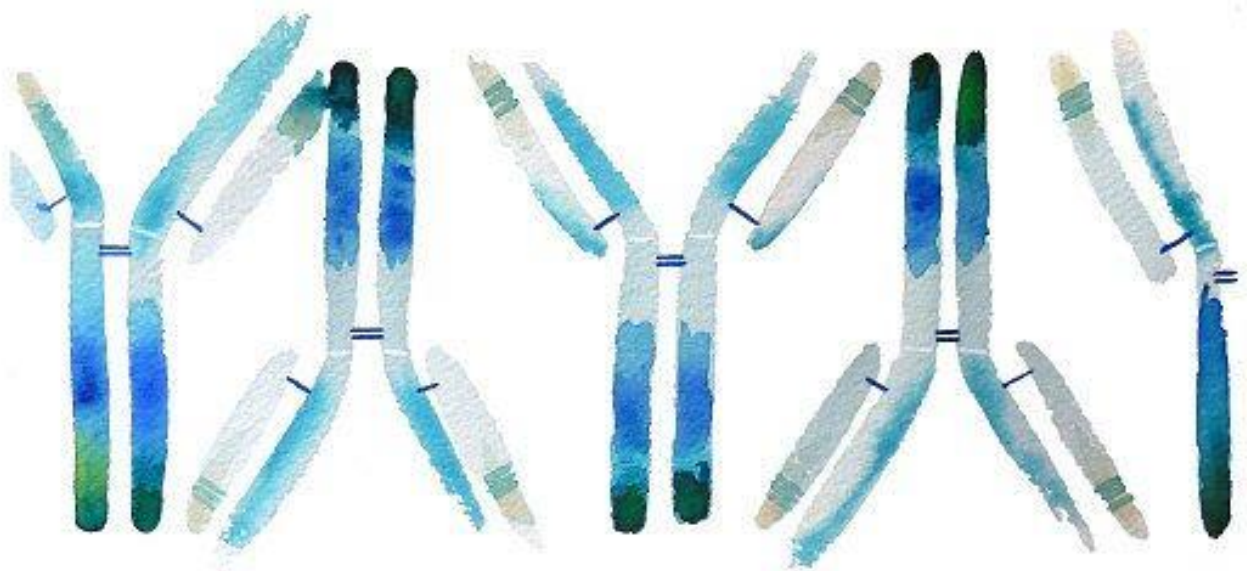
Medical Immunology



Anas Abu-Humaidan
M.D. Ph.D.

Lecture 6

Molecules of the immune system

- 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.

- In this lecture we will discuss some of those molecules.

- Main topics:

Soluble PRR
The complement system
Interferons

- 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 molecular structures that are **characteristic of microbial pathogens but not mammalian cells.**
- The innate immune system recognizes microbial products that are often **essential for survival of the microbes.**
- The microbial substances that stimulate innate immunity are called **pathogen-associated molecular patterns (PAMPs).**
- Different classes of microbes (e.g., viruses, gram-negative bacteria, gram positive bacteria, fungi) express different PAMPs.

- The innate immune system also recognizes endogenous molecules that are produced by or released **from damaged and dying cells**. These substances are called **damage associated molecular patterns (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**.

- 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.

TABLE 4–2 Examples of PAMPs and DAMPs

Pathogen-Associated Molecular Patterns		Microbe Type
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG	Virus, bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fungi, bacteria
	Dectin glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	
Crystals	Monosodium urate	
Nuclear proteins	HMGB1	

CpG, cytidine-guanine dinucleotide; dsRNA, double-stranded RNA; HMGB1, high-mobility group box 1; HSPs, heat shock proteins; LPS, lipopolysaccharide; ssRNA, single-stranded RNA.

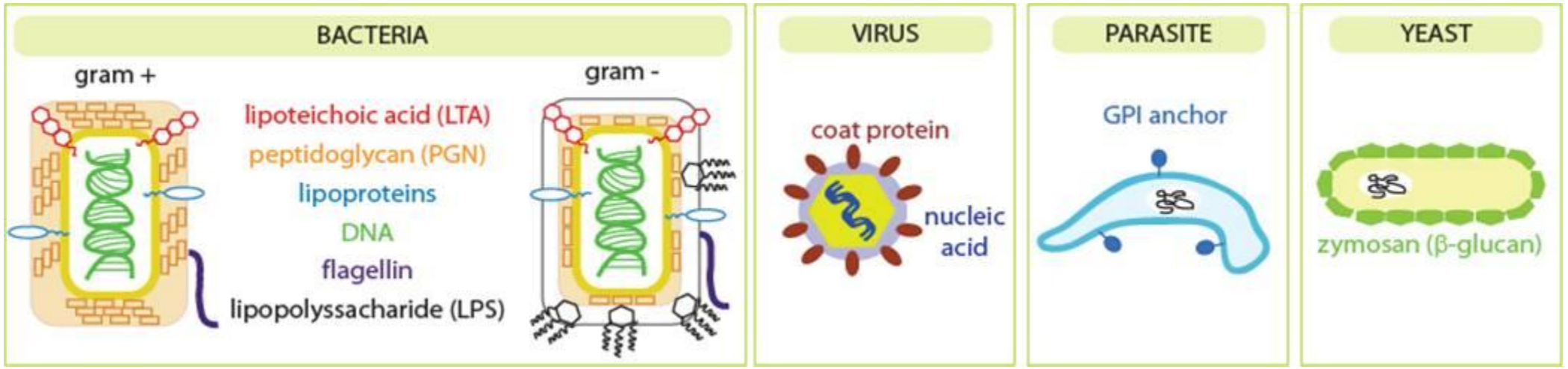
- **Pattern recognition receptors (PRRs)** play a crucial role in the proper function of the innate immune system. PRRs are germline-encoded host sensors, which detect molecules typical for the pathogens.

- 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**.

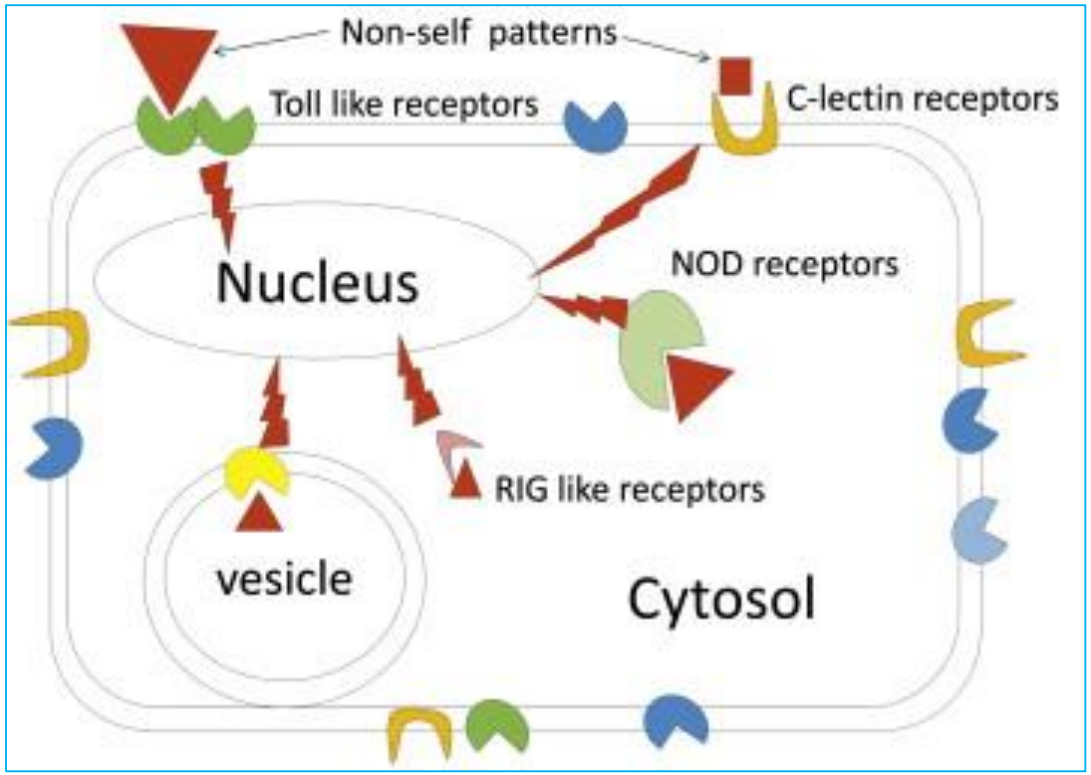
- PRR can be **cell bound** or **soluble**.

- Cell bound PRR can be found on **different compartments of the cell**. (membrane, cytosol)

Examples of PAMPs



Examples of cellular PRR

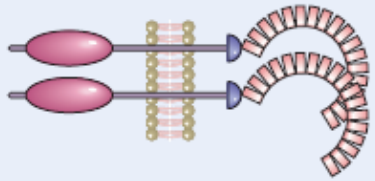
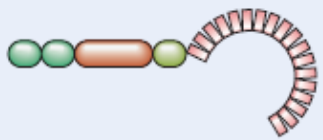
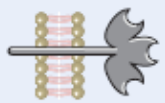


Examples of soluble PRR are:

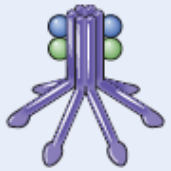
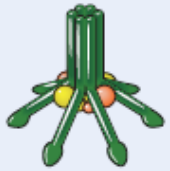
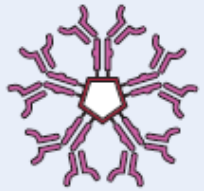
- **Natural antibodies**
- **complement proteins**

Molecules of the immune system / cell bound PRR

TABLE 4–3 Pattern Recognition Molecules of the Innate Immune System

Cell-Associated Pattern Recognition Receptors	Location	Specific Examples	PAMP/DAMP Ligands
Toll-like receptors (TLRs) 	Plasma membrane and endosomal membranes of dendritic cells, phagocytes, B cells endothelial cells, and many other cell types	TLRs 1-9	Various microbial molecules including bacterial LPS and peptidoglycans, viral nucleic acids
NOD-like receptors (NLRs) 	Cytoplasm of phagocytes epithelial cells, and other cells	NOD1/2 NALP family (inflammasomes)	Bacterial cell wall peptidoglycans Flagellin, muramyl dipeptide, LPS; urate crystals; products of damaged cells
RIG-like receptors (RLRs) 	Cytoplasm of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
C-type lectin–like receptors 	Plasma membranes of phagocytes	Mannose receptor Dectin	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues

Molecules of the immune system / Soluble PRR

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	*N*-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

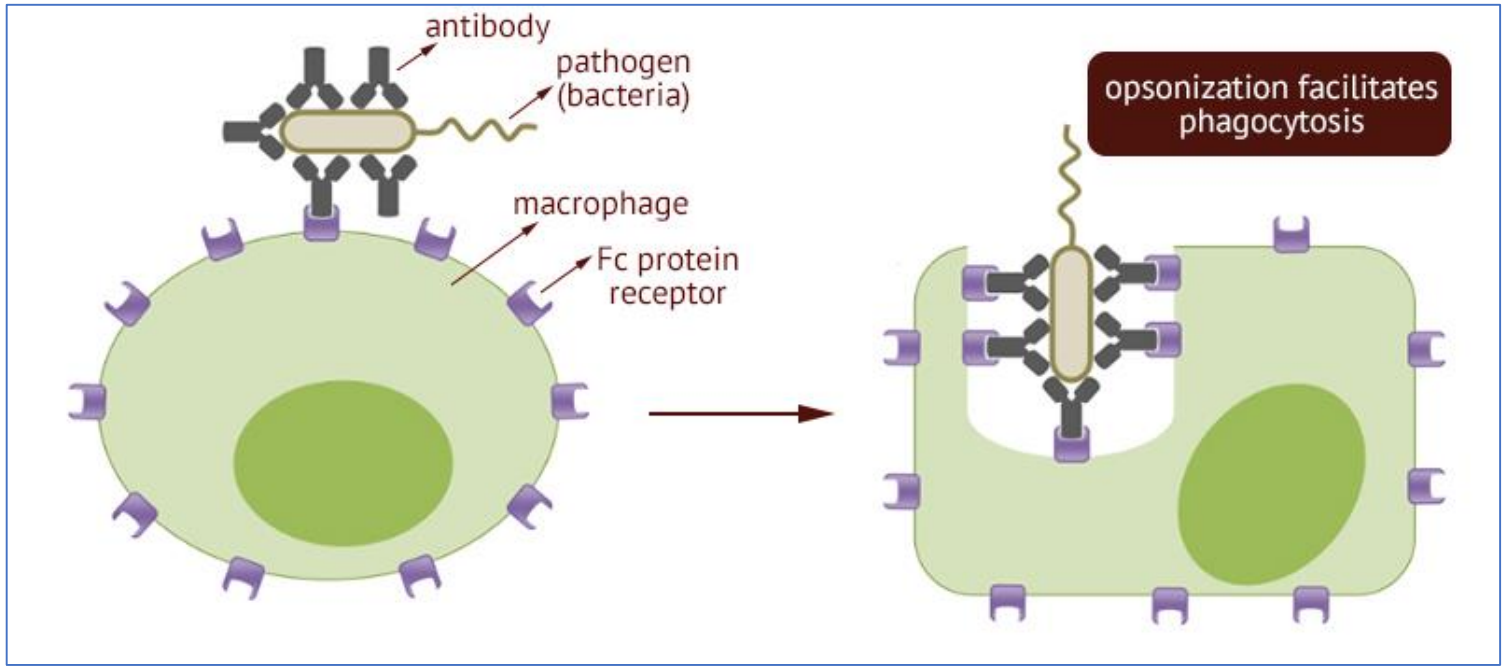
- These molecules provide early defense against pathogens that are present outside host cells at some part of their life cycle. The soluble effector molecules function in two major ways:

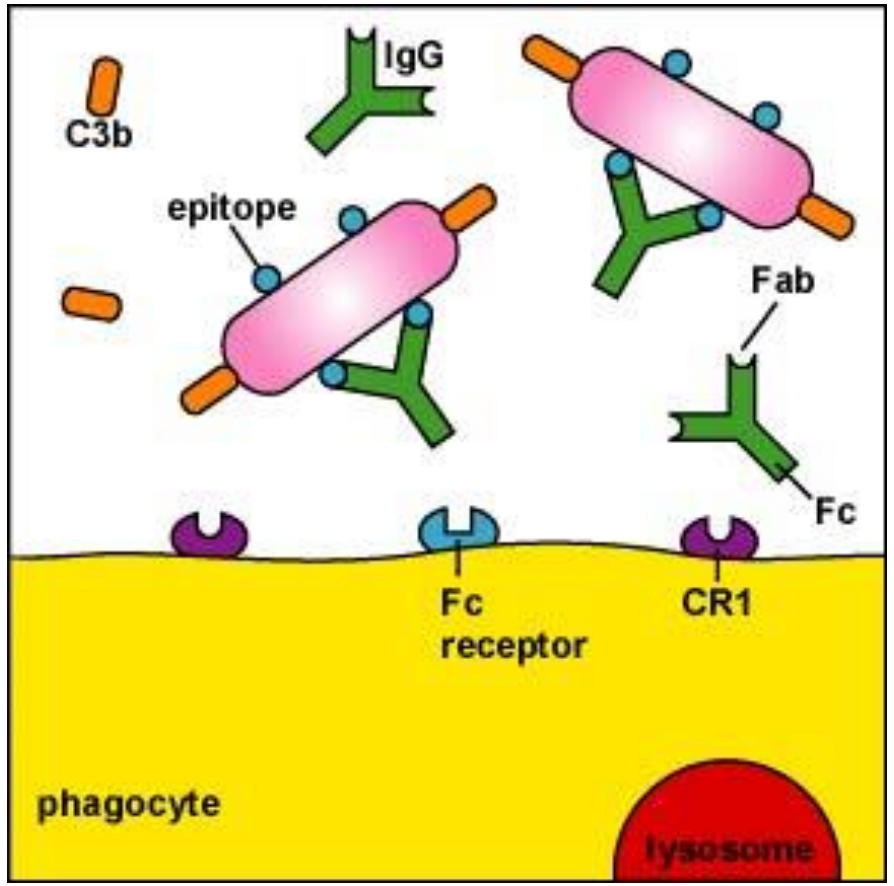
- By binding to microbes, they act as **opsonins** and enhance the ability of macrophages, neutrophils, and dendritic cells to phagocytose the microbes. This is because the phagocytic cells express membrane receptors specific for the opsonins.

- 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**

- **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.





- 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.

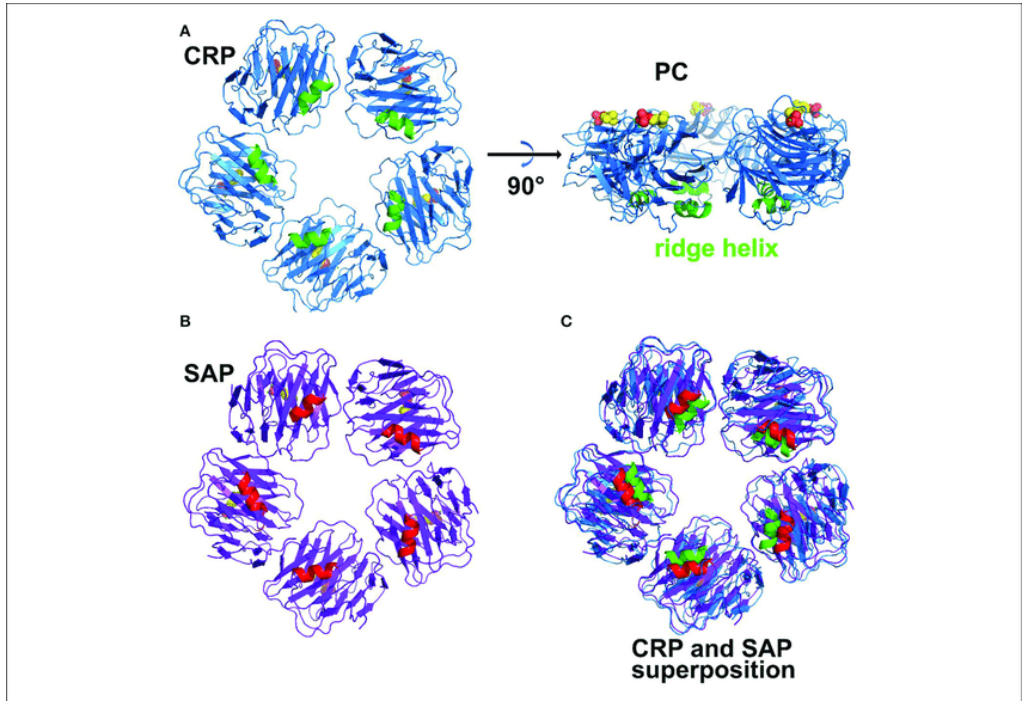
- Natural antibodies are usually specific for carbohydrate or lipid molecules but not proteins, and most are IgM antibodies, one of several structural classes of Ig molecules.

- 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 (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 **TNFα**. The **liver** responds by producing a large number of **acute-phase reactants**.

- Measurement of acute-phase proteins, especially **C-reactive protein**, is a useful marker of inflammation in medical clinical pathology.

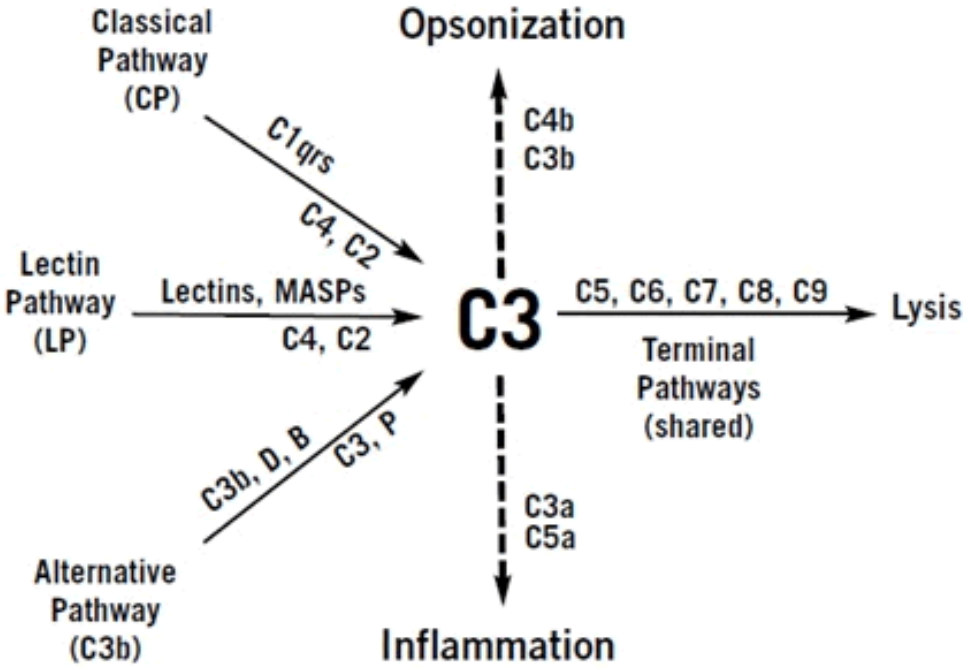
Positive APPs	Negative APPs
C-reactive protein (CRP)	Albumin
Serum Amyloid A (SAA)	Transferrin
Haptoglobin (Hp)	Transthyretin
Ceruloplasmin	Retinol-binding protein
α2-Macroglobulin	**Note: Negative Acute Phase Protein "decreases" in inflammation**
α1-Acid glycoprotein (AGP)	
Fibrinogen	
Complement (C3, C4)	

The 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**.

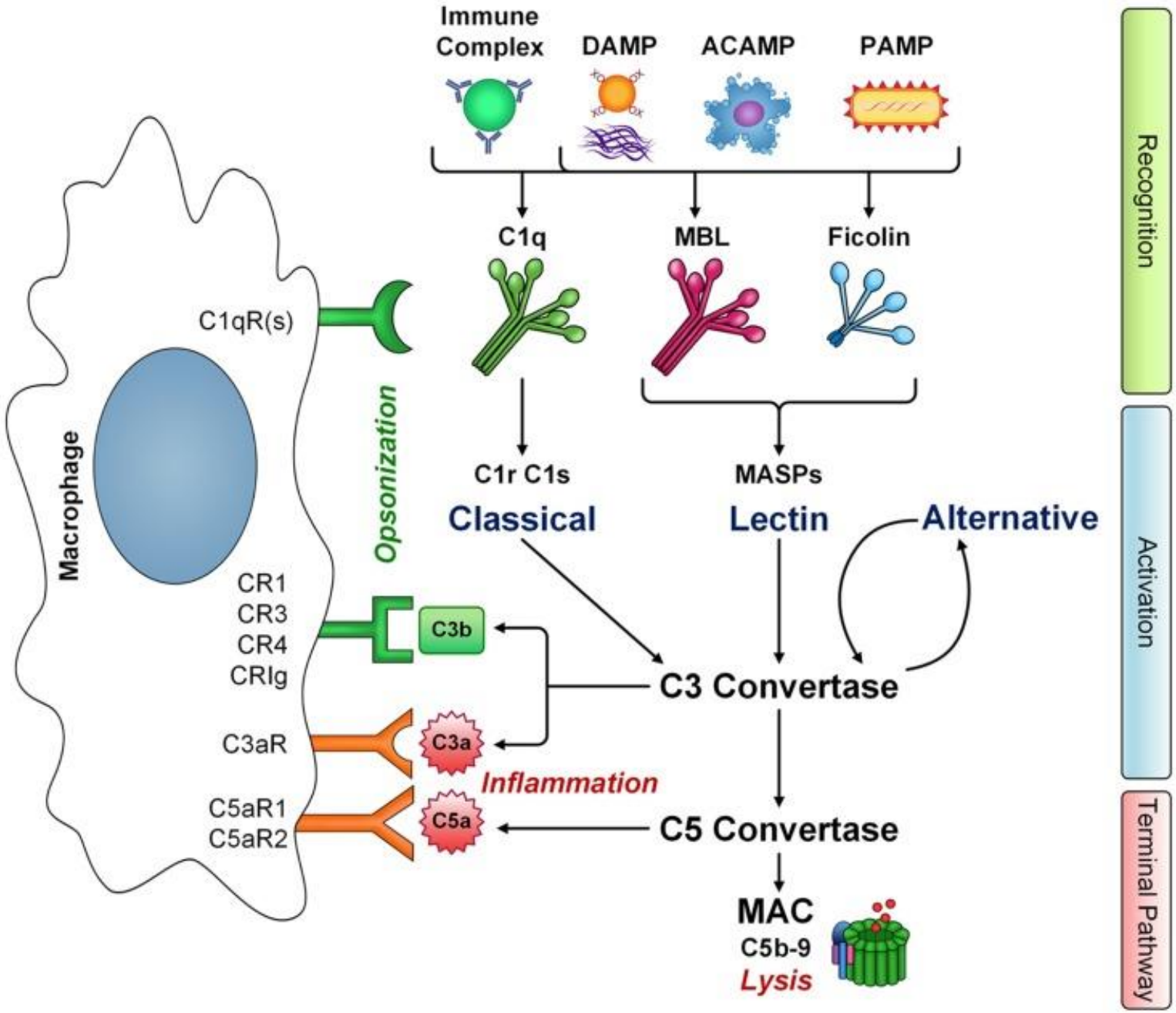
- Complement has the following functions:
  - **Opsonization** of the pathogen (or a dead cell) to ease phagocytosis (**C3b**, **C4b**).
  - Generation of **anaphylatoxins** (**C3a** and **C5a**) to draw in leukocytes and potentiate the immune reponse.
  - Formation of **a pore in the bacterial cell 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**.

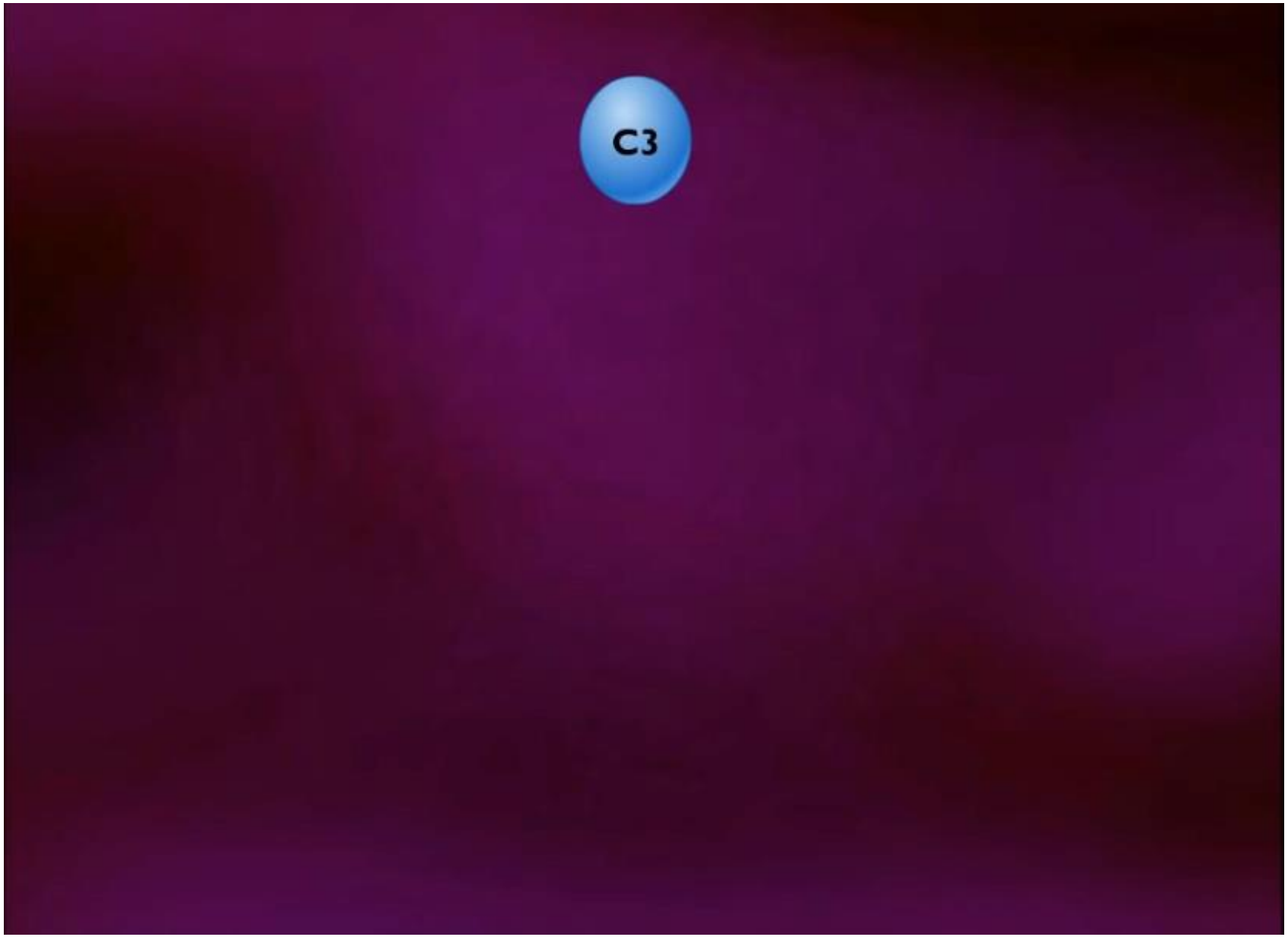


The complement system

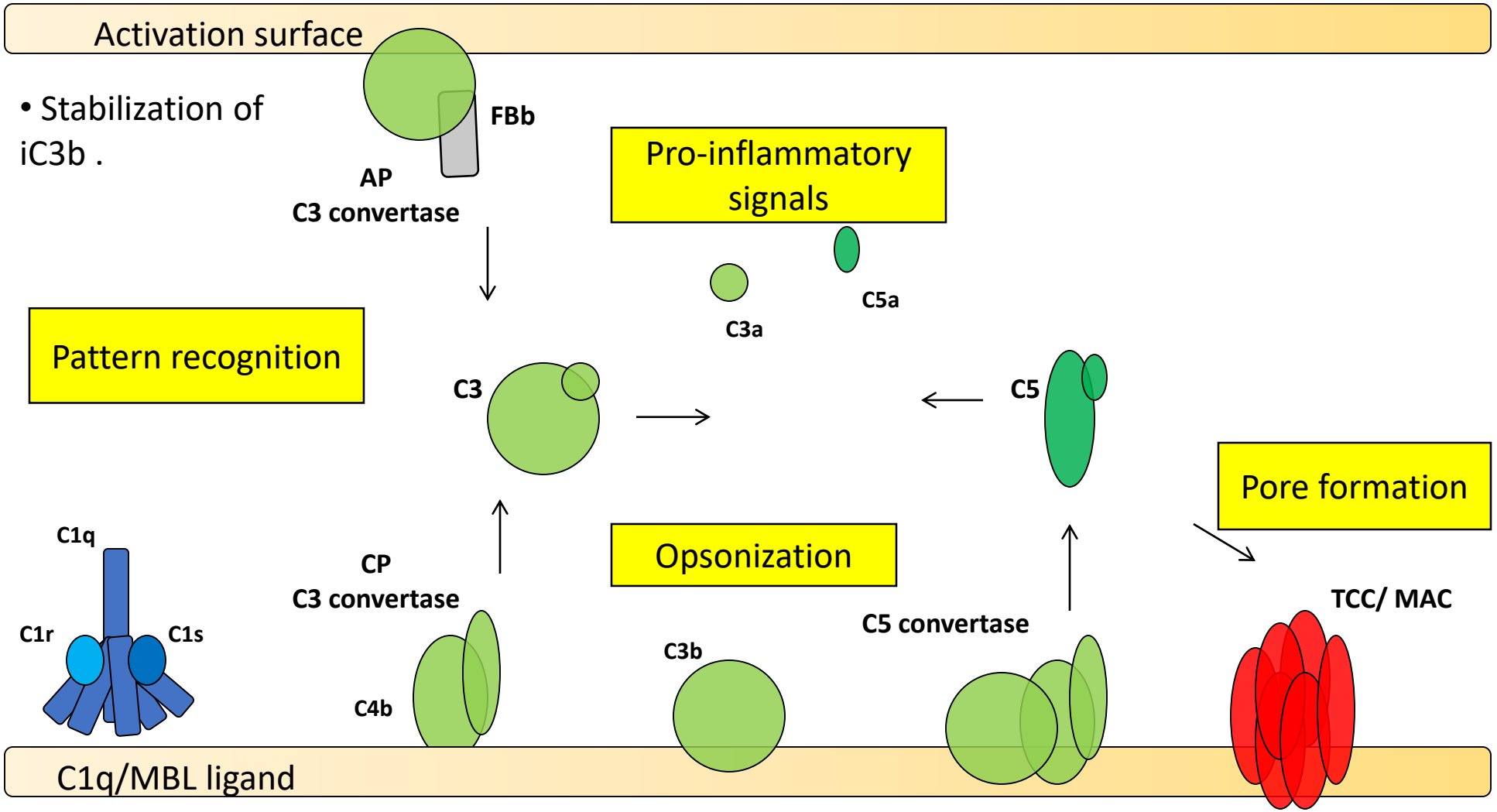
- 3 pathways of complement activation depend on different **PRR** but converge at C3 activation.
- A C3 convertase is formed from activated complement proteins, In the classical and lectin pathways, C3 convertase is made from C2bC4b, while in the alternative pathway, it's made from C3bFb.
- Each step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

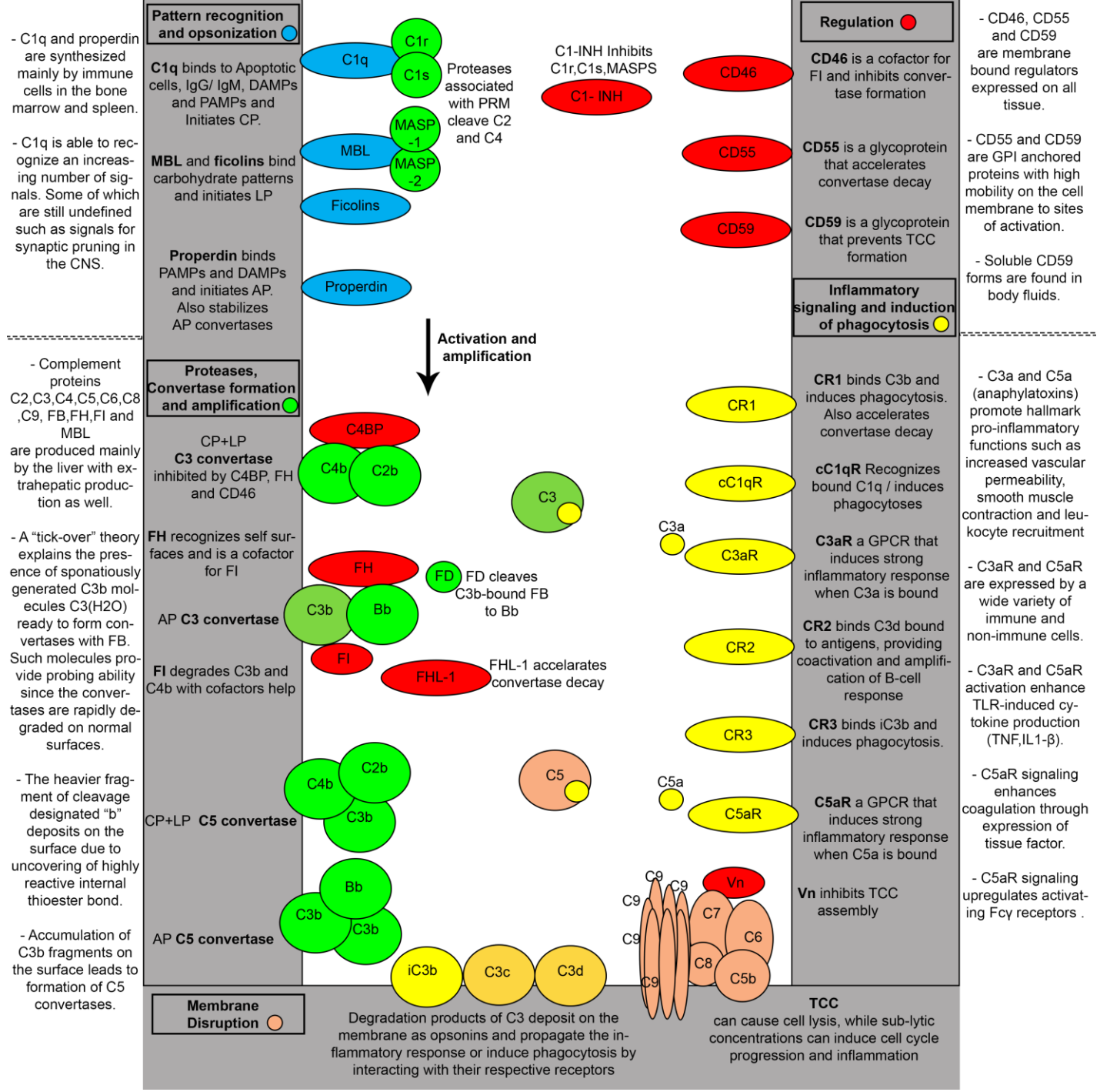



C1
Complement: a group of serum proteins
Inflammation
Cell lysis
Opsonization


C3

Complement activation

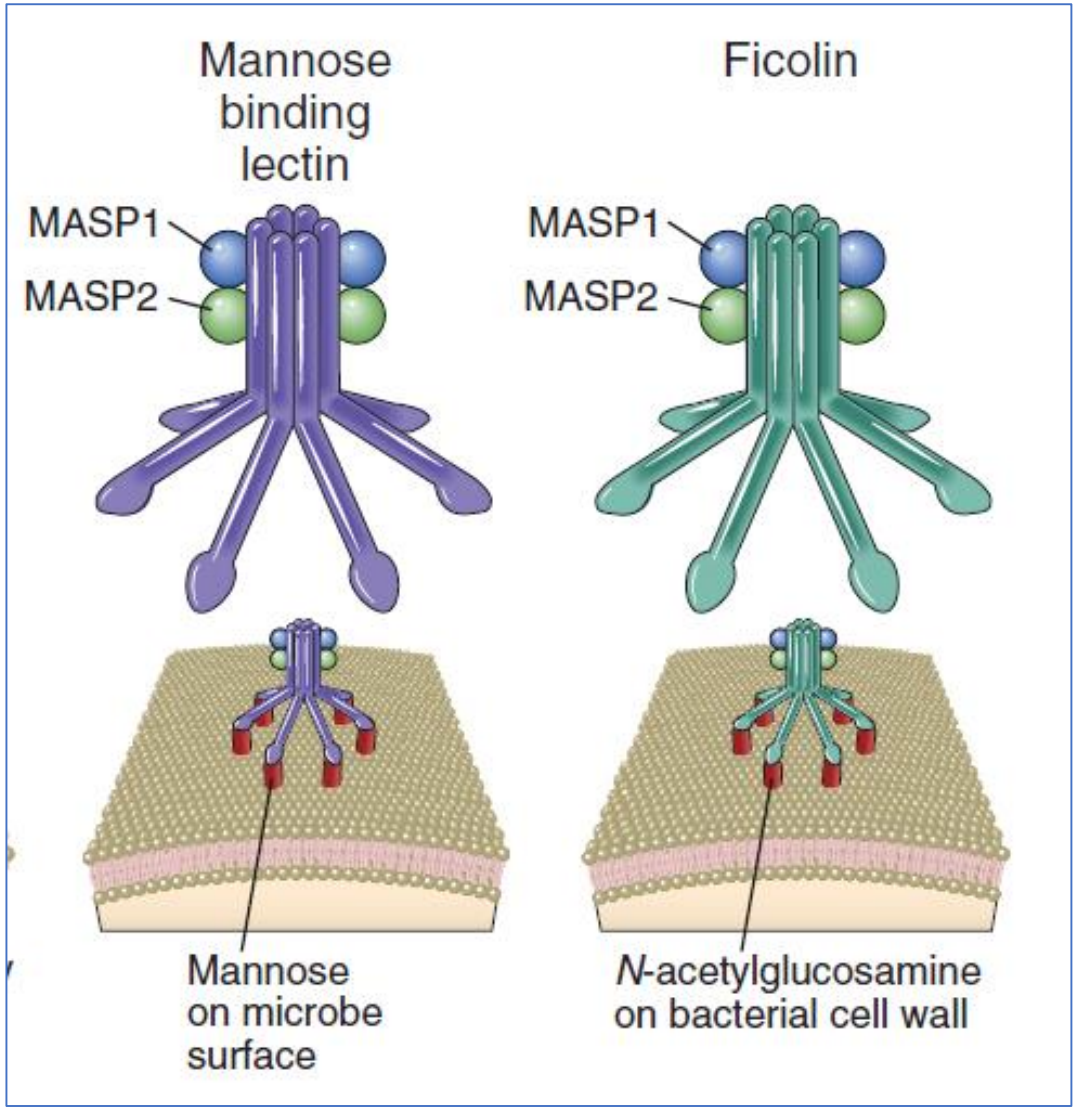



- C1q and properdin are synthesized mainly by immune cells in the bone marrow and spleen.
- C1q is able to recognize an increasing number of signals. Some of which are still undefined such as signals for synaptic pruning in the CNS.
Pattern recognition and opsonization
C1q binds to Apoptotic cells, IgG/ IgM, DAMPs and PAMPs and Initiates CP.
MBL and ficolins bind carbohydrate patterns and initiates LP
Properdin binds PAMPs and DAMPs and initiates AP. Also stabilizes AP convertases
C1r
C1q
C1s
Proteases associated with PRM cleave C2 and C4
MASP -1
MBL
MASP -2
Ficolins
Properdin
C1-INH Inhibits C1r,C1s,MASPS
C1- INH
Regulation
CD46
CD55
CD59
CD46 is a cofactor for FI and inhibits convertase formation
CD55 is a glycoprotein that accelerates convertase decay
CD59 is a glycoprotein that prevents TCC formation
- CD46, CD55 and CD59 are membrane bound regulators expressed on all tissue.
- CD55 and CD59 are GPI anchored proteins with high mobility on the cell membrane to sites of activation.
- Soluble CD59 forms are found in body fluids.
Inflammatory signaling and induction of phagocytosis
Activation and amplification
- Complement proteins C2,C3,C4,C5,C6,C8 ,C9, FB,FH,FI and MBL are produced mainly by the liver with extrahepatic production as well.
- A “tick-over” theory explains the presence of sponatiously generated C3b molecules C3(H2O) ready to form convertases with FB. Such molecules provide probing ability since the convertases are rapidly degraded on normal surfaces.
- The heavier fragment of cleavage designated “b” deposits on the surface due to uncovering of highly reactive internal thioester bond.
- Accumulation of C3b fragments on the surface leads to formation of C5 convertases.
Proteases, Convertase formation and amplification
CP+LP C3 convertase inhibited by C4BP, FH and CD46
FH recognizes self surfaces and is a cofactor for FI
AP C3 convertase
FI degrades C3b and C4b with cofactors help
CP+LP C5 convertase
AP C5 convertase
C4BP
C4b
C2b
C3
FH
FD
FD cleaves C3b-bound FB to Bb
C3b
Bb
FI
FHL-1
FHL-1 accelarates convertase decay
C5
iC3b
C3c
C3d
CR1
cC1qR
C3aR
CR2
CR3
C5aR
C3a
C5a
CR1 binds C3b and induces phagocytosis. Also accelerates convertase decay
cC1qR Recognizes bound C1q / induces phagocytoses
C3aR a GPCR that induces strong inflammatory response when C3a is bound
CR2 binds C3d bound to antigens, providing coactivation and amplification of B-cell response
CR3 binds iC3b and induces phagocytosis.
C5aR a GPCR that induces strong inflammatory response when C5a is bound
Vn inhibits TCC assembly
- C3a and C5a (anaphylatoxins) promote hallmark pro-inflammatory functions such as increased vascular permeability, smooth muscle contraction and leukocyte recruitment
- C3aR and C5aR are expressed by a wide variety of immune and non-immune cells.
- C3aR and C5aR activation enhance TLR-induced cytokine production (TNF,IL1-β).
- C5aR signaling enhances coagulation through expression of tissue factor.
- C5aR signaling upregulates activating Fcγ receptors .
C9
Vn
C7
C6
C8
C5b
Membrane Disruption
Degradation products of C3 deposit on the membrane as opsonins and propagate the inflammatory response or induce phagocytosis by interacting with their respective receptors
TCC
can cause cell lysis, while sub-lytic concentrations can induce cell cycle progression and inflammation

- 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.



Molecules of the immune system / Important cytokines

Cytokines are a broad and loose category of small proteins that are important in cell signaling.

Cytokines include **chemokines**, **interferons**, **interleukins**, lymphokines, and **tumour necrosis factors**

Cytokines are produced by a broad range of cells, including **immune** and **non-immune cells**

TABLE 2–2 Some Cytokines Acting in Infection

	CELL SOURCE	FUNCTIONS
Interleukins (IL)		
IL-1	Macrophages, endothelium, fibroblasts, epithelial	Differentiation and function of immune effectors, PMN response (T_H17)
IL-2	T cells (T_H1)	T-cell proliferation, cytolytic activity of natural killer (NK) cells
IL-4	T cells (T_H2), macrophages, B cells	Differentiation of naïve T cells to helper T cells, proliferation of B cells
IL-5	T cells (T_H2)	Eosinophil activation
IL-8	Macrophages, endothelial, T cells, keratinocytes, PMNs	Chemoattractant for PMNs and T cells, PMN degranulation, migration of PMNs
IL-17	T cells (T_H17)	Inflammation, PMN response
IL-22	T cells (T_H17)	Antimicrobial peptides
Interferons (IFN)		
IFN-α/β	T cells, B cells, macrophages, fibroblasts	Antiviral activity, stimulates macrophages, MHC class I expression
IFN-γ	T cells (T_H1, CTLs), NK cells	T-cell activation, macrophage activation, PMNs, NK cells, antiviral, MHC class I and II expression
Tumor Necrosis Factor (TNF)		
TNF-α	T cells, macrophages, NK cells	Expression of multiple cytokines, (growth and transcription factors), stimulates inflammatory response, cytotoxic for tumor cells
TNF-β	T cells, B cells	Same as TNF-α

MHC, Major histocompatibility complex; PMN, Polymorphonuclear neutrophil

- The major way by which the innate immune system deals with **viral infections** is to induce the expression of type I interferons. Type I interferons are a large family of structurally related cytokines that mediate the **early innate immune response to viral infections**.

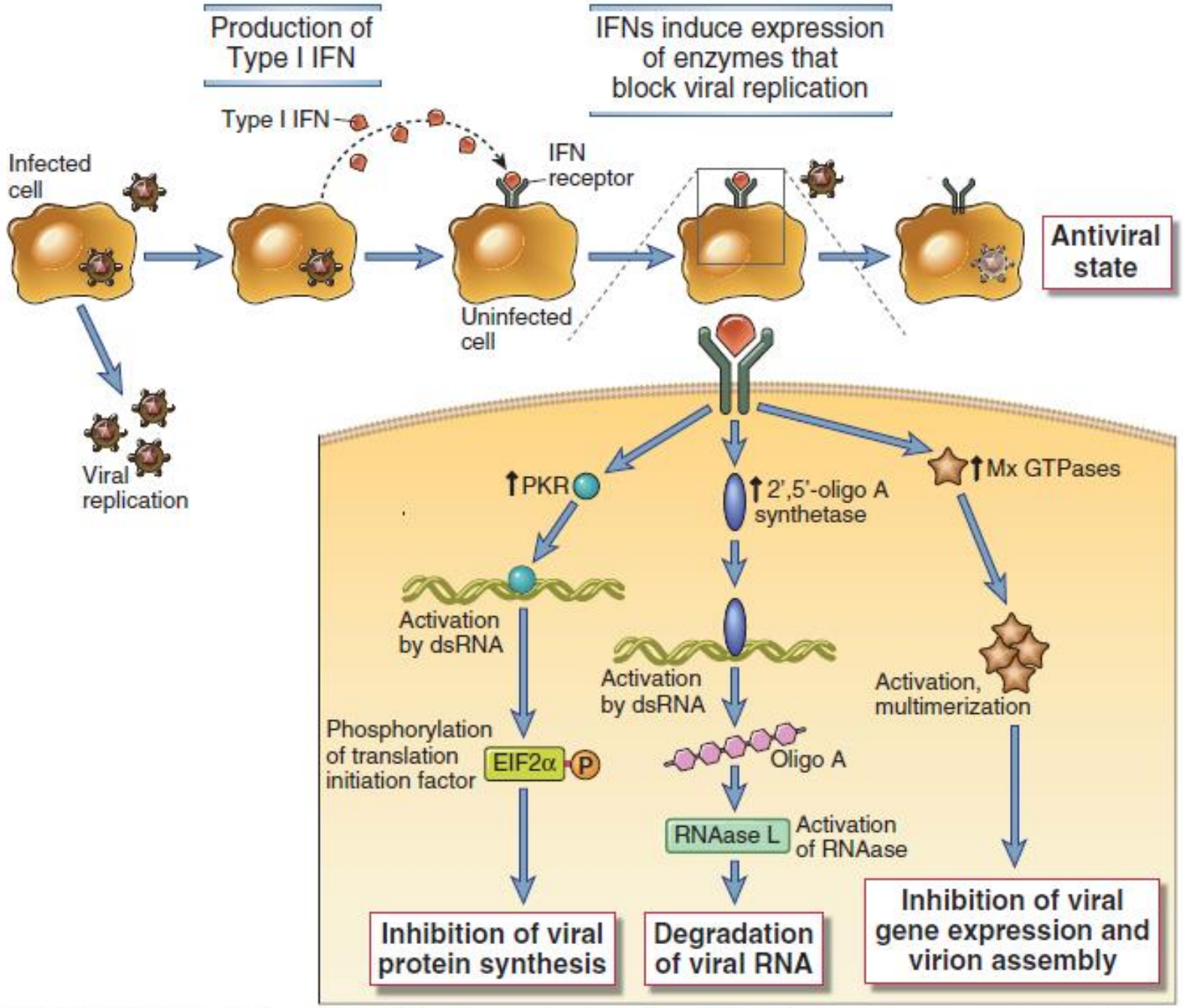
- Type I interferons, signaling through the type I interferon receptor, activate transcription of several genes that confer on the cells a resistance to viral infection, called an **antiviral state**.

- Type I interferons cause **sequestration of lymphocytes in lymph nodes**, thus maximizing the opportunity for encounter with microbial antigens.

- Type I interferons **increase the cytotoxicity of NK cells** and **CD8+ CTLs**

- **Upregulate expression of class I MHC** molecules and thereby increase the probability that virally infected cells will be recognized and killed by CD8+ CTLs.

Molecules of the immune system / Important cytokines / Interferons



Further reading:

- Cellular and Molecular Immunology. 7th Edition..

Chapter 4. Innate immunity

CELL-ASSOCIATED PATTERN RECOGNITION RECEPTORS OF INNATE IMMUNITY

SOLUBLE RECOGNITION AND EFFECTOR MOLECULES OF INNATE IMMUNITY