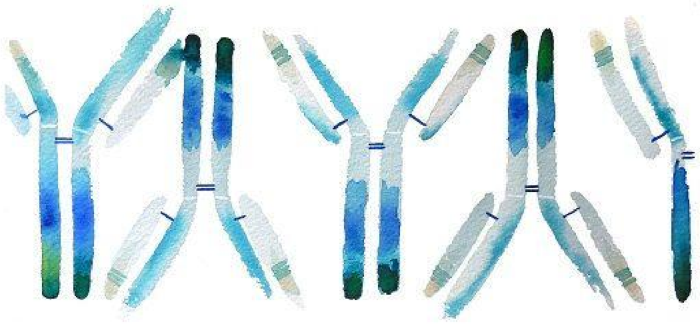
بسم الله الرحمن الرحيم

Medical Immunology



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M.D. Ph.D.

Lecture 9

Innate immunity

أستغفر الله العظيم وأتوب إليه
لا حول ولا قوة إلا بالله العظيم

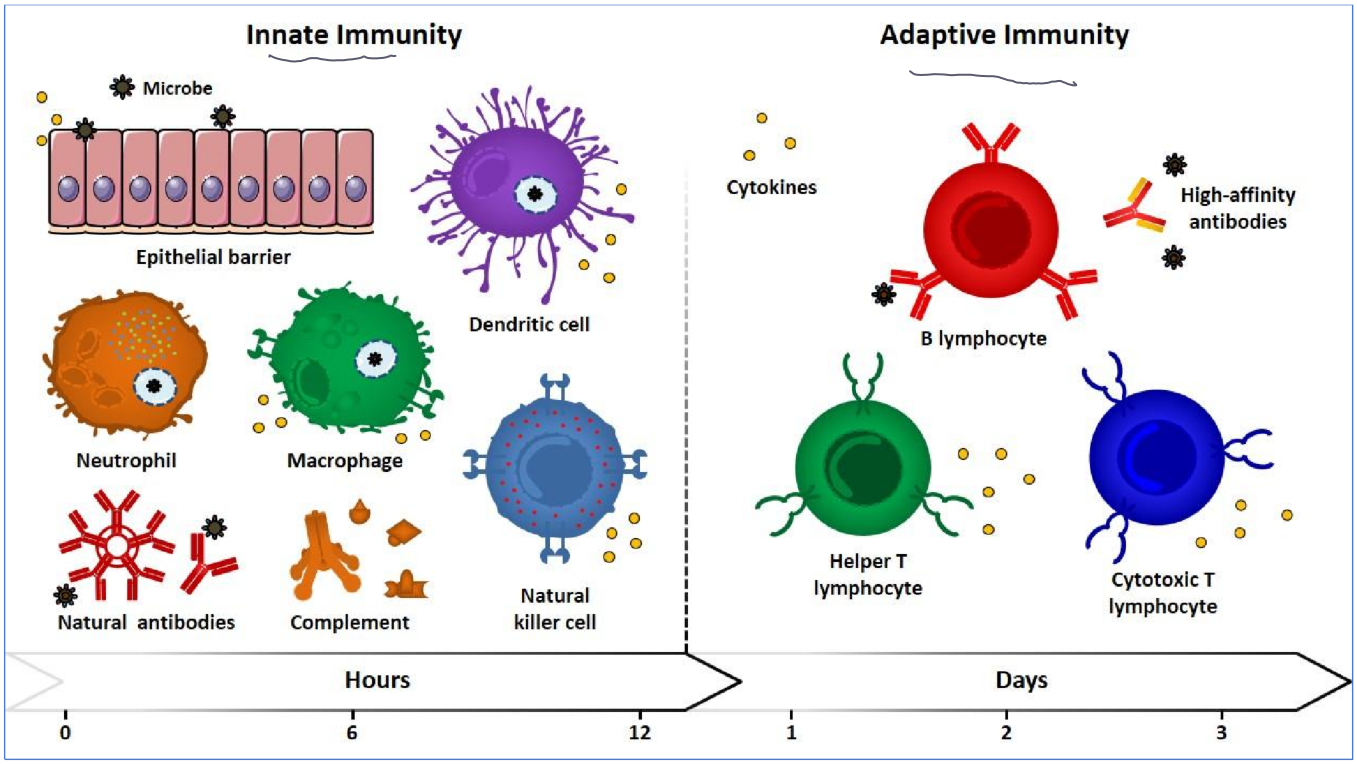
- In this lecture we will discuss

- Main topics:

Immune responses to extracellular

أستغفر الله العظيم وأتوب إليه

Innate immunity



* الدكتور حكا هدول السلايدين وقال قراءة

GENERAL FEATURES OF IMMUNE RESPONSES TO MICROBES

أستغفر الله العظيم وأتوب إليه

- Defense against microbes is mediated by the effector mechanisms of innate and adaptive immunity

- The immune system responds in distinct and specialized ways to different types of microbes to most effectively combat these infectious agents.

- The survival and pathogenicity of microbes in a host are critically influenced by the ability of the microbes to evade or resist the effector mechanisms of immunity.

- Many microbes establish latent, or persistent, infections in which the immune response controls but does not eliminate the microbe and the microbe survives without propagating the infection.

- In many infections, tissue injury and disease may becaused by the host response to the microbe and its products rather than by the microbe itself.

Immunity to Extracellular Bacteria

أستغفر الله العظيم وأتوب إليه

- The principal mechanisms of innate immunity to extracellular bacteria are :

- **Phagocytosis**: Phagocytes use various surface receptors, including **mannose receptors** and **scavenger receptors**, to recognize extracellular bacteria, and they use **Fc receptors** and **complement receptors** to recognize bacteria **opsonized** with antibodies and complement proteins, respectively. As well as TLRs and other PRR.

- **The inflammatory response**: dendritic cells and phagocytes that are activated by the microbes secrete cytokines, which induce leukocyte infiltration, and initiates and propogates adaptive immune responses.

- **complement activation**.

Innate immunity

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Table 1

Innate immune system components

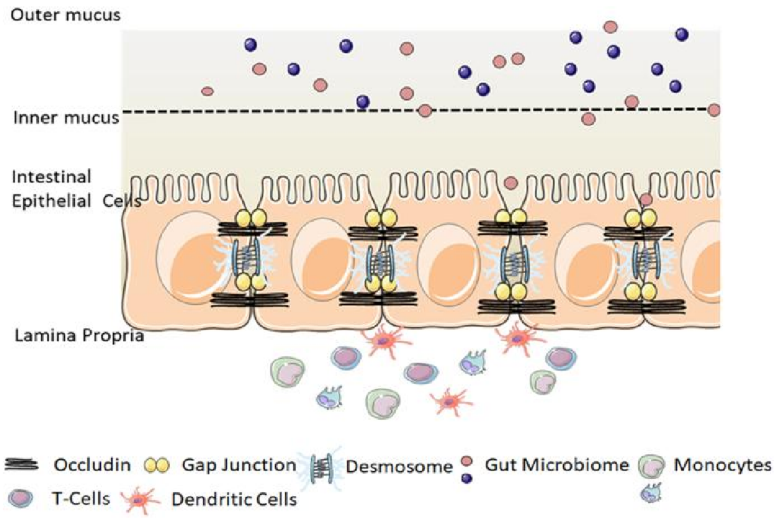
Natural barriers	Cells	Pattern-recognition receptors	Cytokines	Natural antimicrobial products
Skin, mucosal epithelia	Neutrophils, macrophages/dendritic cells, natural killer cells, natural killer T cells, γδ T cells, B1 lymphocytes	Mannose-banding lectins, Toll-like receptors, ...	IL-1, IL-6, IL-8, IL-12, IL-15, IL-18, G-CSF, M-CSF, GM-CSF, TNF-α, IFN-γ, ...	Defensins, lactoferrin, lysozyme, natural antibodies, complement, reactive oxygen species

IFN interferon; IL interleukin; *G-CSF* granulocyte colony-stimulating factor; *GM-CSF* granulocyte-macrophage colony-stimulating factor; *M-CSF* macrophage colony-stimulating factor; *TNF* tumor necrosis factor

Innate immunity / epithelial barriers/ tight junctions

لا حول ولا قوة إلا بالله العظيم

- Intact epithelial surfaces (in the skin and the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary) form **physical barriers** between microbes in the external environment and host tissue.
- **Tight junctions** are crucial for the maintenance of barrier integrity



Innate immunity / epithelial barriers/ mucus

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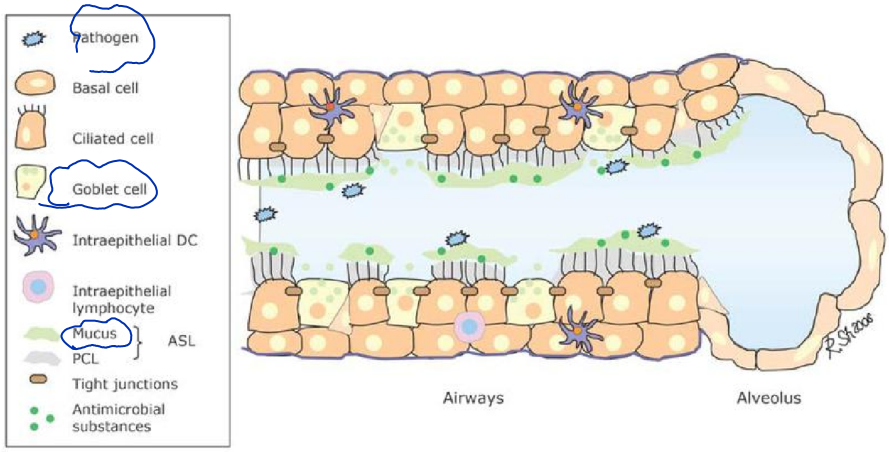
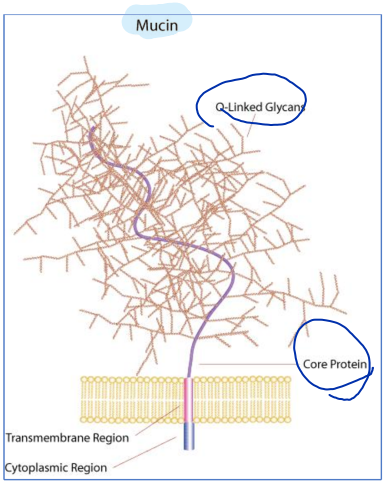
Mucus, a viscous secretion containing inorganic salts, antimicrobial enzymes (such as **lysozymes***), **immunoglobulins**, and glycoproteins such as **lactoferrin** and **mucins**. Mucus **physically impairs microbial invasion** and facilitates microbe removal by **ciliary action** in the bronchial tree and **peristalsis** in the gut. *Lysozyme is a **naturally occurring enzyme** found in bodily secretions such as tears, saliva, and milk. It functions as an antimicrobial agent by cleaving the peptidoglycan component of bacterial cell walls

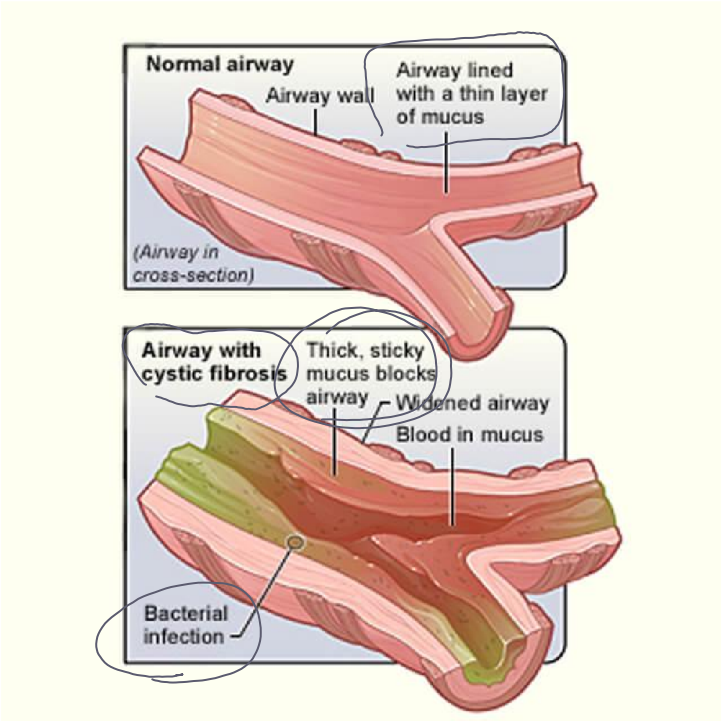




أستغفر الله العظيم وأتوب إليه

Innate immunity / epithelial barriers/ mucus

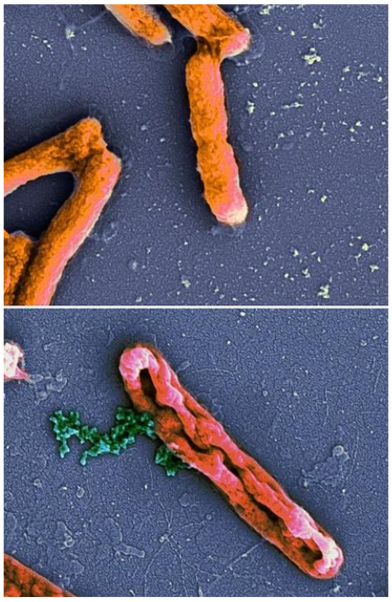
In cyctic fibrosis (CF) Defective CFTR protein impacts the function of several organs and **alters the consistency of mucosal secretions**. The latter of these effects probably plays an important role in the **defective resistance of CF patients to many pathogens**.



أستغفر الله العظيم وأتوب إليه

Innate immunity / epithelial barriers/ antimicrobial peptides

- **Antimicrobial peptides (AMPs),** also called host defense peptides (**HDPs**) are part of the innate immune response found among all classes of life.
- **Defensins** are **small cationic peptides**, produced by epithelial cells of mucosal surfaces and by granule-containing leukocytes, including neutrophils, natural killer cells, and cytotoxic T lymphocytes.
- **Cathelicidins** are produced by neutrophils and various barrier epithelia, after cleavage they have **bactericidal** and **immunomodulatory functions**.
- Antimicrobial peptides possessing a net positive charge are attracted and **incorporated** into negatively charged **bacterial membranes** thus disturbing them.



BELOW: Disrupted cell membranes and leakage of bacterial chromosome (green) in the treated group.

Innate immunity / epithelial barriers/ microbiota

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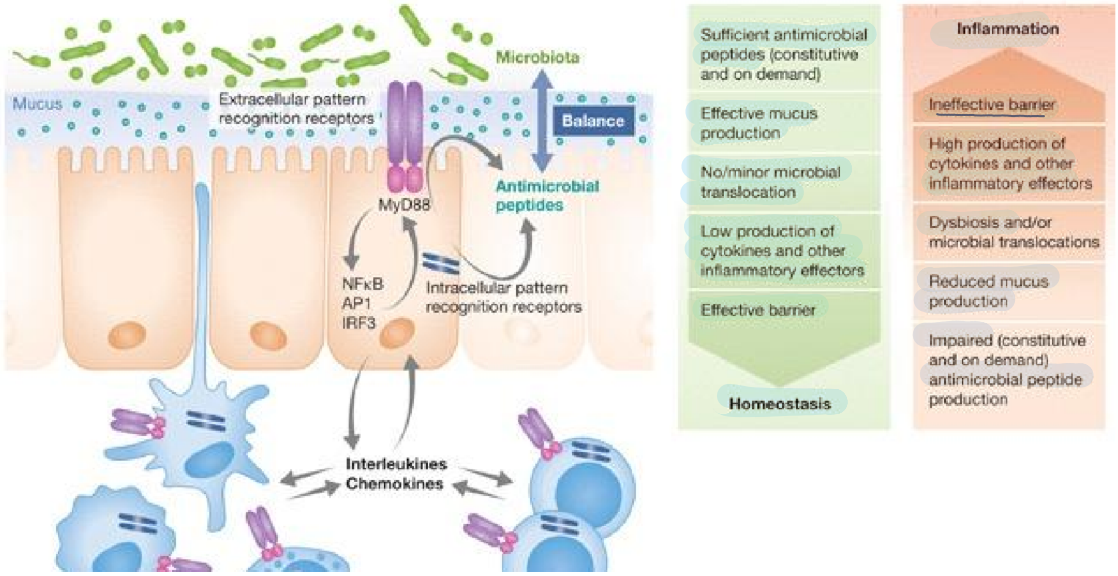
- The gut **microbiota** is key to the efficient development and maintenance of the intestinal barrier.



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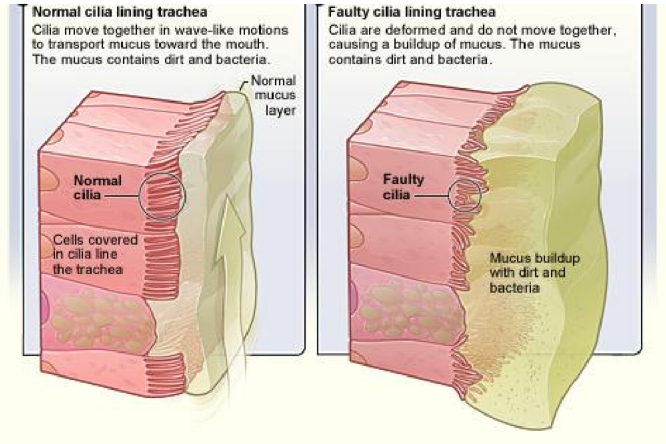
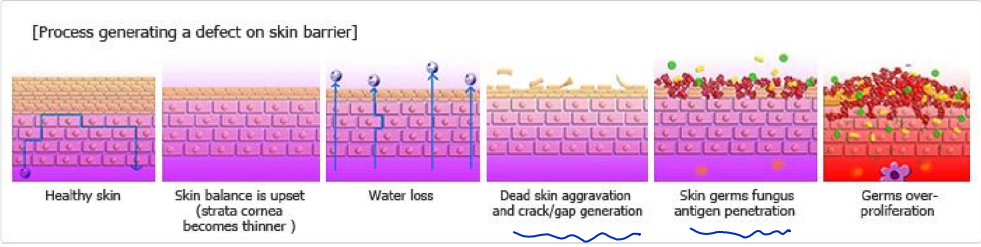
Innate immunity / epithelial barriers



- **primary ciliary dyskinesia**, an inherited disorder that leads to impaired mucociliary clearance,and repeated **chest infections.**

- In **eczema** a defective skin barrier leads to recurrent infections.



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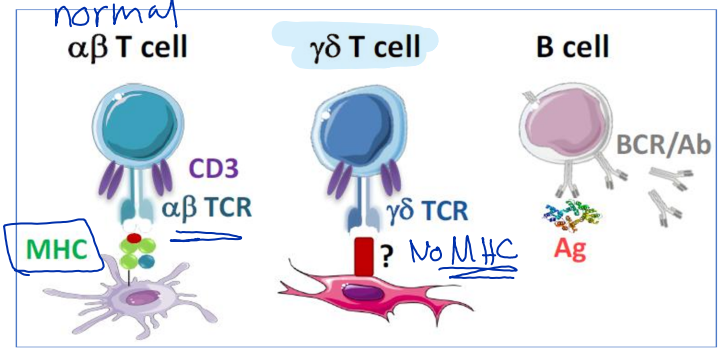
Innate immunity / epithelial barriers/ intraepithelial T lymphocytes

- Barrier epithelia contain certain types of lymphocytes, including **intraepithelial T lymphocytes**, that recognize and respond to commonly encountered microbes, Most of them do not express CD4 nor CD8 and differentiate in the thymus.

- T cells in epithelia express a form of antigen receptor called the **γδ receptor** that may recognize peptide and nonpeptide antigens. A common characteristic of these T cells is the **limited diversity** of their antigen receptors compared with most T cells in the adaptive immune system. And **do not depend on MHC** presentation.



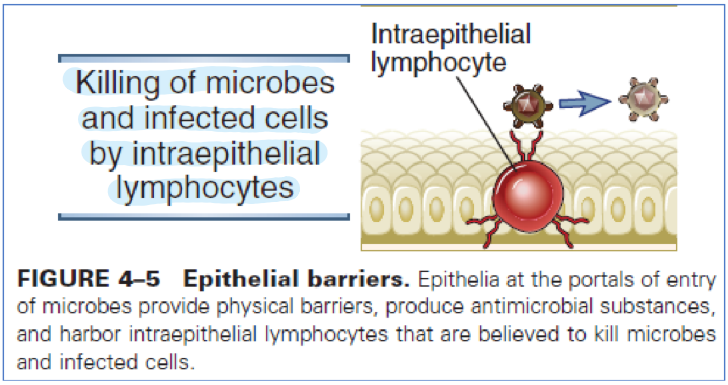


FIGURE 4–5 Epithelial barriers. Epithelia at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor intraepithelial lymphocytes that are believed to kill microbes and infected cells.

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Innate immunity / Leukocyte Migration into Tissues مراجعة

- Major immune cellular components move through the blood, into tissues (leukocyte homing/ recruitment), and often back into the blood again.

- Example: Delivery of leukocytes **from their sites of maturation** (bone marrow or thymus) **to injured tissue** (or **secondary lymphoid organs** where they encounter antigens and differentiate into effector lymphocytes and are delivered into sights of infection).

- Leukocytes that have not been activated by external stimuli (i.e. considered to be in a resting state), normally located in the circulation and lymphoid organs.

- Endothelial cells at sites of infection and tissue injury are also activated, mostly in response to cytokines secreted by macrophages and other tissue cells at these sites.

- The recruitment of leukocytes and plasma proteins from the blood to sites of infection and tissue injury is called **inflammation**.

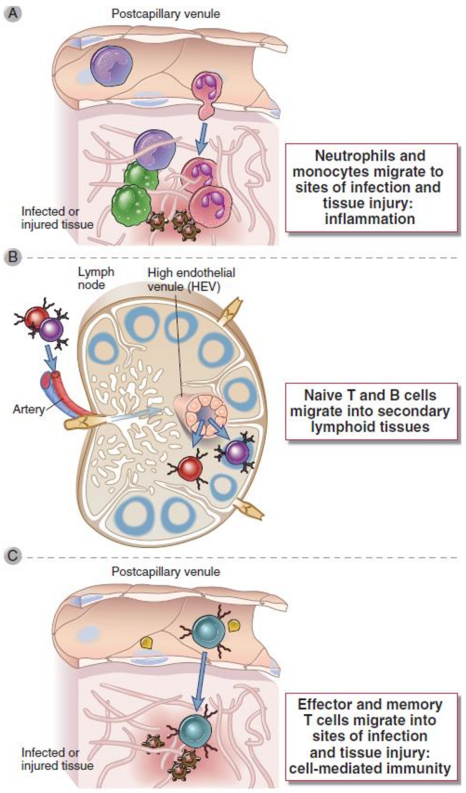
Innate immunity / Leukocyte Migration into Tissues

لا إله إلا الله

- Leukocyte recruitment from the blood into tissues depends first on adhesion of the leukocytes to the endothelial lining of postcapillary venules and then movement through the endothelium and underlying basement membrane into the extravascular tissue.

- This **adhesion** is mediated by two classes of molecules, called **selectins** and **integrins**, and their **ligands**.

- The leukocytes **home** into tissue following signals from different **chemokines**.



Leukocyte Migration into Tissues/ adhesion molecules

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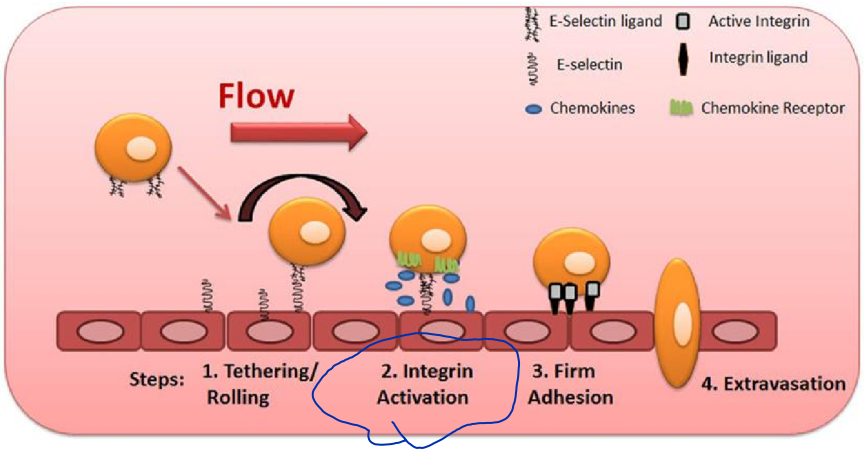
- **Selectins** are **plasma membrane carbohydrate-binding adhesion molecules** that mediate an initial step of **low affinity adhesion** of circulating leukocytes to endothelial cells lining postcapillary venules. Expressed within 1 to 2 hours in response to the cytokines IL-1 and TNF.

- The ligands on leukocytes that bind to **E-selectin** and **P-selectin** on endothelial cells are **complex sialylated carbohydrate**



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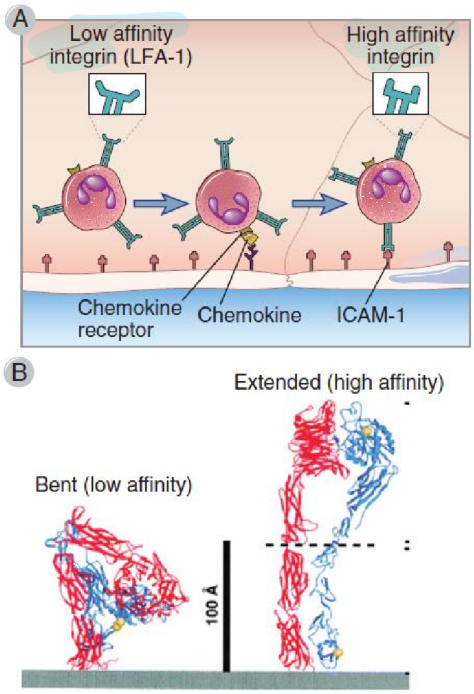
Leukocyte Migration into Tissues/ adhesion molecules

- **Integrins** are heterodimeric **cell surface proteins** that **mediate adhesion** of cells to other cells or to extracellular matrix, through specific binding interactions with various **ligands**.

- **Integrins** respond to intracellular signals by rapidly **increasing their affinity** for their ligands.
  ↳ they change their shape

- An important integrin is called **LFA-1** (leukocyte function-associated antigen 1) expressed on leukocytes and it's ligand (intercellular adhesion molecule) **ICAM-1**.

- **Chemokines** also induce membrane clustering of integrins leading to **increased avidity of integrin interactions** with ligands on the endothelial cells, and therefore tighter binding of the leukocytes to the endothelium.



Leukocyte Migration into Tissues/ Chemokines

- Chemokines are a large family of structurally homologous cytokines that **stimulate leukocyte movement** and **regulate the migration** of leukocytes from the blood to tissues.
- There are about 50 human chemokines, all of which are 8- to 12-kD polypeptides.
- The two major families are the **CC** chemokines, in which the **cysteine residues are adjacent**, and the **CXC** family, in which these **residues are separated by one amino acid**
- The chemokines of the CC and CXC subfamilies are produced by **leukocytes** and by **several types of tissue cells**, such as endothelial cells, epithelial cells, and fibroblasts.
- The receptors for chemokines belong to the seven transmembrane, guanosine triphosphate (GTP)–binding (G) protein–coupled receptor (**GPCR**) superfamily
- **Interleukin 8** (IL-8 or chemokine (C-X-C motif) ligand 8, CXCL8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells

Leukocyte Migration into Tissues/ Chemokines

قراءة مرهم

- IL-8, also known as neutrophil chemotacti factor, has two primary functions. It **induces chemotaxis in target cells, primarily neutrophils** but also other granulocytes, causing them to migrate toward the site of infection. IL-8 also **stimulates phagocytosis** once they have arrived.
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- Recently, it has been found that tumors very frequently co-opt the production of this chemokine, which in this malignant context exerts immunosuppression.

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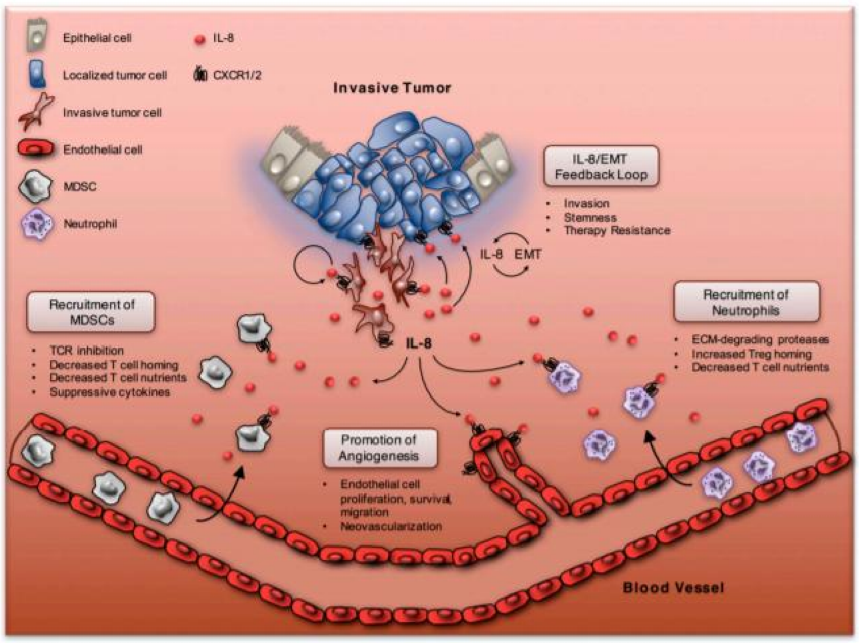

Figure 1. Effects of IL-8 on the tumor and microenvironment. Depiction of IL-8 promoting autocrine and paracrine tumor cell EMT, enhancing angiogenesis, and remodeling the tumor microenvironment through attraction of neutrophils and myeloid-derived suppressor cells (MDSCs).

Leukocyte Migration into Tissue

- Which chemokine family member is primarily responsible for recruiting neutrophils to sites of infection or tissue injury?

- a) CXC chemokines
- b) CC chemokines
- c) CX3C chemokines
- d) XC chemokines

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Leukocyte Migration into Tissues/ Clinical correlate

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- **Leukocyte adhesion deficiency (LAD)** is an immunodeficiency disorder involving both B and T cells and is characterized by an inability of leukocytes to migrate to the site of infection to kill offending microbes.

BCs no Adhesion

Major immunologic features:

- There is an inability to form pus.
- There is a deficiency of various glycoproteins including LFA-1/Mac-1, glycoprotein 150/95.
- Leukocytes cannot migrate to infection sites to kill invading microorganisms due to mutations in the CD18 glycoprotein.
- Adhesion molecules deficiency results in an abnormal inflammatory response and eventually recurrent bacterial infections.

Leukocyte Migration into Tissues/ summary

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- In response to microbes and cytokines produced by encounter with microbes, endothelial cells lining postcapillary venules at the site of infection **rapidly increase** surface expression of **selectins**. **Slowing down** leukocytes.
- **Chemokines** bind to specific chemokine receptors on the surface of the rolling leukocytes, resulting in increased avidity of binding of leukocyte **integrins** to their ligands on the endothelial surface. leukocytes attach firmly to the endothelium, their cytoskeleton is reorganized, and they spread out on the endothelial surface.
- Leukocytes **transmigrate** between the borders of endothelial cells, a process called **paracellular transmigration**, to reach extravascular tissues. Paracellular transmigration depends on leukocyte integrins and their ligands on the endothelial cells

Leukocyte Migration into Tissues/ summary

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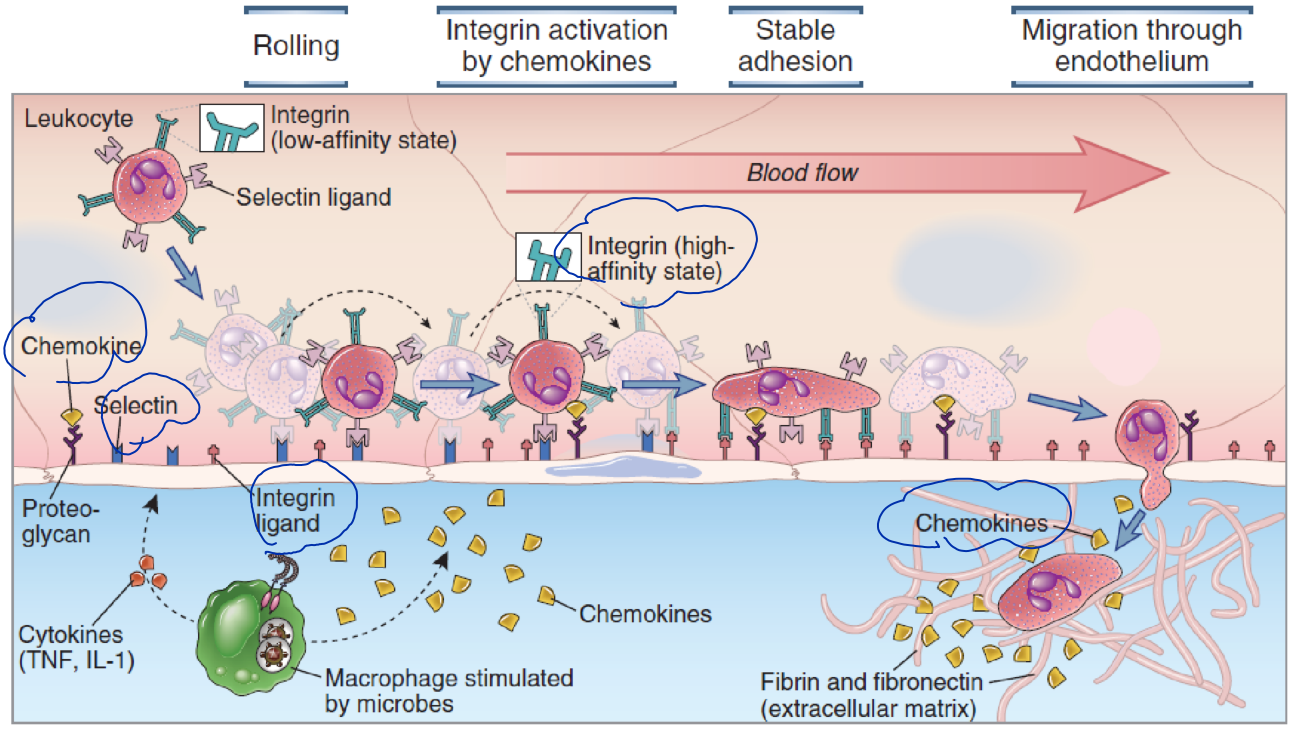

Immunity to Extracellular Bacteria / professional phagocytes

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- Cells that have specialized phagocytic functions, primarily macrophages and neutrophils, are the **first line of defense against microbes** that breach epithelial barriers.

- They serve several functions: 1) **Internalize and kill microbes**. Neutrophils macrophages are particularly good at this function. 2) Phagocytes respond to microbes by **producing various cytokines** that promote inflammation. Macrophages are particularly good at this.

- The essential role that phagocytes play in innate immune defense against microbes is demonstrated by the **high rate of lethal bacterial and fungal infections** in patients with **low blood neutrophil counts** caused by bone marrow cancers or cancer therapy, or inherited deficiencies.

Immunity to Extracellular Bacteria / professional phagocytes

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- IgG subtypes that bind best to Fc receptors (IgG1 and IgG3) are the most efficient opsonins for promoting phagocytosis. Binding of **FcγRI** receptors on phagocytes to multivalent antibody-coated particles leads to **engulfment** of the particles and the **activation** of phagocytes.

- Activation leads to:

➢ Production of the enzyme **phagocyte oxidase**, which catalyzes the intracellular generation of **reactive oxygen species** that are cytotoxic for phagocytosed microbes. This process is called the **respiratory burst**.
➢ Activation of an enzyme called **inducible nitric oxide synthase** (iNOS), which triggers the production of **nitric oxide** that also contributes to the killing of pathogens.
➢ Secretion of **hydrolytic enzymes** and reactive oxygen intermediates into the external milieu that are capable of killing extracellular microbes too large to be phagocytosed. The same toxic products may **damage tissues.**

Immunity to Extracellular Bacteria / professional phagocytes

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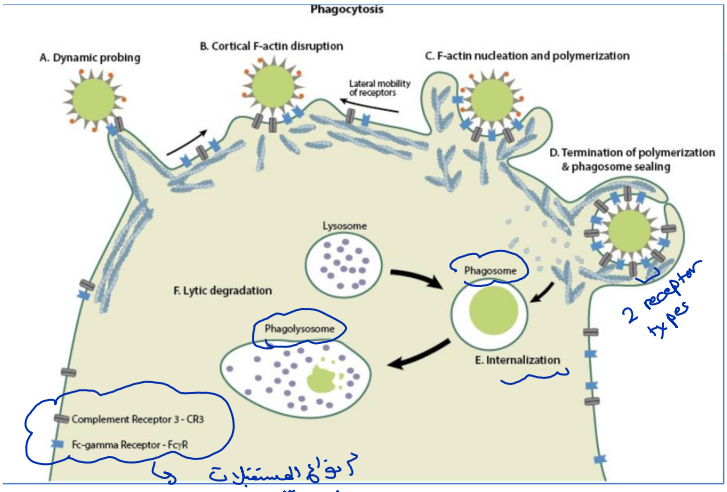
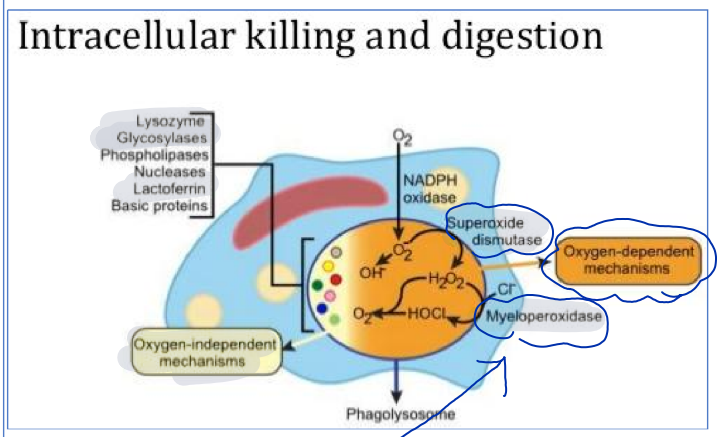


2 receptor types

أنواع المستقبلات

يلي تتحلى الـ Pathogen

على الـ Phagocyte



Binding of **Fc receptors** causes an increase in **oxygen uptake** by the phagocyte called the **respiratory burst**. This influx of oxygen is used in a variety of mechanisms to cause damage to microbes inside the phagolysosome, but the common theme is the creation of **highly reactive small molecules** that damage the biomolecules of the pathogen.

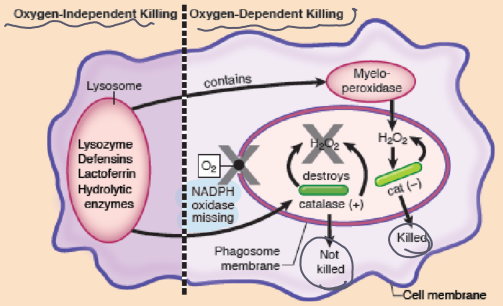
Immunity to Extracellular Bacteria / professional phagocytes

Clinical Correlate

When defects prevent phagocytes from performing their critical functions as first responders and intracellular destroyers of invading antigens, clinically important pathologic processes ensue. Such defects tend to make the patient susceptible to severe infections with **extracellular bacteria** and **fungi**.

Chronic granulomatous disease (CGD) is an inherited deficiency in the production of one of several subunits of **NADPH oxidase**. This defect eliminates the phagocyte's ability to produce many critical oxygen-dependent intracellular metabolites ($\cdot O_2^-$, $\cdot OH$, $^1O_2$, and H_2O_2). The 2 other intracellular killing mechanisms remain intact (myeloperoxidase + $H_2O_2 \rightarrow$ HOCl and lysosomal contents).

- If the patient is infected with a catalase-negative organism, the H_2O_2 waste product produced by the bacterium can be used as a substrate for myeloperoxidase, and the bacterium is killed.
- If the patient is infected with a catalase-positive organism (e.g., *Staphylococcus*, *Klebsiella*, *Serratia*, *Aspergillus*), the myeloperoxidase system lacks its substrate (because these organisms destroy H_2O_2), and the patient is left with the oxygen-independent lysosomal mechanisms that prove inadequate to control rampant infections. Thus, CGD patients suffer from chronic, recurrent infections with catalase-positive organisms.



Intracellular Killing in CG

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Effector mechanisms of humoral immunity

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TABLE 12–3 Fc Receptors

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High ($K_d < 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils; eosinophils, platelets	Phagocytosis; cell activation (inefficient)
FcγRIIB (CD32)	Low ($K_d > 10^{-7}$ M)	B lymphocytes	Feedback inhibition of B cells
FcγRIIC (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low ($K_d > 10^{-6}$ M)	NK cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low ($K_d > 10^{-6}$ M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High ($K_d > 10^{-10}$ M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low ($K_d > 10^{-6}$ M)	Neutrophils, eosinophils, monocytes	Cell activation?

GPI, glycophosphatidylinositol; NK, natural killer.

Immunity to Extracellular Bacteria / complement activation

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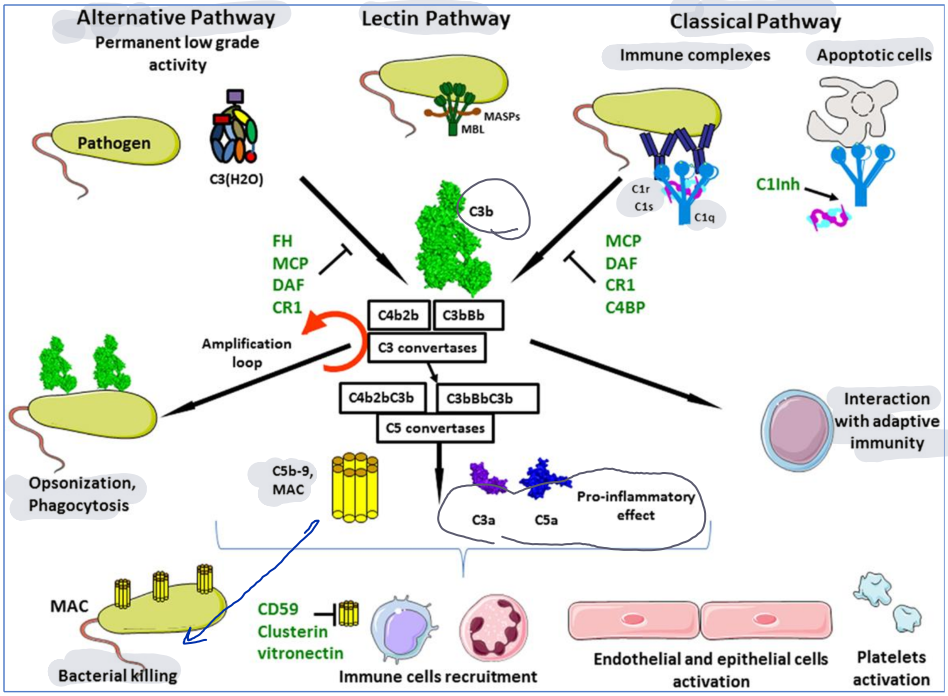

Immunity to Extracellular Bacteria / Antigen presentation حسبنا الله ونعم الوكيل

- Macrophages and dendritic cells function as antigen-presenting cells (APCs). They present peptide antigens derived from digested bacteria on the major histocompatibility complex class II and activate acquired immunity by activating helper T cells.

- While macrophages present antigens within tissues, dendritic cells present antigens in the lymph node. **Only dendritic cells can activate naïve T cells to become effector T cells, and are the most powerful APCs**

Immunity to Extracellular Bacteria / Antigen presentation

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- Dendritic cells are a heterogeneous family of bone marrow–derived cells with long dendrite-like cytoplasmic processes are constitutively present in epithelia and most tissues of the body.

- Most versatile sensors of PAMPs and DAMPs among all cell types in the body.

they are the best APCs

- TLR signaling induces dendritic cell expression of molecules, including **costimulatory molecules and cytokines**, that are needed, in addition to antigen, **for the activation of the naive T cells.** Activation into **effector T cell** subtypes depends on the nature of the pathogen.

Adaptive Immunity to Extracellular Bacteria

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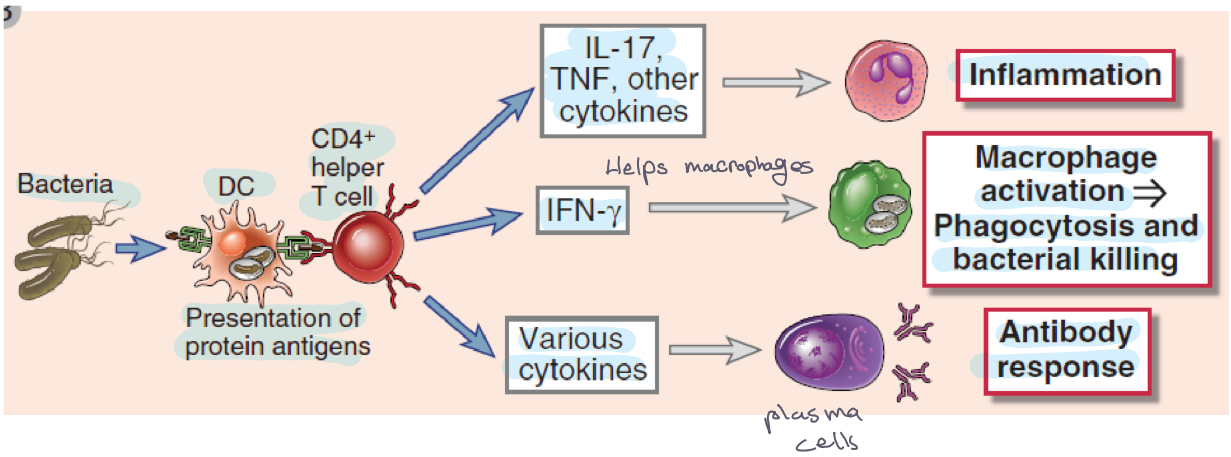

- The protein antigens of extracellular bacteria also **activate CD4+ helper T cells**, which **produce cytokines that induce local inflammation, enhance the phagocytic and microbicidal activities of macrophages and neutrophils**, and **stimulate antibody production**

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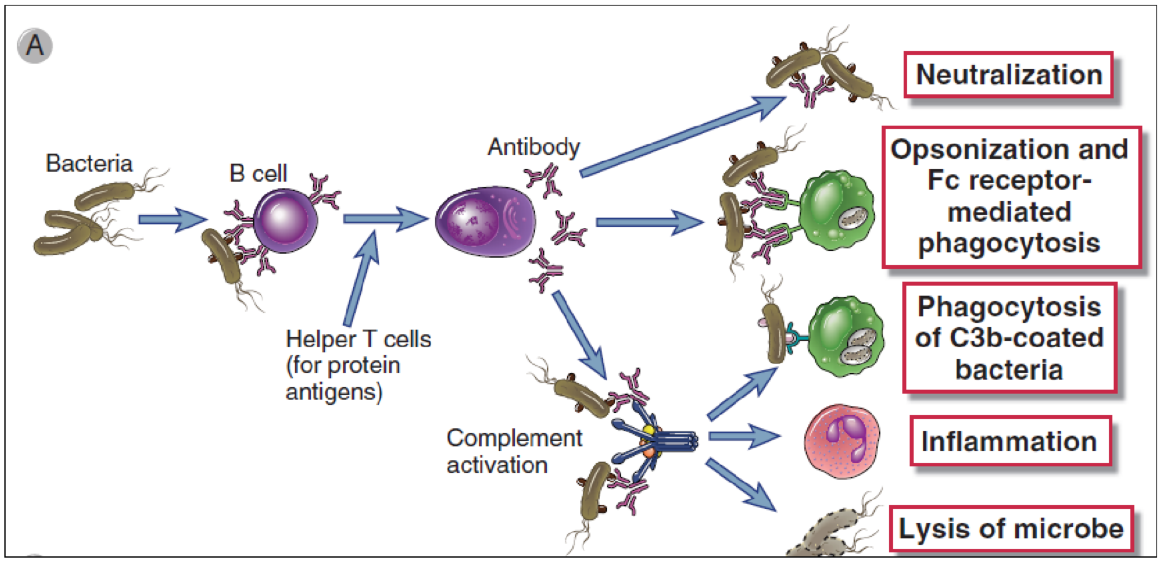
Adaptive Immunity to Extracellular Bacteria

- **Humoral immunity** is a major protective immune response against extracellular bacteria, and it functions to block infection, to eliminate the microbes, and to neutralize their toxins.



Injurious effects following immunity to extracellular

الحمد لله عدد ما خلق الحمد لله ملء ما خلق

- Inflammatory reactions are usually self-limited and controlled. Cytokines secreted by leukocytes in response to bacterial products also stimulate the production of acute-phase proteins and cause the systemic manifestations of the infection. **Sepsis** is a severe pathologic consequence of disseminated infection by some gram-negative and gram-positive bacteria.

- A late complication of the humoral immune response to bacterial infection may be the **generation of disease-producing antibodies**. The best-defined examples are two rare **sequelae of streptococcal infections** of the throat or skin. Infection leads to the production of antibodies against a bacterial cell wall protein (M protein). Some of these antibodies cross-react with self antigens.

Injurious effects following immunity to extracellular

- Certain bacterial toxins stimulate all the T cells in an individual that express a particular family of Vβ T cell receptor (TCR) genes. Such toxins are called **superantigens** because they resemble antigens in that they bind to TCRs and to class II MHC molecules (although not to the peptide-binding clefts) but **activate many more T cells than do conventional peptide antigens**.

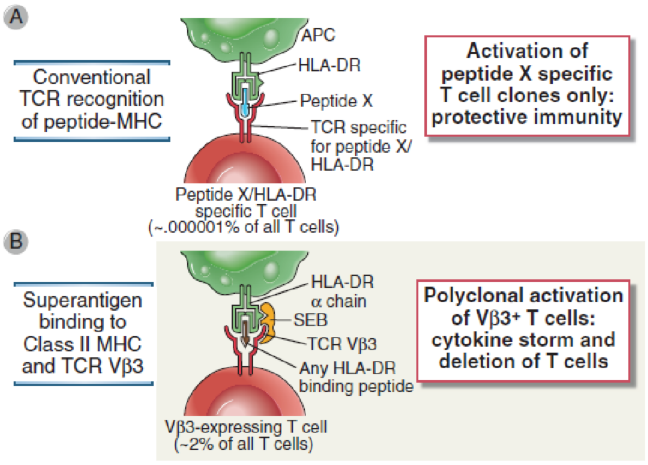


FIGURE 15–2 Polyclonal activation of T cells by bacterial superantigens. A, Conventional microbial T cell antigens, composed of a peptide bound to the peptide-binding groove of an MHC molecule, are recognized by a very small fraction of T cells in any one individual, and only these T cells are activated to become effector T cells that protect against the microbe. **B,** In contrast, a superantigen binds to class II MHC molecules outside the peptide-binding groove and simultaneously binds to the variable region of any TCR β chain, as long as it belongs to a particular V_β family, regardless of the peptide-MHC specificity of the TCR. In this way, superantigens activate T cells to secrete cytokines and also induce apoptosis of T cells. Different superantigens bind to TCRs of different V_β families. Because thousands of clones of T cells will express a TCR β chain from a particular V_β family, superantigens can induce massive cytokine release (cytokine storm) and cause deletion of many T cells. In the example shown, staphylococcal enterotoxin B (SEB) is the superantigen, which binds mainly to HLA-DR and the V_β segments of TCRs belonging to the V_β3 family. APC, antigen-presenting cell.

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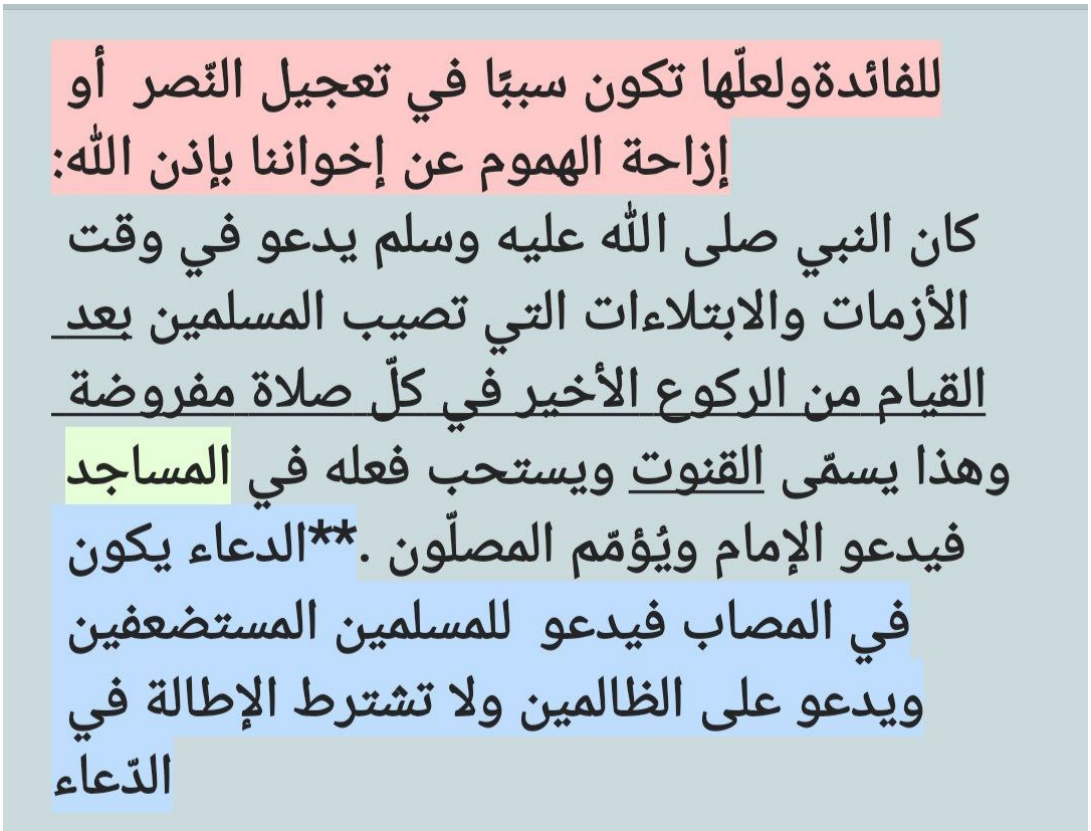
Further reading:

- Cellular and Molecular Immunology. 7th Edition..

Chapter 4. Innate immunity
Chapter 15. Immunity to microbes



والسلام عليكم عباد الله الصالحين