PATHOLOGY

بسم الله الرحمن الرحيم

MID – Lecture #9

Inflammation 4

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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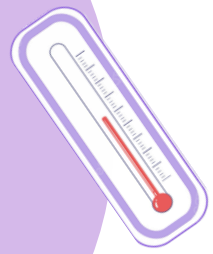

Assess your understanding of the previous lecture!

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TERMINATION OF ACUTE IR

- ✓ In the previous lecture, we have discussed how our vascularised tissues respond to injury (inflammatory response), by recognising the offending agent then recruitment of WBCs and other circulating molecules, then removing the enemy by phagocytosis.
- ✓ In this lecture we are going to talk about the **4th R in the inflammation response.**

Recognising –> Recruitment –> Remove –> Regulate –> Repair

- ✓ All of the previous steps of inflammation were in need for active mediators and stimulators, but we don't want these mediators to continue their processes; because they lead to side effects such as pain, general tiredness, fever and tissue damage.

➢ Let's discuss these mechanisms in detail

MECHANISMS OF TERMINATION

- Our body has seven major mechanisms to terminate (control/decrease/regulate) the inflammatory response after the three Rs (recognition, recruitment and removal of the enemy).
- All 7 mechanisms work together to achieve the desired effect.

Mediators are produced in rapid bursts
Release is stimulus dependent
Short half-lives
Degradation after release
PMNs short life (apoptosis)
Stop signals production (TGF-ß, IL-10)
Neural inhibitors (cholinergic): inhibits TNF

MECHANISMS OF TERMINATION

- **Mediators are produced in rapid bursts:**

Mediators are released quickly and not continuously, mainly by the inflammatory cells. Our body does not have a sustained release of these mediators.

- **Release is stimulus-dependent:**

The release of these mediators is stimulus-dependent, meaning that they are not released if it is not present. Hence, no stimulus means no release.

- **Short half-lives:**

Most of these mediators, which include proteins and gases, have a short half-life ranging from seconds to days (maximum of 1-2 days), similar to neutrophils. Then they are degraded or neutralized by enzymes and other factors.

- **Degradation after release:**

The tissue at the site of injury contains digestive enzymes that promptly degrade these mediators.

- **PMNs short life (apoptosis):** (Not all inflammatory cells have short life.)

Mediators are produced and released primarily by inflammatory cells, such as neutrophils and monocytes (inactive macrophages). During the acute phase, neutrophils are a key source of these mediators. Most neutrophils have a short half-life, with their DNA programmed to undergo apoptosis, or programmed cell death, within 1-2 days, and a maximum of 3 days.

- **Stop signals production (TGF-ß, IL-10):** Very important

Certain mediators can inhibit and degrade those released in earlier phases. For example, transforming growth factor-beta **(TGF-ß)** is one of the strongest repair factors along with interleukin-10 **(IL-10)**. These mediators are released during the last phase of inflammation **(R5)** and work to supress earlier mediators while promoting their degradation.

- **Neural inhibitors (cholinergic) inhibit TNF:**

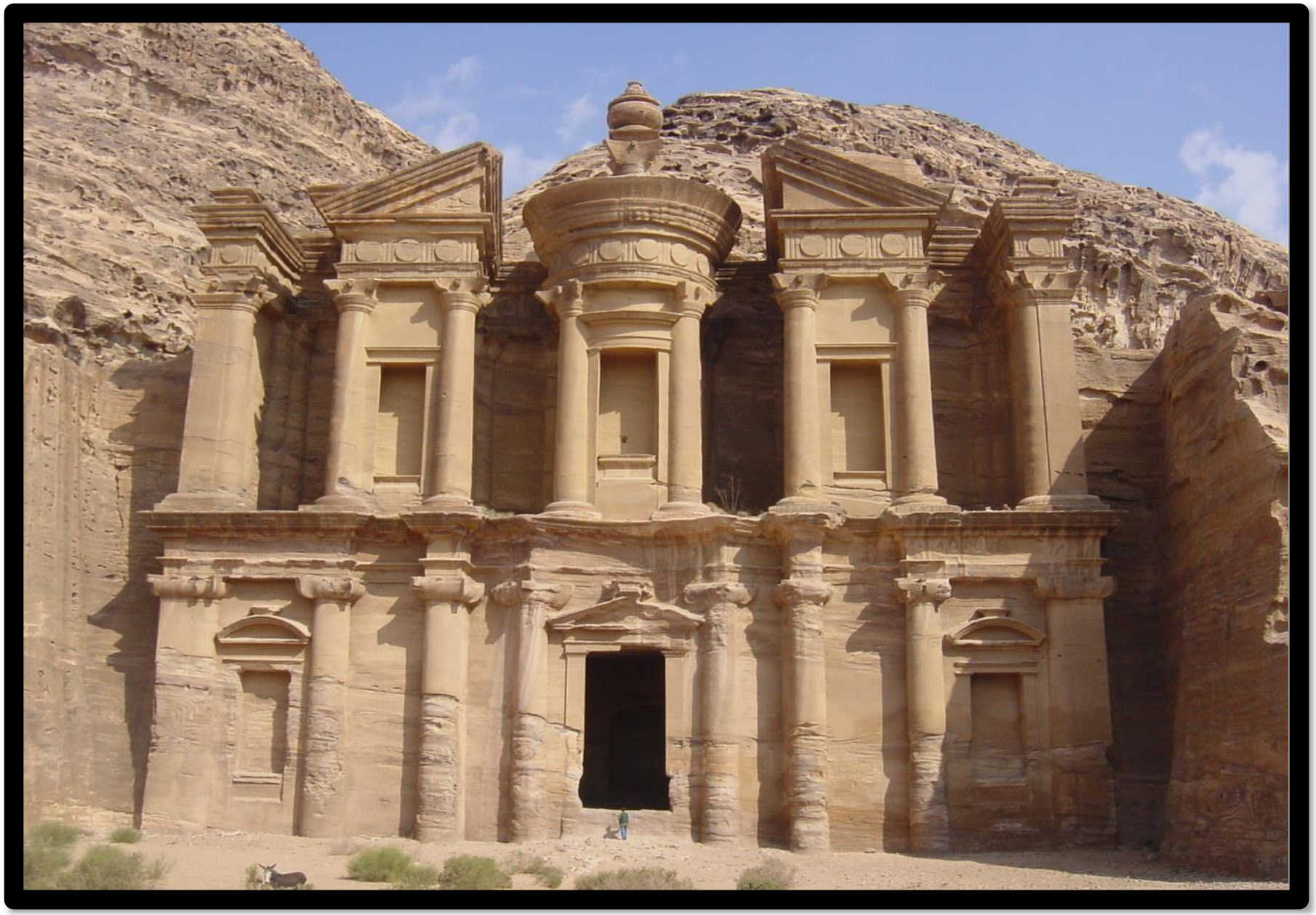
Certain neural inhibitors, which are called cholinergic inhibitors, can inhibit the release of certain mediators such as tumour necrosis factor **(TNF).**

Summary

Leukocyte Activation and Removal of Offending Agents

- Leukocytes can eliminate microbes and dead cells by phagocytosis, followed by their destruction in phagolysosomes.
- Destruction is caused by free radicals (ROS, NO) generated in activated leukocytes and by granule enzymes.
- Neutrophils can extrude their nuclear contents to form extracellular nets that trap and destroy microbes.
- Granule enzymes may be released into the extracellular environment.
- The mechanisms that function to eliminate microbes and dead cells (the physiologic role of inflammation) also are capable of damaging normal tissues (the pathologic consequences of inflammation).
- Anti-inflammatory mediators terminate the acute inflammatory reaction when it is no longer needed.

فاصل حضاري :)



MEDIATORS OF ACUTE INFLAMMATION

Tissue macrophages, dendritic cells & mast cells

✓ Those are the 4 major groups of inflammatory mediators (they are not the only ones).

Vasoactive amines	**Histamine, serotonin**
Lipid products	**PGs and LTs**
Cytokines	**IL, TNF and Chemokines**
Complement activation	**C1-9**

➢ TO BE CONTINUED

GENERAL FEATURES OF MEDIATORS

- **Cell derived at the site: from granule release or synthesized upon stimulation**

  - Mediators are rapidly released from intracellular granules or synthesized in response to a stimulus. The cell machinery is prepared to synthesize mediators immediately upon stimulation. Therefore, if there is no injury, there will be no release of these mediators. Excluding auto-immune diseases.

- **Plasma proteins needs activation**

  - The complement proteins which are present in small amounts in the plasma (in the circulation), do not exert any function unless they are activated, so they need stimulus (mediators) to be active.

- **Active mediators need stimulation**

- **Most mediators have a short life span**

  - We don't want the inflammatory process to be prolonged in order to not cause tissue injury. Most of them are produced by cells that have a short life span.

- **One can activate the other (and one can inhibit the other)**

  - Some mediators can activate or inhibit the release of other mediators depending on the timing and the location.

➢ In the table in the next slide, there are important mediators that we **should know and MEMORIZE their source and action**

TABLE 3.5 Principal Mediators of Inflammation

Mediator	Source	Action
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion, and activation
Cytokines (TNF, IL-1, IL-6)	Macrophages, endothelial cells, mast cells	Local: endothelial activation (expression of adhesion molecules). Systemic: fever, metabolic abnormalities, hypotension (shock)
Chemokines	Leukocytes, activated macrophages	Chemotaxis, leukocyte activation
Platelet-activating factor	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Complement	Plasma (produced in liver)	Leukocyte chemotaxis and activation, direct target killing (membrane attack complex), vasodilation (mast cell stimulation)
Kinins	Plasma (produced in liver)	Increased vascular permeability, smooth muscle contraction, vasodilation, pain

- **NOTICE** that there is overlapping between these mediator in terms of action. So, you have to remember the mediator, its source and its actions

- **At least two or three questions on the exam will be about this table**

MEDIATORS OF INFLAMMATION

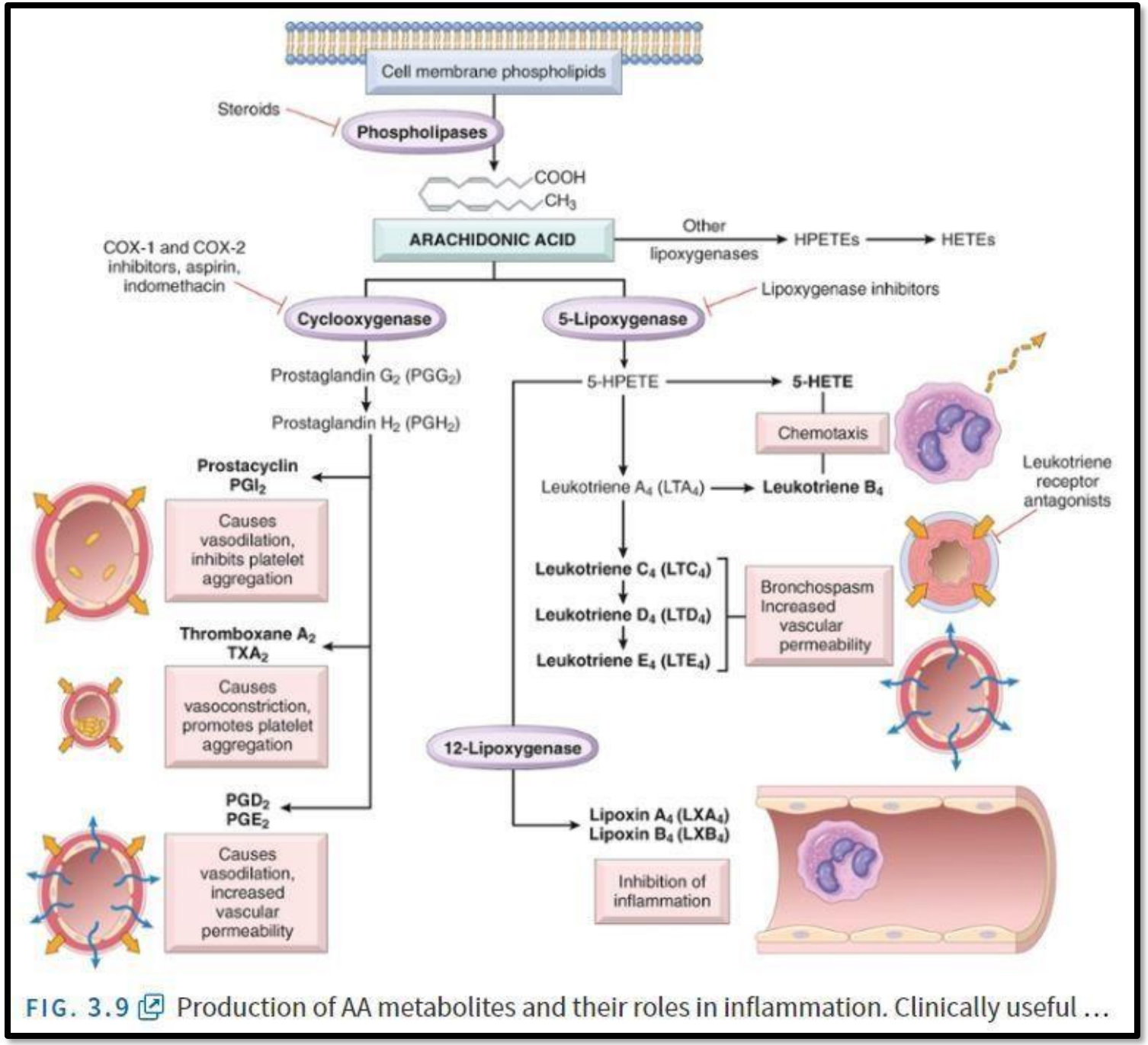


FIG. 3.9 Production of AA metabolites and their roles in inflammation. Clinically useful …

ARACHIDONIC ACID METABOLITES (EICOSANOIDS)

- Arachidonic Acid (AA) is a 20-carbon polyunsaturated fatty acid that is derived from dietary sources. It is present in membrane phospholipids. When the cell membrane phospholipids (phospholipid bilayer) are degraded by phospholipases, they produce multiple products that have important and critical chemical inflammation functions such as mediators.

- So, firstly an enzyme called **phospholipase** (The father enzyme) destroys phospholipids producing Arachidonic acid, then the arachidonic acid goes into 2 different pathways (depending on the situation, the intensity and the type of inflammation or the environment):

✓ **Cyclooxygenase pathway:** the two cyclooxygenase enzymes, COX-1 and COX-2 will destroy arachidonic acid, producing a big group of mediators called prostaglandins.

✓ **5-lipoxygenase pathway:** lipoxygenase enzyme will destroy arachidonic acid, producing another big group of mediators called leukotrienes (Leuko means white (WBCs) and trienes means movers or stimulators)

INHIBITORS INVOLVED IN AA METABOLISM

- ✓ **Steroidal inhibitors:** Phospholipases, which degrade phospholipids to produce AA, are inhibited by drugs called steroids (cortisone). Cortisone is very critical, strong and commonly used as anti-inflammatory drug. If a patient is given steroids, they will inhibit phospholipase which will consequently inhibit the production of all the leukotrienes and the prostaglandins.

- ✓ **Lipoxygenase inhibitors:** Inhibit the production of all leukotrienes. These inhibitors are commonly used in acute asthmatic conditions. Remember that leukotriene **(B4)** is a strong chemotactic agents. All of the other leukotrienes (like C4, D4, E4) are involved in the production of the signs and symptoms of acute asthmatic attack (cause bronchospasms and edema (increase vascular permeability) in the bronchus).

- ✓ **COX-1 and COX-2 inhibitors (anti-prostaglandin):** non-steroidal anti-inflammatory drugs inhibit the cyclooxygenase pathway, which will only inhibit the production of prostaglandins. Consequently, they are called anti-prostaglandins.

PROSTAGLANDINS (PGS)

Produced by mast cells, macrophages, or endothelial cells by the actions of two cyclooxygenase enzymes in response to an inflammatory stimulus. They are produced in order:

- ✓ **Prostaglandin G2 (PGG2)**
- ✓ **Prostaglandin H2 (PGH2)**

- ✓ **Prostacyclin (PGI2)**: It's an important chemical mediator of inflammation because of its functions as a vasodilator –like histamine– and it inhibits platelet aggregation.

- ✓ **Thromboxane A2 (TXA2)**: It has the opposite function of prostacyclin. It causes vasoconstriction and stimulates platelet aggregation, causing blockage of the coronary arteries (Myocardial Infarction) or brain arteries (strokes or cerebrovascular accidents).

- ✓ **PGD2/PGE2**: They have a less critical function, but they can cause vasodilation which leads to increased vascular permeability (similar to PGI2).

TABLE 3.6 Principal Actions of Arachidonic Acid Metabolites in Inflammation

Summarize them

Action	Eicosanoid **AA metabolites**	
Vasodilation	Prostaglandins PGI_2 (prostacyclin), PGE_1, PGE_2, PGD_2	important
Vasoconstriction	Thromboxane A_2, leukotrienes C_4, D_4, E_4	important
Increased vascular permeability	Leukotrienes C_4, D_4, E_4	
Chemotaxis, leukocyte adhesion	Leukotriene B_4	
Smooth muscle contraction	Prostaglandins PGC4, PGD4, PGE4	

- As an example, (physiological process) on smooth muscles contraction: the uterus requires prostaglandins to contract. If contractions start when it's not time for birth, some medications can be given to the mother either to delay or stimulate smooth muscle contractions.

POINTS TO REMEMBER ABOUT AA METABOLISM

They are important

- Aspirin – cycloxygenase

- **Aspirin, indomethacin, ibuprofen.** All are non-steroidal anti-inflammatory drugs (NSAIDs) . More common and less side effects - for arthritis, pain, muscle pain.
- Aspirin can inhibit cyclooxygenase, thus decreasing the production of prostaglandins (in the cyclooxygenase pathway).

- Steroids – phospholipase and anti inflamm

- Steroids are a major inhibitor to the major enzyme which is phospholipase, and it will inhibit the production of all prostaglandins and all leukotrienes, this is why steroids are a very potent, strong, and sometimes dangerous anti-inflammatory drug (you should not give it to the patient unless you have to).

- Prostacyclin (PGI2): vasodilator and –Pl aggreg

(-) Means inhibit

- PGI2 is a potent vasodilator and inhibits platelet aggregation.

- Thrombaxane A2: vasoconstrictor and +pl aggreg (+) Means stimulate

- **TXA2** is a significant vasoconstrictor and promotes platelet aggregation.

- TXA2-PGI2 imbalance: IHD & CVA

- **PGI2, TXA2** have opposite functions and the imbalance between those two prostaglandins is thought to play a major role in the pathogenesis of ischemic heart disease and cerebrovascular accident (strokes) in the brain. The reason why TXA2 is stimulated more than PGI2 during ischemic heart disease or stroke remains unknown.

- PG (PGE2): pain & fever

كلام الدكتور هون كان عام ، يعني ما خصصه بالـ E2

- **PG** is a major mediator of pain and fever
- **PGs** also mediate smooth muscle contraction during delivery .

➢ At least one or two questions on the exam will be about these six points

CYTOKINES

- Cyto: produced by cells
- Kines: kinetic function

- **Proteins secreted by many cells (activated lymphocytes, activated macrophages, dendritic cells and endothelial cells or platelets sometimes).**

- **They are a big group of chemicals that mediate and regulate (stimulate or inhibit) immune and inflammatory response.**

TABLE 3.7 Cytokines in Inflammation

Cytokine	Principal Sources	Principal Actions in Inflammation
In Acute Inflammation		
TNF	Macrophages, mast cells, T lymphocytes	Stimulates expression of endothelial adhesion molecules and secretion of other cytokines; systemic effects
IL-1	Macrophages, endothelial cells, some epithelial cells	Similar to TNF; greater role in fever
IL-6	Macrophages, other cells	Systemic effects (acute phase response)
Chemokines	Macrophages, endothelial cells, T lymphocytes, mast cells, other cell types	Recruitment of leukocytes to sites of inflammation; migration of cells in normal tissues
IL-17	T lymphocytes	Recruitment of neutrophils and monocytes
In Chronic Inflammation		
IL-12	Dendritic cells, macrophages	Increased production of IFN-γ
IFN-γ	T lymphocytes, NK cells	Activation of macrophages (increased ability to kill microbes and tumor cells)
IL-17	T lymphocytes	Recruitment of neutrophils and monocytes

The most important cytokines involved in inflammatory reactions are listed. Many other cytokines may play lesser roles in inflammation. There is also considerable overlap between the cytokines involved in acute and chronic inflammation. Specifically, all the cytokines listed under acute inflammation may also contribute to chronic inflammatory reactions.

IFN-γ, Interferon-γ; *IL-1*, interleukin-1; *NK*, natural killer; *TNF*, tumor necrosis factor.

- ✓ **T lymphocytes and neutrophils can have functions in both chronic and acute inflammation**
- ✓ **However, neutrophils are the major driver of acute inflammation**
- ✓ **Macrophages, plasma cells, and T-lymphocytes are the major drivers of chronic inflammation**

- **NOTE regarding the previous table**

- During the COVID-19 pandemic, an anti-IL-6 drug was used to mitigate the effects of what is known as a cytokine storm and decrease inflammatory response. When severe acute inflammation occurs, such as in cases caused by the coronavirus, it can trigger the release of numerous cytokines that can potentially harm the body, including the lungs and heart (causing thrombosis, bleeding, cardiac output inhibition). The goal of this drug is to reduce the impact of the cytokine storm.

- When they studied the lungs of dead patients who died because of coronavirus, they found that 1/3 have diffuse alveolar damage, 1/3 bleeding and 1/3 have thrombosis. There are a lot of things that we don't know about coronavirus.

MAJOR ROLES OF CYTOKINES IN ACUTE INFLAMMATION

- Whenever we have an inflammatory response, there will be local signs, symptoms, or effects of inflammation (Local actions), as well as distant non-local effects (Systemic actions) which happen because of the release of many of these mediators into the bloodstream. Systemic actions of inflammation can be either protective or pathological.

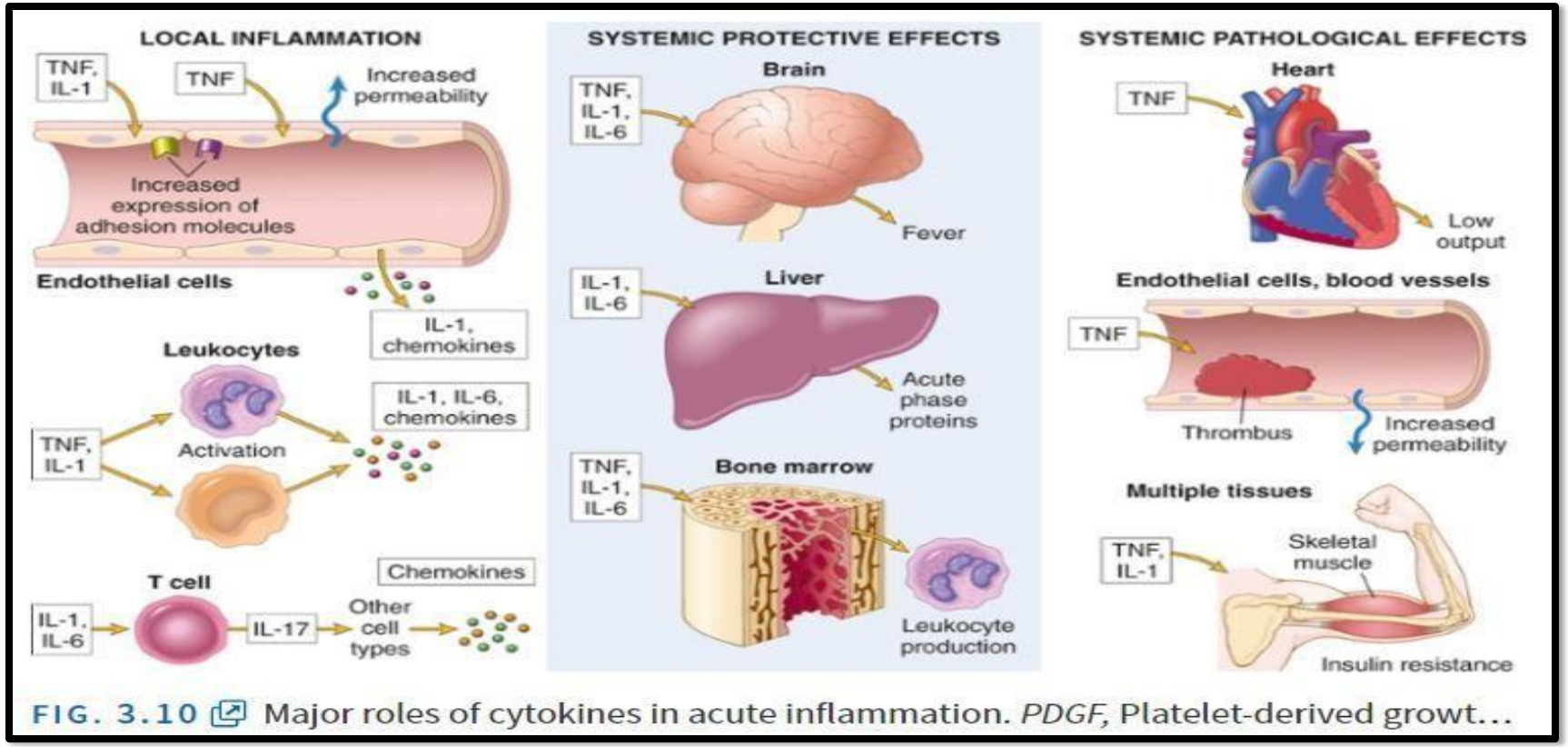


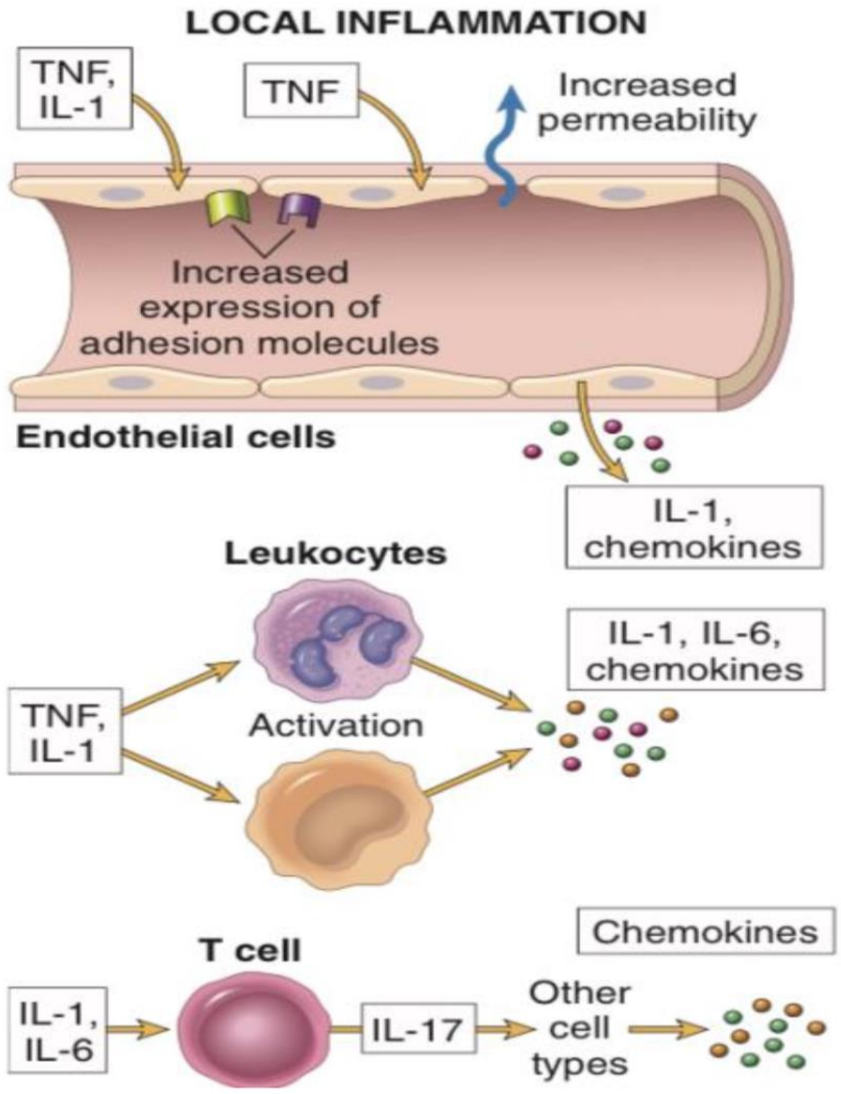
FIG. 3.10 Major roles of cytokines in acute inflammation. *PDGF,* Platelet-derived growt...

Local inflammation

- Cytokines can act locally (in tonsillitis you will have pain in the tonsils area).

- So locally, there will be swelling, pain, edema, and redness which are induced by chemical mediators at the local level.
- They help the clinician identify the source of inflammation, especially in children.

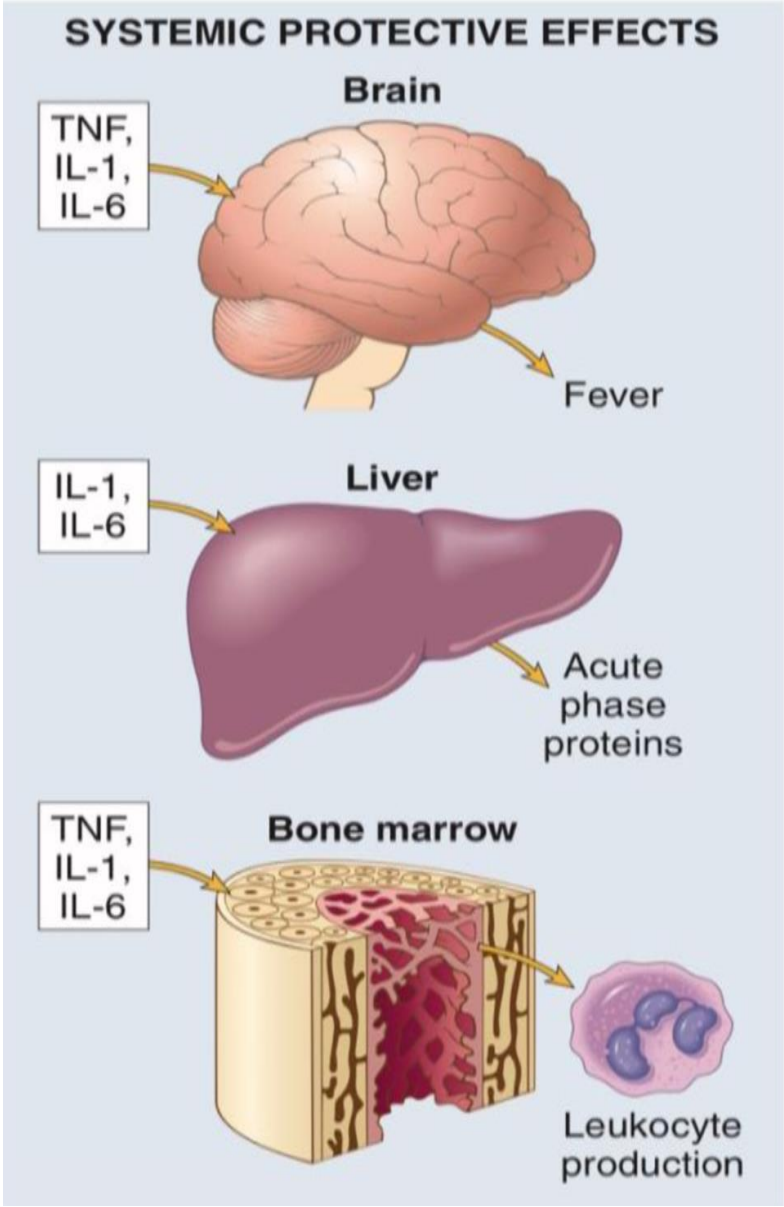
- Tumor necrosis factor (TNF) and interleukin-1 (IL-1) trigger the endothelial cells → increase the expression of adhesion molecules, then leukocytes adhere to the vessel wall. They also increase the permeability of blood vessels, so molecules move into the affected tissue.
- Leukocytes, activated by TNF and IL-1, begin releasing other cytokines and chemokines (e.g. IL-1, IL-6) to amplify the inflammatory response
- T-cells respond to IL-1 and IL-6 by producing IL-17, another cytokine to sustain inflammation and recruit additional cell types via chemokines, further enhancing the immune response.



Systemic protective effects

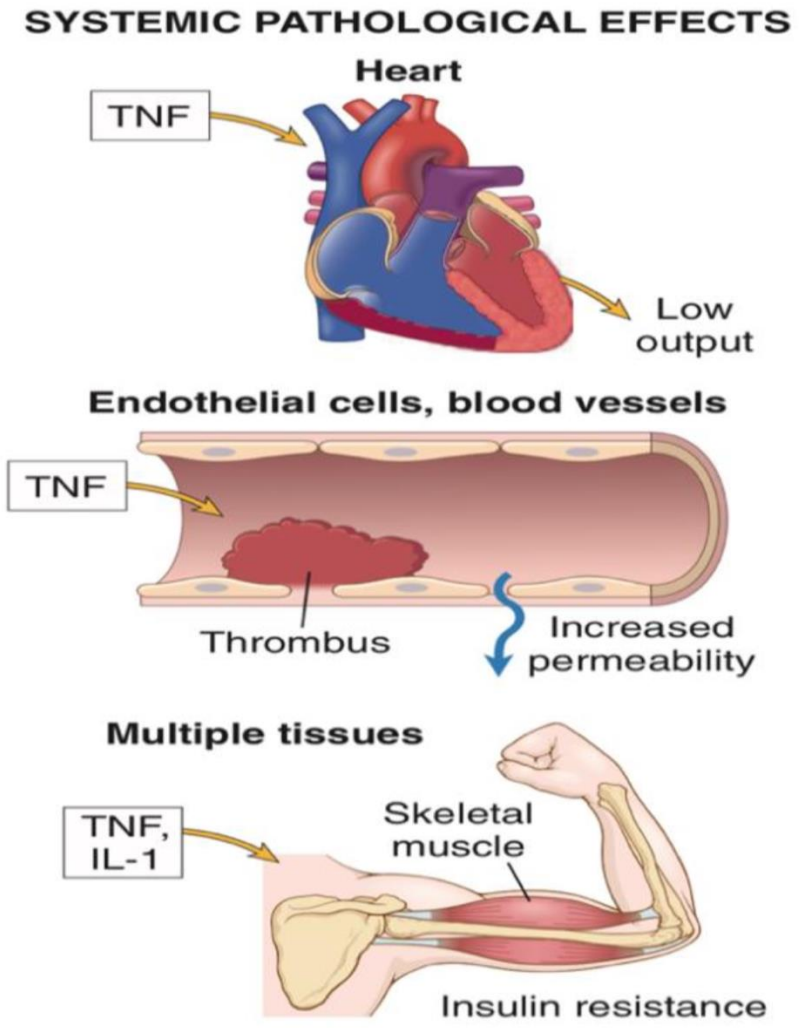
- Cytokines like **TNF, IL-1,** and **IL-6** have systemic protective effects. They travel through the bloodstream and reach various parts of the body, including the brain, toes, and everywhere else.

- In the brain, cytokines go to the thermal center and induce fever, creating a high temperature that is unfavorable for bacteria and viruses. However, it may also impact the body's enzymes.
- Phagocytes release cytokines **(IL-1 and IL-6)** which then signal the liver to produce acute phase proteins (non-specific parameters), such as **CRP** (C-reactive protein). Monitoring the concentration of **CRP** over a few days can indicate the intensity of inflammation. If the concentration increases, it means the inflammation is worsening. Conversely, a decrease in **CRP** levels over time indicates that the medication is effective, and the inflammation is improving.
- Cytokines **(such as TNF, IL-1, IL-6)** will go to the bone marrow and stimulate the production of more WBC (increase in the WBC counts –> more inflammation)



Systemic pathological effects

- Sometimes, an excessive response or high levels of mediators can have systemic pathological effects.
- Some cytokines, such as **TNF**, can travel to the heart and decrease cardiac output, potentially leading to heart failure due to severe acute inflammation.
- **TNF** can induce platelet aggregation and vasodilation in endothelial cells leading to thrombosis. So, we give the patient **anti-TNF.**



- In a stressed condition, many steroids, cytokines, and mediators can cause insulin resistance, even if you don't have diabetes. This can result in skeletal muscles not responding effectively to insulin.
- Diabetic patients with pneumonia, arthritis, or other acute inflammations are expected to have high blood sugar levels

CHEMOKINES

- **Small proteins (small family of cytokines) , mainly chemoattractants**
- **There are more than 40 different chemokines and 20 receptors**
- **They are grouped in 4 groups: C-X-C; C-C; C; CX3-C . You DON'T have to memorize them**
- **They have G-protein coupled receptors**

- One important aspect of chemokines is that they exert their effects through G-protein coupled receptors. These receptors play a crucial role in mediating the signaling pathways triggered by chemokines.

- **2 main functions: inflammation & maintain tissue architecture**

Chemokines have two primary functions: In acute inflammation, they recruit white blood cells to the site of injury. Additionally, they help maintain tissue architecture. For example, in conditions like pneumonia, chemokines prevent acute inflammation from damaging structures such as the alveoli.

COMPLEMENT SYSTEM

- **3rd big group of mediators of inflammation**

- **Soluble proteins (inactive), need activation**

- They are soluble proteins that are primarily produced by the liver, and they circulate in the bloodstream in small amounts. They play a crucial role in host defense against microbes and in pathological inflammatory reactions. They are an essential part of the immune system's response to infection and help regulate the inflammatory process.
- These proteins are present in the body in an inactive form, so they require stimulation or activation to carry out their functions. Once activated, they can initiate a cascade of reactions that lead to the elimination of pathogens and the modulation of the immune response.

- **There are more than 20 complements , the most important are C1-C9**
- **They are important in Innate & adaptive immunity (like vaccines)**
- **Functions: vascular permeability, chemotaxis & opsonization.**
- **C3 is most abundant; cleavage of which is the critical in all pathways .**

➢ TO BE CONTINUED

- **C3 is most abundant; cleavage of which is the critical in all pathways .**

- **C3** has practical clinical applications since it's the most abundant in the serum. It's important to know that **C3** cleavage is critical in all pathways, making it the main gatekeeper. What this means is that when **C3** is cleaved, all the pathways that activate the complement system will be activated.

- **Complement fixation = complement system activation.** So, when we say this drug fixes the complement, that means this drug stimulates the cascade of the complement system.

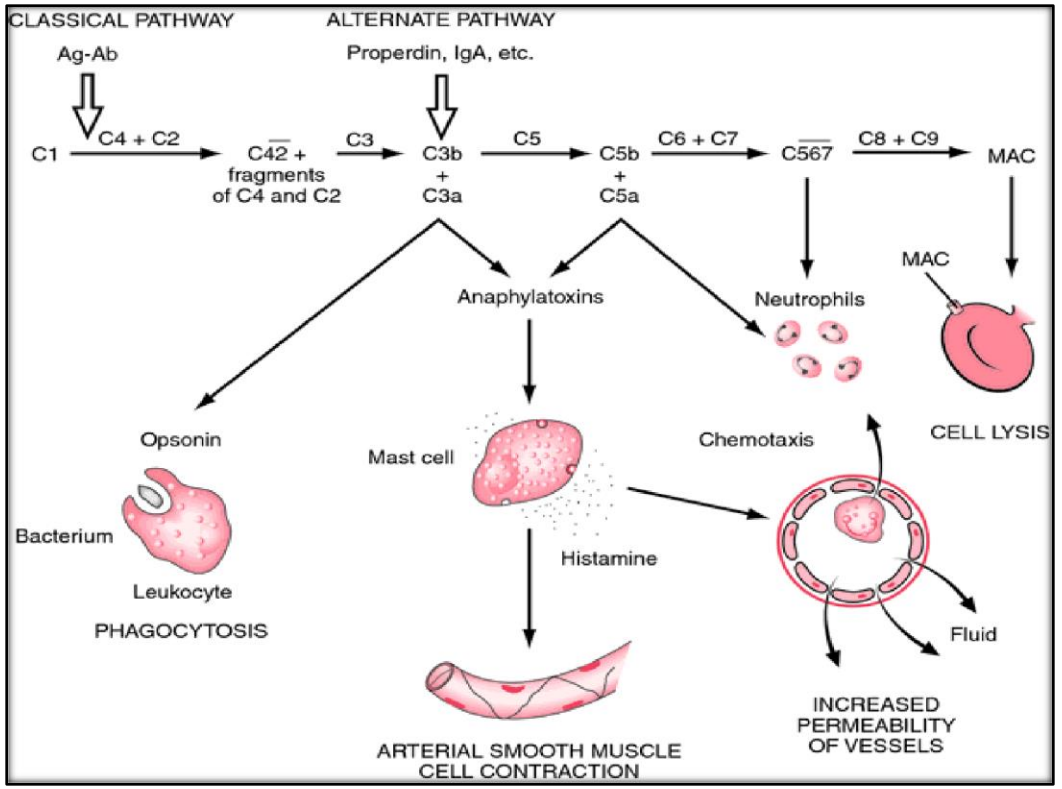
- Measuring the changes in C3 levels can provide valuable insights into immune function and the presence of inflammation or infection.
- **What is the main driver of fixing the complements?** C3

Explanation regarding this illustration

- Previously, there were only two ways to activate the complement system:

1. The classic pathway: By antigen-antibody complex, that stimulates the complement (fixing it) and activation of **C3** (the main driver).

2. The alternate pathway: Activated by various substances, such as IgA, secretions, properdin and some cell membrane components from bacteria and viruses.



There are three Major mechanisms to activate the complements via activation of the C3 (Alternative pathway, Classical pathway, Lectin pathway)

- Now we have three different stimulants for the activation of the complement system via activation C3
- ✓ When C3 is activated (when cleaved), we will have C3a and C3b
- ✓ C3a and C5a→ Strong inflammatory reactants (vascular permeability and dilatation) they are also the strongest chemotactic agents.
- ✓ C3b→ Major opsonizing agent; as it helps the macrophages and the neutrophils in the engulfment of infectious agents (phagocytes).
- ✓ Opsonization: "coating" pathogens, making them recognizable and easier to be eliminated by immune cells (to enhance phagocytosis).
- ✓ C3b is the strongest opsonizing agents in the complement system.
- ✓ MAC: is a **M**embrane **A**ttack **C**omplex composed of multiple fragments of c9 → makes holes in the bacterial wall to enhance its killing.

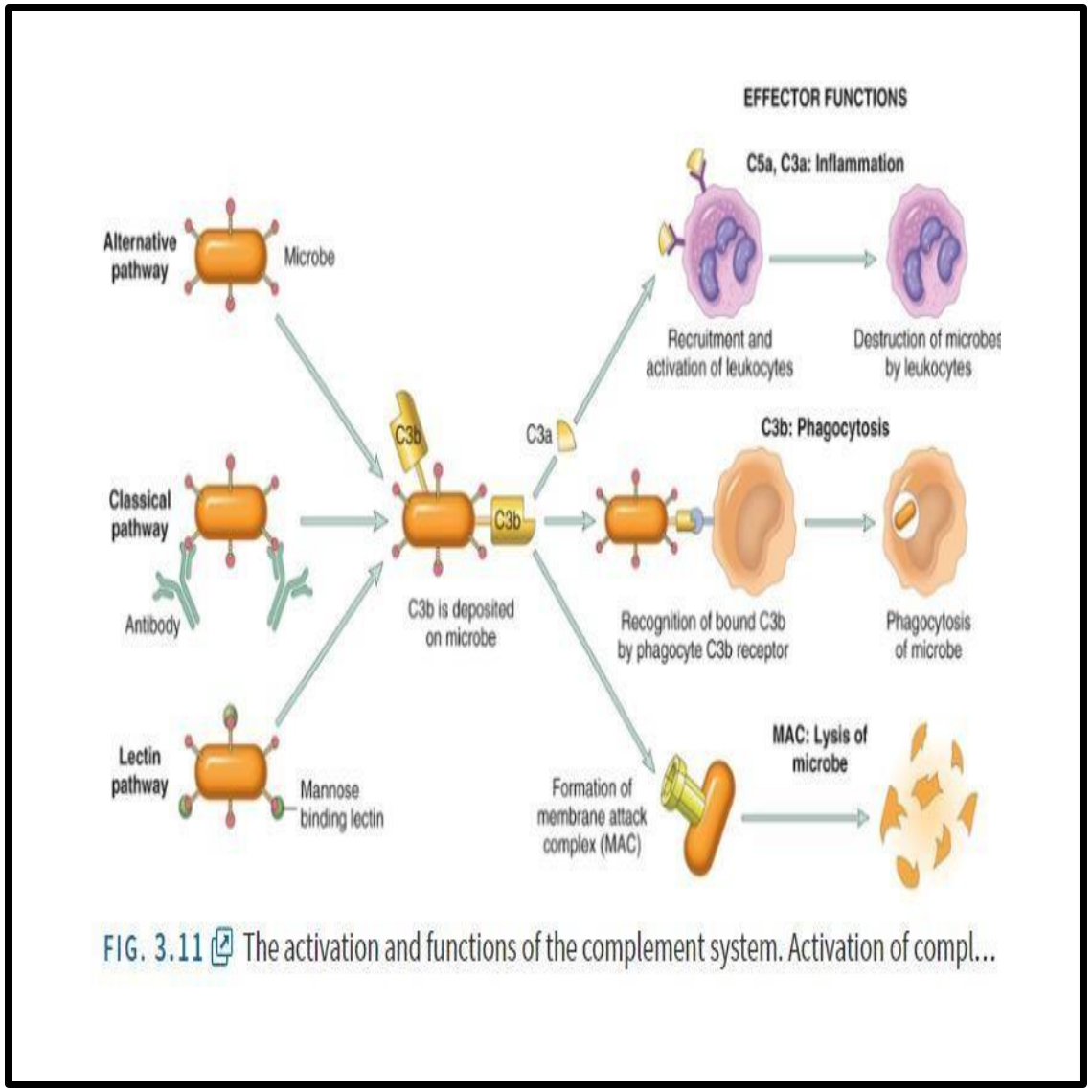


FIG. 3.11 The activation and functions of the complement system. Activation of compl...

Complement System FUNCTIONS

- **Inflammation: histamine like, anphylatoxins (initial anaphylaxis (extreme allergic reaction), initial allergic reaction, initial vascular phase) (C5a).**
- **Opsonization & phagocytosis: enhance phagocytosis (C3b)**
- **Cell lysis: Membrane Attack Complex (MAC) (Ending complement), C9 multiples. Makes small holes in thin membrane of microbial wall.**

REGULATORY PROTEINS FOR CS

- **C1 inhibitor: if deficient →hereditary angioedema**

A lack of C1 inhibitor leads to excessive activation of the complement system, resulting in a condition called hereditary angioedema. This causes episodes of severe swelling, especially in the skin and mucous membranes.

- **Decay accelerating factor (DAF), which inhibit C3 convertases and CD59 inhibits MAC. Abnormalities cause PNH (Paroxysmal Nocturnal Hemoglobinuria)**

DAF inhibits C3 convertases, CD59 inhibits membrane attack complex (MAC). A deficiency in DAF or CD59 can leads to Paroxysmal Nocturnal Hemoglobinuria (PNH). A rare disease characterized by the destruction of red blood cells (hemolysis), leading to symptoms like dark-colored urine, fatigue, and increased risk of blood clots.

- **Factor H: proteolysis of C3 convertase; mutations cause hemolytic uremic syndrome**

Factor H prevents the formation of C3 convertase on host cell surfaces, protecting cells from being targeted by the complement system. Mutations in Factor H can lead to improper regulation of the complement system, resulting in a disease called Hemolytic Uremic Syndrome (HUS). This syndrome causes the destruction of red blood cells, acute kidney failure, and low platelet counts.

- **CS protein deficiencies can occur leading to infection susceptibility**

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	16	COX-1 and COX-2 inhibitors are non steroidal **inflammatory** drugs	COX-1 and COX-2 inhibitors are non steroidal **anti- inflammatory** drugs
	26	Cytokines like IL-1 and IL-6 travel to the liver and stimulate phagocytes **to produce** acute phase proteins/reactants	Phagocytes releases cytokines (IL-1 and IL-6) which then **signal the liver to produce** acute phase proteins such as C-reactive protein (CRP)
	34	-	Extra inflammation added to enhance your understanding
V1 → V2	27	Vasoconstriction	Vasodilation

Additional Resources:

Reference Used:

- Robbin’s Basic Pathology 11th edition

رسالة من الفريق العلمي:

لن تُوَلّد الأُمّة العظماء دون ثَمَن.

ولن يُخَلّد التاريخ أسماء الفارغين، ولن يسير في درب الله أيّ خائفٍ مرتجف ! الأيّام القاسية تَصقل نُفوسًا صَلبة، وشدائد الامتحان تُثَبّت الإيمان ، مَن رامَ المَعالي يُدرِك شَوكَ الطّريق ، والذي يَلزَم ثَغره كأنّه الأمل الوحيد فيه ؛ لن يقف!
يا طول الطريق أعدَدنا لك نَفَسًا يَسبِقُك، ويَقينًا يَغلِبُك، نحن اليوم نُعَدّ، وغدًا نَشتَدّ، يقينًا بالله.

اللهم أنت ربي لا إله إلا أنت خلقتني وأنا عبدك وأنا على عهدك ووعدك ما استطعت، أعوذ بك من شر ما صنعت، أبوء لك بنعمتك علي وأبوء بذنبي فاغفر لي فإنه لا يغفر الذنوب إلا أنت.