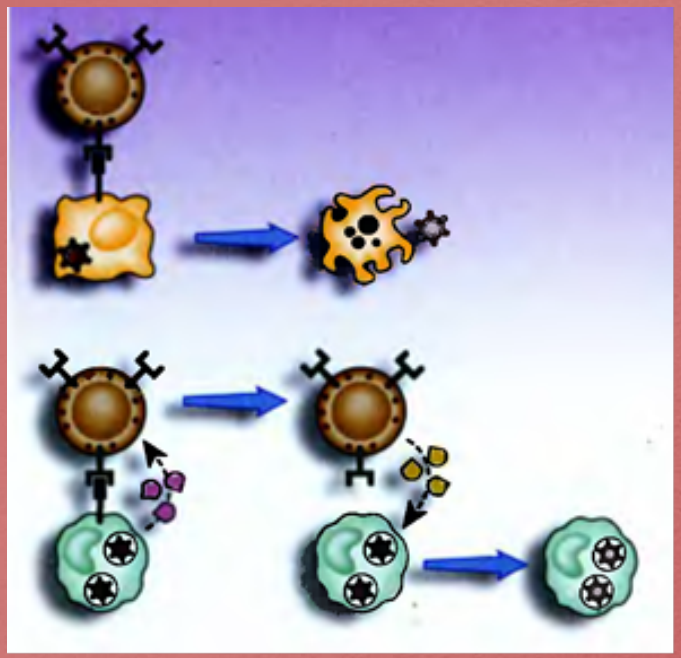
Innate Immunity
Defense System against Infections



Course 2

Assoc. Prof. Emrah Şefik Abamor

Innate vs. Adaptive Immunity

Innate

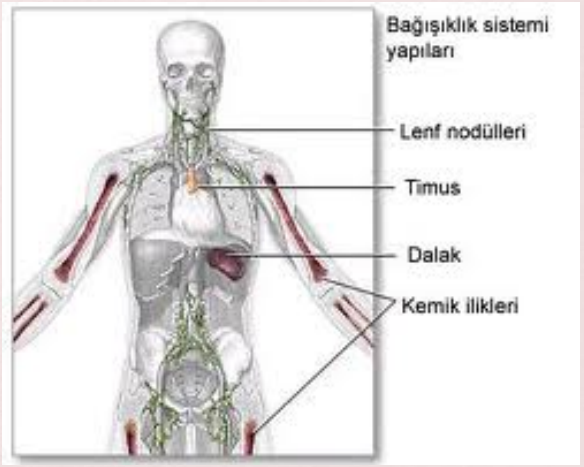
- Primitive (found in all multicellular organisms)
- Directed towards types of molecules
- Effectors are broadly reactive
- Response is immediate
- No anamnestic responses
- Effectors: epithelial cells, phagocytes, endothelial cells, fibroblasts

Adaptive

- Only in vertebrates
- Directed towards specific epitopes
- Response is slow
- Effectors are highly specific
- Memory persists
- Effectors: Lymphocytes, APCs

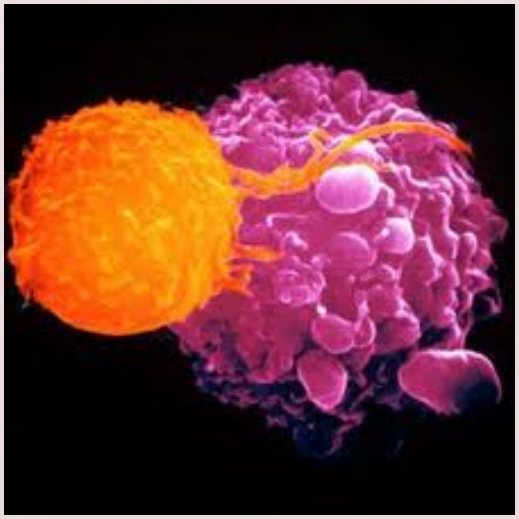
Innate Immunity

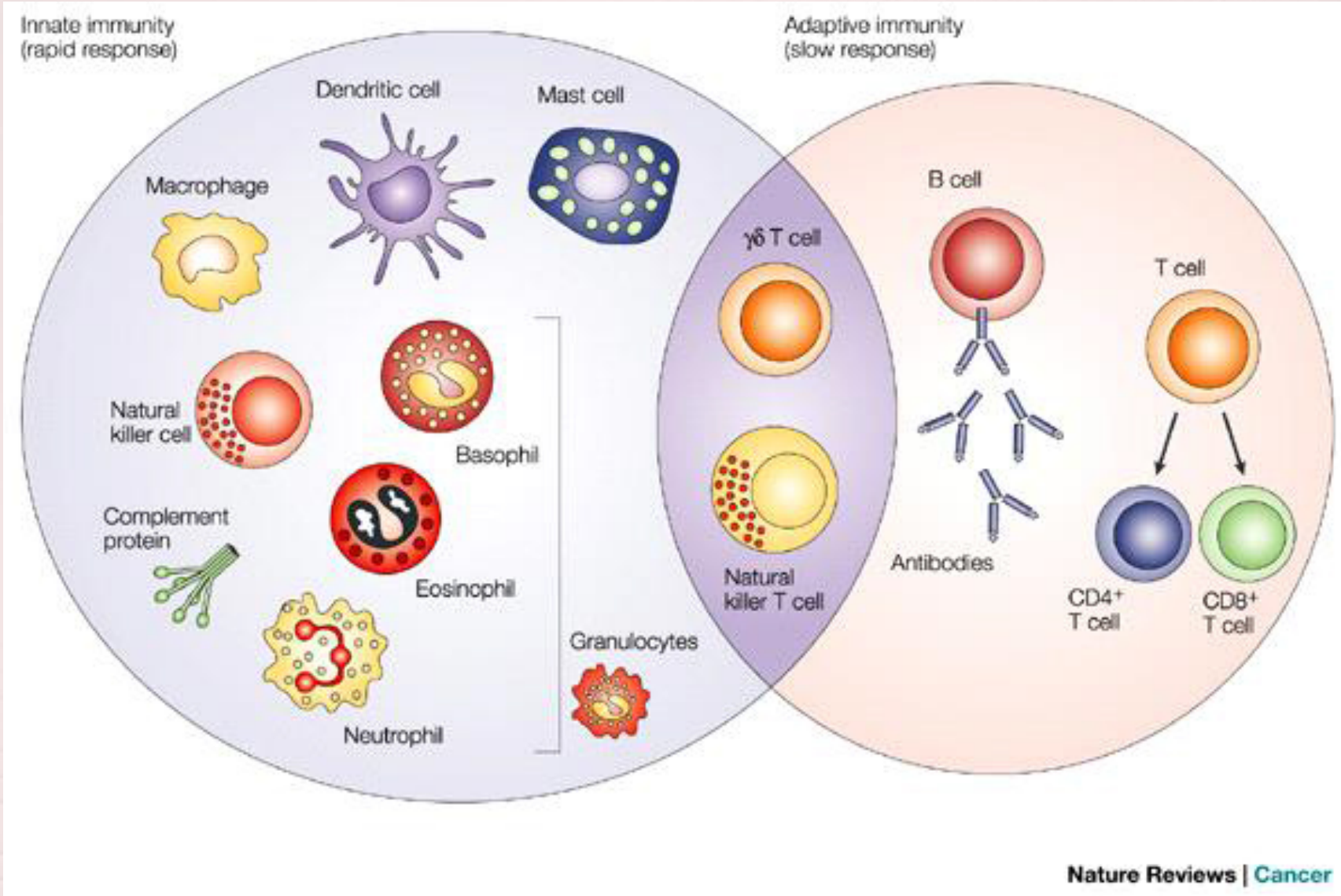
- All multicellular organisms such as vertebrates, invertebrates and plants have defense mechanisms to protect against infections caused by microorganisms.

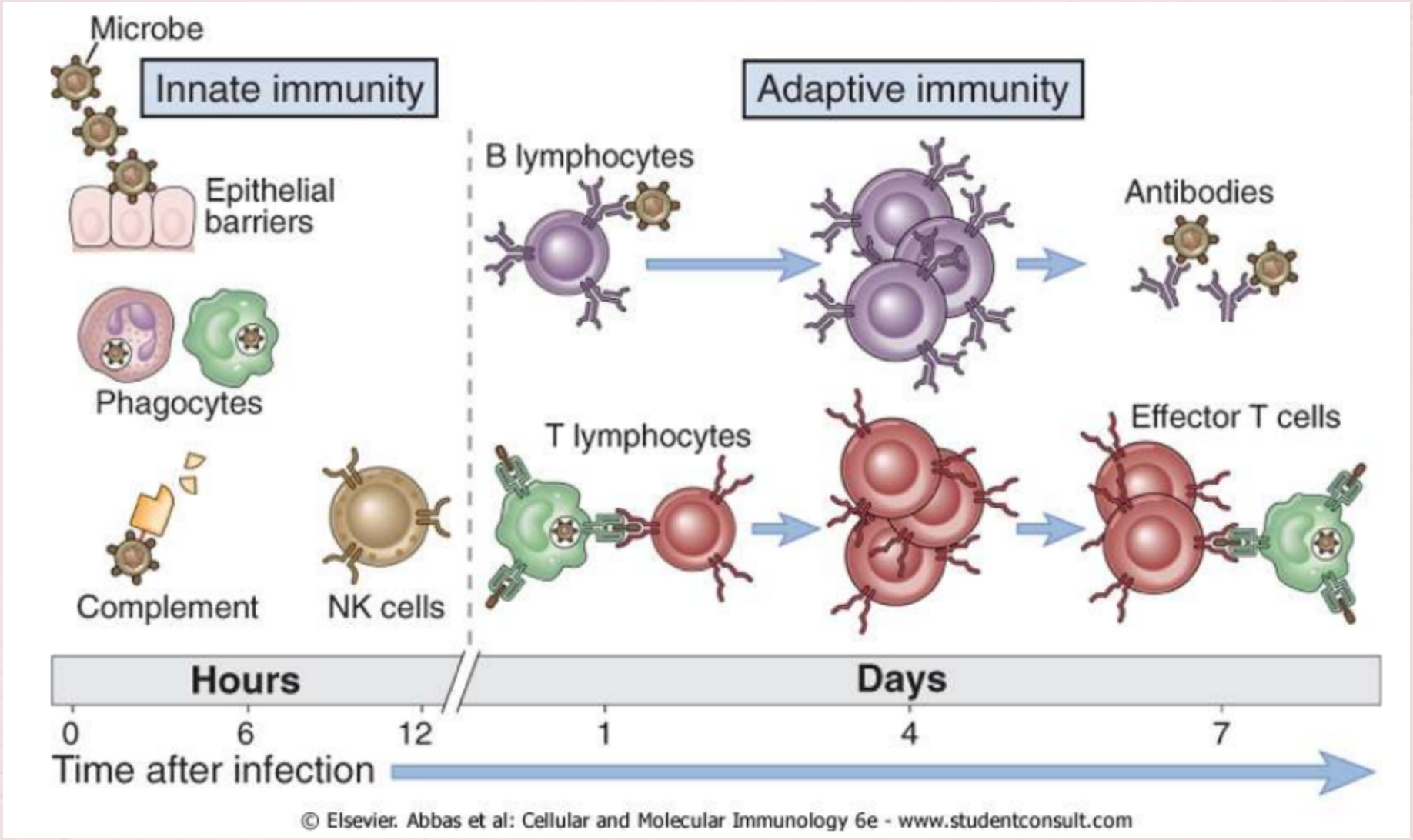


Innate Immunity

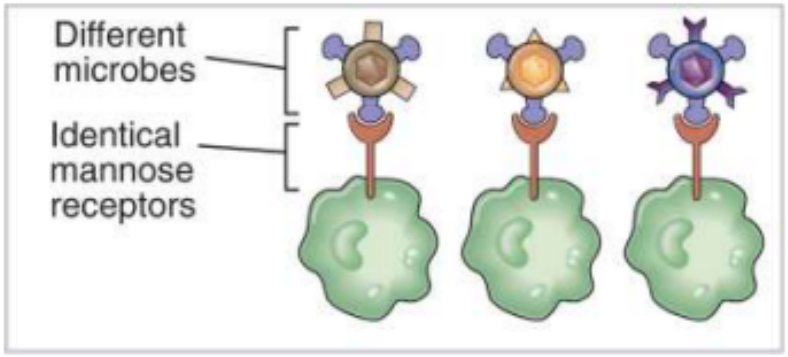
- Since these defense mechanisms are naturally present in organisms to recognize microorganisms and destroy them, they are called natural resistance or natural immunity.

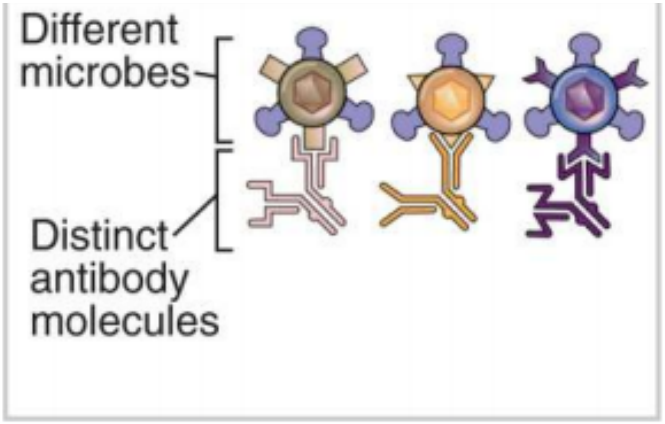


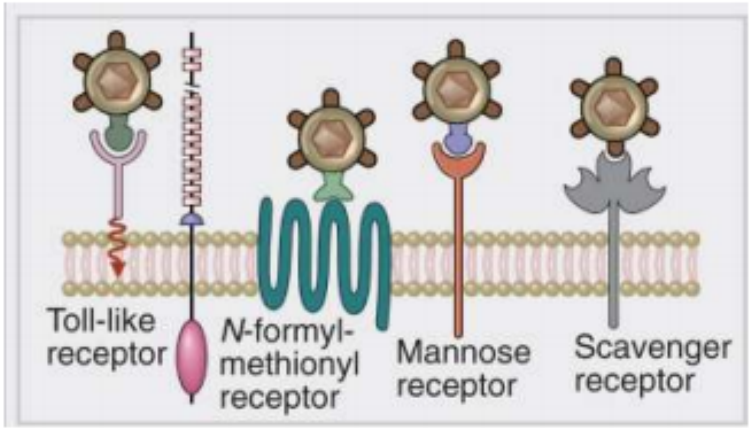

Innate immunity
(rapid response)
Adaptive immunity
(slow response)
Dendritic cell
Mast cell
Macrophage
γδ T cell
B cell
T cell
Natural
killer cell
Basophil
Complement
protein
Eosinophil
Natural
killer T cell
Antibodies
CD4+
T cell
CD8+
T cell
Granulocytes
Neutrophil
Nature Reviews | Cancer

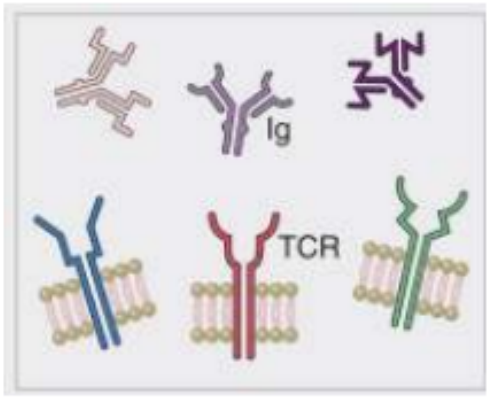


Identification of Microorganisms









Nonklonal
Self-nonself ayırım iyi

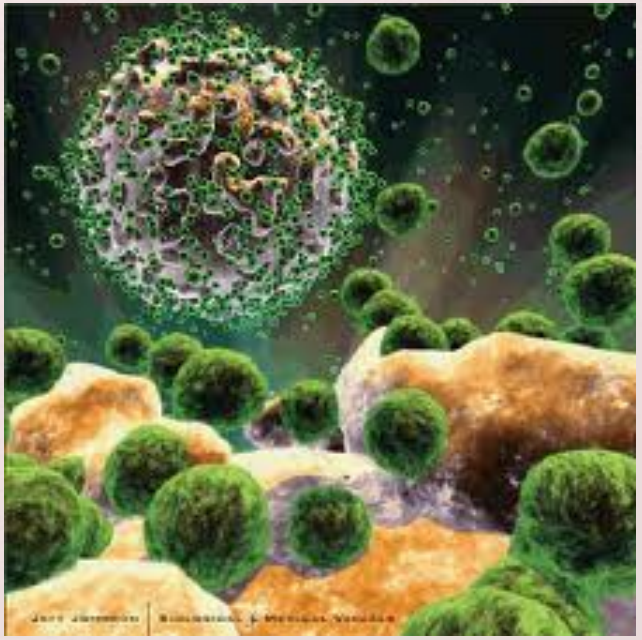
Klonal
Self-nonself ayırım ?

Innate Immunity

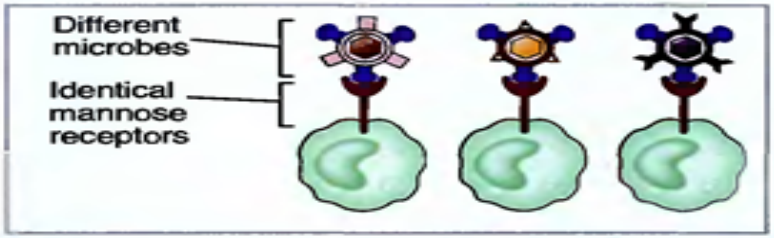
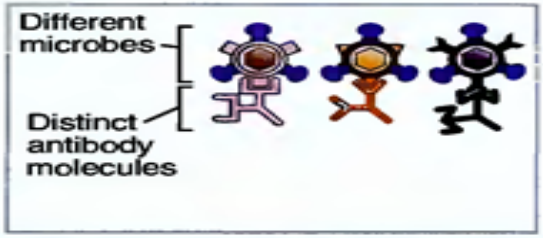
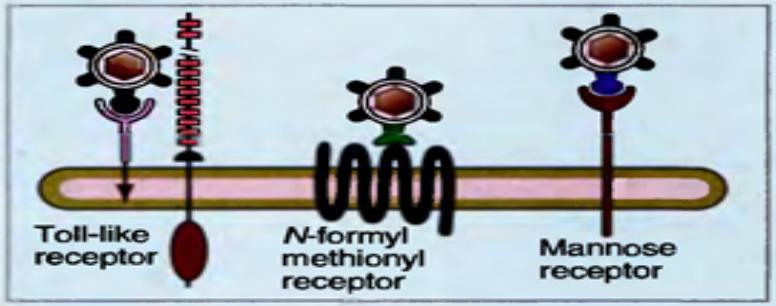
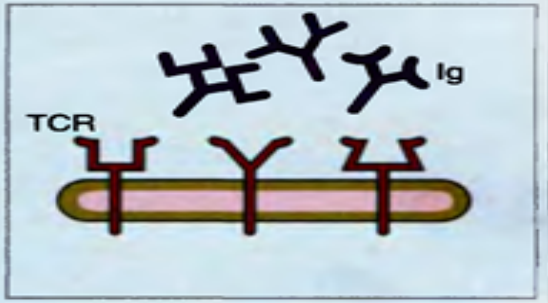
- Common feature of all building blocks of natural immunity is,
  - to recognize and respond to microorganisms,
  - they do not react against substances other than microorganisms

Innate Immunity

- In addition to infectious agents, the natural immune system also recognizes host cells that have been exposed to the harmful effects of microorganisms.



Doğal Bağışıklığın Mikroorganizmaları Tanıması

	Innate immunity	Adaptive immunity
Specificity	For structures shared by classes of microbes ("molecular patterns") 	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Receptors	Encoded in germline; limited diversity 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Discrimination of self and nonself	Yes; host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on selection against self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

Recognition of Microorganisms

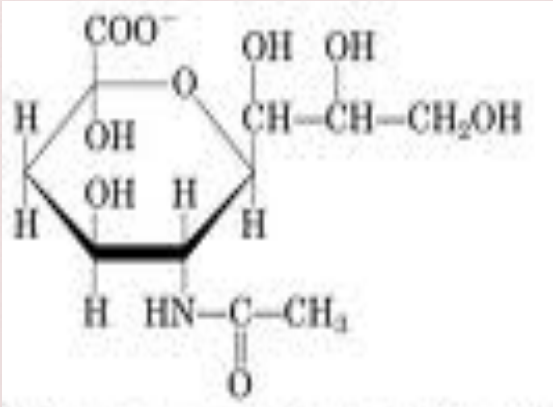
- The building blocks of innate immunity recognize structures that are not found in the host cell but are common to different microorganisms.
  - bacteria,
  - viruses
  - fungus

Recognition of Microorganisms

- For example, phagocytic cells carry receptors for bacterial lipopolysaccharides (LPS or endotoxin) that many bacterial cells share in common but are not found in mammalian cells.

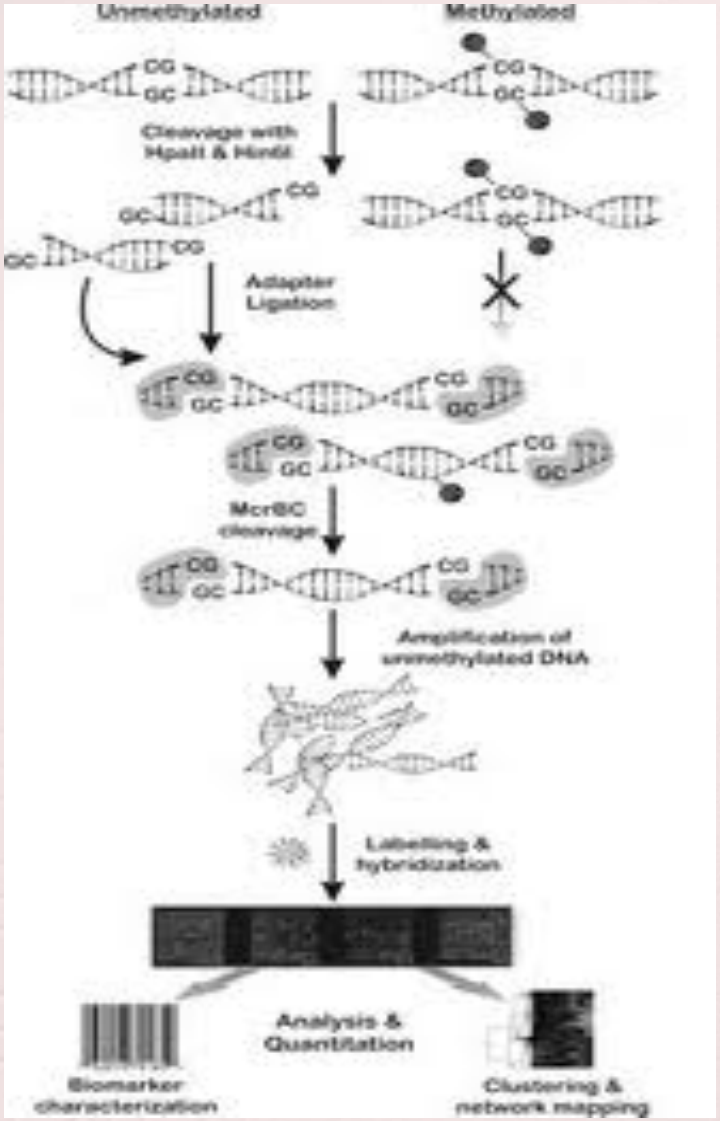
Recognition of Microorganisms

- Another group of receptors possessed by phagocytic cells recognize mannose structures located in the terminal region of glycoproteins.
- As is known, in the terminal region of mammalian glycoproteins sialic acid or N-acetylgalactosamine is found.



N-Acetylneuraminic acid (sialic acid)
(Neu5Ac)

Recognition of Microorganisms



- Phagocytic cells recognize the **double-stranded RNA structure** that mammalian cells do not have but are seen in many viruses.
- They also recognize **unmethylated CpG nucleotides** that are not seen in mammalian DNA but are present in the structure of bacterial DNA.

Recognition of Microorganisms

- Phagocytic cells,
- Mononuclear leukocyte,
- Polymorphonuclear (PMN) leukocyte

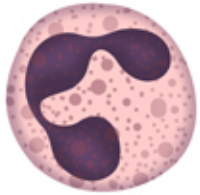
All are produced in the bone marrow.

They migrate to the infection area upon the effect of the chemoactractant substance in the infection area.

TYPES OF WHITE BLOOD CELLS

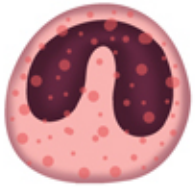
Granulocytes

1. Neutrophil



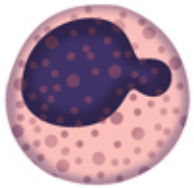
Helps in phagocytosis

2. Eosinophil



Fights against parasitic infection

3. Basophil



Produces inflammatory and allergic reactions

Agranulocytes

4. Lymphocyte

Produces specific immune responses

B Lymphocyte

T Lymphocyte

Natural Killer Cell

5. Monocyte

Fights off bacteria, viruses and fungi

Recognition of Microorganisms

- Neutrophils, macrophages, monocytes, eosinophils and basophils participate in the immune defense of the host organism by fighting infections with their common receptors.

Recognition of Microorganisms

- **Molecular patterns:** These are microbial molecules that are the target of natural immunity and are found on the same type of microorganisms.
- The receptors of natural immunity, which have the ability to recognize these common structures, are called pattern recognition receptors.

Recognition of Microorganisms

- Some building blocks of natural resistance exhibit host cell binding properties, but activation does not occur despite binding.
- Even if the complement plasma proteins adhere to the host cell, the activation of the complement proteins is blocked by the effect of the regulator molecules not found in microorganisms but located on the surface of the host cell.

Recognition of Microorganisms

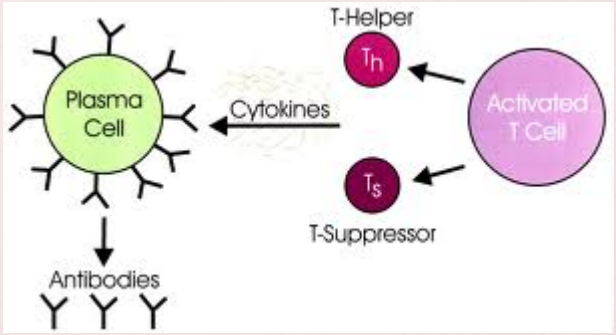
- However,
- Contrary to natural immunity, acquired immunity shows specificity to structures called antigens, which differ even in the same type of microorganisms and are not found in common with them.

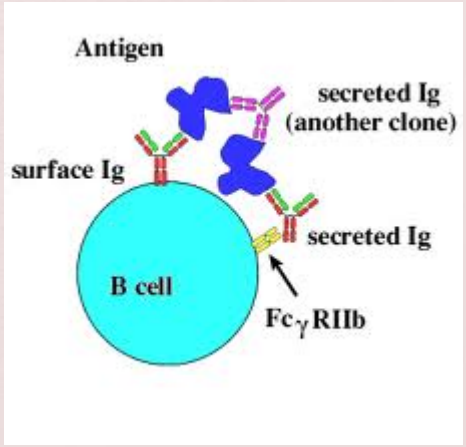
Recognition of Microorganisms

- Acquired immunity has the ability to recognize many more chemically different structures.
- Despite recognizing billions of different antigens in an entire lymphocyte pool, the number of strands of microorganisms recognized by all receptors of natural immunity can only be expressed in thousands.

Recognition of Microorganisms

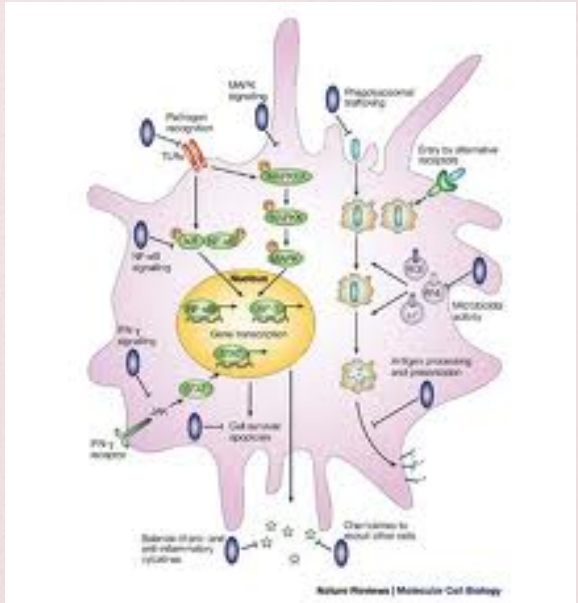
- As a result, the receptors of acquired immunity are distributed clonally, and each lymphocyte clone (B and T cells) consists of cells with a different and specific receptor for a particular antigen.

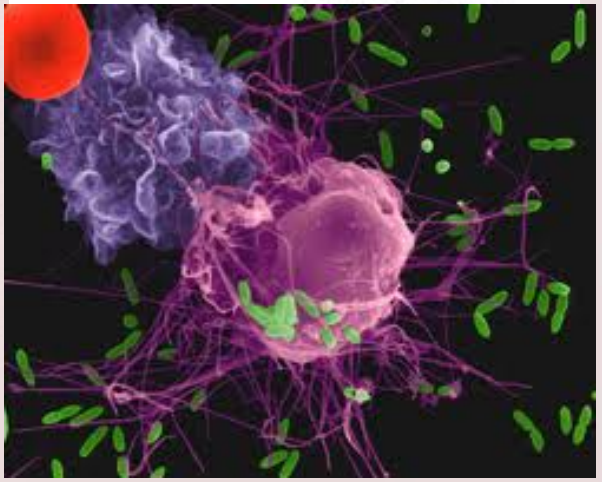




Recognition of Microorganisms

- In contrast, in innate immunity, the receptors do not show clonal differentiation and the same common receptor is expressed on the surface of each cell type, for example macrophages.
- Because of this feature, many cells of natural immunity recognize the same microorganism.





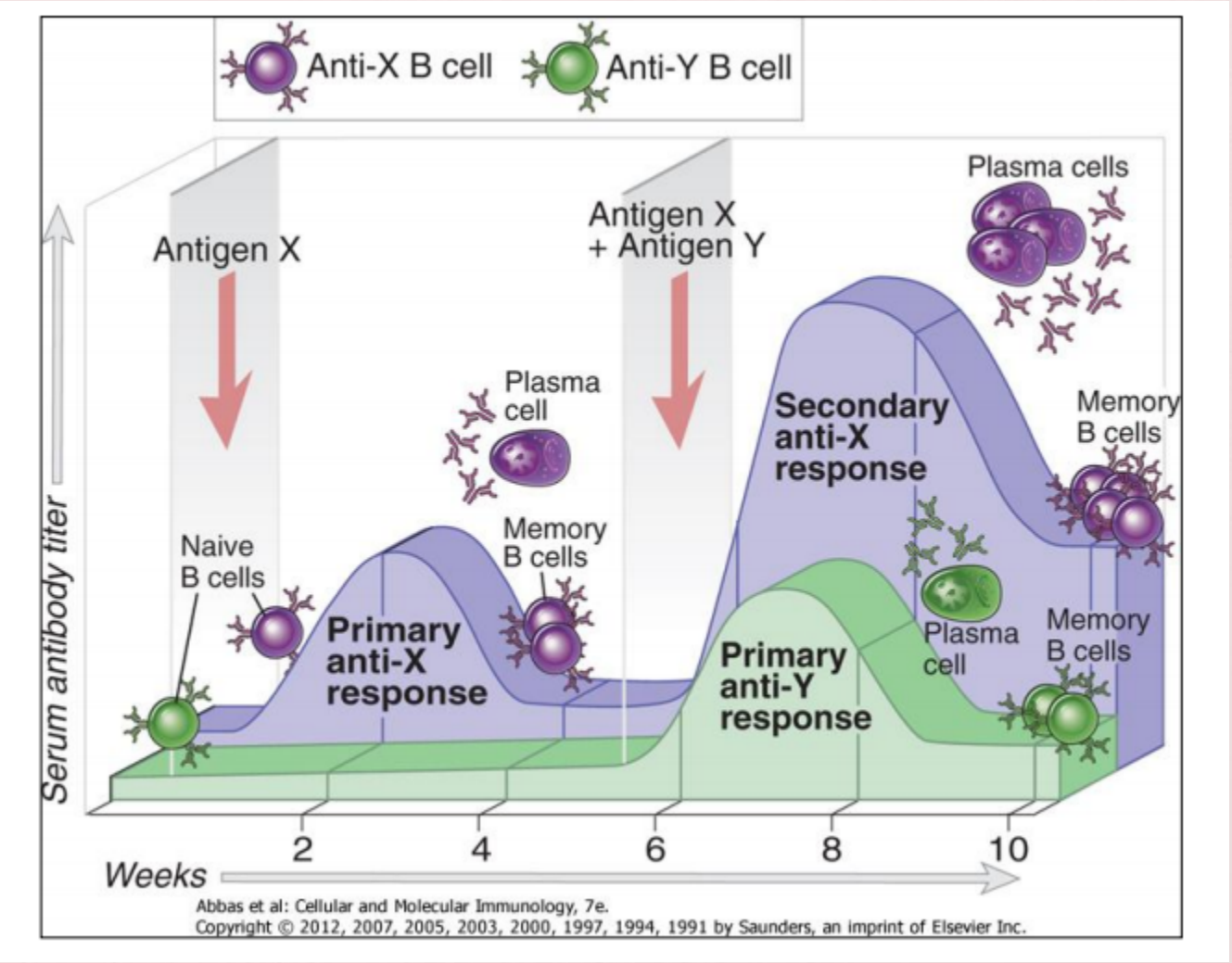
Recognition of Microorganisms

- Natural immunity creates a similar response every time it encounters the same factor,
- Acquired immunity creates a much more effective response to the next encounter with the microorganism it meets.

Memory

- In other words, the acquired immunity remembers that it encounters the microorganism and acts accordingly: this event is called the memory of the immunity, and it makes the host's defense responses more severe in the case of recurrent and persistent infections.

Memory


Anti-X B cell
Anti-Y B cell
Antigen X
Antigen X
+ Antigen Y
Plasma cells
Plasma
cell
Secondary
anti-X
response
Memory
B cells
Naive
B cells
Memory
B cells
Primary
anti-X
response
Primary
anti-Y
response
Plasma
cell
Memory
B cells
Serum antibody titer
2
4
6
8
10
Weeks
Abbas et al: Cellular and Molecular Immunology, 7e.
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Components of Innate Immunity

- Natural immunity; creating a barrier to infections
  - epithelial layer,
  - cells found in tissues and circulation, and
  - a series of plasma proteins.
  - These building blocks have different but complementary functions in preventing the entry of microorganisms into the tissues of the host and removing those that have entered.

Epithelial layer

- Epithelial layer
- It is a cover that forms the common entrance gates of microorganisms to the body, is found in the skin, digestive and respiratory systems, and creates a physical and chemical barrier against infections.

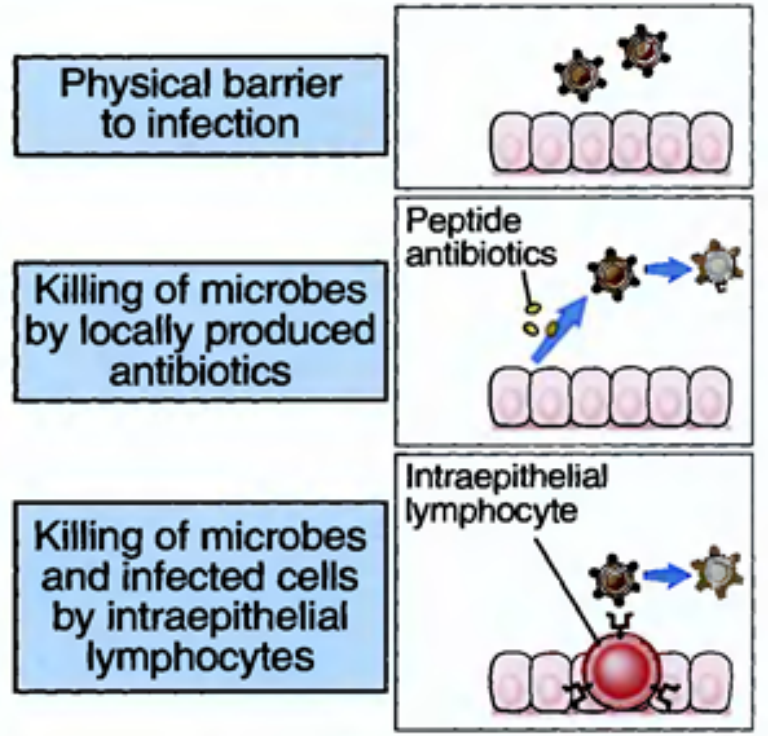
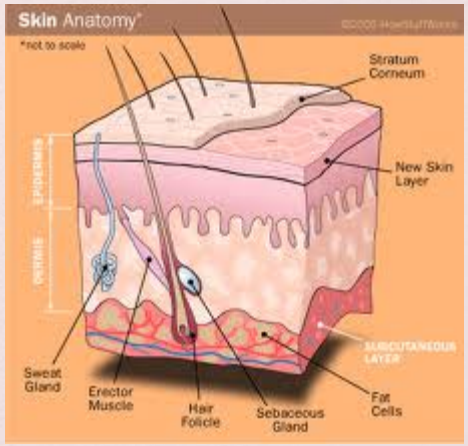
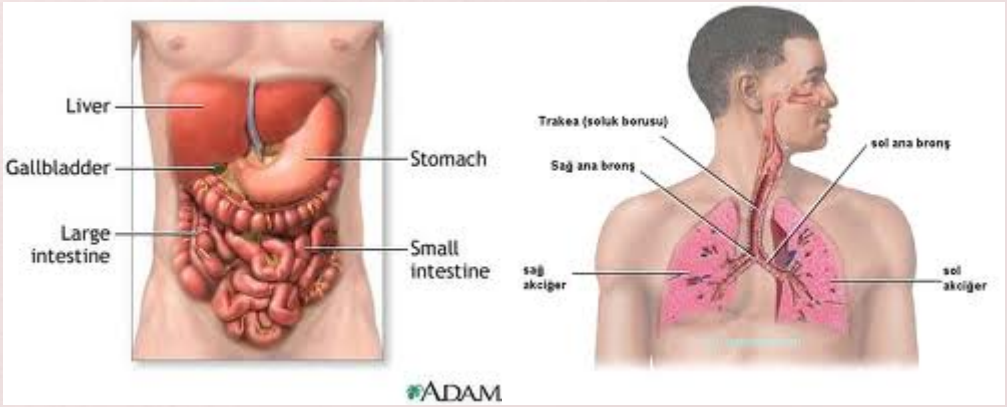


Figure 2–2 Functions of epithelia in innate immunity. Epithelia present at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor lymphocytes that are believed to kill microbes and infected cells.

Epithelial layer

- There are three main areas where the body comes into contact with the external environment:
  - skin,
  - digestive system and
  - respiratory system





Epithelial layer

- All three regions are physically covered by the epithelial layer that tries to prevent the entry of microorganisms.
- In addition, epithelial cells produce peptide antibiotics that can kill bacteria.

Epithelial layer

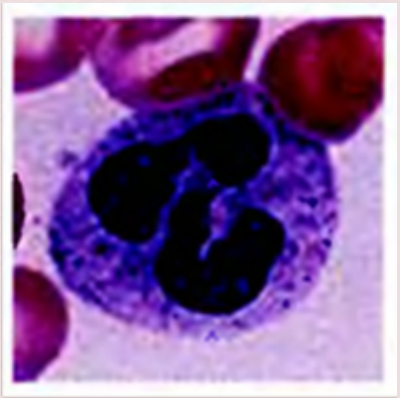
- A special group of B lymphocytes, defined as B-1 cells, are similar to intraepithelial lymphocytes in terms of the limited number of antigen receptors.
- B-1 cells, which are not found in the epithelial layer, act in the peritoneal cavity against microorganisms or toxins that manage to penetrate the intestinal wall.

Epithelial layer

- Most of the IgM class antibodies found in the blood of healthy individuals and called natural antibodies are products of B-1 cells
- They generally show specificity to carbohydrate molecules found in the cell wall of many bacteria.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Neutrophils and monocytes, as phagocytic cells in circulation, go to the infection site and recognize microorganisms there and perform the intracellular destruction function by taking them inside.



Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Neutrophils (Polymorph nuclear leukocytes, PNL) are the most densely found cells in the blood, with 4000 to 10000 per mm3.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- During infection, neutrophil production increases in the bone marrow and their number in the blood reaches 20000 per mm3.
- Neutrophil synthesis produced by many cell types in response to infection;
- They are stimulated by cytokines called colony stimulating factors that play a role in the growth and maturation of neutrophil precursors in the bone marrow.

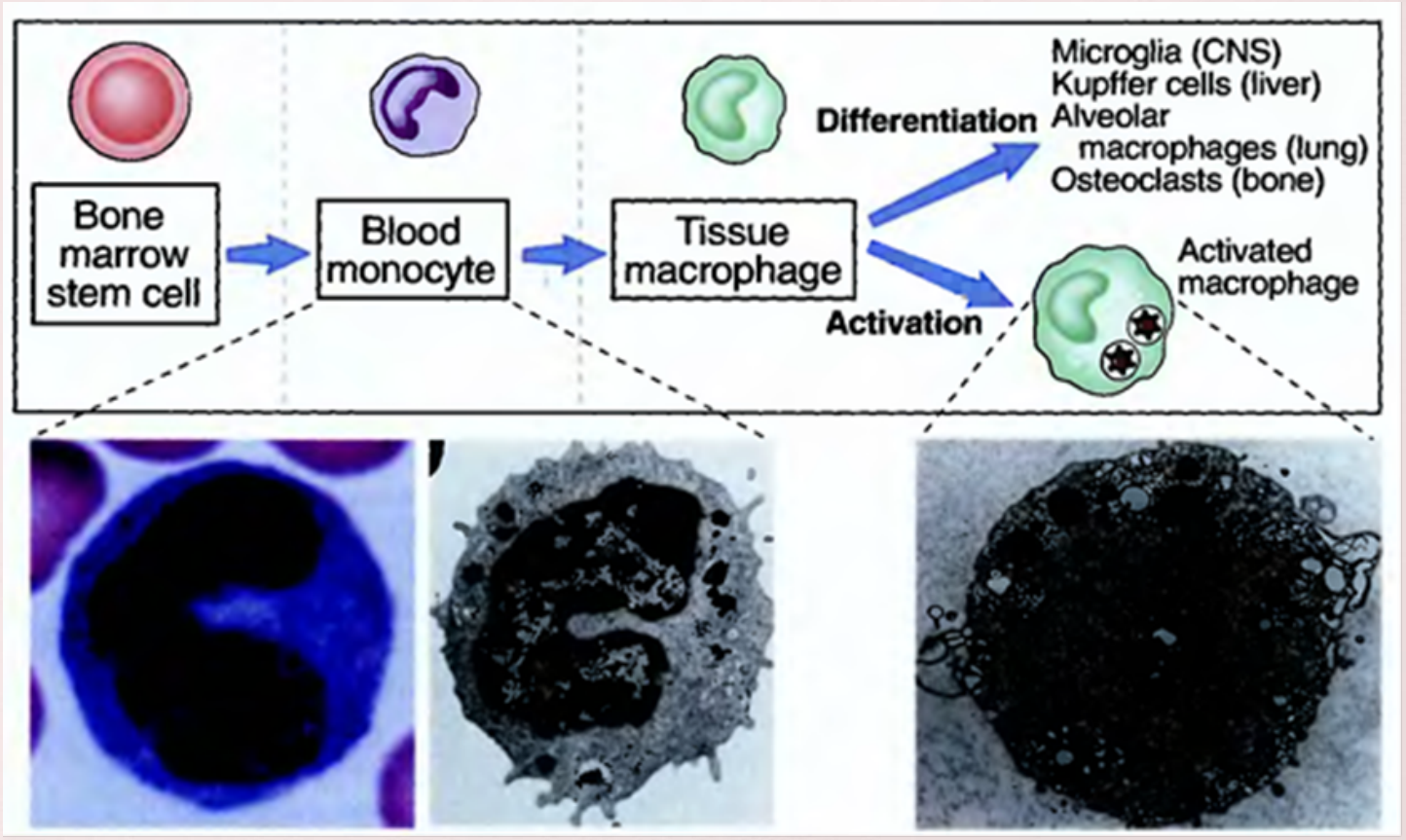
Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Neutrophils are the most important cells that play a role in the response to many infections, especially bacterial and fungal infections.
- In addition to the circulating microorganisms, they move rapidly towards the infection focus outside the vein, digest the microorganisms present there and die within a few hours.

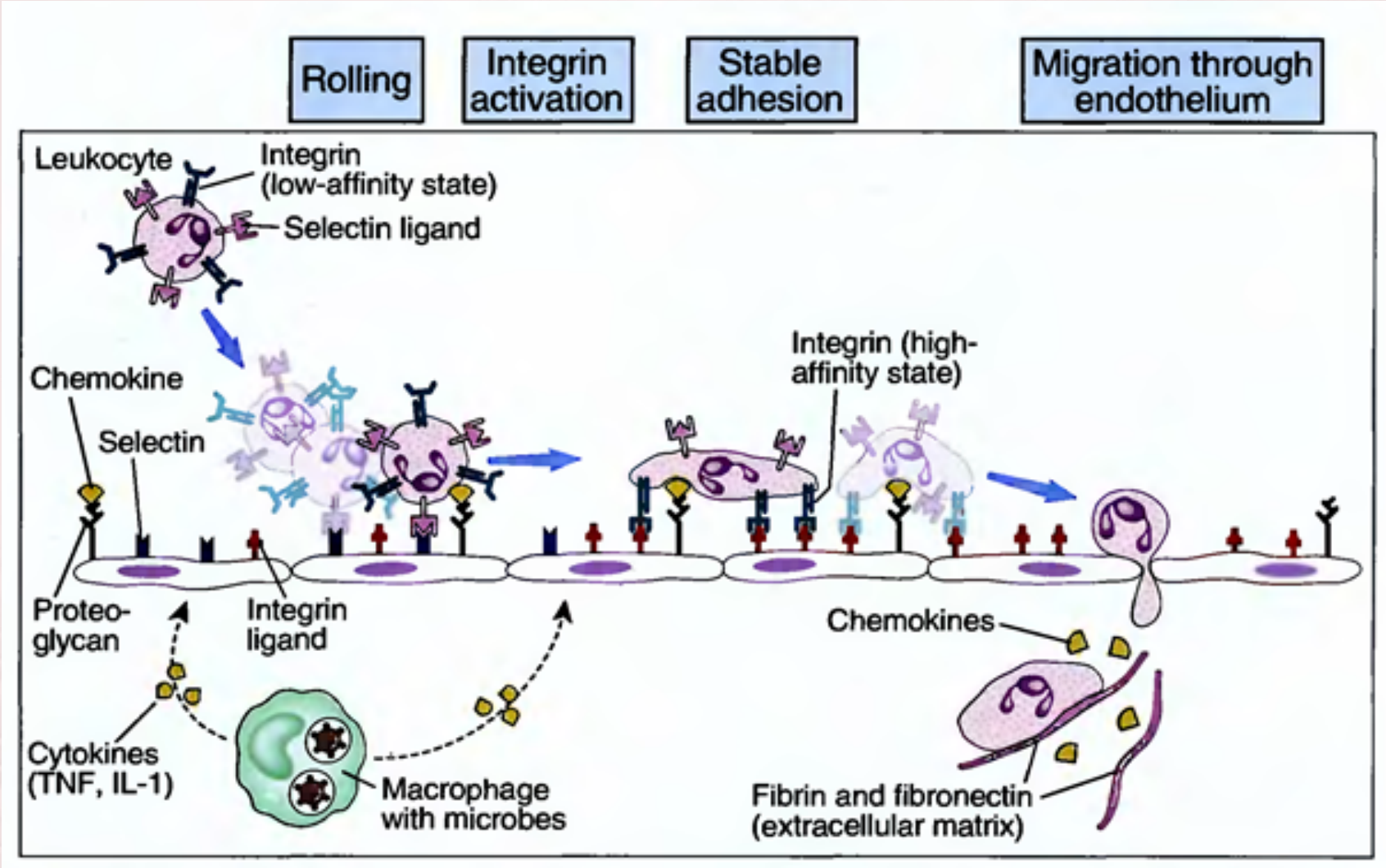
Phagocytic cells: Neutrophils and Monocytes / Macrophages

- The concentration of monocytes in blood, which are less in number than neutrophils, is between 500 and 1000 in mm3.
- These cells are also effective against microorganisms in circulation and tissues;
- Unlike neutrophils, they live longer in extravascular tissues and the monocytes that settle in the tissues differ and become macrophages.

Phagocytic cells: Neutrophils and Monocytes / Macrophages



Phagocytic cells: Neutrophils and Monocytes / Macrophages



Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Neutrophils and monocytes bind to the adhesion molecules in the endothelium and head to the infection site outside the circulation upon the call of chemoattractants synthesized in the presence of microorganisms.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- When an infectious microorganism crosses the epithelial layer and enters subepithelial tissues, the macrophages located in that area recognize the microorganism and produce dissolved proteins called cytokines.
- Two of these cytokines, **tumor necrosis factor (TNF)** and **interleukin-1 (IL-1)**, affect the endothelium of capillaries in the infection area.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- These cytokines stimulate the expression of two groups of adhesion molecules called **E-selectin** and **P-selectin** as a result of their interaction with endothelial cells.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- While selectins allow neutrophils to bind poorly to the endothelium, allowing it to roll, integrins are responsible for tight binding of neutrophils.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Circulating neutrophils and monocytes have carbohydrate molecules on their surface that bind poorly to selectins.
- Although neutrophils attach to the endothelium, flowing blood dissolves this weak attachment.
- Integrins have low affinity on the surface of non-activated leukocytes.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- As cells roll over the endothelium, endothelial cells receive TNF and IL-1 stimulation produced by macrophages that encounter microorganisms and synthesize a special group of cytokines called chemokines (chemoattractant cytokines).

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Chemokines allow the leukocytes rolling in the endothelium to reach a high concentration.
- Chemokines also rapidly increase the affinity of leukocyte integrins to their ligands in the endothelium.
- On the other hand, TNF and IL-1 stimulate the expression of integrin ligands by affecting the endothelium.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

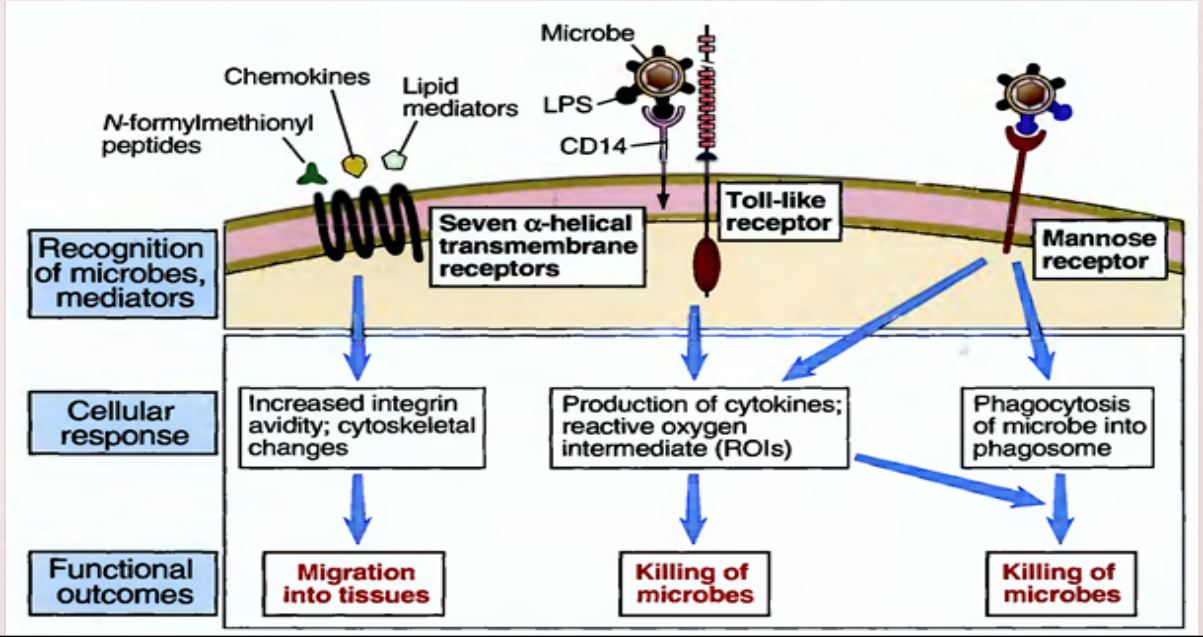
- As a result of the strong binding of integrins to their ligands, the rolling of leukocytes on the endothelial surface ends.
  - As a result, leukocytes follow the direction of the chemokines, cross the vessel wall and move to the site of infection.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- The accumulation of leukocytes in the infection area, meanwhile, the expansion of the vessels and the increase in their permeability, is called inflammation.
- Hereditary deficiency of integrin and selectin ligands disrupts the passage of leukocytes to the infection site and increases susceptibility to infections. Such negativities are defined as leukocyte adhesion deficiency.

Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Microorganisms in circulating and extravascular tissues are recognized by neutrophils and macrophages through special receptors.



Phagocytic cells: Neutrophils and Monocytes / Macrophages

- TLR-2 -----> bacteria glycolipids
- TLR-4 -----> bacteria LPS or endotoxin,
- TLR-5 -----> flagellin protein
- TLR-9 -----> unmethylated CpG nucleotides

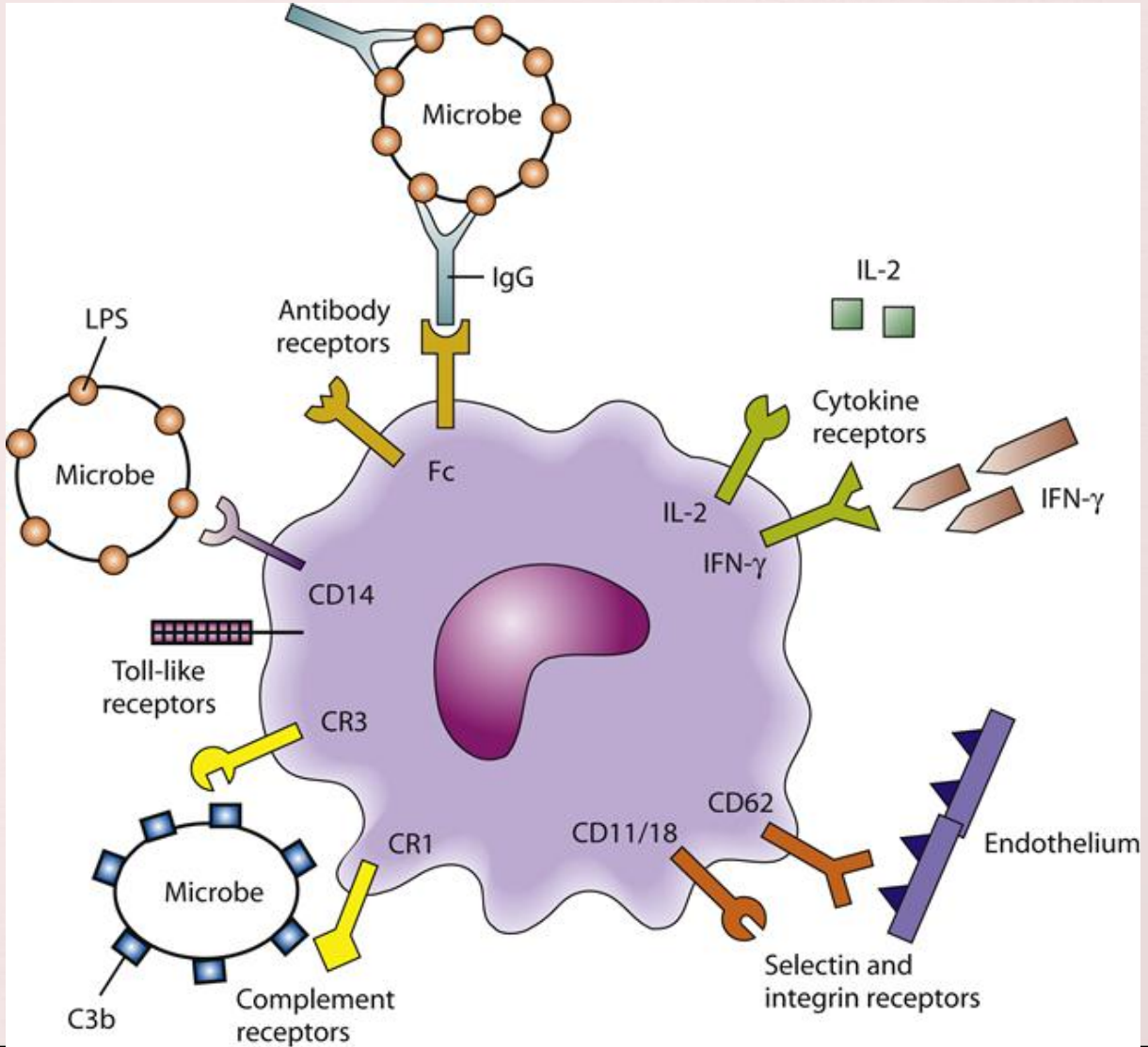
Phagocytic cells: Neutrophils and Monocytes / Macrophages

- The signal that results from the activation of TLRs activates the transcription factor, briefly referred to as NF-kB (nuclear factor kB),
- thereby stimulating the production of cytokines and other proteins that play a role in the anti-microbial activities of activated phagocytes.

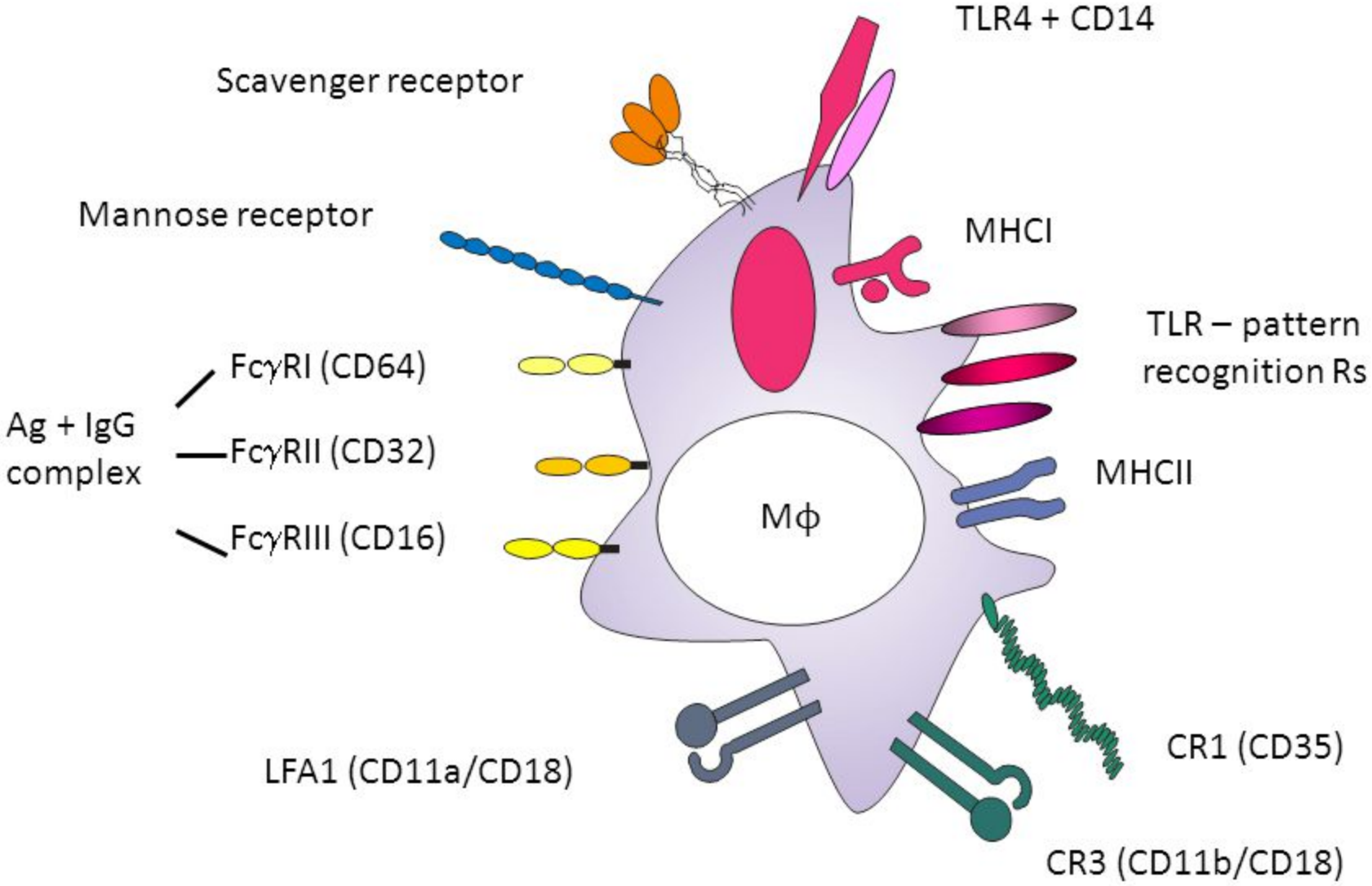
Phagocytic cells: Neutrophils and Monocytes / Macrophages

- Neutrophils and macrophages play a role in killing microorganisms by the process of swallowing called phagocytosis through receptors that perceive other structural features of microorganisms.

Phagocytic cells: Neutrophils and Monocytes / Macrophages



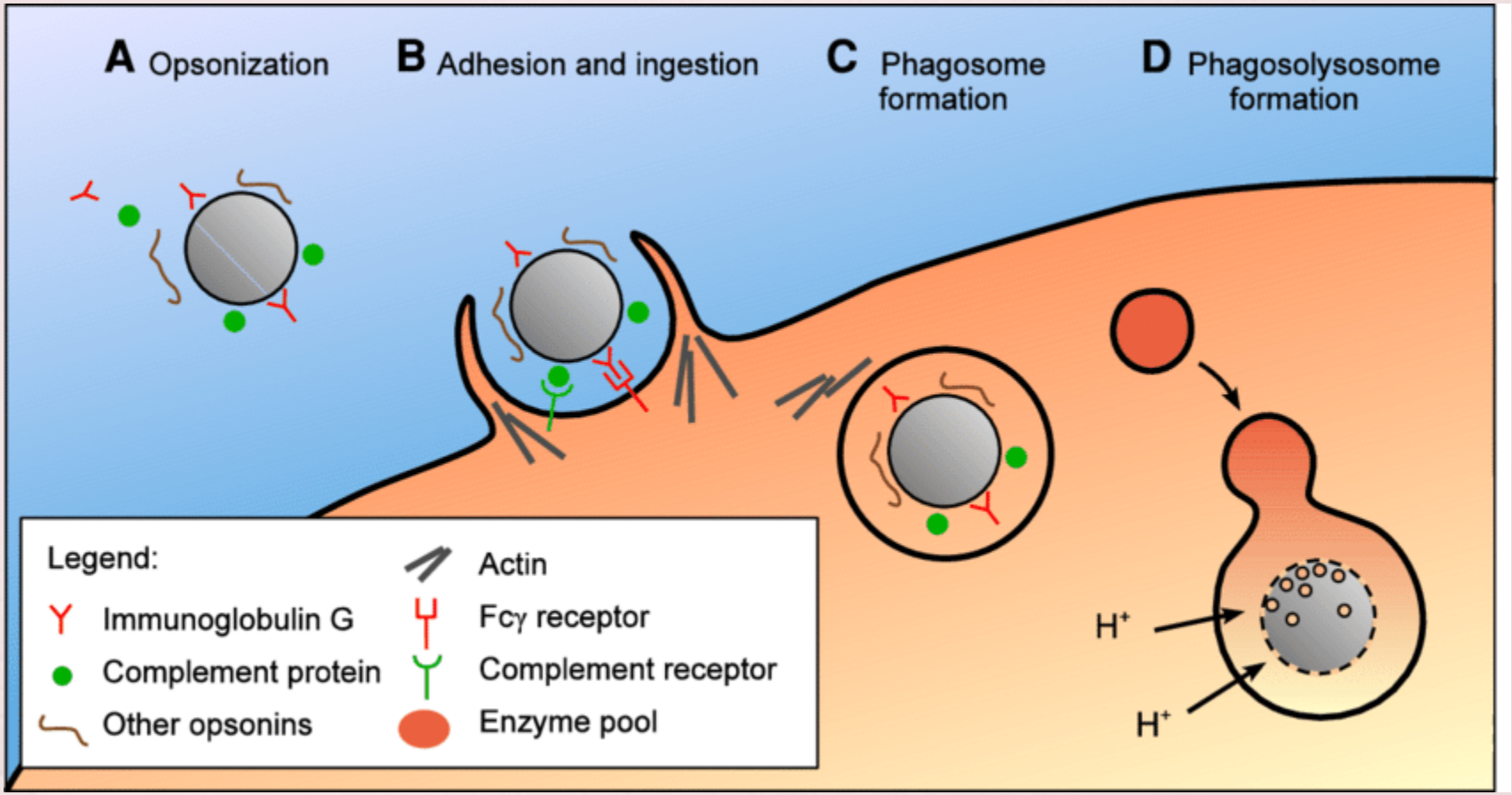
RECEPTORS AND CELL-SURFACE MOLECULES OF MACROPHAGES



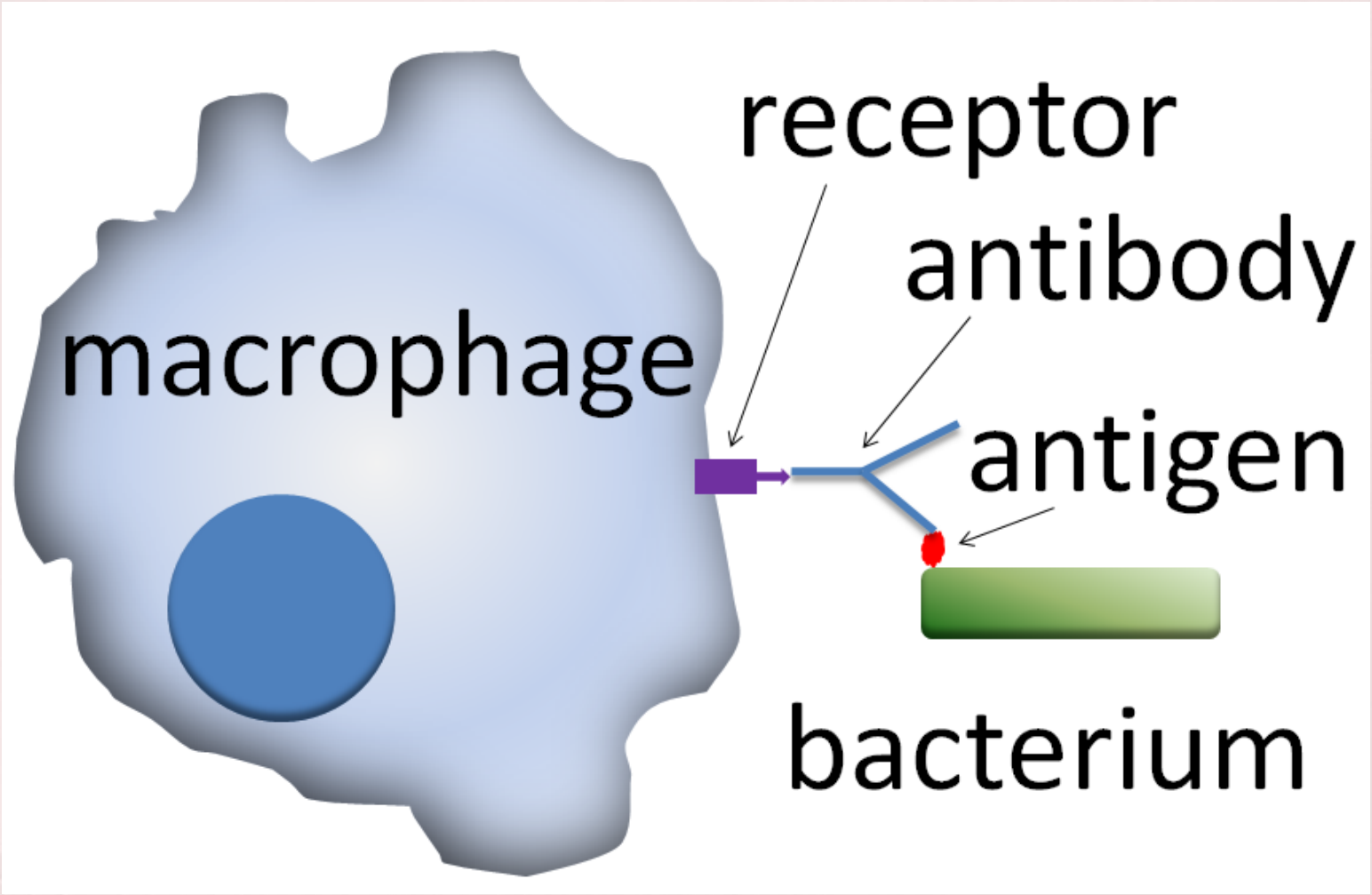
Phagocytic cells: Neutrophils and Monocytes / Macrophages

- This phenomenon, which enables microorganisms to collect some substances on their surfaces and to be recognized more strongly by phagocytic cells, is called opsonization

Opsonization

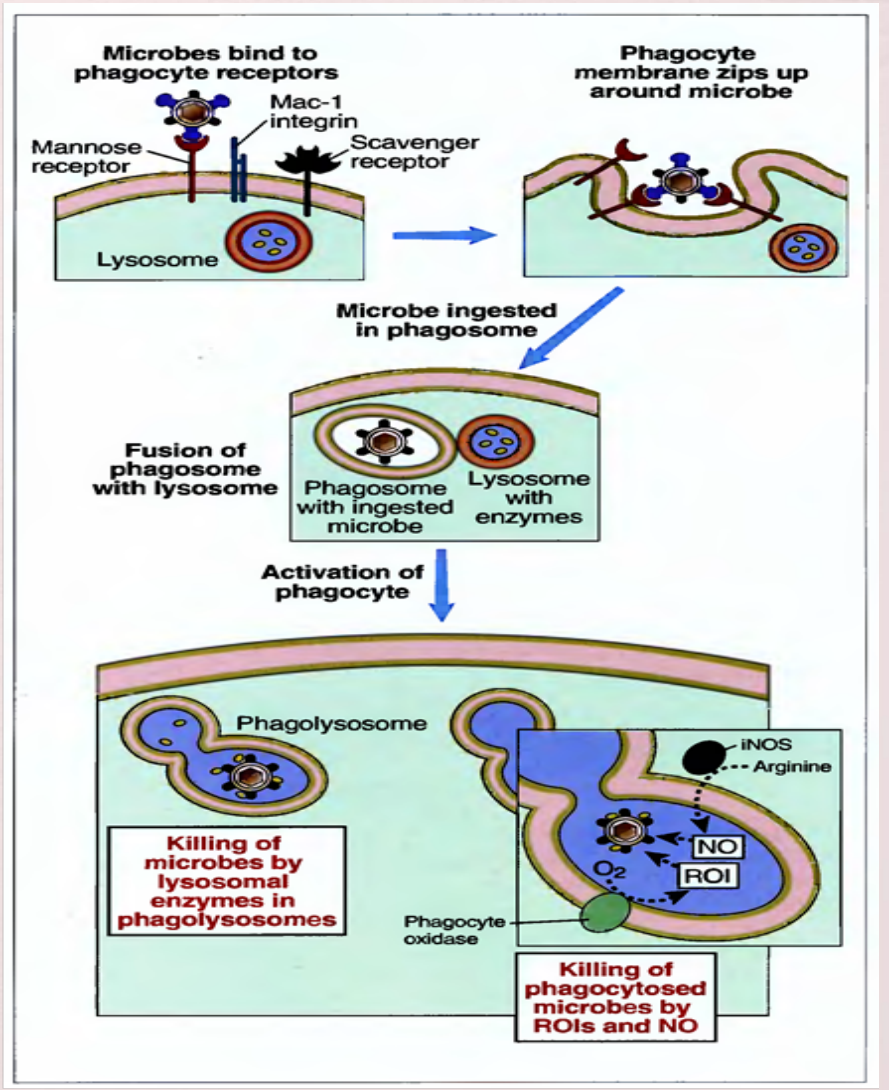


Opsonization



Phagocytic cells: Neutrophils and Monocytes / Macrophages

- The phagocytosis phase follows the recognition of microorganisms by neutrophils and macrophages, and the activation of phagocytic cells ends with the destruction of microorganisms.



Phagocytosis

- Phagosomes combine with lysosomes to form phagolysosomes.
- Receptors, which recognize microorganisms and cause them to bind to cells and be trapped inside the cell, send signals that cause the stimulation of certain enzymes within the phagolysosomes.
- The enzyme, defined as **phagocyte oxidase**, converts molecular oxygen into superoxide anion and free radicals.

Phagocytosis

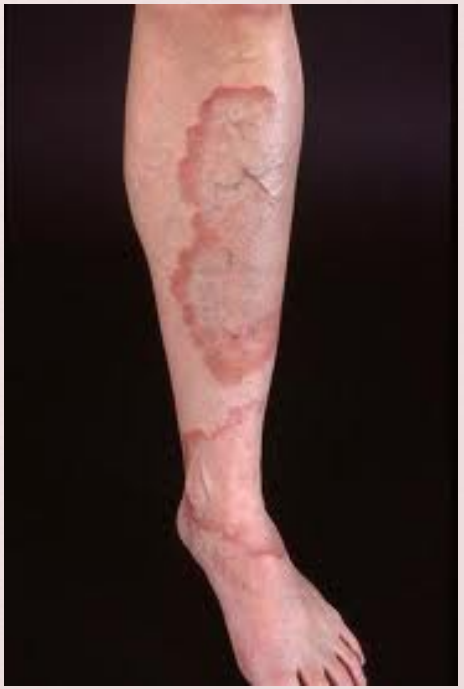
- A second enzyme called nitric oxide synthetase enables the conversion of arginine to nitric oxide (NO), another microbiocidal substance.
- Lysosomal proteases, another enzyme group, cause the breakdown of microbial proteins.

Phagocytosis

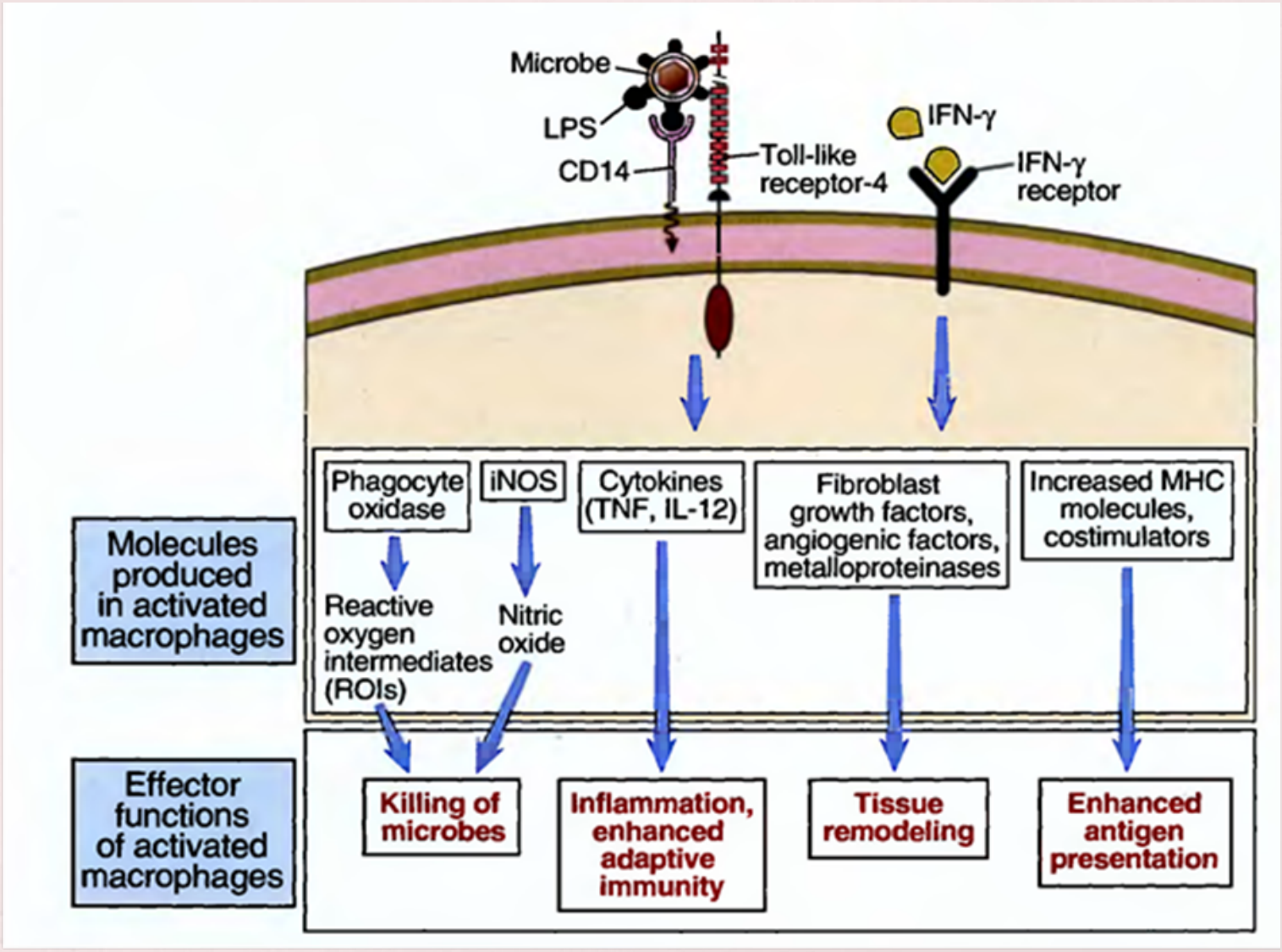
- The inherited deficiency of phagocytic oxidase enzyme is the cause of immune deficiency disease defined as chronic granulomatous disease.

Phagocytosis

- In this case, phagocytic cells do not destroy intracellular microorganisms;
- As a result, a structure called granuloma emerges which is formed by macrophages and lymphocytes concentrating around microorganisms emerges.

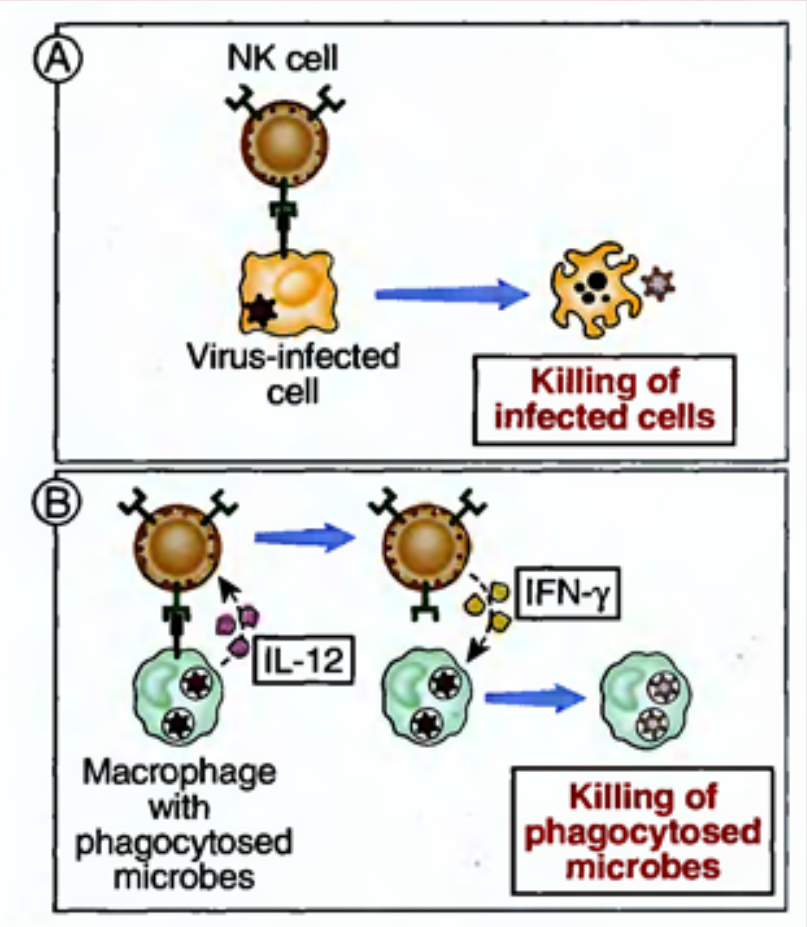


Molecules produced by macrophages



Natural killer (NK) Cells

- NK cells are specialized lymphocyte series cells that fight against intracellular microorganisms by killing infected cells and secreting cytokine **(IFN-γ),** which activates macrophages.



Natural killer (NK) Cells

- NK's constitute 10% of lymphocytes in circulating and peripheral lymphoid organs.
- These cells with dense cytoplasmic granules have their own surface antigens.
- However, they do not carry antigen receptors specific to B and T cells, such as immunoglobulin or T cell receptors.

Natural killer (NK) Cells

- Activation receptors
- Inhibition receptors

Natural killer (NK) Cells

- Activation receptors include receptors that recognize molecules on the surface of cells infected with the virus or on the surface of cells that have phagocyted intracellular bacteria or viruses;
- They also have receptors on their surface that cause the destruction of normal cells and recognize uninfected host cell surface molecules.

Natural killer (NK) Cells

- However, it is the inhibitory receptors of NKs that recognize normal cell molecules that will prevent this situation and stop the activation of NKs.
- These inhibitory receptors show specificity for the various alleles of MHC class 1 molecules carried by the nucleated cells of each individual.

Natural killer (NK) Cells

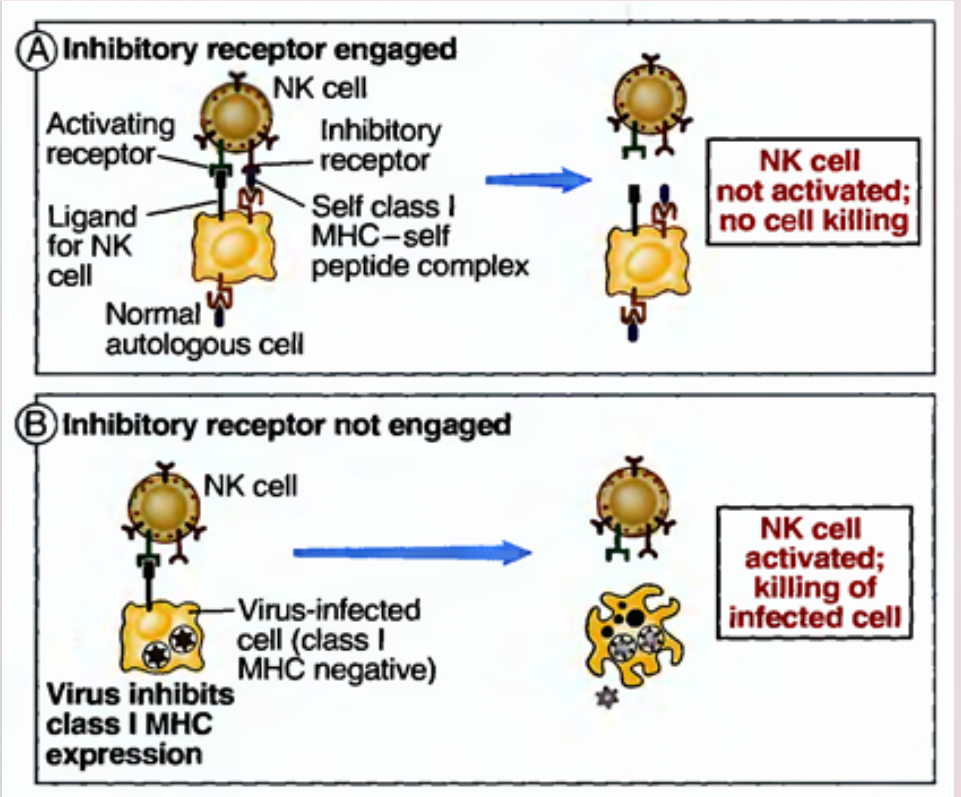
- Inhibitory receptors carry tyrosine-rich inhibitory-motif immunoreceptors (ITIM) in their cytoplasmic domains;
- This structure undergoes phosphorylation in the tyrosine-rich regions of the receptor following fusion with the MHC class 1 molecule.

Natural killer (NK) Cells

- Phosphorylated ITIMs; they bind to and activate tyrosine phosphatases, a protein belonging to the cytoplasm; As a result of this development, phosphate groups are separated from the tyrosine region and The activity initiated by activator receptors is blocked.

Natural killer (NK) Cells

- In short, when inhibitory receptors of NKs are effective and recognize MHCs, NK cells are disabled.



Natural killer (NK) Cells

- Many viruses block MHC expression on the surfaces of the cells they infect, thus trying to escape the influence of virus-specific CD8 + cytotoxic T lymphocytes.

Natural killer (NK) Cells

- In the face of this development, the inhibitory receptors of NKs are not effective and the destruction of virus-infected cells by NKs begins.
- NK cells need cytokine stimulation to be secreted by macrophages in order to be effective against infections.

Natural killer (NK) Cells

- Interleukin-12 (1L-12), which is one of the cytokines produced by macrophages, has NK stimulating properties.
- NK cells have Fc receptors on their surface that serve to bind some IgG molecules; Through these receptors, cells coated with antibodies are recognized by NKs.

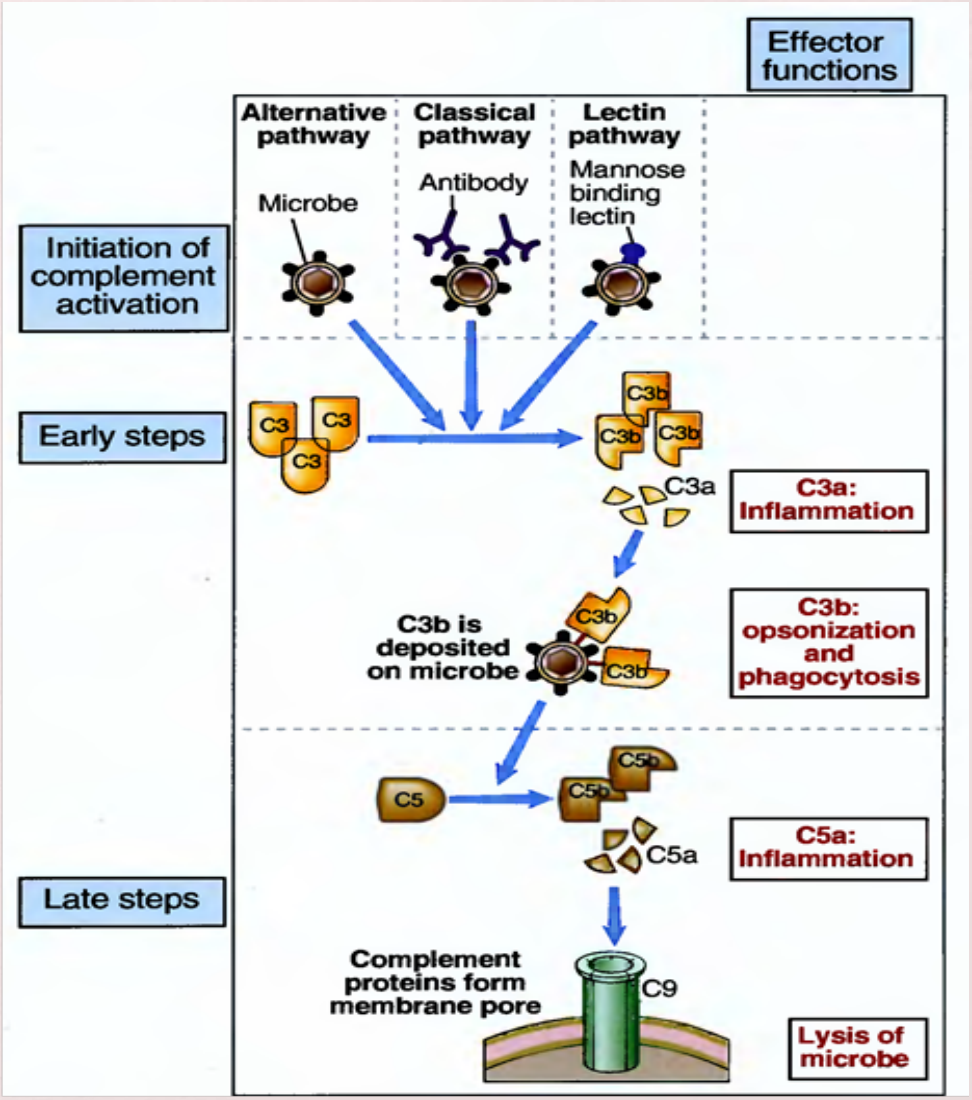
- After all, the host and microorganism are in similar evolutionary effort:
- while the host is trying to recognize the viral antigens presented with their CTLs and MHC,
- viruses try to destroy MHC molecules; In this case, NK, which has an effect on MHC deficiency, will come into play.

Complement System

- The complement system, which forms an important mechanism in defense against microorganisms, consists of proteins in the circulation and membrane.
- Most of the complement proteins are proteolytic enzymes and their activation takes place as a result of the activation of these enzymes one after the other (enzymatic cascade).

Complement System

- The system can be activated in three different ways.



Complement System

- In the alternative way, some complement proteins are activated on the surfaces of microorganisms.
- The regulatory proteins of the system that are not found on microorganisms (found in the host cell) do not play a role in this mechanism, which is considered within the scope of natural resistance.

Complement System

- The classical pathway is induced by the binding of antibodies to microorganisms or other antigens, and this mechanism is evaluated within the humoral mechanism of action of acquired immunity.

Complement System

- The lectin pathway is activated after a plasma protein defined as a mannose-binding lectin binds to the terminal mannose region of the surface glycoproteins of microorganisms.

Complement System

- The lectin pathway, which stimulates the proteins of the classical activation pathway, is considered within the scope of natural resistance since there are no antibodies involved.
- Activated complement proteins act like proteolytic enzymes to break down other proteins of the system.

Complement System

- The complement system has three main functions in defense:
- First of all, while C3b combines with microorganisms on one side, on the other hand, they cling to special receptors located on the phagocytic cell surface and ultimately act as a bridge in the binding of microorganisms to phagocytic cells.

Complement System

- Secondly, some intermediates of the system act as chemoattractants for neutrophils and monocytes and ultimately contribute to the formation of inflammation in that area.

Complement System

- Third, a polymeric protein complex is formed in the cell membrane of microorganisms as a result of the activation of the complement system;
- This structure ends with the formation of a hole that allows water and ions to enter and exit the cell, and thus the destruction of the microorganism occurs.

Cytokines of Natural Resistance

- Macrophages and other cells stimulated by microorganisms secrete proteins defined as cytokines that direct cellular reactions that take place within the scope of natural resistance.

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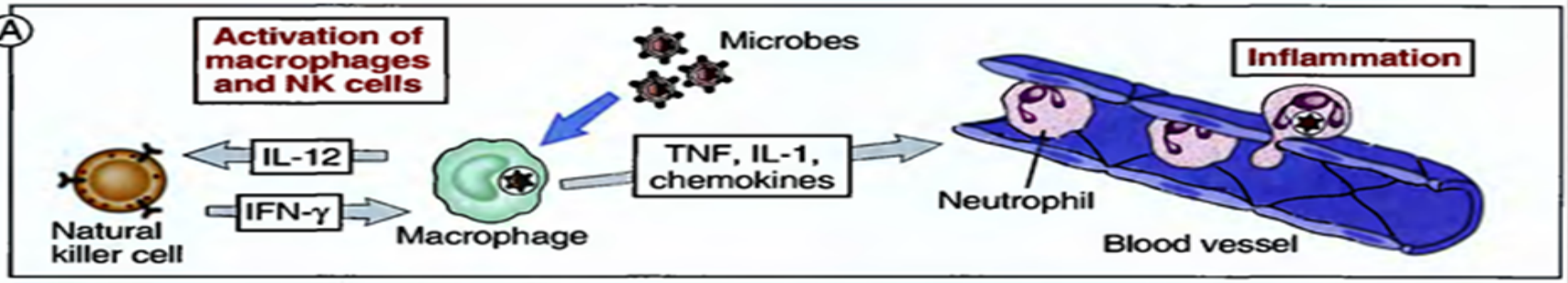

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Cytokine	Principal cell source(s)	Principal cellular targets and biologic effects
Tumor necrosis factor (TNF)	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute phase proteins Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
Interleukin (IL-1)	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute phase proteins
Chemokines	Macrophages, endothelial cells, T lymphocytes, fibroblasts, platelets	Leukocytes: chemotaxis, activation
Interleukin-12 (IL-12)	Macrophages, dendritic cells	NK cells and T cells: IFN-γ synthesis, increased cytolytic activity T cells: T_H1 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: Macrophages IFN-β: Fibroblasts	All cells: antiviral state, increased class I MHC expression NK cells: activation
Interleukin-10 (IL-10)	Macrophages, T cells (mainly T_H2)	Macrophages: inhibition of IL-12 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

Invasion of Natural Resistance by Microorganisms

- Pathogenic microorganisms tend to settle in the host they enter and survive by resisting natural resistance.

Mechanism of immune evasion	Organism (example)	Mechanism
Resistance to phagocytosis	*Pneumococcus*	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 meningitides*	Sialic acid expression inhibits C3 and C5 convertases
	Streptococcus	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 peptide antibiotics