Lecture 3

TRANSMIGRATION:

- CD 31 (PECAM-1), platelet endothelial cell adhesion molecule expressed both on leukocytes and endothelial cells
- WBC pierce through wall by collagenases

* We Finished the initial vascular phase From the vascular dilatation due to histamine, then the more active process increased vascular permeability, then the movement of the inFlammatory cells mainly neutrophils and macrophages From intravasular to extravascular compartment and we detailed this process From margination, rolling, initial select and weak attachement to stronger integral attachement to the wall of the endothelial cells, then the active process of transmigration or diapedesis due to CD31 action utilizing the collagenase destroying the basement membrane and movement of the cells to outside

* "itis" is a term we end by it to indicate inFlammation like tonsillitis, appendix and appendicitis

* We will be hearing in all our life when we are dealing with patients with inflammation, pneumonia, dermatitis

CHEMOTAXIS:

* If the site of action is tonsils, there's chemotaxis to the tonsils by WBCs
* If the injury in your skin, it movement of WBCs to your skin

- **WBCs moving to injury tissue site**
- **Due to CHEMOATTRACTANTS (exogenous and endogenous):**

* It is an active process and it in induced by certain group of mediators which are called all together collectively chemoattractants

* these chemoattractants or the mediators or the agents which induce and help move the cells or chemotaxes WBCs plaster to the site of injury and they could be exogenous (outside the body) or endogenous (inside the body)

Bacterial products	**Peptides (N-…)** — N-terminal are actually strong chemoattractants, they will induce and help move the WBCs from the intravascular compartment into the site of injury
Cytokines — * it is a big group of mediators, released by inflammatory cell mainly lymphocytes and macrophages epecifically	**Chemokine family** — * they are a subgroup of the cytokines, they are strong chemoattractant group
Complement system	**C5a** — * It is a certain part * It is the strongest chemoattractants among those plasma protein or complement system
Lipoxygenase pathway	**LTB4** (Leukotriene B4) — * they are the strongest chemoattractant among the arachidonic acid metabolised
AA — * It is arachidonic acid metabolised which is part of cell membrane component in the lipo oxygenase pathway	

* These are the major chemoattractants will induce and help move of WBCs from the intravascular compartment to site of injury

WBCs infiltrate in tissue:

* After these inflammatory cells move into the tissue, sometime as pathologists we receive skin excision, appendix removed, tonsils removed, so we examine them and section them, then we stain them and we look at the tissue infiltrate and decide what type of inflammation is there, so if we see neutrophils, this indicate acute inflammation and those are short-lived and they don't stay more than 6 to 24 hours

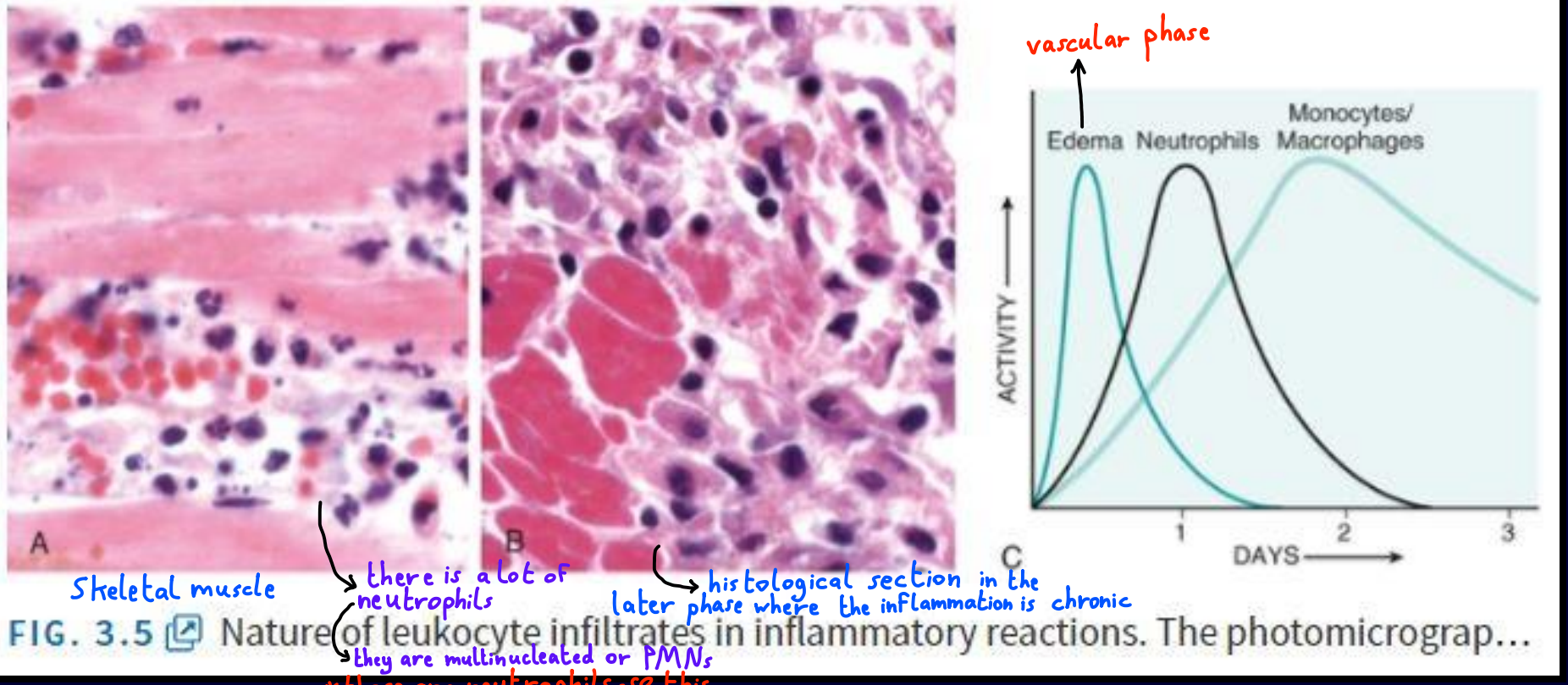
- **Depends on the age of inflammatory response and the type of stimulus**

Neutrophils (PMNs)	**6-24 hours, acute phase**
Macrophages and lymphocytes and plasma cells	**24-48 hours and then may stay**
Allergic reactions	**Eosinophils**

the whole mark of acute inflammatory cells and tissue

they come after the first day and sometimes they stay for a couple of days and weeks

they considered as allergic reaction tissue infiltrate, so whenever we have a nasal body from allergy, we see alot of nutrients there



Skeletal muscle

there is a lot of neutrophils

they are multinucleated or PMNs

histological section in the later phase where the inflammation is chronic

FIG. 3.5 Nature of leukocyte infiltrates in inflammatory reactions. The photomicrograp…

*those are neutrophils, so this is an acute inflammatory process which indicates that those changes have been there just the last 24 hours followed by leukocytes or macrophages

*We will postpone it because it is very complicated

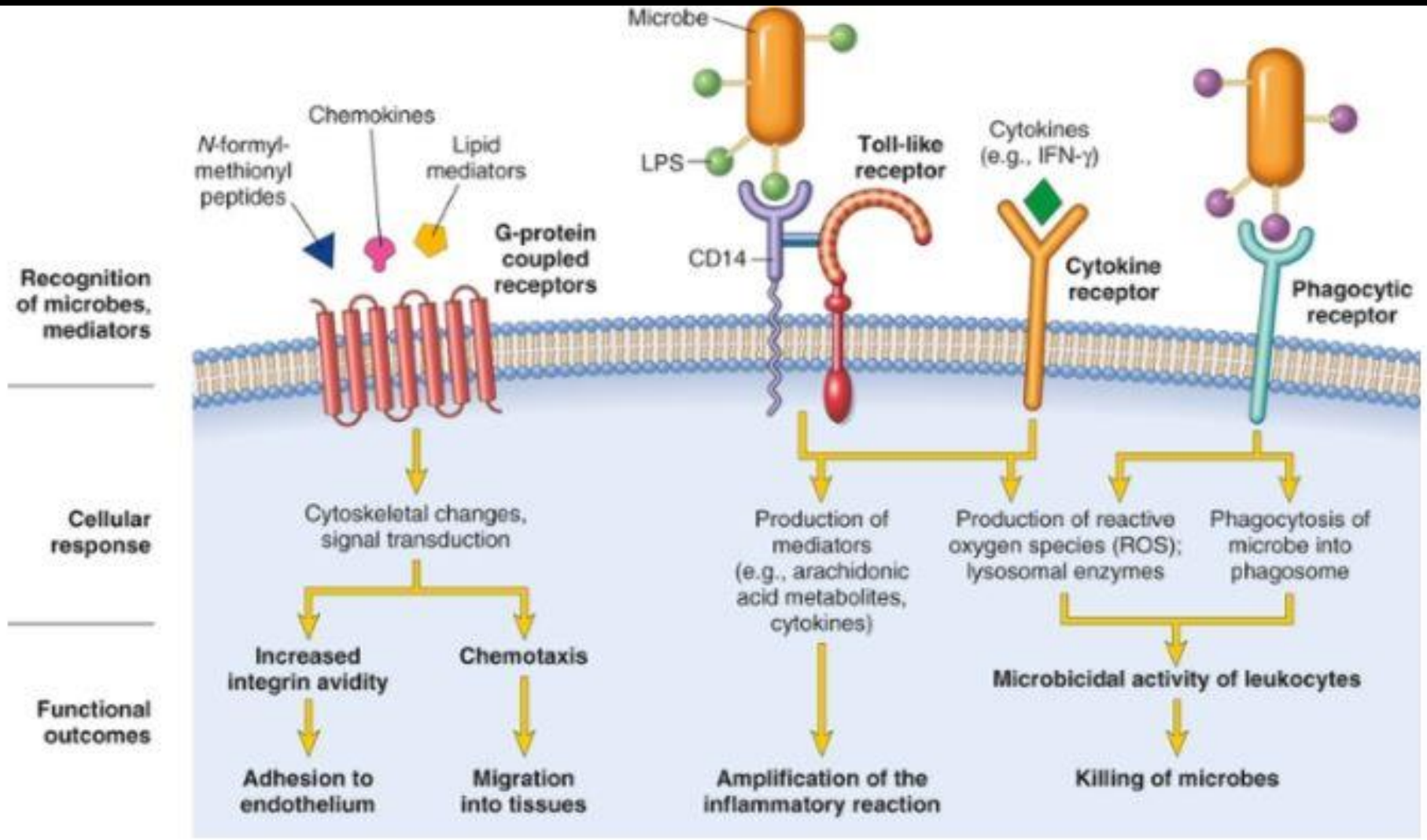


FIG. 3.6 Leukocyte activation. Various types of leukocyte cell surface receptors recogni…

LEUKOCYTE ACTIVATION:

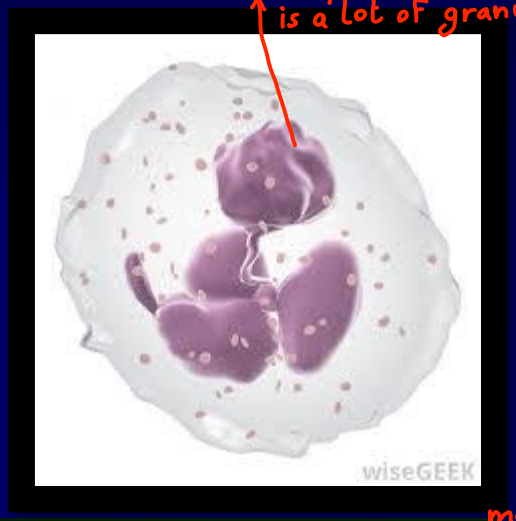
*It is an important Function in the initial phase of inFlammation

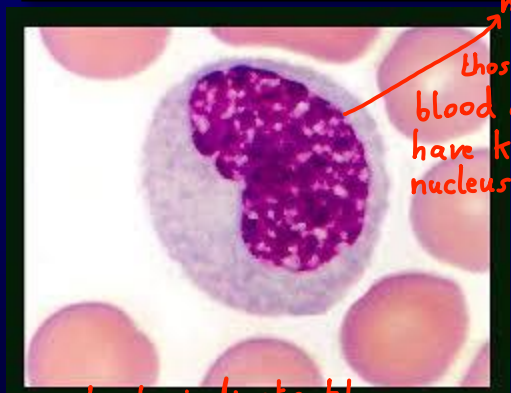
- **Phagocytosis and intracellular killing**

these are the main Function of those two cells when they recognizing the injury and enemy

*The two major cells of the initial phase of inFlammation;-

- **Neutrophils** ①
- **and monocytes** ②





*The macrophage is the same like monocyte, but we use the term monocyte to indicate the presence of circulating macrophages

PHAGOCYTOSIS:

*It is multi step process, it's not just one quick action, they include:-

- 1. Recognition and attachment of the enemy: mannose receptors; opsonins (IgG, C3b)
- 2. Engulfment forming phagocytic vacuole: phagosome
- 3. Killing & degradation: reactive oxygen species (ROS); NO. H2O2-MPO-halide is the most potent bactericidal system of neutrophils

(by specific receptors on the neutrophils or macrophages)
(invading foreign agent)
immunoglobulin
complement system
surface receptor
of foreign material and they will be sacked in phagosome
(a vacuole inside the cytoplasm of the inflammatory cell whether it's a neutrophil or a macrophage) (there is a bacteria inside it, they killing and degrdation then starts by an active process through recruitment of reactive oxygen species

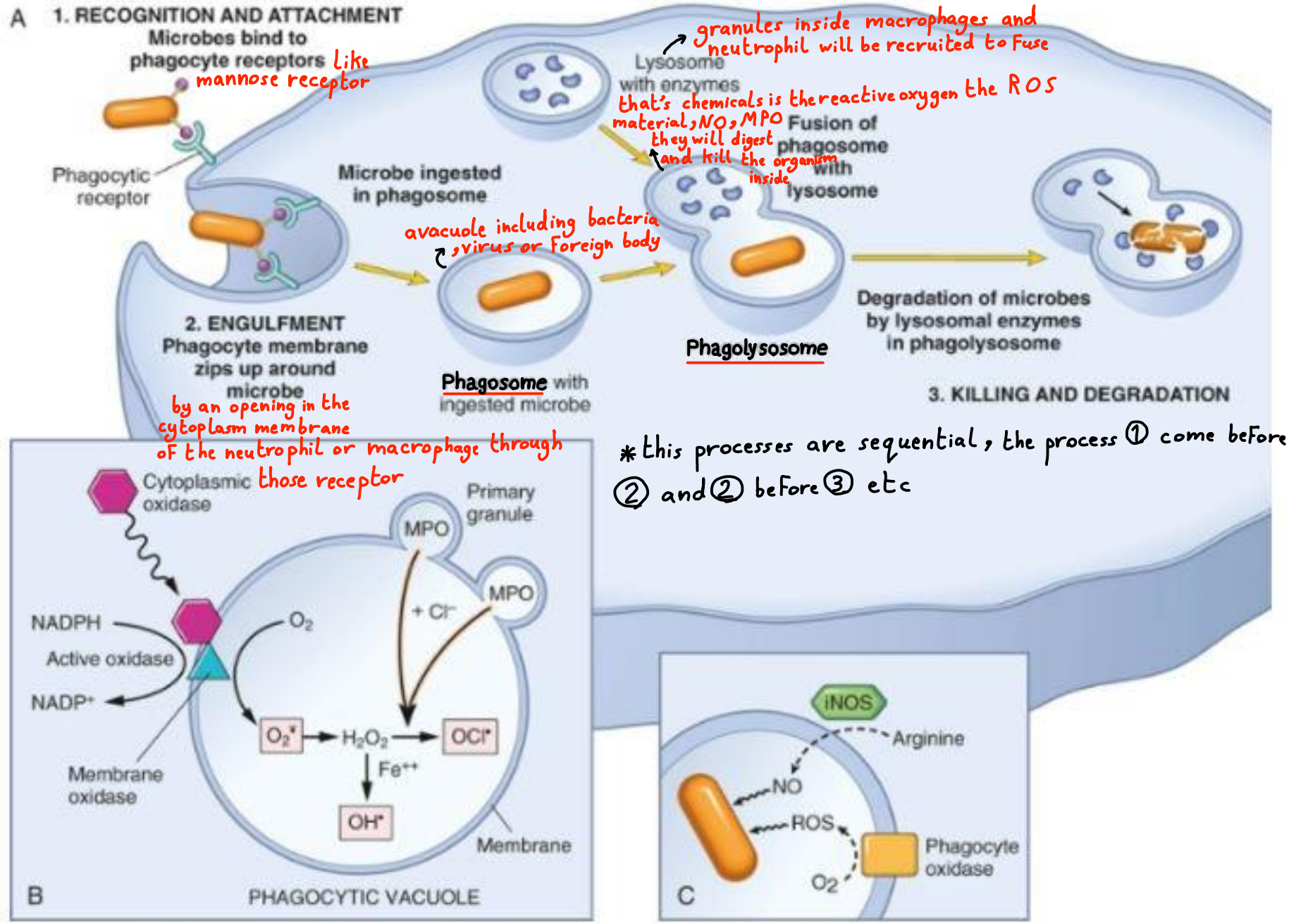


FIG. 3.7 Phagocytosis and intracellular destruction of microbes. (A) Phagocytosis of a p…

*In the last 10-15 year this attached alot of attention and research and there was alot of knowledge originated From the discovery of NO which is basically is a soluble gas which produced From arginine which is an amino acid by an enzyme called nitric oxide synthase (NOS), there are three types of nitric oxide enzymes

NITRIC OXIDE (NO)

- **Soluble gas produced from Arginine by NO synthase (NOS)**
- **NOS 3 types: eNOS, nNOS, iNOS**
- **iNOS: intracellular killing stimulated by cytokines mainly IFN-ɣ** (inter Feron - gamma) (it's one of the cytokins) (there's alot of research on the inter Feron) (now, they are being utilized also as a target therapy For certain inFlammatory autoimmune diseases and certain cancer treatment)
- **NO reacts with superoxide (O2-*) to form ONOO* radical peroxynitrite**
  ↳ one of the strong reactive oxygen radicals

* both neutrophil and macrophages have granules
* the neutrophil is one which is more heavily granulated, there are PMN

GRANULE ENZYMES

- Present in PMNs and monocytes
- In PMNs: 2 types; large azurophil (primary) and smaller (secondary) granules.
- Primary G: MPO, other enzymes
- Secondary G: lysozyme, and others
- These are usually neutralized by anti-proteases (such as ά-1 antitrypsin: inhibits elastase)…deficiency…diseases

azurophil was given because of the color of the dye reaction, but they are called primary granules, they are big

they have lysozymes and others and they are once which utilized after the production of the phagolysozyme in the later stages of phagocytosis

* they contain myoperoxidase and some enzymes which are needed in the intracellular killing

* those can be injurious if they aren't controlled, so we always have check and balances over them, so they are usually neutralized by antiproteases such as alpha-1-antitrypsin which inhibit elastases

* IF you have a certain diseases you're going to take especially in the GI tract α-1-antitrypsin, there are consequences because there will be no inhibition of those lysozymes and enzymes released by the neutrophils and the macrophages and this will induce injury and chronic diseases

*It is known in last 10 years

NEUTROPHIL EXTRACELLULAR TRAPS (NETs)

*very thick

- Viscous meshwork of nuclear chromatin binds peptides and anti-microbial agents after PMN death (NETosis)
- Sepsis
- Maybe involved in SLE

*IF the neutrophil died and ruptured the chromatin material and the intracytoplasmic and nuclear material outside, they will cause a very thick viscous meshwork of material which will help trap those bacteria or invaders at the site of injury, so that then they will be killed by other still viable neutrophil so this is an additional Function of neutrophil even after it dies, the products of chromatin material in particular will cause a viscous meshwork of nuclear of proteins helping to trap organisms at the site of injury, so they can be localized and killed by other colleagues which are still alive

*Recently, they discovered that neutrophil extracellular traps, they play a major role in the pathogenesis of sepsis and also they Found that there is a role in a disease which is called auto immune disease called systemic lupus erythematosus

مرض التذاؤب الإحترازي

affected young Females, they will have rash in their cheeks and it's multi system disease autoimmune disease attacks kidney and

hearts, skin and joint etc

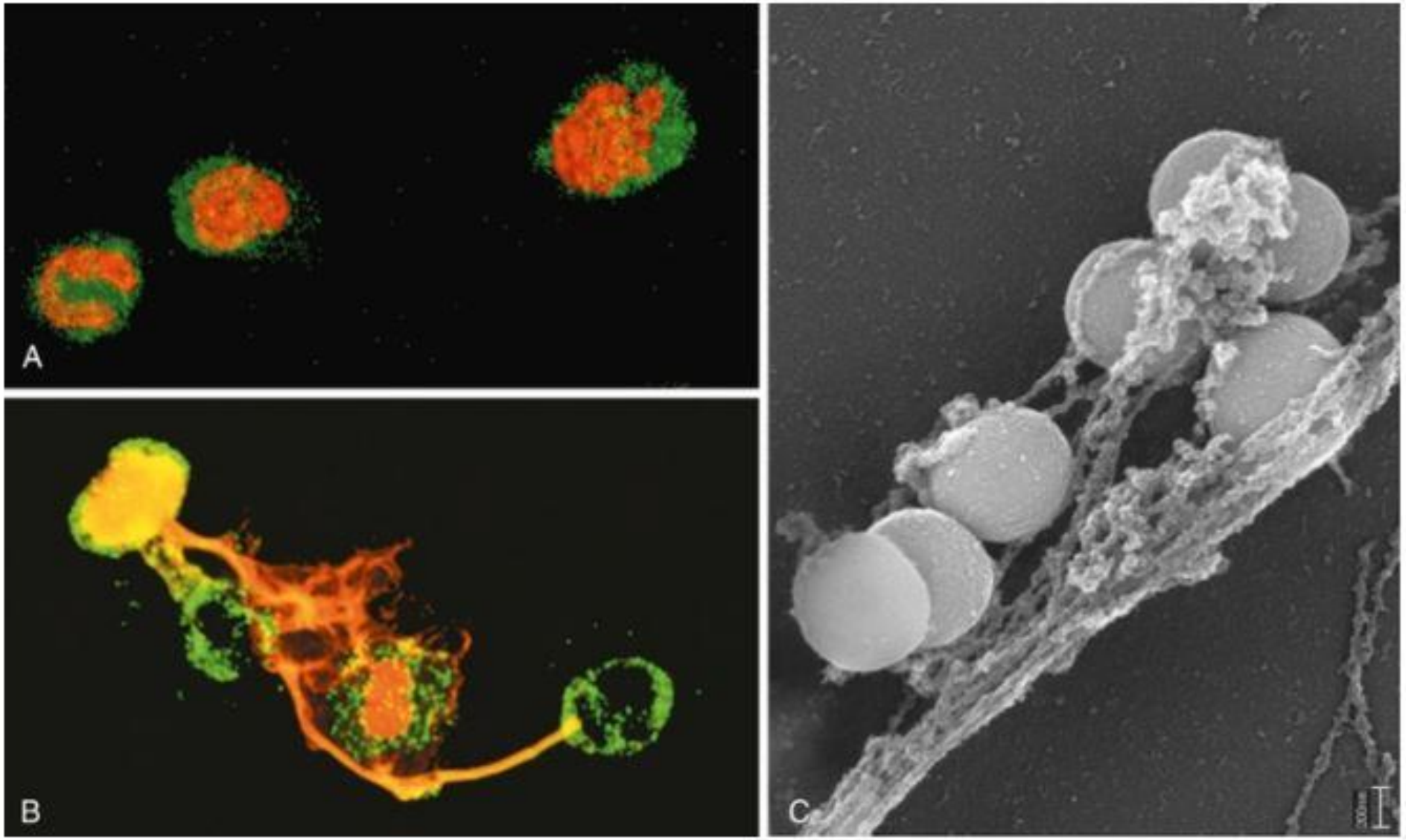


FIG. 3.8 Neutrophil extracellular traps (NETs). (A) Healthy neutrophils with nuclei stain…

*We need WBCs For your deFence mechanisms to help you get rid oF organisms
*however, there are somtimes injury related to the inFiltration oF leukocytes in tissue
*although we need them to protect us From invaders, viruses and bacterias, however iF there is too much response there will be some injury, each inFlammatory process can leave some side eFFect or tissue injury For example iF there is prolonged inFlammation specifically iF there is very virulent and strong organism Like tuberculosis (TB)

LEUKOCYTE-MEDIATED TISSUE INJURY

*How leukocytes can induce tissue injury:-

- **A. Prolonged inflammation (TB an Hepatitis)** → second example:- it will cause chronic liver disease From this prolonged inFlammation
- **B. Inappropriate inflammatory response (auto-immune diseases)** ↳ it induce your own tissue damage
- **C. Exaggerated response (asthma and allergic reactions)** تضخم

OTHER FUNCTIONS OF ACTIVATED WBCs

- **Amplify or limit reaction (cytokines)**
- **Growth factors secretion (repair)**
- **T-lymphocytes has also a role in acute inflammation (T-HELPER-17); produce cytokine IL-17 (deficiency cause disease)**

IF we need For example, in second, third phase of inflammation, we need actual amplification of the cytokines, we need amplification of the inflammatory process I need more solders, more recruitment, more chemotaxis, there are many bacterias, the enemy numbers is high

*IF the enemys was dead and I don't need any more army in the streets, they will produce cytokines to limit and contain and terminate the inflammatory reaction

*They are important actually in the last phase of inflammation when repair start

(CD For T cells)

a deficient where the immunity is decreased

which are the helper, they help in acute inflammation, not just T-helper-17 is a major player in acute inflammation, so opposite to what we thought initially that the T lymphocytes don't play a role in acute inflammation actually they discovered that T-helper plays a major role in acute inflammation and they produce a cytokine called interleukin-17

Lecture 4

TERMINATION OF ACUTE IR

*AFter we killed almost all the organisms in the First couple oF days oF inFlammation, I need to terminate because there are side eFFects, those enzymes, mediators can injure the tissue

*this processing is a major mechanisms to control inFlammatory responce eFFect

Mediators are produced in rapid bursts

Release is stimulus dependent

*iF there is no stimulus, there are no secretion oF mediator
*iF there is stimulus, there are secretion oF mediator

Short half-lives

* From seconds to minute
* It has a short halF-lives

Degradation after release

* there is mechanisms to digest or degradate these mediators

PMNs short life (apoptosis)

programed cell death
* RBCs has 120 days halF-live

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

secreted aFter step 3or4 to stopage the signal that's make stimulation
interLeukin
transFormig growth Factor

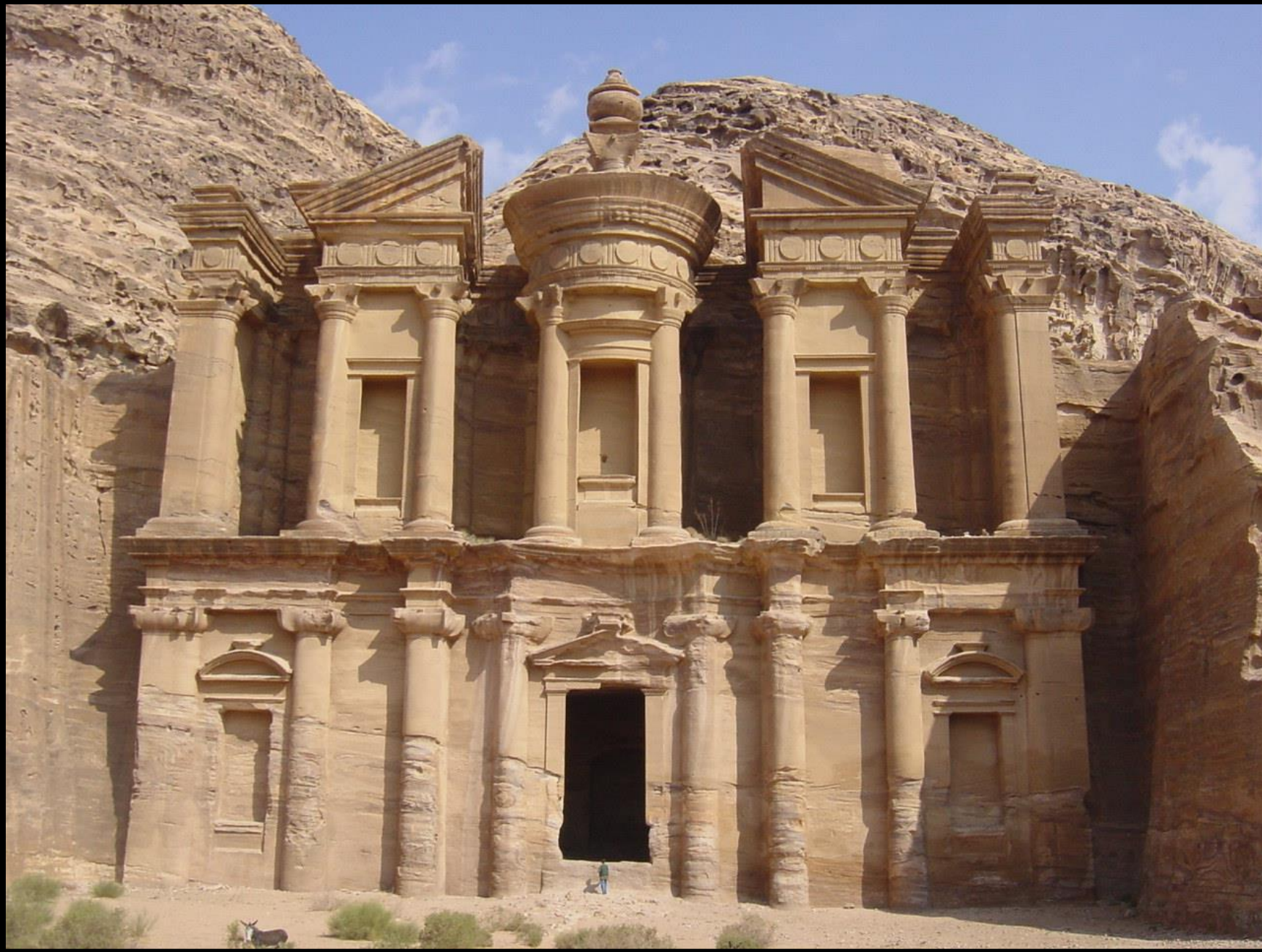
Neural inhibitors (cholinergic): inhibits TNF

↳ it's slightly passive
tumer necrosis Factor

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.



* there is no responce without mediators

MEDIATORS OF A. INFLAMMATION:

Tissue macrophages, dendritic cells & mast cells

* These are major group of mediators

Vasoactive amines	Histamine, serotonin
Lipid products	prostaglandin Leukotrienes PGs and LTs
Cytokines * it has a hundreds of mediators	interLeukin tumer necrosis Factor IL, TNF and chemokines
Complement activation	C1-9

GENERAL FEATURES OF MEDIATORS:

- Cell derived at the site: from granule release or synthesized upon stimulation
- Plasma proteins: needs activation
- Active mediators needs stimulation
- Most mediators have short life span
- One can activate the other

site of injury
present in PMNs and macrophages
*It secretes mediators
they are circulating in our blood in our serum
(not active)
(not passive)
and also one can inhibit the other

TABLE 3.5 Principal Mediators of Inflammation مهم/مطلوب حفظ

Mediator	Source	Action
Histamine (the main driver of vascular phase)	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Prostaglandins (that originate from arachidonic acid)	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

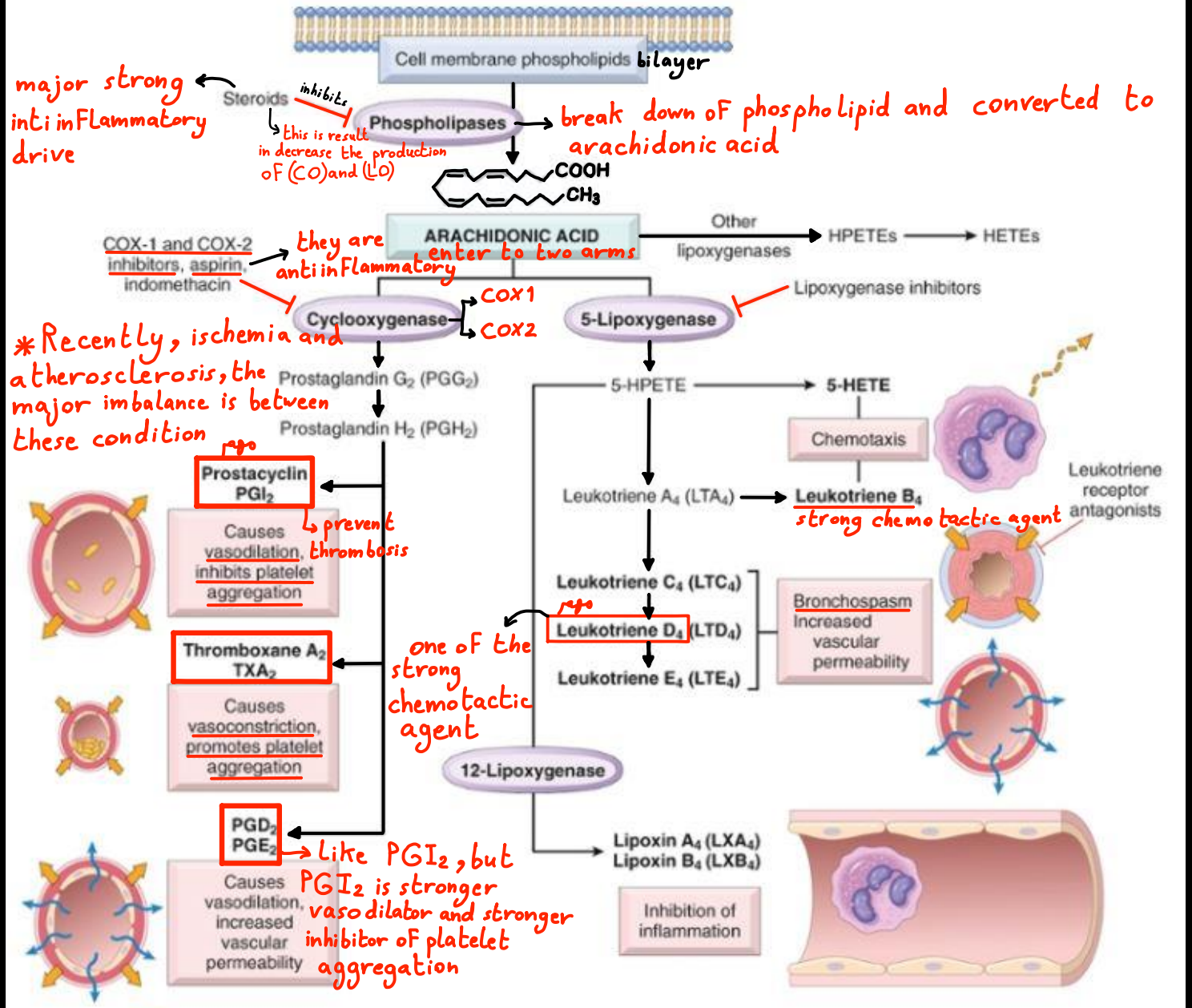


FIG. 3.9 Production of AA metabolites and their roles in inflammation. Clinically useful …

TABLE 3.6 Principal Actions of Arachidonic Acid Metabolites in Inflammation

Action	Eicosanoid (arachidonic acid metabolized)
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 (occur in child birth)	Prostaglandins PGC4, PGD4, PGE4

* an imbalance in production of PGI_2 and thromboxane A_2 is probably the major pathogentic mechanism of ischemic diseases in heart, brain etc
* among the arachidonic acid metabolised, the strongest chemotactic is leukotriene D_4

POINTS TO REMEMBER ABOUT AA METABOLISM:

- Aspirin – cycloxygenase (inhibition)
- Steroids – phospholipase and anti inflamm
- Prostacyclin (PGI2): vasodilator and – Pl aggreg
- Thrombaxane A2: vasoconstrictor and + pl aggreg

opposite to each other

* the imbalance between them is the ischemic heart disease and celebru vascular accident

- TXA2-PGI2 imbalance: IHD & CVA

ischemic heart disease

- PG (PGE2): pain & fever

CYTOKINES:

- **Proteins secreted by many cells (activated lymphocytes, macrophages and dendritic cells)**
- **Mediate and regulate 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.

*The mediators that are secreted are mainly cytokines

ago

dangerous

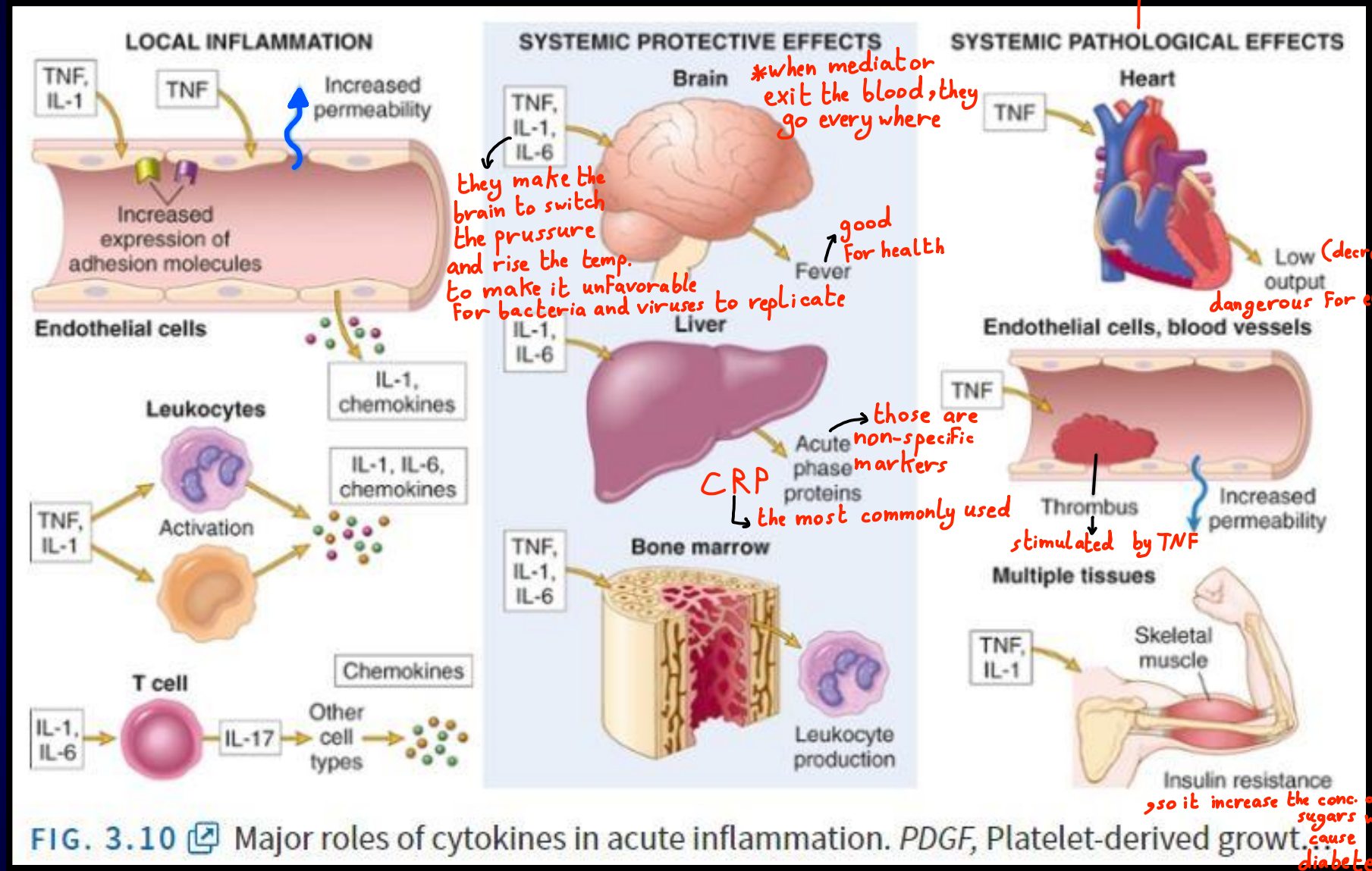


FIG. 3.10 Major roles of cytokines in acute inflammation. *PDGF*, Platelet-derived growt...

*a small Family From cytokines

CHEMOKINES:

*they called chemokines because it's a strong chemoattractants agent

- Small proteins, mainly chemoattractants
- 40 different and 20 receptors
- 4 groups: C-X-C; C-C; C; CX_3-C
- They have G-protein coupled receptors
- 2 main functions: A inflammation & maintain tissue architecture

*some drugs synthesized to block the receptors

*they are big Family

*It is very important

*It has intracellular called G-receptor and extracellular

*the mediators stimulate For extramembrane component and transFer the message to intra component

(make it preserve)

COMPLEMENT SYSTEM:

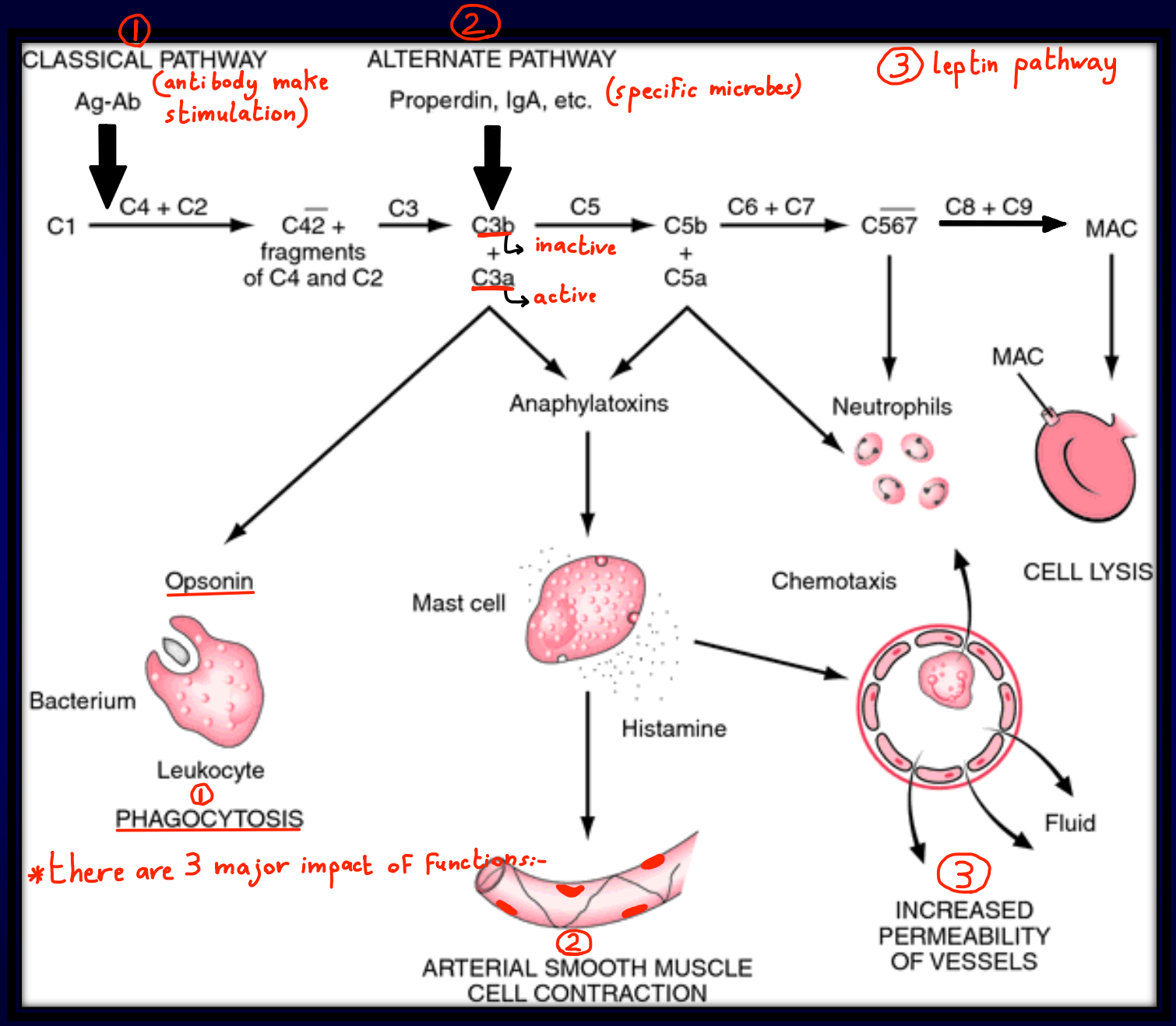
* It is part of our innate immunity
* they are small protein circulating in blood

- Soluble proteins (inactive) needs activation
- More than 20, C1-C9 (1970)
- Innate & adaptive immunity
- Functions: vascular permeability, chemotaxis & opsonization (in serum)
- C3 is most abundant; cleavage of which is the critical in all pathways

* those are mediators From any where, they are part of complement system, they help neutrophile and marcophage to enhance phagocytosis activity and intensity, so if we have defeciency of complement system the killing and phagocytosis will be weak and a diseases happened

It is the main gate keeper among three stimulation

* C3 activate the complement system in 3 mechanisms :-



* there are 3 major impact of functions :-

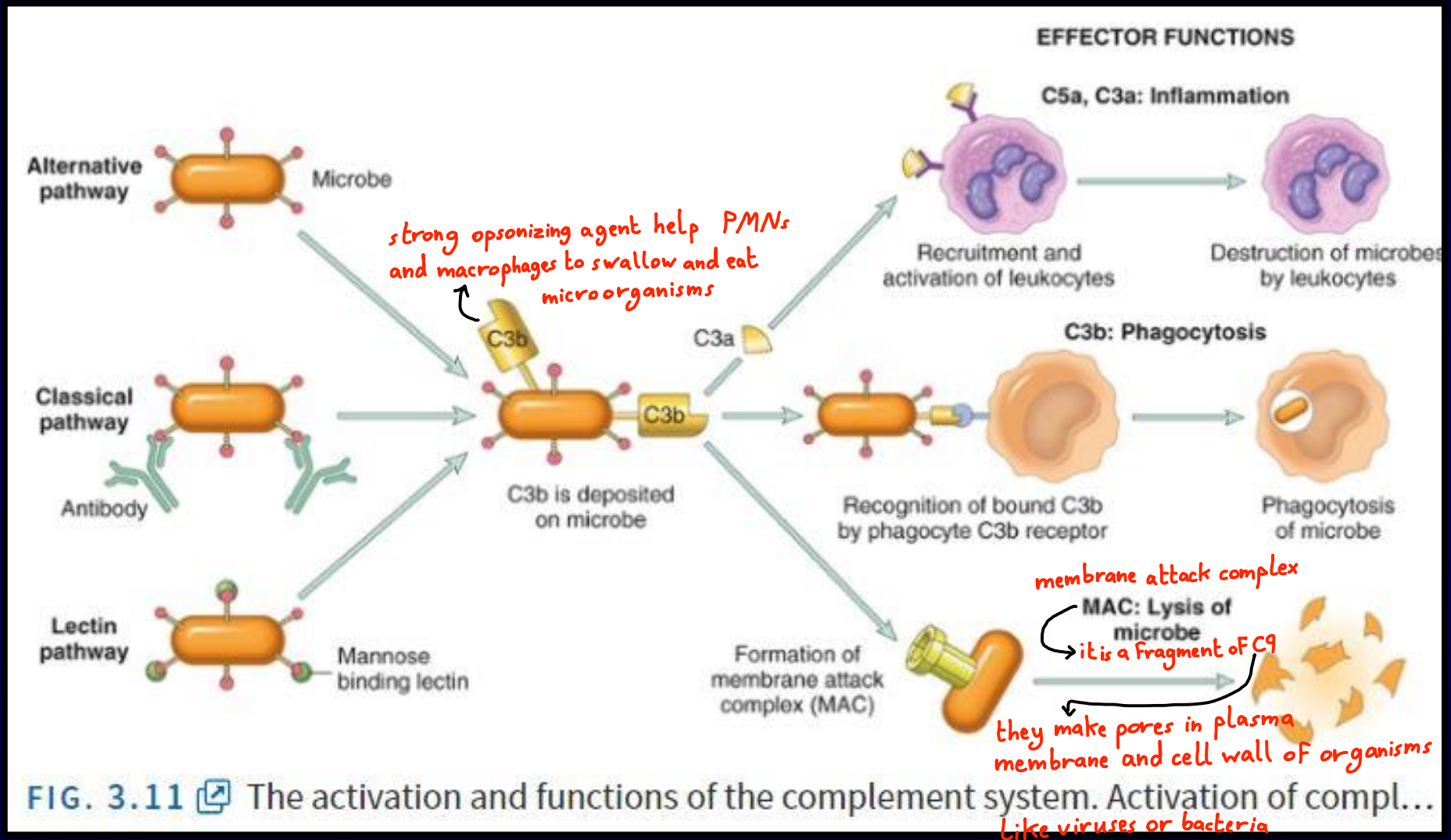


FIG. 3.11 The activation and functions of the complement system. Activation of compl…

C S FUNCTIONS:

- **Inflammation: histamine like, anphylatoxins (C5a).** الحساسية
- **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** (severe edema in the upper of respiratory system)
- **Decay accelerating factor (DAF), which inhibit C3 convertases and CD59 inhibits MAC Abnormalities cause PNH** paroxysmal nocturnal hemoglobinuria
- **Factor H: proteolysis of C3 convertase; mutations cause hemolytic uremic syndrome**
- **CS protein deficiencies can occur leading to infection susceptibility**

اللهم انصر أهل غزة وثبت أقدامهم. اللهم احرس أهل غزة بعينك التي لا تنام. اللهُم كُن لأهل غزة عونًا ونصيرًا، وبدّل خوفهم أمنًا. اللهم اجعل لأهل غزة النصرة والعزة والغلبة والقوة والهيبة.