Cells of Innate Immunity

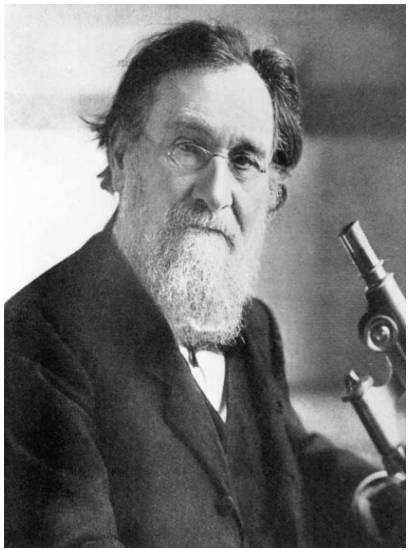
Chunguang Yan Ph.D.

Department of Pathogenic Biology and Immunology

Medical school, Southeast University

Cells of Innate Immunity

Pioneers of Innate Immunity

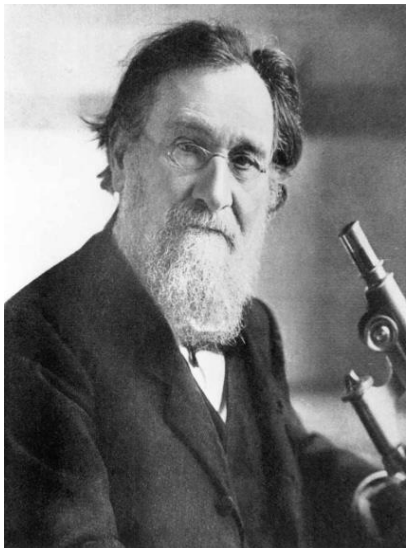


Metchnikoff
(1845~1916)

- In 1883, Elie Metchnikoff demonstrated that cells also contribute to the immune state of an animal.
- He observed that certain white blood cells, which he termed phagocytes, were able to ingest microorganisms and other foreign material.

Cells of Innate Immunity

Pioneers of Innate Immunity



Metchnikoff
(1845~1916)

- Metchnikoff hypothesized that cells, rather than serum components, were the major effector of immunity.
- The active phagocytic cells identified by Metchnikoff were, likely, blood monocytes and neutrophils.

- In 1908, Mechnikoff won NOBEL PRIZES

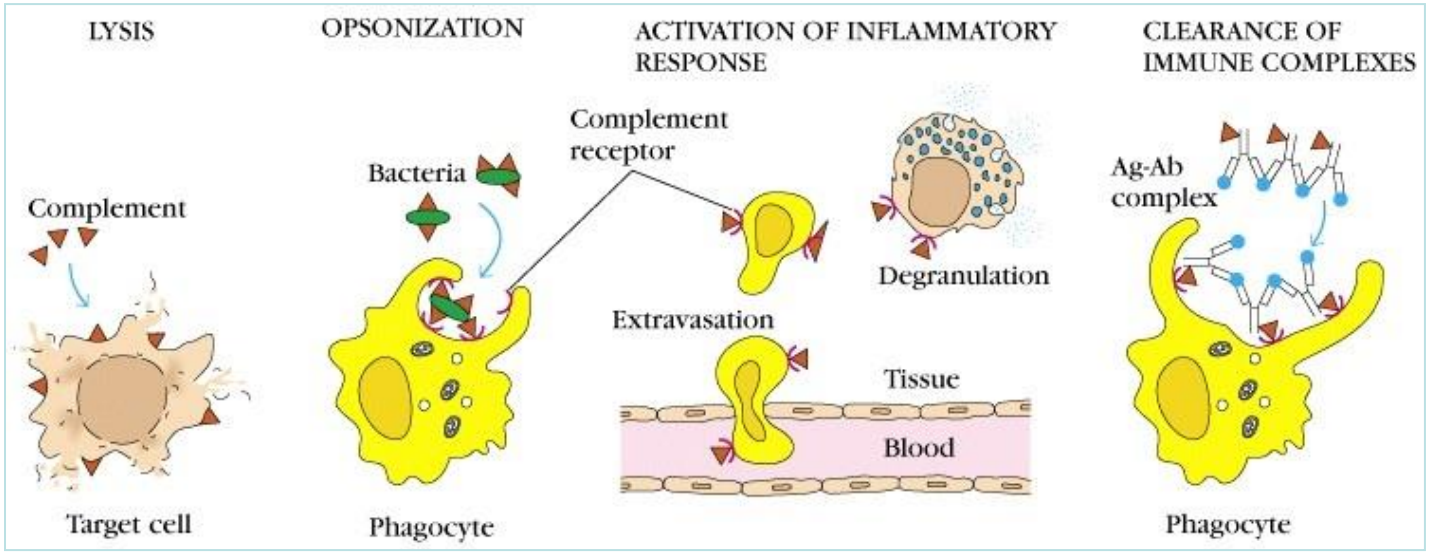
Cells of Innate Immunity

Pioneers of Innate Immunity

Bordet

(1870 – 1961)

- **In 1919, Bordet won NOBEL PRIZES due to his discovery of Complement and Complement-mediated bacteriolysis**



Cells of Innate Immunity

Milestones in innate immunity ⌖ 100 years later

Hoffmann discovered the function of the fruit fly Toll gene in innate immunity.

Toll-like receptors identify constituents of other organisms like fungi and bacteria, and trigger an immune response, explaining how septic shock can be triggered by bacterial remains

Jules A. Hoffmann

Lemaitre B, Nicolas E, Michaut L, Reichhart JM, **Hoffmann JA**. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. **Cell**. **1996** Sep 20;86(6):973-83.

http://en.wikipedia.org/wiki/Jules_A._Hoffmann

Cells of Innate Immunity

Milestones in innate immunity

- **Charles Janeway (left) were the first to identify Toll-like receptors in mammalian cells.**

  [mæ'meljən]

A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature. 1997;388(6640):394-7.*

Cells of Innate Immunity

Milestones in innate immunity

Charles Janeway Professor

Section of Immunobiology,

Yale University School of Medicine

PRR: pattern recognition receptor

PAMP: pathogen associated molecular pattern

Structures that are characteristic of microbial pathogens and are not present on normal mammalian cells.

Innate immune cells can distinguish self from non-self with recognizing PAMP by PRR

Cells of Innate Immunity

Milestones in innate immunity

Beutler demonstrating that one of the mammalian Toll-like receptors, **TLR4**, acts as the membrane-spanning component of the mammalian LPS receptor complex. The TLRs (of which ten are now known to exist in humans) are now widely known to function in the perception of microbes, each detecting signature molecules that herald infection.

['hɛrəld]

Bruce A. Beutler

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, **Beutler B**. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in TLR4 gene. **Science. 1998** Dec 11;282(5396):2085-8.

http://en.wikipedia.org/wiki/Bruce_Beutler

Cells of Innate Immunity

Milestones in innate immunity

Jules A. Hoffmann

Bruce A. Beutler

Charles Janeway

passed away on April 12, 2003

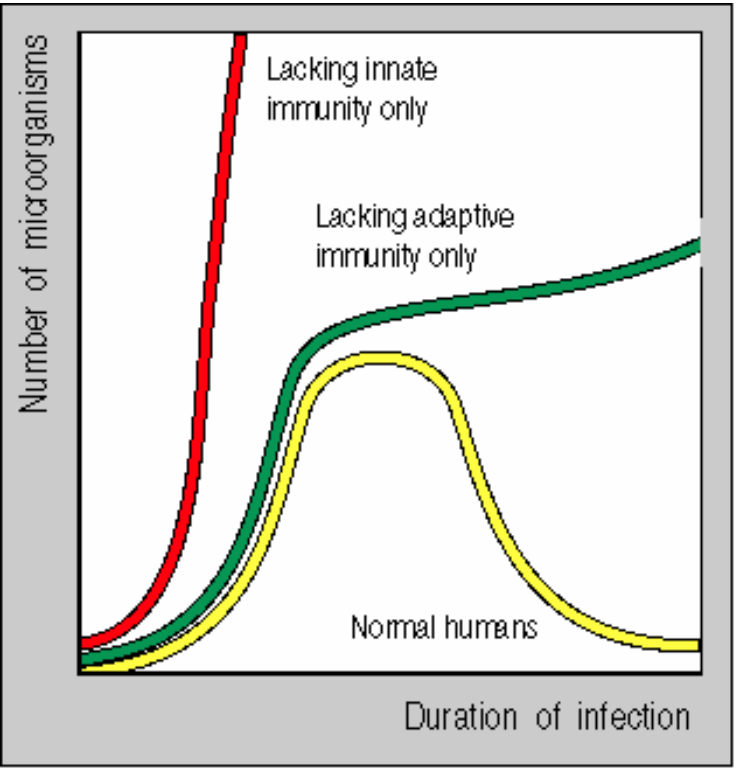
Jules A. Hoffmann & Bruce A. Beutler received one-half of the **2011 Nobel Prize** in Physiology or Medicine, for "their discoveries concerning the activation of innate immunity"

Phase of Innate Immunity

- **The microorganisms**

  that are encountered daily in life of a normal healthy individual

- **only occasionally cause perceptible disease.**



Most are detected and destroyed within hours by defense mechanisms

that are not antigen-specific and

do not require a prolonged period of induction

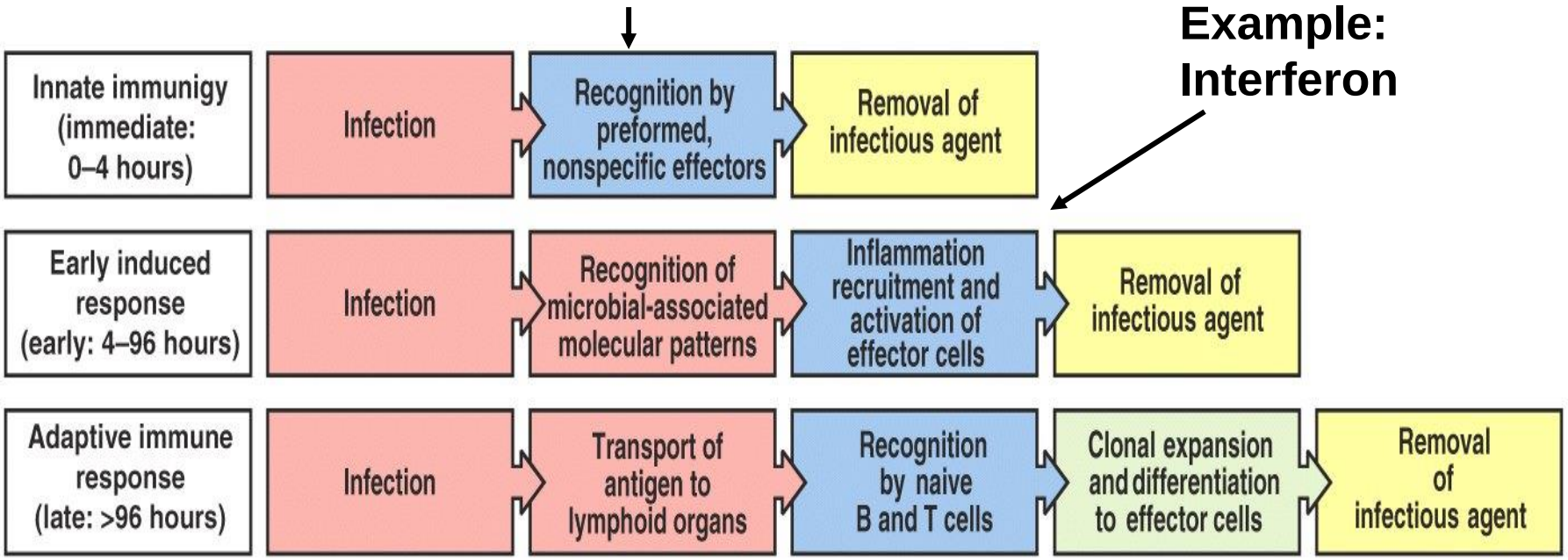
These are the mechanisms of innate immunity.

Phase of Innate Immunity

Innate defense is both preformed and inducible

Example: phagocytes, NK cells, complement

Example: Interferon



Cells of Innate Immunity

Epithelium

- Our whole body surfaces are defended by epithelia,
- which provide a physical barrier between the internal milieu and the external world containing pathogens.

- These epithelia comprise our
  - skin
  - Mucous membranes
    - Gastrointestinal tract
    - Respiratory tract
    - Genital-urinary tract

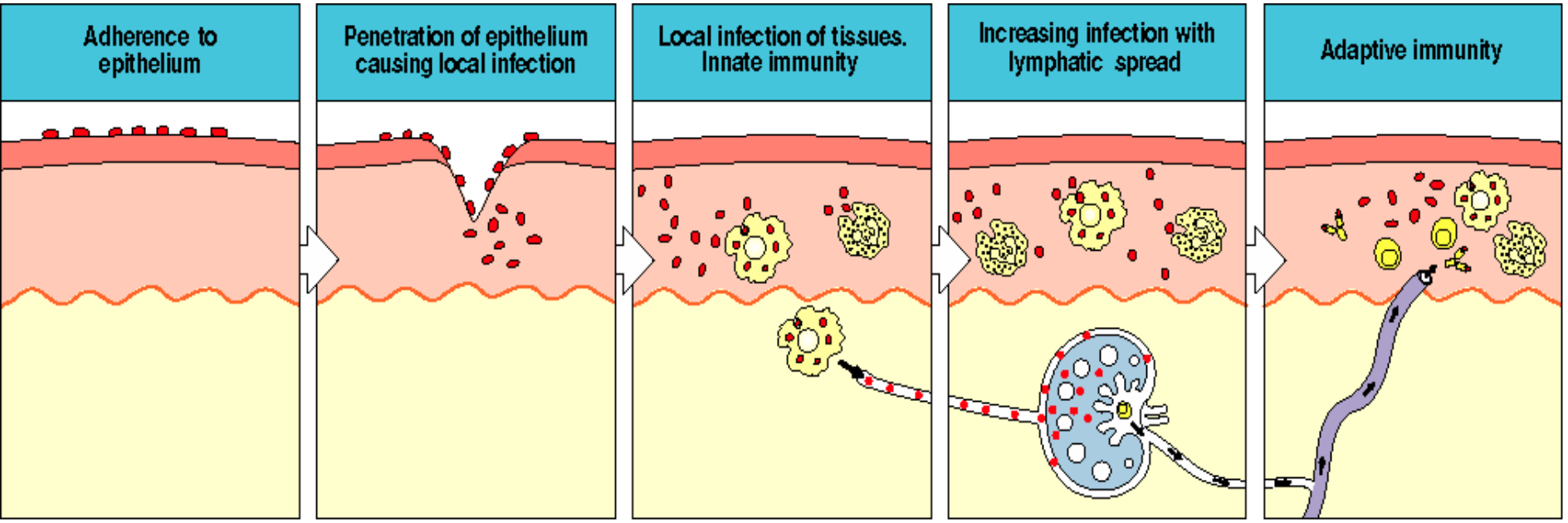
Cells of Innate Immunity

❑ Epithelium

Epithelial barriers to infection	
Mechanical physical barrier	Epithelial cells joined by tight junctions Flow of air or fluid over epithelial surface Movement of cilia
Chemical	Fatty acids (skin) Low pH (stomach) Enzymes:lysozyme(saliva,sweat,tears),pepsin(gut) Antibacterial peptides, crytidins(intestine) Defensine(epithelium)
Microbiological Normal flora	Compete with pathogenic microorganism for nutrients and for attachment sites on epithelium Produce antibacterial substances

Cells of Innate Immunity

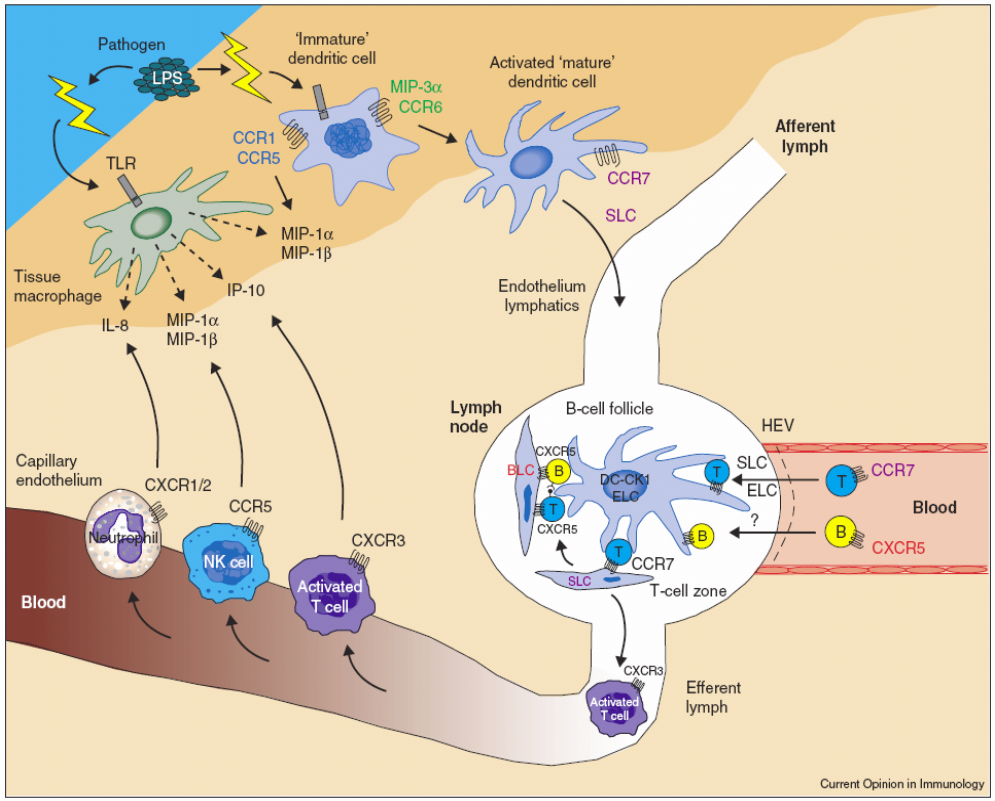
❑ Epithelium



When a pathogen penetrates an epithelial barrier and begins to replicate in the tissues of the host and causes local infection, the innate immune mechanisms (cells and molecules) act immediately.

Cells of Innate Immunity

- **Epithelium**



The innate immune cells (Macrophages and Dendritic Cells)

migrate into lymph nodes and induce adaptive immune response.

Cells of Innate Immunity

❑ Phagocytes

- A group of white blood cells is collectively referred to as granulocytes or polymorphonuclear leukocytes (PMNs).
- They can easily be identified by their multi-lobed nucleus and by the abundant storage granules in their cytoplasm.
- Granulocytes are composed of three cell types identified as neutrophils, eosinophils and basophils, based on their staining characteristics with certain dyes.

Cells of Innate Immunity

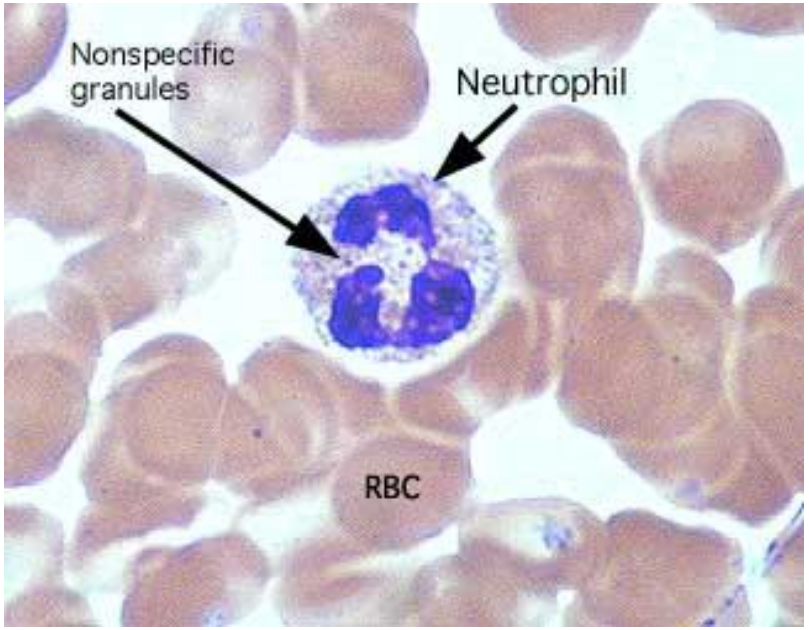
❑ Phagocytes

- These cells are predominantly important in the removal of bacteria and parasites from the body.
- They engulf these foreign bodies and degrade them using their powerful enzymes.

Phagocytes - Neutrophils

Neutrophils

- multi-lobed nucleus.
- 50%-70% of circulating WBC (higher numbers suggestive of bacterial infection).



- Neutrophils are the 1st cells to arrive at a site of infection.
- A number of substances produced during an inflammatory response recruit neutrophils to a site of inflammation.

Phagocytes - Neutrophils

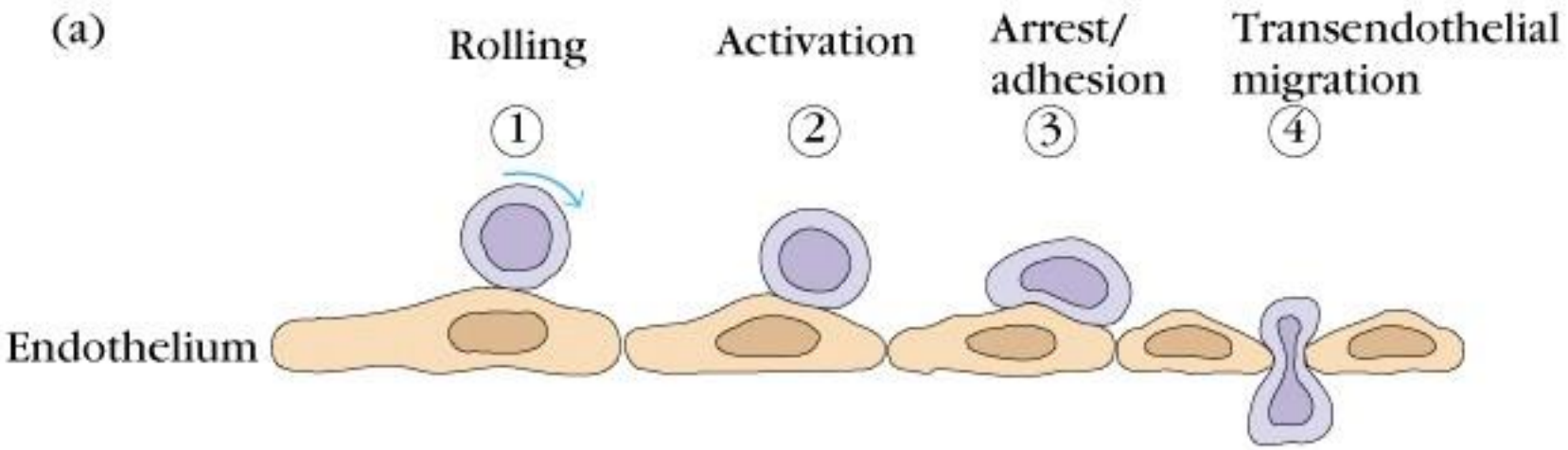
Margination and Emigration

- Once released from the marrow, neutrophils normally circulate continuously in the blood throughout the brief lives.

- If their journey carries them into an inflamed tissue, however, the cells rapidly
  - adhere to the activated endothelium of local venules,
  - migrate through the vessel walls, and
  - invade the affected tissues, where they may
  - accumulate in vast numbers.

Emigration of Neutrophils

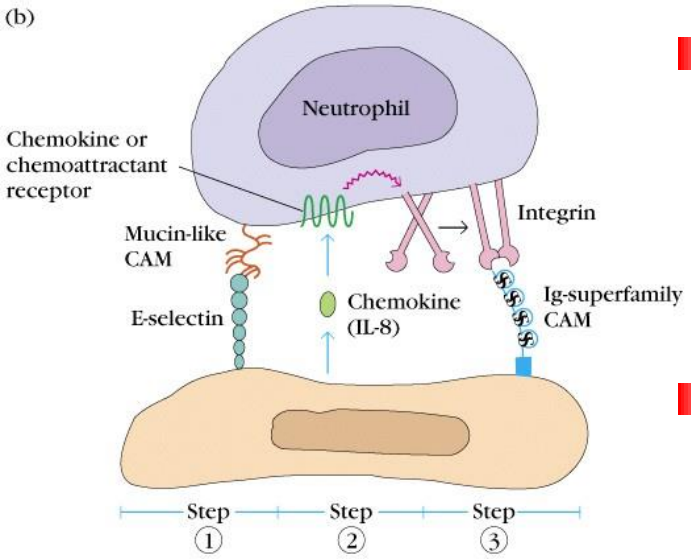
❑ Process of emigration



- As an inflammatory response develops, the vascular endothelium is then said to be activated, or inflamed.
- Neutrophils are generally the first cell type to bind to inflamed endothelium from where neutrophils extravasate into the inflamed tissue.

Emigration of Neutrophils

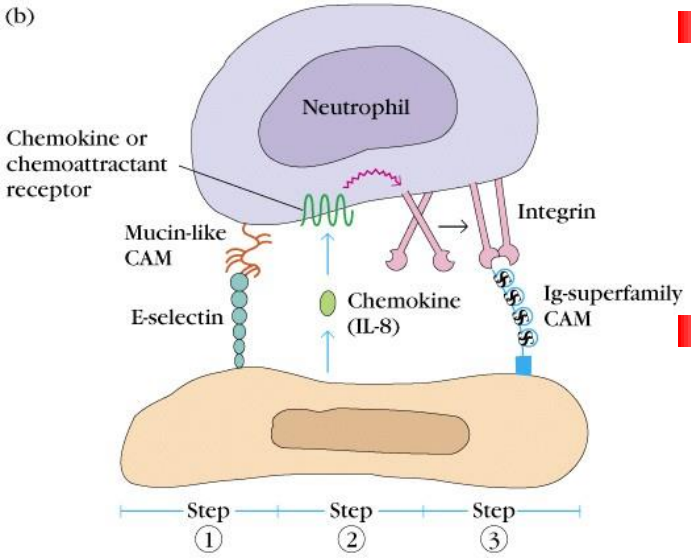
❑ First step: Rooling mediated by Selectin



- In the first step, neutrophils attach loosely to the endothelium by a low affinity selectin-carbohydrate interaction.
- During an inflammatory response, cytokines and other mediators act upon the local endothelium, inducing expression of adhesion molecules of the selectin family.

Emigration of Neutrophils

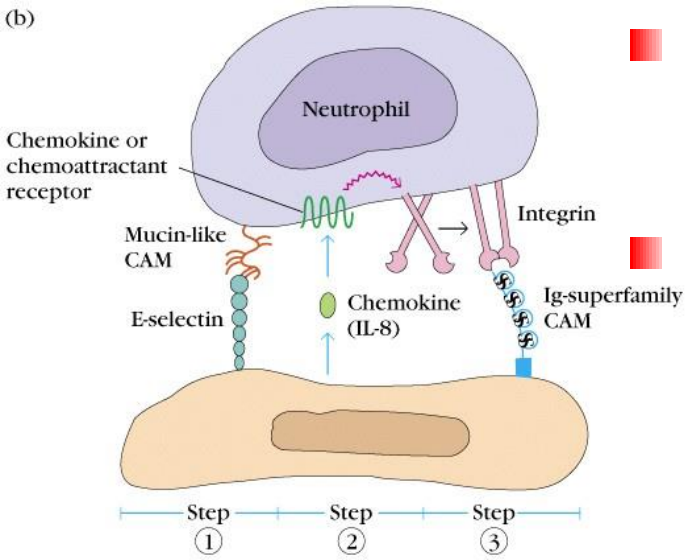
❑ First step: Rooling mediated by Selectin



- These E- and P-selectin molecules bind to mucin-like cell-adhesion molecules on the neutrophil membrane.
- This interaction tethers the neutrophil briefly to the endothelial cell, but the force of the circulating blood soon detaches the neutrophil.
- Selectin molecules on another endothelial cell again tether the neutrophil; this process is repeated so that the neutrophil tumbles end-over-end along the endothelium, a type of binding called rolling .

Emigration of Neutrophils

❑ Second step: Activation mediated by Chemoattractants

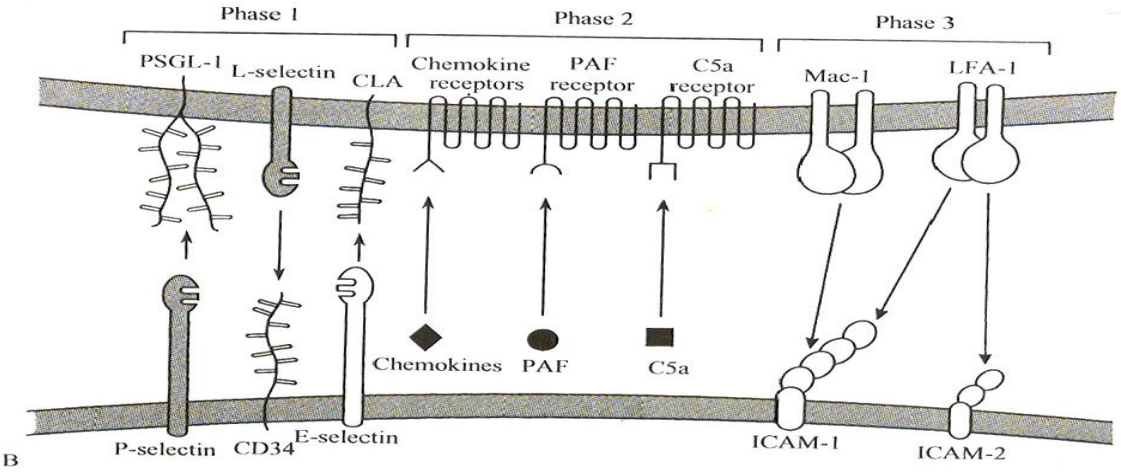


- As the neutrophil rolls, it is activated by various chemoattractants;
- These are either permanent features of the endothelial cell surface or secreted locally by cells involved in the inflammatory response.

- Among the chemoattractants are members of a large family of chemoattractive cytokines called chemokines.
- Two chemokines involved in the activation process are interleukin 8 (IL-8) and macrophage inflammatory protein (MIP-1) .

Emigration of Neutrophils

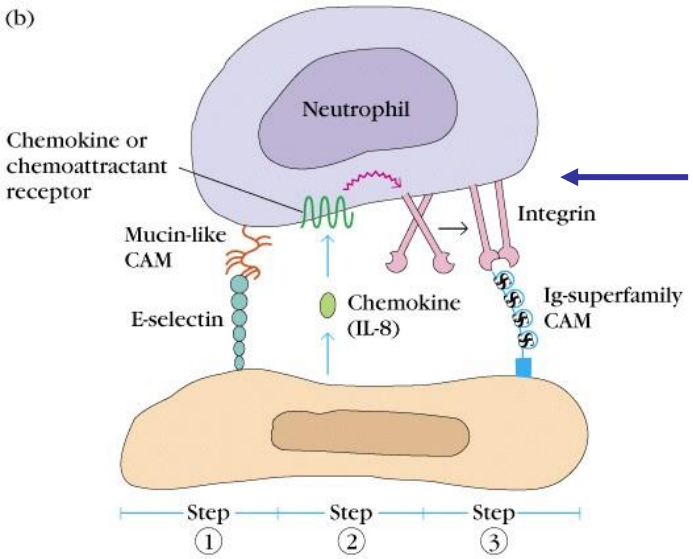
- **Second step: Activation mediated by Chemoattractants**



- **However, not all chemattractants belong to the chemokine group.**
- **Other chemoattractants are platelet-activating factor (PA F) , the complement split products C5a, C3a produced by the break down of bacterial proteins during an infection .**
- **Binding of these chemoattractants to receptors on the neutrophil membrane triggers an activating signal mediated by G protein associated receptors.**

Emigration of Neutrophils

❑ Third step: Attachment mediated by integrins

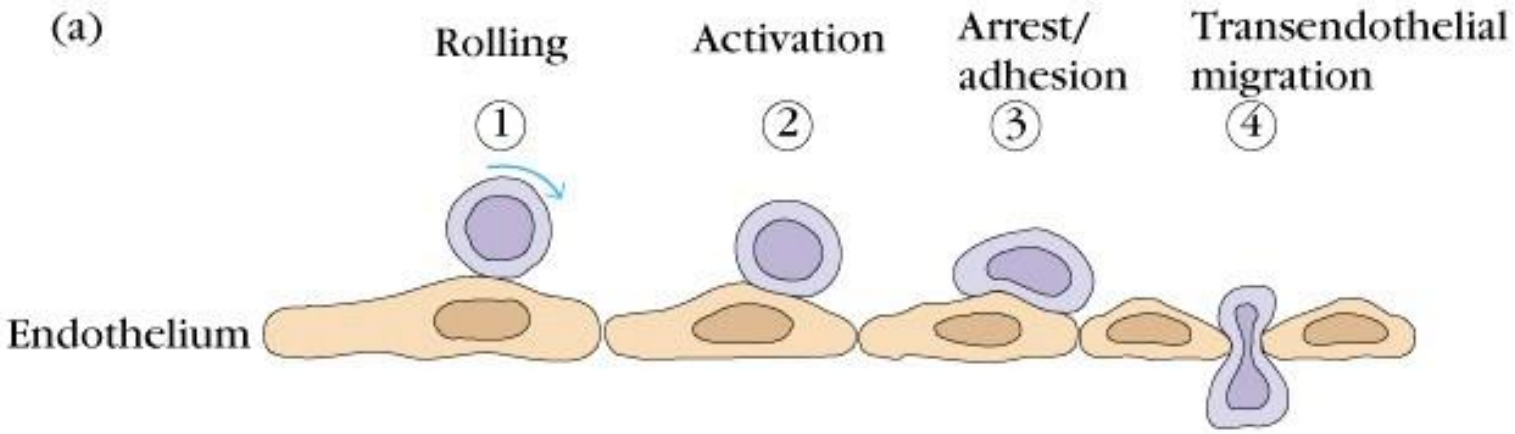


- This signal induces a conformational change in the integrin molecules in the neutrophil membrane, increasing their affinity for the Ig-superfamily adhesion molecules on the endothelium.

- Subsequent ***interaction*** between integrins and Ig-superfamily CAMs stabilizes adhesion of the neutrophil to the endothelial cell, enabling the cell to adhere firmly to the endothelial cell .

Emigration of Neutrophils

- **Last step: Emigration**



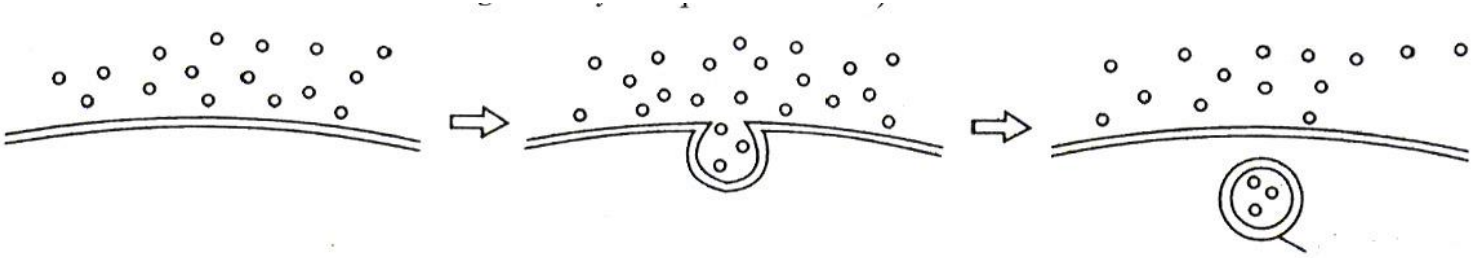
- **Subsequently, the neutrophil migrates through the vessel wall into the tissues.**

Phagocytes - Neutrophils

- **Engulfment of pathogens**
  - **Pinocytosis: unmodified fluid**
  - **Endocytosis: modified fluid and particle**
  - **Phagocytosis: particle (usually >100nm in diameter)**

Engulfing of pathogens

- **Pinocytosis**



Pinocytotic vesicle

Pinocytosis occur through formation of minute surface vesicles filled with unmodified extra cellular fluid.

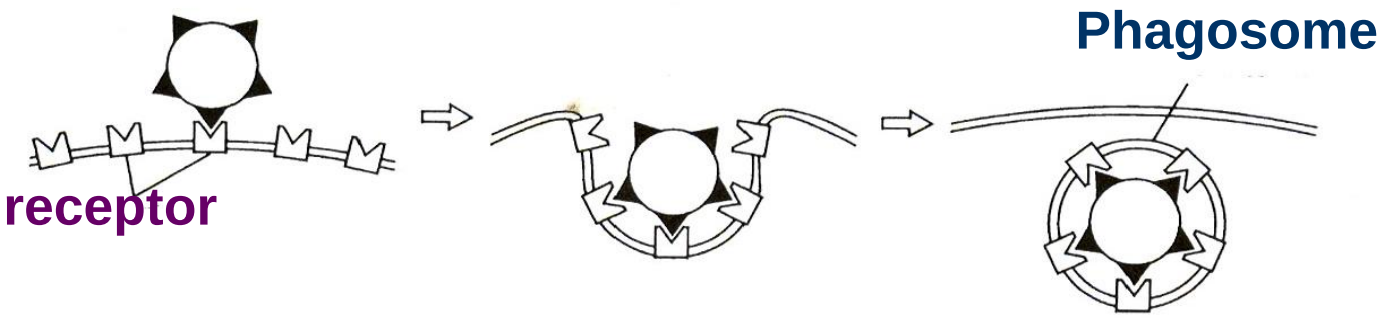
Engulfing of pathogens

❑ Receptor-mediated endocytosis

- **Be triggered by the binding of a soluble ligand to one or more specific surface receptors,**
- **Resulting polymerization of clathrin protein on the cytoplasmic aspect of the plasma membrane** [,pɔliməraiˈzeiʃən]
- **Leads to invagination of the receptor and Formation of a coated vesicle.** [inˌvædʒiˈneiʃən]

Engulfing of pathogens

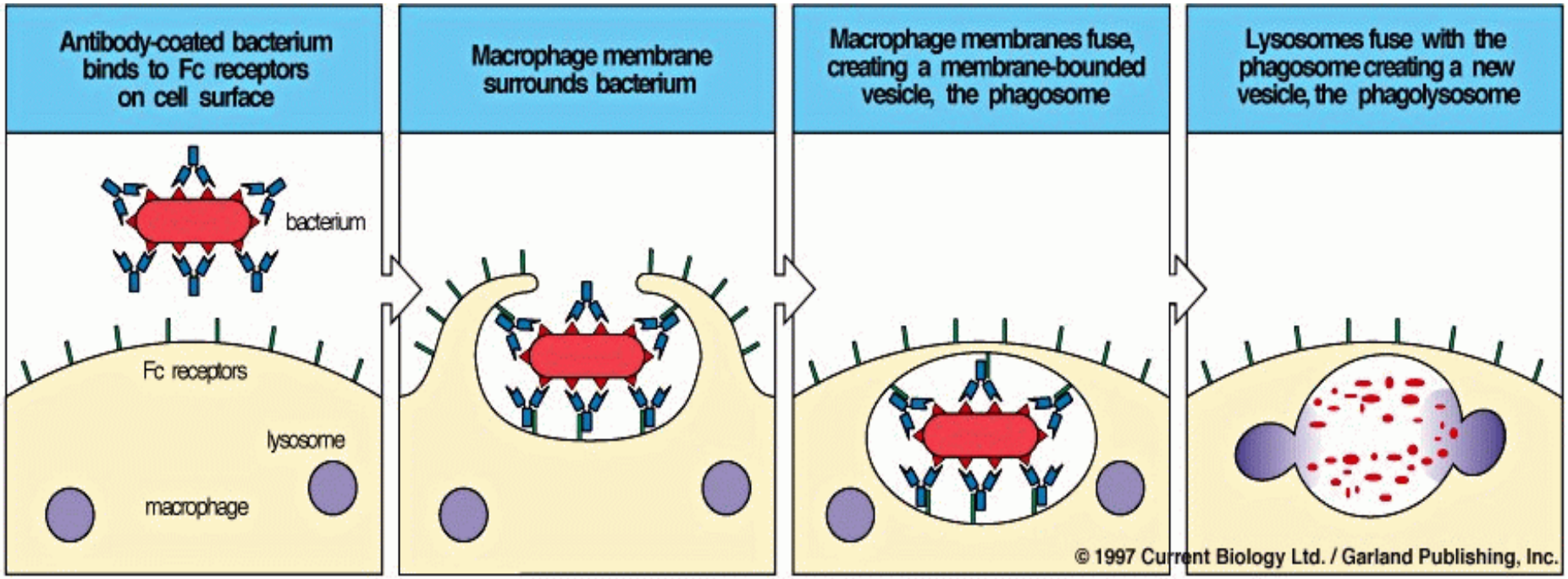
❑ Phagocytosis



- Multiple surface receptors sequentially engage the surface of a target particle, usually >100nm in diameter, such as bacteria.
- Phagosomes are lined by a sing lipid bilayer derived from the plasma membrane.

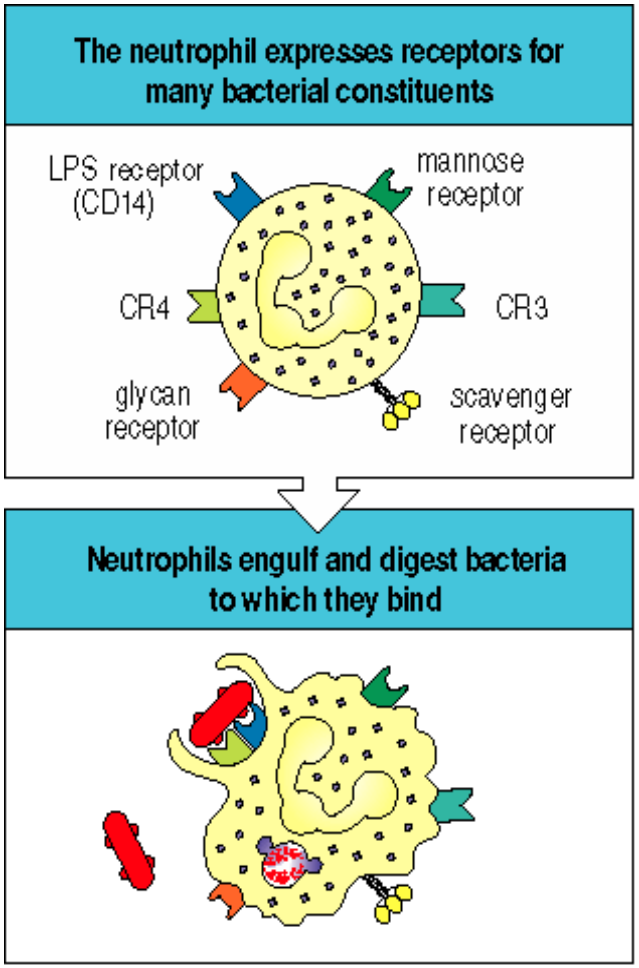
Engulfing of pathogens

- **Phagocytosis**



Engulfing of pathogens

- **PRR:pattern recognition receptor on the neutrophils**



LPS receptor:

CD14

toll-like receptor-4

CR3,4:

Complement (C') receptors (C3b)

Scavenger receptor:

sialic acid-bearing protein

Mannose receptor:

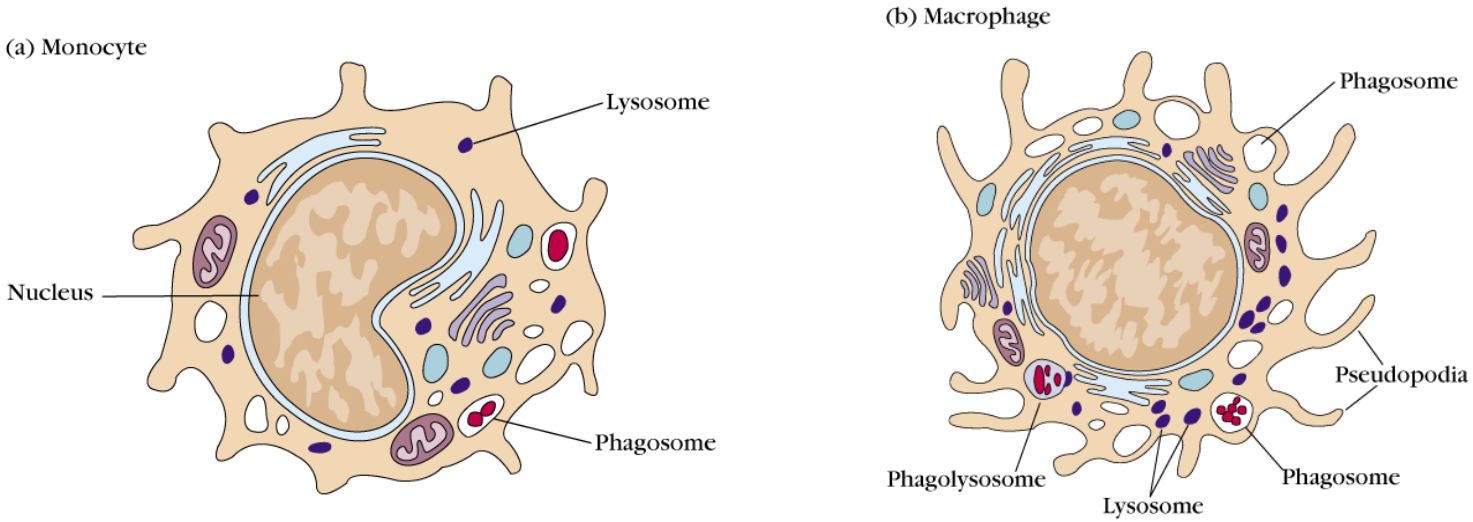
Binds mannose on bacteria, activates C'

Glycan receptor:

Polysaccharides

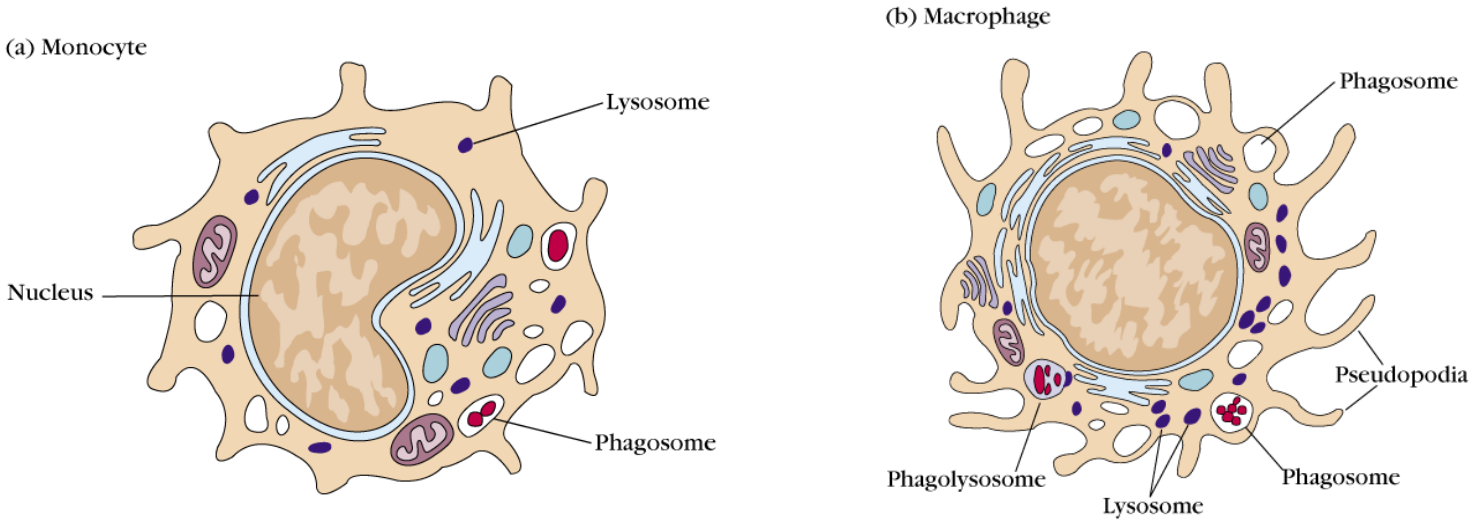
IN ADDITION: TLRs

Mononuclear phagocytes & Macrophage system



- The mononuclear phagocytic system consists of monocytes circulating in the blood and macrophages settling in the tissues.
- During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes,
- which leave the bone marrow and enter the blood,
- where they further differentiate into mature monocytes.

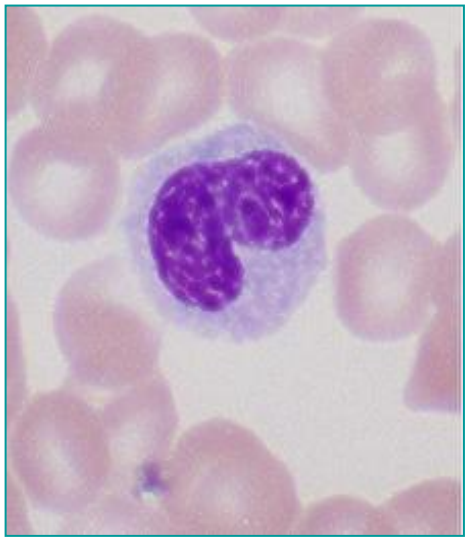
Mononuclear phagocytes & Macrophage system



- Monocytes circulate in the bloodstream for about 8 h, during which time they enlarge;
- They then migrate into the tissues and differentiate into specific tissue macrophages.
- Tissue macrophages live for about 2-4 months, during this time some macrophages remain immobile, inactive stage.

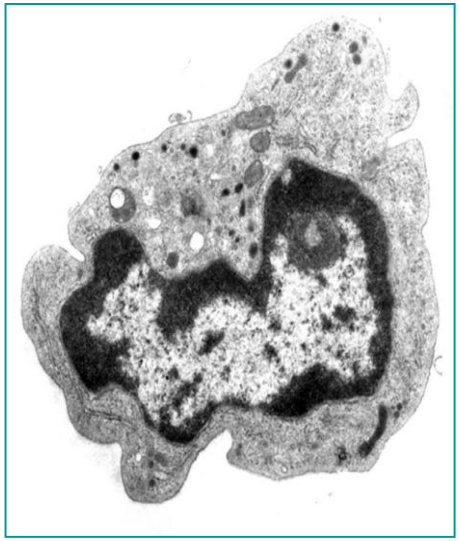
Mononuclear phagocytes & Macrophage system

- Nearly all tissues, organs and cavities harbor a population of resident phagocytes.
- Most contain only a diffuse scattering of individual phagocytic cells that remain inconspicuous under normal conditions and are very similar to one another in appearance and function.



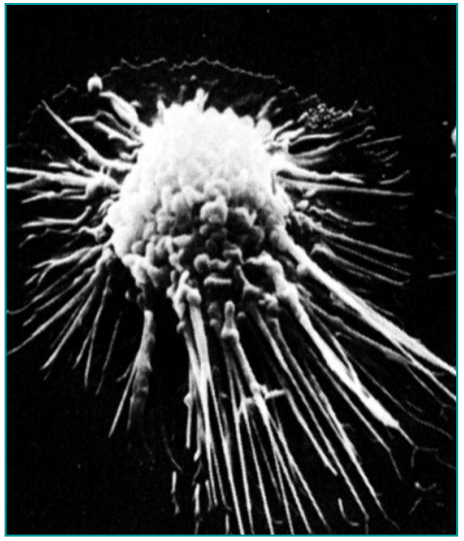
Micrograph

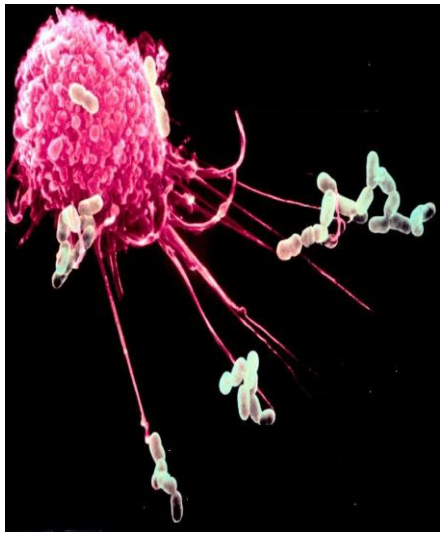
by microscope



Electron micrograph

by electron microscope





Scanning micrograph

by electron microscope

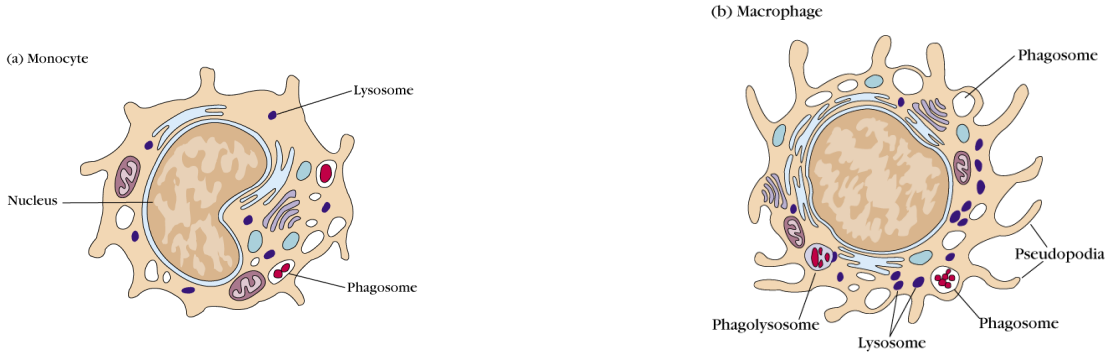
Mononuclear phagocytes & Macrophage system

monocytes circulating in the blood and

macrophages remaining in the tissues

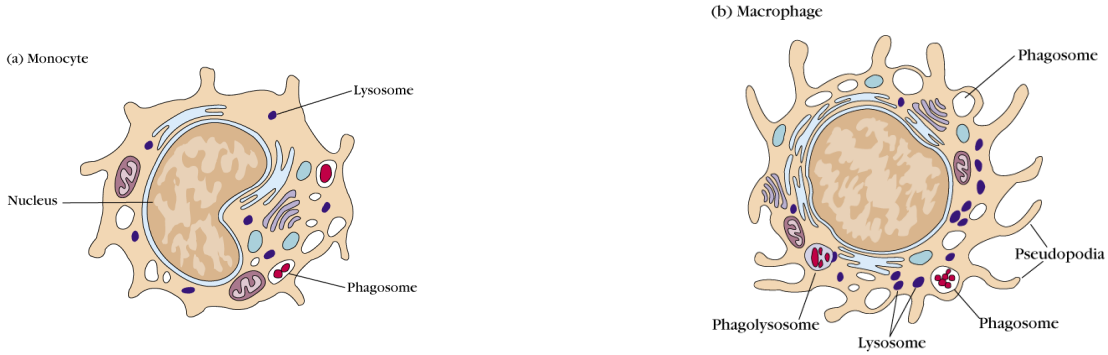
Tissue	Cell Type Designation
Blood	**Monocytes**
Bone Marrow	**Monocytes and Monocyte percursors(monoblasts)**
Any solid tissue	**Resident macrophages and myeloid dendritic cells**
Skin	**Langrehans cells**
Liver	**Kupffer cells**
Lung	**Alveolar Macrophages**
Bone	**Osteoclasts**
Synovium	**Type A synovial cells**
Central nervous system	**Microglia**
Pleural Cavity	**Pleural Macrophages**
Peritoneal Cavity	**Peritoneal Macrophages**

Mononuclear phagocytes & Macrophage system



- **Differentiation of a monocyte into a tissue macrophage involves a number of changes:**
  - **Enlarges five- to ten folds;**
  - **Increase in both number and complexity of their intracellular organelles;**
  - **Acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes,**
  - **Begins to secrete a variety of soluble factors.**

Mononuclear phagocytes & Macrophage system



- Macrophages are dispersed [dis'pə:st] throughout the body.
- Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain mobile and are called free, or wandering, macrophages.
- Free macrophages travel by amoeboid movement throughout the tissues.

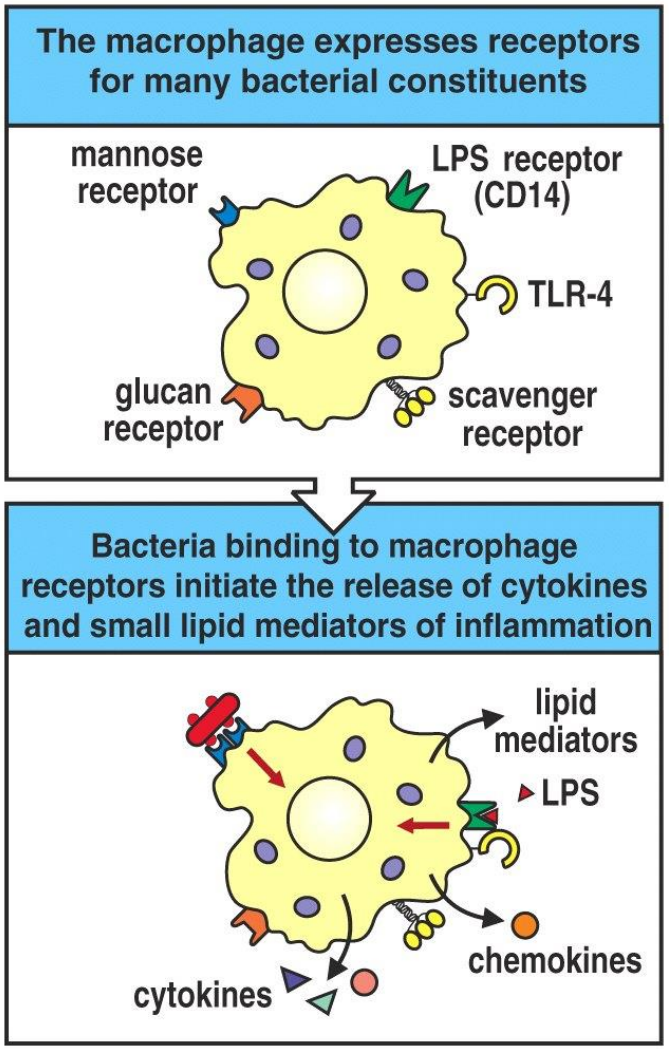
Mononuclear phagocytes & Macrophage system

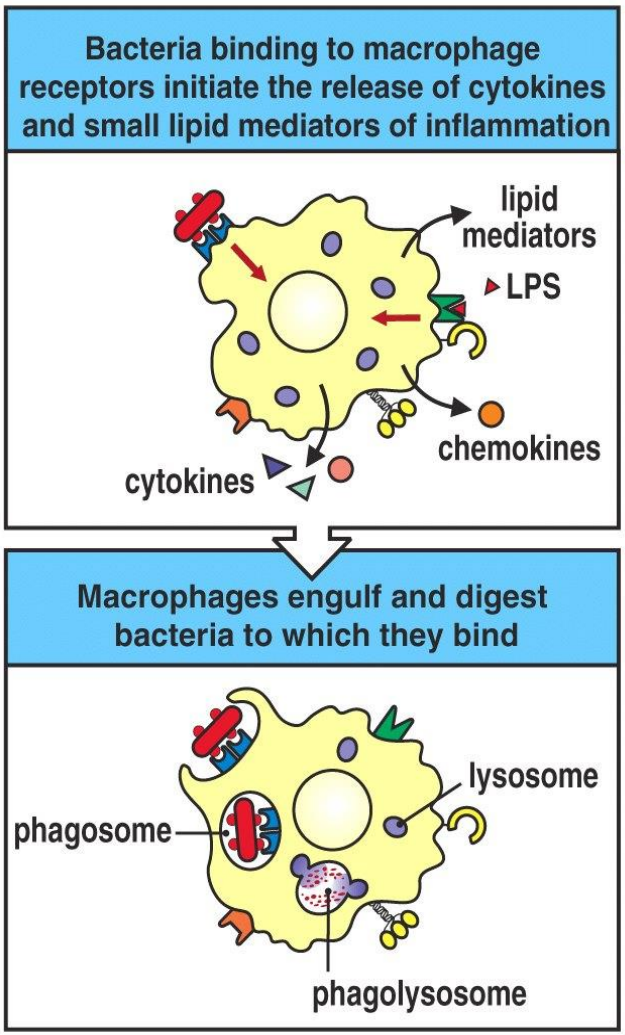
MACROPHAGES ACTIVATION

- Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response.
- Phagocytosis of particulate antigens serves as an initial activating stimulus.

Mononuclear phagocytes & Macrophage system

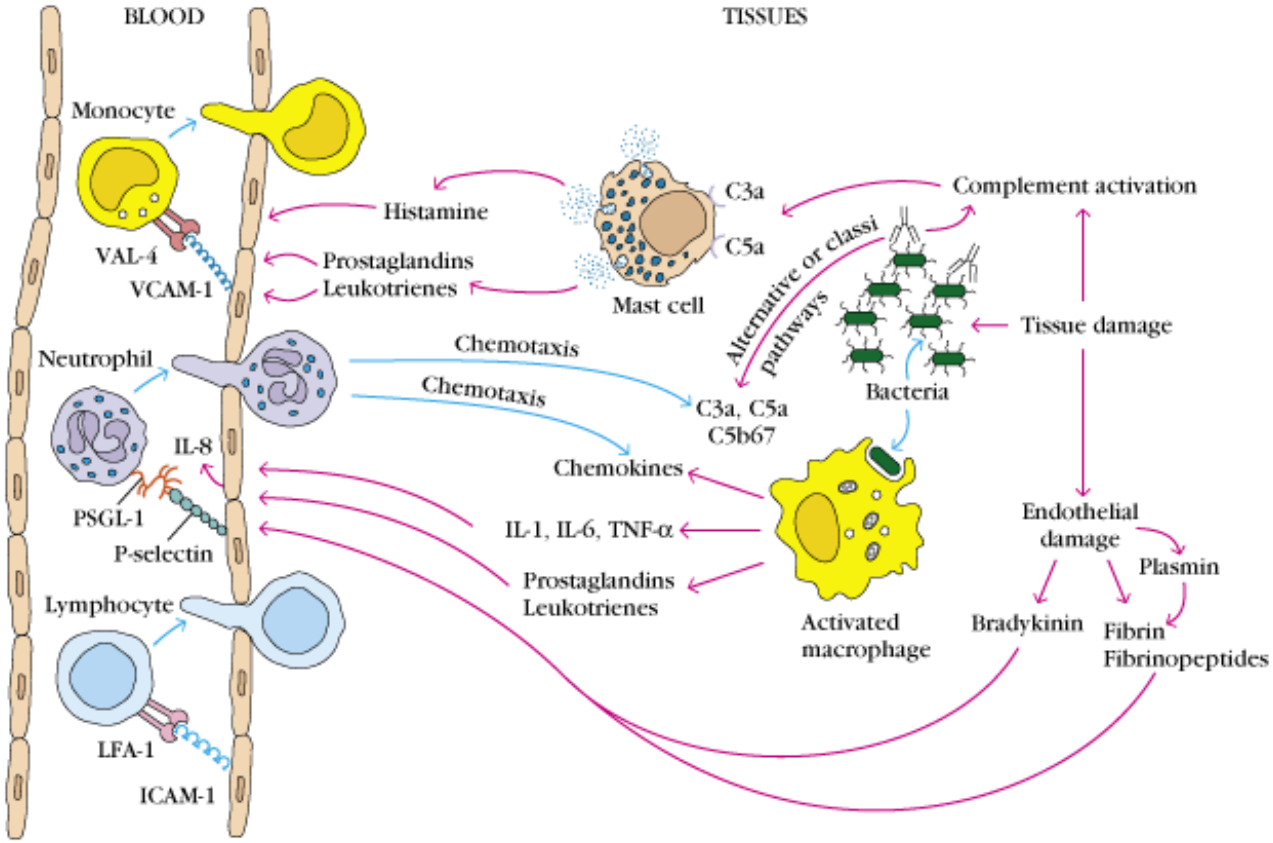
MACROPHAGES ACTIVATION





Mononuclear phagocytes & Macrophage system

Increased secretion of inflammatory mediators by activated macrophages



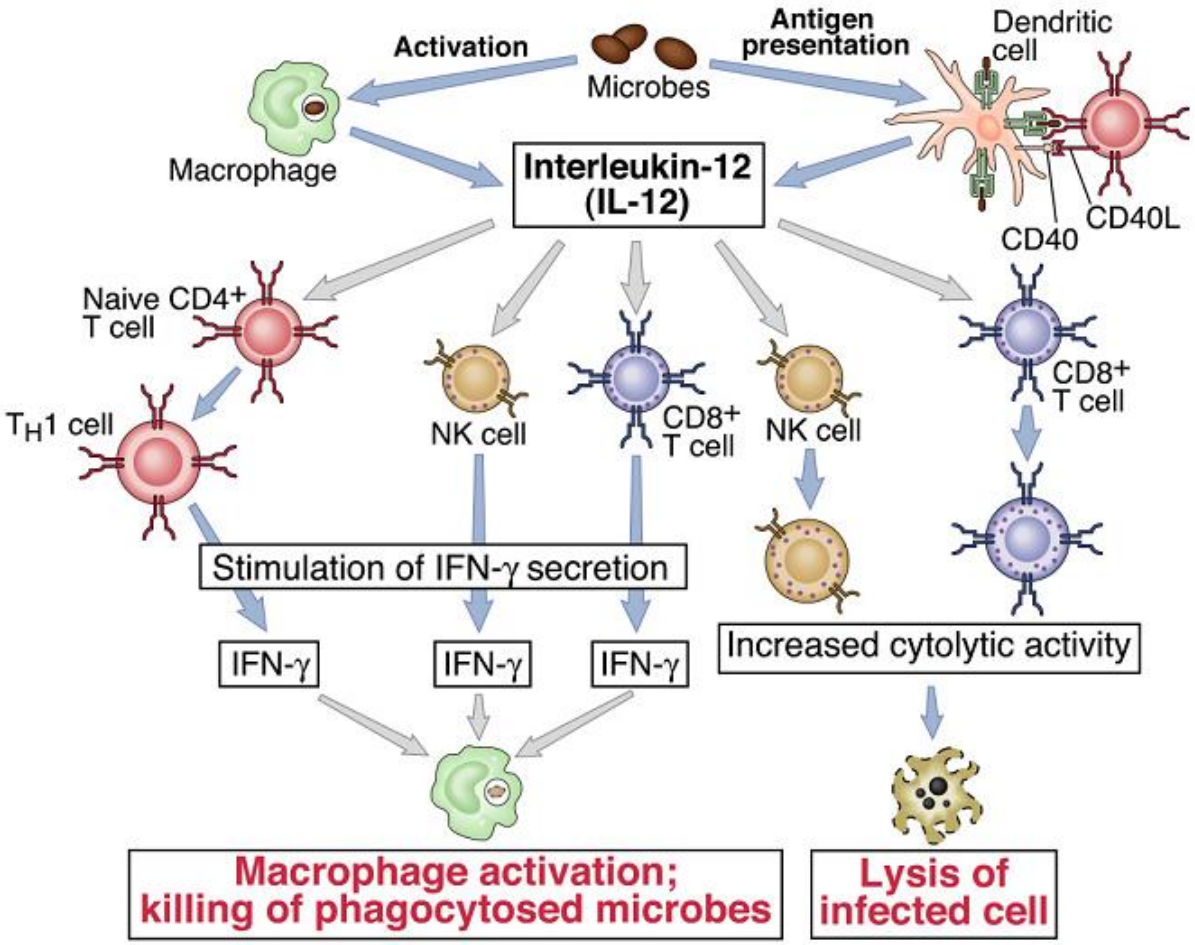
Mononuclear phagocytes & Macrophage system

MACROPHAGES ACTIVATION

- Macrophage activity can be further enhanced
  - By mediators of the inflammatory response, and
  - By components of bacterial cell walls.
  - One of the most potent activators of macrophages is interferon gamma (IFN-γ) secreted by activated Th cells.

Mononuclear phagocytes & Macrophage system

MACROPHAGES ACTIVATION



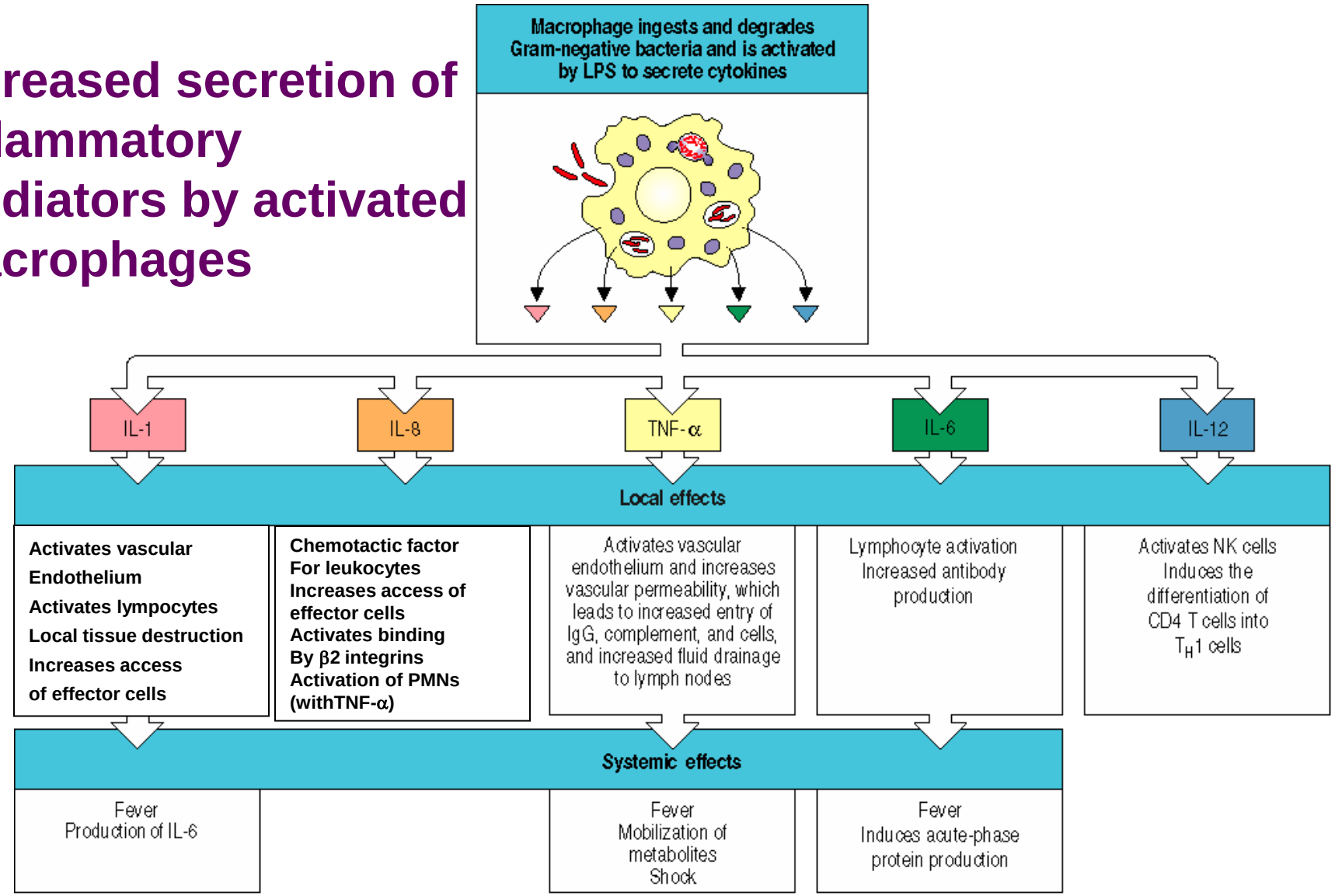
Mononuclear phagocytes & Macrophage system

MACROPHAGES ACTIVATION

- Activated macrophages are more effective than resting ones in eliminating potential pathogens, because they
  - exhibiting greater phagocytic activity,
  - an increased ability to kill ingested microbes,
  - an increased secretion of inflammatory mediators,
  - an increased ability to activate T cells.

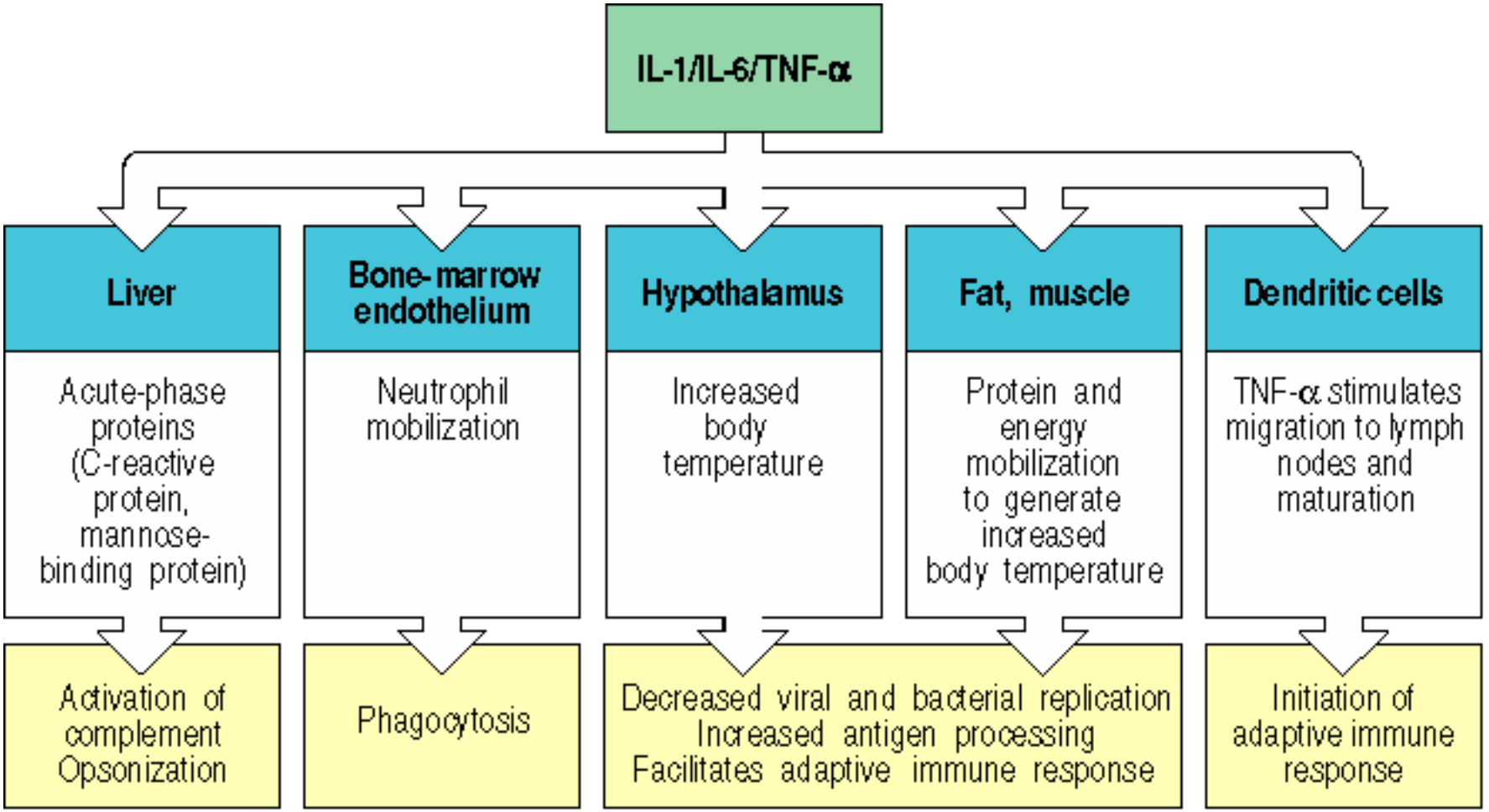
Mononuclear phagocytes & Macrophage system

increased secretion of inflammatory mediators by activated macrophages



Mononuclear phagocytes & Macrophage system

increased secretion of inflammatory mediators by activated macrophages



network

Mononuclear phagocytes & Macrophage system

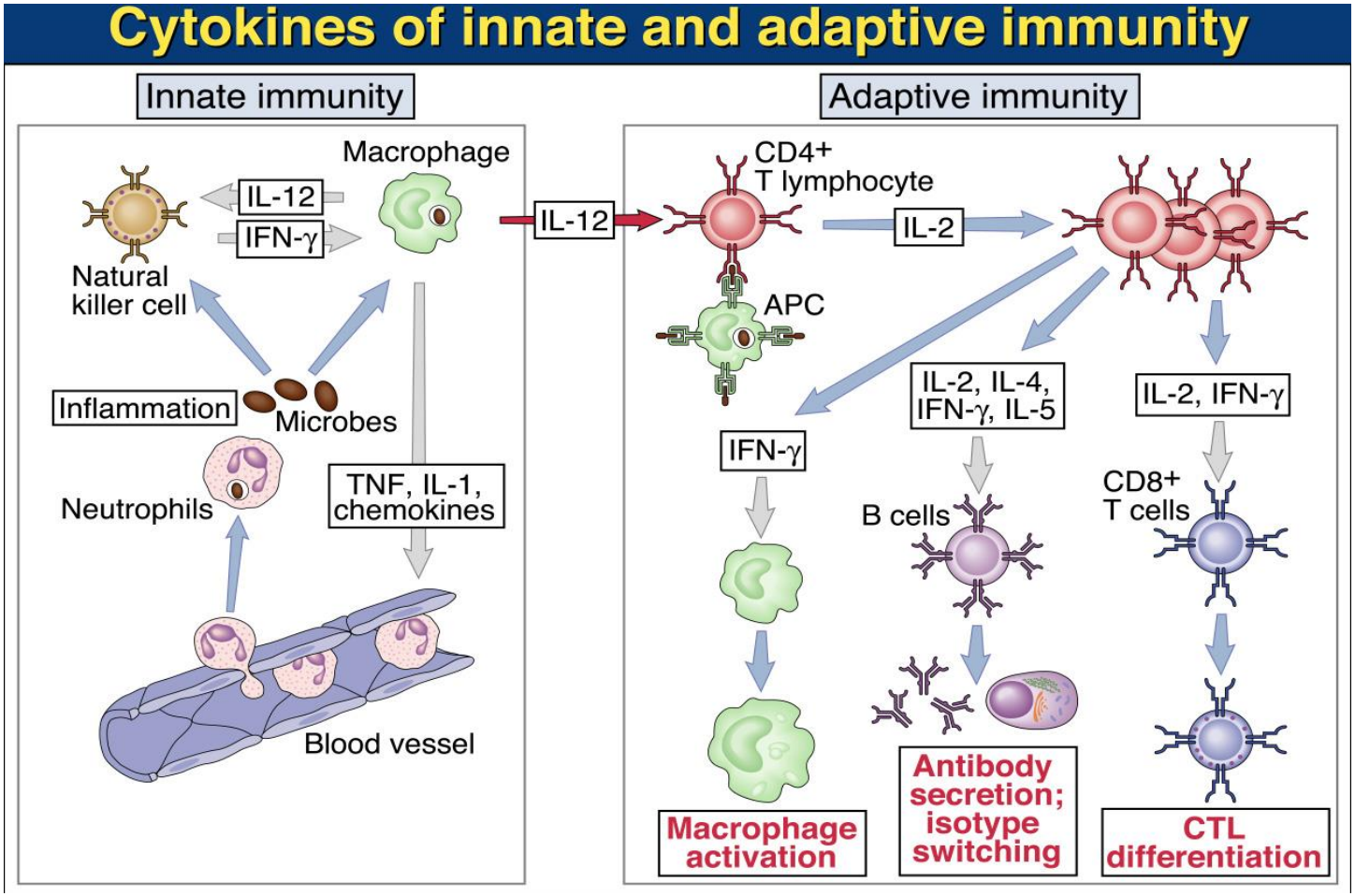
MACROPHAGES ACTIVATION

- In addition, activated macrophages, but not resting ones, secrete various cytotoxic proteins that help them eliminate a broad range of pathogens, including
  - virus-infected cells,
  - tumor cells,
  - intracellular bacteria.

Mononuclear phagocytes & Macrophage system

During the immune response

macrophages and Th cells facilitate each other's activation



Natural killer cells & NK cells

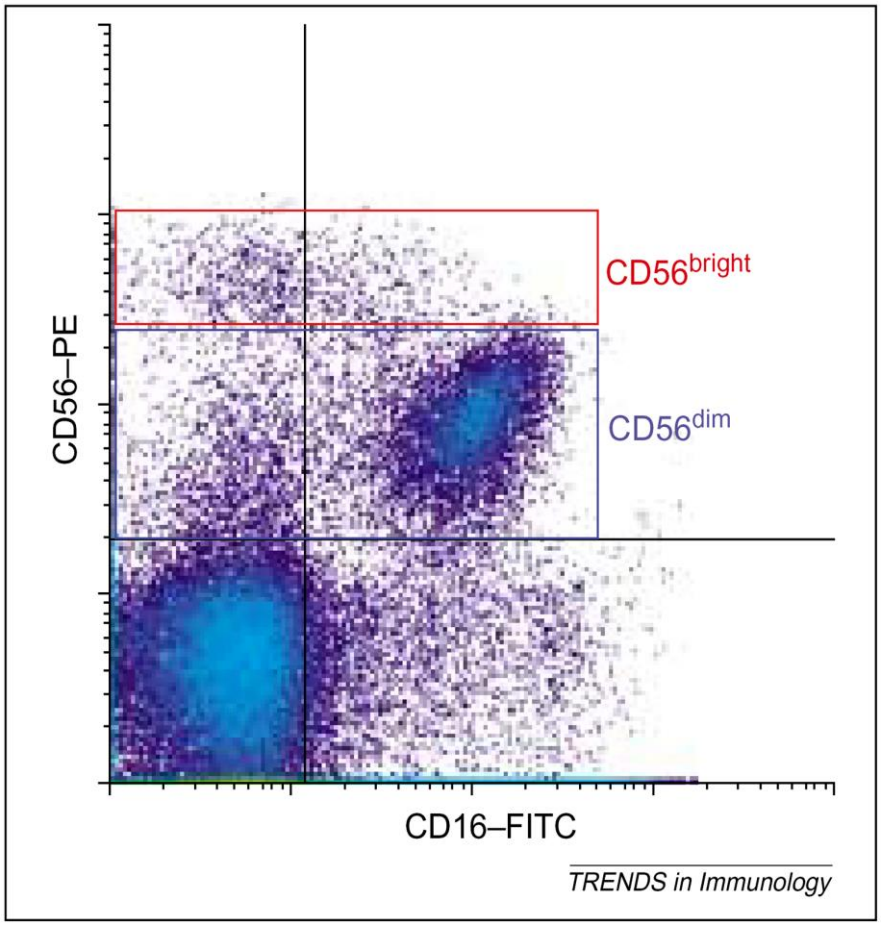
NK cells do not require a thymus for their development but have several similarities to activated CD8 T cells.

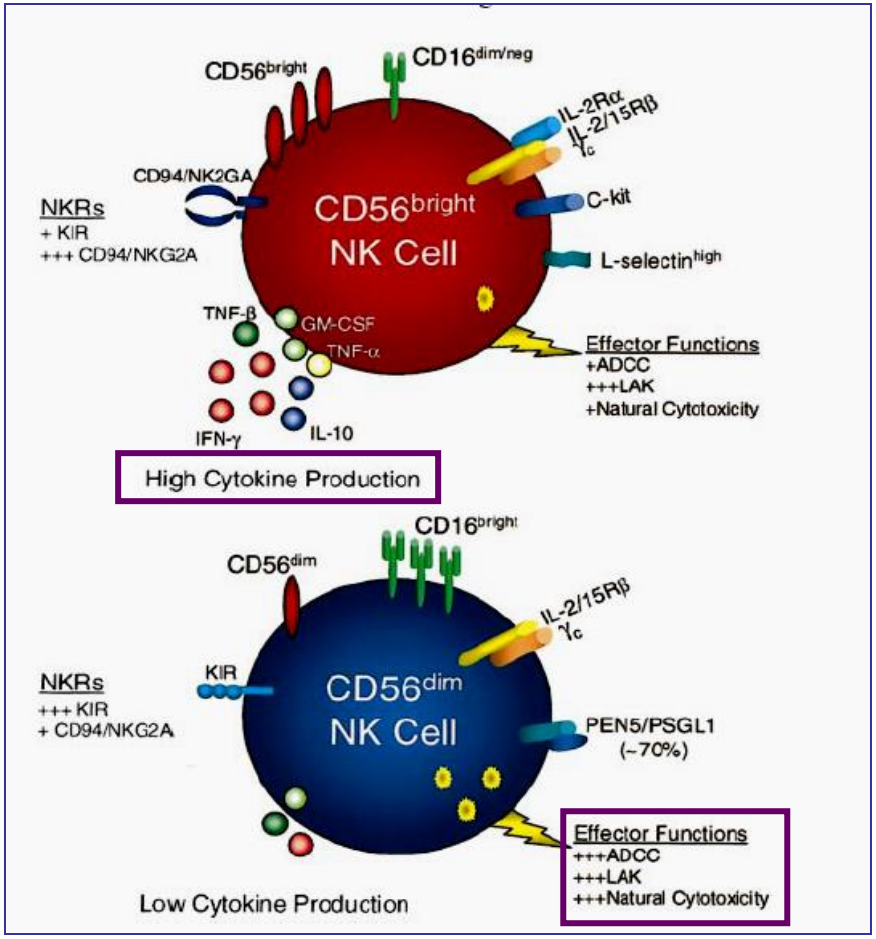
- They look like large lymphocytes and contain granules.
- They are ready to kill target cells without clone expansion.
- They kill target cells using perforin.
- They can rapidly produce cytokines upon ligand recognition.
- They seem to be especially important in resistance to intracellular infections of viruses or bacteria.

Natural killer cells & NK cells

Surface markers identified as human NK cells:

TCR^{-}、mIg^{-}、$CD56^{+}$、$CD16^{+}$

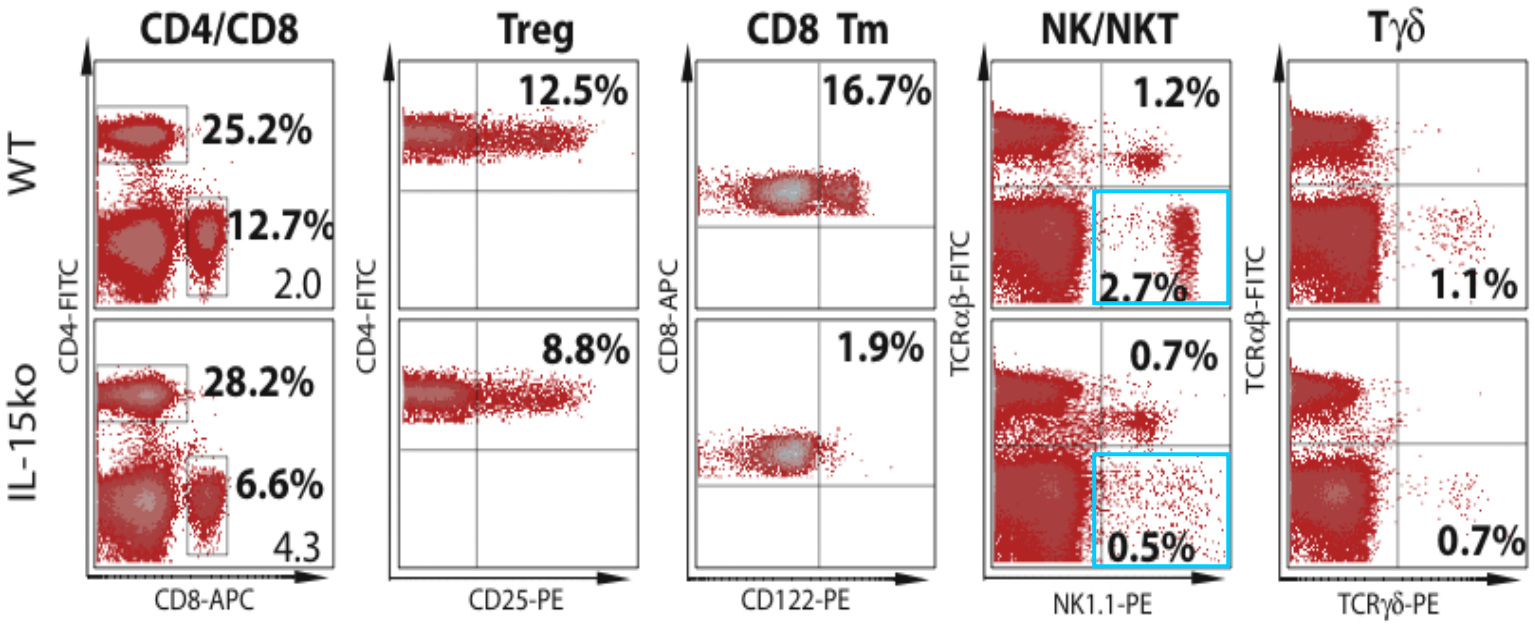




Natural killer cells & NK cells

Surface markers identified as murine NK cells:

$TCR^{-}/CD3^{-}$、mIg^{-}、 $NK1.1^{+}$



Blood samples taken from wild-type mice and IL-15KO mice

Activating and inhibitory receptors of NK cells

- NK cells express together both inhibitory and stimulatory receptors.
- Stimulatory receptors see self molecules some of which are often stress-induced "non-classical" MHC class I - like molecules that are not polymorphic.
  - MICA :MHC class Ⅰ chain-related molecules A/B
- NK cells also express inhibitory receptors which see self MHC, *both* classical and some non-classical MHC molecules.

Activating and inhibitory receptors of NK cells

KIR:

killer immunoglobulin-like receptor

NK-cell receptors					
Inhibitory			Activating		
Receptor	Structural type	Ligand	Receptor	Structural type	Ligand
KIR2DL	Ig	HLA-C	KIR2DS	Ig	HLA-C
KIR3DL	Ig	HLA-B,C	KIR3DS	Ig	HLA-B?

HLA-C/B

KIR3DL

ITIM:immunoreceptor tyrosine-based inhibitory motif

DAP12

HLA-,Pathogen

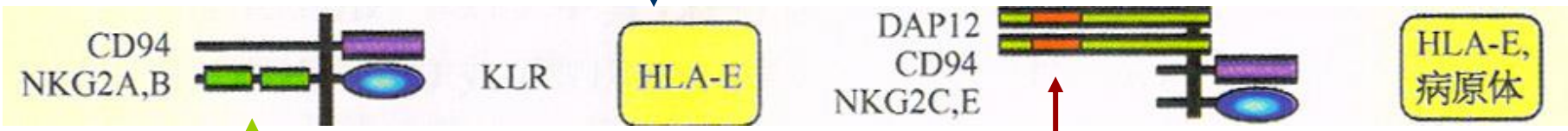
KIR2DS

ITAM:immunoreceptor tyrosine-based activation motif

Activating and inhibitory receptors of NK cells

KLR:
killer lectin-like receptors

NK-cell receptors					
Inhibitory			Activating		
Receptor	Structural type	Ligand	Receptor	Structural type	Ligand
LILRB1,2	Ig	HLA class I	LILRA3	Ig	?
CD94:NKG2A	Lectin	HLA-E	CD94:NKG2C/E	Lectin	HLA-E



HLA-,Pathogen

ITIM:immunoreceptor tyrosine-based inhibitory motif

ITAM:immunoreceptor tyrosine-based activation motif

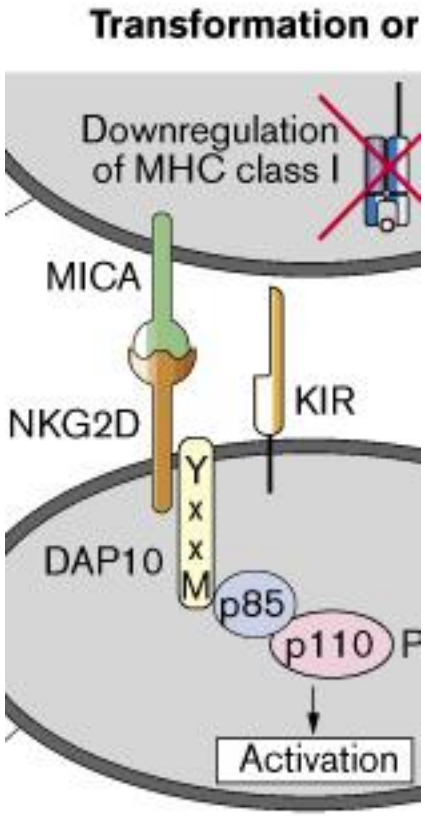
Activating and inhibitory receptors of NK cells

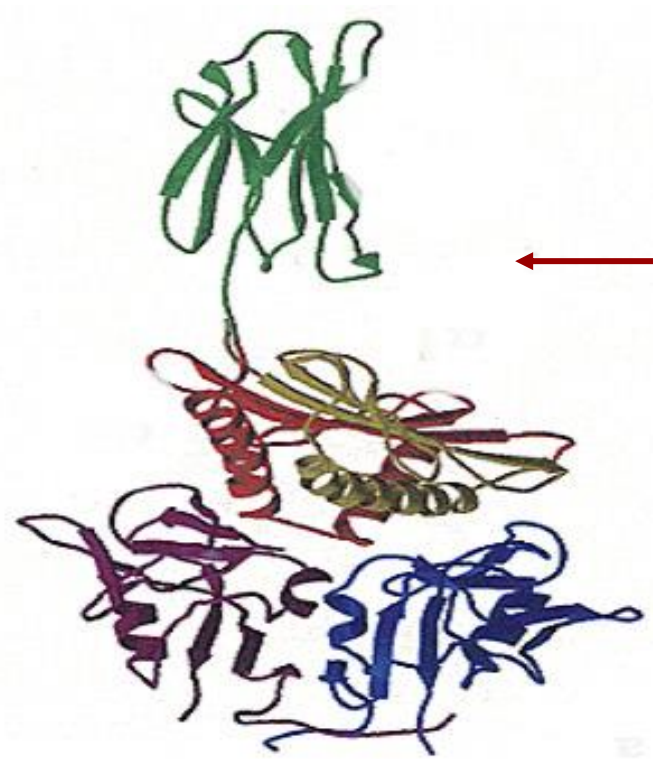
KLR: NKG2D

Ligand: MICA/B:

MHC class Ⅰ chain-related molecules A/B

Up-expressing on the surface of some cancer cells

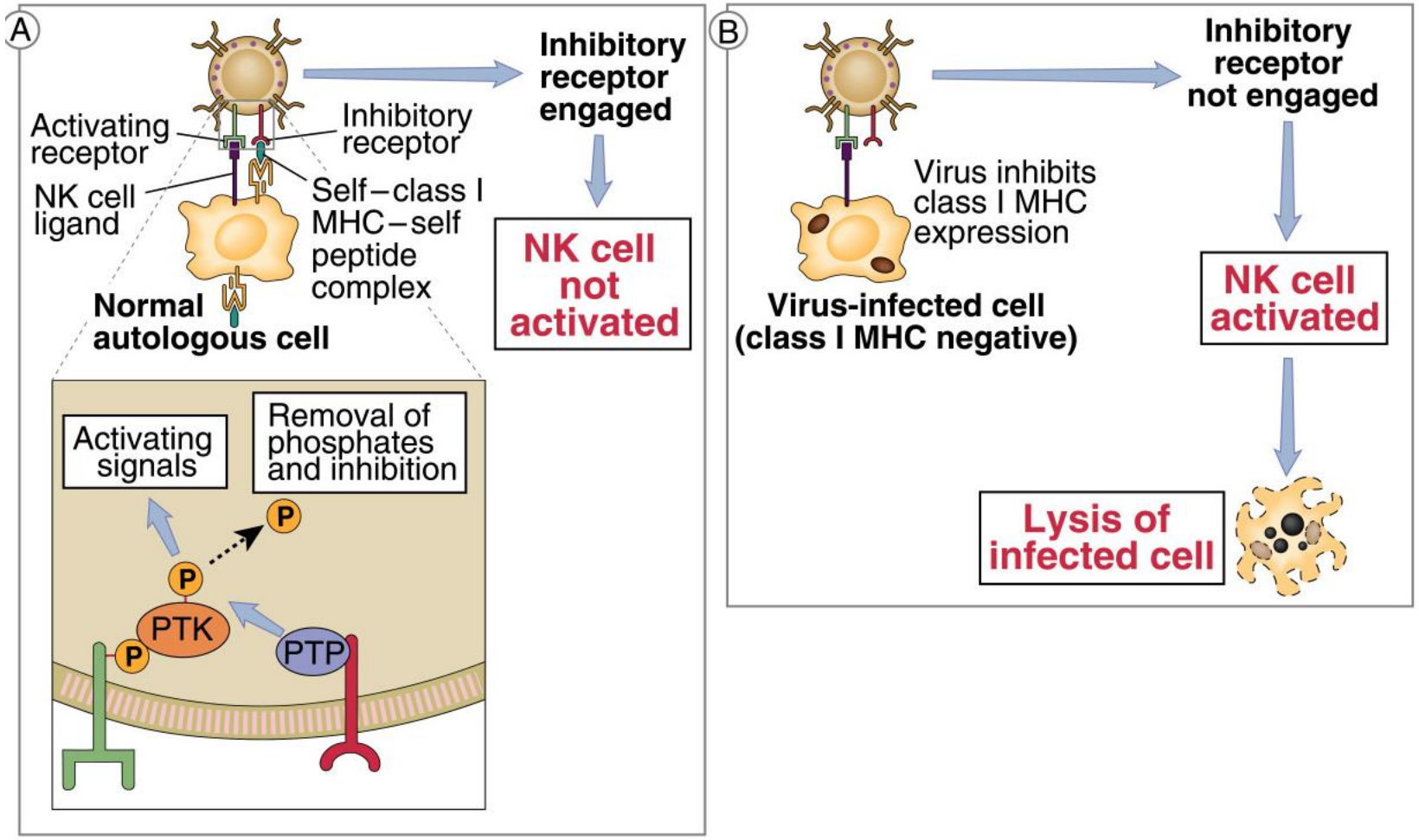




receptors

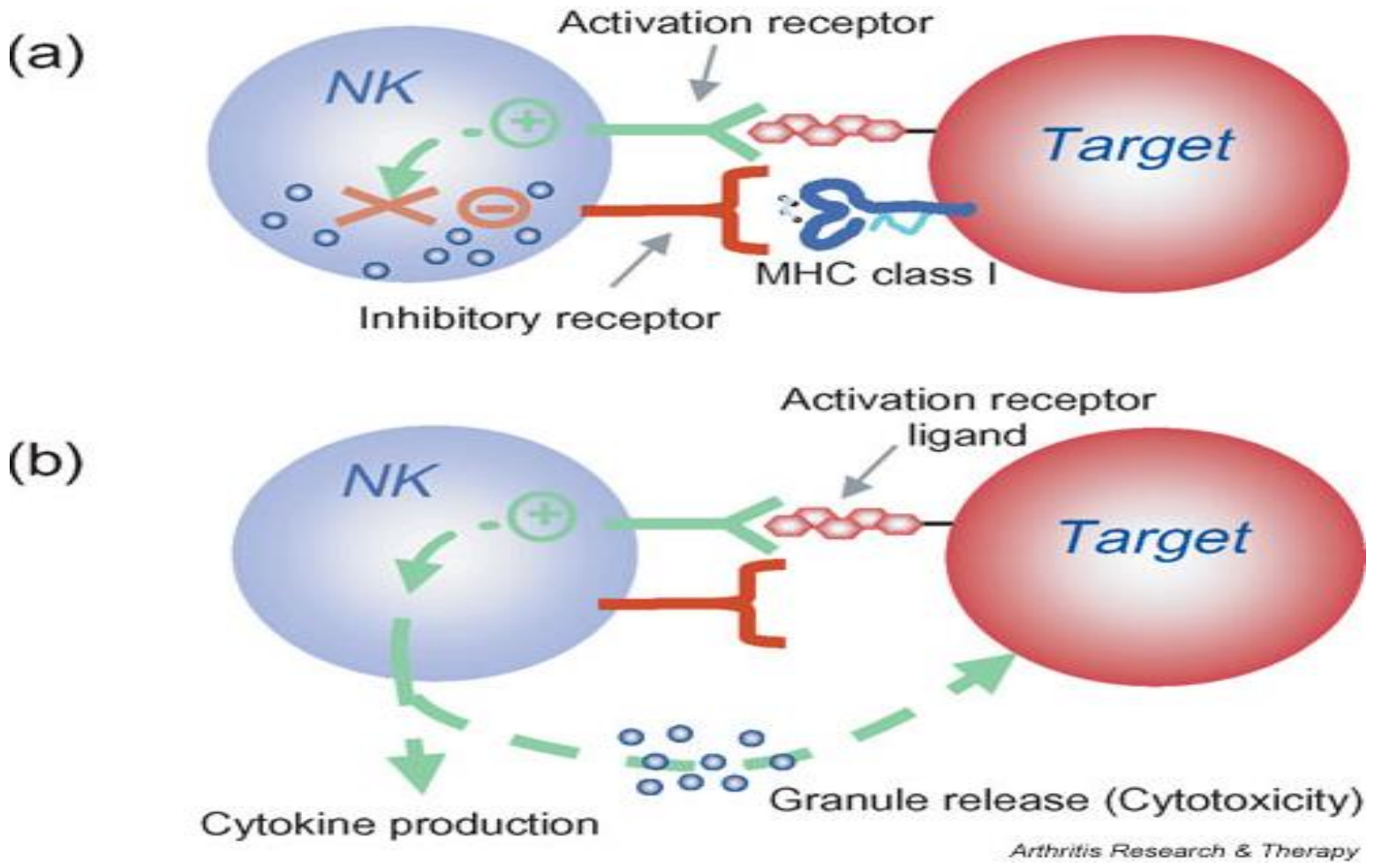
Activating		
Receptor	Structural type	Ligand
KIR2DS	Ig	HLA-C
KIR3DS	Ig	HLA-B?
LILRA3	Ig	?
CD94:NKG2C/E	Lectin	HLA-E
LAIR-2	Ig	?
NKG2D	Lectin	MIC-A,B and others
NKp30	Ig	?
NKp44	Ig	?
NKp46	Ig	?
CD16	Ig	Fc

Missing self recognition by NK cells

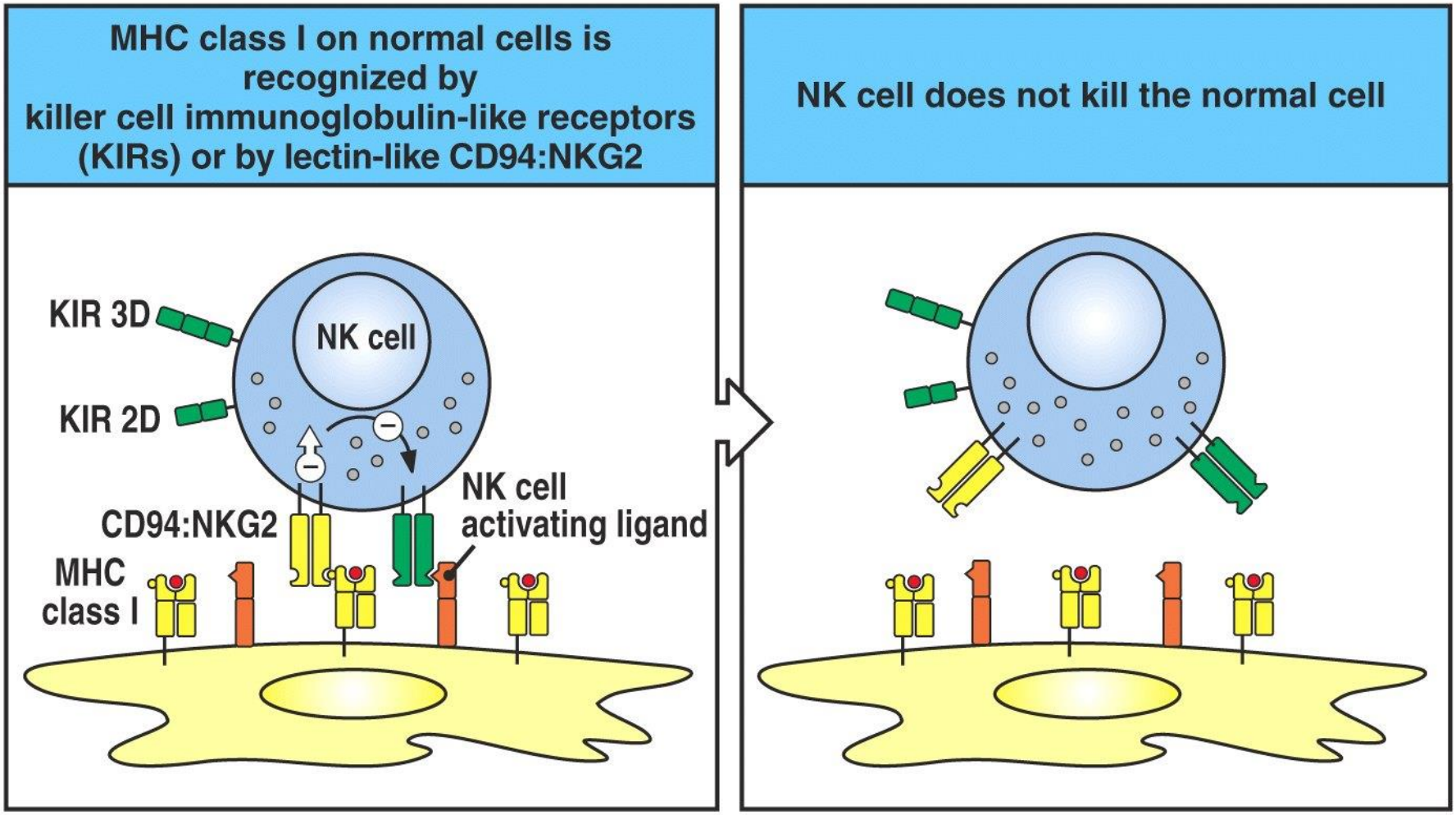


Missing self activates NK cells

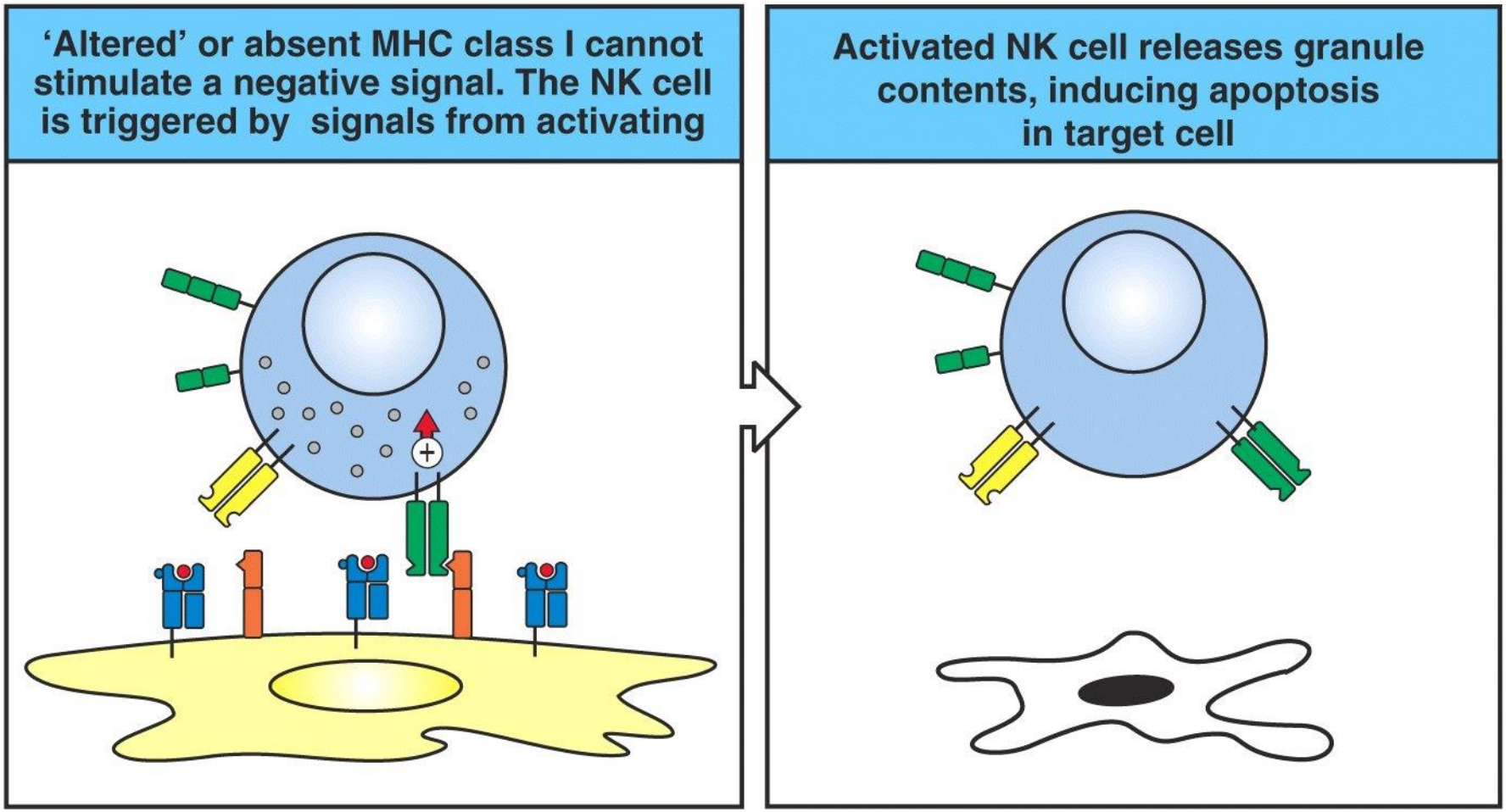
(some tumors surveillance from NK cells)



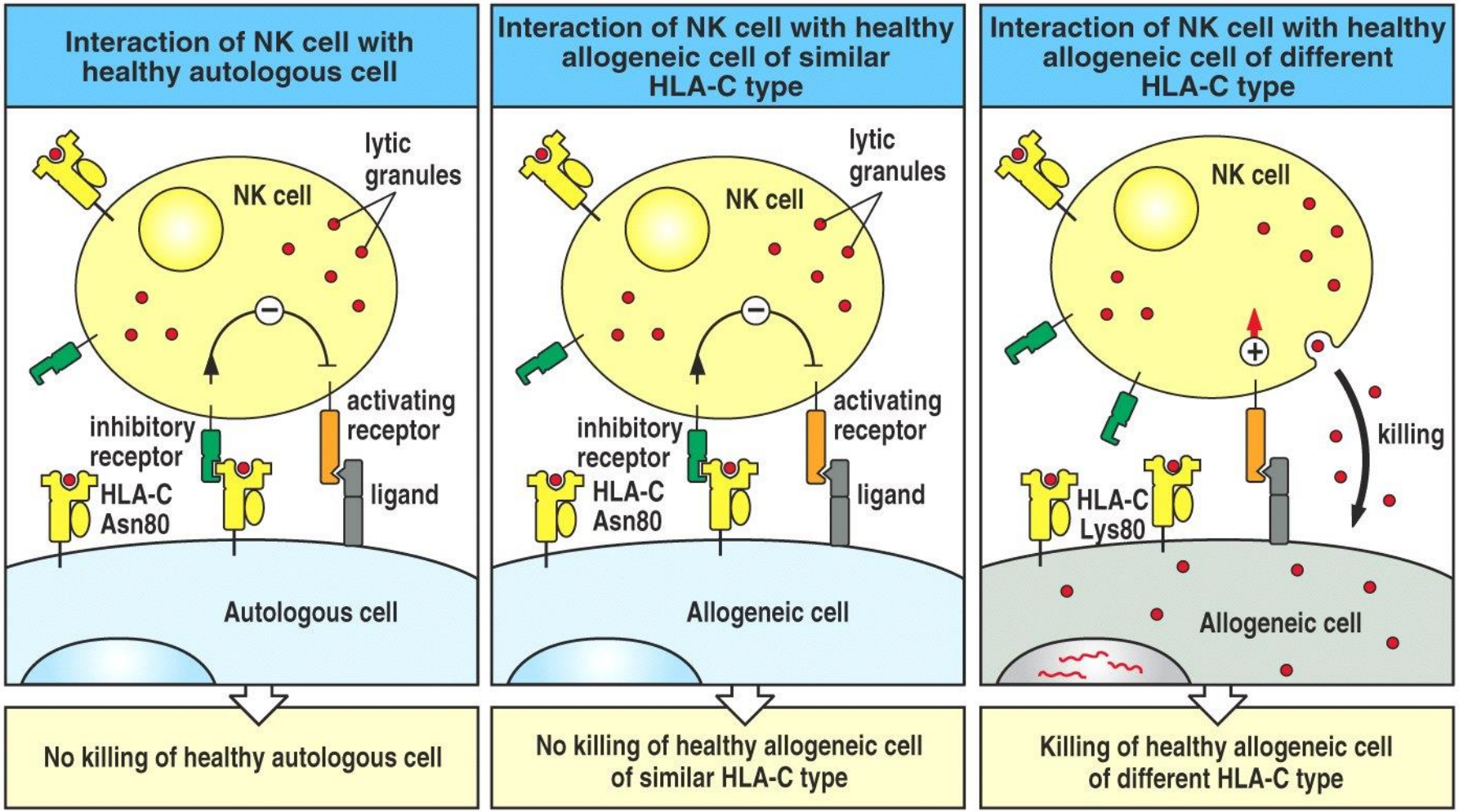
Missing self recognition by NK cells



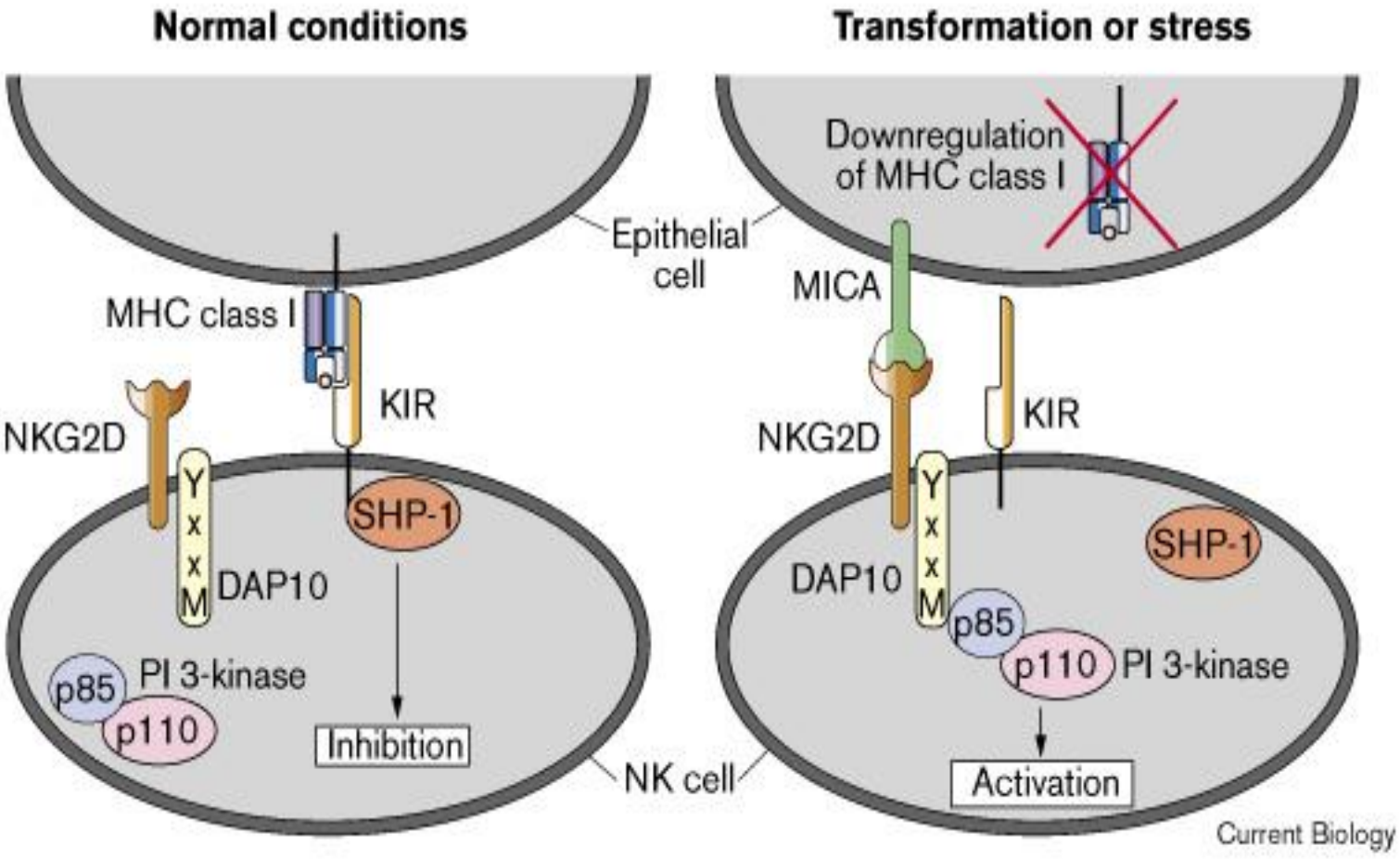
Missing self recognition by NK cells



NK cells can kill healthy cells from histo-incompatible individuals



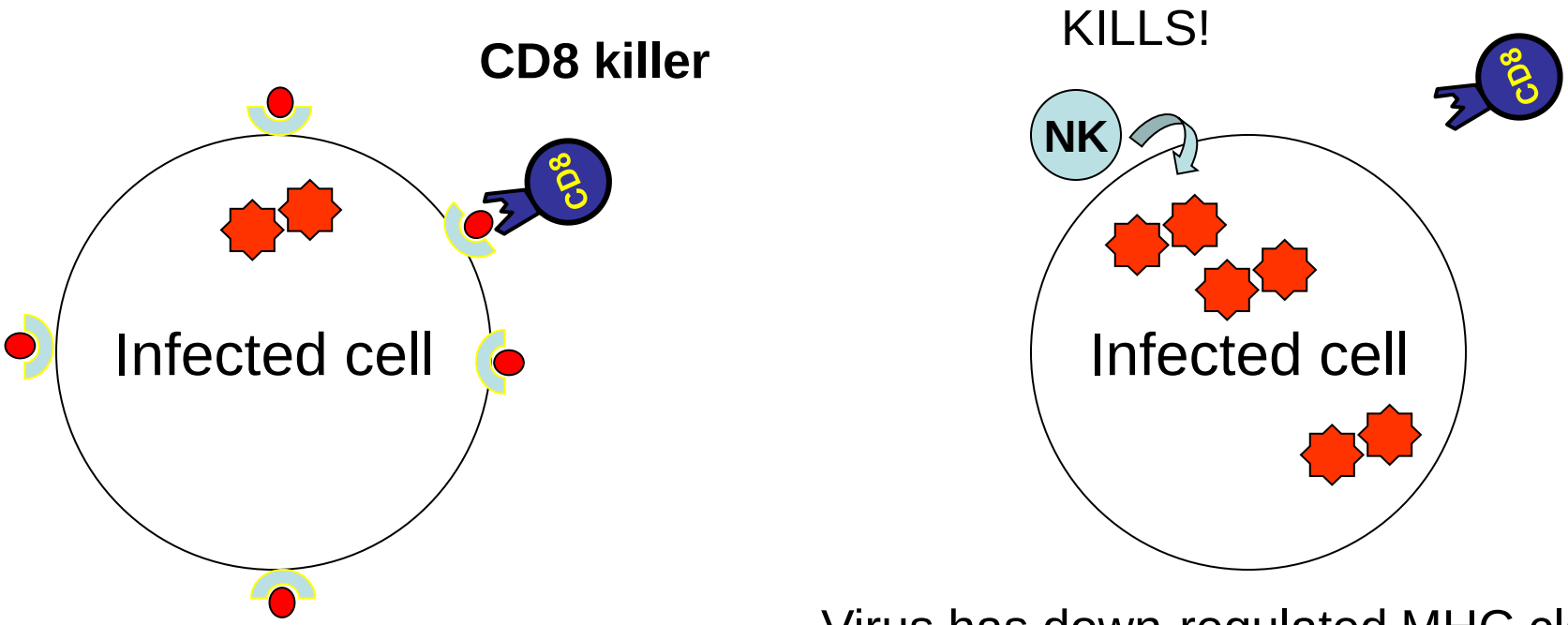
MICA recognition - “Stress”



MHC class Ⅰ chain-related molecules A/B

CD8 T cells & NK cells

NK cells play an important role in "backing up" CD8 T cells

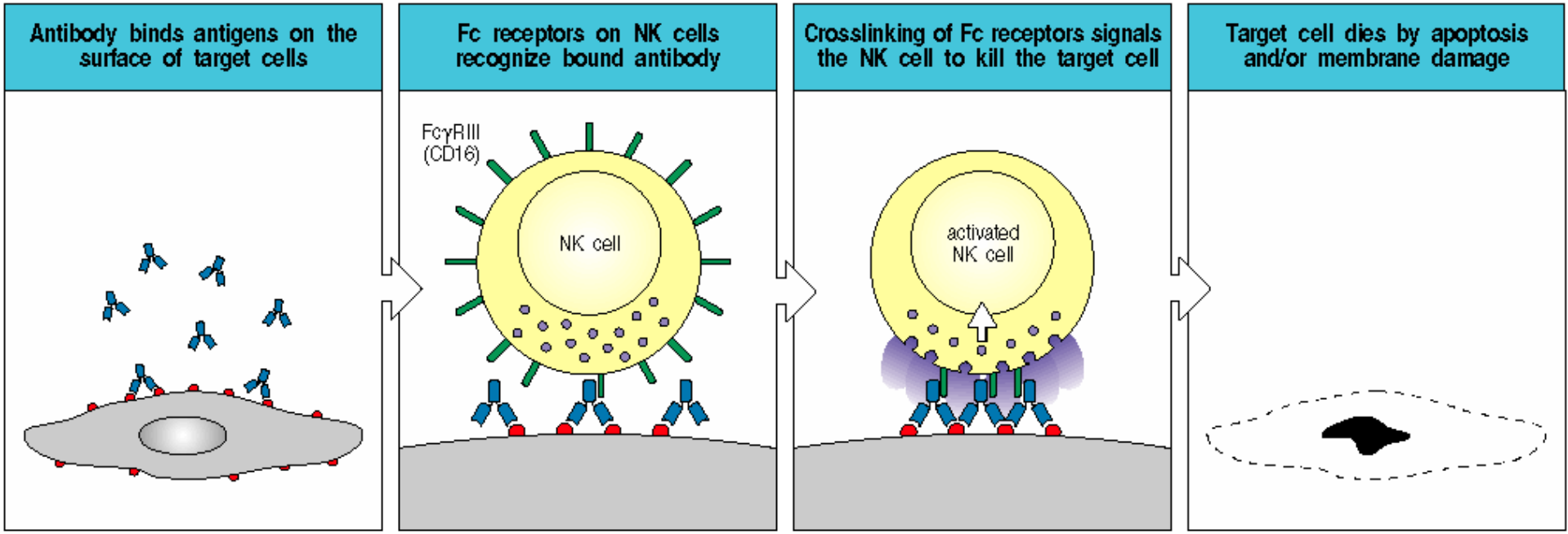


Virus has down-regulated MHC class I

Natural killer cells & NK cells

One way that NK cells recognize their targets

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)



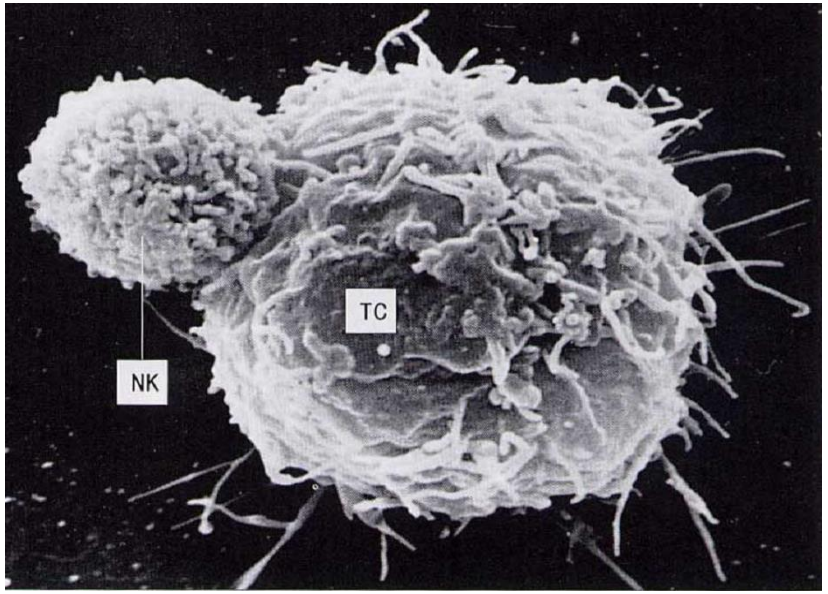
NK cells kill rapidly when they detect targets, for example, antibody coated cells.

In this case the cells are activated by a low affinity Fc receptor tha recognizes clustered antibody decorating a cell surface.

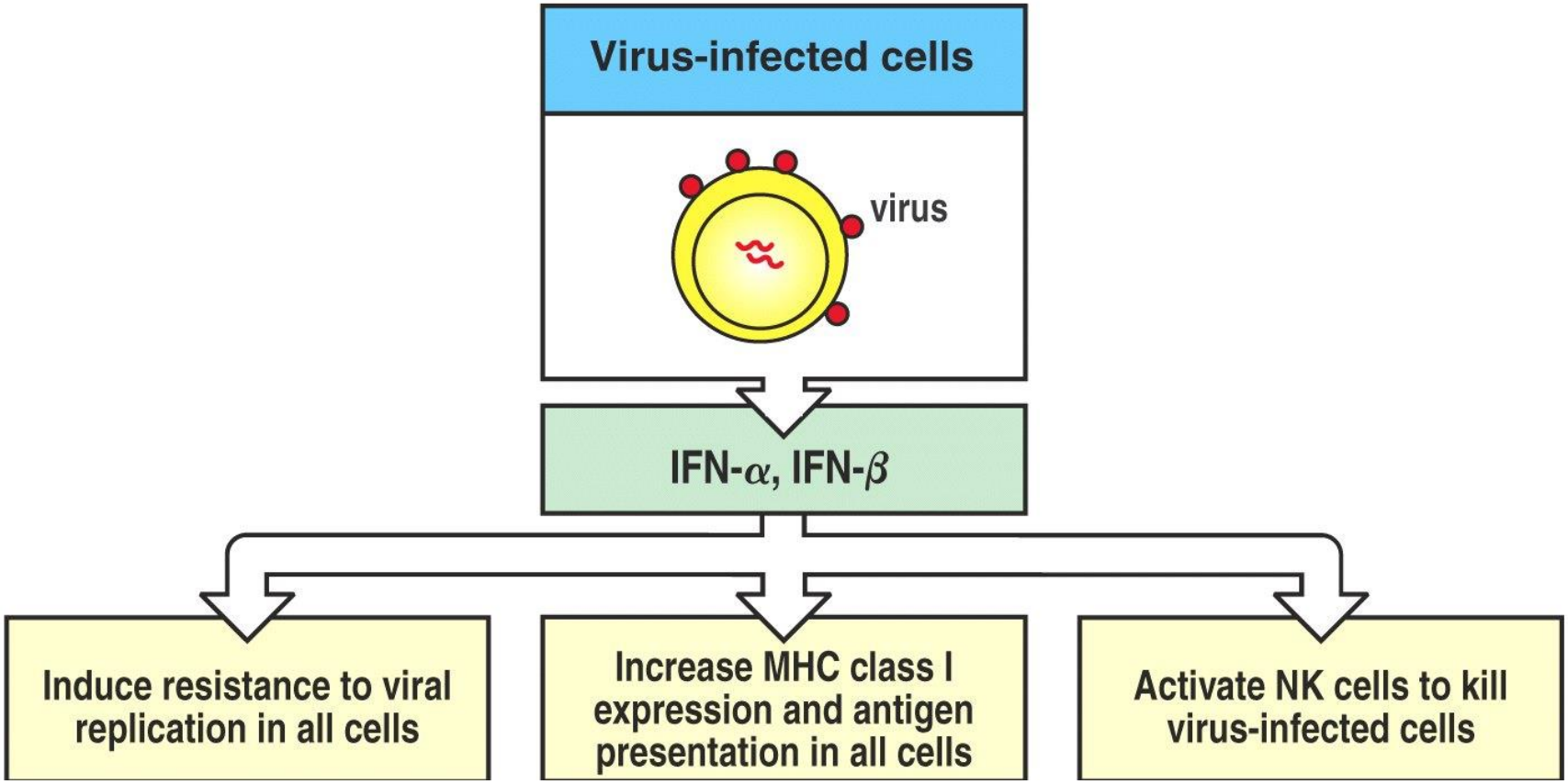
NK tolerance by the mother to the fetus

- MHC class I antigens are expressed at very low levels in embryonic tissue.

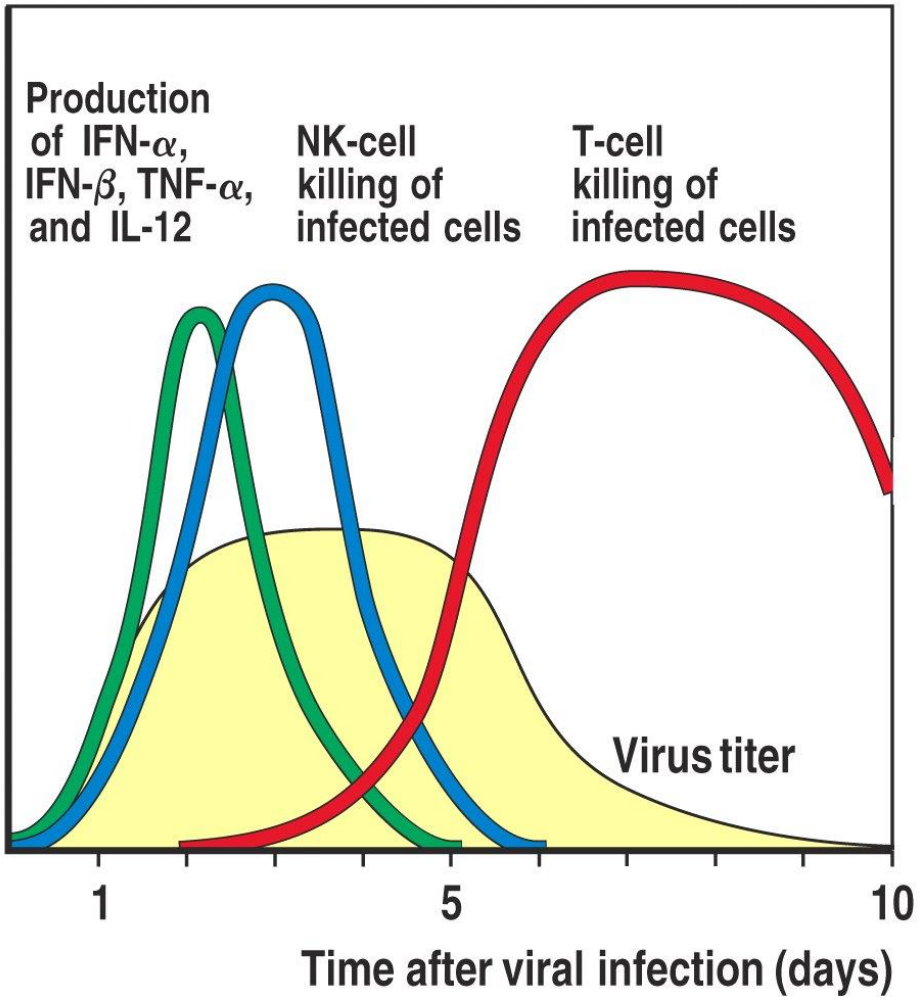
- HLA-G is highly expressed by the placenta, suppressing NK function.



Early in an inflammatory reaction, NK cells are activated by IFN-γ and can eliminate cells that down regulate MHC class I



Early in an inflammatory reaction, NK cells are activated by IFN-g and can eliminate cells that down regulate MHC class I



NK cell recognition Concepts

- NK cells are effector cells, not naïve.
- NK cells must learn self when immature.
- Recognition
  - NK "missing self" recognition of cells that lose MHC class I expression
  - NK “stress”recognition – of cells that express molecules without on normal cells
  - NK cells can recognize targets Fc receptor
- Missing self recognition is based on the presence of both inhibitory and activating receptors on NK cells.

Summary

1. Immune Organs and their main functions
2. Cells of Innate Immunity
3. Mononuclear phagocytes and their main functions
4. NK cells and their main functions