

# MEDIATORS OF INFLAMMATION



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# MEDIATORS OF INFLAMMATION

- ▶ The mediators of inflammation are the substances that initiate and regulate inflammatory reactions.
- ▶ The most important mediators of acute inflammation are:
  - 1 ▶ vasoactive amine.
  - 2 ▶ lipid products (prostaglandins and leukotrienes).
  - 3 ▶ cytokines (including chemokines),
  - 4 ▶ products of complement activation

# General properties of the mediators of inflammation

*resident cells*

- ❑ 1. Mediators may be produced locally by cells at the site of inflammation, or may be derived from circulating inactive precursors that are activated at the site of inflammation.
- Cell-derived mediators *Cellular component (جزء خلوي) من resident cell of site of inflammation* are rapidly released from intracellular granules or synthesized de novo in response to a stimulus

They are most important for reactions against offending agents in tissues.

- Plasma-derived mediators (e.g., complement proteins) are present in the circulation .  
They are produced mainly in the liver, are effective against circulating microbes

- ▶ 2. Active mediators are produced only in response to various molecules that stimulate inflammation, including microbial products and substances released from necrotic cells.
- ▶ 3. Most of the mediators are short-lived.
- ▶ 4. One mediator can stimulate the release of other mediators.

# Major groups.

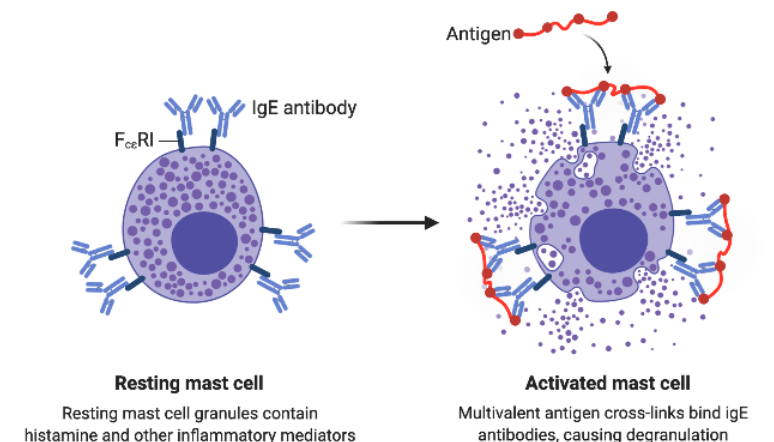
- ▶ 1. Vasoactive Amines: Histamine and Serotonin.
- ▶ 2. Arachidonic Acid Metabolites. *lipid metabolites*
- ▶ 3. Cytokines and Chemokines.
- ▶ 4. Complement System.

most common & fastest

# 1. Vasoactive Amines: 1. Histamine

- ▶ Vasoactive means: act on blood vessels.
- ▶ *why histamine is fast? =>* They are stored as preformed molecules in cells and are therefore among the first mediators to be released during inflammation.
- ▶ Released from mast cells, which are normally present in the connective tissue adjacent to blood vessels.
- ▶ Histamine also is found in blood basophils and platelets

IgE Cross-linking Induces Mast Cell Activation and Degranulation



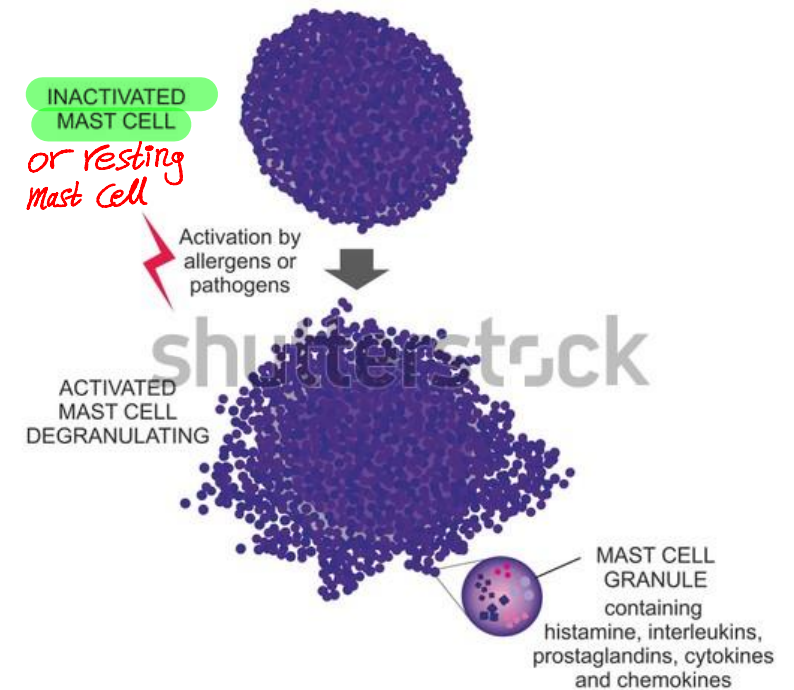
▶ It is stored in mast cell granules and is released by degranulation in response to a variety of stimuli, including :

▶ (1) physical injury, such as trauma, cold, or heat, by unknown mechanisms.

*IgE antibody*

▶ (2) binding of antibodies to mast cells, which underlies immediate hypersensitivity (allergic) reactions.

▶ (3) products of complement called anaphylatoxins (C3a and C5a)



# ❖ ACTION OF HISTAMINE:

▶ Its vasoactive effects are mediated mainly via binding to H<sub>1</sub> receptors.

▶ Histamine actions:

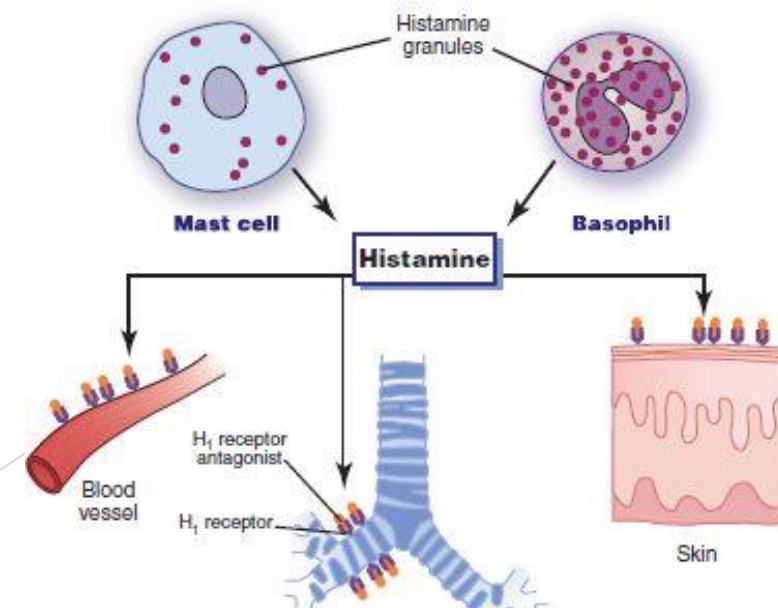
• Vasodilation.

• increases vascular permeability of venules, producing interendothelial gaps in postcapillary venules.

• contraction of some smooth muscles

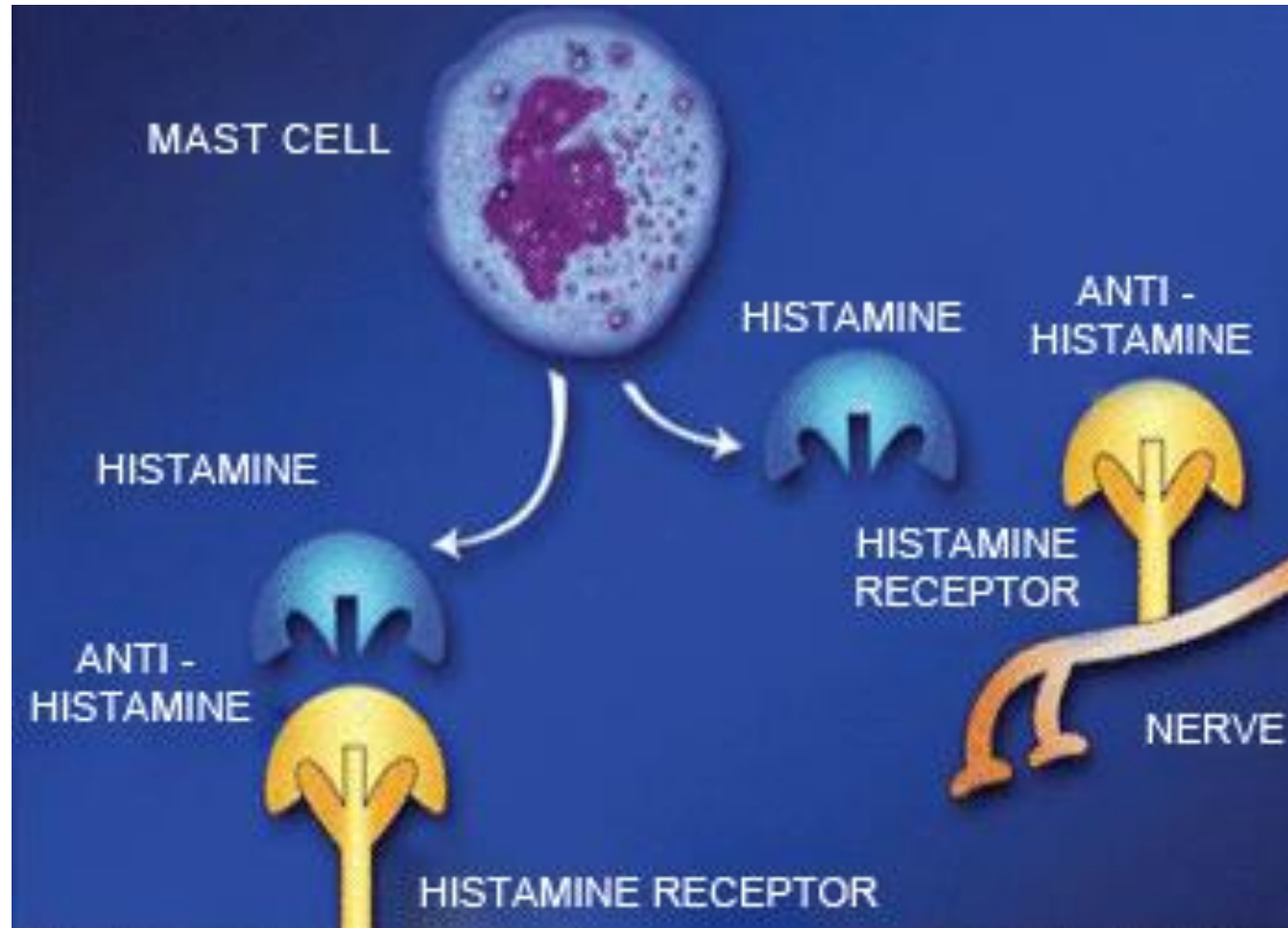
*in asthma ⇒ Contraction of bronchial smooth muscles*

*person died with acute asthma, the bronchi would become contracted & filled with secretion, this secretion due to shortness in breath due to contraction & the airway filled with secretion*





- ▶ The antihistamine drugs that are commonly used to treat some inflammatory reactions, such as allergies, are H1 receptor antagonists that bind to and block the receptor.



## 2. Serotonin

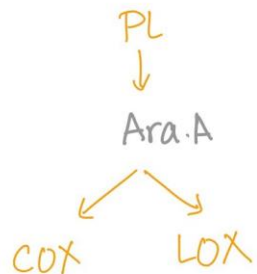
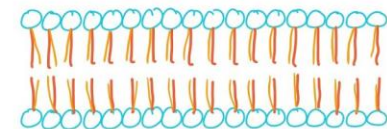
- ▶ Serotonin (5-hydroxytryptamine) is a preformed vasoactive mediator present in platelets and certain neuroendocrine cells, such as in the gastrointestinal tract, and in mast cells in rodents but not humans.
- ▶ Its primary function is as a neurotransmitter in the gastrointestinal tract.
- ▶ It also is a vasoconstrictor, but the importance of this action in inflammation is unclear

*the important action is for histamine*

## 2. Arachidonic Acid Metabolites

- ▶ The lipid mediators prostaglandins and leukotrienes are produced from arachidonic acid present in membrane phospholipids, and they stimulate vascular and cellular reactions in acute inflammation.
- ▶ Arachidonic acid is a 20-carbon polyunsaturated fatty acid that is derived from dietary sources or by conversion from the essential fatty acid linoleic acid.

### Arachidonic Acid pathway



lipid metabolite??  
the origin from cell membrane phospholipid

وادي أعالج المريض من fever  
يس في نفس الوقت ما وادي آخر  
action ال COX-1

selective COX-2 inhibitor  
block only COX-2

Steroids  
enzyme

Convert phospholipid  
to arachidonic acid



Cell membrane phospholipids

Phospholipases



ARACHIDONIC ACID

Other lipoxygenases → HPETEs → HETEs

COX-1 and COX-2  
inhibitors, aspirin  
indomethacin

enzyme  
Cyclooxygenase

1  
5-Lipoxygenase  
increase inflammation

Lipoxygenase inhibitors

prevent formation of  
prostaglandins

non steroidal  
group

Prostaglandin G<sub>2</sub> (PGG<sub>2</sub>)  
Prostaglandin H<sub>2</sub> (PGH<sub>2</sub>)

general not specific

prevent thrombus

Prostacyclin  
PGI<sub>2</sub>

1  
2  
Causes  
vasodilation,  
inhibits platelet  
aggregation

وادي صلاحه صغبر  
واحي يسر ال Lumen

thrombosis.

Thromboxane A<sub>2</sub>  
TXA<sub>2</sub>

1  
2  
Causes  
vasoconstriction,  
promotes platelet  
aggregation

Causing edema

chemoattractant  
for neutrophils.

PGD<sub>2</sub>  
PGE<sub>2</sub>

1  
2  
Causes  
vasodilation,  
increased  
vascular  
permeability

the same function

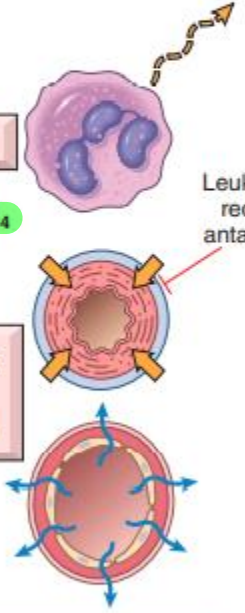
Leukotriene C<sub>4</sub> (LTC<sub>4</sub>)  
Leukotriene D<sub>4</sub> (LTD<sub>4</sub>)  
Leukotriene E<sub>4</sub> (LTE<sub>4</sub>)

Bronchospasm  
Increased  
vascular  
permeability

2  
12-Lipoxygenase  
stop inflammation

Lipoxin A<sub>4</sub> (LXA<sub>4</sub>)  
Lipoxin B<sub>4</sub> (LXB<sub>4</sub>)

Inhibition of  
inflammation



Leukotriene  
receptor  
antagonists



Very important

**Table 3.6 Principal Actions of Arachidonic Acid Metabolites in Inflammation**

Action	Eicosanoid
Vasodilation	Prostaglandins PGI <sub>2</sub> (prostacyclin), PGE <sub>1</sub> , PGE <sub>2</sub> , PGD <sub>2</sub>
Vasoconstriction	Thromboxane A <sub>2</sub> , leukotrienes C <sub>4</sub> , D <sub>4</sub> , E <sub>4</sub>
Increased vascular permeability	Leukotrienes C <sub>4</sub> , D <sub>4</sub> , E <sub>4</sub>
Chemotaxis, leukocyte adhesion	Leukotriene B <sub>4</sub>
Smooth muscle contraction	Prostaglandins PGC <sub>4</sub> , PGD <sub>4</sub> , PGE <sub>4</sub>

# A. Prostaglandins.

- ▶ Prostaglandins (PGs) are produced by mast cells, macrophages, endothelial cells, and many other cell types.
- ▶ They are involved in the vascular and systemic reactions of inflammation.
- ▶ They are generated by the actions of two cyclooxygenases called COX-1 and COX-2.
- ▶ Prostaglandins are named based on structural features coded by a letter (e.g., PGD, PGE, PGF, PGG, and PGH) and a subscript numeral (e.g., 1, 2), which indicates the number of double bonds in the compound.
- ▶ The most important prostaglandins in inflammation are PGE2, PGD2, PGF2a, PGI2 (prostacyclin), and TXA2 (thromboxane A2).

رج نكتب عن الـ PGs  
المهمة كالمادة

## Characteristics comparison between COX-1 and COX-2

	COX-1	COX-2
Synthesis	intrinsic	induced
Functions	<p><i>always</i> ←</p> <p>physiological:</p> <ol style="list-style-type: none"><li>1 gastrointestinal protection</li><li>2 platelet aggregation regulation</li><li>3 vascular resistance regulation</li><li>4 renal blood flow regulation</li></ol>	<p>* <u>physiological:</u></p> <ol style="list-style-type: none"><li>1 production of PG</li><li>2 elevated during pregnancy</li></ol> <p>* <u>pathological:</u></p> <ol style="list-style-type: none"><li>1 producing proteinase, PG, and other inflammatory mediators</li></ol>

*Cause of fever, pain & manifestation is mediators (Prostaglandins)*

- ▶ In addition to their local effects, prostaglandins are involved in the pathogenesis of pain and fever, two common systemic manifestations of inflammation. PGE2 makes the skin hypersensitive to painful stimuli, and causes fever during infections

← B<sub>2</sub> بالى حافى لى



# 2. Leukotrienes.

- ▶ Leukotrienes are produced in leukocytes and mast cells by the action of lipoxygenase and are involved in vascular and smooth muscle reactions and leukocyte recruitment.

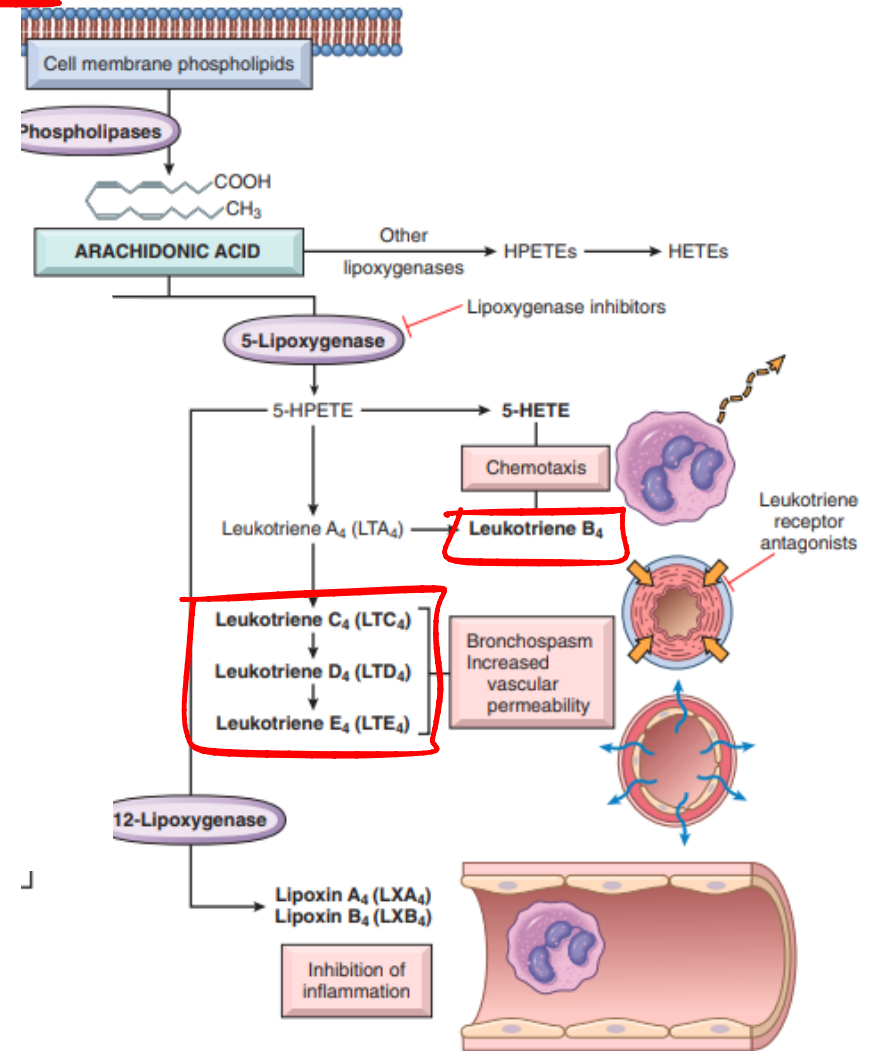
## ❖ LTB<sub>4</sub>:

- Produced by neutrophils and some macrophages.
- Potent chemotactic agent and activator of neutrophils, causing aggregation and adhesion of the cells to venular endothelium.
- Generation of ROS.
- Release of lysosomal enzymes.

## ❖ LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>:

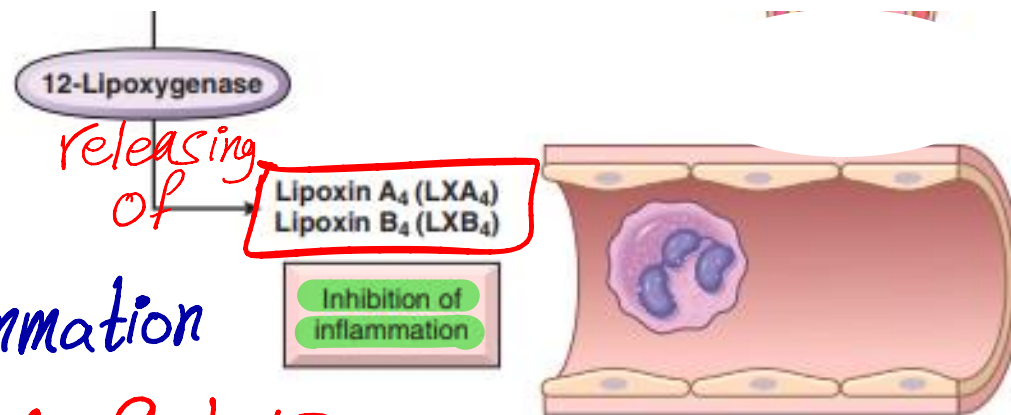
- Produced mainly in mast cells.
- Cause intense vasoconstriction, bronchospasm (important in asthma), and increased permeability of venules.

*if I want to treat an asthma patient  
I will block to lipoxygenase enzyme*



# 3. Lipoxins

- ▶ Are generated from arachidonic acid by the lipoxygenase pathway.
- ▶ Unlike prostaglandins and leukotrienes, the lipoxins suppress inflammation by inhibiting the recruitment of leukocytes.
- ▶ They inhibit neutrophil chemotaxis and adhesion to endothelium.



Stop the inflammation  
ET9F, IL-10, LXA<sub>4</sub> & LXB<sub>4</sub>

# Pharmacologic Inhibitors of Prostaglandins and Leukotrienes

- ▶ The importance of eicosanoids in inflammation has driven attempts to develop drugs that inhibit their production or actions and thus suppress inflammation. These anti-inflammatory drugs include the following:

# 1. Cyclooxygenase inhibitors

- ▶ It include aspirin and other (NSAIDs), such as ibuprofen.
- ▶ They inhibit both COX-1 and COX-2 and thus block all prostaglandin synthesis (hence their efficacy in treating pain and fever).
- ▶ Selective COX-2 inhibitors:
- ▶ are 200- to 300-fold more potent in blocking COX-2 than COX-1.
- ▶ COX-1 is responsible for the production of prostaglandins that are involved in both inflammation and physiologic functions such as protecting gastric epithelial cells from acid-induced injury, whereas COX-2 generates prostaglandins that are involved only in inflammation.
- ▶ So in patient with gastric ulceration ....?

*if patient has a peptic ulcer I will give him selective COX-2 inhibitor*

*→ it is expensive*

▶ selective COX-2 inhibitors may increase the risk of cardiovascular and cerebrovascular events, possibly because they impair endothelial cell production of prostacyclin (PGI<sub>2</sub>), which prevents thrombosis, while leaving intact the COX-1-mediated production by platelets of TXA<sub>2</sub>, which induces platelet aggregation. Thus, selective COX-2 inhibition may tilt the balance toward vascular thrombosis

treat pain & fever → block COX-1  
treat asthma → block lipoxygenase

## ▶ 2. Lipoxygenase inhibitors:

- Pharmacologic agents that inhibit leukotriene production (e.g., zileuton) are useful in the treatment of asthma.

## ■ 3. Corticosteroids:

- are broad-spectrum anti-inflammatory agents that : block
- reduce the transcription of genes encoding COX-2, phospholipase A2, proinflammatory cytokines (e.g., IL-1 and TNF), and iNOS.

## ■ 4. Leukotriene receptor antagonists:

- It block leukotriene receptors and prevent the actions of the leukotrienes.
- These drugs (e.g., Montelukast) are useful in the treatment of asthma.

# 3. Cytokines and Chemokines

## ▶ Cytokines:

- ▶ are proteins secreted by many cell types (principally activated lymphocytes, macrophages, and dendritic cells, but also endothelial, epithelial, and connective tissue cells) that mediate and regulate immune and inflammatory reactions.

# TNF

## ▶ A. Tumor Necrosis Factor and Interleukin-1:

- ▶ Serve critical roles in leukocyte recruitment by promoting adhesion of leukocytes to endothelium and their migration through vessels.
- ▶ Produced by activated macrophages and dendritic cells under the effect of . Microbial products, foreign bodies, necrotic cells.
- ▶ The most important roles of these cytokines in inflammation are the following:
  - ▶ 1. Endothelial activation.
  - ▶ 2. Activation of leukocytes and other cells.
  - ▶ 3. Systemic acute-phase response.
  - ▶ 4. TNF regulates energy balance by promoting lipid and protein catabolism and by suppressing appetite.



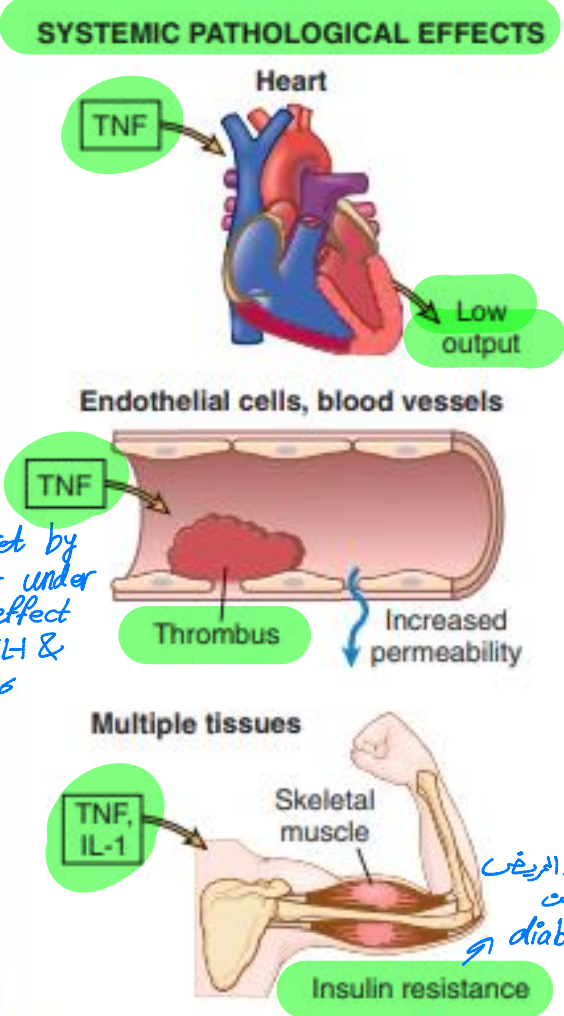
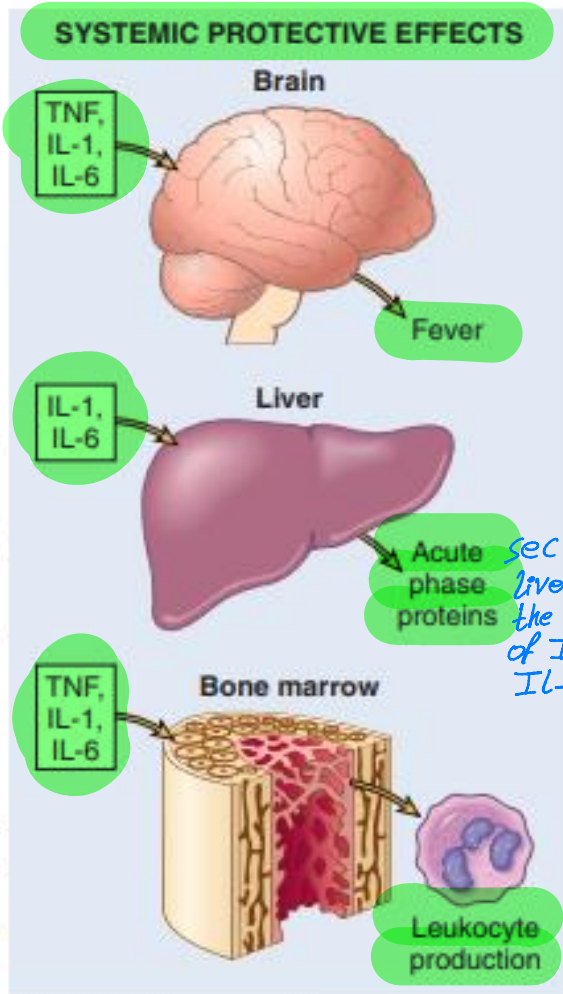
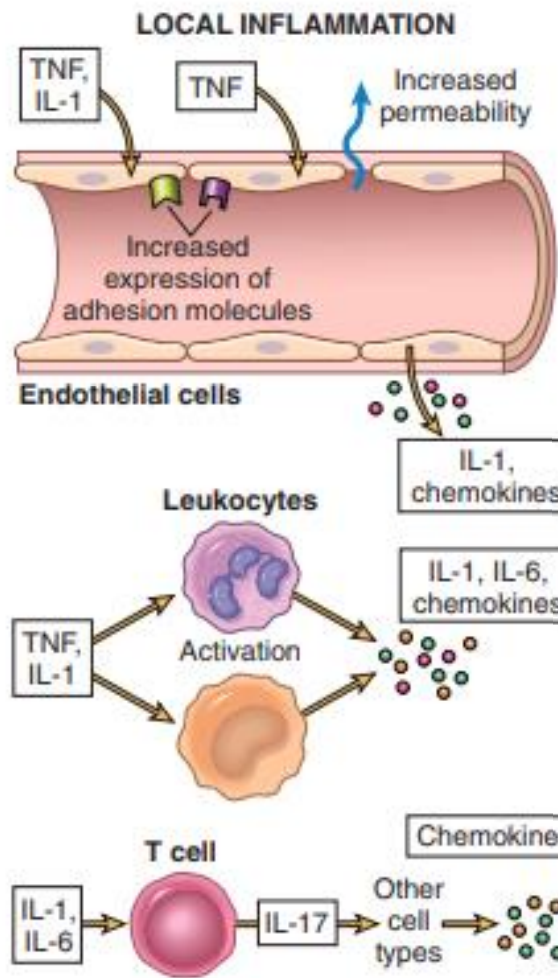
- ▶ TNF antagonists have been remarkably effective in the treatment of chronic inflammatory diseases:
- ▶ rheumatoid arthritis.
- ▶ التهاب الجلد  
psoriasis.
- ▶ some types of inflammatory bowel disease.

هنریل

- Sustained production of TNF contributes to **cachexia**, a pathologic state characterized by weight loss, muscle atrophy, and anorexia that accompanies some chronic infections and cancers



very important



secret by liver under the effect of IL-1 & IL-6

مع الوقت المريض  
يحس بوجع  
ال diabetes

Fig. 3.10 Major roles of cytokines in acute inflammation. PDGF, Platelet-derived growth factor; PGE, prostaglandin E; PGI, prostaglandin I.

► B. Chemokines:

► Chemokines are a family of small proteins that act primarily as chemoattractants for specific types of leukocytes.

► Chemokines mediate their activities by binding to seven-transmembrane G protein-coupled receptors.

► They are classified into four major groups, according to the arrangement of cysteine (C) residues in the proteins:

*Contain 4 Cysteine*

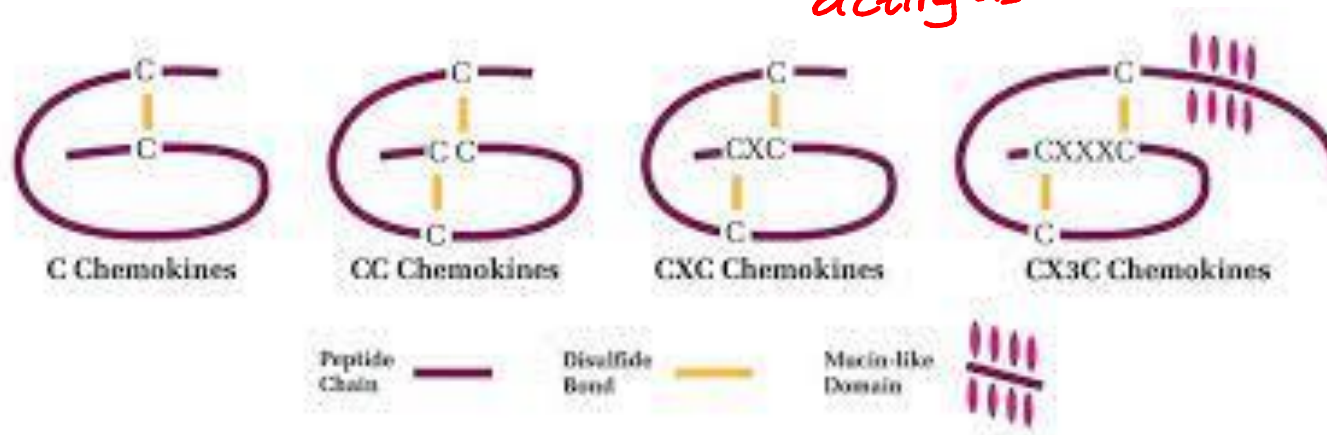
*amino acid between 1st & 2nd cysteine*

► **1. C-X-C chemokines :**

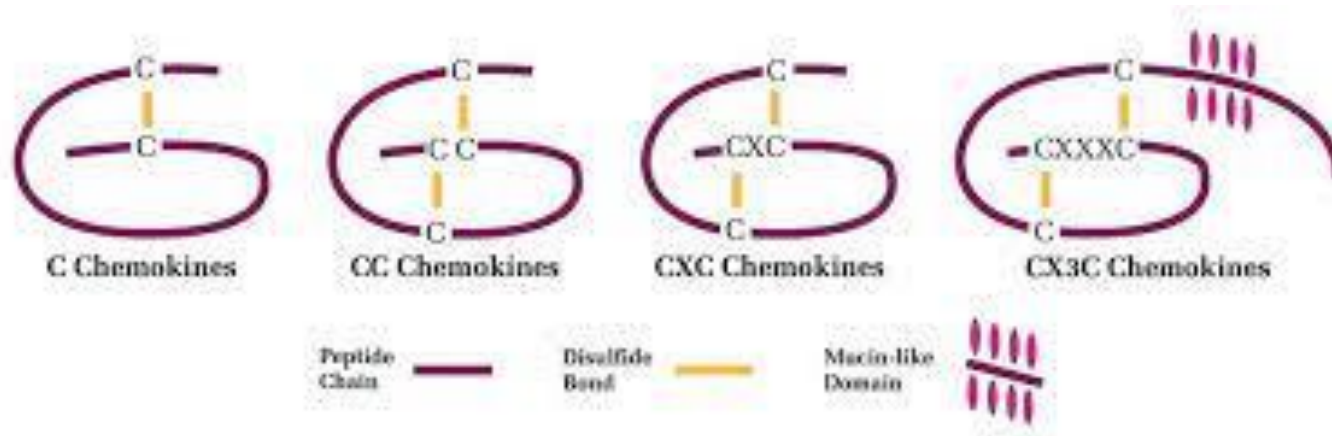
► have one amino acid residue separating the first two of the four conserved cysteines.

► These chemokines act primarily as neutrophils chemoattractant. **IL-8** (now called CXCL8) is typical of this group.

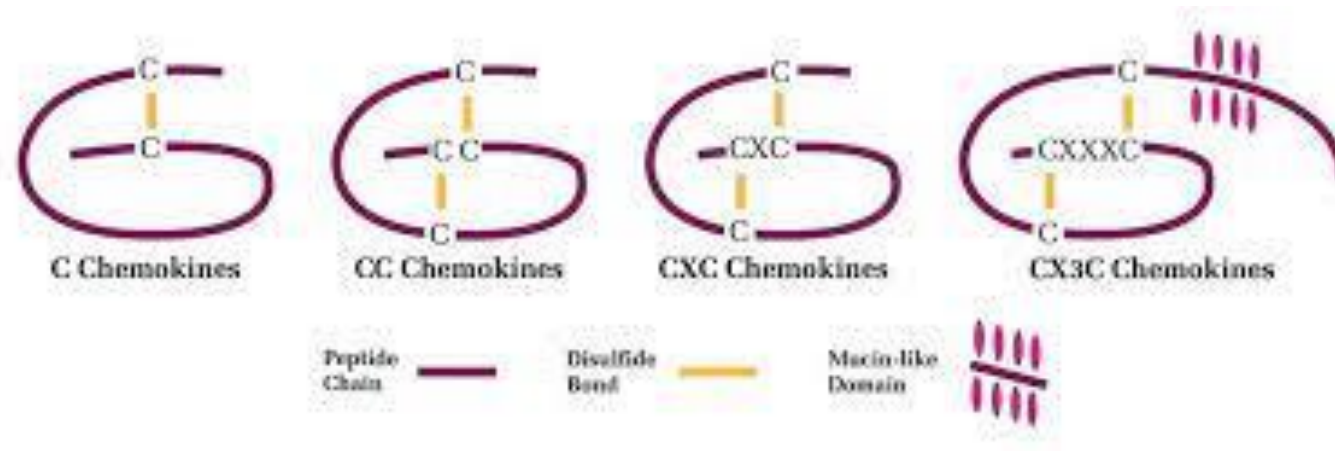
*acting as*



- ▶ 2. C-C chemokines:
- ▶ have the first two conserved cysteine residues adjacent.
- ▶ include monocyte chemoattractant protein (MCP-1, CCL2), eotaxin (CCL11), and macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ , CCL3).
- ▶ mainly serve as chemoattractants for monocytes, eosinophils, basophils, and lymphocytes.



- ▶ 3. C chemokines :
  - ▶ lack the first and third of the four conserved cysteines.
  - ▶ The C chemokines (e.g., **lymphotactin**, XCL1) are relatively specific for lymphocytes.
- ▶ 4. CX3C chemokines :
  - ▶ contain three amino acids between the first two cysteines
  - ▶ The only known member of this class is called **fractalkine** (CX3CL1).



- ▶ Chemokines bind to proteoglycans and are displayed at high concentrations on the surface of endothelial cells and in the extracellular matrix. They have two main functions:
- ▶ • Acute inflammation:
- ▶ Most chemokines stimulate leukocyte attachment to endothelium by acting on leukocytes to increase the affinity of integrins, and also serve as chemoattractants, thereby guiding leukocytes to sites of infection or tissue damage.
- ▶ • Maintenance of tissue architecture:
- ▶ organize various cell types in different anatomic regions of the tissues, such as T and B lymphocytes in discrete areas of the spleen and lymph nodes

homing  
ال home تبه

# 4. Complement System

- ▶ The complement system is a collection of soluble proteins and their membrane receptors that function mainly in host defense against microbes and in pathologic inflammatory reactions.
- ▶ There are more than 20 complement proteins, some of which are numbered C1 through C9. *C<sub>1</sub>-C<sub>9</sub>*
- ▶ In the process of complement activation, several cleavage products of complement proteins are elaborated that cause increased vascular permeability, chemotaxis, and opsonization

→ tagging the microbes for macrophage to recognize them



- ▶ The critical step in complement activation is the proteolysis of the third (and most abundant) component, C3. Cleavage of C3 can occur by one of three pathways:

- ▶ 1. The classical pathway:

- ▶ which is triggered by fixation of C1 to antibody (IgM or IgG) that has combined with antigen.

- ▶ 2. The alternative pathway:

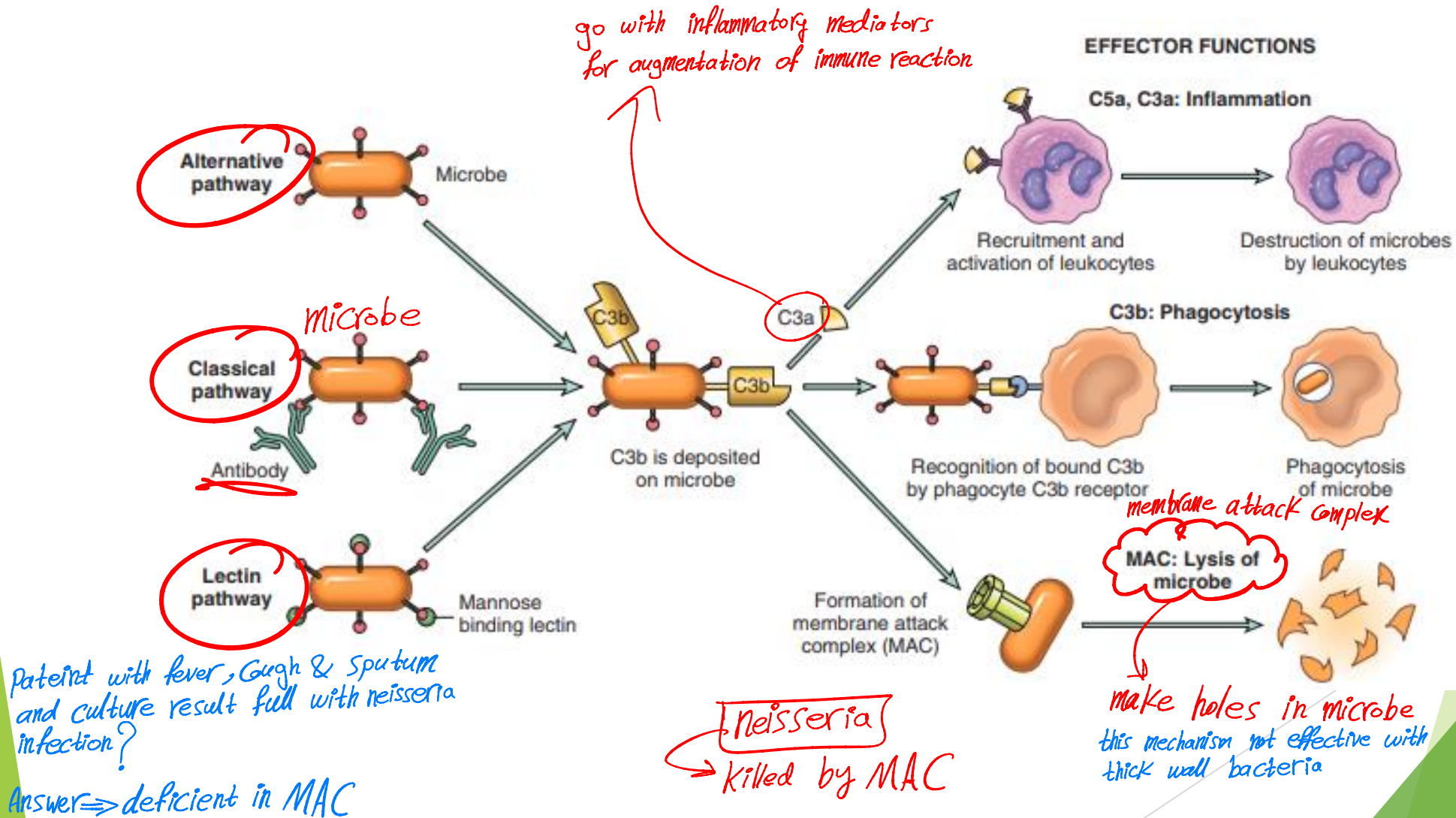
- ▶ which can be triggered by microbial surface molecules (e.g., endotoxin), complex polysaccharides, and other substances, in the absence of antibody.

- ▶ 3. The lectin pathway:

- ▶ in which plasma mannose-binding lectin binds to carbohydrates on microbes and directly activates C1. *sugar on the surface of microbe*

- ▶ All three pathways of complement activation lead to the formation of an enzyme called the C3 convertase, which splits C3 into two functionally distinct fragments, C3a and C3b.
- ▶ C3a is released, and C3b becomes covalently attached to the cell or molecule where the complement is being activated.
- ▶ More C3b then binds to the previously generated fragments to form C5 convertase, which cleaves C5 to release C5a and leave C5b attached to the cell surface.
- ▶ C5b binds the late components (C6-C9), culminating in the formation of the membrane attack complex (MAC), composed of multiple C9 molecules).

❖ Complement proteins are present in inactive forms in the plasma, and many of them are activated to become proteolytic enzymes that degrade other complement proteins.



# The complement system has three main functions:

- ▶ 1. Inflammation.:
- ▶ C5a, C4a and C3a are called anaphylatoxins.
- ▶ They stimulate histamine release from mast cells and thereby increase vascular permeability and cause vasodilation.
- ▶ C5a also is:
  - a chemotactic agent for neutrophils, monocytes, eosinophils, and basophils.
  - activates the lipoxygenase pathway of arachidonic acid metabolism in neutrophils and monocytes, causing release of more inflammatory mediators.

▶ 2. Opsonization and phagocytosis:

- ▶ C3b and its cleavage product iC3b (inactive C3bact as opsonins.
- ▶ promote phagocytosis by neutrophils and macrophages, which bear cell surface receptors for these complement fragments.

▶ 3. Cell lysis:

- ▶ The deposition of the MAC on cells drills holes in the cell membrane, making the cells permeable to water and ions and resulting in their osmotic death (lysis).
- ▶ This function of complement is important mainly for the killing of microbes with thin cell walls, such as Neisseria bacteria.

# Regulatory proteins for complement system

- ▶ 1. C1 inhibitor :
- ▶ blocks the activation of C1.
- ▶ Inherited deficiency of this inhibitor is the cause of hereditary angioedema.
  
- ▶ 2. Decay accelerating factor (DAF) and CD59 :
- ▶ DAF prevents formation of C3 convertases.
- ▶ CD59 inhibits formation of the MAC. → over expression of CD59  
no MAC → Grit killed neisseria
- ▶ An acquired deficiency of these regulators and excessive complement activation and lysis of red cells This gives rise to a disease called paroxysmal nocturnal hemoglobinuria (PNH)

▶ 3. Factor H:

- ❑ is a plasma protein that serves as a cofactor for the proteolysis of the C3 convertase.
- ❑ its deficiency results in excessive complement activation. *why???*
- ❑ Mutations in Factor H are associated with hemolytic uremic syndrome, as well as in wet macular degeneration of the eye *HUS*

▶ The complement system contributes to disease in several ways.:

- ❑ The activation of complement by antibodies or antigen-antibody complexes deposited on host cells and tissues.
- ❑ Inherited deficiencies of complement proteins cause increased susceptibility to infections.
- ❑ deficiencies of regulatory proteins cause a variety of disorders.

