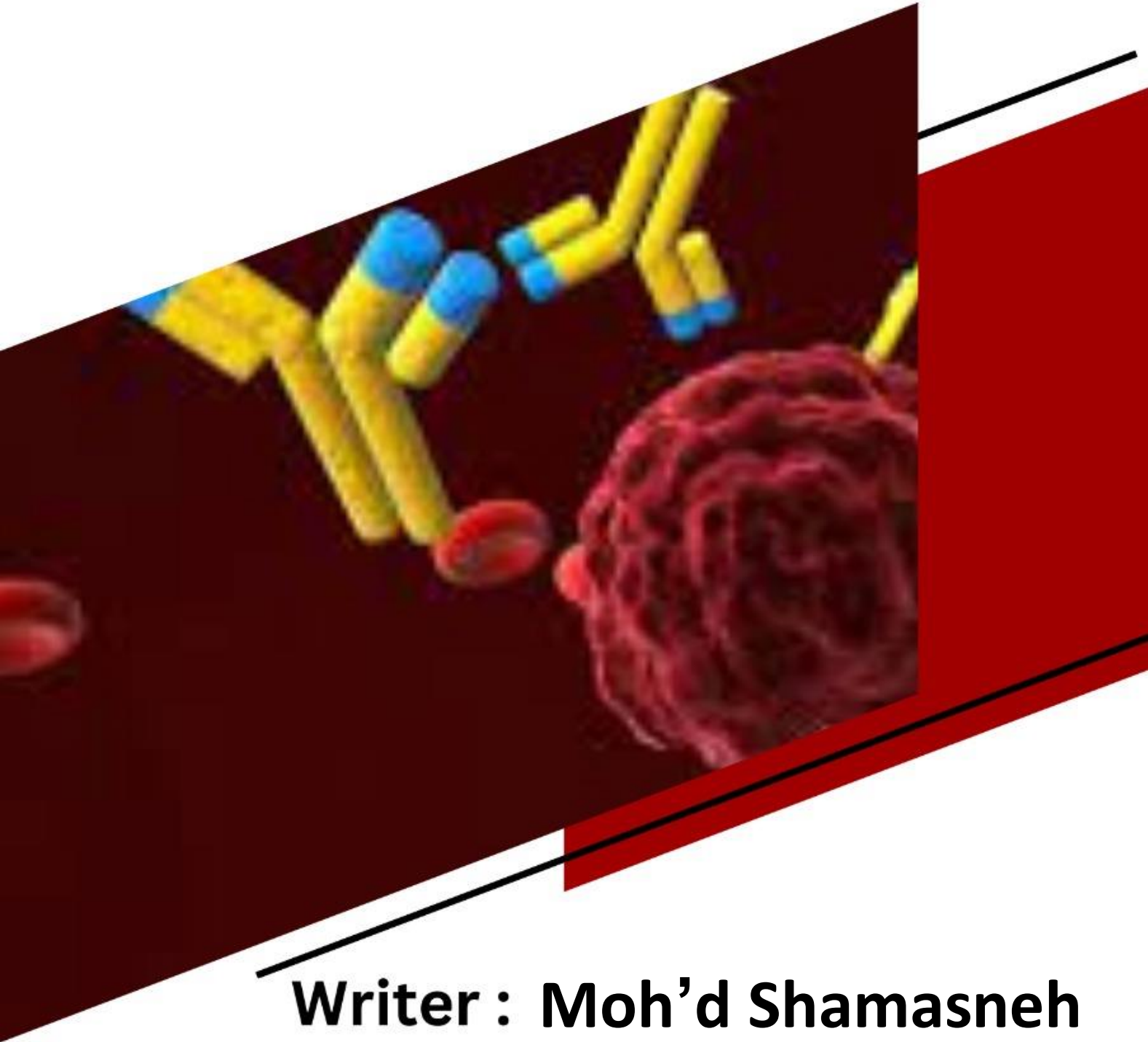


Doctor 021

IMMUNOLOGY

Sheet no.6



Writer : Moh'd Shamasneh

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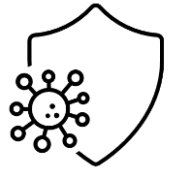
Doctor : Anas Abu Hmaidan

INTRODUCTION

In the previous lectures we talked about the cell & tissues of the immune system , in the last lecture we started discussion about the molecules and mediators of the immune system that orchestrate (arrange) the immune response between the immune system & invading foreign microbes or the dead tissue .

Remember : two main types of immunity :

1. The innate immunity (not specific).
2. The adaptive immunity (specific to pathogen).



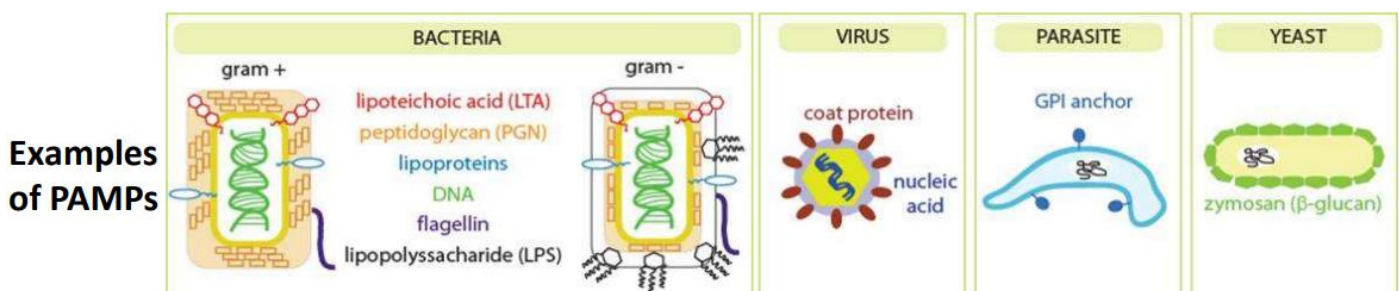
These 2 types cannot be separated , they are connected , for example , by APC cells (as you remember) .

- **Much of the interactions between cells of the immune system, and between the immune system and foreign introducers depend on the action of cell bound and secreted molecules.**
- **The cells and soluble molecules of innate immunity either exist in a fully functional state before encounter with microbes or are rapidly activated by microbes.**
- **The innate immune system recognizes microbial products that are often essential for survival of the microbes**
- **The innate immune system recognizes molecular structures that are characteristic of microbial pathogens but not mammalian cells.**

For ex. cell wall is essential for the survival of bacteria, because it contains structural proteins & glycoproteins & lipids that are important for survival of bacteria, so bacteria can't change them .

We call these molecular structures “**molecular patterns**” , and they consist of two types :

1. **Pathogen associated molecular patterns (PAMPs)** ; antigens coming from pathogens that is recognized by the body.



2. **Damage associated molecular patterns (DAMPs)** ; endogenous molecules found in the nucleus, such as transcription factors, to be located extracellularly indicates that there's damage going on . the immune system recognize these transcription factors as DAMPS.

- **DAMPs may be produced as a result of cell damage caused by infections, but they may also indicate sterile injury to cells caused by any of myriad reasons, such as chemical toxins, burns, trauma, or decreased blood supply.**

Different classes of microbes (e.g., viruses, gram-negative bacteria, gram positive bacteria, fungi) express different PAMPs.

- **DAMPs are generally not released from cells dying by apoptosis. In some cases, healthy cells of the immune system are stimulated to produce and release DAMPs, which enhances an innate immune response to infections.**

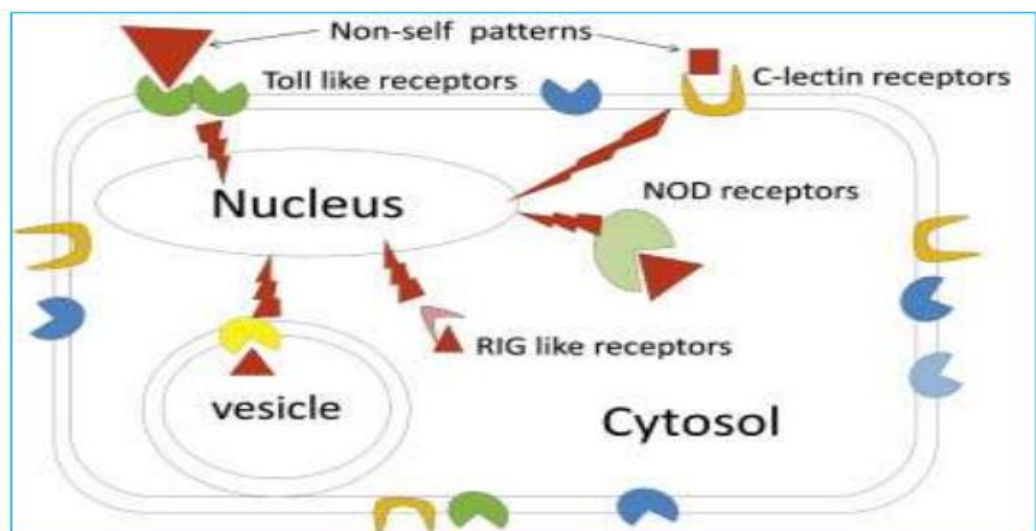
The immune system sense these patterns through **Pattern Recognition Receptors (PRRs)** ;

They play a crucial role in the proper function of the innate immune system. PRRs are germline-encoded host sensors, which detect molecules typical for the pathogens.

They are proteins expressed, mainly, by cells of the innate immune system, such as dendritic cells, macrophages, monocytes, neutrophils and epithelial cells, to identify two classes of molecules: PAMPs and DAMPs.

PRR can be cell bound or soluble (in the cytosol \ endosomes).

Examples of cellular PRR



Examples of soluble PRR are:

- Natural antibodies
- complement proteins.



هون بئس الجد

SOLUBLE PRR & EFFECTOR

General look

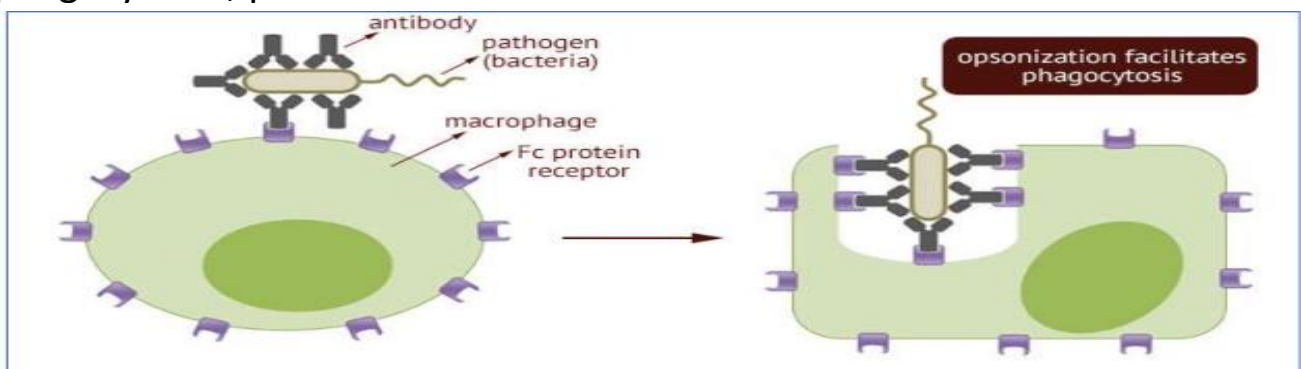
FUNCTION:


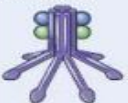



- These molecules provide early defense against pathogens that are present outside host cells at some part of their life cycle. The soluble effector molecules function in two major ways:
- By binding to microbes, they act as opsonins and enhance the ability of macrophages, neutrophils, and dendritic cells to phagocytose the microbes. This is because the phagocytic cells express membrane receptors specific for the opsonins.
- After binding to microbes, soluble mediators of innate immunity promote inflammatory responses that bring more phagocytes to sites of infections, and they may also directly kill microbes.

Wait a minute, what do you mean by **Opsonins** or **Opsonization** ?

- **Opsonization** is the molecular mechanism whereby molecules, microbes, or apoptotic cells are chemically modified to have a stronger attraction to the cell surface receptors on phagocytes and NK cells.
- **Opsonins** include antibodies and complement proteins.

More explanation ; the antibody consists of two portions (upper portion that contains the light chains and lower portion containing the heavy chains) ,When the pathogen come , it is covered by the upper portion of several antibodies , leaving the lower portion free to interact with the phagocyte or macrophage's receptor to enhance the process of phagocytosis , please look downward.



Soluble Recognition Molecules	Location	Specific Examples	PAMP Ligands
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	<i>N</i> -Acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	C3	Microbial surfaces
Natural antibodies 	Plasma	IgM	Phosphorylcholine on bacterial membranes and apoptotic cell membranes

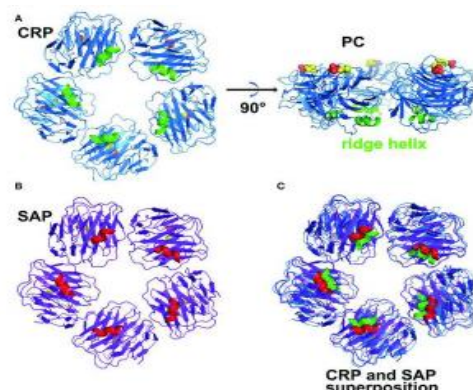
1. Natural antibodies .

These antibodies differ from antibodies which are produced by B cells after recognition to antigen (which is a part of adaptive immunity) , this kind of antibodies is a part of innate immunity .

- There are subsets of B cells that produce antibodies with only a limited number of specificities without overt exposure to foreign antigens, and these are called natural antibodies. (different from adaptive immunity antibodies).
- They recognize common molecular patterns on microbes or stressed and dying cells. Their specificity is similar to PRR, recognize a few and common PAMPs and DAMPs.
- Natural antibodies are usually specific for carbohydrate or lipid molecules but not proteins, and most are IgM antibodies, one of several structural classes of Ig molecules.

2. Pentraxin family (pent = 5 subunits) .

- The pentraxin family, which is a phylogenetically old group of structurally related pentameric



proteins. Prominent members of this family include the short pentraxins C-reactive protein (CRP) and serum amyloid P (SAP) and the long pentraxin PTX3.

- Both CRP and SAP bind to a few PAMPs and DAMPs, and can bind C1q and initiate the classical pathway.
- Plasma concentrations of CRP are very low in healthy individuals but can increase up to 1000-fold during infections and in response to other inflammatory stimuli.
- Some of those proteins that increase in concentration following inflammation are called acute phase reactants / acute phase proteins.

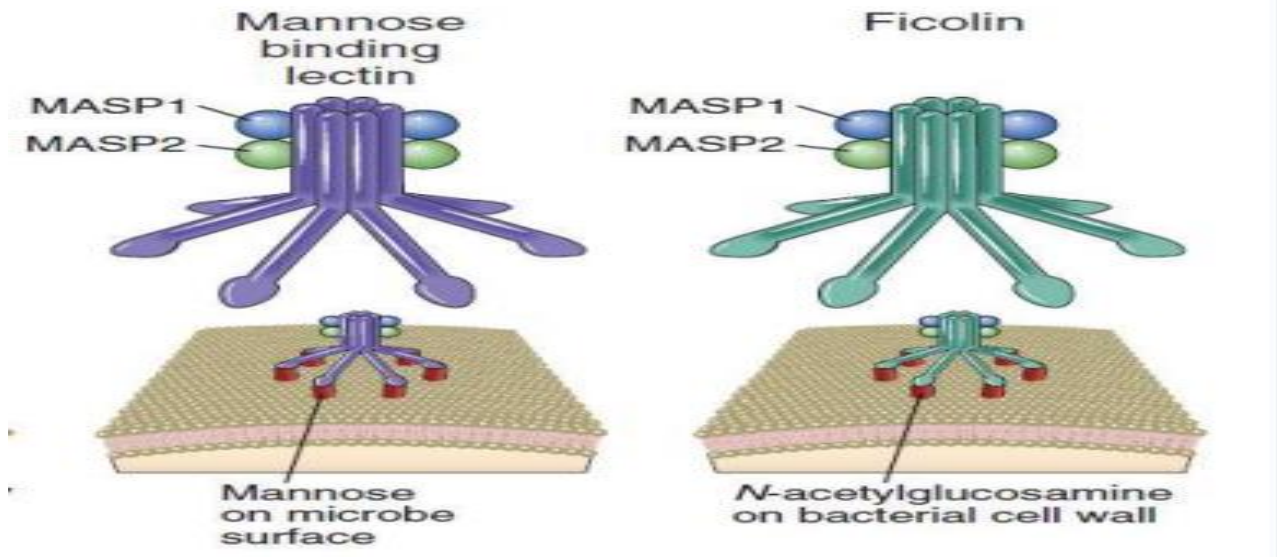
➤ **Acute phase proteins :**

- **Acute-phase proteins (APPs) are a class of proteins whose plasma concentrations increase in response to inflammation. This response is called the acute-phase reaction.**
- **In response to injury or infection, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL1, and IL6, and TNF α . The liver responds by producing a large number of acute-phase reactants.** These cytokines accumulate in the blood and have systemic effects that are typically seen in infection:
 - Brain; have an effect on the hypothalamus causing fever.
 - Liver; induce hepatocytes to release acute phase proteins.
- **Measurement of acute-phase proteins, especially C-reactive protein, is a useful marker of inflammation in medical clinical pathology.**

3. Collectins and ficolins: recognize sugars and activate complement system .

- The collectins are a family of trimeric or hexameric proteins, each subunit of which contains a collagen-like tail connected by a neck region to a calcium-dependent (C-type) lectin head.

- MBL, which is a soluble pattern recognition receptor that binds carbohydrates with terminal mannose and fucose, activates the lectin pathway of complement activation.
- Ficolins are plasma proteins that are structurally similar to collectins, possessing a collagen-like domain, but instead of a C-type lectin domain, they have a fibrinogen-type carbohydrate recognition domain.



Doctor Anas did not go into details , he just said that “ **they are soluble pattern recognition receptors , recognize special type of PAMPs which is SUGAR (mannose , does not exist in mammalian cells) . MBL & ficolin can both activate complement system**”.

4. Complement system

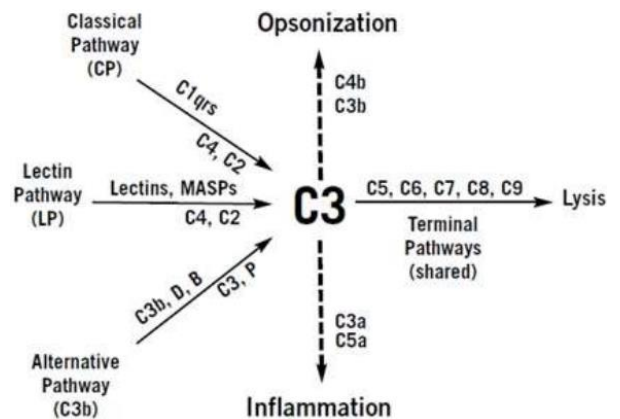
The complement system is a group of proteins that circulate the blood in inactive form, until a pattern is sensed with proteins like (C1q, Lectins) which leads to a series of reactions of protein cleavage and activation.

- It has functions that go beyond the borders of innate immunity, it has a general role in homeostasis (which is also a function of the immune sys.).
- One protein activates the other and so on, in some cases two proteins form an enzyme in order to become more efficient in cleaving downstream proteins, these cleaved and activated proteins are the effectors; opsonization, promote inflammation and induce lysis.

➤ Complement has the following functions:


- Opsonization of the pathogen (or a dead cell) to ease phagocytosis (C3b, C4b).
- Generation of anaphylatoxins (C3a and C5a) to draw in leukocytes and potentiate the immune response.
- Formation of a pore in the bacterial cell wall called MAC (membrane attack complex, C5b-9).

➤ Complement deficiencies lead to increased susceptibility to infections. And is also associated with autoimmune diseases like systemic lupus erythematosus (SLE), indicating a role for complement in maintaining homeostasis.



Important

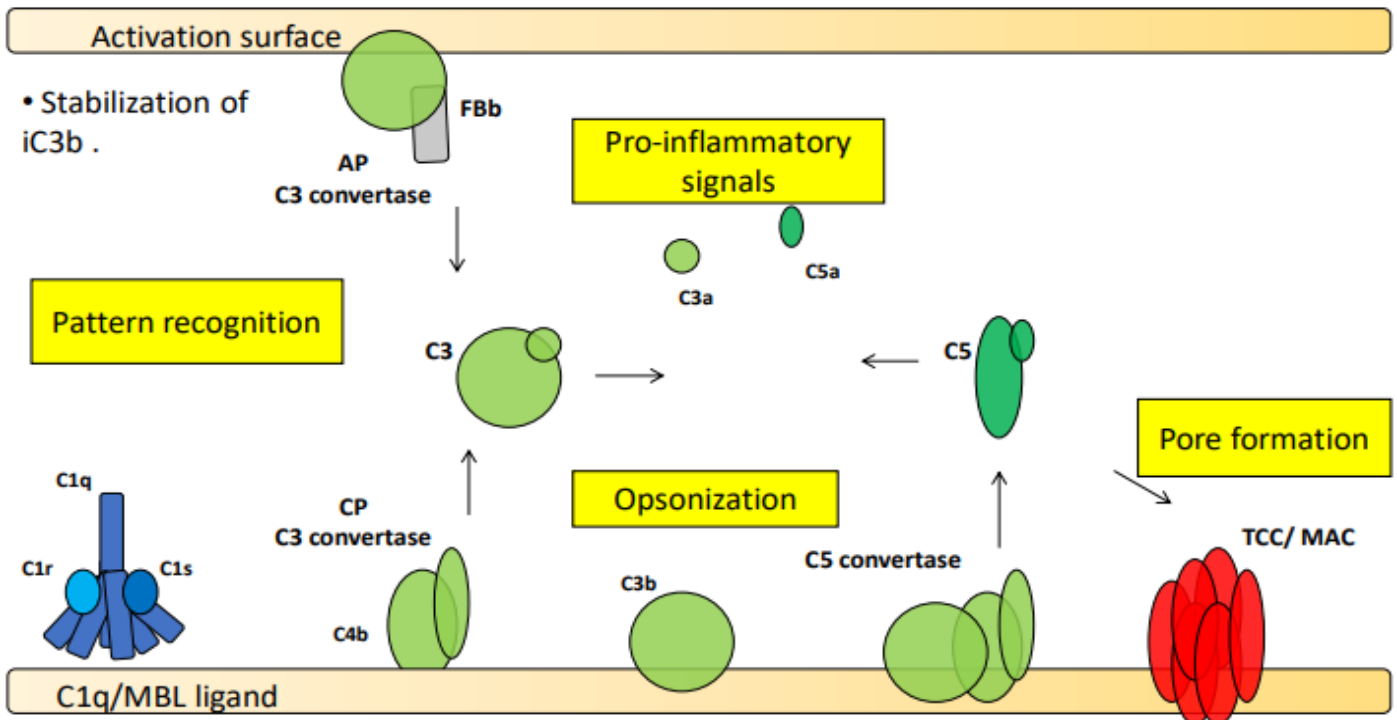
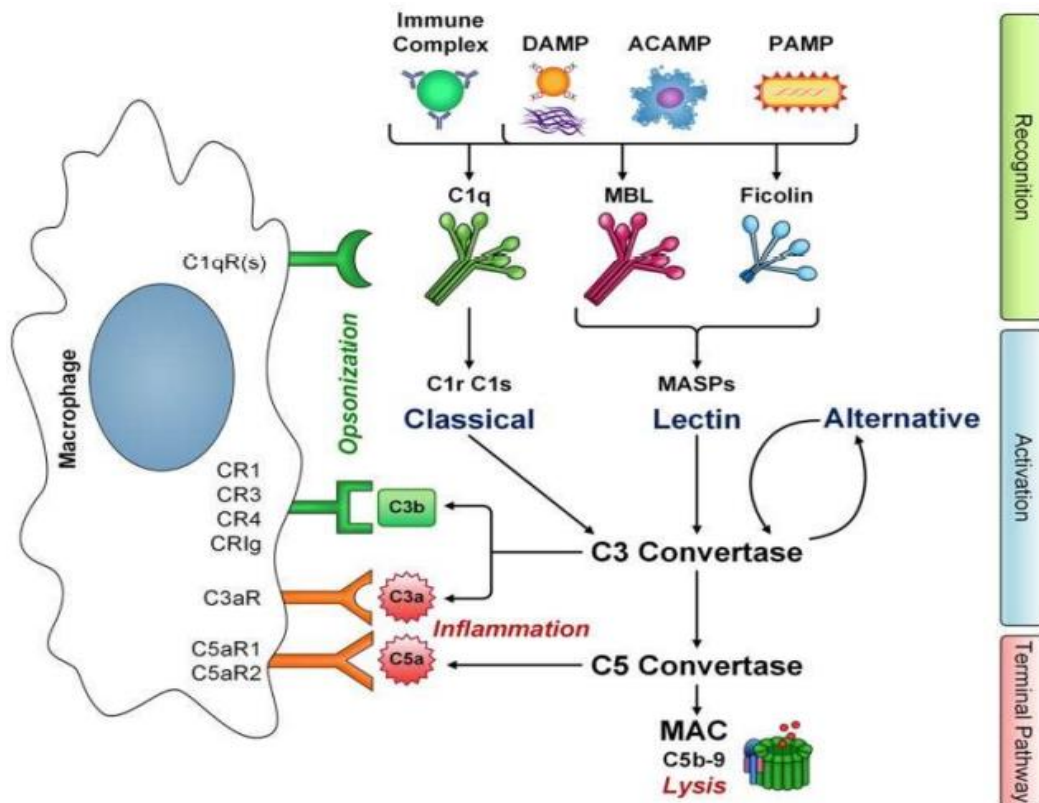
Hi doctors , how is my sheet going with you ?

This following topic (complement system) will not be correctly understood if you do not watch the recorded lecture . so make some effort  and back to the 6th lec. On JU medicine 021 , best wishes .



- 3 pathways of complement activation depend on different PRR but converge at C3 activation.
- A C3 convertase is formed from activated complement proteins, In the classical and lectin pathways, C3 convertase is made from C2bC4b, while in the alternative pathway, it's made from C3bFb.
- Each step of complement activation is regulated by soluble and cell surface proteins.

These 2 coming diagrams will improve your knowledge



More explanation about **the classical pathway** :-

PAMPs and the other proteins associated, work together to activate **C1q**, **MBL & Ficolin** (all structurally similar).

C1q binds to FC portion of the antibody, activating C1r & s that cleave C2, C4 forming C3 convertase (classical pathway).

MBL and **Ficolin** detect PAMPs & DAMPs, activating MASPs (associated serine proteases) forming the C3 convertase.

the C3 convertase cleaves C3 into 2 fragments. If the pathway continues after C3, a C5 convertase will be formed which cleaves C5 into 2 fragments as well:

- **C5a**, lighter fragment involved in inflammation .
- **C5b-9**, heavier fragment which binds to cell surface forming a scaffold (C5,C6,C7,C8) for C9 proteins attachment which form a pore inducing lysis. TCC or MAC, membrane attack complex.

The alternative pathway : (It works if another pathway works)

C3b formed can attach to blood factor B, forming C3 convertase, **differs** in composition from the one formed in classical pathway.

It can work independently by the unstable form of C3b, iC3b, (differs from the inactive form) which doesn't attach to normal host cells but to bacterial or any activated surface which stabilize it. The iC3b in some way is a pattern recognition molecule since it recognizes different surfaces.

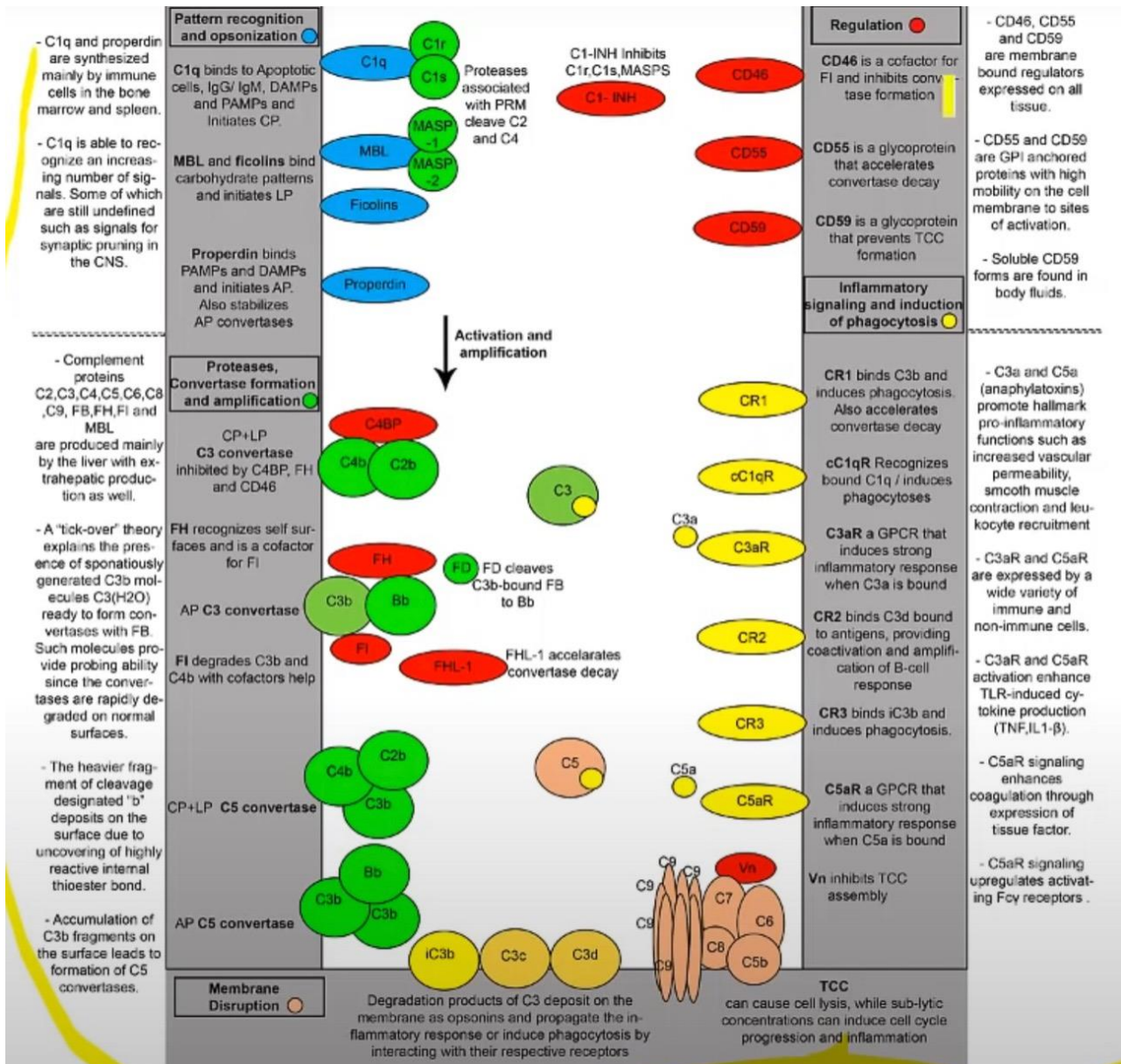
- ❖ The previous 2 pathways keep activating themselves by C3b and convertases formation on bacterial surfaces but if they occur on host surface, complementary regulatory proteins will be found .

➤ The following is used as a reference.


✓ **The red circles** in the right are the regulators of complement system :

CD46 (receptor for entry of certain viruses) and **CD55** both inhibit convertases, **CD59** (cell bound) Factor H, inhibits convertases and involved in other inflammatory diseases .


- ✓ **The yellow circles** (on right side) are the receptors involved in inflammation: C5a, C3a and C3b for phagocytosis since it is an opsonin.



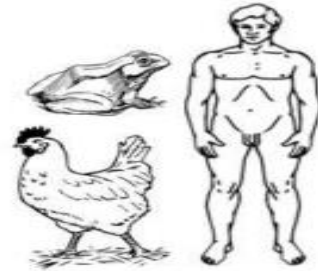
These coming figures were not mentioned by Dr. Anas during the lecture , he just insert them in his slide , so عشان أبري ذمتي I insert them here too .



Sea urchins present around 500 million years ago have 2 components with significant homology to vertebrate C3 and factor B (Bf), called SpC3 and SpBf, respectively.
Those components found in body fluid can be induced in response to immune challenge and are thought to represent a primitive alternative pathway.

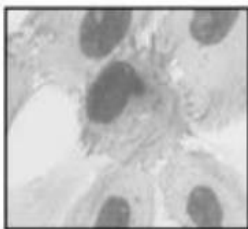


Lamprey is an early vertebrate that lacks immunoglobulins and thus a classical pathway.
An orthologue of C1q (LC1q) that acts as a GlcNAc-specific lectin is expressed in lamprey.
LC1q does not have components to bind to immunoglobulins and could represent a predecessor to the classical pathway



Higher vertebrates including Mammals, Aves and Amphibia share a very similar set of complement genes, with sporadic absence of some genes like C2 and C9 in chicken or the amphibian C1 inhibitor in frogs.

Cancer



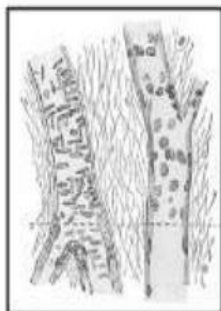
- Deposition of activation fragments including TCC in breast cancer tissue, but not benign lesions.
- Upregulation of complement regulatory proteins in several solid tumors.
- Increased levels of activation fragments including TCC in serum of oral carcinoma patients compared to healthy subjects.

Acute macular degeneration (AMD)

- Genetic variations in certain complement proteins like factor H confers risk to develop AMD.
- Drusen in AMD (subretinal pigment epithelial deposits) contain complement activation fragments.
- Increased levels of activation fragments in plasma of AMD patients compared to controls.



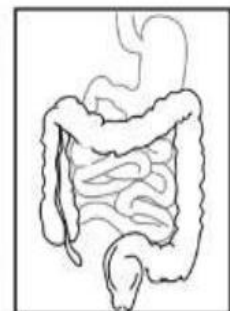
Ischemia/ reperfusion (I/R) injury



- Local deposition of complement fragments and release of anaphylatoxins following I/R.
- Complement inhibition decreases necrotic damage following myocardial infarction in pig models.
- Factor B knockout mice have less functional and morphological renal damage following I/R.

Inflammatory bowel disease

- Increased secretion of C3 and C4 in Crohn's patients intestine, including non-lesional parts.
- Abnormal complement activity in relatives of Crohn's patients.
- Increased levels of complement regulator CD55 in stool of ulcerative colitis patients correlates with disease activity.



THANK YOU