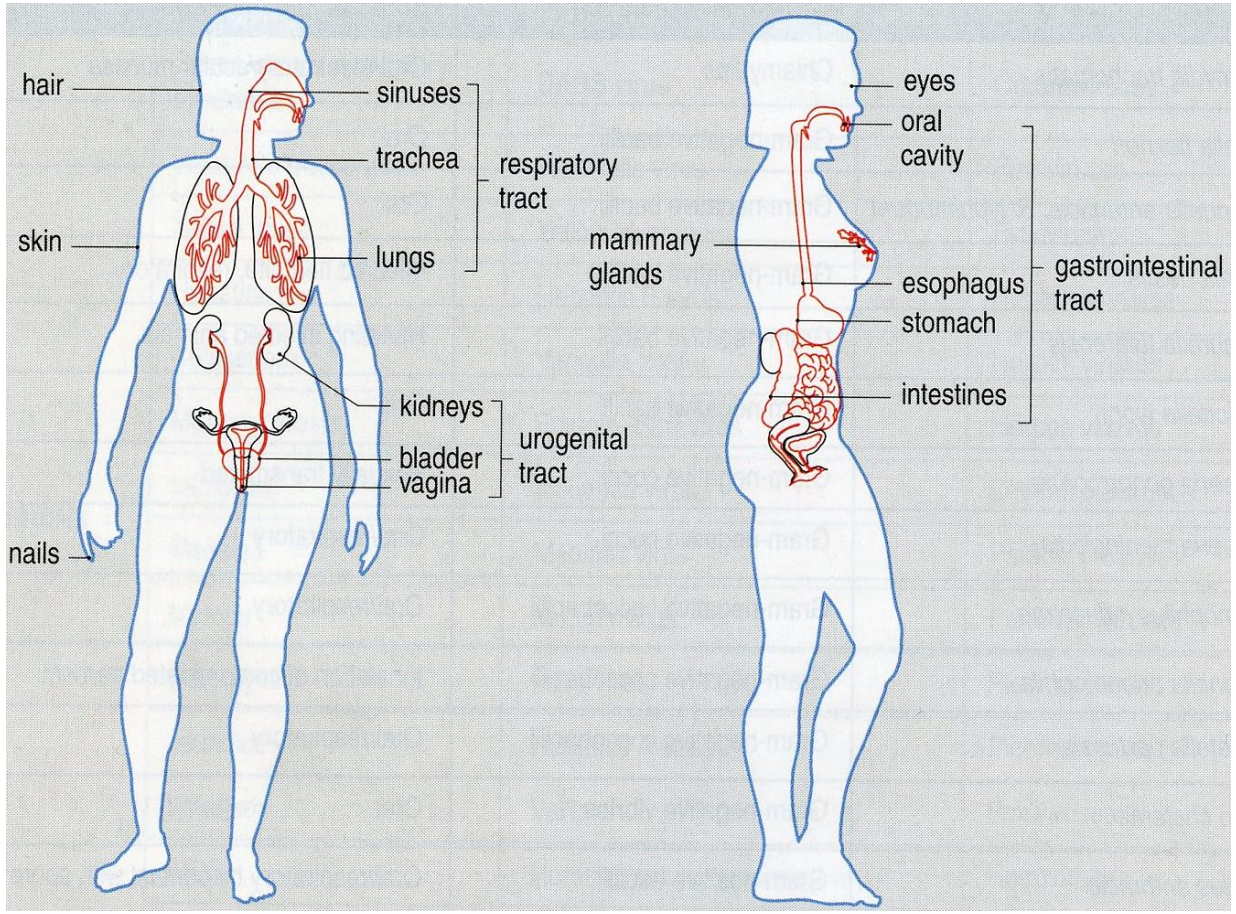
First line of defense: Innate immunity

- Ancient protection system present in all multicellular organisms
- Set of ubiquitous and germ line encoded resistance factors
    - Physical factors (anatomical barriers)
    - Physiological factors (Humoral components)
    - Cells factors (hematopoietic and non hematopoietic origin)
    - Limited repertoire of invariable germline encoded receptors
- Very fast reactions (within minutes to hours).

First line of defense: Innate immunity

- Direct detection of pathogens (non specific)
  - Conserved molecular motives shared by large groups of pathogens (PAMP).
- Indirect detection of pathogens
  - Damages associated molecular patterns (DAMP).
- Critical for the ignition of the second line of defense of the adaptive immunity.

Innate immunity: the physical barriers



- Skin: Epithelium protected by a layer of keratinized cells (robust and impermeable)
- Mucosae: respiratory track, gastro-intestinal, urogenital, and mammary glands.
  - Not keratinized epithelia specialized in the communication (less impermeable, more vulnerable).

From Parham 4th edition

Innate immunity: The physiological barriers

Mucus: Thick fluid that protect the mucosa surfaces

- Glycoproteins
- Proteoglycans (Mucins)
- Enzymes

Secreted substances:

- Sebum
- Lactic acid
- Defensins
- Lysozyme, phospholipase A2
- **Cytokines**
- **Complement system**
- Acute phase response proteins
- Interferons and cytokines
- Flora (microbiota 10^{14} cells)

Acidic pH (skin, stomach and vagina) inhibit bacterial growth

Patient with severe cutaneous burns and primary ciliary dyskinesia

- extremely susceptible to infections
  - Innate and adaptive immunity not enough

The complement system

Large number of plasma proteins produced by the liver

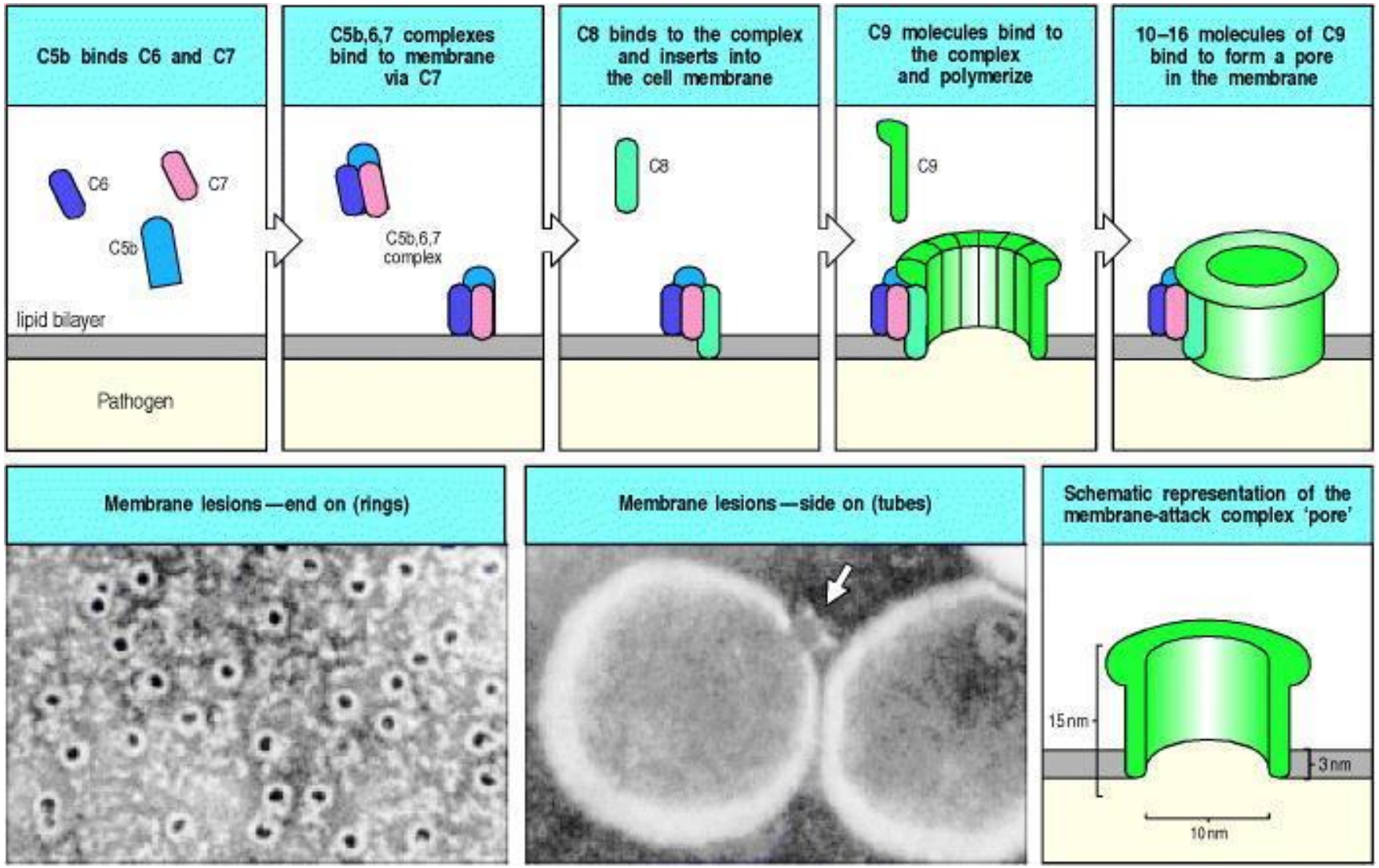
Release in the circulation in an inactive (precursors)
Widely distributed throughout body fluids and tissues

Activated by pathogens (PAMP or DAMP).

React with one another through proteolytic cascade

- At the start a small number of complement proteins
- huge amplification at each successive enzymatic reaction
- Rapid and disproportionately large complement response.
  - Opsonize pathogens
  - Induce inflammatory responses
  - Fight infection

Complement Membrane attack complex



Janeway CA Jr et al. New York: Garland Science; 2001.

C3a, C4a, and C5a → Local inflammation

- Large among of C3a,C4a or C5a **(anaphylotoxins)**
  - Anaphylactic shock
    - Smooth muscle contraction
    - Increase vascular permeability
- Activate endothelial cells to induce adhesion molecules (C5a and C3a)
- Activate Mast cells (C3a and C5a)
  - Release of histamine and TNF-α
  - Amplification loop
- Recruitment of Ab and phagocytes
- Increased fluid in the tissues
- Hastens the movement of pathogen/Antigen to the local lymph nodes
- Promote initiation of the adaptive immune response
- C5a and to a lower extent C3s act as chemokines
  - activates neutrophils and monocytes and increase their
    - adherence to vessel walls,
    - migration toward sites of Ag deposition,
    - ability to ingest particles,
    - expression of CR1 and CR3

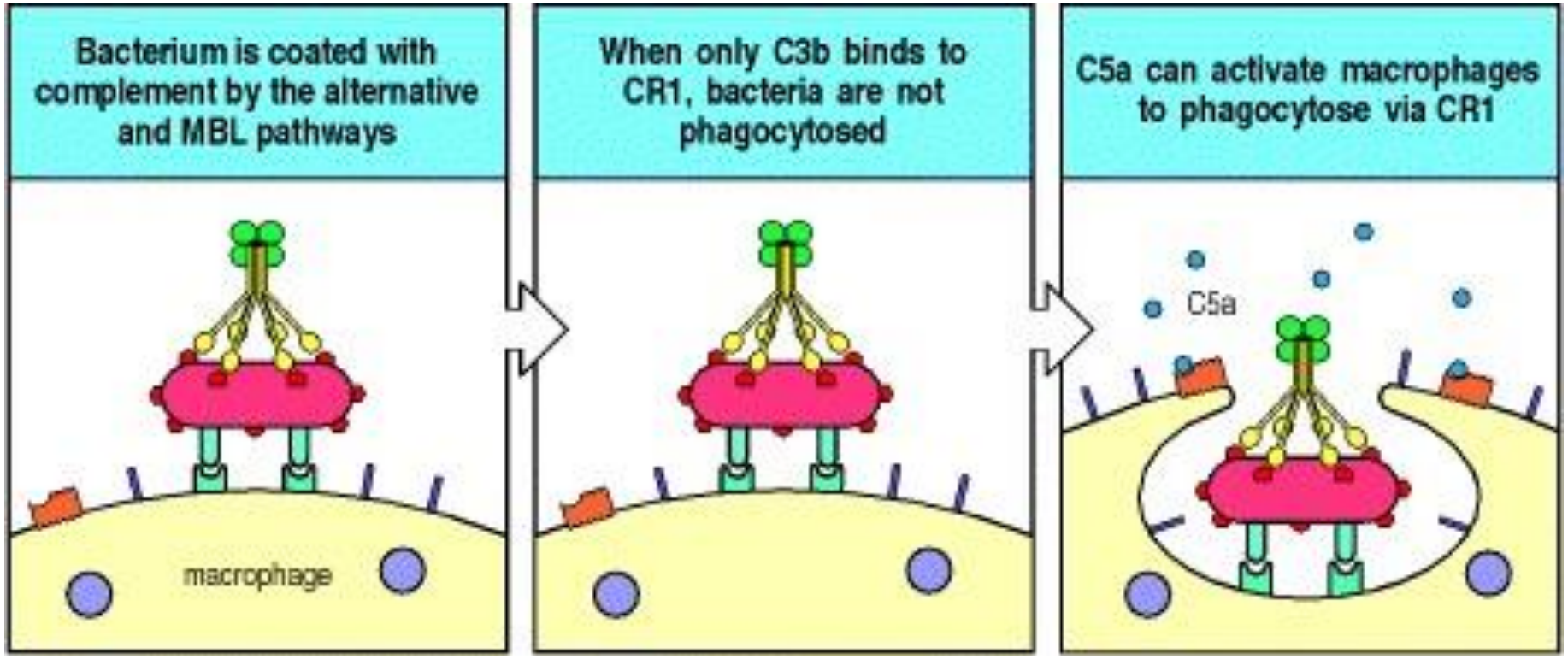
Complement receptors

CR2/CD21
CR3/(CD11b:CD18)
CR4/(CD11c:CD18)

Bind to Intact C3b bound to pathogen surface
Fragment of C3b iC3b and C3Dg

Phagocytosis of opsonized pathogens

The complement facilitates antigens phagocytosis



Janeway CA Jr et al. New York: Garland Science; 2001.

Phagocytes:

- Monocytes/macrophages
- Neutrophils,
- Dendritic cells

3 means anti-microbial mechanisms

- **Phagocytosis**

- **Soluble signals such as cytokines**
  - Recruited other cells to fight the infection
  - Repair damages cause by pathogens
  - Activate immune reaction (**inflammation**)
- **Macrophages and dendritic cells**,
  - Antigen presentation
  - Connection innate and adaptive immunity

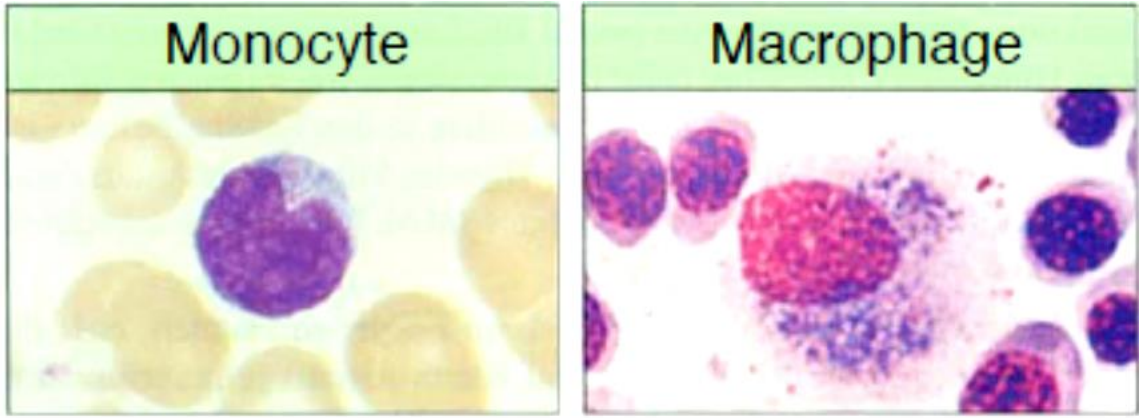
Monocyte/Macrophage

Monocytes

- circulate in the blood
- Differentiate into macrophage in peripheral tissues

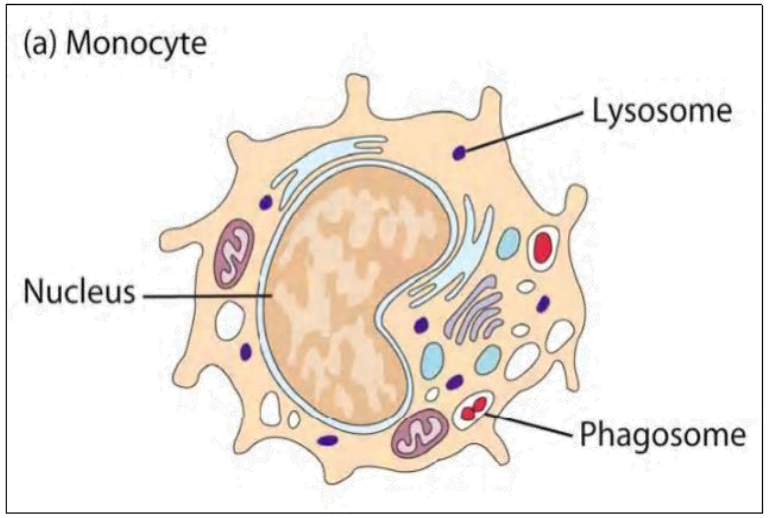
Macrophage have different name in tissues

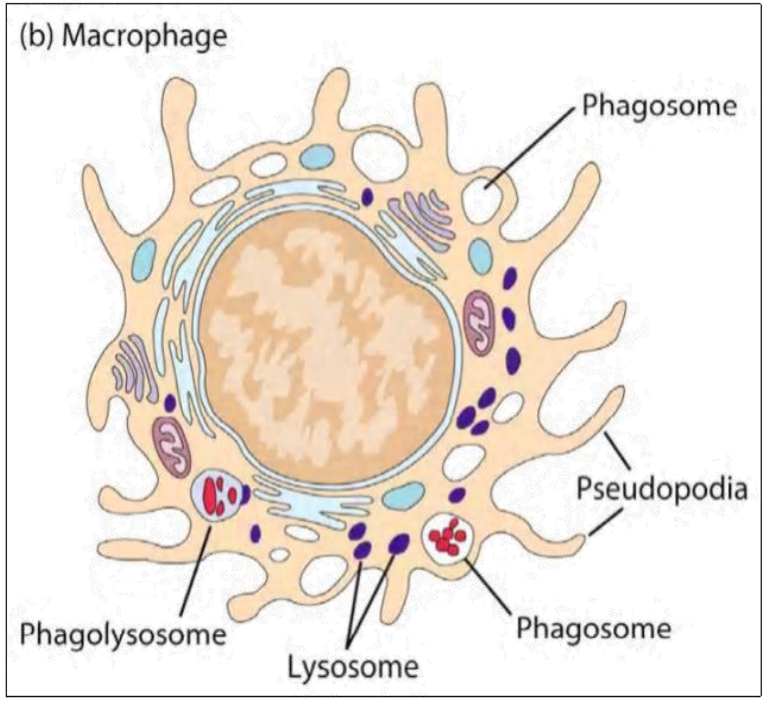
- Lung: Alveolar macrophages
- Liver: Kupffer cells
- Boon: Osteoclasts
- Kidney: Mesangial cells
- Brain: Microglia cells
- Same function: Phagocytosis and cytokine production



Monocyte/Macrophage

- Increase size 5-10 fold
- Increase number of cytoplasmic organelles: Lysosomes
- Increase production of Hydrolytic enzymes
- Increase phagocytic activity
- Increase secretion of soluble factors
  - Cytokines: interleukins 1, 6 and TNFa
  - Lysosomal components

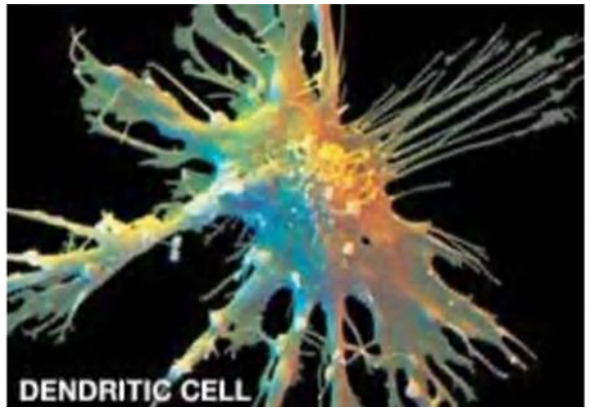


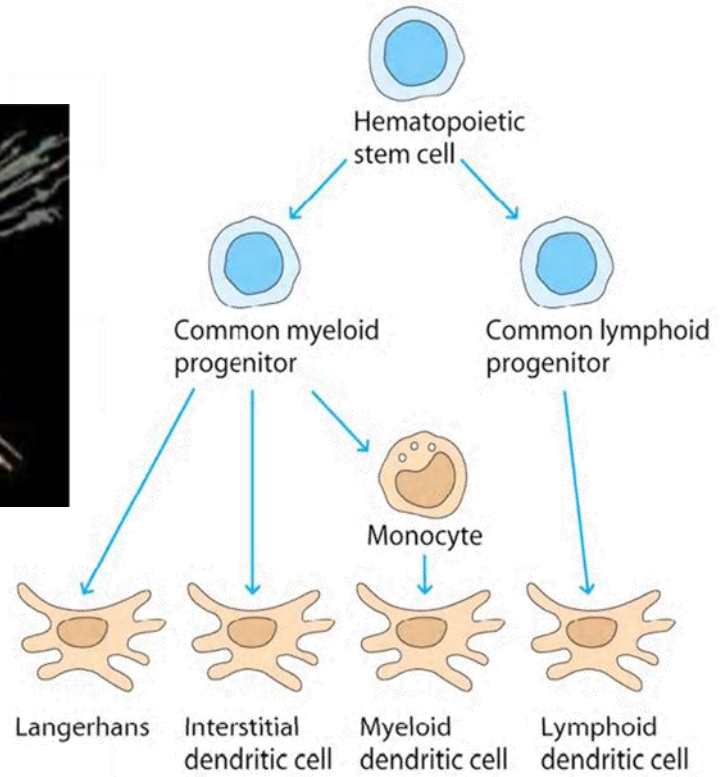


Dendritic cells

Four types

- Langerhans cells
- Interstitial
- Myeloid
- Lymphoid





Immature

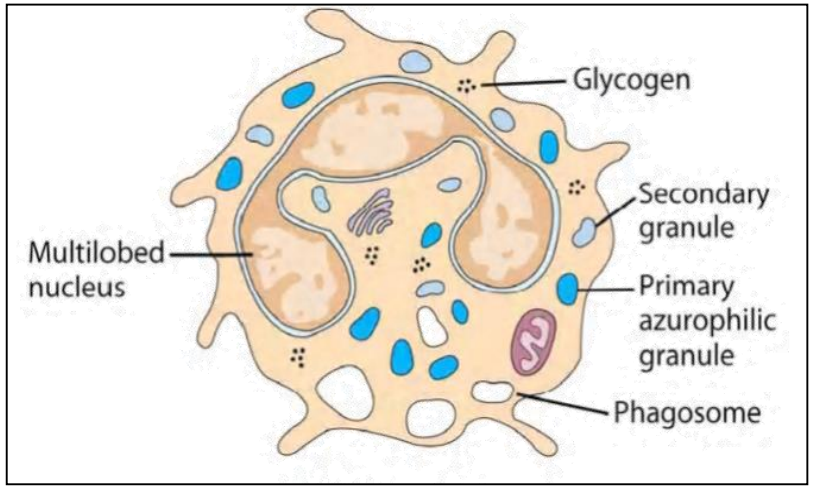
- High phagocytic capacity
- High ability to digest antigens

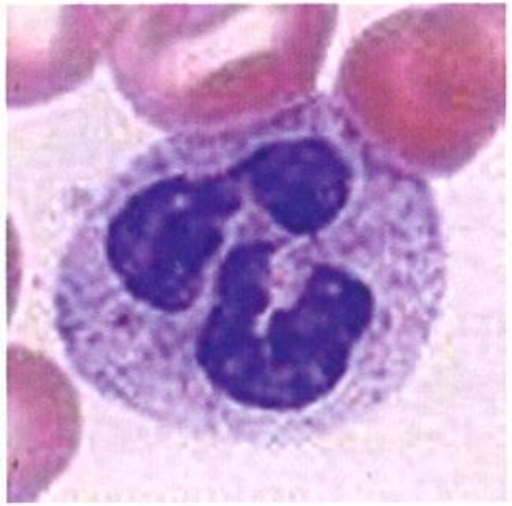
Activated

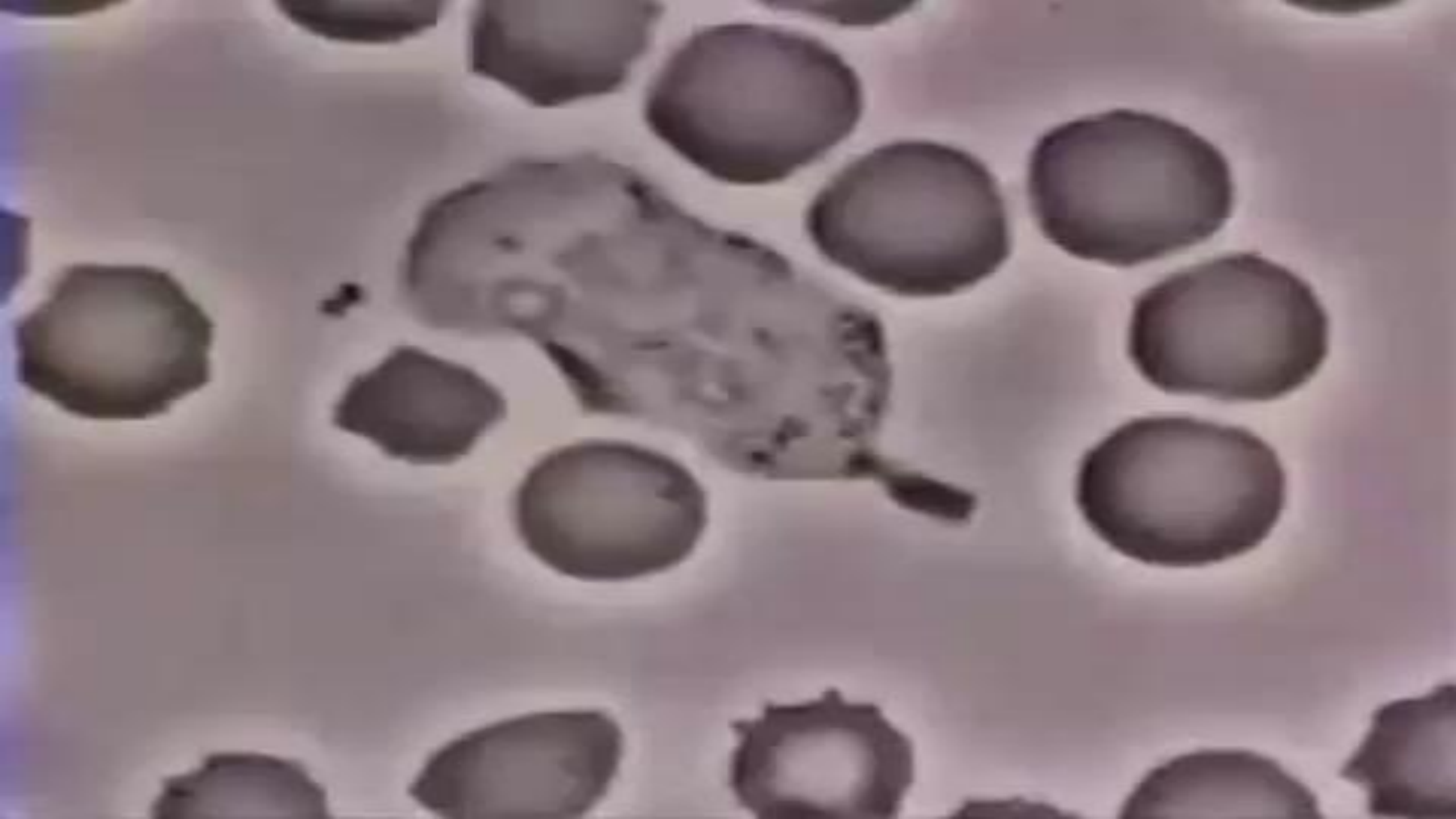
- High migratory capacity
  - (Tropism for lymph nodes: CCR7-CCL21)
- High antigen presentation capacity (Class I & II expression up)
- High expression of costimulatory molecules

Neutrophils

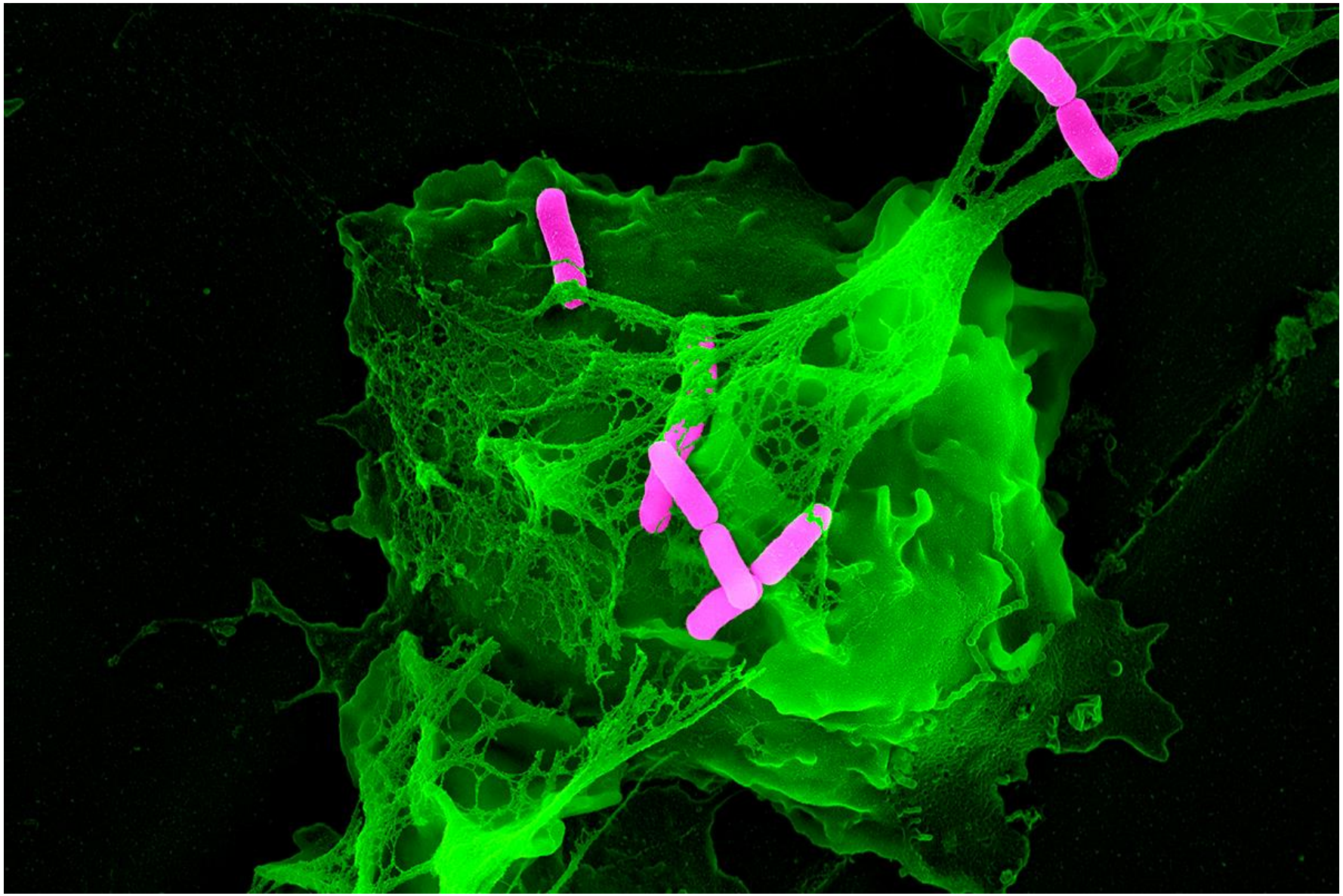
Multi-lobular nucleus
High phagocytic capacity
Cytoplasmic granules containing lytic enzymes
Granules fuse with phagosome
Neutrophils Extracellular Trap (NET)
Increase production of Hydrolytic enzymes
Increase phagocytic activity
Increase secretion of soluble factors
    Cytokines: interleukins 1, 6 and TNFa
    Lysosomal components





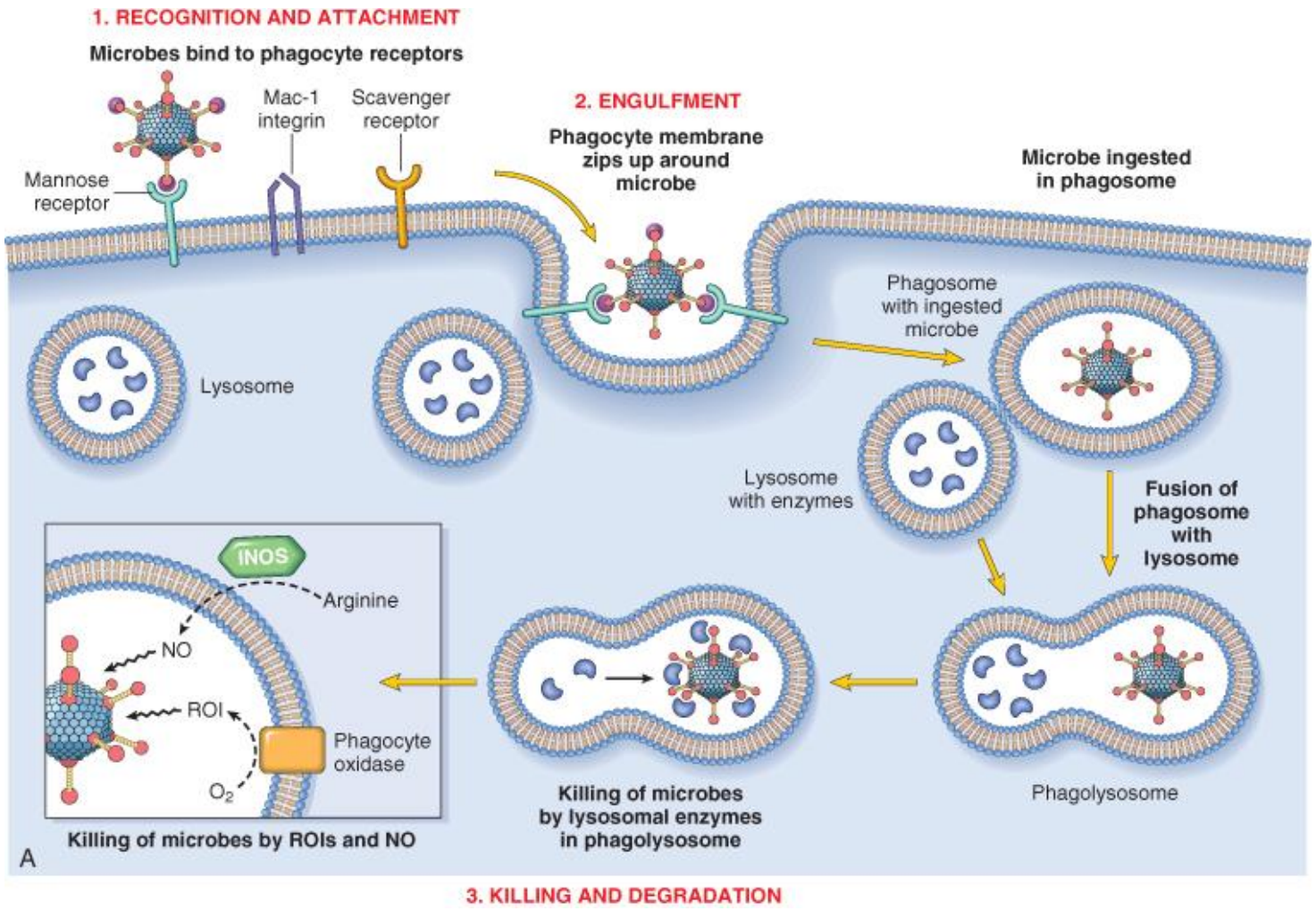


Neutrophil Extracellular Traps (NETs)



Phagocytosis and killing of intracellular microorganisms

Engulfment of microorganisms, foreign particles or cell debris by phagocytes (neutrophils, monocytes, macrophages and dendritic cells)



Respiratory burst:

Most powerful antimicrobial mechanism of the phagocytes

Rapid increase in O_2 consumption in phagosomes, occurs.

NADPH oxidase

Six subunits, which are separated under physiological conditions

Activation causes the formation of the multi-subunit enzyme

ROS (H_2O_2, $O_2^{\cdot -}$, $O^{\cdot}$) + H^+ translocation

Acidification of phagosome, activation of hydrolases

ROS react with all major classes of biomolecules

Destruction of bacterial cell walls,
membranes
genomes.

The inducible nitric oxide synthase (iNOS)

Formation of NO

NO + $O_2^{\cdot -}$ to form peroxynitrite ($ONOO^-$) extremely toxic

Activated neutrophils undergo fusion of the **cytoplasmic azurophilic granules** with the phagolysosome with release of the granule content.

Azurophilic granules are loaded with the **enzyme myeloperoxidase (MPO).** MPO is an abundant heme protein that accounts for 5% of the total neutrophil protein content.
MPO produces hypochlorous acid from hydrogen peroxide and chloride anion.

The granulocytes

- Eosinophils
- Basophils,
- Mastocytes

Anti-parasitic response

- Very potent reaction
- Degranulation
  - Lytic granule content to kill parasites
  - Vasoactive factor to expel parasites

Allergic reaction

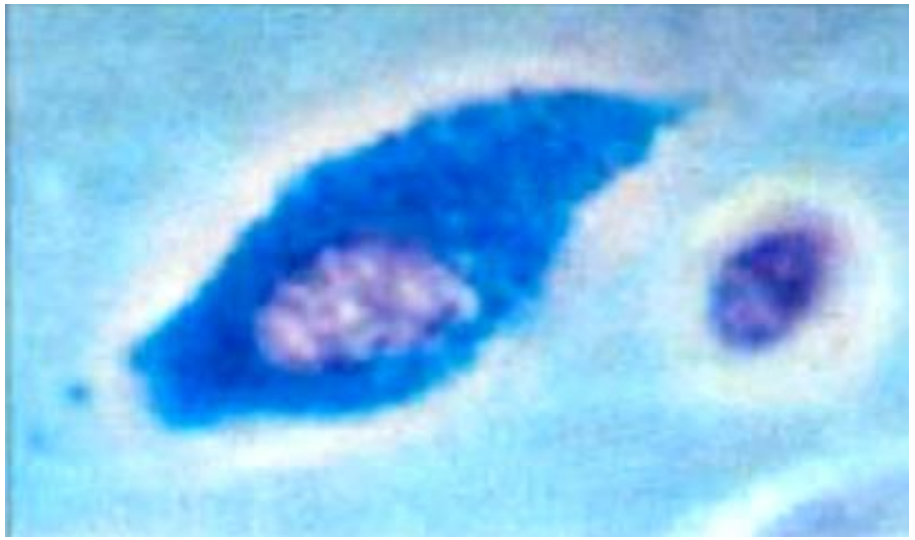
- Potent reaction anti-parasitic reaction against harmless agent

Mastocytes

- Unlobed nucleus
- Non-motile
- Non-phagocytic
- Heavy granulated cytoplasm

Secretion of soluble factors

- Cytokines
- Lytic granule content
- Vasoactive factors (histamine, prostaglandins, heparin leukotriene)

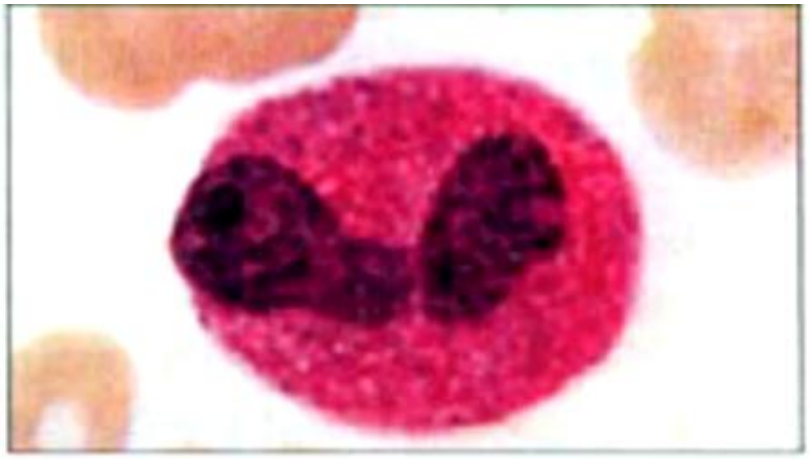


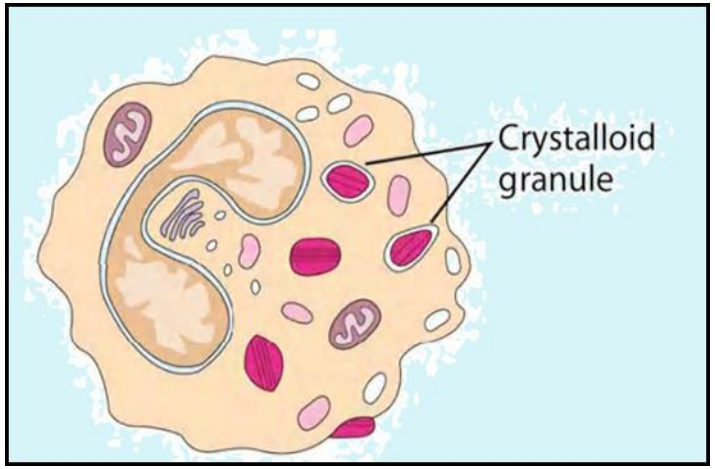
Eosinophils

- Bilobed nucleus
- Motile
- Non-Phagocytic

Secretion of soluble factors

- Cytokines
- Lytic granule content
- Vasoactive factors (histamine, prostaglandins, leukotriene)



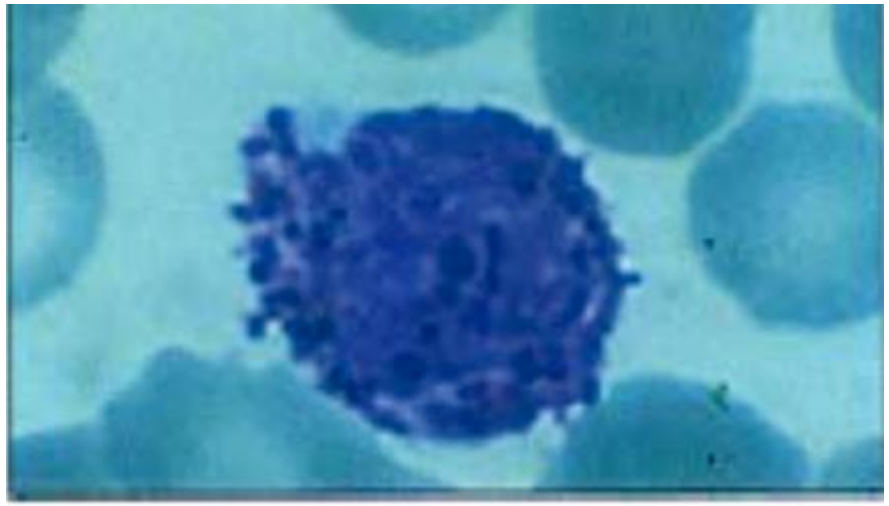


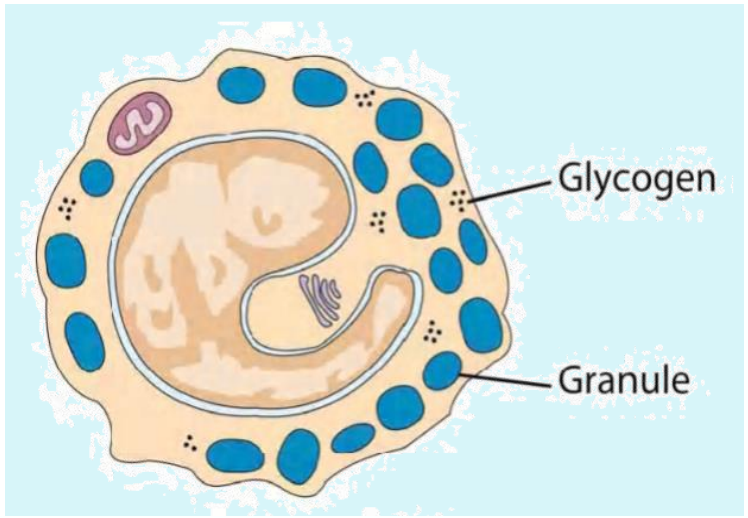
Basophils

- lobed nucleus
- Motile
- Non-phagocytic
- Heavy granulated cytoplasm

Secretion of soluble factors

- Cytokines
- Lytic granule content
- Vasoactive factors (histamine, heparin, leukotriene)

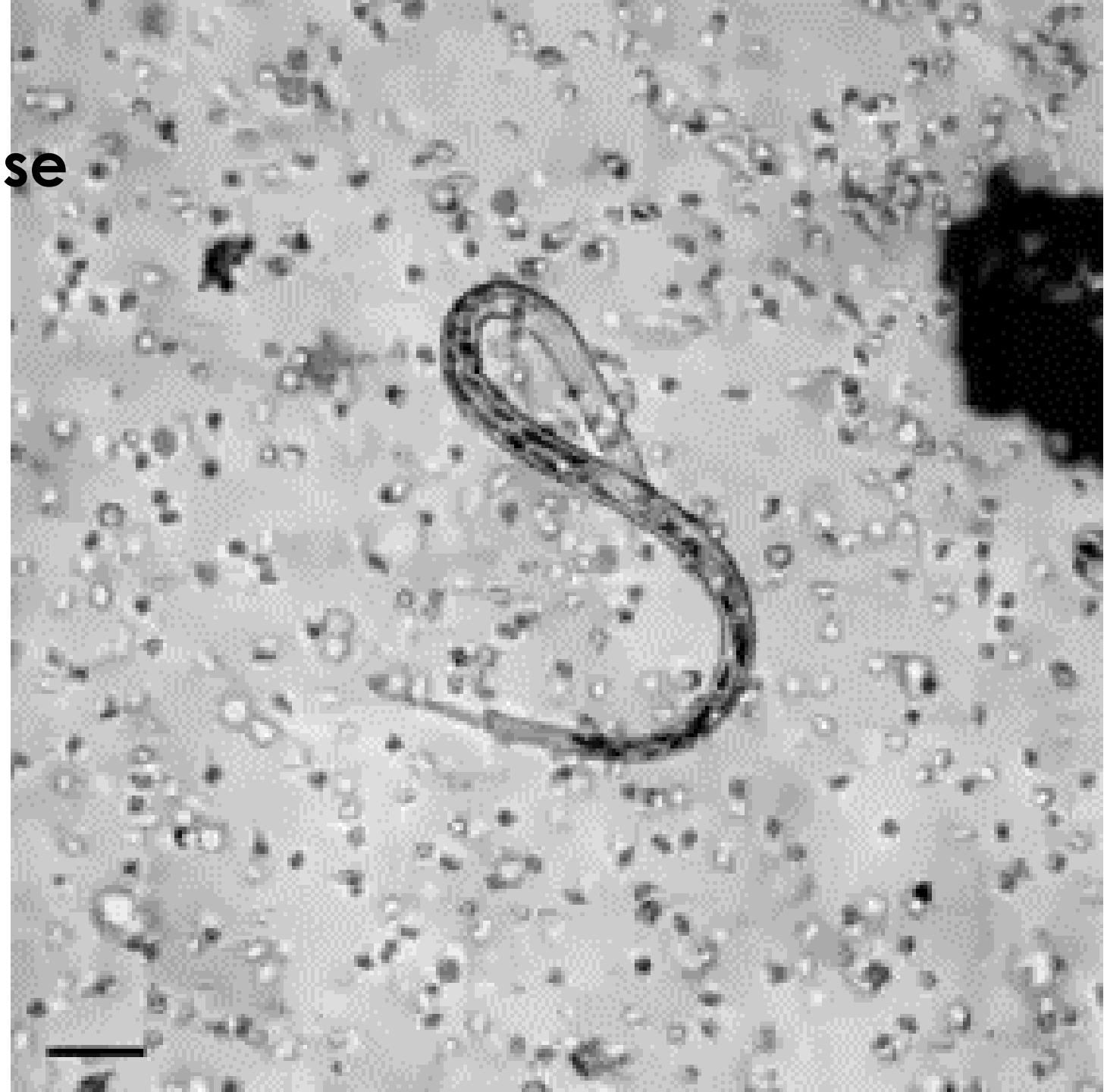




The granulocytes:

Anti-parasitic response

- Eosinophils
- Basophils,
- Mastocytes



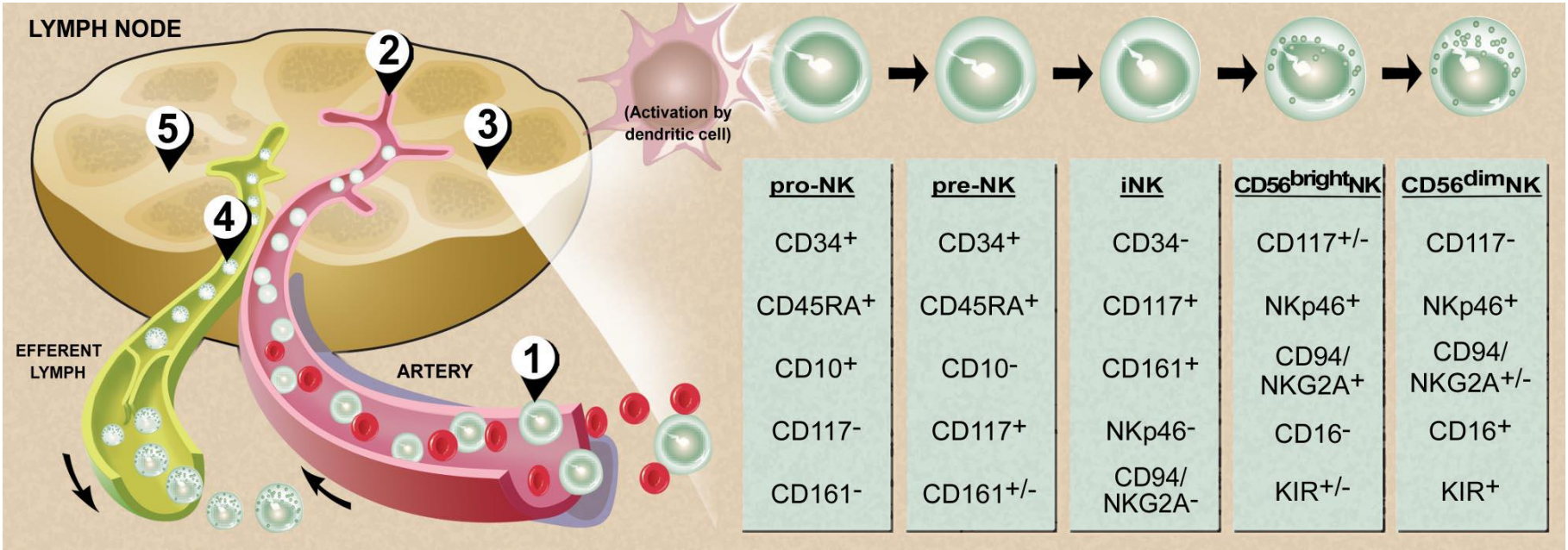
Leukocytes in blood

	Mean number per microliter	Normal range
White blood cells (leukocytes)	7400	4500–11,000
Neutrophils	4400	1800–7700
Eosinophils	200	0–450
Basophils	40	0–200
Lymphocytes	2500	1000–4800
Monocytes	300	0–800

Natural Killer cells differentiates in secondary lymphoid tissues

- CD34$^+$ CD45RA$^+$ pre-NK cells
  - < 1% of BM CD34$^+$
  - < 10% of Blood CD34$^+$

- Abundance of DC and other APC expressing mIL-15
- CD3$^-$CD56$^+$NKp46$^+$ NK cells are phenotypically and functionally heterogeneous



Caligiuri, M. A. Blood 2010

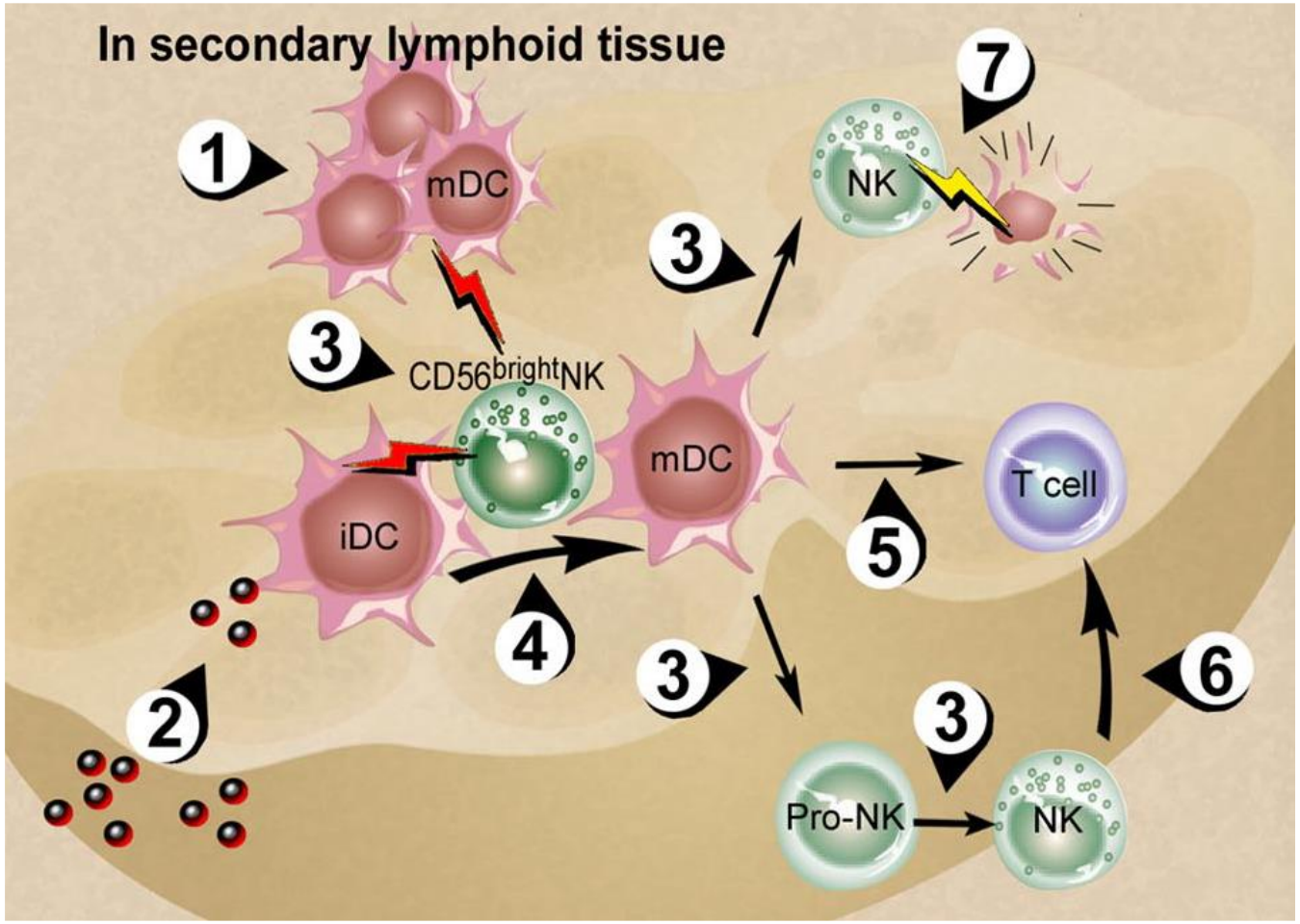
Natural Killer cell functional heterogeneity

- NK cells $CD3^{-}$ $CD56^{bright}$ $NKp46^{bright}$ $CD16^{dim}$
  - 100% in SLT, only 10% in blood
  - Produce abundant amount of cytokines and chemokines
  - Little cytotoxicity

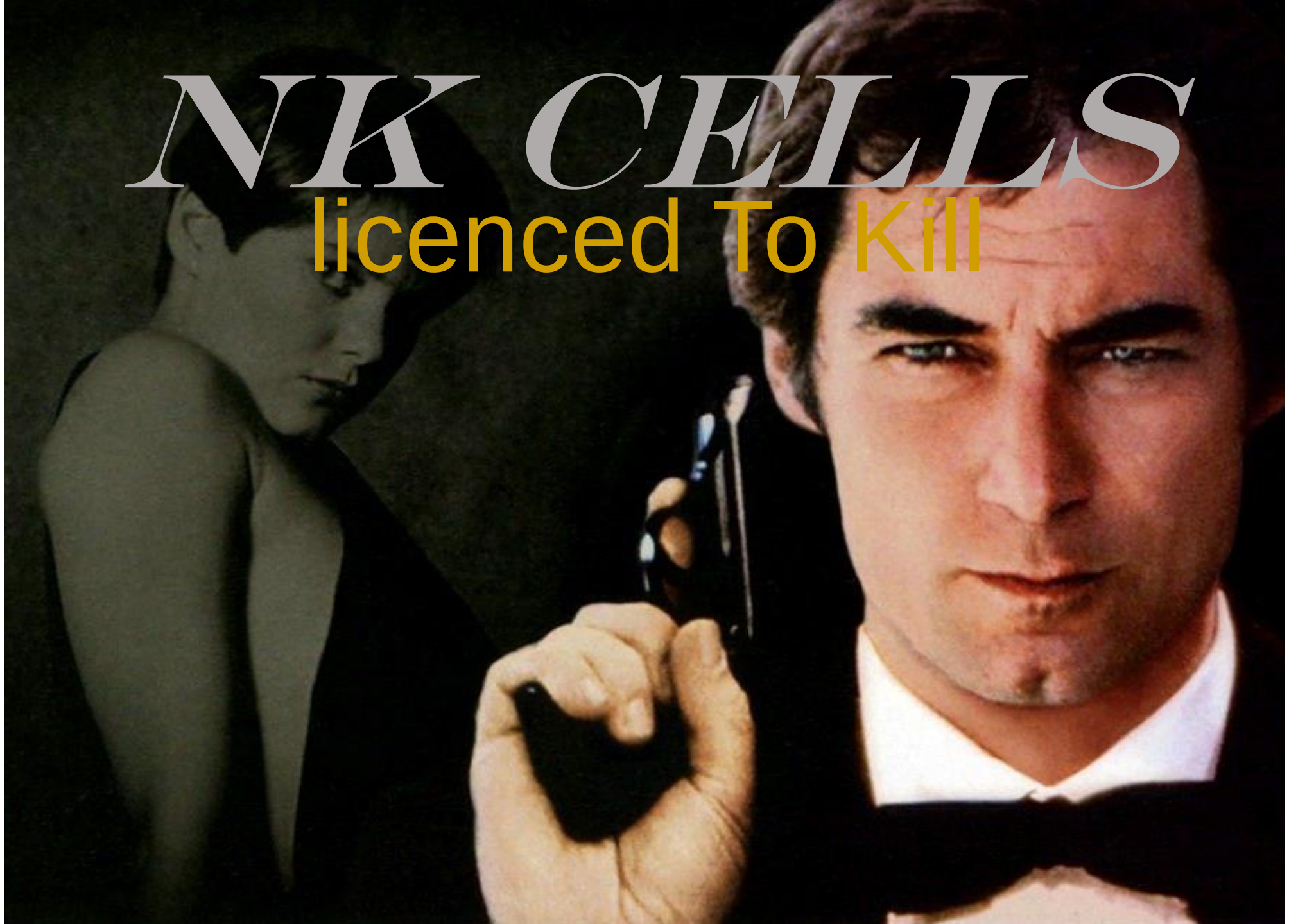
- NK cells $CD3^{-}$ $CD56^{dim}$ $NKp46^{dim}$ $CD16^{bright}$
  - Mostly circulating
  - Little cytokines and chemokines production
  - Highly cytotoxic

Natural Killer cell activation in SLT

- Bridges innate and adaptive immunity
  - Proximity with APC and T cell



Caligiuri, M. A. Blood 2010


NK CELLS
licenced To Kill

Natural Killer cell activating and inhibitory receptors

	Receptor	Ligand
MHC	KIR2DL1 (CD158a)	HLA-C group 2
	KIR2DL2/3 (CD158b)	HLA-C group 1
	KIR3DL1	HLA-B alleles
	KIR3DL2	HLA-A alleles
	LIR-1/ILT2 (CD85j)	Multiple HLA class I
Non-MHC	ITIM	
	NKG2A (CD94/CD159a)	HLA-E
	KLRG1	E/N/P-cadherin
	NKR-P1 (CD161)	LLT1
	Siglec-7 (CD328)	sialic acid
	Siglec-9 (CD329)	sialic acid
	IRp60 (CD300a)	?
	ITAM	
	CD16 (FcgRIIIA)	IgG
	NKp30 (CD337)	?
	NKp46 (CD335)	Viral hemaglutinin
	KIR2DS1–2	HLA-C (low affinity)
	KIR2DS3–6	?
	KIR3DS1	?
	NKG2C (CD94/159c)	HLA-E
	Non–ITAM	
	NKG2D (CD314)	ULBPs, MICA, MICB
	2B4 (CD244)	CD48
	CD2	LFA-3 (CD58)
	CRACC (CD319)	CRACC (CD319)
	NTB-A	NTB-A
	DNAM-1 (CD226)	PVR (CD155), CD112
	CD7	SECTM1, Galectin
	CD59	C8, C9
	BY55 (CD160)	HLA-C
	KIR2DL4 (CD158d)	HLA-G (soluble)
	CD44	Hyaluronan
	Integrin	
	LFA-1 (αLβ2, CD11a/18)	ICAM-1–5
	MAC-1 (αMβ2, CD11b/18)	ICAM-1,iC3b,Fibrinogen
	CD11c/18	ICAM-1, iC3b
	VLA-4 (α4β1, CD49d/29)	VCAM-1, Fibronectin
	VLA-5 (α5β1, CD49e/29)	Fibronectin

Bryceson Y. T Immunological Reviews 2006

Natural Killer activation relies on the balance between inhibition and activation signaling