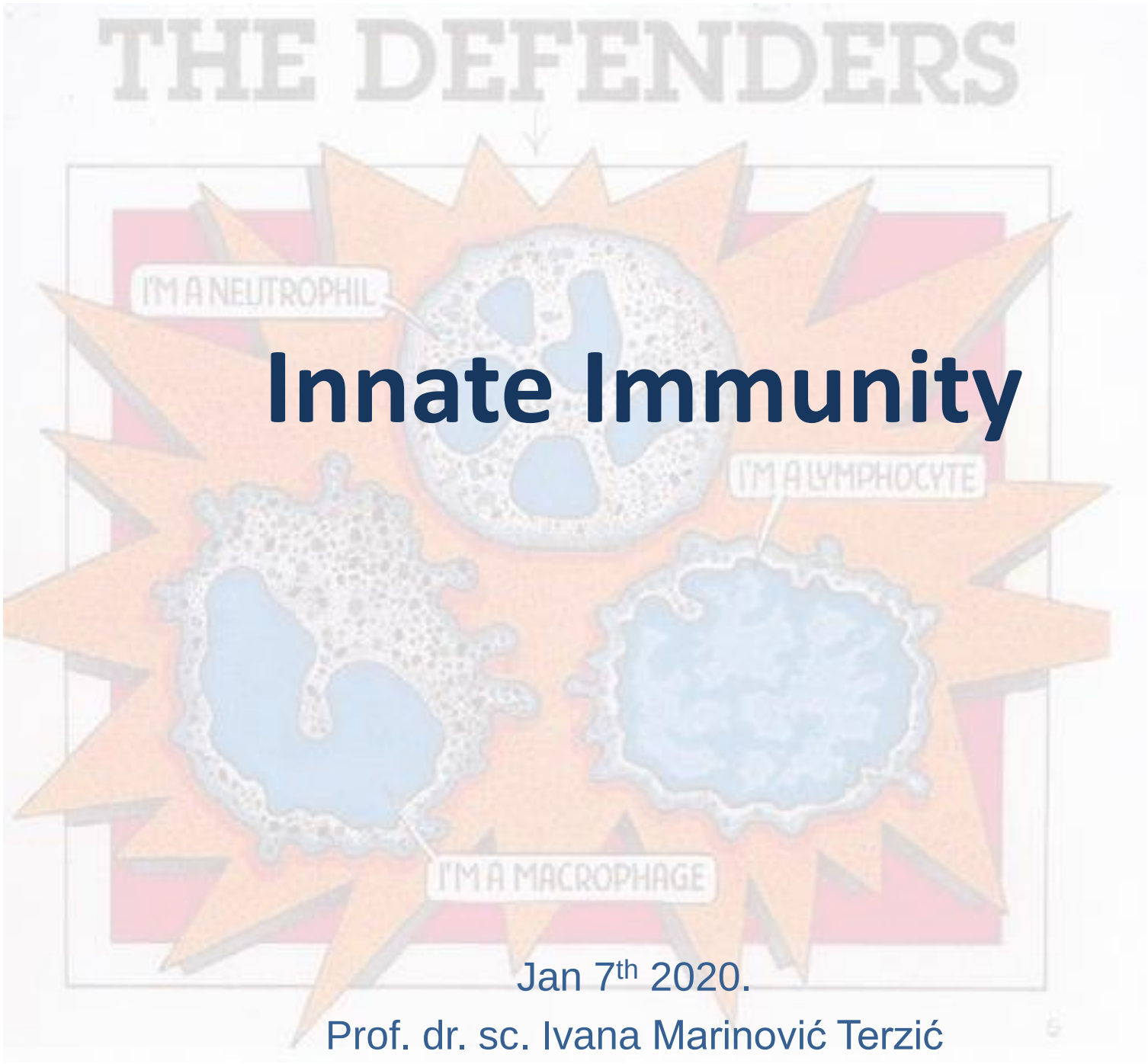
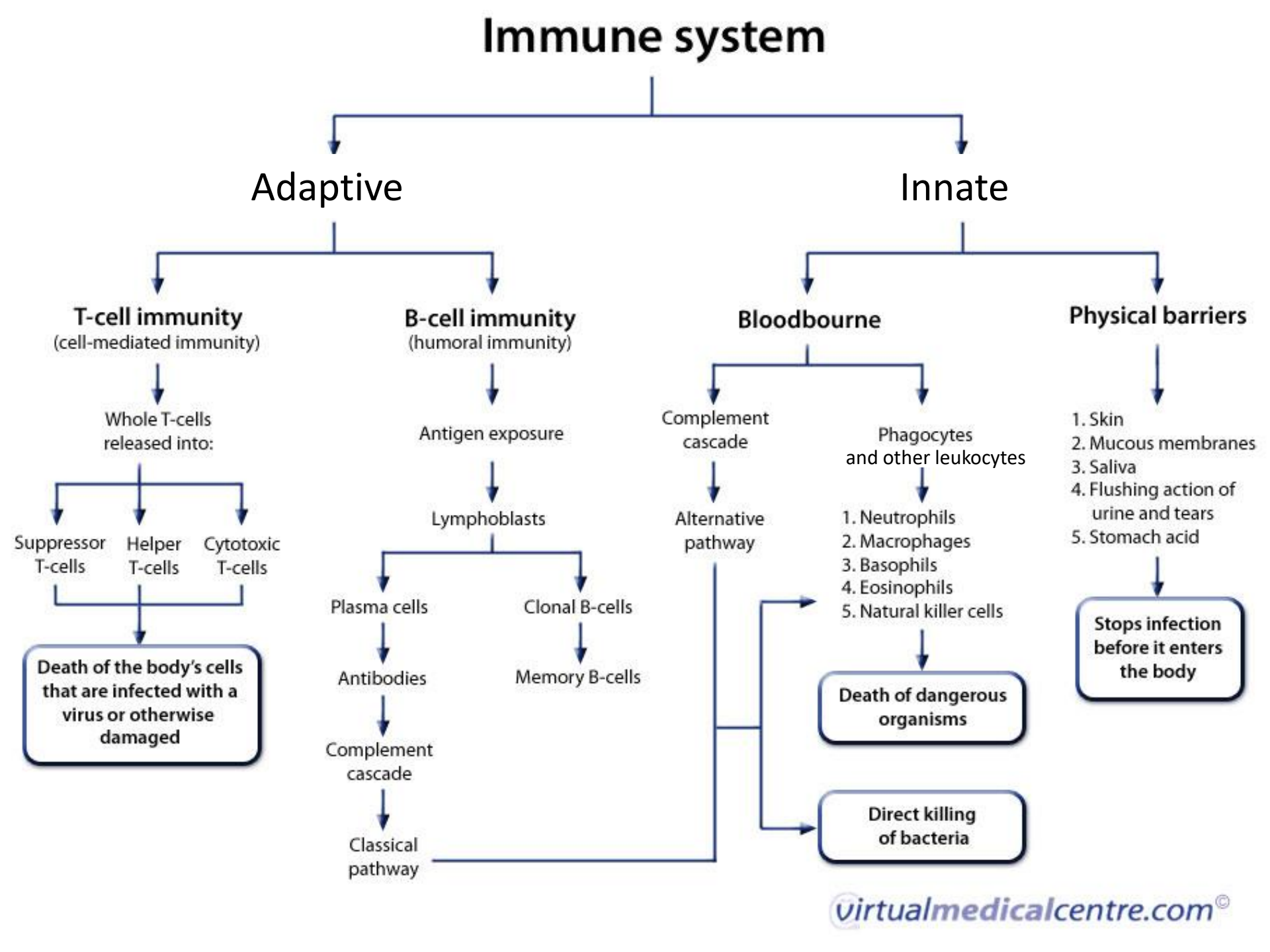


Innate Immunity

Jan 7th 2020.

Prof. dr. sc. Ivana Marinović Terzić


Immune system
Adaptive
Innate
T-cell immunity
(cell-mediated immunity)
B-cell immunity
(humoral immunity)
Bloodbourne
Physical barriers
Whole T-cells released into:
Antigen exposure
Complement cascade
Phagocytes and other leukocytes
1. Skin
2. Mucous membranes
3. Saliva
4. Flushing action of urine and tears
5. Stomach acid
Suppressor T-cells
Helper T-cells
Cytotoxic T-cells
Lymphoblasts
Alternative pathway
1. Neutrophils
2. Macrophages
3. Basophils
4. Eosinophils
5. Natural killer cells
Plasma cells
Clonal B-cells
Antibodies
Memory B-cells
Complement cascade
Classical pathway
Death of the body's cells that are infected with a virus or otherwise damaged
Death of dangerous organisms
Direct killing of bacteria
Stops infection before it enters the body
virtualmedicalcentre.com©

Immune system overview

- **1st line of defense**
  - **skin** (2m^2) and **mucosal membranes** (~400m^2):

    physical barrier, lymphoid cells, antimicrobial molecules
- **2nd line of defense**
  - **Innate immune system**: macrophages, NK cells, neutrophils, complement, cytokines
- **3rd line of defense**
  - **Adaptive immune system**: T cells, B cells, antibodies, cytokines

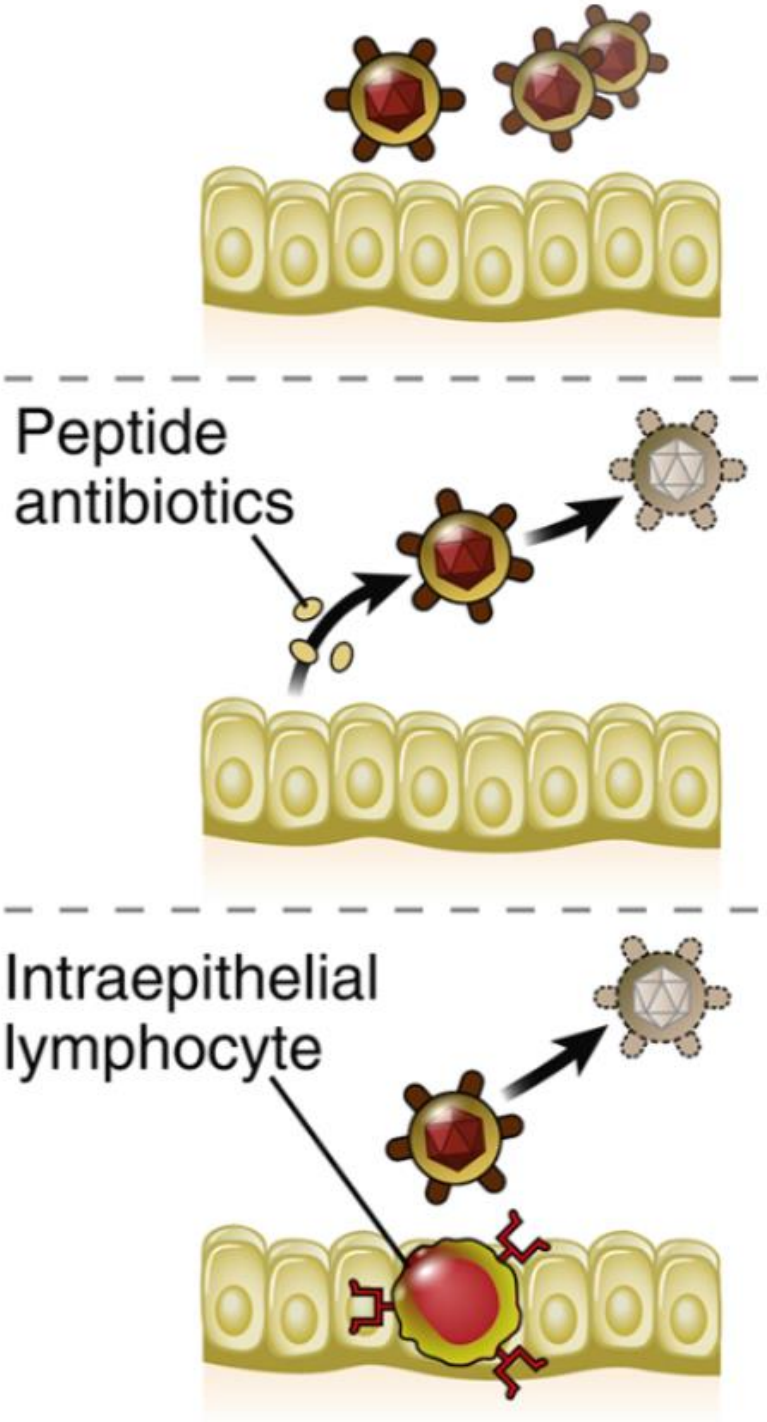
Epithelial barriers

- Skin, gastrointestinal tract, respiratory tract, urogenital tract
  - epithelia that provide physical and chemical barrier against infection
- Epithelial and immune cells produce peptide antibiotics
  - human beta-defensin HBD1 i HBD2 – interferes with bacterial membrane cations
- Intraepithelial lymphocytes
  - recognize microbial lipids
  - not very specific

Physical barrier to infection

Killing of microbes by locally produced antibiotics

Killing of microbes and infected cells by intraepithelial lymphocytes



Innate immune system

…or natural or native immunity

- **2nd line of defense** -- works against invaders that breach physical barriers of skin and mucosa – microbes (and dead cells, debris)
- "Innate" - shared by all animals

  (vertebrates and invertebrates)

Innate immune system

…or natural or native immunity

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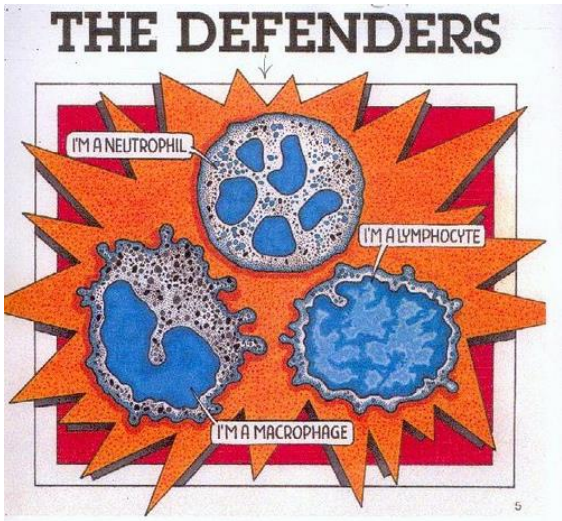
  (vertebrates and invertebrates)

Innate immune system

- It is NOT specific as adaptive!
- It is NOT weak!
- **Specifically targets microbes**
- **Powerful early defense mechanism**
- **Removes infections**
- **Instructs adaptive immunity**



The innate immune system responds more quickly than adaptive immune system. Why is a quick response important?

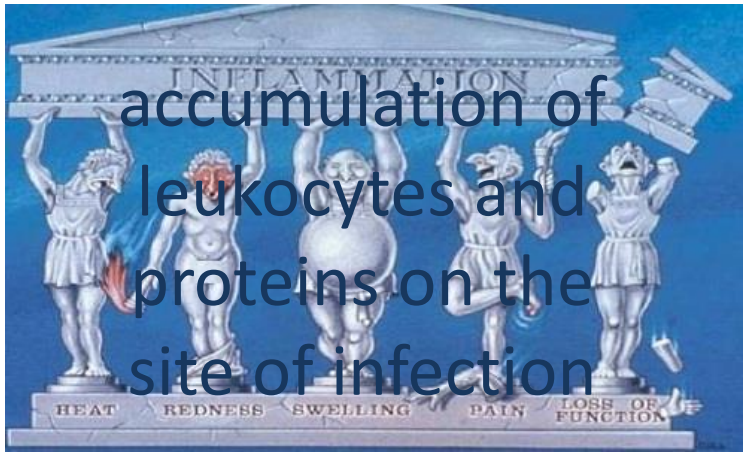
- Starting with one bacterium that doubles every thirty minutes --> 10^{14} bacteria in one day
- 10^{14} bacteria equivalent to ~100 liters of a dense culture
- Total volume of blood in human ~5 liters
- **VERY** important to check a bacterial infection quickly!

Major questions

- How does the innate immune system recognize microbes?
- How do the different components of innate immunity function to combat different microbes?
- How do innate immune reactions stimulate adaptive immune responses?

2 major types of innate immune responses

Inflammation



accumulation of leukocytes and proteins on the site of infection

Antiviral defense

killing of virus infected cells

Recognition of microbes

- Structures shared by various microbes

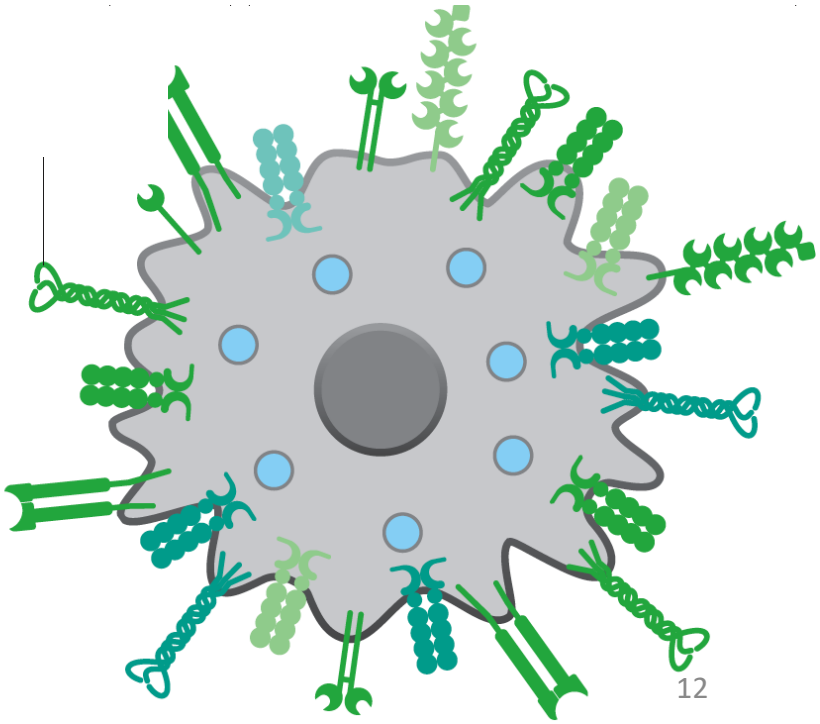
  (bacteria, viruses, fungi,…)

  But! Not present on the host cells

- Recognized by receptors on

  the surface or inside of

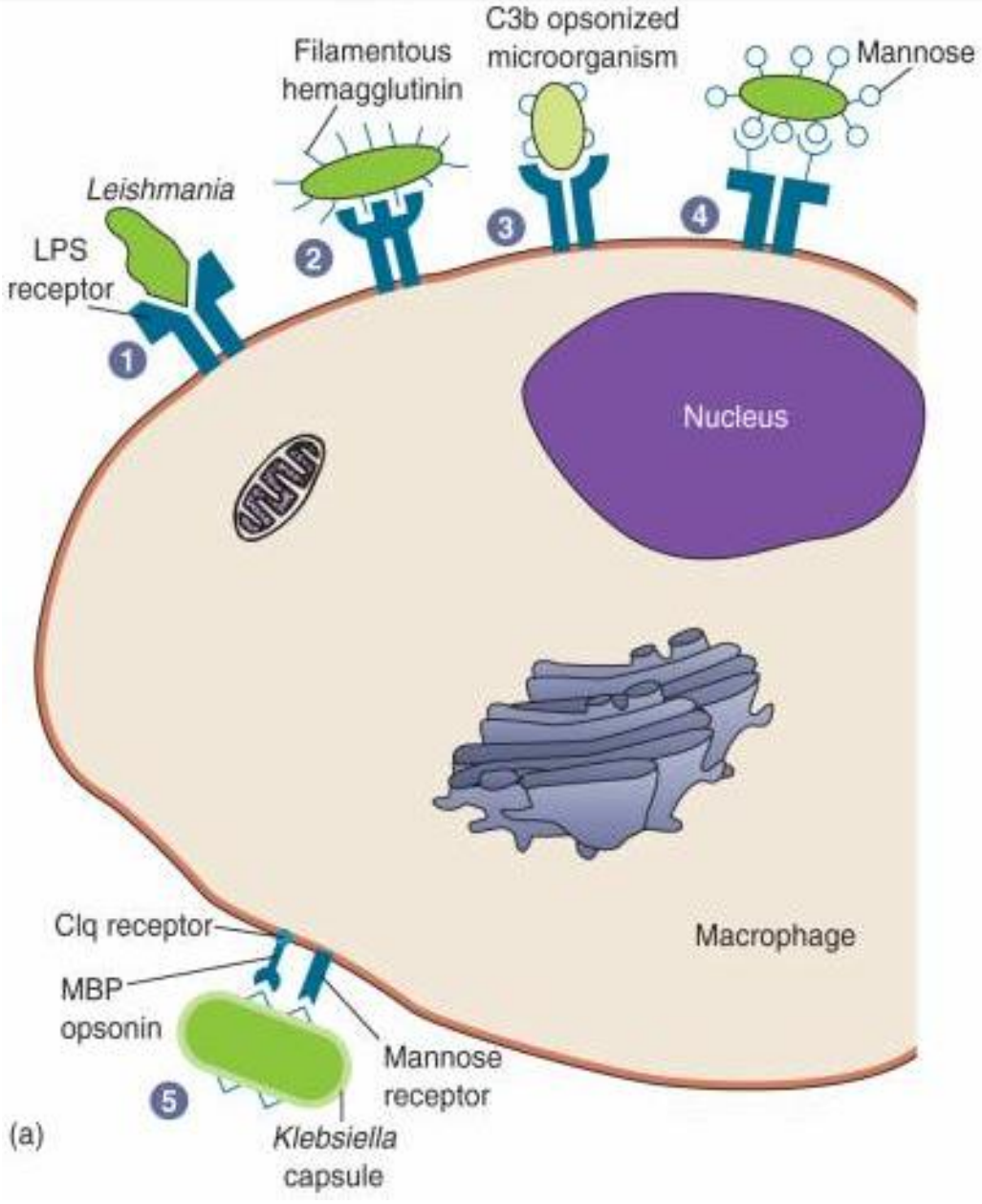
  the innate immunity cells



Receptors on the phagocyte surface

LPS (lipopolysaccharide)
Mannose
dsRNA
Unmethylated CpG nt
Hemagglutinin
= **pathogen-associated molecular patterns, PAMPS**

➡ **pattern recognition receptors**



Innate immunity receptors

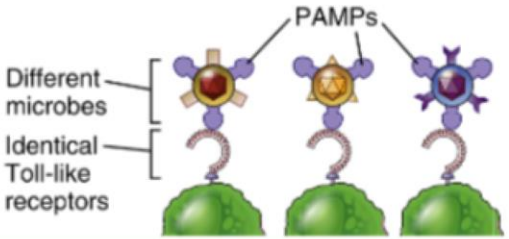
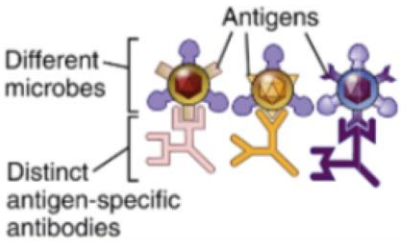
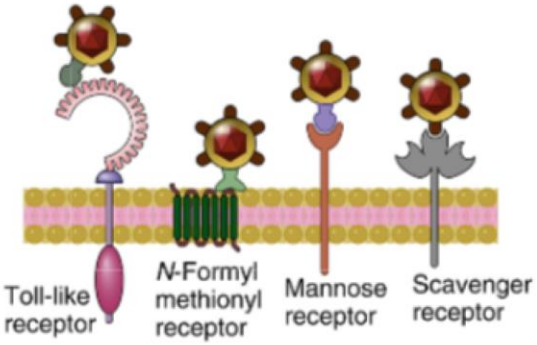
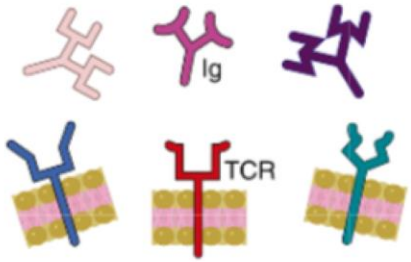
- Encoded in the germline
- Not produced by somatic recombination genes
- All of the receptors of the innate immunity recognize less than a thousand patterns (adaptive: > billion)
- Nonclonally distributed – identical receptors on all cells of particular type

Innate immunity receptors (cont.)

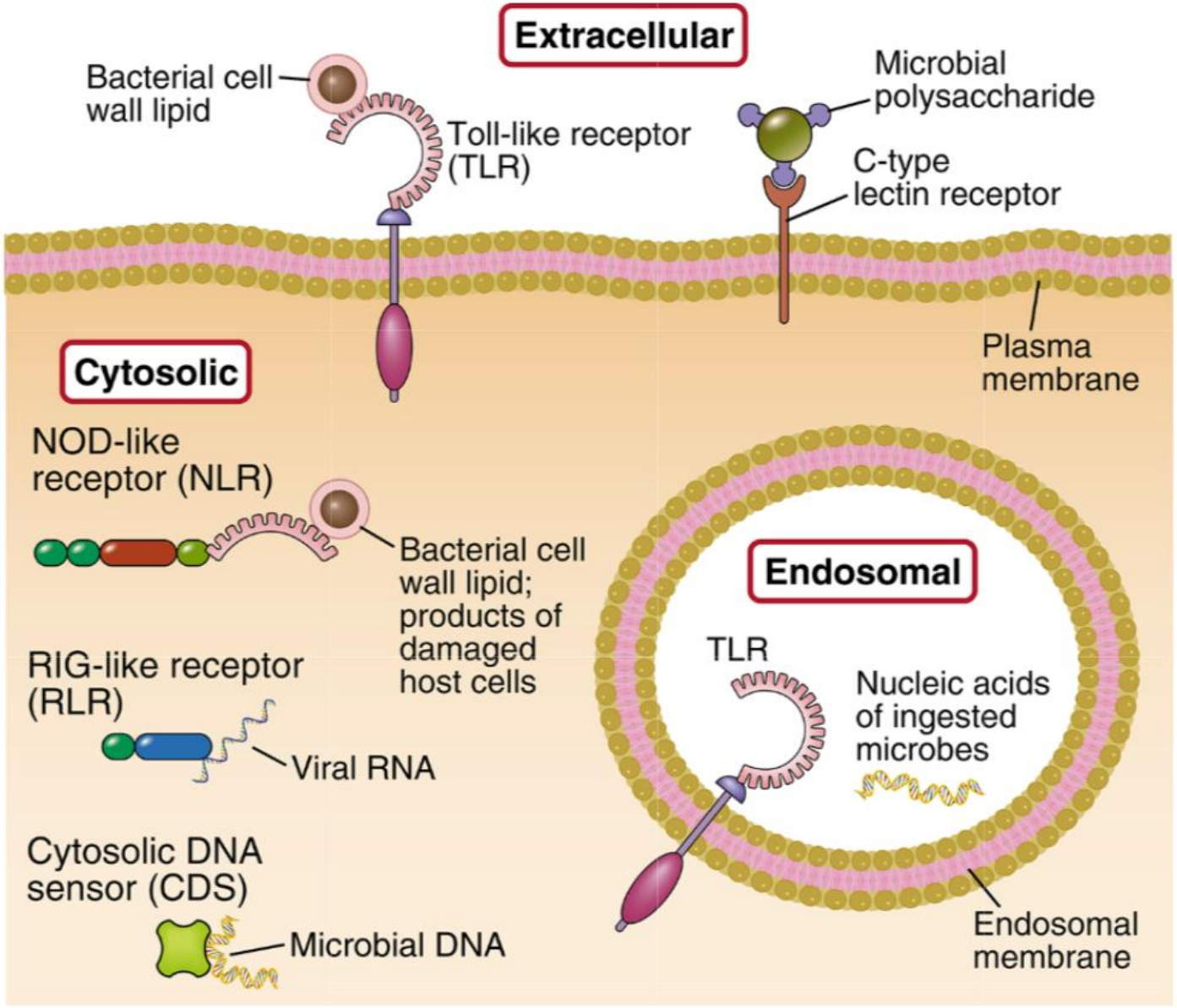
- No reaction against “healthy self” (host)

Why?

1. Specificity for microbial structures and damage components
2. Expression of regulatory molecules on the surface of the mammalian cells to prevent innate immune reactions

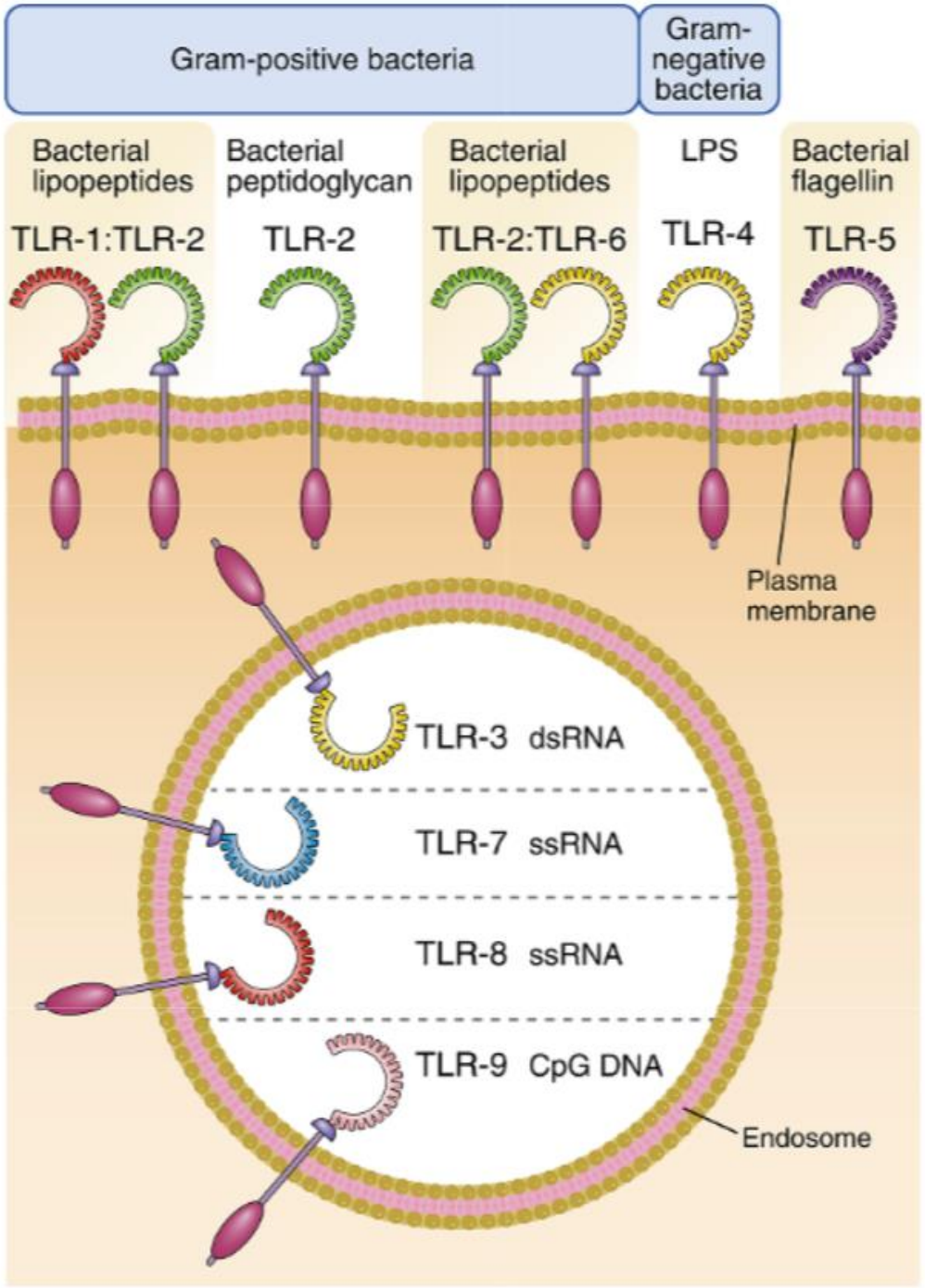
Feature	Innate immunity	Adaptive immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns) 	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Number of microbial molecules recognized	About 1000 molecular patterns (estimated)	>10^7 antigens
Receptors	Encoded in germline; limited diversity (pattern recognition receptors) 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Number and types of receptors	<100 different types of invariant receptors	Only 2 types of receptors (Ig and TCR), with millions of variations of each
Distribution of receptors	Nonclonal: Identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Genes encoding receptors	Germline encoded, in all cells	Formed by somatic recombination of gene segments only in B and T cells
Discrimination of self and nonself	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (hence the possibility of autoimmunity)

Cellular receptors for microbes



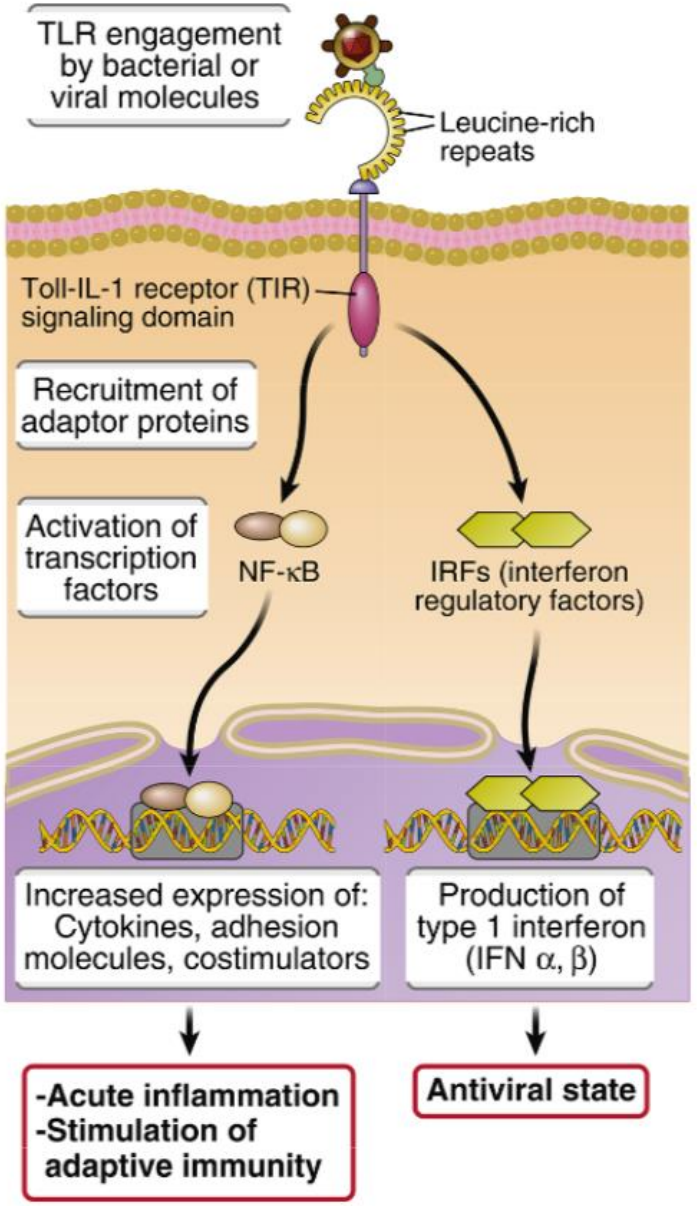
Toll-like receptors (TLRs)

- Specific for different microbial component
- TLR-1, TLR-2 & TLR-6: bacterial lipoglycans
- TLR-3, TLR-7, TLR-8 & TLR-9: viral nucleic acids
- TLR-4: bacterial LPS
- TLR-5: bacterial flagellin
- TLR-9: unmethylated CpG-rich nt


Gram-positive bacteria
Gram-negative bacteria
Bacterial lipopeptides
Bacterial peptidoglycan
Bacterial lipopeptides
LPS
Bacterial flagellin
TLR-1:TLR-2
TLR-2
TLR-2:TLR-6
TLR-4
TLR-5
Plasma membrane
TLR-3 dsRNA
TLR-7 ssRNA
TLR-8 ssRNA
TLR-9 CpG DNA
Endosome

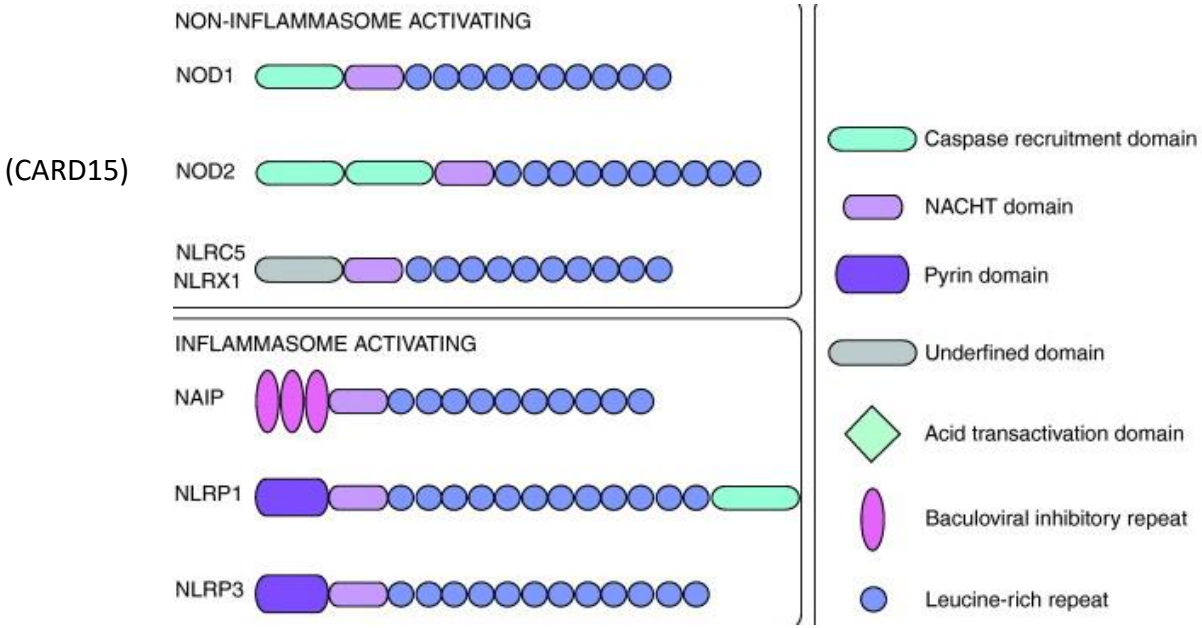
Toll-like receptor activation

- Activate transcription of various cytokines, enzymes, adhesion molecules, …
- transcription factors:
  - NF-κB (nuclear factor κB)
  - IRF-3 (interferon regulatory factor 3)



NOD-like receptors

- The **nucleotide-binding oligomerization domain receptors – NOD**
- Intracellular sensors of PAMPs (fagocytosis) and DAMPs (cellular stress)
- lymphocytes, macrophages, dendritic cells, non-immune cells (epithelial)

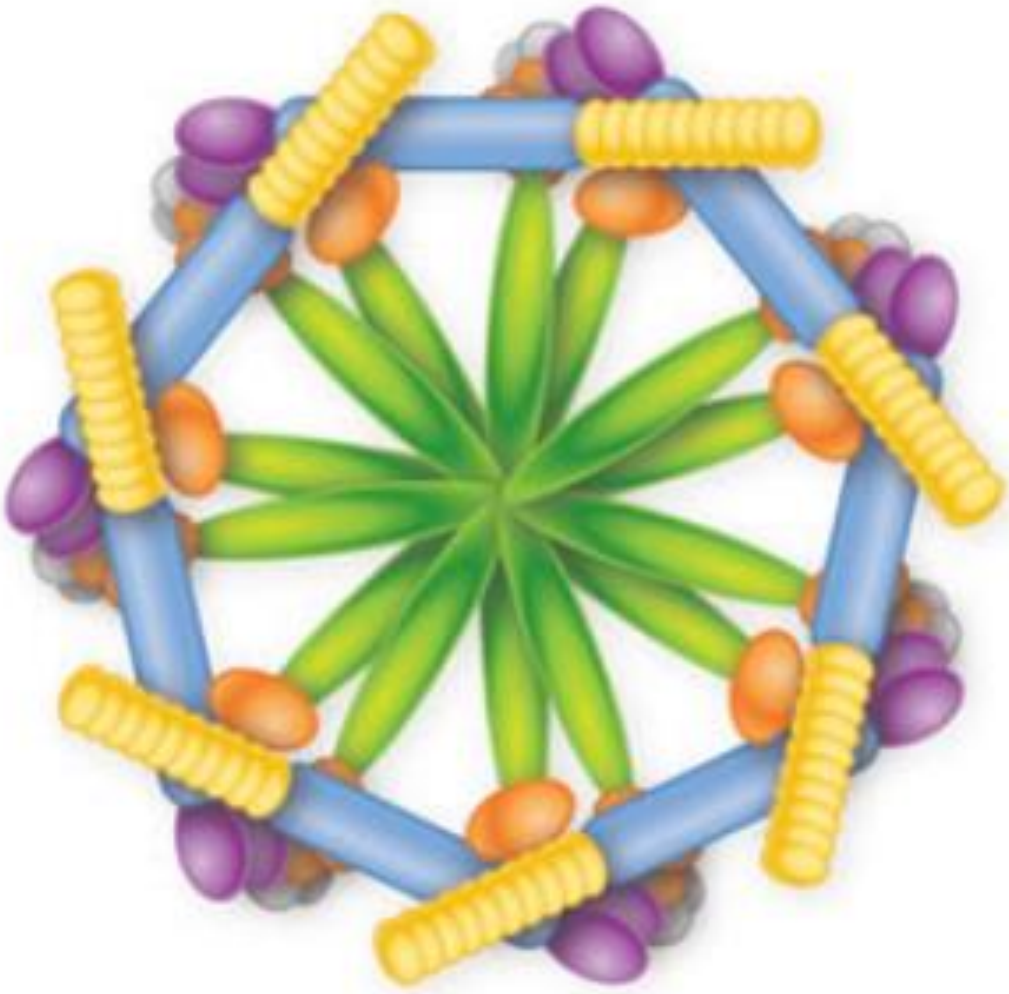


PAMP – pathogene associated mollecular patterns

DAMP – damage associated mollecular patterns

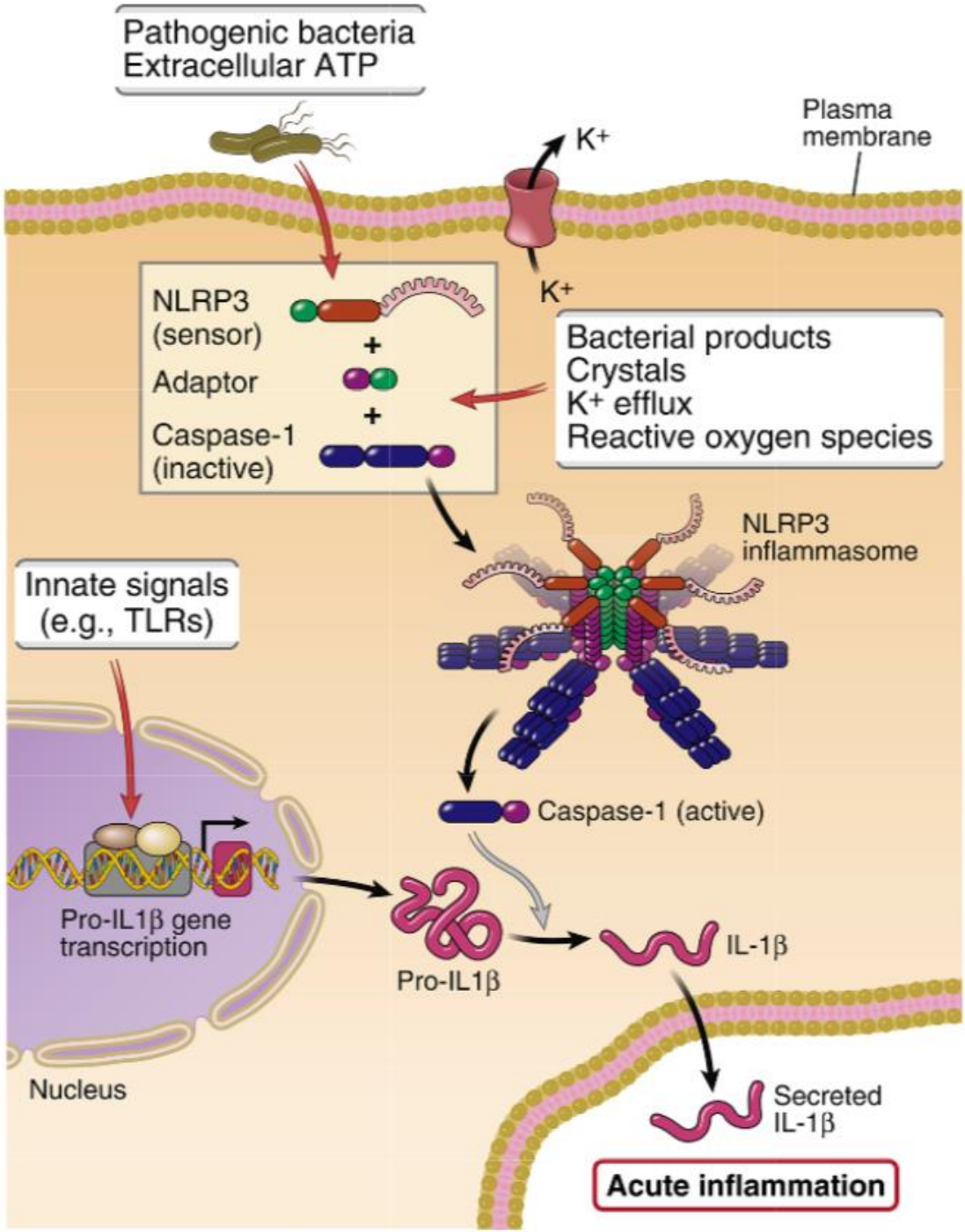
Inflammasome

Protein complex that recognizes diverse set of inflammation-induced stimuli, including PAMPs and DAMPs



Where are inflammasomes?

In cytoplasm!



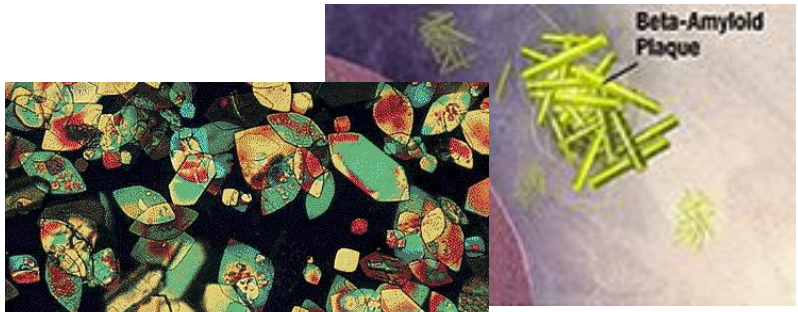
What are inflammasomes sensing?

Pathogenic signals:

- Viral RNA
- Messenger RNA microbial origin
- Toxins

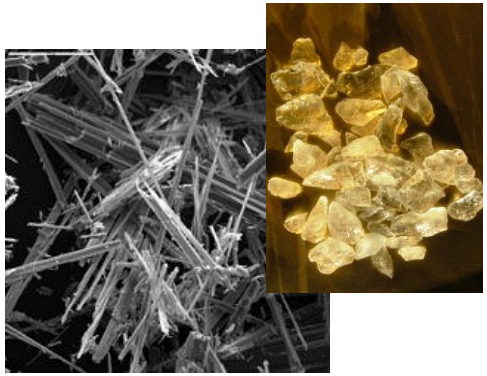
Endogenous signals:

- extracellular ATP
- hyaluronic acid
- β-amyloids
- Uric acid crystals



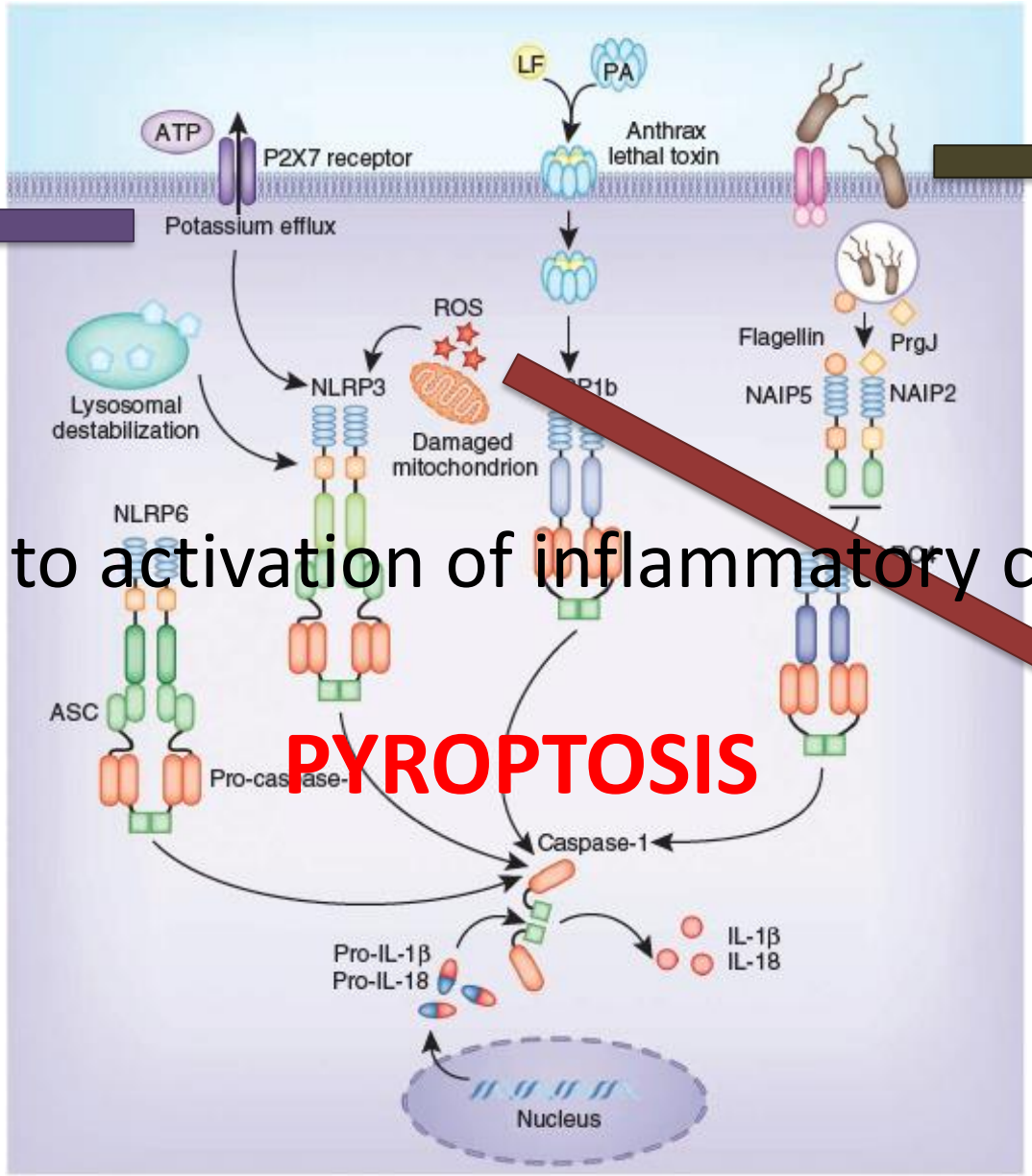
Environmental signals:

- Asbestos
- Silica
- Alum



Inflammasome activation

Potassium efflux: Induced by bacterial toxins, extracellular ATP



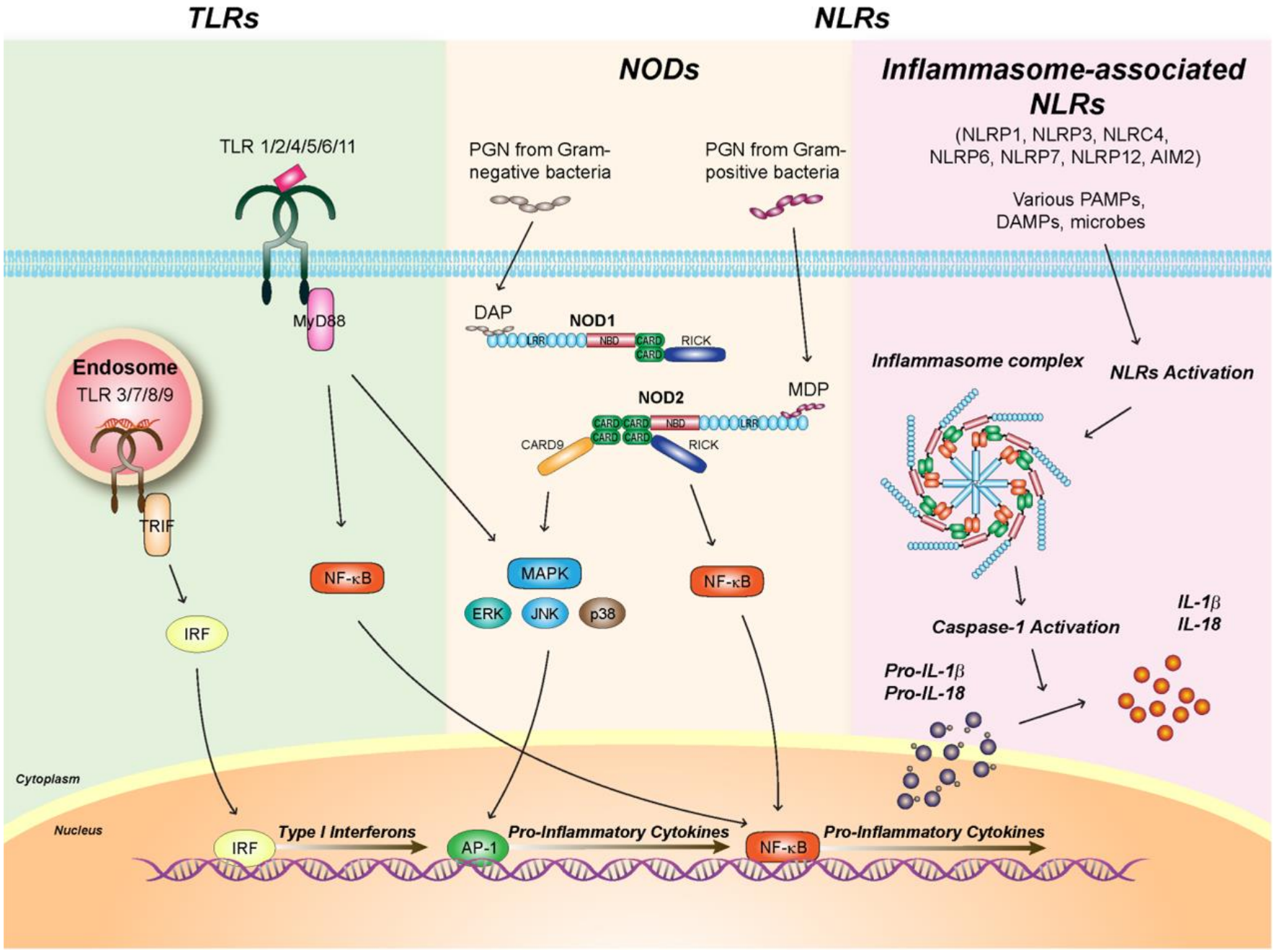
Microbial digestion products – flagellin,…

All leads to activation of inflammatory caspases

PYROPTOSIS

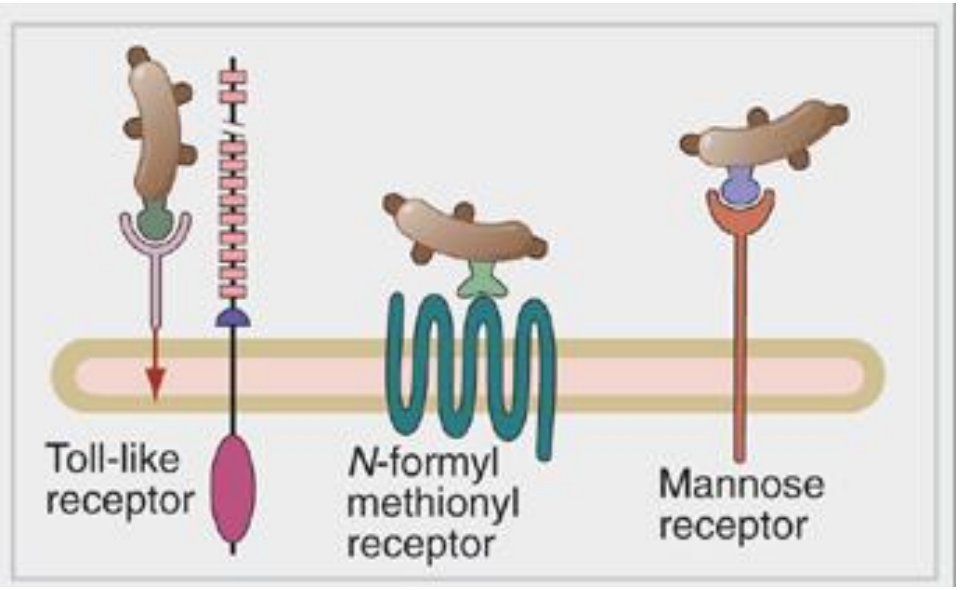
ROS, asbestos, silica, Aluminum, …..

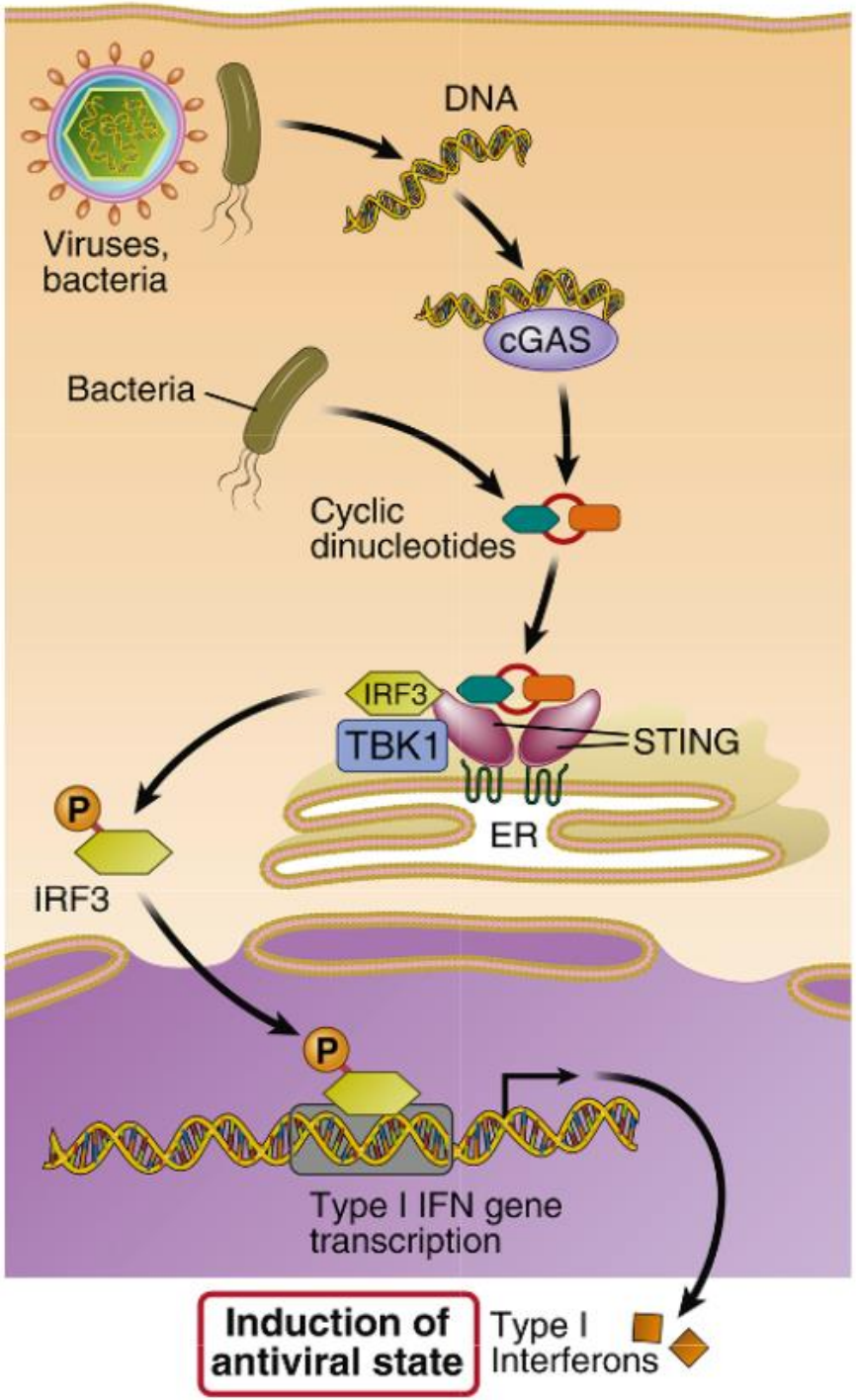
TOLL-like receptors and NOD-like receptors



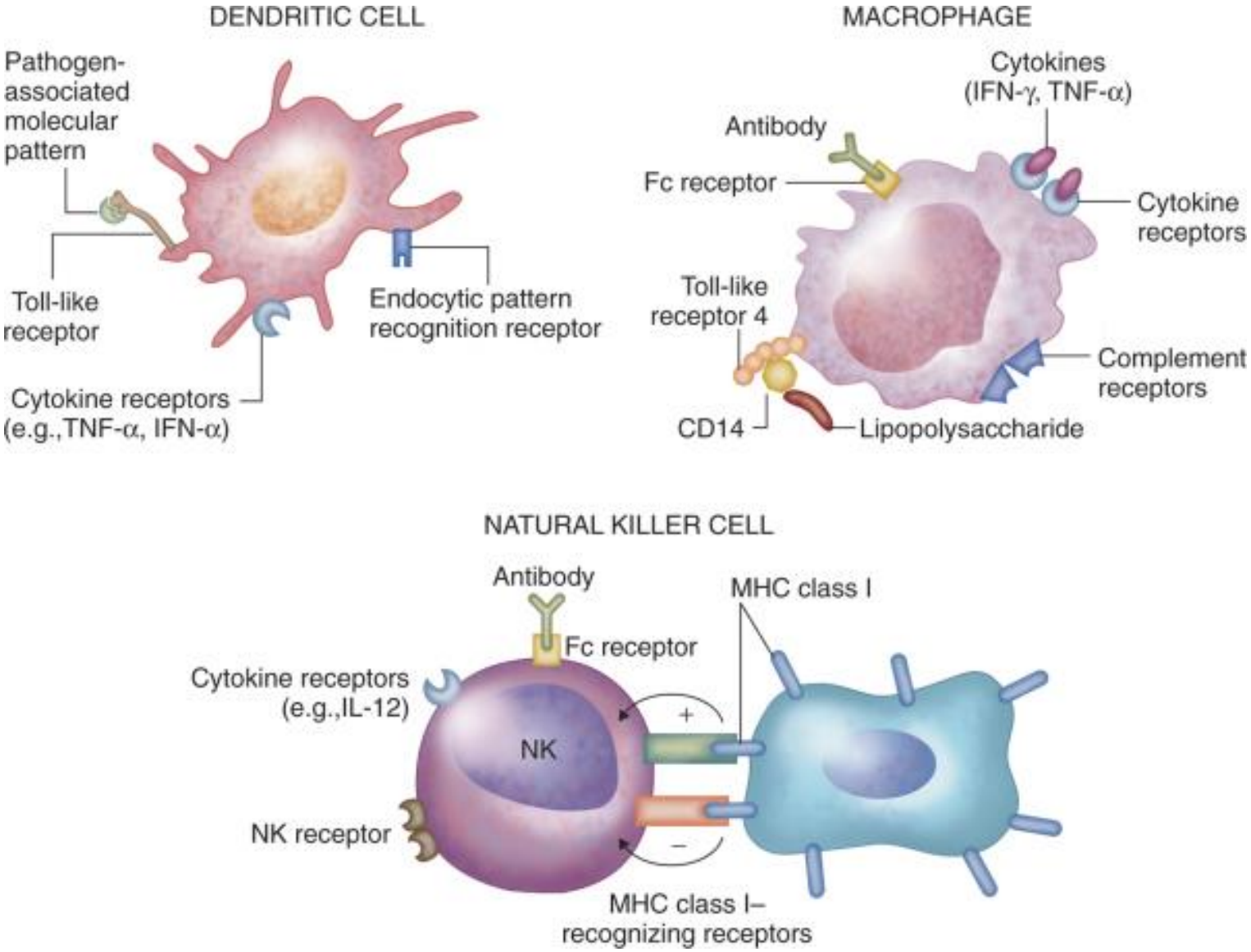
Other receptors

- Mannose receptor, lectin receptors
- N-formylmethionine receptor
- Scavenger receptors (modified LDL, atherosclerosis)
- RIG-like helicases (viral RNA)
- Cytosolic DNA sensor (CDS)



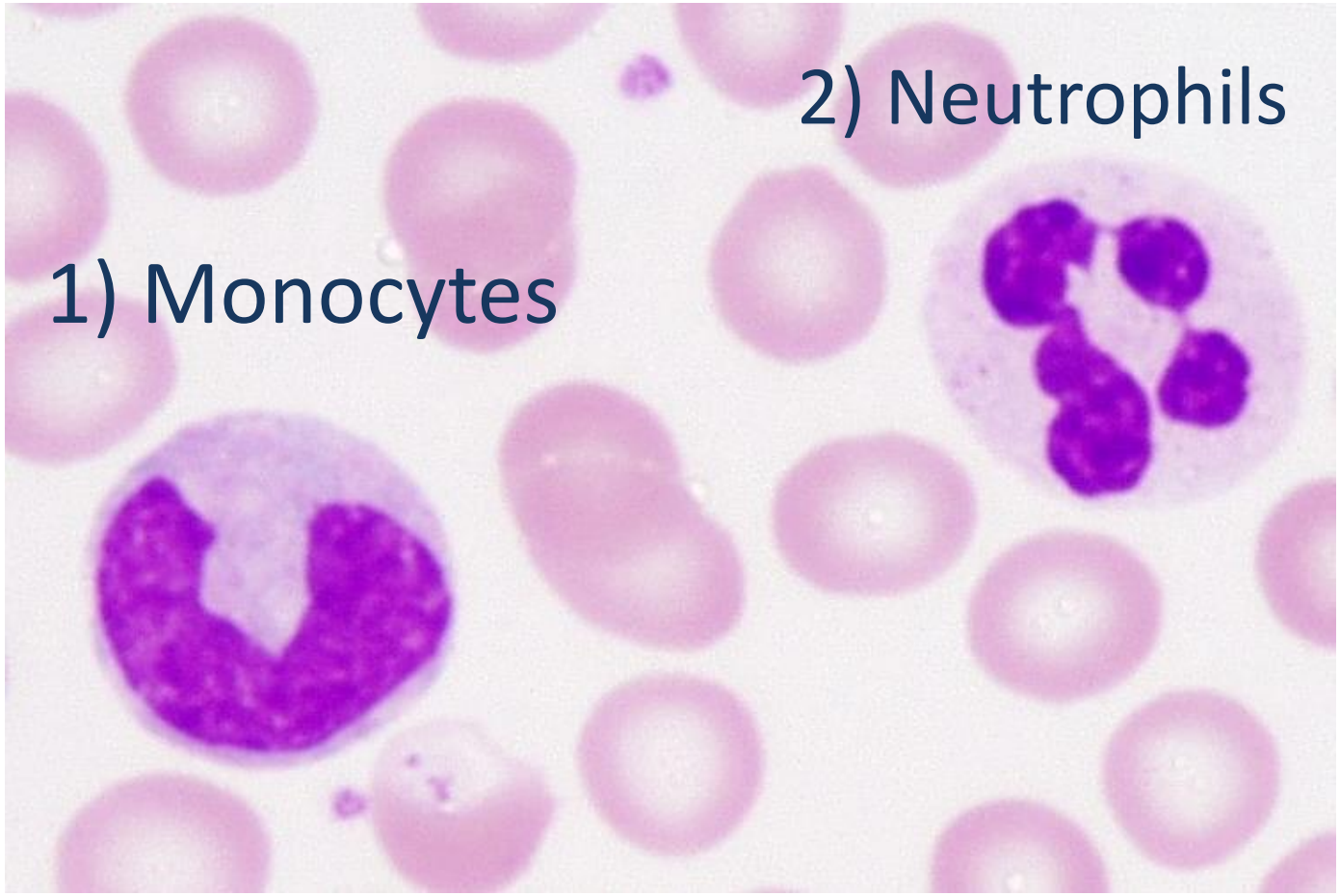

DNA
Viruses,
bacteria
cGAS
Bacteria
Cyclic
dinucleotides
IRF3
TBK1
STING
ER
P
IRF3
P
Type I IFN gene
transcription
Induction of
antiviral state
Type I
Interferons

Immune cells



Phagocytes

- 2 types of circulating phagocytes:

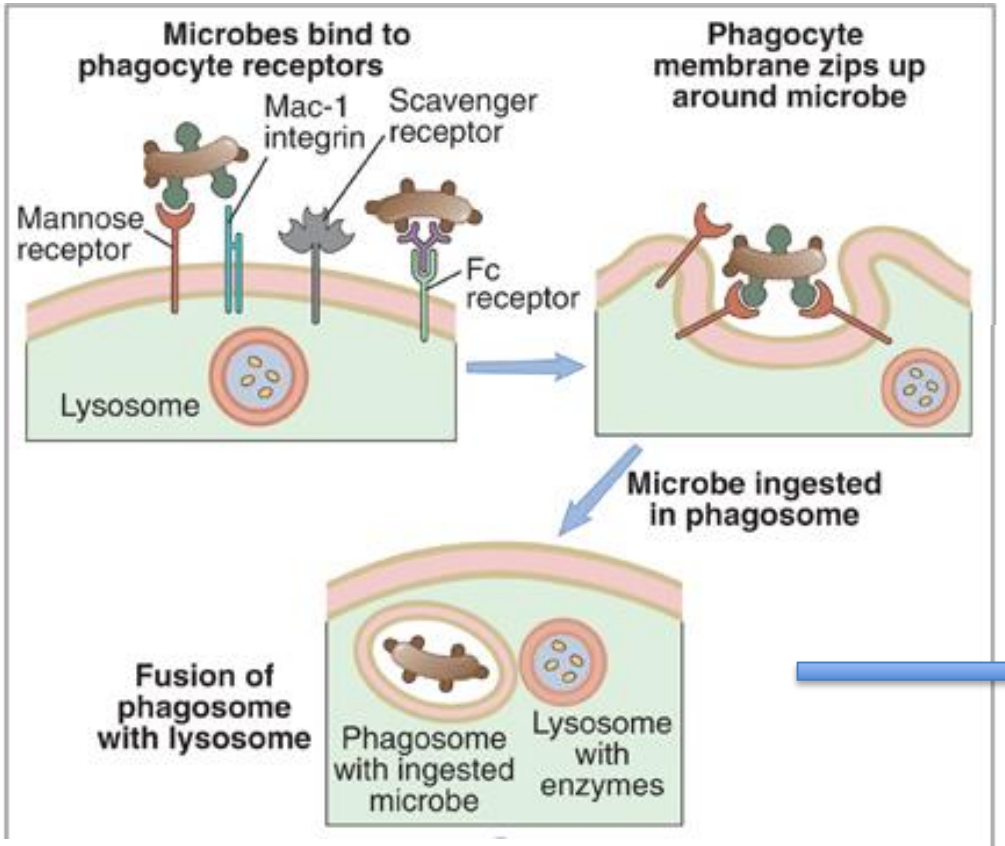


Feature	Neutrophils	Macrophages
Origin	HSCs in bone marrow	HSCs in bone marrow (in inflammatory reactions) Many tissue-resident macrophages: stem cells in yolk sac of fetal liver (early in development)
Life span in tissues	1–2 days	Inflammatory macrophages: days or weeks Tissue-resident macrophages: years
Responses to activating stimuli	Rapid, short lived, enzymatic activity	More prolonged, slower, often dependent on new gene transcription
Phagocytosis	Rapid ingestion of microbes	Prolonged ability to ingest microbes, apoptotic cells, tissue debris, foreign material
Reactive oxygen species	Rapidly induced by assembly of phagocyte oxidase (respiratory burst)	Less prominent
Nitric oxide	Low levels or none	Induced following transcriptional activation of iNOS
Degranulation	Major response; induced by cytoskeletal rearrangement	Not prominent
Cytokine production	Low levels per cell	Major functional activity, large amounts per cell, requires transcriptional activation of cytokine genes
Extracellular traps	Rapidly induced, by extrusion of nuclear contents	Little
Secretion of lysosomal enzymes	Prominent	Less

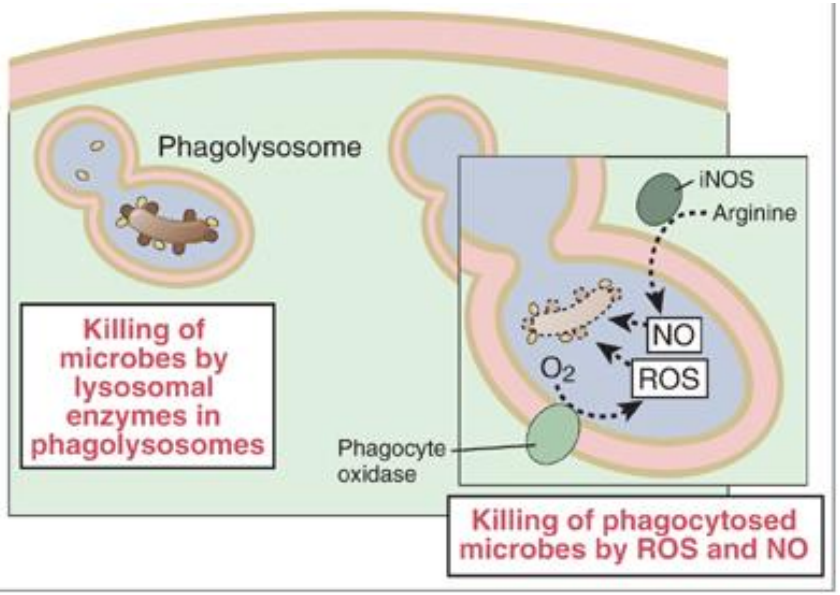
Phagocytosis

- Neutrophils and macrophages ingest and destroy microbes
- Steps:
  1. Receptors binding to microbe
  2. Membrane extension
  3. Closing and pinching of phagosome
  4. Fusion with lysosome for digestion

Phagocytosis



Enzymes: phagocyte oxidase & nitric oxide synthase



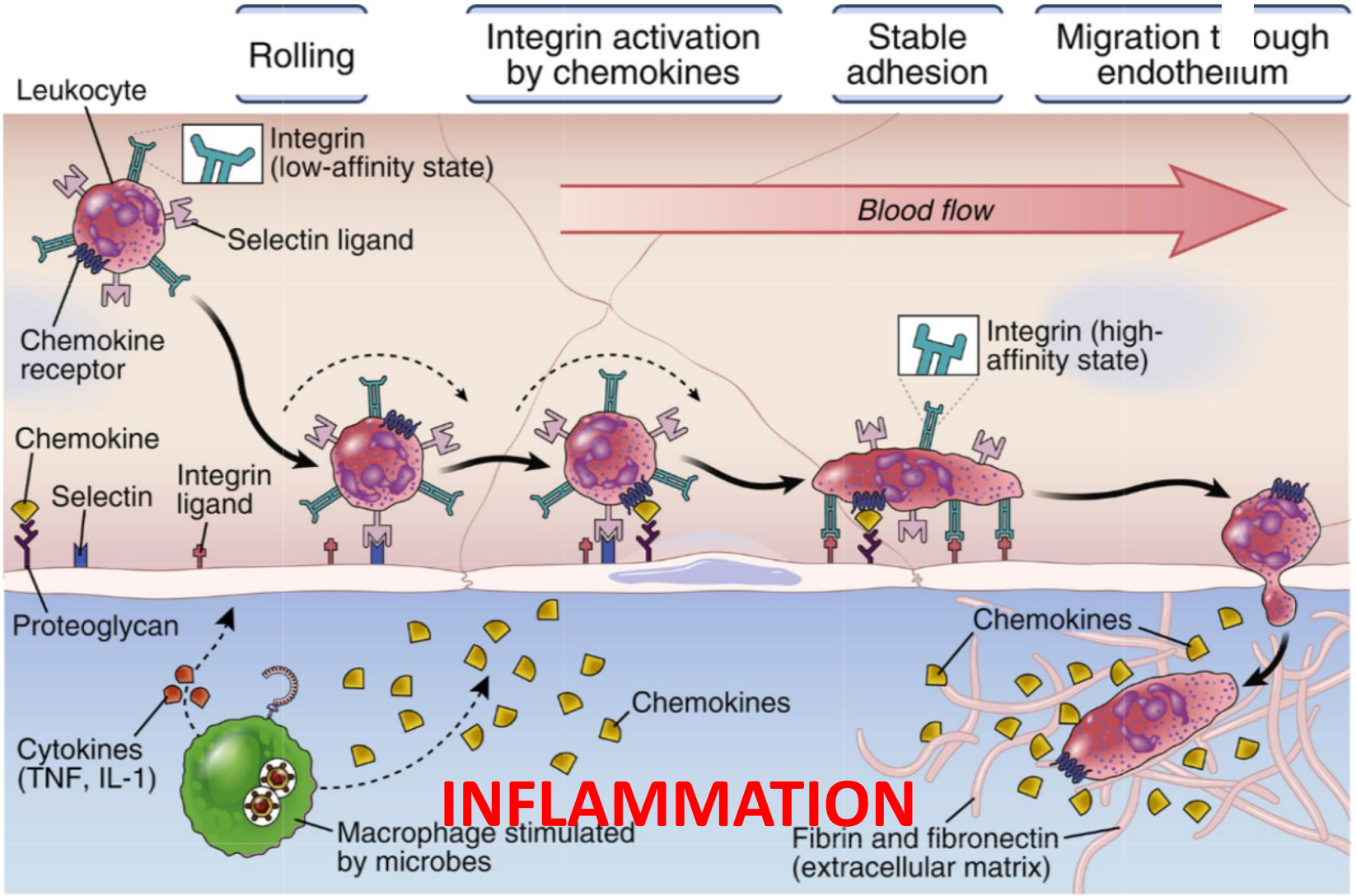
Neutrophils
(or polymorphonuclear leukocytes – PMNs)

- Most abundant leukocytes in blood (4-10 x 10^9/L)
- Upon infection – production (in bone marrow) increases rapidly (up to 20 x 10^9/L)
- Production stimulated by CSF secreted by many cell types
- First cell type to respond to infection

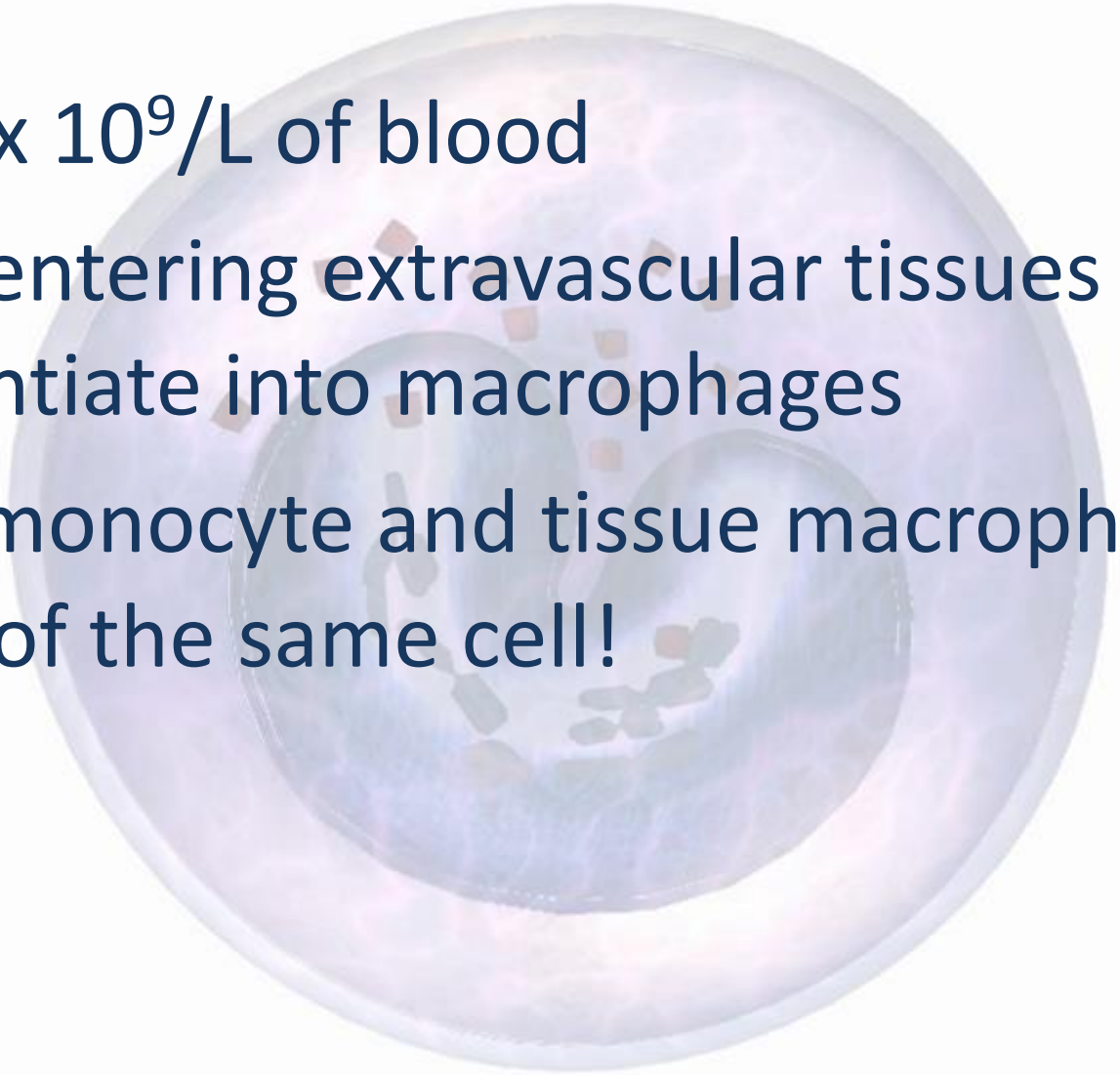
Leukocyte migration

- Multistep process
- Microbe breach through epithelium – recognized by macrophages – production of cytokines: TNFα and IL-1 to stimulate endothelial cells to rapidly express selectins
- Selectins necessary for leukocyte adhesion
- Production of chemokines by endothelial cells and macrophages

Leukocyte migration



Monocytes



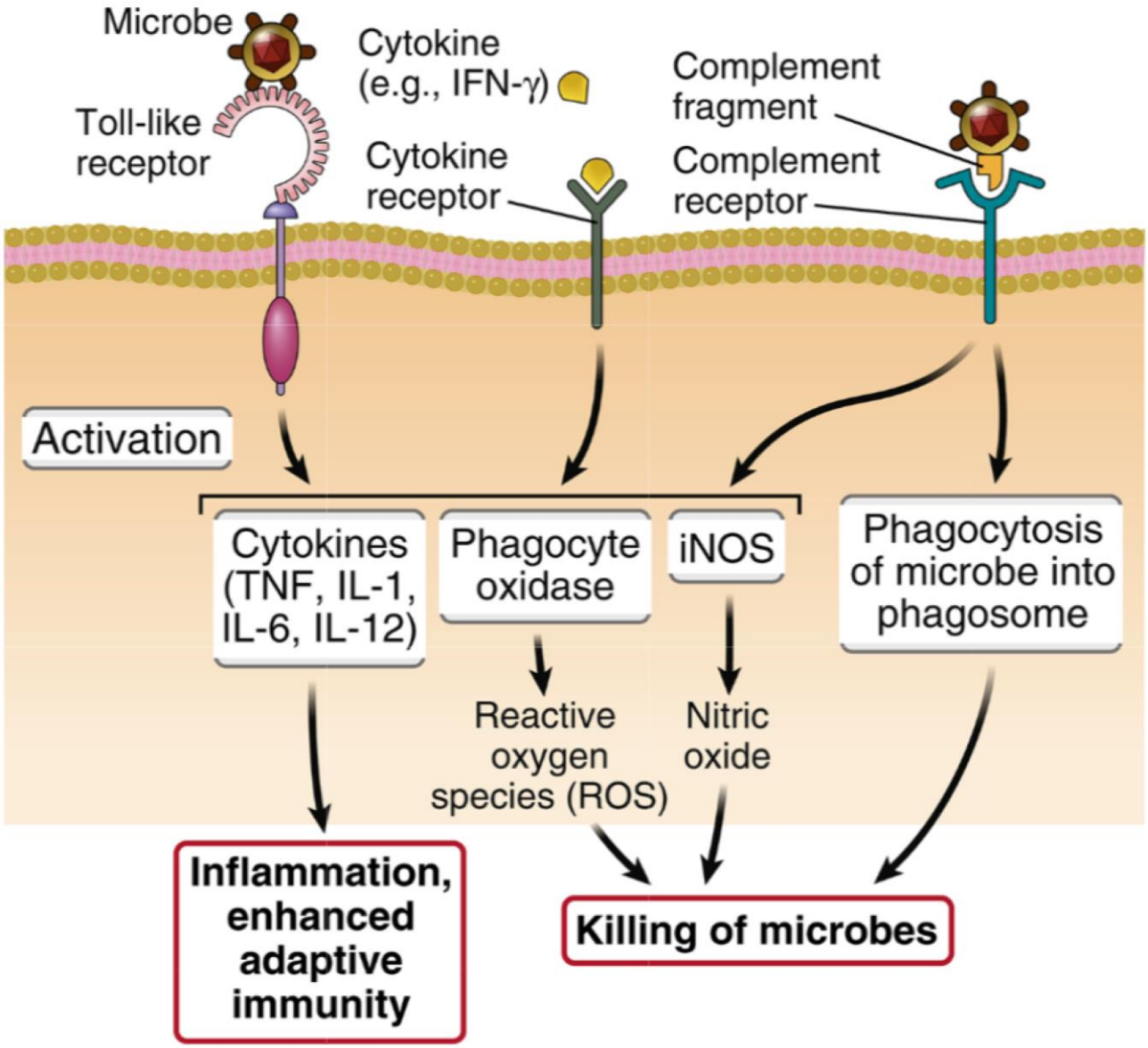
- 0,5 - 1 x 10^9/L of blood
- When entering extravascular tissues differentiate into macrophages
- Blood monocyte and tissue macrophage are 2 stages of the same cell!

Macrophages

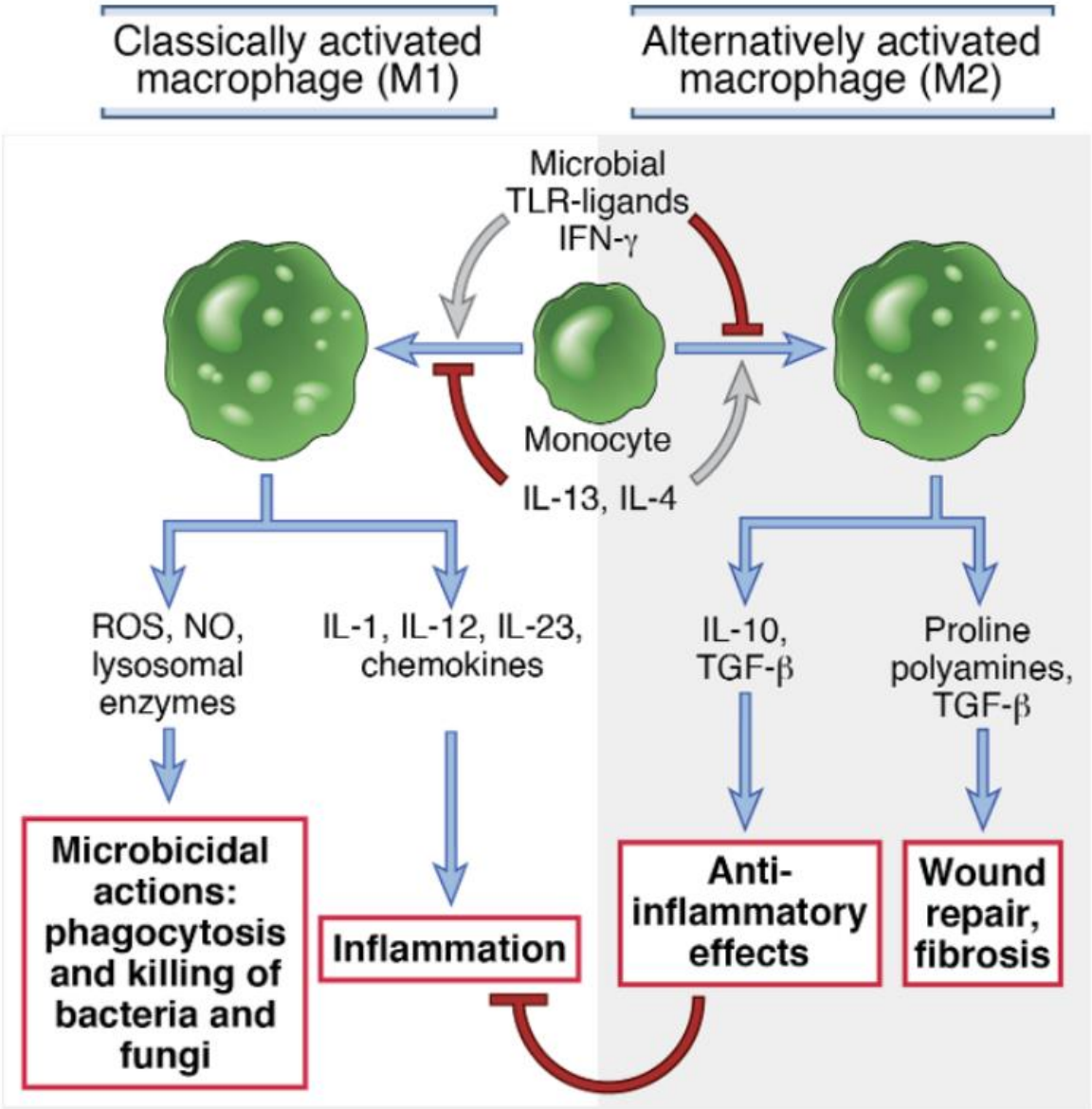
- Perform several functions:

1. Phagocytosis
2. Production of cytokines to activate leukocytes
3. Secrete growth factors and enzymes to repair injured tissue
4. Stimulate T lymphocytes to enhance adaptive immunity
5. Respond to products of T cells to function in cell-mediated immunity

Classical macrophage activation (M1)



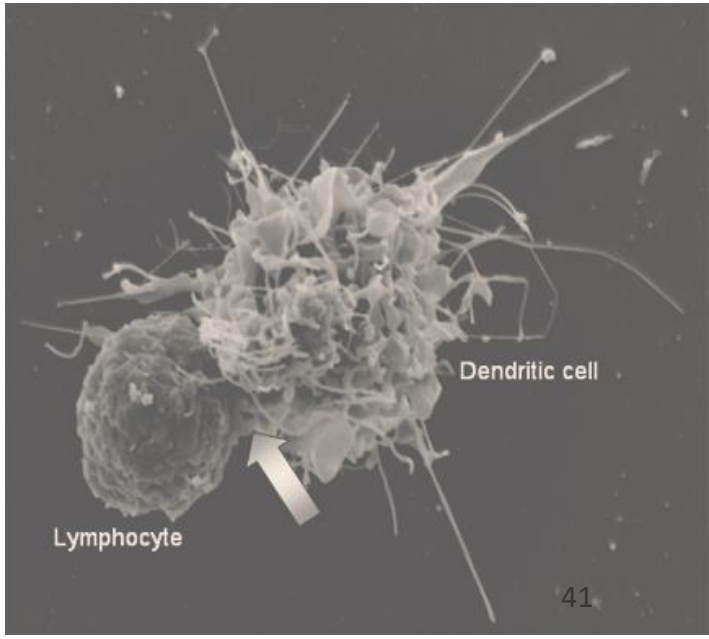
Macrophage activation balance



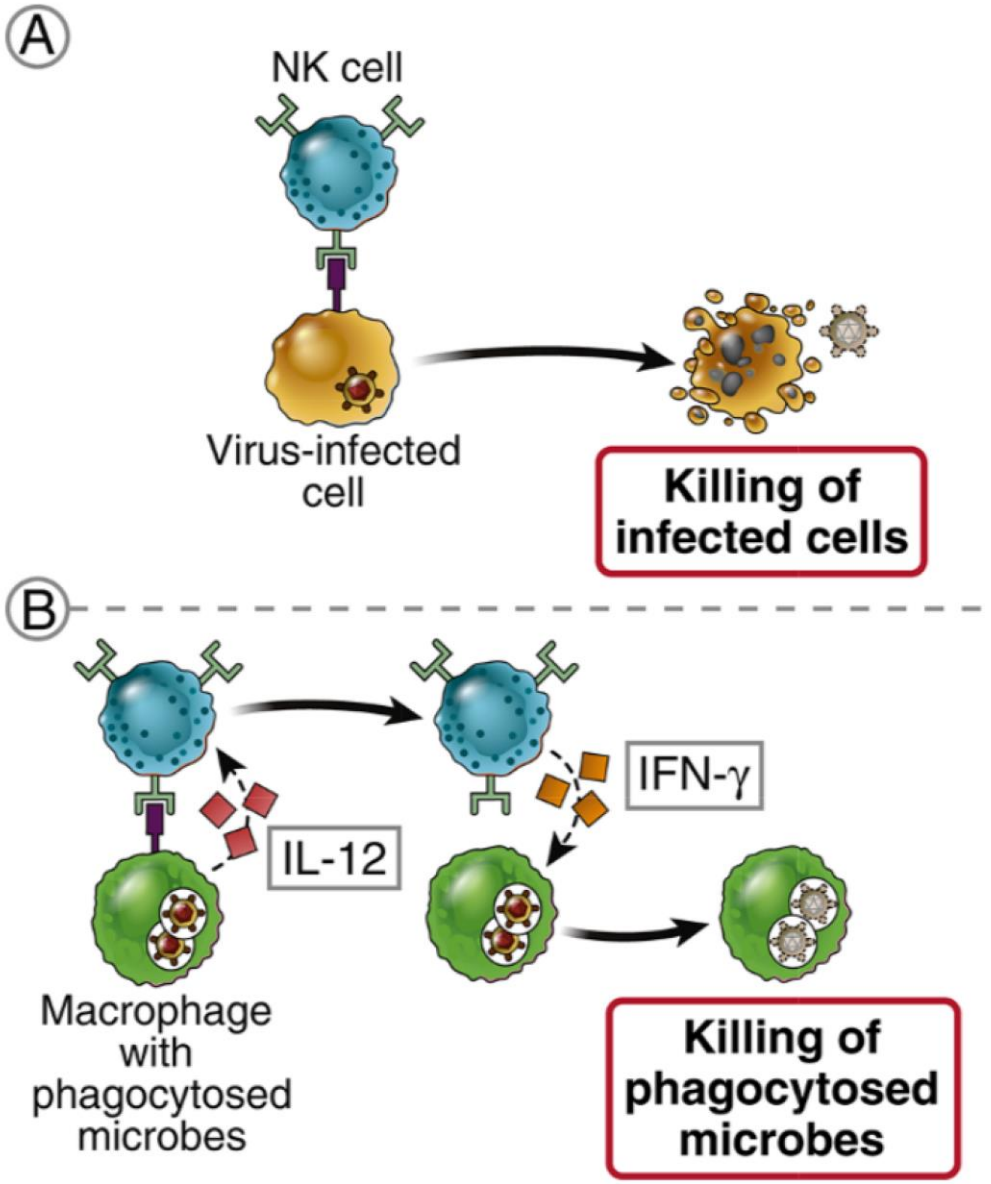
Dendritic cells

- Produce cytokines to recruit leukocytes and initiate adaptive immune response
- Important bridge between innate and adaptive response
- Major function:

  Antigen display!



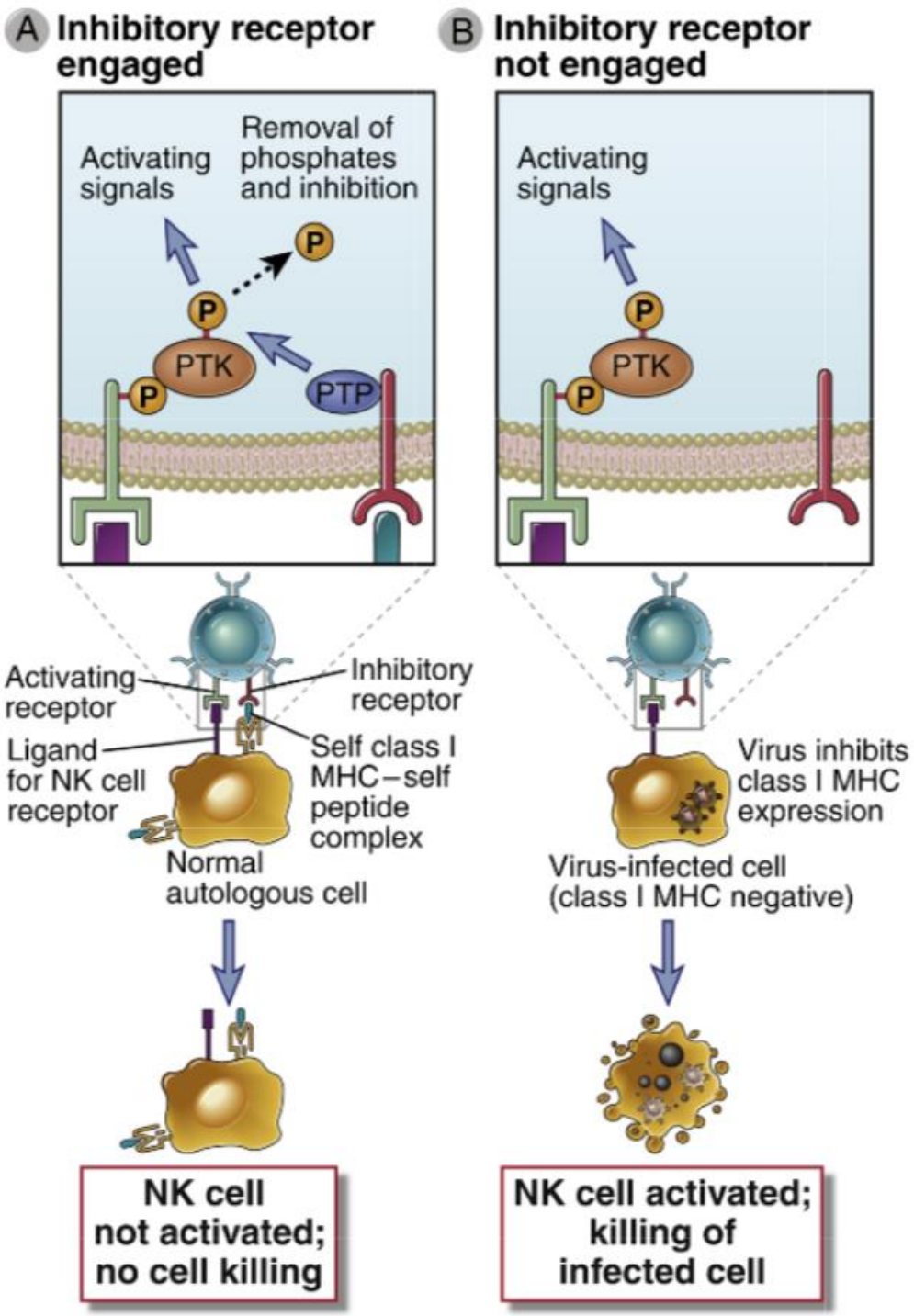
Natural killer cells – NK cells



- Innate lymphoid cell, subtype of lymphocytes
- 10% of lymphocytes in blood and organs
- cytoplasmic granules
- specific surface markers
- NO TCR & Ig

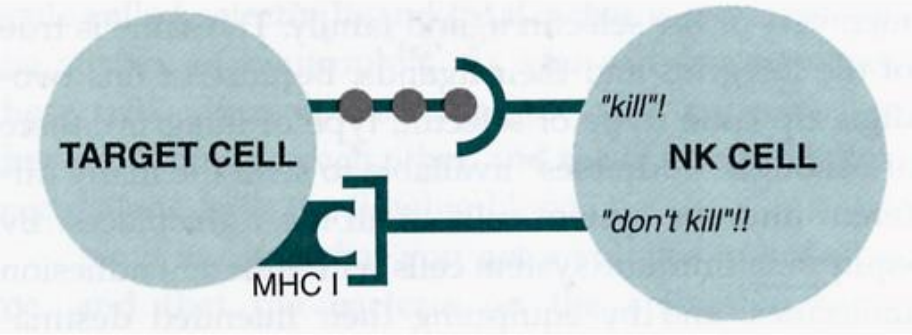
- IFN-γ – activate macrophages
- IL-12 produced by macrophage activates NK cell (+ IL-15, INF-γ)

NK cells On/Off receptors



NK cells "ON/OFF" receptors

- Identify targets based on "missing self"
  - Two types of NK receptors: **inhibitory** and **activating**
  - If inhibitory receptor recognizes a self protein (a class I MHC molecule) on a target cell, the NK cell is turned **OFF** even if activating receptor binds a ligand on the same target cell
  - If activating receptor binds a ligand, but inhibitory receptor does not, NK cells is turned **ON** and kills
  - Many virally-infected cells and tumor cells down-regulate expression of class I MHC molecules



NK cells “ON/OFF” receptors

- **Inhibitory** receptors (eg.)

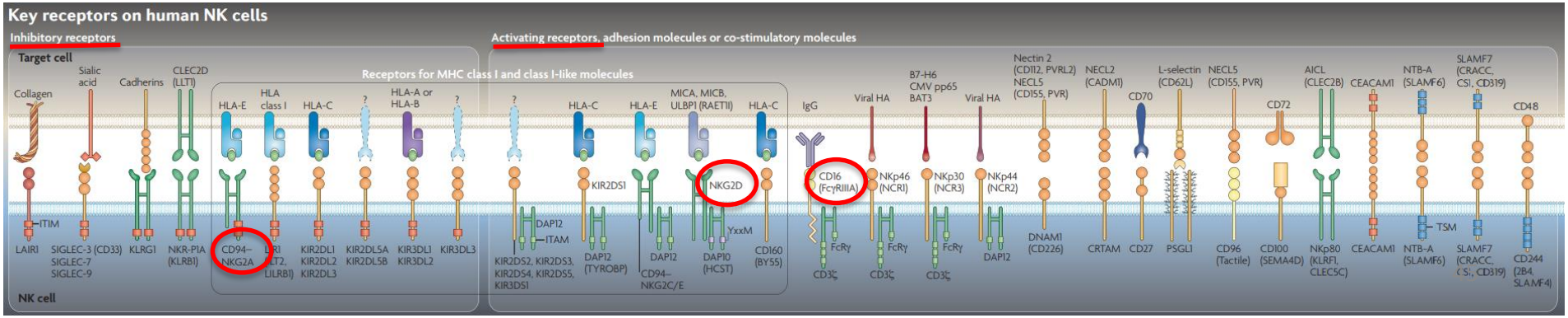
1. KIR (killer cell immunoglobulin like receptors) – recognize MHC-I molecules
2. CD-94 + NKG2A receptor

- Contain **ITIMs** (immunoreceptor tyrosine-based **inhibitory** motifs)

- **Activating** receptors (eg.)

1. CD-94 + NKG2 typeC –stress induced proteins
2. NKG2D – MHC1
3. Receptors recognizing IgG antibodies (CD16 → ADCC)

- Contain **ITAMs** (immunoreceptor tyrosine-based **activation** motifs)



Mast cells

- Tissue residing
- Filled with citoplasmic granules containing histamin, heparin, prostaglandins and proteolytic enzymes
- Important for helminth infestation and allergies
- Similar to basophiles in appearance and function (but have different origin)

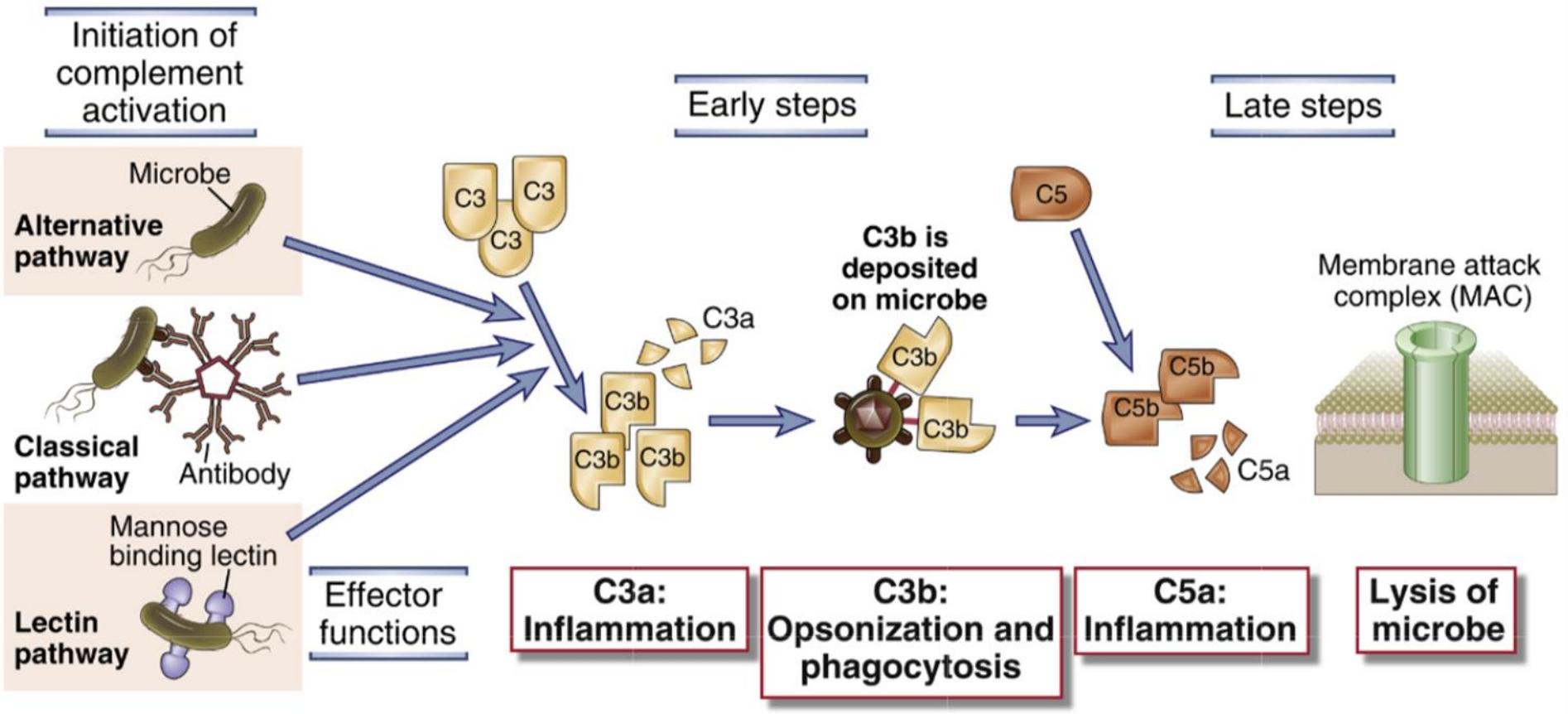
Eosinophils

- Type of granulocyte
- Important in defence against parasites
- Role in alergic reactions (astma)
- Role in antitumor defense (with CTL):
  - Secrete chemoatractans to recruite lymphocytes to tumor site
  - Atract macrophages

Other cells

- **γδ T cells** (epithelial)
- **NK-T cells** – CD1 (recognize microbial lipids and glicolipids bound to CD1d – non classical MHC)
- **Innate lymphoid cells** – ILC (Natural helper cells)
- **B-1 cells** (population of B lymphocytes – produce IgM) – react to lipids and carbohydrates
- Marginal zone B cells (spleen) – react to polysaccharide antigens

Complement system



Complement system

- 3 activation pathways:

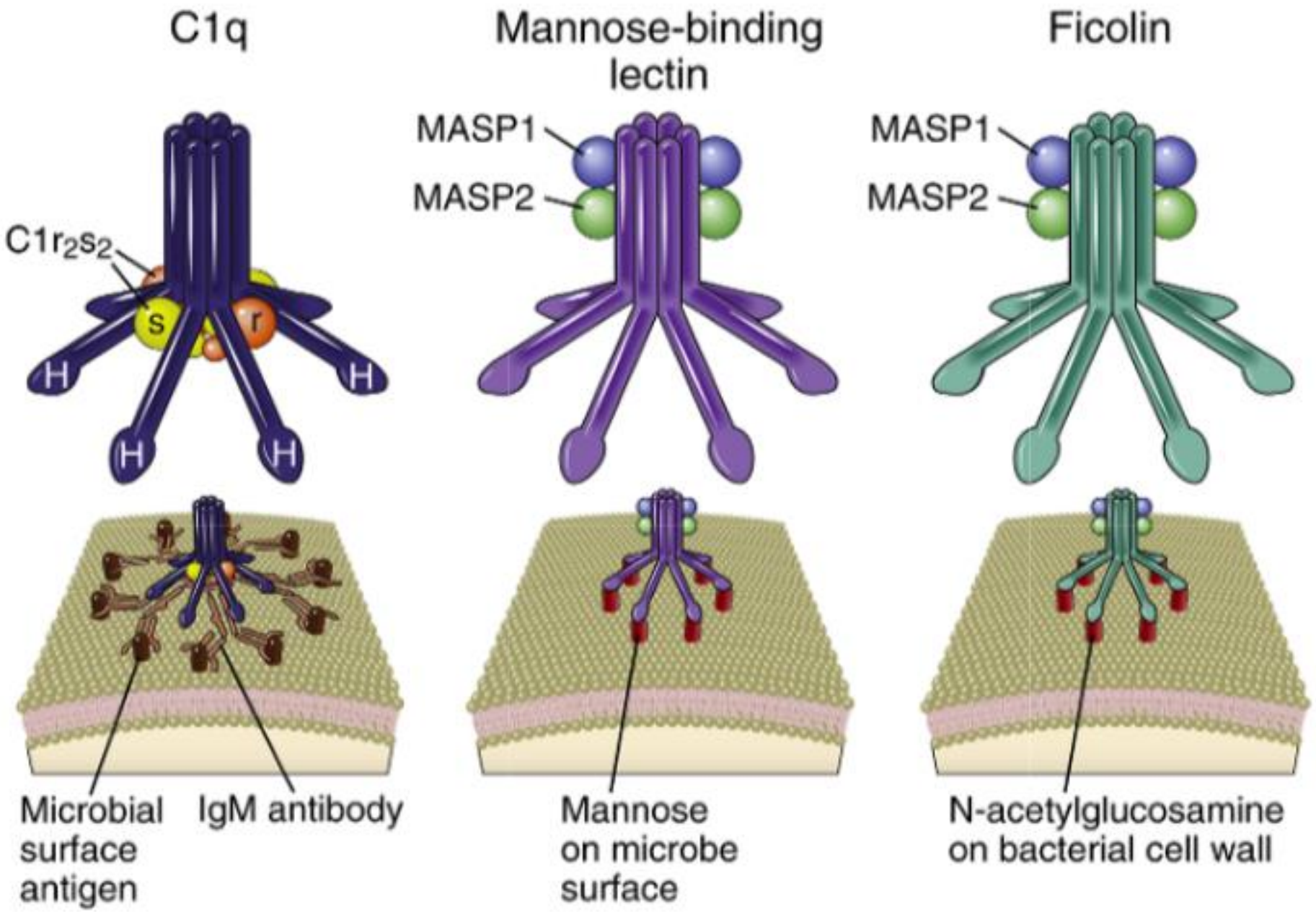
1. Alternative
2. Classical

   (OBS! adaptive)
3. Lectin (and Ficolin)

- 3 functions:

1. C3b coated microbe activates phagocytosis
2. Chemoattraction
3. Pore in the microbe

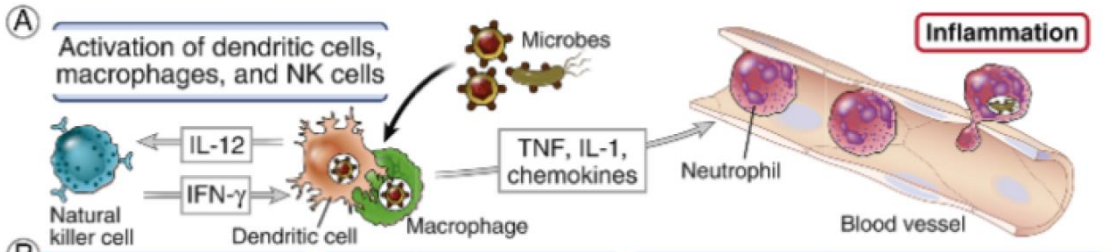
Complement system



Other plasma proteins

- C-reactive protein, CRP (opsonizes microbes)
- Mannose binding lectin
- Lung surfactant proteins
- Acute phase proteins: (CRP, serum amiloid P and A, complement factors, MBP, fibrinogen, prothrombin, factor VIII, alpha 2 macroglobulin, ferritin, ceruloplasmin,…)

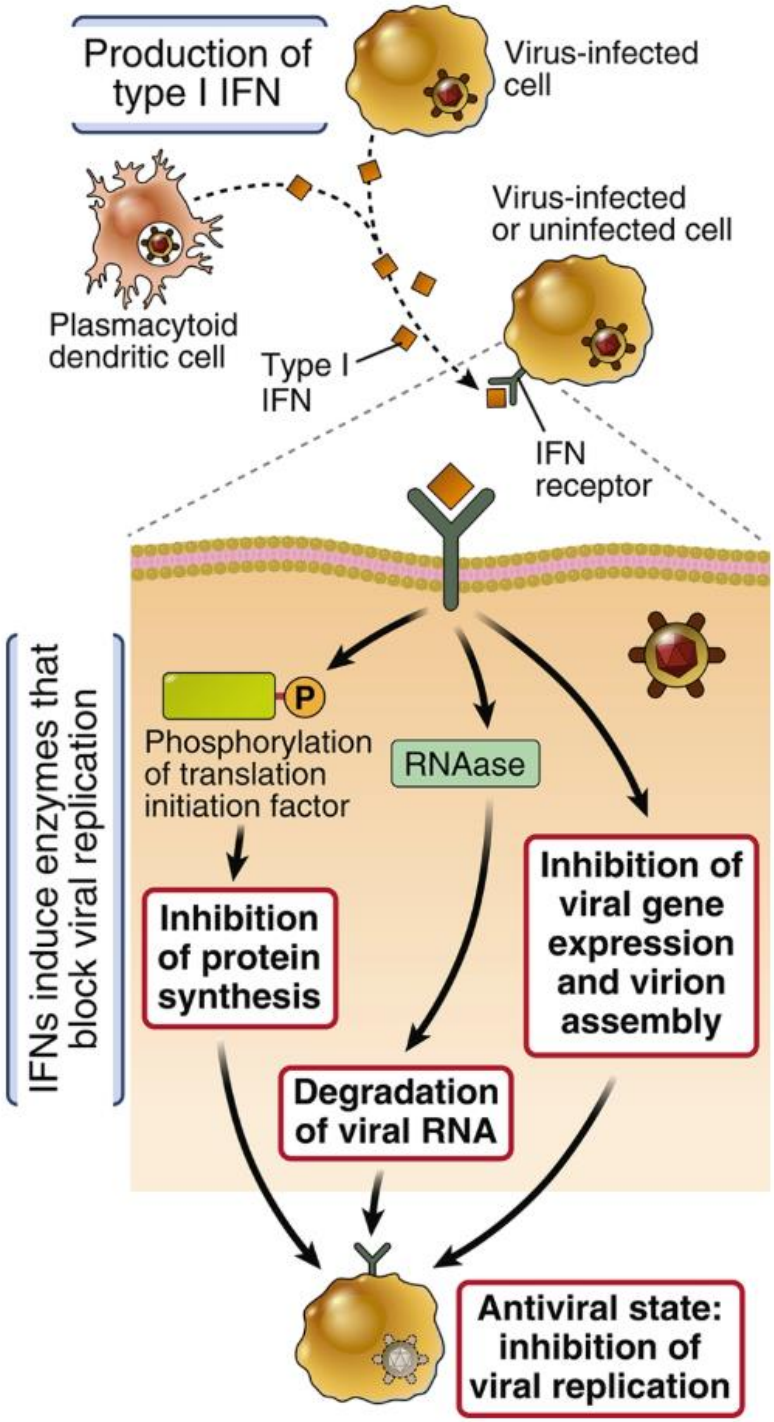
Innate immunity cytokines

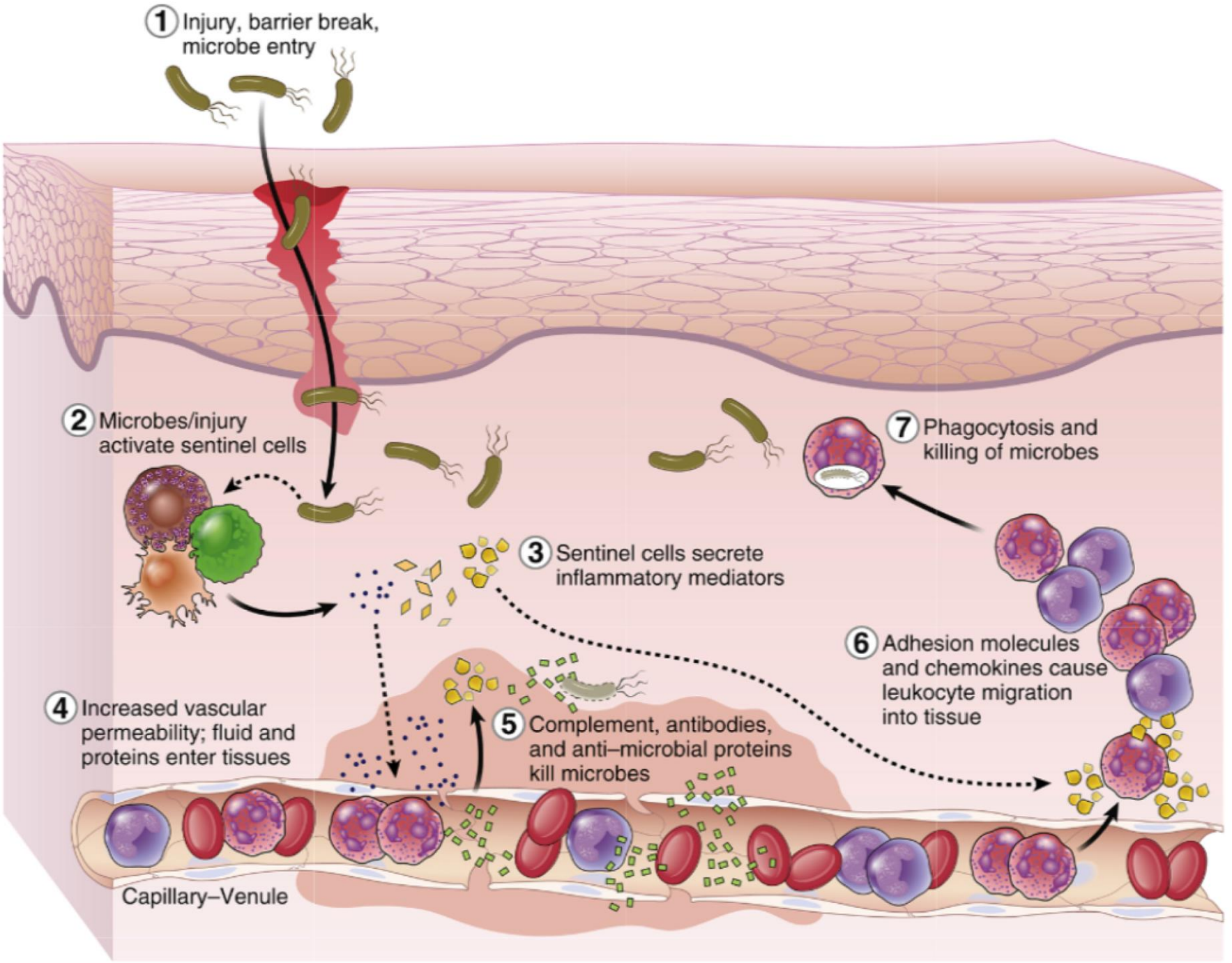


B

Cytokine	Principal cell source(s)	Principal cellular targets and biologic effects
Tumor necrosis factor (TNF)	Macrophages, T cells, mast cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute-phase proteins Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
Interleukin-1 (IL-1)	Macrophages, dendritic cells, endothelial cells, some epithelial cells, mast cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute-phase proteins T cells: Th17 differentiation
Chemokines	Macrophages, dendritic cells, endothelial cells, T lymphocytes, fibroblasts, platelets	Leukocytes: increased integrin affinity, chemotaxis, activation
Interleukin-12 (IL-12)	Dendritic cells, macrophages,	Natural killer (NK) cells and T cells: IFN-γ production,increased cytotoxic activity T cells: Th1 differentiation
Interferon-γ (IFN-γ)	NK cells, T lymphocytes	Activation of macrophages Stimulation of some antibody responses
Type I IFNs (IFN-α, IFN-β)	IFN-α: Dendritic cells, macrophages IFN-β: Fibroblasts, epithelial cells	All cells: antiviral state, increased class I major histocompatibility complex (MHC) expression NK cells: activation
Interleukin-10 (IL-10)	Macrophages, dendritic cells, T cells	Macrophages, dendritic cells: inhibition of cytokine and chemokine production, reduced expression of costimulators and class II MHC molecules
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	Liver: synthesis of acute-phase proteins B cells: proliferation of antibody-producing cells
Interleukin-15 (IL-15)	Macrophages, others	NK cells: proliferation T cells: proliferation
Interleukin-18 (IL-18)	Macrophages	NK cells and T cells: IFN-γ synthesis
TGF-β	Many cell types	Inhibition of inflammation T cells: differentiation of Th17, regulatory T cells

Interferon type 1

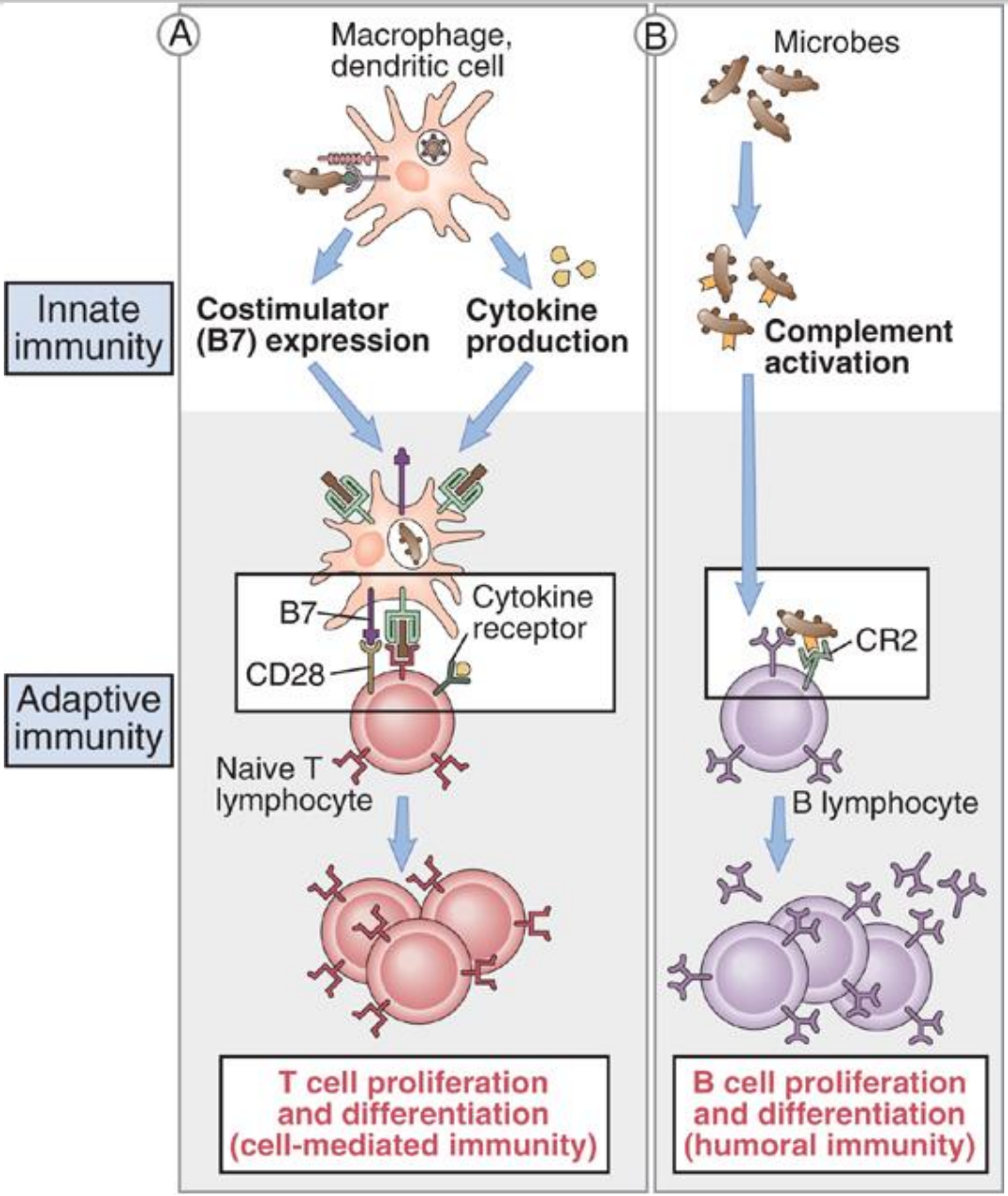



1 Injury, barrier break, microbe entry
2 Microbes/injury activate sentinel cells
3 Sentinel cells secrete inflammatory mediators
4 Increased vascular permeability; fluid and proteins enter tissues
5 Complement, antibodies, and anti–microbial proteins kill microbes
6 Adhesion molecules and chemokines cause leukocyte migration into tissue
7 Phagocytosis and killing of microbes
Capillary–Venule

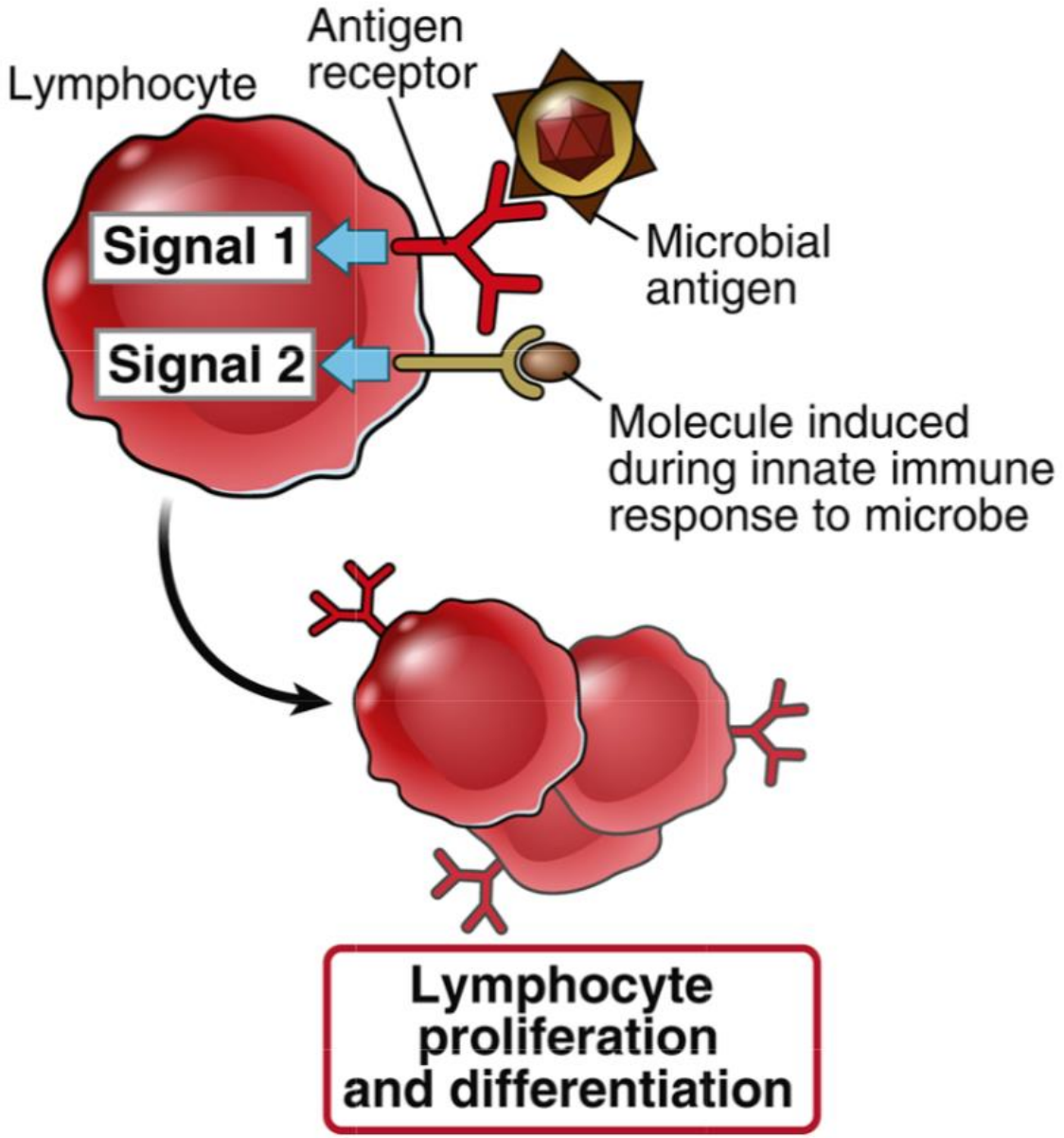
Evasion of innate immunity by microbes

Mechanism of immune evasion	Organism (example)	Mechanism
Resistance to phagocytosis	Pneumococci	Capsular polysaccharide inhibits phagocytosis
Resistance to reactive oxygen intermediates in phagocytes	Staphylococci	Production of catalase, which breaks down reactive oxygen intermediates
Resistance to complement activation (alternative pathway)	*Neisseria meningitidis*	Sialic acid expression inhibits C3 and C5 convertases
	Streptococci	M protein blocks C3 binding to organism, and C3b binding to complement receptors
Resistance to antimicrobial peptide antibiotics	*Pseudomonas*	Synthesis of modified LPS that resists action of anti-bacterial peptides

Innate activates adaptive!



Two signal requirement for lymphocyte activation



Summary – Innate immunity

1. Provides a barrier to prevent the spread of infection
2. Identifies and eliminates pathogens
3. Initiates an inflammatory response
4. Provides signals to activate and regulate the type of adaptive immune response generated
5. Does not produce protective immunity
6. Non-antigen-specific immunity