8

Pathology

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Sheet Slides

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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 vasoactive amines, lipid products (prostaglandins and leukotrienes), cytokines (including chemokines), and products of complement activation.

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

General features of mediators of inflammation

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.

- Cells derived at the site (Cell-derived mediators) are rapidly released from intracellular granules or synthesized upon stimulation.

2) Plasma proteins: needs activation

- Plasma-derived mediators (e.g., complement proteins) are present in the circulation as inactive precursors that must be activated, usually by a series of proteolytic cleavages, to acquire their biologic properties. They are produced mainly in the liver, are effective against circulating microbes, and can also be recruited into tissues.

3) Active mediators: needs stimulation (to amplify their activity)

Active mediators are produced only in response to various molecules that stimulate inflammation, including microbial products and substances released from necrotic cells.

4) Most of the mediators are short-lived. (minutes and hours).
They quickly decay, or are inactivated by enzymes, or they are otherwise scavenged or inhibited. There is thus a system of checks and balances that regulates mediator actions. These built-in control mechanisms are discussed with each class of mediator.

5) One mediator can stimulate (or inhibit) the release of other mediators.

For instance, products of complement activation stimulate the release of histamine, and the cytokine TNF acts on endothelial cells to stimulate the production of another cytokine, IL-1, and many chemokines. The secondary mediators may have the same actions as the initial mediators **but also may have different and even opposing activities**, thus providing mechanisms for amplifying—or, in certain instances, counteracting—the initial action of a mediator.

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

Major Vasodilator

Very important table that must be memorized. However, since we have overlap, focus on the unique actions.

Major mediator for pain and smooth muscle contraction

1) Vasoactive Amines: Histamine and Serotonin

Histamine causes dilation of arterioles and increases the permeability of venules. Histamine is considered the principal mediator of the immediate transient phase of increased vascular permeability, producing interendothelial gaps in postcapillary venules. **(Histamine is the major vasoactive amine).**

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 is also a vasoconstrictor, but the importance of this action in inflammation is unclear. **(Sometimes called Histamine of the animals)**

- **However,** the doctor said: nowadays they claim that serotonin also has a function in humans.

2) Arachidonic Acid Metabolites

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. Most cellular arachidonic acid is esterified and incorporated into **membrane phospholipids.**

Mechanical, chemical, and physical stimuli or other mediators (e.g., C5a) trigger the release of arachidonic acid from membranes by activating cellular **phospholipases**, mainly phospholipase A2.

Once freed from the membrane, arachidonic acid is rapidly converted to bioactive mediators. These mediators, Arachidonic Acid metabolites, which are also called **eicosanoids** (because they are derived from 20-carbon fatty acids; Greek eicosa = 20), are **synthesized** by two major classes of enzymes:

1) Cyclooxygenases (which generate prostaglandins).

2) Lipoxygenases (which produce leukotrienes and lipoxins)

Eicosanoids bind to G protein-coupled receptors on many cell types and can mediate virtually every step of inflammation.

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

This table was given in the slides

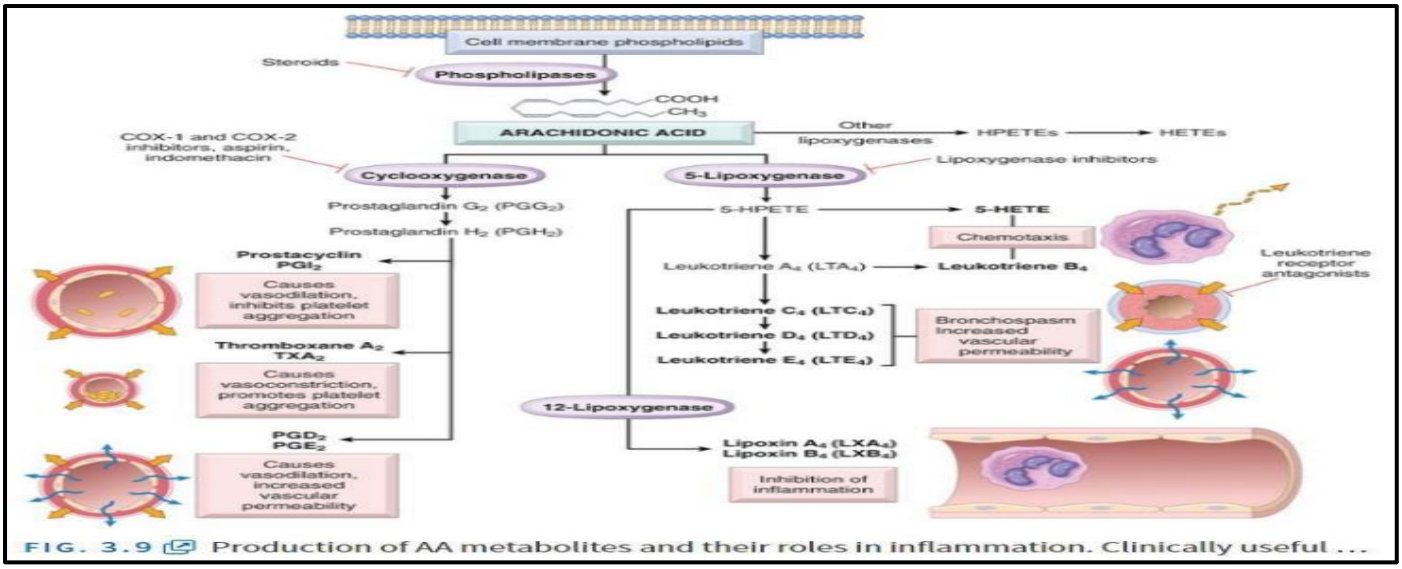


FIG. 3.9 Production of AA metabolites and their roles in inflammation. Clinically useful ...

Study every detail of this figure, the following table and info in page 5 should help you understand it.

Prostaglandins

The most important prostaglandins in inflammation are PGE2, PGD2, PGF2a, PGI2 (prostacyclin), and TXA2 (thromboxane A2), each of which is derived by the action of a specific enzyme on an intermediate in the pathway. Some of these enzymes have restricted tissue distribution and functions.

1) **PGD2** is the major prostaglandin made by mast cells**; along with PGE2** (which is more widely distributed), **it causes vasodilation and increases the permeability of postcapillary venules**, thus potentiating exudation and resultant edema. PGD2 also is a chemoattractant for neutrophils.

2) **TXA2**: Platelets contain the enzyme thromboxane synthase, which is responsible for synthesizing TXA2, the major platelet eicosanoid. **TXA2 is a vasoconstrictor and a potent platelet-aggregating agent, and thus promotes thrombosis.**

3) **PGI2:** In contrast, vascular endothelium contains prostacyclin synthase, which is responsible for the formation of **prostacyclin (PGI2)**. Prostacyclin **is a vasodilator and a potent inhibitor of platelet aggregation, and thus serves to prevent thrombus formation** on normal endothelial cells. **A thromboxane– prostacyclin imbalance has been implicated in coronary and cerebral artery thrombosis**

Note: 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.

Leukotrienes and Lipoxins

The synthesis of leukotrienes involves multiple steps, the first of which generates leukotriene A4 (LTA4), which in turn gives rise to LTB4 or LTC4.

1) **LTB4** is produced by neutrophils and some macrophages and is a **potent chemotactic agent** and activator of neutrophils, causing aggregation and adhesion of the cells to venular endothelium, generation of ROS, and release of lysosomal enzymes.

2) **The cysteinyl-containing leukotriene LTC4 and its metabolites, LTD4 and LTE4**, are produced mainly in mast cells and cause intense vasoconstriction, **bronchospasm (important in asthma),** and increased permeability of venules.

Lipoxins are also generated from arachidonic acid by the lipoxygenase pathway.

The doctor didn't separate lipoxins from leukotrienes but for those who are interested, here is some beneficial information regarding how they differ.

Unlike prostaglandins and leukotrienes, lipoxins suppress inflammation by inhibiting the recruitment of leukocytes. **They inhibit neutrophil chemotaxis and adhesion to endothelium**. Leukocytes, particularly neutrophils, produce intermediates in lipoxin synthesis, and these are converted to lipoxins by platelets interacting with the leukocytes.

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: (Check them on the photo in page 4)

1) Cyclooxygenase (COX) inhibitors includes **aspirin** and other **non-steroidal anti-inflammatory drugs (NSAIDs)**, such as ibuprofen. They inhibit both COX-1 and COX-2 and, therefore, block all prostaglandins. (hence, their efficacy in treating pain and fever).

2) Lipoxygenase inhibitors. 5-lipoxygenase is not affected by NSAIDs. Many new inhibitors of this enzyme pathway have been developed. 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 reduce the transcription of genes encoding COX-2, phospholipase A2, proinflammatory cytokines (e.g., IL-1 and TNF).

4) Leukotriene receptor antagonists block leukotriene receptors and prevent the actions of the leukotrienes. These drugs (e.g., Montelukast) are useful in the treatment of asthma.

Points to remember about AA metabolism

- ✓ **Aspirin** – inhibits cyclooxygenase.
- ✓ **Steroids** – inhibit phospholipase (anti-inflammatory).
  You should be very careful when giving steroids to a patient since it is a very potent drug with big risks of complications, it's like paralyzing one arm of inflammatory response which are AA metabolites.
- ✓ **Prostacyclin (PGI2):** vasodilator and inhibits Platelet aggregation.
- ✓ **Thromboxane A2**: vasoconstrictor and induces Platelet aggregation.
- ✓ **TXA2-PGI2 imbalance**: is implicated in IHD & CVA. (Ischemic Heart Disease & Cerebrovascular Accident which are the major causes of death in adults).
- ✓ **PG (PGE2):** main mediator for pain & fever.
  *If you have noticed, we said that **kinin** is also a **major mediator** for **pain**, that's why they won't be given as two separate answers in the exam.*
- ✓ **HETE** (hydroxyl-eicosa-tetraenoic acid): is involved in chemotaxis. **However**, the **strongest** chemotactic agent is **LTB4.**

3) Cytokines

- **Cytokines** are **proteins** secreted by many cell types (mainly activated lymphocytes, macrophages, and dendritic cells. However, endothelial, epithelial, and connective tissue cells are also included).
- **Mainly inflammatory cells**, **but** even epithelial cells can secrete them.
- They **mediate** and **regulate** (activate/inhibit) immune and inflammatory **response**.
  - That's why there are many drugs that work on these cytokines.

Extra beneficial information from the book

inflammatory stimuli can stimulate the secretion of TNF and IL-1.
The production of TNF is induced by signals through TLRs and other microbial sensors, and the synthesis of IL-1 is stimulated by the same signals, but the generation of the biologically active form of this cytokine **is dependent** on the inflammasome.

TNF regulates energy balance by promoting lipid and protein catabolism and by suppressing appetite. Therefore, 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.

TNF antagonists have been remarkably effective in the treatment of chronic inflammatory diseases, particularly rheumatoid arthritis, psoriasis, and some types of inflammatory bowel disease.

If u have noticed IL-17 is present in both acute and chronic inflammation. Recall from sheet 7 that lymphocytes (example T-helper-17) **DO** have a role in acute inflammation.

Another very important table, make sure you memorize it.

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.

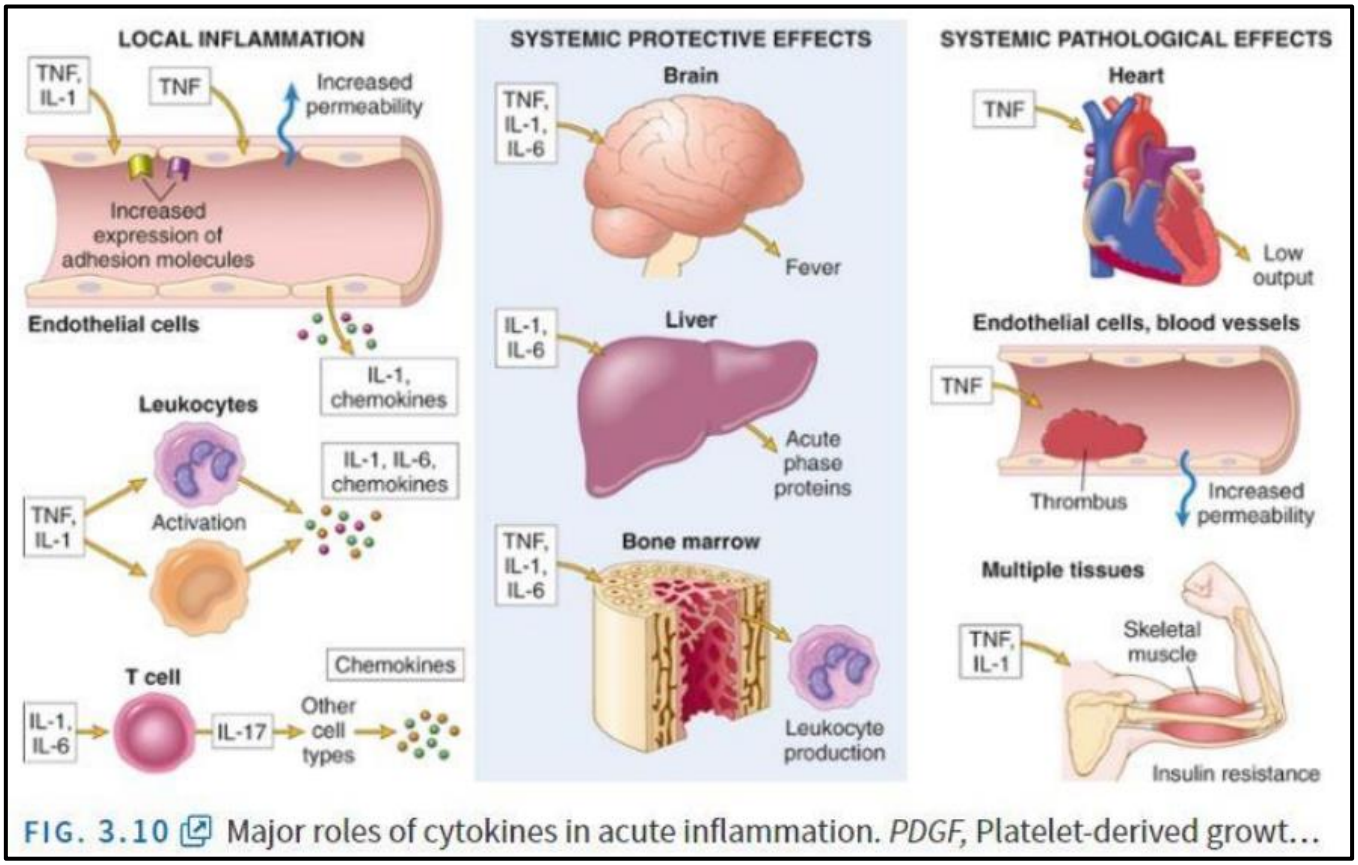


FIG. 3.10 Major roles of cytokines in acute inflammation. *PDGF*, Platelet-derived growt…

The actions of **TNF** and **IL-1** contribute to the **local and systemic reactions** of inflammation (notice from the picture above).

Moreover, the most important roles of these cytokines in inflammation are the following:

1) Local inflammation: involves vascular phase, chemotaxis, etc.. , which will eventually cause redness, swelling, pain, tenderness.

Note: the following points about local inflammation are mentioned in the book

- **Endothelial activation:** Both TNF and IL-1 act on endothelium to induce a spectrum of changes referred to as endothelial activation. These changes include increased expression of endothelial adhesion molecules, mostly E- and P-selectins and ligands for leukocyte integrins; increased production of various mediators, including other cytokines and chemokines, and eicosanoids; and increased procoagulant activity of the endothelium.
- **Activation of leukocytes and other cells:** TNF augments responses of neutrophils to other stimuli such as bacterial endotoxin and stimulates the microbicidal activity of macrophages. IL-1 activates fibroblasts to synthesize collagen and stimulates proliferation of synovial cells and other mesenchymal cells. IL-1 and IL-6 also stimulate the generation of a subset of CD4+ helper T cells called TH17 cells.

2) Systemic Effects; which can be:

a) Systemic protective effects: ***(Examples are found in the picture, page 7***

- IL-1 and TNF (as well as IL-6) induce the systemic acute-phase responses associated with infection or injury (responses far from the site of inflammation), including fever.
- IL-1 and IL-6 also go to the liver and stimulate the production of acute phase proteins or acute phase reactants (CRP or ESR), which is a general indication that gives an idea for general inflammatory conditions.
- IL-1, IL-6 and TNF also go to the bone morrow and regulate hematopoiesis.

b) Systemic pathological effects: (harmful) ***(Examples are found in the picture, page 7)***

Cytokines are also involved in the pathogenesis of the systemic inflammatory response syndrome (SIRS), resulting from disseminated bacterial infections (sepsis) and other serious conditions.

- Affecting the heart leading to a **low cardiac output**; Sever **acute** inflammatory response leads to a **cardiogenic shock**
- Thrombosis can lead to an injury (which happens due to imbalance between TXA2 and PGI2 as mentioned earlier). For instance, a myocardial infarction in the heart or a stroke in brain.
- In addition to multiple harmful cases such as muscle pain.

When you have an acute inflammation, you can notice an increase in white blood count, (WBC range was about 12,000 to 20,000), which is part of the protective role in acute inflammatory process to increase the immunity when there is, for example, a bacterial inflammation. **However, what do we conclude if there is an exaggerated response?** Normal white blood count is 8,000 to 11,000 cells per microliter of blood. If a blood test (i.e. CBC; Complete Blood Count,) indicated that the white blood count is 50,000, this would **NOT** be an **acute inflammation** *(we should have a range of 12,000 to 20,000 in acute inflammation)* **BUT** this indicates that we **might** have **cancer**, specifically, **leukemia**. By using **cytometry**, we can indicate if this is leukemia or not.

- ❖ If those 50,000 are monoclonal, this indicates a tumor since they originated from one cell.
- ❖ But if they are polyclonal, this is good news, it **indicates a leukemoid reaction**, an exaggerated inflammatory response.

Monoclonal cells are a group of cells produced from a **single** ancestral cell by repeated cellular replication, and that what commonly happens in cancer.

(This example was given by the doctor and will be discussed thoroughly in Neoplasia)

Chemokines (a small Family of Cytokines)

- **Chemokines** are a family of small proteins (8–10 kD) that act **mainly as chemo-attractants** for specific types of leukocytes. About 40 different chemokines and 20 different receptors for them have been identified.
- They are classified into four major groups, according to the arrangement of cysteine (C) residues in the proteins:
  C-X-C, **C-C**, **C** and **CX3-C**. (The doctor didn't explain how each group works)
- They function through G-protein coupled receptors**.** Chemokines mediate their activities by binding to seven-transmembrane G protein–coupled receptors.
- They have 2 main functions:

1) 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. Because they mediate aspects of the inflammatory reaction, they are sometimes called inflammatory chemokines. Their production is induced by microbes and other stimuli.

2) Maintenance of tissue architecture: since the architecture of the tissue is important for the tissue to function.

- Some chemokines are produced constitutively by stromal cells in tissues and are sometimes called homeostatic chemokines. These organize various cell types in different anatomic regions of the tissues. **(from the book)**

4) Complement System

The complement system is a collection of soluble circulating proteins and their membrane receptors that function mainly in host defense against microbes and in pathologic inflammatory reactions. (They are soluble inactive proteins made in the liver that need activation to be functional).

There are more than **20 complement proteins**, some of which are numbered C1 through C9 (most important ones).

They function in both innate immunity (mostly from the mother) and adaptive immunity for defense against microbial pathogens.

Functions: In the process of complement activation, several cleavage products of complement proteins are elaborated that **cause increased vascular permeability**, **chemotaxis,** and **opsonization**.

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, thus forming an enzymatic cascade capable of tremendous amplification. The critical step of ALL PATHWAYS in complement activation **is the proteolysis of the third (and most abundant) component, C3. (remember these 2 important things about C3)**

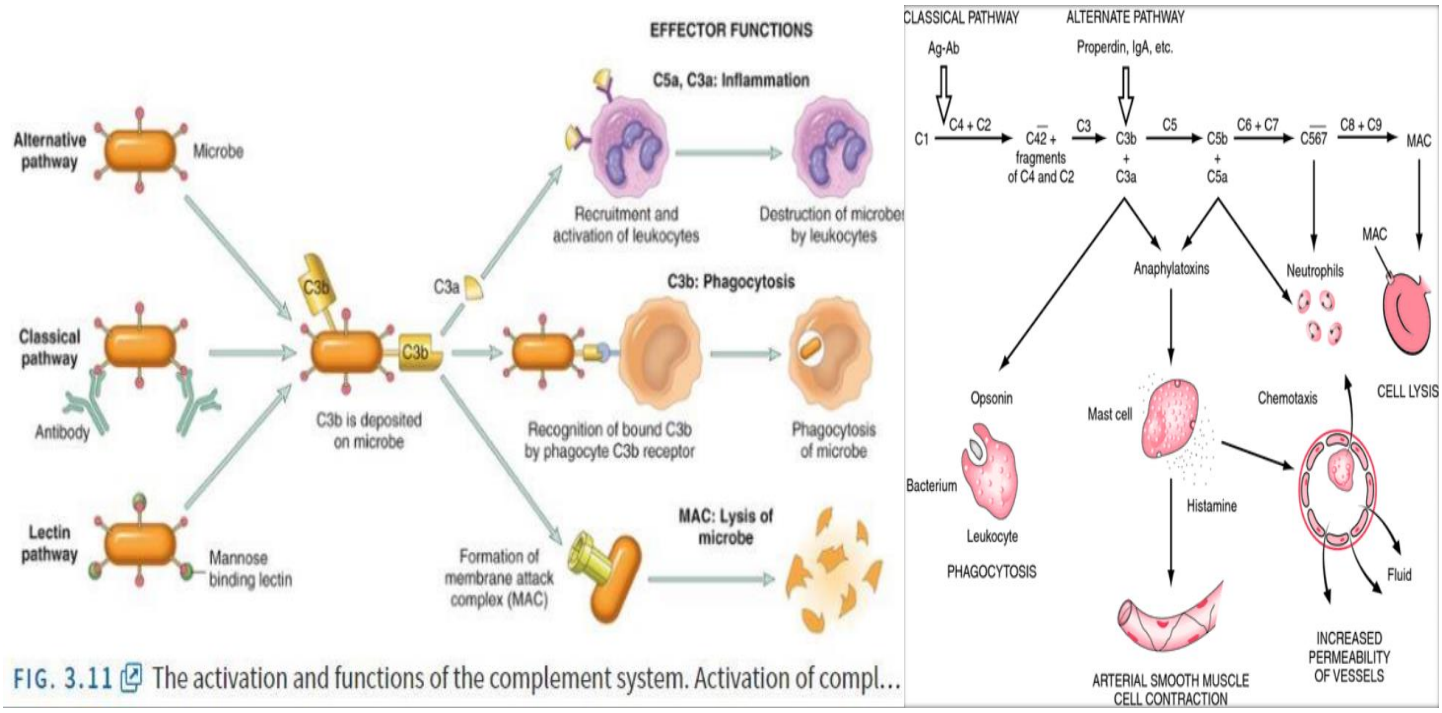


FIG. 3.11 The activation and functions of the complement system. Activation of compl...

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. (When u take a vaccination, Adaptive immunity)

2) The alternative pathway, which can be triggered by microbial surface molecules (e.g., endotoxin, or LPS), 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.

- 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.

Mechanism

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). MAC is essential in cell lyses; its mechanism is discussed below.**

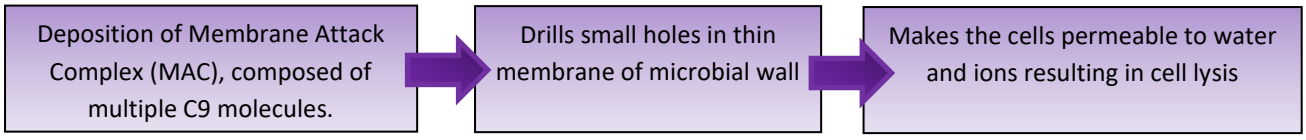
Complement System Functions:

1) Inflammation: histamine like **anphylatoxins** (C5a).

- C5a, and, to a lesser extent, C4a and C3a are cleavage products of the corresponding complement components that stimulate histamine release from mast cells and thereby increase vascular permeability and cause vasodilation. **They are called anaphylatoxins because** they have similar effects to those of mast cell mediators which are involved in **acute** major inflammatory **reactions** called **anaphylaxis.** C5a is also a chemotactic agent for neutrophils, monocytes, eosinophils, and basophils. In addition, C5a 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 C3b), when fixed to a microbial cell wall, act as opsonins and **promote/enhance** phagocytosis by neutrophils and macrophages, which bear cell surface receptors for these complement fragments. **(C3b is a strong opsonizing agent)**

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).



Regulatory Proteins for Complement System:

The **activation of complement** is tightly **controlled** by cell-associated & circulating **regulatory proteins**.

- Different regulatory proteins inhibit the production of active complement fragments or remove fragments that deposit on cells.
- These regulators are expressed on normal host cells and thus prevent healthy tissues from being injured at sites of complement activation.
- Regulatory proteins can be overwhelmed when large amounts of complement are deposited on host cells and in tissues, as happens in autoimmune diseases, in which individuals produce complement-fixing antibodies against their own cell and tissue antigens.
- **CS** (Complement system) proteins **deficiencies** can occur leading to **infection** susceptibility.

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1) C1 inhibitor:

- C1 inhibitor blocks the activation of C1; the first protein of the classical complement pathway.
- Inherited **deficiency** of this inhibitor is the cause of hereditary **angioedema**.

2) Decay accelerating factor (DAF) and CD59

- DAF and CD59 are two proteins linked to plasma membrane by a glycophosphatidyl (GPI) anchor.
  - DAF **prevents formation of C3 convertases.**
  - CD59 **inhibits formation of the MAC.**
- **Abnormalities cause PNH** *(Paroxysmal Nocturnal Hemoglobinuria)**:*** **An acquired deficiency** of the **enzyme** that **creates GPI** anchors leads to **deficiency** of these regulators and **excessive** complement **activation** and **lysis of red cells** (which are sensitive to complement-mediated cell lysis). This, ultimately, results in PNH disease.

3) Factor H:

- A **plasma protein** that serves as a **cofactor** for the **proteolysis of the C3 convertase**. ***(its deficiency results in excessive complement activation).***
- **Mutations** in Factor H are associated with a **kidney disease** called the **Hemolytic Uremic Syndrome.**