PATHOLOGY

Sheet no.

Writers : الفريق العلمي-طوفان الأقصى

Corrector: Sra’a Ali

Doctor : Mousa Al-Abbadi

Inflammation and Repair

Lecture 4

In previous lectures , we have talked about how our vascularized tissues response to injury (inflammation response), by recognizing 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

recognize—> Recruitment—> Remove —> Regulate—> Repair.

TERMINATION OF THE ACUTE INFLAMMATORY RESPONSE

All previous steps of inflammation were in need for active mediators and stimulators, but we don't want these mediators to continue their process; because that leads to side effects and tissue damage.

****Our body has 7 major mechanisms to Terminate (control/ decrease/ regulate) the inflammatory response after the three Rs (recognition, recruitment, removal of the enemy):**

1.Mediators are produced in rapid bursts:

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

2.Release is stimulus-dependent

The release of these mediators is stimulus-dependent, they're released if the stimulus is there. So, (no stimulus – no release).

3.Short half-lives:

These mediators have a short half-life (seconds, minutes, and maybe days), then they get degraded, neutralized by enzymes and other factors.

4.Degradation after release:

The tissue at the site of injury is equipped with certain enzymes that are ready and capable of destroying these mediators. For example, histamine is degraded by histaminase.

5.PMNs short life (apoptosis):

Mediators are released and produced by cells, mainly inflammatory cells. For example, during the acute phase, neutrophils produce these mediators. Neutrophils have a short half-life, and their DNA is programmed to undergo programmed cell death, also known as apoptosis, after 1-2 days.

6.Stop signals production (TGF-ß, IL-10):

Certain mediators can inhibit other mediators like transforming growth factor-beta (TGF-ß), it's one of the strongest fibrogenic factors that make repair, and also we have interleukin-10 (IL-10). Those mediators are released in the last phase of inflammation (R4 and R5) and they are capable of stopping the signals that are responsible for releasing the initial mediators.

7.Neural inhibitors (cholinergic): inhibits TNF:

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

***Once again, these 7 mechanisms help your body control and gradually reduce the inflammatory response after R3 (after phagocytosis).**

وَأَنْ لَيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَى وَأَنَّ سَعْيَهُ سَوْفَ يُرَى ثُمَّ يُجْزَاهُ الْجَزَاءَ الْأَوْفَى

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.

MEDIATORS OF INFLAMMATION

There are hundreds and thousands of mediators that are produced by inflammatory cells, tissue macrophages, dendritic cells, and mast cells which initiate and regulate inflammatory reactions. These mediators are divided into four major groups:

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

GEGENERAL FEATURES OF MEDIATORS

1.Cell-derived at the site of injury

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.

2.Plasma proteins needs activation

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

3.Active mediators need stimulation

4.Most mediators have short life span

We don't want the inflammatory process to be prolonged in order not to cause tissue injury.

5.One can activate the other (and one can inhibit the other)

One can activate the other, and one can inhibit the other. Each mediator has the ability to activate or inhibit the release or stimulation of other mediators.

****In the table below 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 In the vascular phase	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

ARACHIDONIC ACID METABOLITES (EICOSANOID)

Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid that is derived from dietary sources. It presents in membrane phospholipids. When the cell membrane phospholipids are degraded by phospholipases, they will produce multiple products that have important and critical chemical inflammation function. (Like mediators)

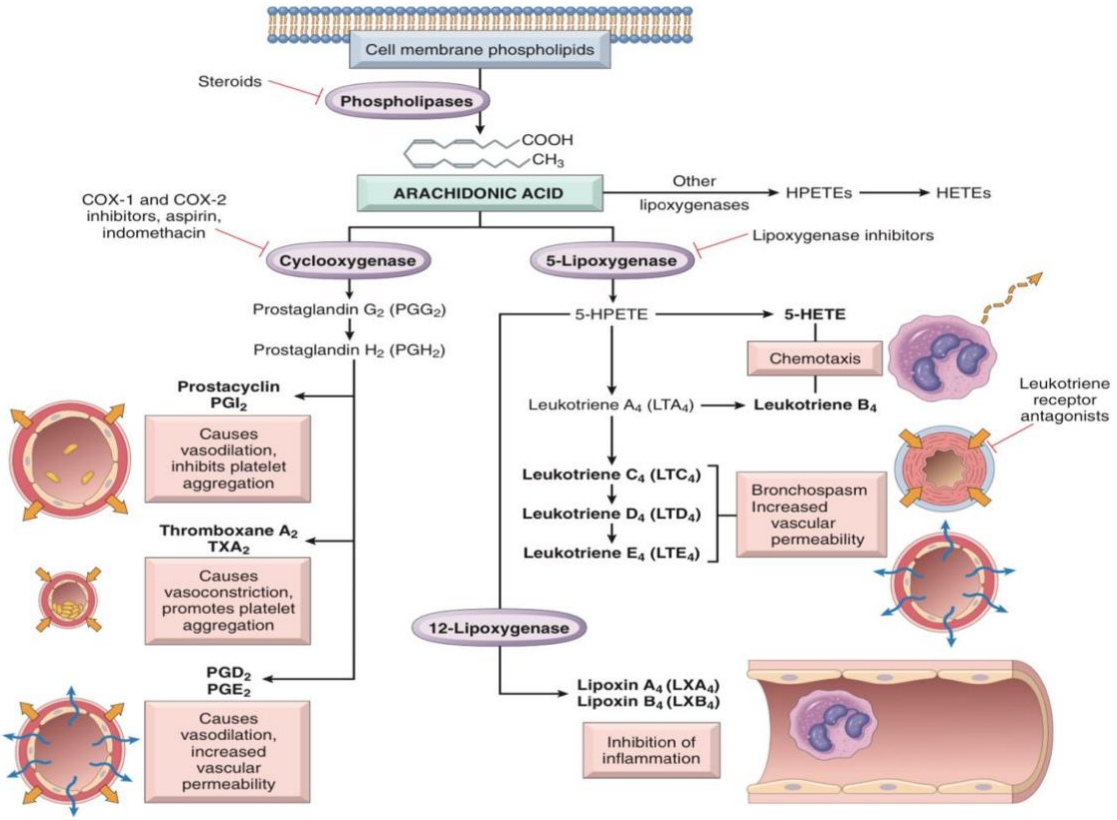


Fig. 3.9 Production of AA metabolites and their roles in inflammation. Clinically useful antagonists of different enzymes and receptors are indicated in *red*. While leukotriene receptor antagonists inhibit all actions of leukotrienes, they are used in the clinic to treat asthma, as shown. *COX-1*, *COX-2*, Cyclooxygenase 1 and 2; *HETE*, hydroxyeicosatetraenoic acid; *HPETE*, hydroperoxyeicosatetraenoic acid.

So, first an enzyme called phospholipase destroys phospholipids producing Arachidonic acid, then the arachidonic acid goes into

2 different pathways:

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

2.5-lipoxygenase pathway: lipoxygenase enzyme will destroy arachidonic acid, producing another big group of mediators called leukotrienes.

Leukotrienes and prostaglandins are important because they have certain clinical implications:

- **Some of leukotrienes (B4) are chemotactic agents, they have role in recruitment of cells.**
- **Other leukotrienes (C4,D4,E4) cause Bronchospasms and edema in the bronchus.**
- **Leukotrienes C4, D4 and E4 are involved in production of acute asthmatic attack..**
- **Lipoxin A4 and Lipoxin B4 make general inhibition of inflammation, particularly anti-chemotaxis.**

****We will talk about them in details and explain everything don't worry**

INHIBITORS INVOLVED IN AA METABOLISM:

All the inhibitors consider as an ANTI-INFLAMMATORY

1. Steroids (anti inflammatory) → Phospholipases "which degrade phospholipids to produce AA", are inhibited by drugs called steroids (cortisone). Cortisone is very critical, strong, important, and commonly used as anti-inflammatory drug. So, if you give a patient steroid, this drug will inhibit phospholipase which will inhibit the production of ALL the leukotrienes and the prostaglandins.

2) cox-1 and cox-2 inhibitors (anti-prostaglandin) → non-steroidal inflammatory drugs, inhibit the cyclooxygenase pathway which will inhibit only the production of all prostaglandins. So, they are called anti-prostaglandins. Ex. aspirin and indomethacin.

3) lipoxygenase inhibitors → Inhibit the production of all leukotrienes. These inhibitors are commonly used in acute asthmatic conditions.

Cox-1, Cox-2, and lipoxygenase inhibitors are less potent and critical compared to steroids.

***Let's go through leukotrienes and prostaglandins more specifically:**

1) PROSTAGLANDINS (PGS):

Produced by mast cells, macrophages, or endothelial cells by the actions of two cyclooxygenases enzyme in response to inflammatory stimulus. They are produced one by one:

1.Prostaglandin G2 (PGG2)

2.Prostaglandin H2 (PGH2)

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

4.Thromboxane A2 (TXA2): It has the opposite function of Prostacyclin.

It causes vasoconstriction and stimulates platelet aggregation.

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

2) LEUKOTRIENES:

Produced by leukocytes and mast cells through the action of lipoxygenase enzyme.

1. 5-HPETE → 5-HETE / Leukotriene A4 (LTA4) → Leukotriene B4 :

Both 5-HETE and Leukotriene B4 act as strong chemotactic agents, with the function of Leukotriene B4 being chemotaxis and recruitment of white blood cells to the site of injury.

2. Leukotriene C4 (LTC4), Leukotriene D4 (LTD4), and Leukotriene E4 (LTE4):

Chemical mediators of inflammation that are believed to play a significant role in bronchospasm, causing constriction of the bronchial diameter and leading to bronchial asthma. They also increase vascular permeability, resulting in more edema.

Antagonists targeting these products are used as a therapy to control acute attacks of bronchial asthma.

3.Lipoxin A4 (LXA4) / Lipoxin B4 (LXB4): They are major inhibitors of inflammation.

This table summarizes the major function produced by arachidonic acid metabolites in inflammation: (Eicosanoid is another name for AA metabolites)

As an example, smooth muscles, such as the uterus, require prostaglandins to contract. If contractions start when it's not time for birth, anti-prostaglandins can be given to the mother.

Table 3.6 Principal Actions of Arachidonic Acid Metabolites in Inflammation

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

POINTS TO REMEMBER ABOUT AA METABOLISM

➢ Aspirin and the non-steroidal anti-inflammatory drugs (NSAID)

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 leukotriene, this is why steroid is a very potent, strong, and sometimes it's a dangerous anti-inflammatory drug.

➢ Prostacyclin (PGI2): vasodilator and – Pl aggregate

PGI2 is a potent vasodilator and inhibits platelet aggregation, making it an effective anti-thrombotic agent.

➢ Thromboxane A2: vasoconstrictor and + Pl aggregate

TXA2 is a significant vasoconstrictor and promotes platelet aggregation, making it a potent pro-thrombotic agent.

➢ 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.

➢ PG (PGE2): pain & fever

PG is a major mediator of pain and fever, contributing to the sensation of pain and the development of fever.

❖ CYTOKINES:

2nd big group of mediators of inflammation

CYTOKINES are proteins secreted by many cells (activated lymphocytes, activated macrophages, and dendritic cells)

They are a big group of chemicals that mediate and regulate the immune and inflammatory response.

Cyto: produced by cells

Kines: kinetic function

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

Chemoattractants major role in chemotaxis

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.

وَأَنْ أَ

During the COVID-19 pandemic, an anti-IL-6 drug was used to mitigate the effects of what is known as a cytokine storm. 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. The goal is to reduce the impact of the cytokine storm.

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 happened because of the release of many of these mediators to the bloodstream. Systemic actions of inflammation can be either protective or pathological.

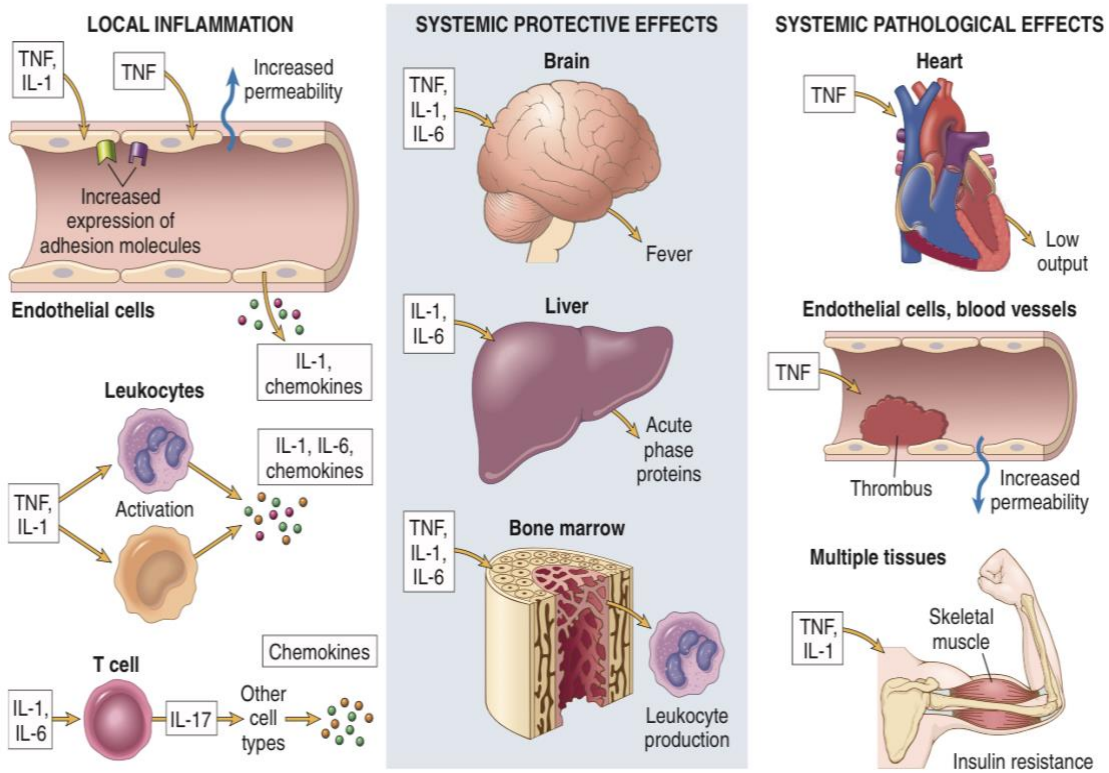


Fig. 3.10 Major roles of cytokines in acute inflammation. *PDGF*, Platelet-derived growth factor; *PGE*, prostaglandin E; *PGI*, prostaglandin I.

1. **Local inflammation**

Cytokines can act locally (in tonsillitis you will have pain in the tonsils area) we will have:

➢increased permeability

➢increased expression of endothelial cells

➢vascular dilatation

➢ erythema

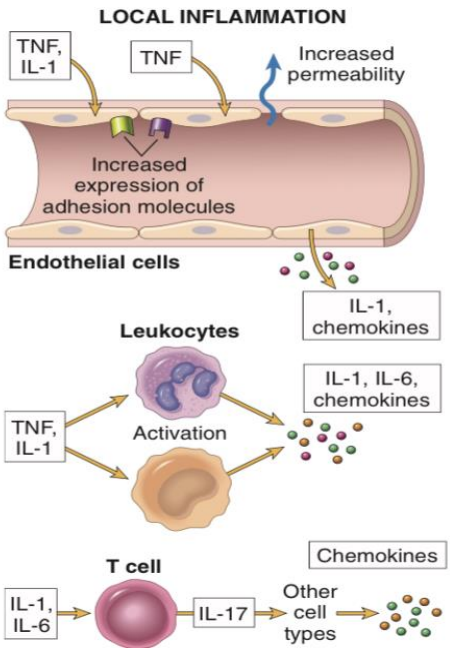
➢recruitment of inflammatory cells ➢activation of leukocytes

➢production of chemokines

➢production of other inflammatory cells

So locally, there will be swelling, edema, and redness which are induced by chemical mediators at the local level.

Part of the local impact of mediators come from cytokines mainly TNF , IL-1



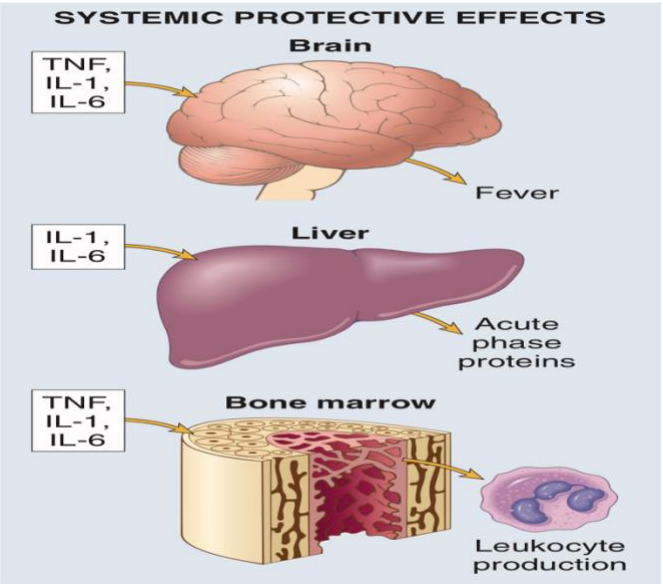
2.Systemic protective effects

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.

Cytokines like IL-1 and IL-6 travel to the liver and stimulate phagocytes to produce acute phase proteins/reactants (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.

Other 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 —> inflammation)



3.Systemic pathological effects

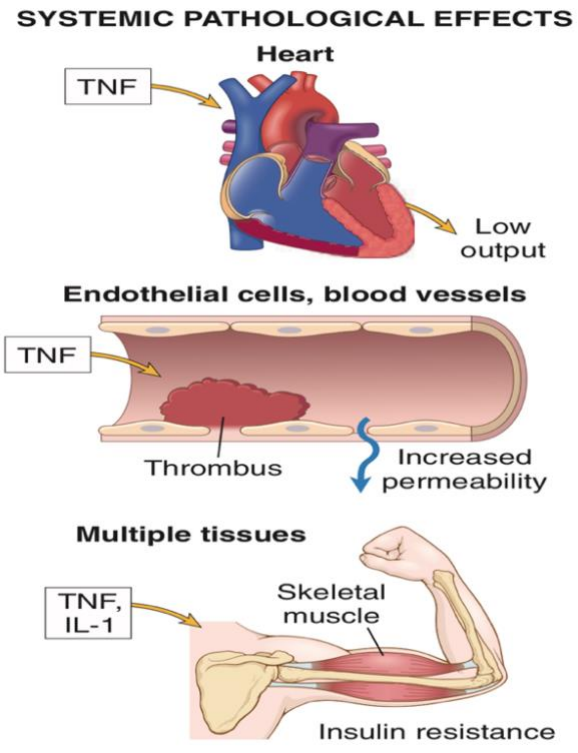
Sometimes, an excessive response or high levels of mediators can have pathological effects. These systemic impacts can indeed be pathological.

- 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 vasoconstriction in endothelial cells leading to thrombosis

- 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



وَأَنْ لَيْسَ لِلإِنسَانِ إِلَّا مَا سَعَى وَأَنَّ سَعْيَهُ سَوْفَ يُرَى ثُمَّ يُجْزَاهُ الْجَزَاءَ الأَوْفَى

CHEMOKINES

- Chemokines are a small family of proteins, known as chemoattractants.

-There are more than 40 chemokines,20 receptors.

-They are grouped in letters (C-X-C; C-C; C; CX3-C). you don't have to memorize them

- One important thing to remember about chemokines is that they exert their effects through a group of receptors known as G-protein coupled receptors. These receptors play a crucial role in mediating the signaling pathways triggered by chemokines.

-They have two major functions:

- In Acute inflammation (they recruit white blood cells to the side of injury).

- In protecting tissue architecture (when we have pneumonia we don't want the acute inflammation to damage the tissues so one of the chemokines functions is to protect the Architecture of the alveoli etc).

COMPLEMENT SYSTEM

3rd big group of mediators of inflammation

➢They are soluble proteins its primarily produced by the liver, 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 C1 - C9).

➢They are important in innate and adaptive immunity.

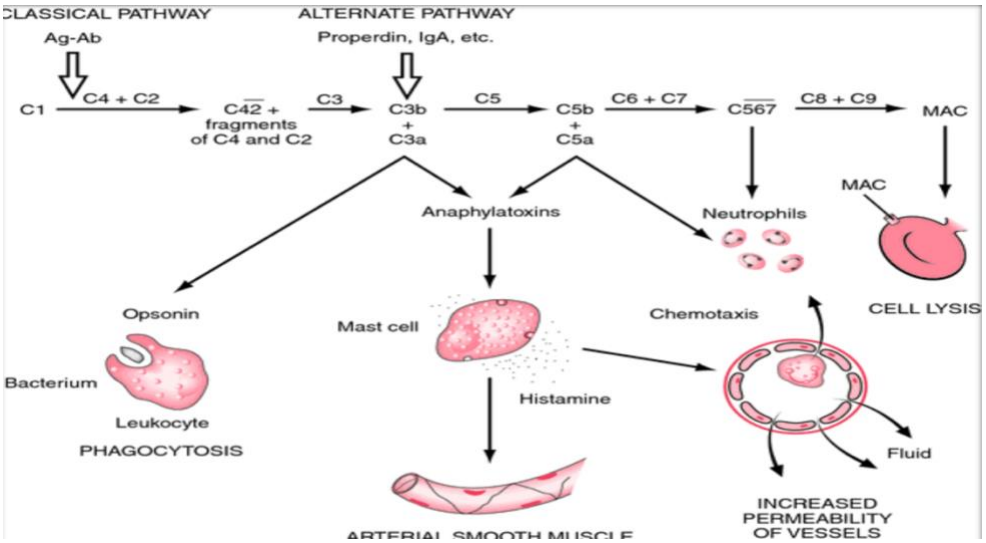
In the old days there were only two ways to activate the complement system

1. The classic pathway

By antigen-antibody complex.

2. The alternate pathway

Other materials from bacterial products



Now we have three different stimulants for the activation of the complement system.

The major functions of the CS are :

- increasing vascular permeability (initial phases of inflammation: edema, swelling, redness),

-chemotaxis

-they are important in a process called opsonization: process in which this factor or this mediator helps the macrophage and the neutrophil to act more actively in the phagocytosis process (without the mediators the process will be slow and ineffective)

➢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 "gatekeeper ". What this means is when C3 is cleaved, all the pathways that activate the complement system will be activated.

When cleaved we will have c3a and c3b

C3b —> -gives us 4 ,5 ,6 ,7 ,8 ,9 (like dominos)

-Major opsonizing agent it helps the macrophage and the neutrophil in engulfment of infectious agents

- C3b is one of the strongest opsonizing agents in the complement system

C3a C5a —> chemotaxis

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

MAC : composed of multiple fragments of c9 —> holes in bacterial wall to enhance its killing

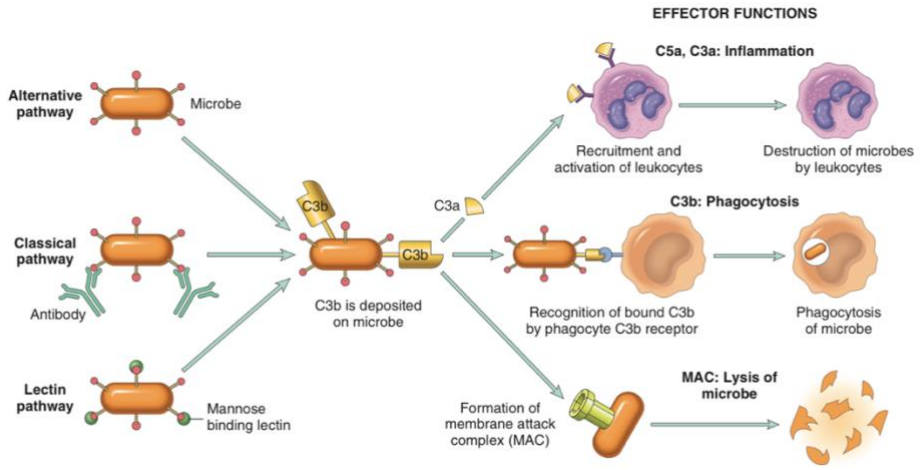


Fig. 3.11 The activation and functions of the complement system. Activation of complement by different pathways leads to cleavage of C3. The functions of the complement system are mediated by breakdown products of C3 and other complement proteins, and by the membrane attack complex (MAC).

C S FUNCTIONS:

• Inflammation: histamine like, anphylatoxins (strong agents in exaggerated immune response)(C5a).

• Opsonization & phagocytosis: enhance phagocytosis (C3b)

• Cell lysis: Membrane Attack Complex (MAC), C9 multiples. Makes small holes in thin membrane of microbial wall

REGULATORY PROTEINS FOR CS:

• C1 inhibitor: if deficient hereditary angioedema

• Decay accelerating factor (DAF), which inhibit C3 convertases and CD59 inhibits MAC Abnormalities cause PNH

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

• CS protein deficiencies can occur leading to infection susceptibility

V2

Page 5:GEGENERAL FEATURES OF MEDIATORS