SHEET NO. 7

IMMUNOLOGY

DOCTOR 2019 | MEDICINE | JU

DONE BY : Doctor 2018

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

DOCTOR : Dr. Anas

Quick recap:

Innate immunity

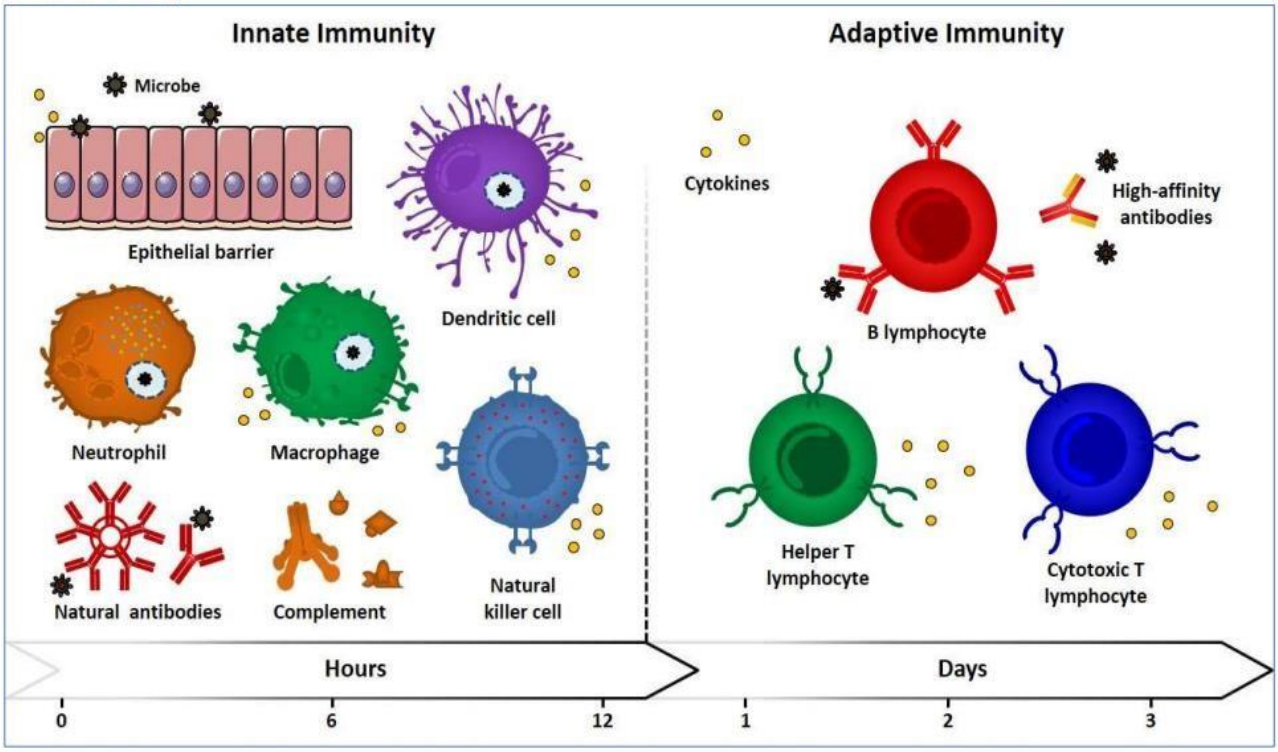


Table 1

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

Note: B1 lymphocytes are considered to be a component of the innate immunity, because they produce natural antibodies (without exposure to antigens).

Main topics that will be discussed in this sheet:

1- Epithelial barriers of innate immunity.

2- Migration of leukocytes into tissue.

Quick introduction:

- The innate immune system is the phylogenically oldest component of the human immune system. Although it is ancient, the innate immune system is highly complex and consists of barriers to infection (**epithelia of skin, gastrointestinal, respiratory, genitourinary tracts**), antimicrobial peptides and proteins, humoral components (i.e**. complement and opsonins**) and cellular components (i.e. **neutrophils, monocytes/macrophages, dendritic cells, and innate lymphoid cells**)

Epithelial barriers:

* **Microorganisms** that cause diseases in humans and animals enter the body through various portals (sites) and cause symptoms of diseases in a variety of ways. The anatomic barriers are fixed defenses against infection, they consist of epithelia that lines the internal and external surfaces of the body along with phagocytes residing beneath all epithelial surfaces.

* Intact epithelial surfaces **(in the skin and the mucosal surfaces of thegastrointestinal, respiratory, and genitourinary**) form **physical barriers** between microbes in the external environment and host tissue.

* Epithelial cells are held together by **tight junctions,** which effectively form a seal against the external environment, they are also crucial for **the maintenance of barrier integrity**.

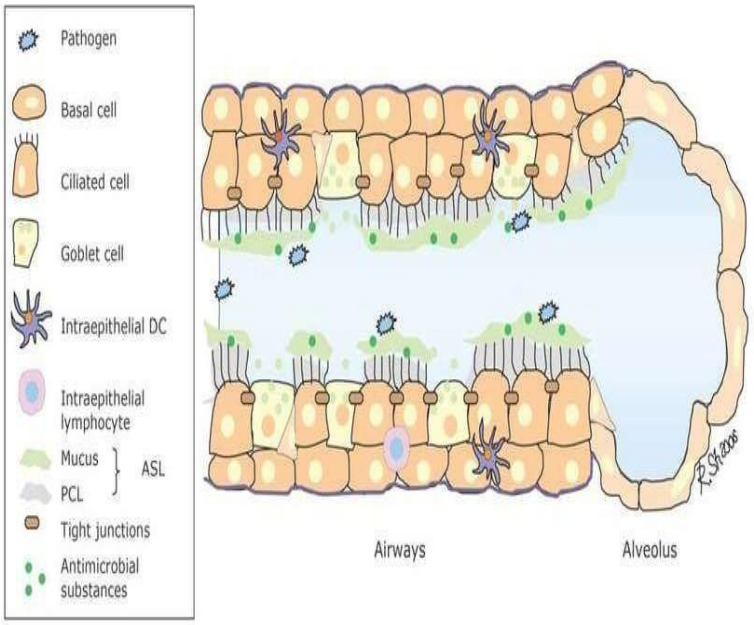
* The internal epithelia are known as **mucosal epithelia**, because they secrete a viscous fluid called **mucus** (a viscous secretion containing **inorganic salts, antimicrobial enzymes (such as lysozymes), immunoglobulins, and glycoproteins such as lactoferrin and mucins**). Mucus physically impairs microbial invasion and facilitates microbe removal **by ciliary action** in the bronchial tree and **peristalsis** in the gut.

*Note: the most **prominent immunoglobin** present in mucus is **IgA**.

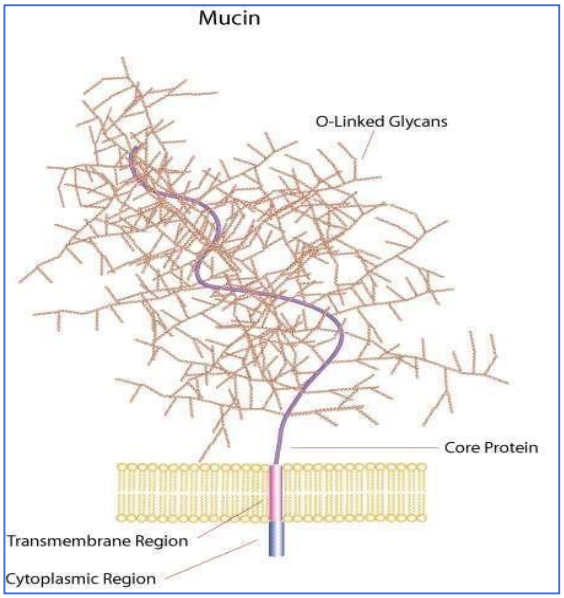
* **Some definitions:**

➥ **Peristalsis in the gut: the involuntary constriction and relaxation of the muscles of the intestine**, creating wavelike movements that push the contents of the canal forward, it is an important mechanism **for keeping both food and infectious agents moving through the body**, **failure** of peristalsis is accompanied with **over-growth** of pathogenic bacteria within the lumen of the gut.

➥ **Mucin**: heavily glycosylated protein.



(Bronchial ciliated epithelium)



(Mucin)

* Epithelial surfaces are more than merely physical barriers to infection, they also secrete a wide variety of chemical substances **that are microbicidal or that inhibit microbial growth**.

* **Antimicrobial peptides (AMPs)** also called **host defense peptides (HDPs),** secreted by **epithelial cells and phagocytes**, are part of the innate immune system found among all classes of life, representing one **of the most ancient forms** of defense against infection.

* Three important classes of antimicrobial peptides in mammals are **defensins**, **cathelicidins** and **histatins (not required)**.

⟹ **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**.

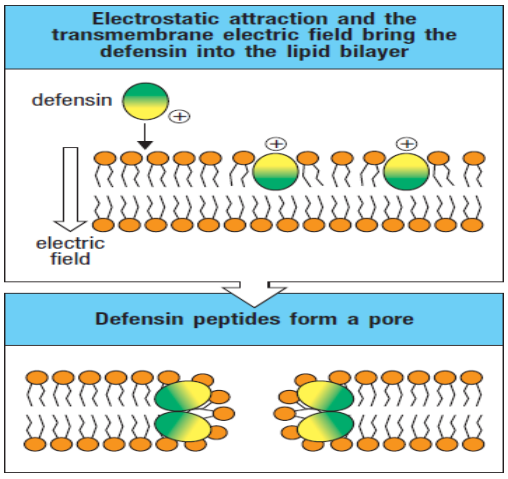
⟹ **Cathelicidins** are produced by **neutrophils and various barrier epithelia**, after cleavage **they have bactericidal and immunomodulatory functions.**

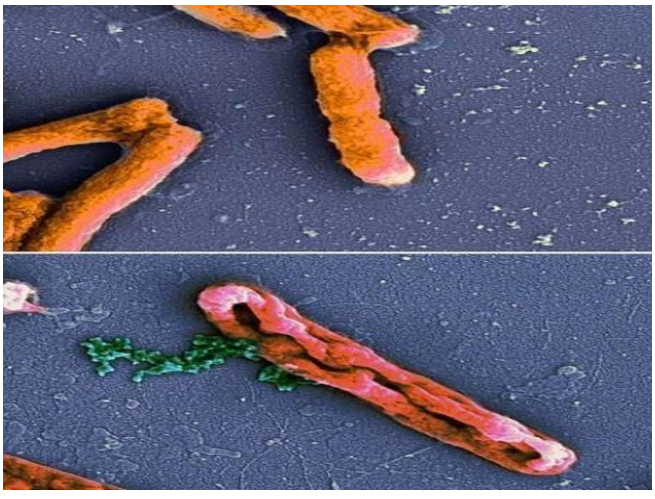
***Good to know about defensins :**

Defensins act within minutes to disrupt the cell membrane of bacteria, fungi and some viruses, the mechanism is thought to **involve insertion of the hydrophobic region** (component of defensins) into the membrane bilayer and formation of a pore that makes the membrane leaky.

* Antimicrobial peptides possessing **a net positive charge** are attracted and incorporated into **negatively charged bacterial membranes** thus disturbing them.

Disrupted cell membrane››leakage of bacterial chromosome (shown in green).

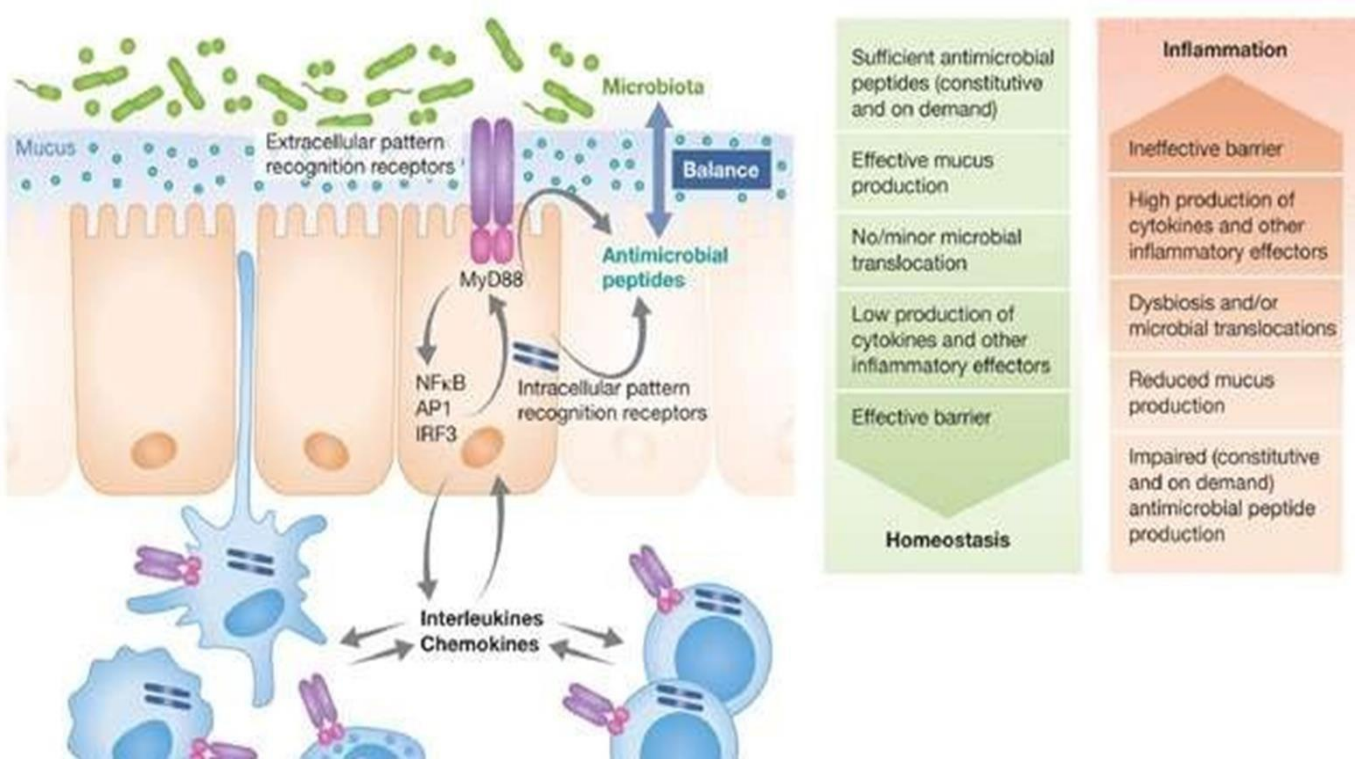




* Healthy epithelial surfaces are usually associated with a large population of normally **nonpathogenic bacteria** known as **commensal bacteria** or **microbiota.**
* Microbiota plays an important role in **innate immune response** against infections, widely distributed in epithelial barriers and **reducing the probability for infectious organisms to colonize there**, when these commensal microorganisms are killed by antibiotics, pathogens frequently **replace them** and **cause diseases**.
* They can also induce responses **that help in strengthen the barrier functions** of epithelia **by stimulating the epithelial cells to produce antimicrobial peptides.**

Factors affecting the integrity of the epithelial barrier

- Continuous secretion of anti microbial peptides
- Continuous secretion of normal mucus
- Presence of the correct microbiota
- Presence of cilia continuously sweeping mucus.
- Presence of sensors continuously sensing microbes.
- Normal adhesion between cells (tight junction, desmosome, …..)



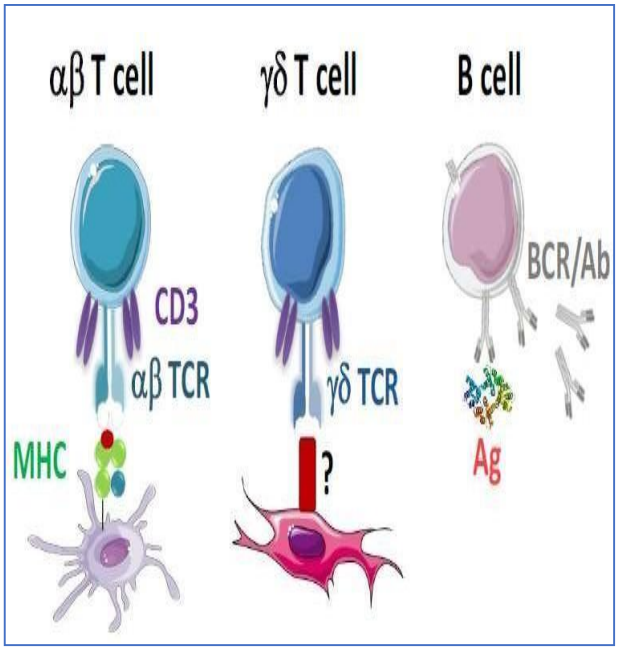
* Barrier epithelia contain certain types of lymphocytes that **are not a component of the adaptive immune system** because they have a limited specifity , including **intraepithelial T lymphocytes**, that **recognize and respond** to commonly encountered microbes.

* This population of T-cells express a distinct type of T-cell receptors made up of **γ: δ (Gamma, delta) heterodimers** rather **than α: β heterodimer of adaptive lymphocytes.**

* 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.**

Intraepithelial T- cells	Adaptive T-cells
γ: δ (Gamma, delta) heterodimers	α: β heterodimer
Limited diversity of antigen receptors	Wide diversity of antigen receptors
Do not recognize antigens as peptides presented on MHC molecules (they recognize them directly), they don't express CD4,CD8 receptors	Recognize antigens as peptides presented on MHC molecules by CD4,CD8 receptors
Respond rapidly	Less than intraepithelial T- cell
Limited known functions (secreting perforin and granzymes inducing apoptosis , secreting cytokines)	Variety of functions (known)

* **The major similarity** between these two types of cells is **that both mature in the thymus.**



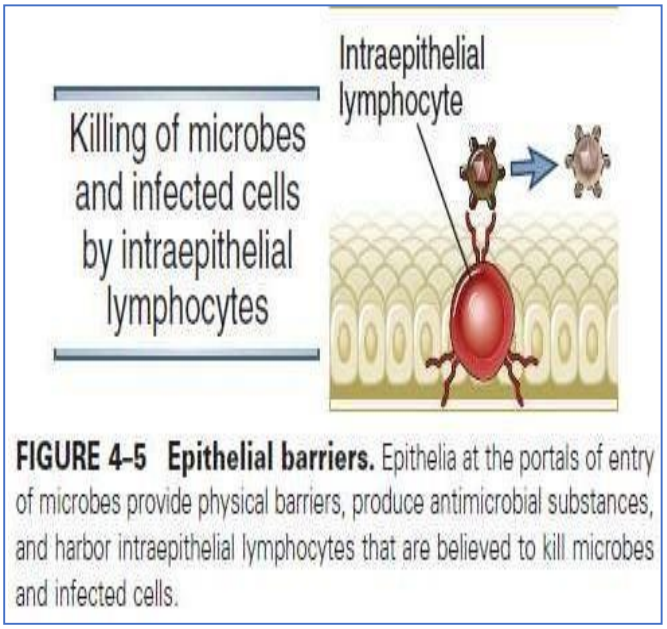


FIGURE 4-5 Epithelial barriers. Epithelia at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor intraepithelial lymphocytes that are believed to kill microbes and infected cells.

*Second part of the lecture (Leukocyte migration to tissues)

* 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., are considered to be in a resting state), **normally located in the circulation and lymphoid organs.**

Remember: **Resident dendritic cell** vs **migratory dendritic cells.**

* Lymphocytes **entry** into a lymph node from the blood occur in distinct stages involving the activity of **adhesion molecules** , **chemokines** and **chemokine receptors**

* **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.

We will study the mechanism of migration of leucocytes to the extravascular tissue(taking T cells as an example):

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

Before going into details, we need to know little about **some adhesion molecules.**

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

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

* are important for specifically guiding leukocytes to particular tissue in a **phenomenon** known as **leucocyte homing** .

* **L- selectin** is expressed on **leukocytes**, where **P- selectin** and **E- selectin** are expressed **on vascular endothelium** at sites of infection.

* **L-selectin** on naive T- cells (not exposed to antigens) guides their exit from the blood into the secondary lymphoid organs by **initiating a light attachment (low affinity)** to the wall **of high endothelial venule** (endothelial cells lining post capillary venule) this will induce **rolling of the cell along the surface of the wall**.

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

Let's now discuss integrins:

* **Naïve T-cells rolling** on the endothelium via selectins require additional types of **cell adhesion molecules** to enter the secondary lymphoid organs, these molecules are called **integrins.**

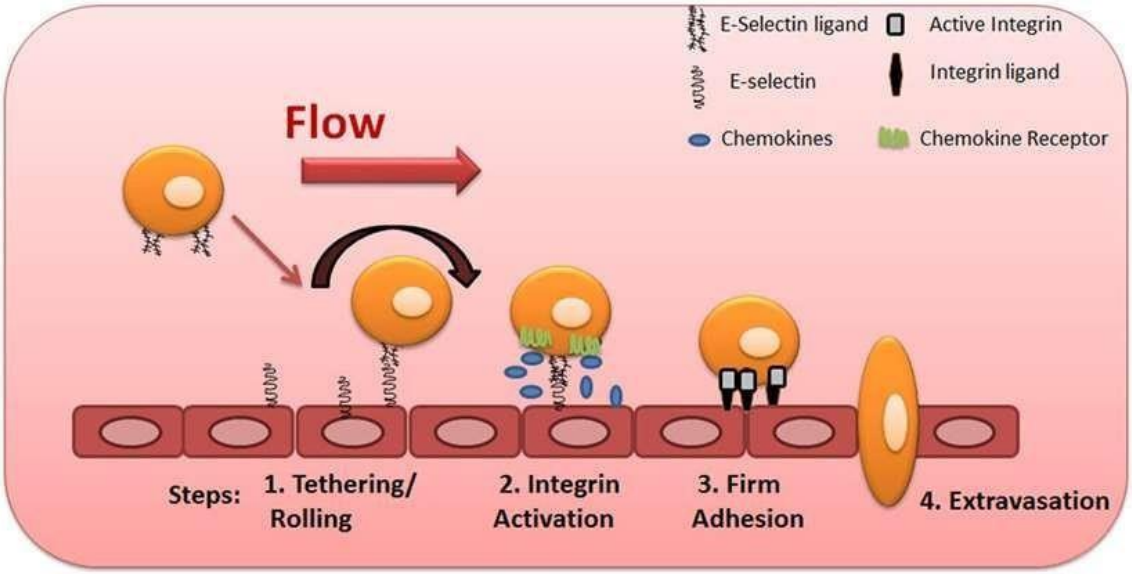
*Integrins **are heterodimeric cell surface proteins** that mediate adhesion of cells to other cells or to extracellular matrix, through specific binding interactions with various ligand.

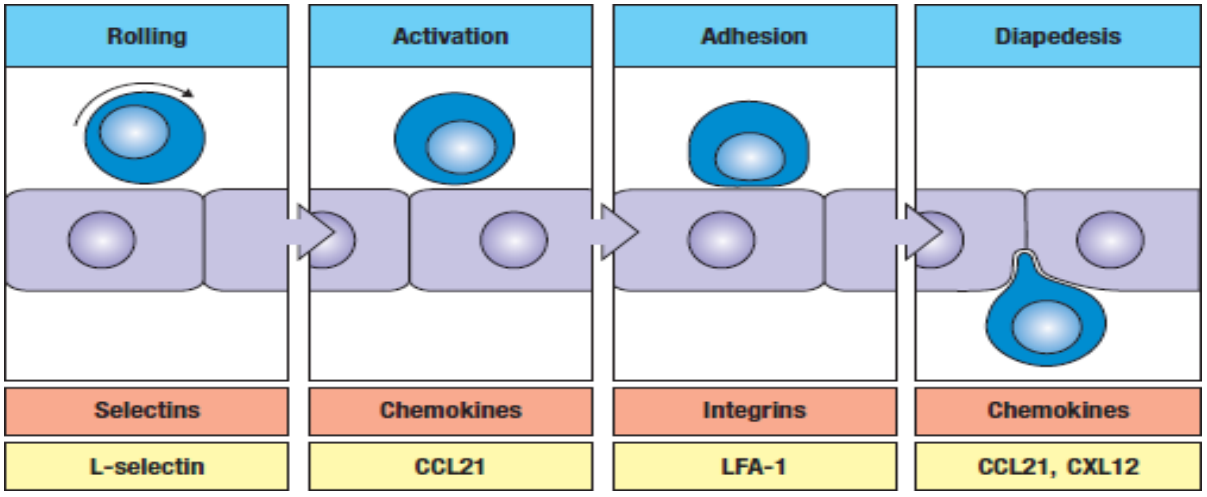
* Integrins **bind tightly** to their ligands **after receiving signals** that induce a change in their conformation , this induces **rapid increasing in their affinity for their ligands.** Signaling by **chemokines** activates integrins on leukocytes to bind tightly to the vascular wall(stop rolling) in preparation of leucocytes migration to sites of inflammation, **chemokines** present at the luminal surface of vascular **wall activate integrins expressed on naïve t cells** during migration to lymphoid organs.

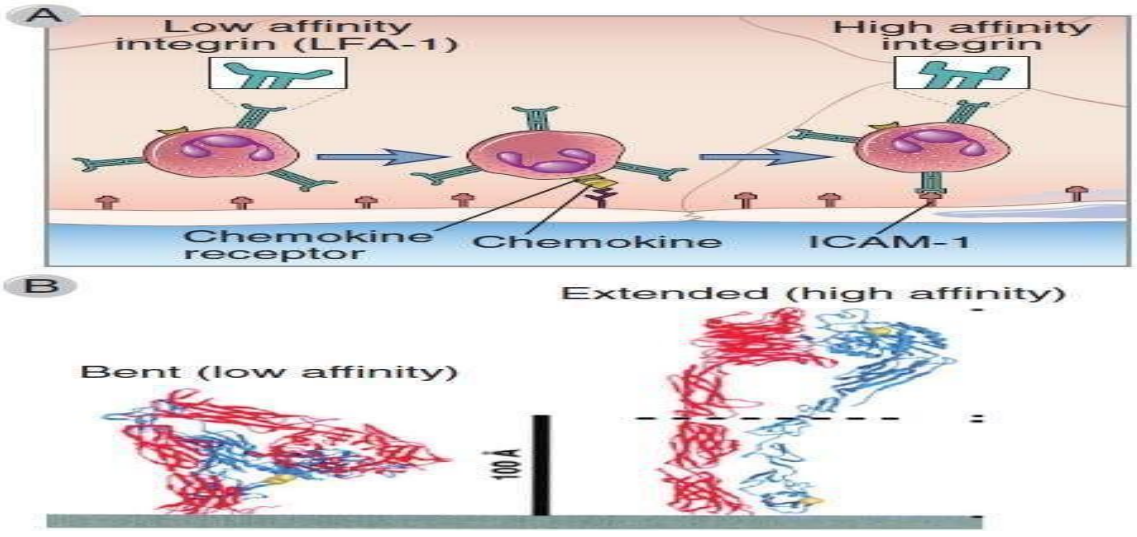
* Chemokines **also induce membrane clustering of integrins** leading to increased avidity of integrin interactions with ligands on the endothelial cells, and therefore tighter binding of leukocytes to the endothelium.

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

* Some pictures







Chemokines are a large family of structurally homologous **cytokines** that **stimulate leukocyte movement** and **regulate the migration** of leukocytes from blood to tissues.

* There are about 50 human chemokines, all of which are 8- to 12-kD (small).

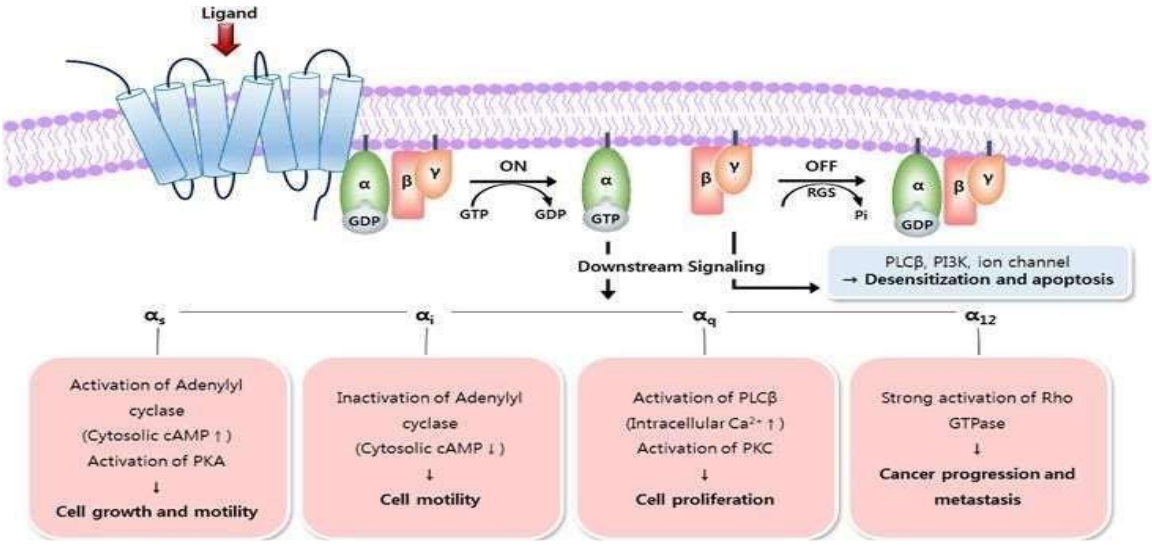
* **Chemokines** fall mainly into two related but distinct groups: **CC chemokines have two adjacent cysteine residues** , where in **CXC chemokines** the **corresponding two cysteine residues are separated by one amino acid**

The role of chemokines in cell recruitment

* They act on the **leukocyte** as it rolls along endothelial cells at sites of inflammation converting this rolling into a stable binding by triggering a conformational change in the adhesion molecules known as **leukocyte integrins,** these conformational changes allow integrins to bind strongly to their ligand on endothelial cells, this binding allows **leukocytes to cross the blood vessel wall (squeezed between the endothelial cells)** in a process called **paracellular transmigration**.

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.**



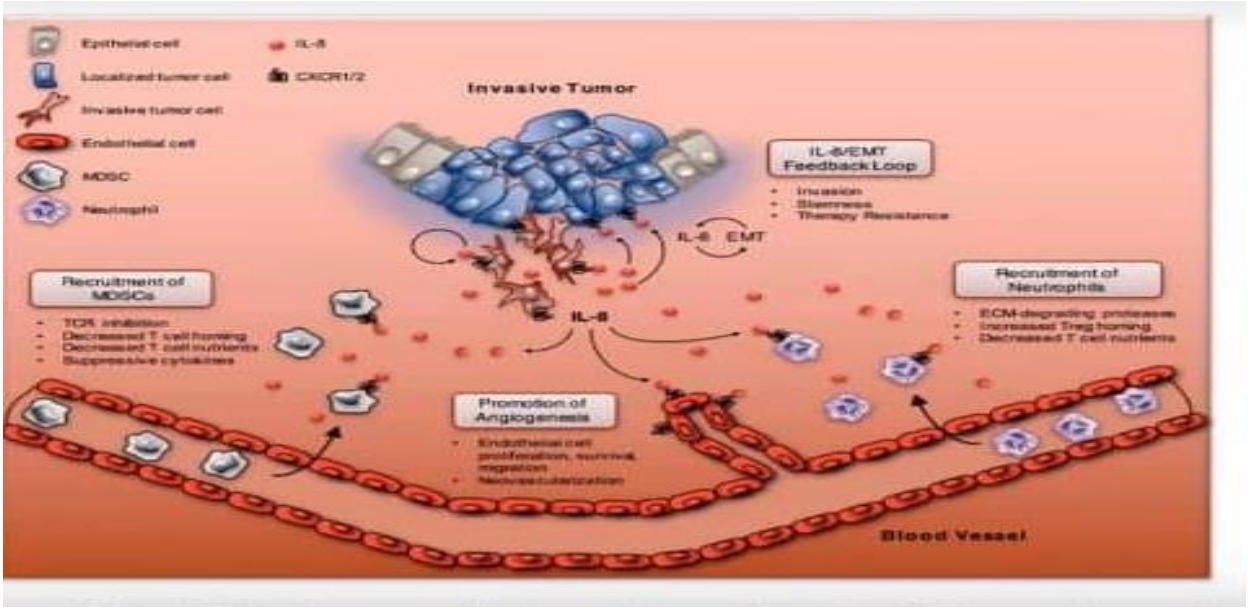
the previous figure shows the molecular mechanism of GPCR signaling .

Slide 18

*Interleukin-8 (CXCL8) plays a role in the attraction of polymorphonuclear inflammatory leukocyte (neutrophils) infiltrate acting on CXCR1/2.

***Chemokines** are redundant and pleiotropic meaning that some of them have **multiple effects on multiple cells.**

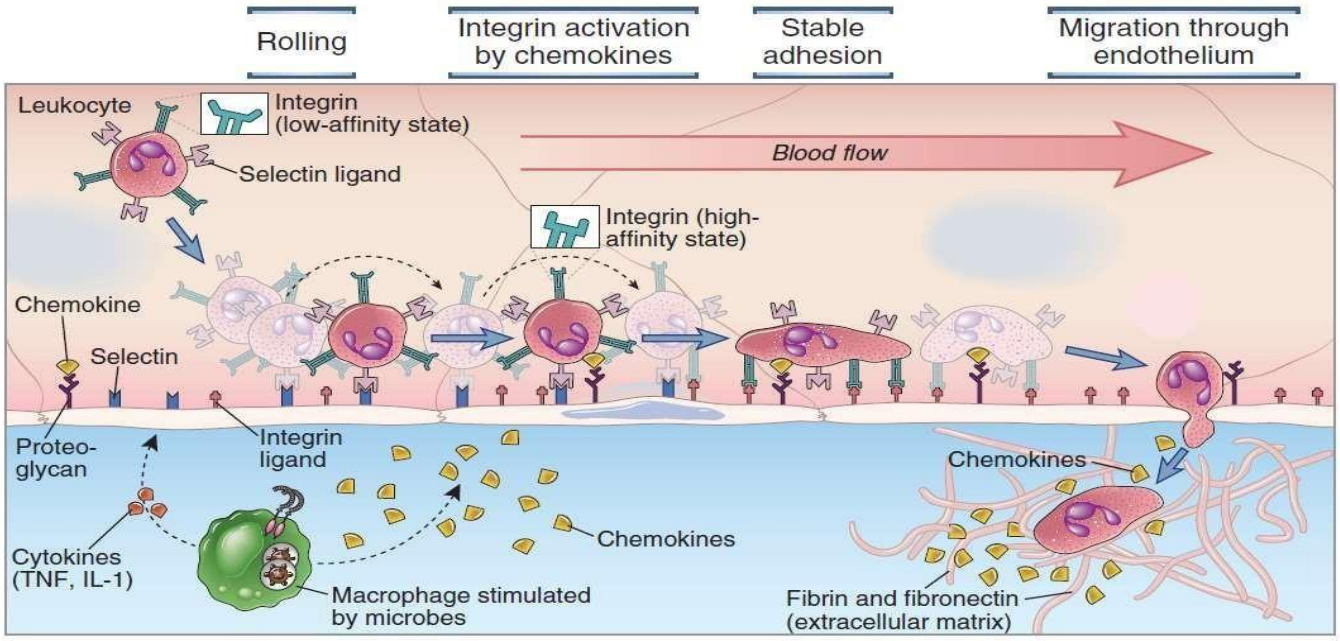
*Recently, it has been found that tumors frequently co-opt **the production of this chemokine**, which in this malignant context exerts different pro-tumoral functions including angiogenesis, survival for cancer stem cells **and** attraction of myeloid cells endowed with the ability to provide growth factors.



We have already explained this in details

- 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

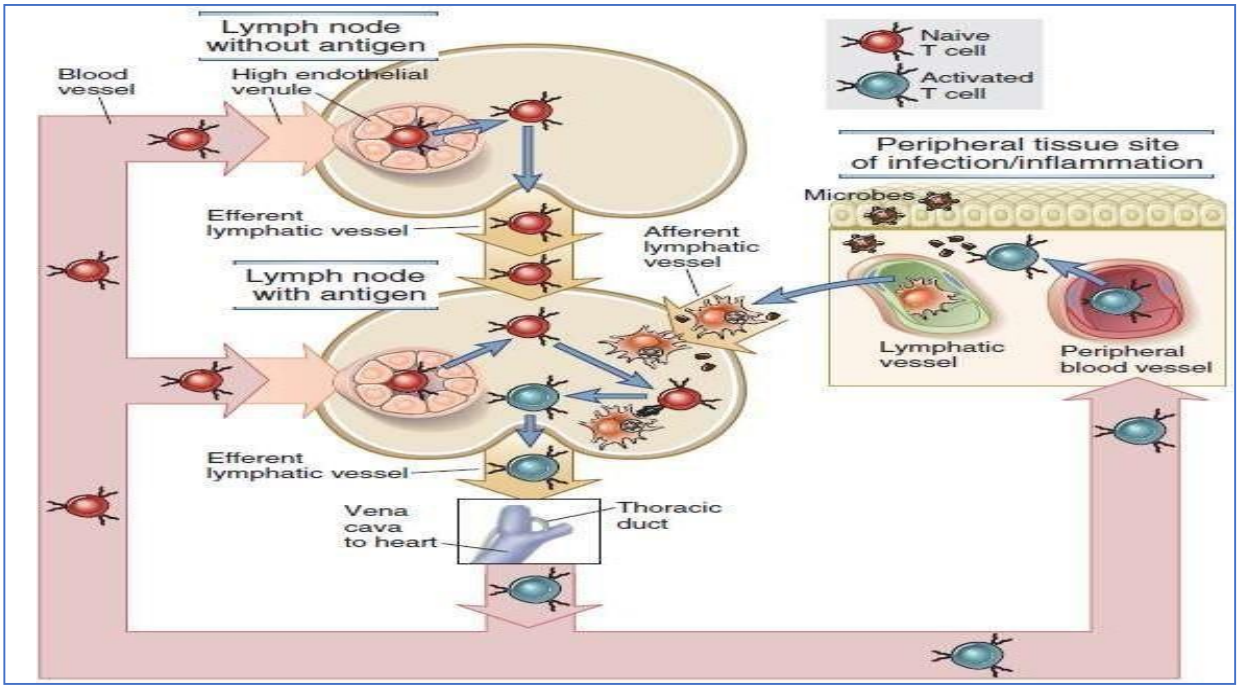
The whole process



*enjoy reading this

- Each lymphocyte goes through one node once a day on average. Peripheral tissue inflammation, which usually accompanies infections, causes a significant increase of blood flow into lymph nodes and consequently an increase in T cell influx into lymph nodes draining the site of inflammation.

- Naive B cells use the same basic mechanisms as do naïve T cells to home to secondary lymphoid tissues throughout the body, which enhances their likelihood of responding to microbial antigens in different sites.

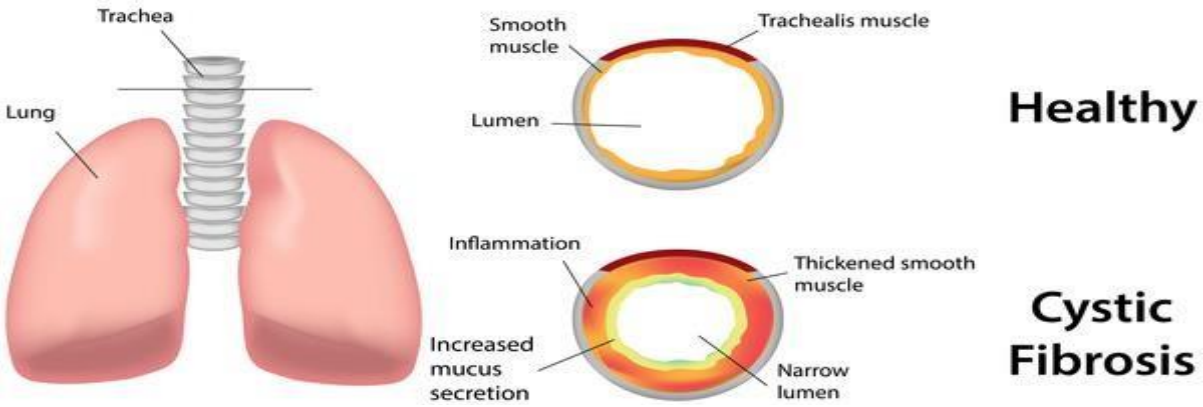


Clinical cases related to what we have discussed

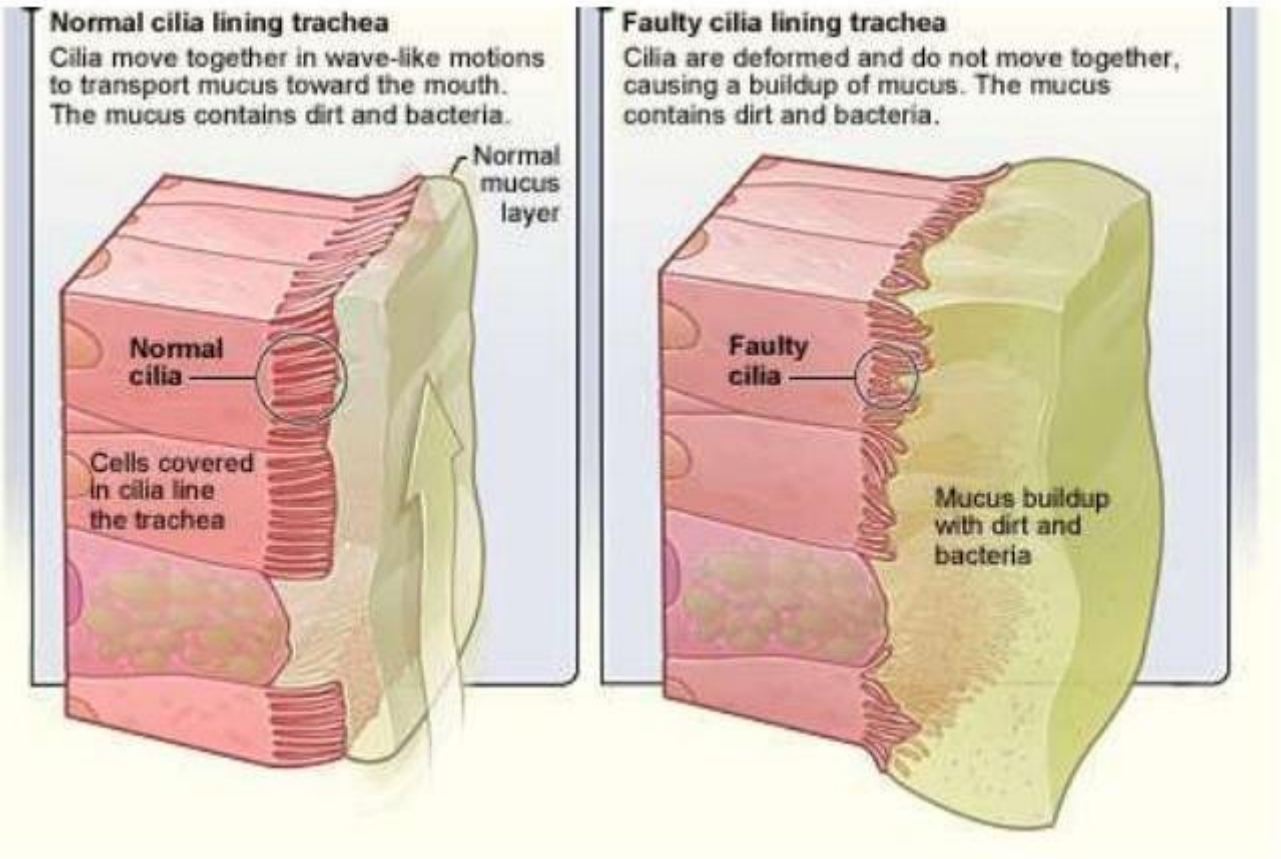
1-cystic fibrosis: is an **inherited disease** occurs because of **mutations in the gene that produces a protein** called **CFTR (cystic fibrosis transmembrane regulator)** causing the body to produce mucus that's extremely **thick and sticky.** This mucus is thicker than the normal one.

In a person **who does not have CF**, **the epithelial cells produce a thin, watery mucus that acts like a lubricant and helps in protecting the body's tissues**. In CF patients, however, the thicker mucus **doesn't move easily**. This thick, sticky mucus **clogs passages** in many of the body's organs causing infection at these sites.

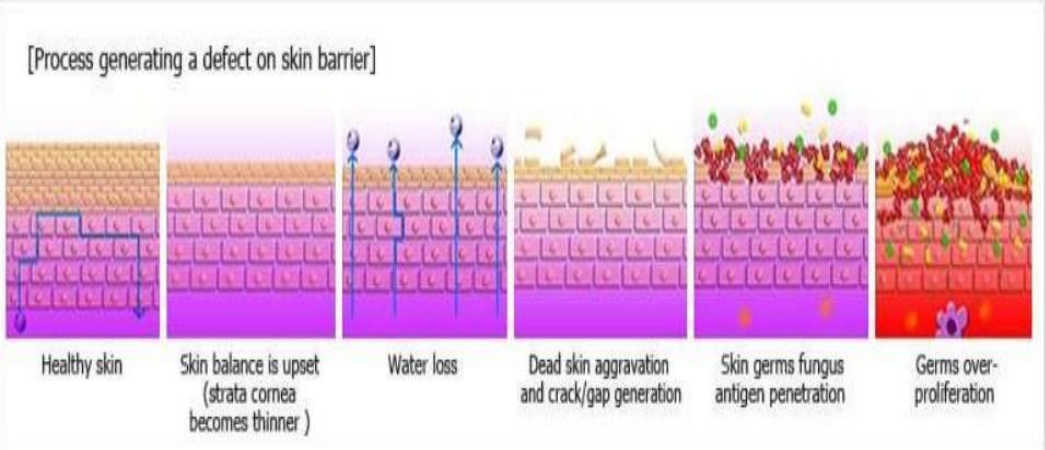
Cystic Fibrosis



2- primary ciliary dyskinesia: is an **inherited condition** in which the microscopic cells in the respiratory **system called cilia do not function normally**. **Ciliary dysfunction prevents the clearance of mucus from lungs**. Bacteria and other irritants in the mucus lead to ***frequent respiratory infections***.



3-eczema: a defective skin barrier leads to **recurrent infections (mainly by staphylococci aureus)** treated by **antibiotics.**



The end