Doctor 021

PATHOLOGY

Sheet no. 4

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In the last lecture, we have talked about phagocytosis, mechanisms of tissue injuries by our own WBCs, and we talked in detail about WBCs functions like **amplifying inflammatory soldiers**, **terminating the inflammatory reaction,** and **secreting growth factors**. And at the end the doctor talked about **T-Helper-17** which is involved in acute inflammation. In today's lecture, we will talk about the **fourth R**, which is **Termination of the acute inflammatory response**.

TERMINATION OF THE ACUTE INFLAMMATORY RESPONSE

All previous steps of inflammation were in need for active mediators and stimulators, but if these mediators stay at the site of inflammation, they will cause huge tissue damage. So, at a certain time the inflammatory response should be terminated and controlled by inhibition of mediators.

- Our body has **seven major mechanisms** to terminate 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. They have short half-life, then their action disappears, after that if the body needs more, other bursts happen. So, our body do not have sustain 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, predominantly inflammatory cells, for example, in acute phase they are produced by neutrophils. And neutrophils have a short half-life, their DNA is programmed to die after (1-days), and this is what we call programmed cell death **(Apoptosis)**.

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

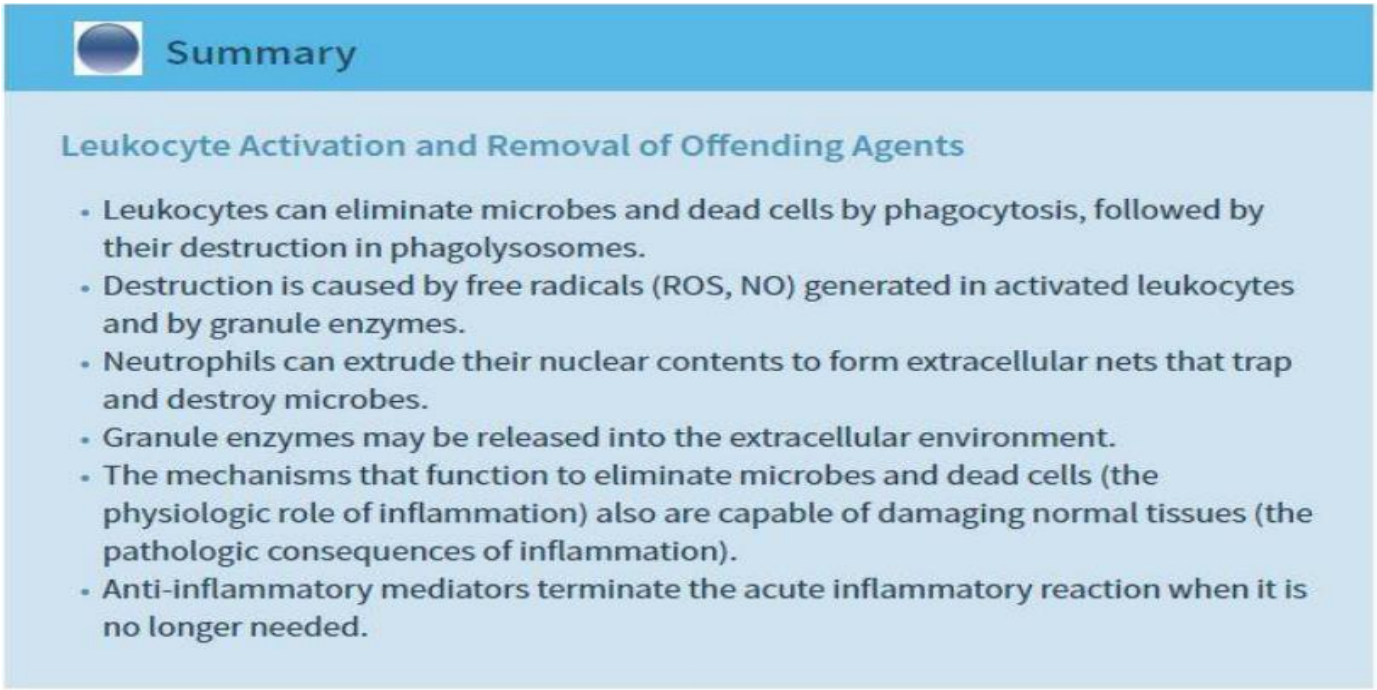
Certain mediators can inhibit other mediators like **transforming growth factor-beta (TGF-ß)**, it's one of the strongest fibro genic factors that make repair, also we have **interleukin-10 (IL-10)**. Those mediators are released toward the last phase of inflammation (R4 and R5) and they are capable 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)**.

So, **mediators** are **produced in rapid bursts** and they are **not continuously released**, they are **stimulus-dependent**, have **short half-lives**, and **degrade** after release by certain enzymes.

And this is how our system can proceed with **R4** which is **regulation and termination** (calm down the inflammatory response).



Note: these blue notes are important, study them.

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:

	MAJOR GROUPS	MOST IMPORTANT OF WHICH
1	Lipid products	PGs and LTs
2	Cytokines	IL, TNF, and chemokines
3	Complement activation	C1-9
4	Vasoactive amines	Histamine, serotonin

GENERAL FEATURES OF INFLAMMATION

1. Cell-derived at the site of injury

Mediators are rapidly released from intracellular granules or are synthesized de novo in response to a stimulus (the cell machinery is ready to synthesize mediators upon stimulation). So, if there is no injury then 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 to be active(mediators).

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)

Each one of the mediators can activate or inhibit the release or the stimulation of others.

PRINCIPLE MEDIATORS OF INFLAMMATION

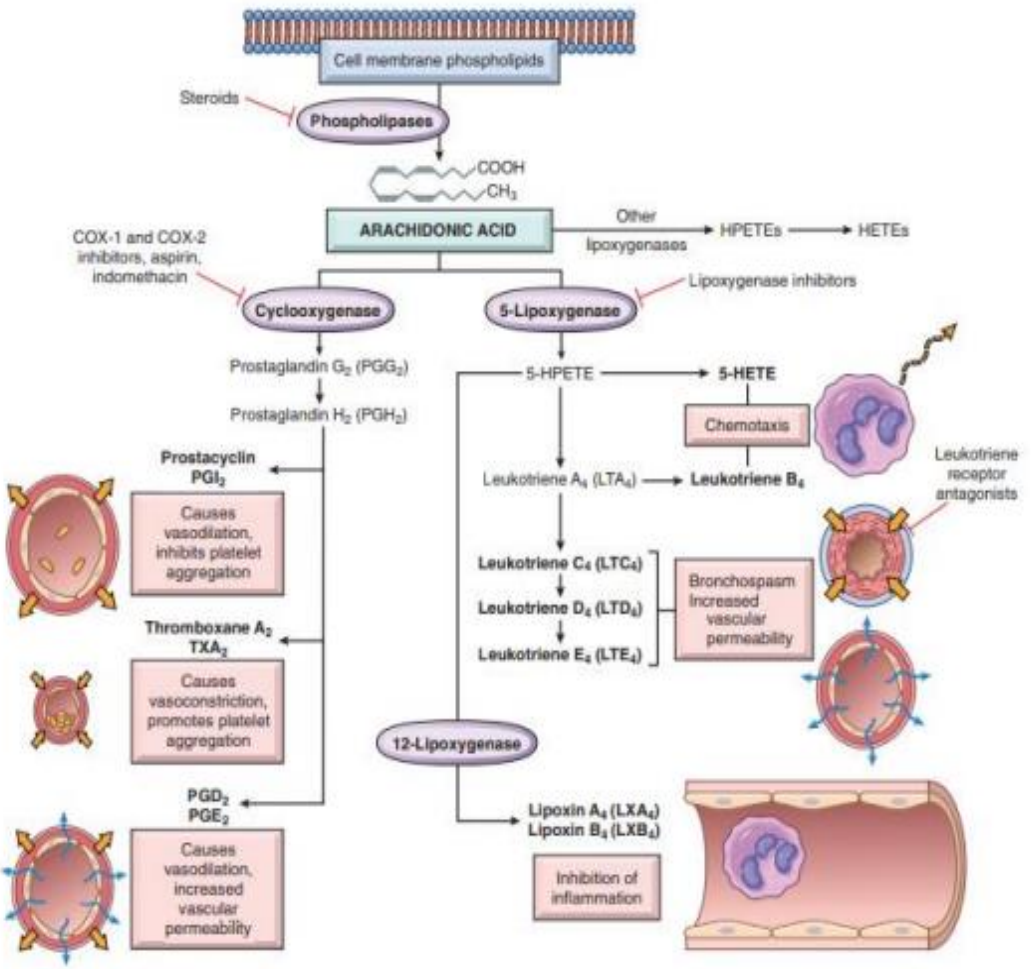
Mediator	Source	Action
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion, 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 the liver)	Leukocyte chemotaxis and activation, direct target killing (membrane attack complex), vasodilation (mast cell stimulation)
Kinins	Plasma (produced in the liver)	Increased vascular permeability, smooth muscle contraction, vasodilation, pain

You have to memorize this table.

- **The major vasodilator is histamine.**

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)



- So, at first >> an enzyme called **phospholipase** destroys phospholipids producing Arachidonic acid, then the arachidonic acid goes into two different pathways:

Check the pic above...

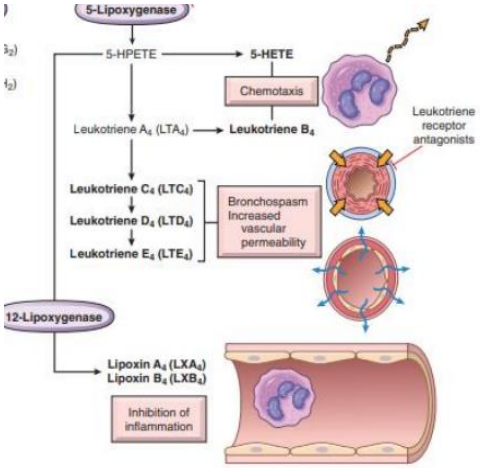
1. Cyclooxygenase pathway (left arm): the two cyclooxygenases enzymes called COX-1 and COX-2 will destroy arachidonic acid, producing a big group of mediators called **prostaglandins**.

2. 5-lipoxygenase pathway (right arm): 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:**

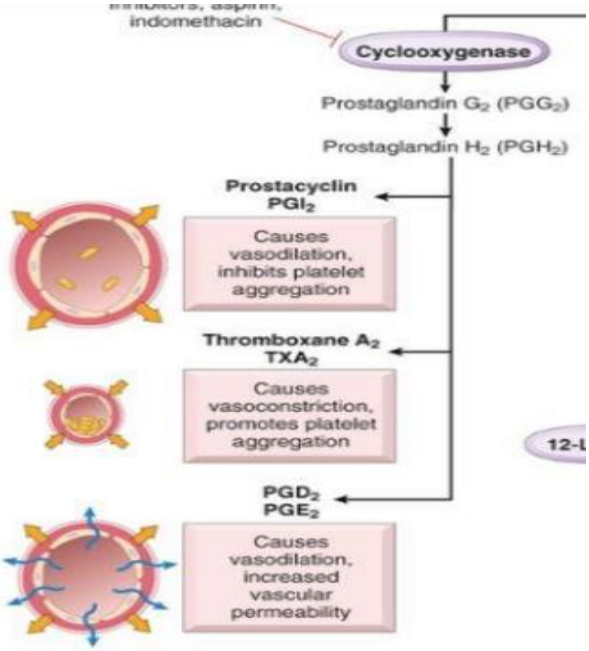
- Some of leukotrienes are **chemotactic agents**, they have role in recruitment of cells.
- Other leukotrienes cause **Bronchospasms and edema** in the bronchus.
- Leukotrienes **C4**, **D4** and **E4** are involved in production of acute asthmatic attack.

If a patient is suffering from **Bronchospasm**, first thing to be noticed that his chest is **wheezing**. What happened is that **Bronchospasm** causes **bronchoconstriction** and **edema** which is what an acute asthmatic attack is. consequently, the lumen of the bronchus narrows to 1cm instead of 2cm, and this what makes his chest wheezing. And in some cases, patients die due to bronchus blocking. In similar cases, doctors give the patient **anti-asthmatic drugs** to manage the situation.



- **Lipoxin A4** and **Lipoxin B4** make general inhibition of inflammation, particularly **anti-chemotaxis**.

- **Cyclooxygenases inhibitors** (COX-1 and COX-2): **Aspirin** and **non-steroidal anti-inflammatory drugs (NSAID)**. when you have headache and take aspirin, it works as strong anti-inflammatory agent through decreasing production of prostaglandins (**PGI2, THROMAXINE A2 and PGE2**).



- Notice that **PGI2** causes **vasodilation** and **inhibition of platelet aggregation**, which helps in **ischemia cases**. While **TXA2** which cause **vasoconstriction** and **platelet aggregation**. So, patients having imbalance between **PGI2** and **TXA2** would suffer from **ischemic heart disease**, **atherosclerosis**, and **cerebrovascular accident (CVA) "STROKE".**

INHIBITORS INVOLVED IN AA METABOLISM:

(QUICK SUMMARY)

1. **cox-1 and cox-2 inhibitors** → 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.
2. **lipoxygenase inhibitors** → inhibit only the production of all the leukotrienes, those inhibitors are usually used in acute asthmatic attacks.
3. **Steroids** → 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.

   Long courses of steroids, especially cortisone, have dangerous side effects such as diabetes, obesity, recurrent infections, muscle pain, and it lowers immunity.

   For example, if someone has severe diabetes or obese, you must try as much as possible to do not give him steroids, unless you have to.

cox-1, cox-2, and lipoxygenase inhibitors less potent and less critical than steroids.

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

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.**

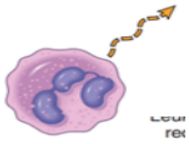
4. **Thromboxane A2 (TXA2)'**

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)**.

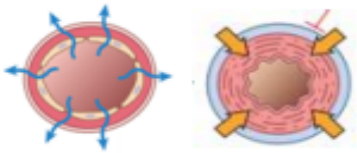
LEUKOTRIENES



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

1. **5-HPETE → 5-HETE / Leukotriene A4 (LTA4) → Leukotriene B4**
   5-HETE and Leukotriene B4 are strong chemotactic agents, the function of leukotriene B4 is chemotaxis and recruitment of white blood cells into the site of injury.

2. **Leukotriene C4 (LTC4) /Leukotriene D4 (LTD4) /Leukotriene E4 (LTE4)**
   Chemical mediators of inflammation are thought to play a major role in the **bronchospasm** (induce constriction of the bronchial diameter and cause bronchial asthma) and increase vascular permeability causing more edema.



The antagonists of these products are utilized as a targeted 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)

ACTION	EICOSANOID
VASODILATION	Prostaglandins PGI2 (prostacyclin), PGE1, PGE2, PGD2
VASOCONSTRICTION	Thromboxane A2, leukotrienes C4, D4, E4
INCREASED VASCULAR PERMEABILITY	Leukotrienes C4, D4, E4
CHEMOTAXIS, LEUKOCYTE ADHESION	Leukotriene B4
SMOOTH MUSCLE CONTRACTION	Prostaglandins PGC4, PGD4, PGE4

POINTS TO REMEMBER ABOUT AA METABOLISM

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

Aspirin and cox1, cox2 inhibitors can inhibit cyclooxygenase, then they inhibit 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 strong **vasodilator** and inhibits **platelet aggregation**.

- **Thromboxane A2: vasoconstrictor and + Pl aggregate**

TXA2 is a major **vasoconstrictor** and **stimulator of 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.

- **PG (PGE2): pain & fever**

Is a major mediator of pain and fever.

Cyto → are produced by cells
kines→they make a kinetic function

❖ CYTOKINES:

2nd big group of mediators of inflammation

CYTOKINES are proteins secreted by many cells, predominantly **activated lymphocytes**, **activated macrophages**, and **dendritic cells**.
They are a big group of chemicals that **mediate** and **regulate** the **immune and inflammatory response**. They are targeted by thousands of studies to help produce some effective medications in the role of cancer angiogenesis and the spread of metastasis of certain cancers.

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.

CYTOKINES IN INFLAMMATION

another table and you have to memorize it ...:)

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; a 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

MAJOR ROLES OF CYTOKINES IN ACUTE INFLAMMATION

Whenever we have an inflammatory response, mediators make actions, we have **local signs, symptoms, or effects of inflammation (Local actions)**, and we have also **distant non-local functions (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.**

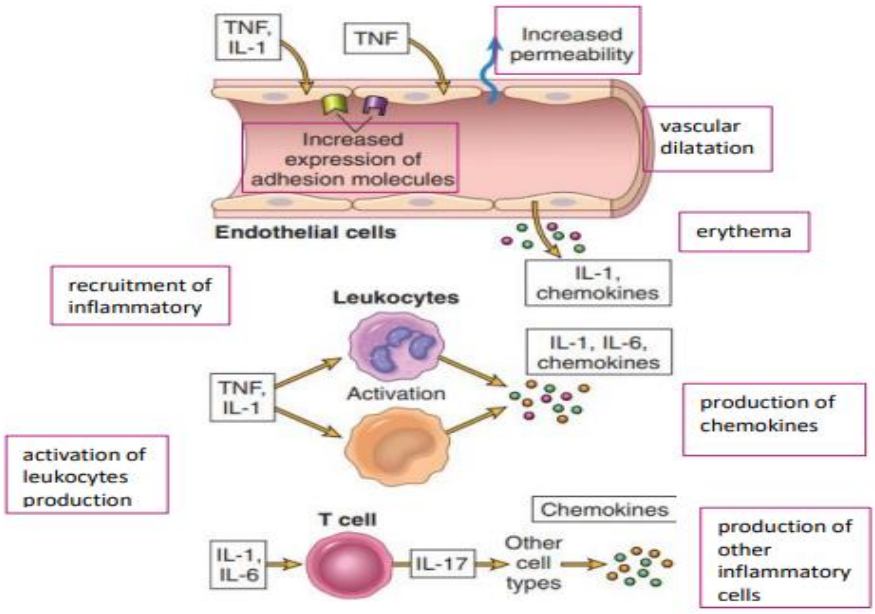
1. Local inflammation

The local actions of mediators can be like:

- **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**

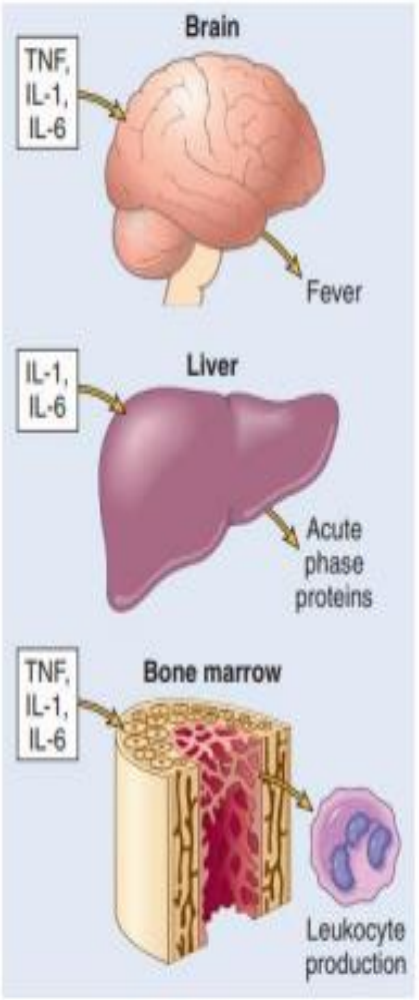
So locally, there will be swelling, edema, and redness which are induced by chemical mediators at the local level.

If a child came to hospital suffering from fever, loss of appetite, not moving a lot, you must look at local changes, for example check his ears and his throat to find out the site of injury.



2.Systemic protective effects

Many of the cytokines **(such as TNF, IL-1, IL-6)** will have systemic protective mechanisms, they go to the brain and produce **fever** which could be sometimes dangerous but it's beneficial **(protective)** because it brings the patient to the clinician where the problem will be treated.



Cytokines **(like IL-1, IL-6)** go to liver and them can stimulate phagocytes to produce **acute phase proteins/reactants (non-specific parameters)** which have a protective effect, they give you a signal that there is intensive inflammatory response. Sometimes we can measure those proteins in the blood to determine if the patient is in acute distress or not.

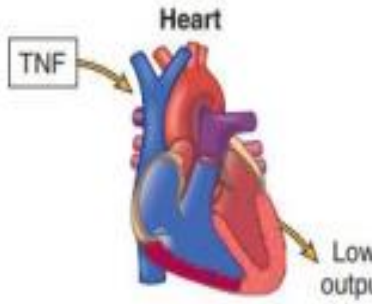
Other cytokines **(such as TNF, IL-1, IL-6)** will go to the bone marrow and stimulate the production of more **hematopoietic cells** which help in the fight against inflammation.

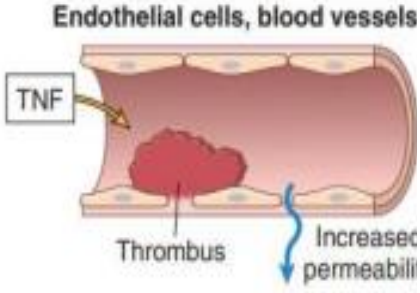
So, all these considered protective systemic effects of inflammation via the production of mediators, which go to the blood vessels and have effects on the systemic body.

3.Systemic pathological effects

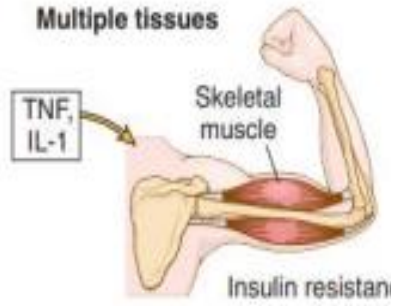
Sometimes, too much response or quantities of mediators can make pathological effects. So, these systemic impacts can be pathological.

Some cytokines **(like TNF, the major cardiogenic function inhibitors)** can go to the heart and depress the cardiogenic function (decrease the cardiac output) which could lead to heart failure from just severe acute inflammation, so. Sometimes patients are given **anti-TNF** which inhibits the impact of this cytokine.

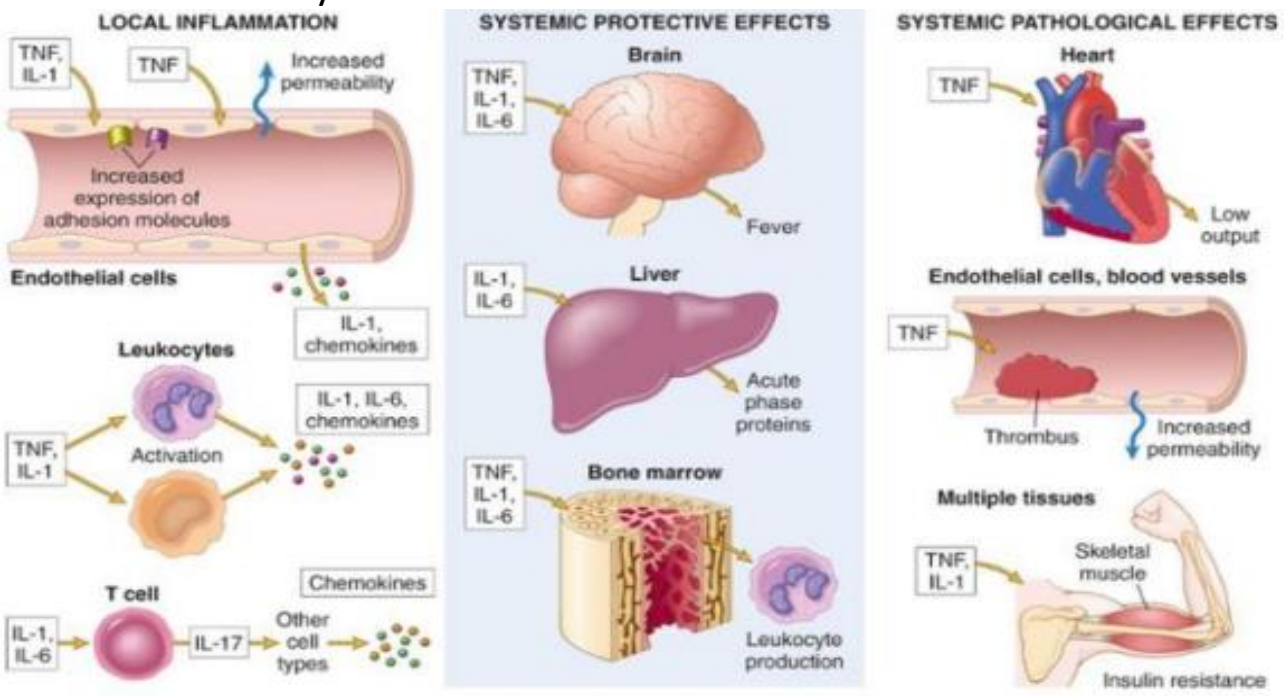




The same cytokines **(TNF)** could cause endothelial cell injury. We said before that the vascular compartment is an important part of the initial phase of inflammation so, TNF can induce **platelet aggregation**, thrombus formation, and shooting emboli which will cause ischemia in the heart and other organs.



TNF, IL-1 may go to the skeletal muscle tissue and causes insulin resistance which is another systemic bad effect.



CHEMOKINES

Chemokines are small family of the big cytokine family; they are very small proteins, and the major action is as chemotactic agents (chemoattractants) "R2". There are more than 40 chemokines, and around 20 receptors called (G-protein coupled 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 act through a group of receptors called G - protein coupled receptors.**
- **They have two major functions:**
  - In Acute inflammation (they recruit white blood cells to the side of injury).
  - In protecting tissue architecture (when you have severe acute inflammation, they try not to damage the skeleton of tissue).

COMPLEMENT SYSTEM

3rd big group of mediators of inflammation

- **They are soluble proteins that are produced by the liver, their function mainly in host defense against microbes and in pathologic inflammatory reactions.**
- **These proteins present in the body in an inactive form, so they need stimulation or activation to do their functions.**
- **There are more than 20 complements. (The most important C1 - C9).**
- **They are important in innate and adaptive immunity.**
- **The major functions of the CS are vascular permeability, chemotaxis (C5a), and they are important in a process called opsonization (C3b).**
- **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 "gatekeeper "to them. What this means is 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's means this drug stimulates the cascade of the complement system.**

COMPLEMENT SYSTEM ACTIVATION (COMPLEMENT FIXATION)

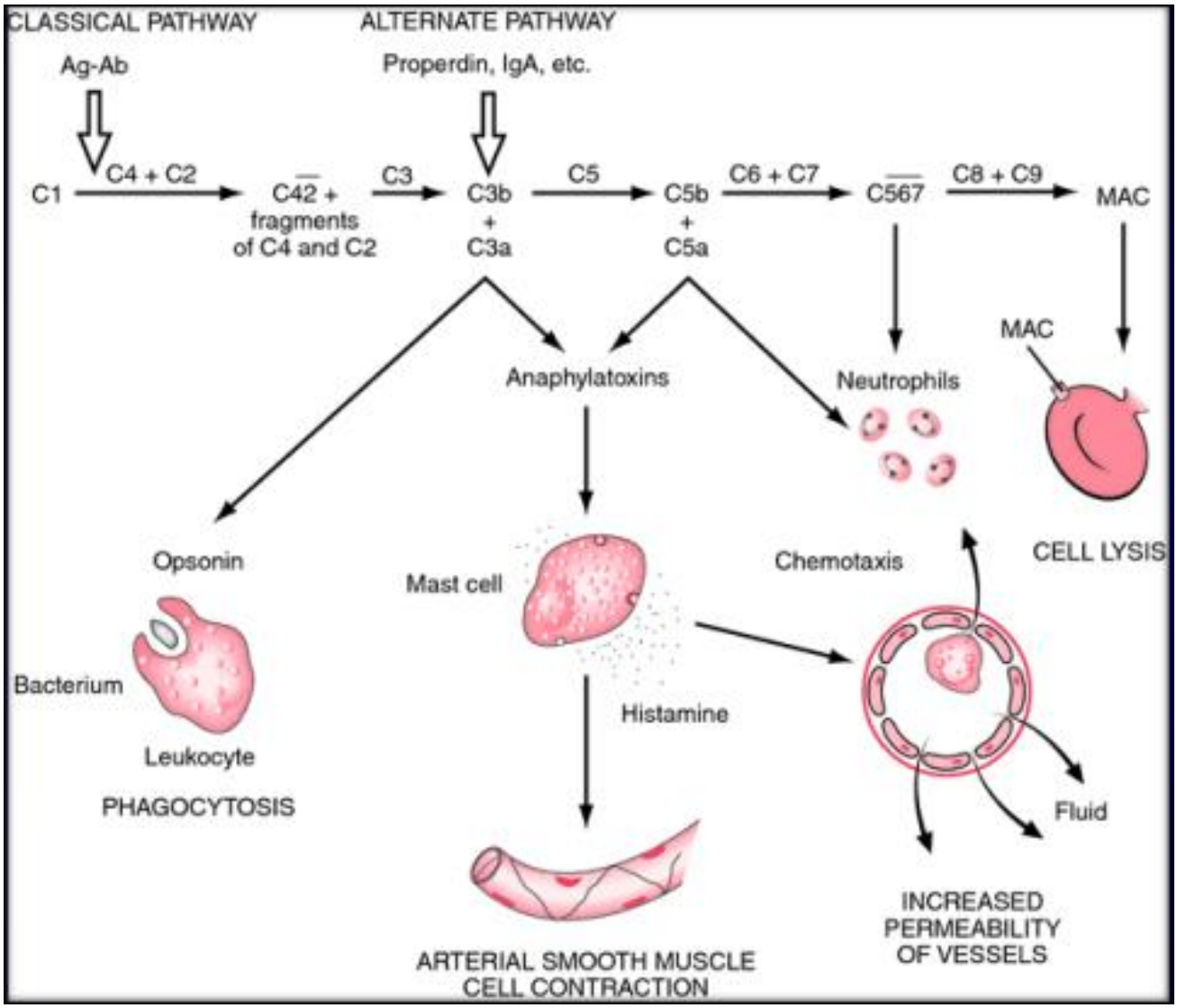
In the old days there were only two ways to activate the complement system

1. **The classic pathway**

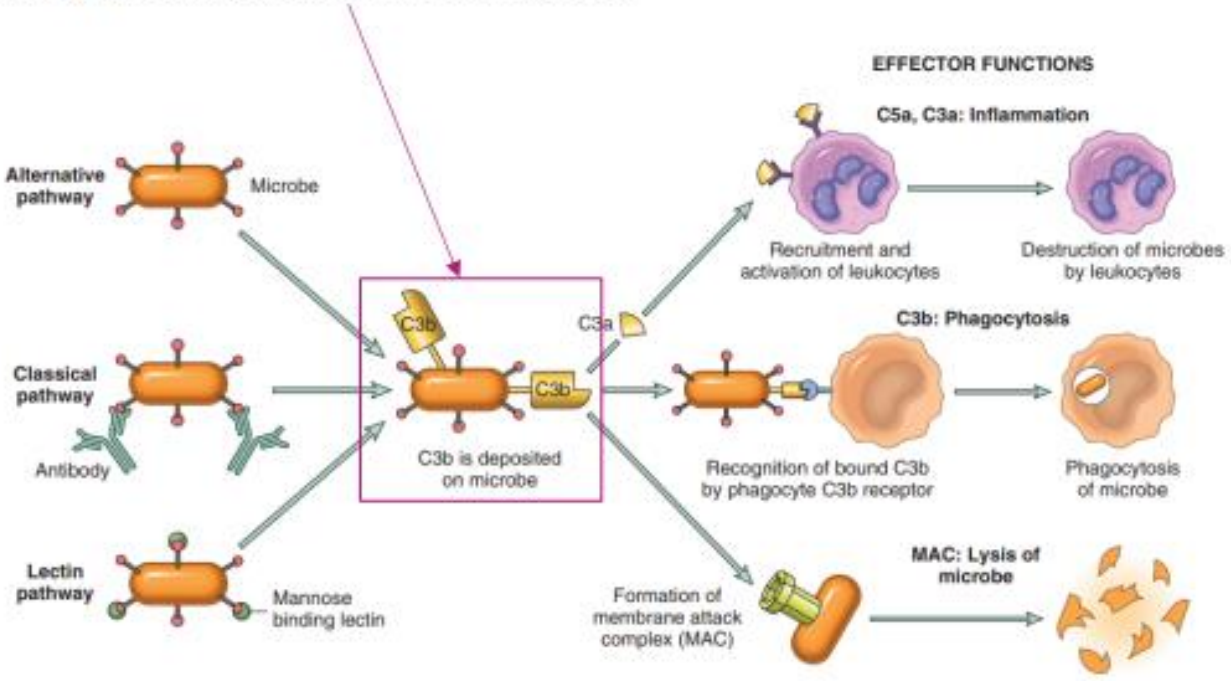
   By antigen-antibody complex. An invader comes →produce antibodies→antibody will join the antigens→the antigen-antibody complex will start activating the complement system.

2. **The alternate pathway**

   Through either IgA or other certain products (like bacteria). The cleavage starts at the activation C3 component, and the cascade will continue inducing multiple functions.



BUT, this is stale knowledge. However, the current knowledge is that we have **three pathways** in which the C3 can be activated:

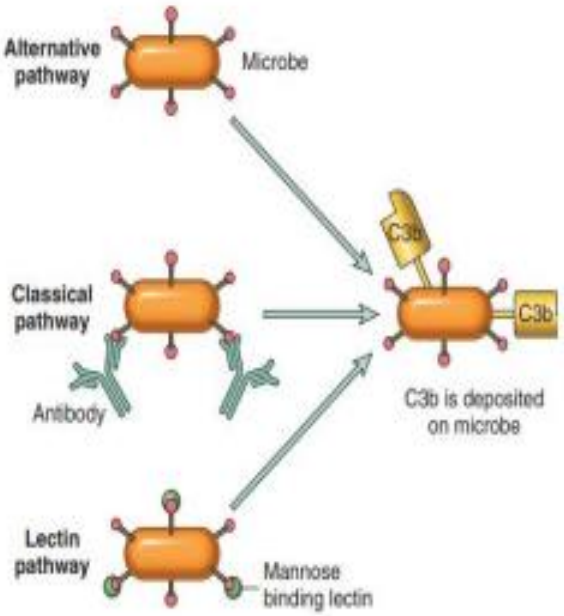


ALL details in this pic are important.

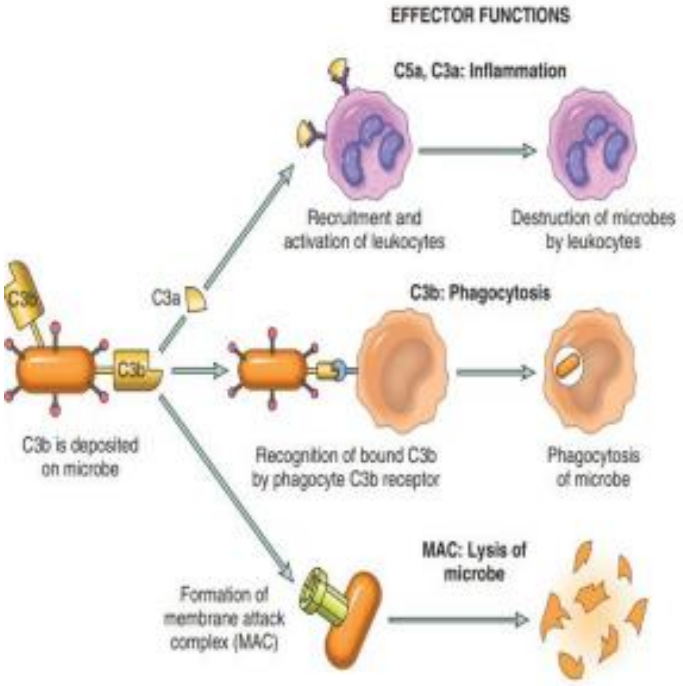
1. **The alternative pathway**
   By certain receptors in the microbial products, activate C3 directly.

2. **The classical pathway**
   By antigen-antibody complex on the surface of bacteria or viruses, the antigen-antibody complex will activate C3.

3. **Lectin pathway**
   In which there a **Mannose receptor** binding to lectin inducing the activation of C3.



After that, **activated C3** goes into 3 pathways:



The active part **C3a** will work as a chemotactic agent (recruitment and activation of leukocytes), this will induce the production of C5a and C3a which are also chemotactic agents.

The **C3b** which is a very important stimulator of phagocytosis helps the macrophage and neutrophil in the phagocytosis process.

The continuous stimulation of the cascade leads to the activation of C5, C6, C7, and then they will produce something called MAC (membrane attack complex)

MAC (membrane attack complex): certain products of the complement system, in the past it used to be a combination of C5, C6, C7, and sometimes C8 or C9 but, nowadays it is recognized that it is multiple of nines (C9s). MAC is important to lice and attacks directly without going through the phagocytosis.

Remember: difficult roads lead to beautiful destinations.

فتح الله علينا وعليكم فتوح العارفين