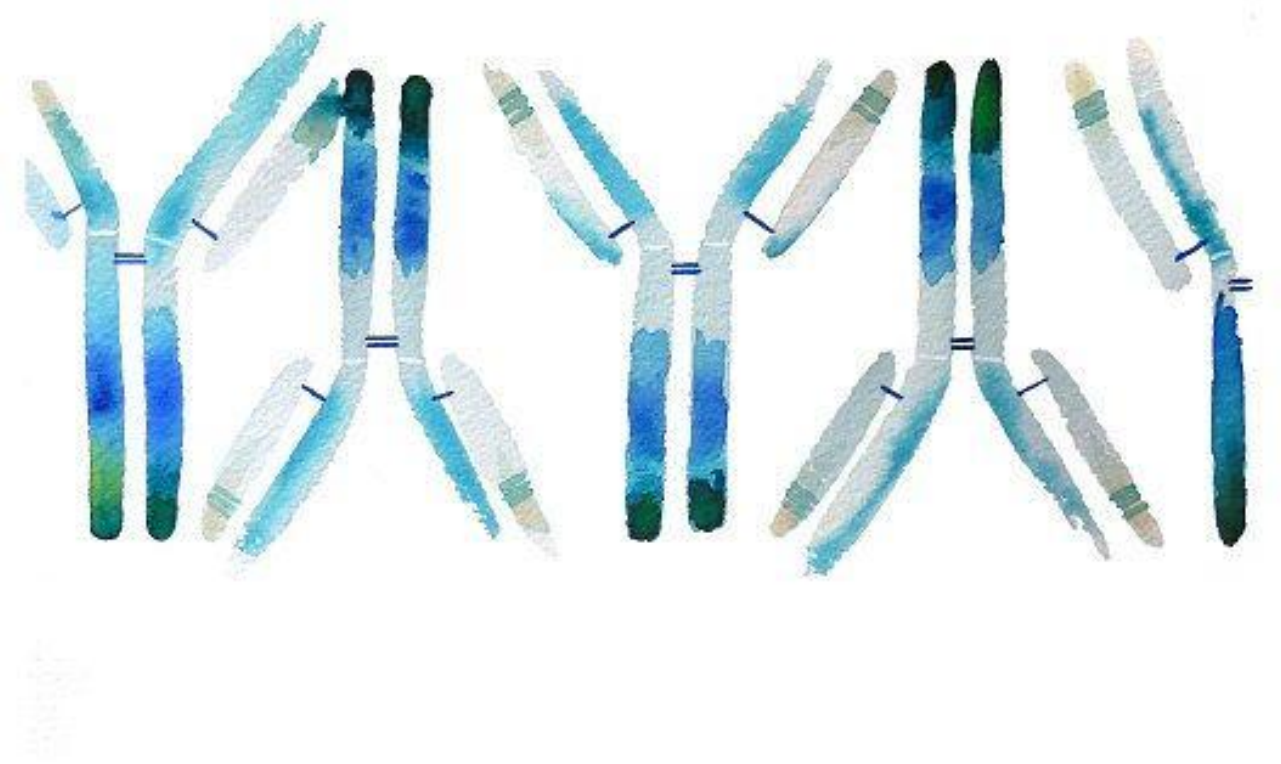


Medical Immunology



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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

Molecules of the immune system / Antigen recognition by innate immunity

- 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.

Molecules of the immune system / Antigen recognition by innate immunity

- 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.

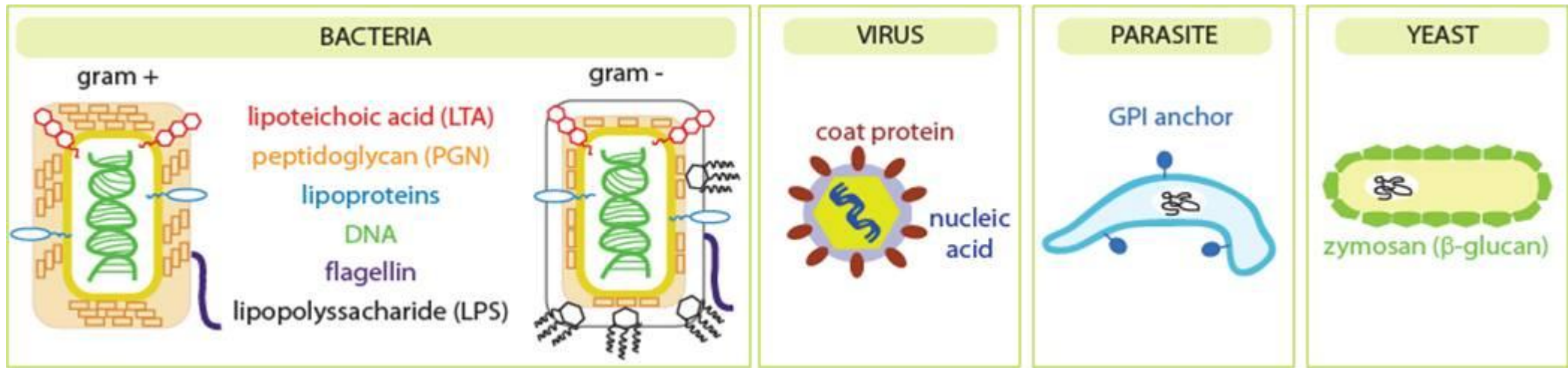
Molecules of the immune system / Antigen recognition by innate immunity

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.		

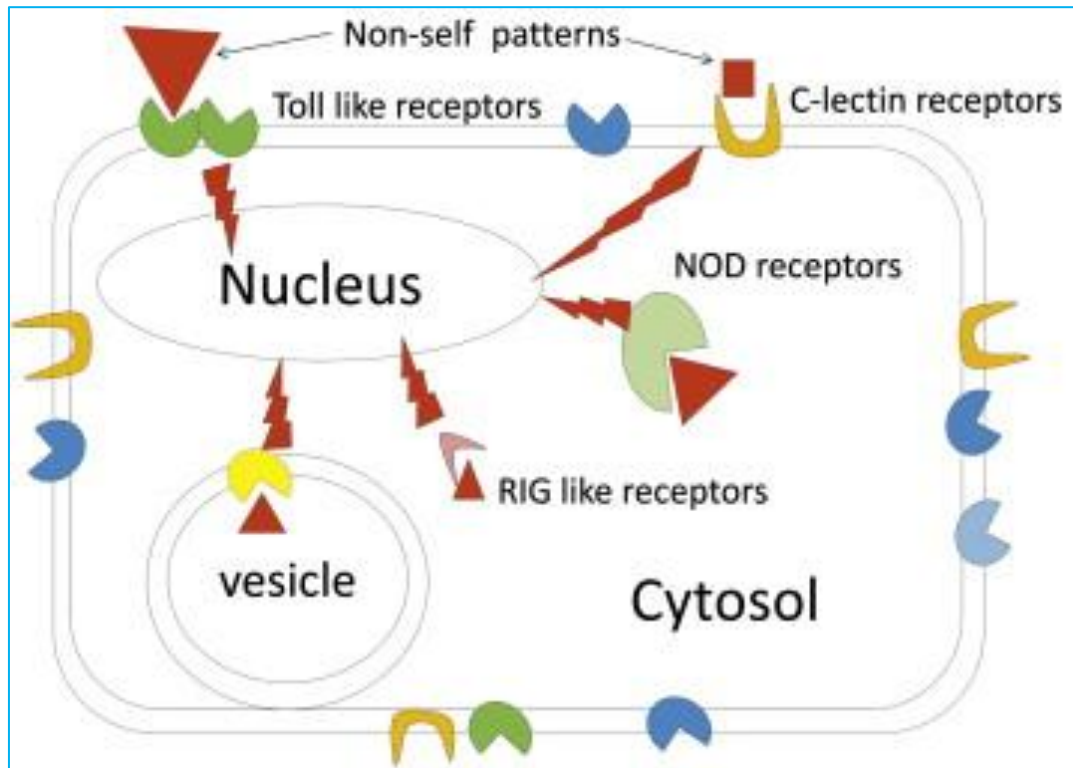
Molecules of the immune system / Antigen recognition by innate immunity

- **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



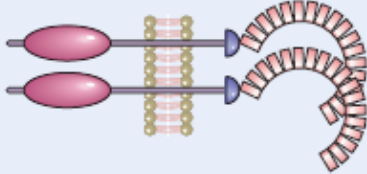
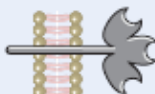
Examples of cellular PRR



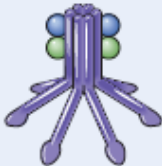
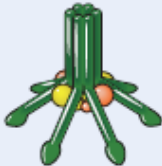
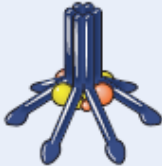
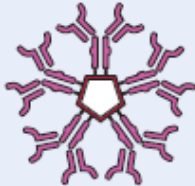
Examples of soluble PRR are:

- 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
<i>N</i>-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing <i>N</i> -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	<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

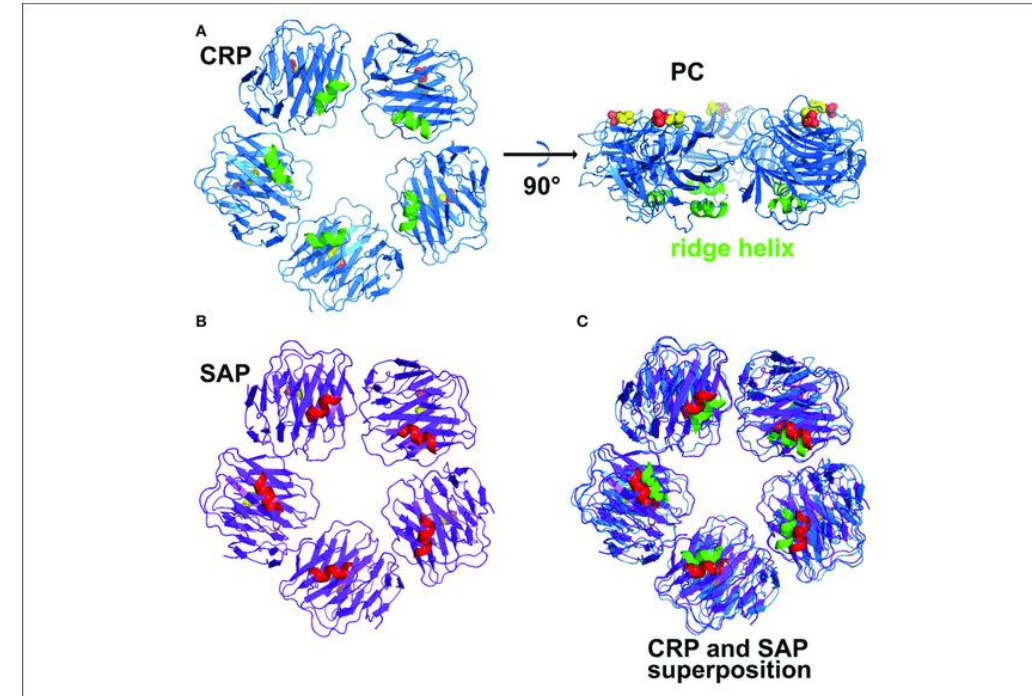
Molecules of the immune system / Soluble PRR

- 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**

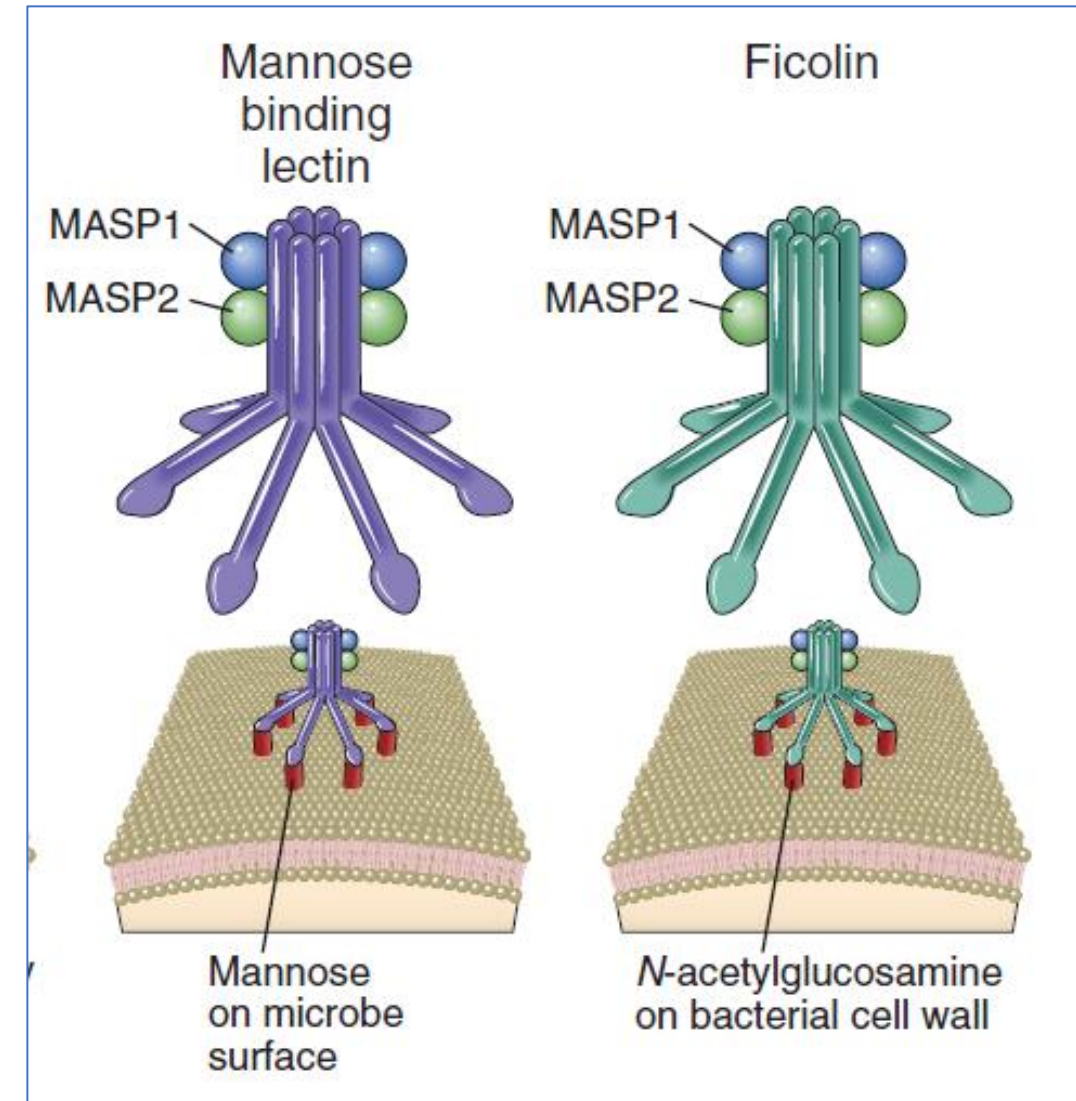
- There are subsets of B cells that produce antibodies with only a **limited number of specificities without overt exposure to foreign antigens**, and these are called natural antibodies. (**different from adaptive immunity antibodies**).
- They recognize common molecular patterns on microbes or stressed 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.

Molecules of the immune system / Soluble PRR/ Pentraxins

- 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**.



- 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.



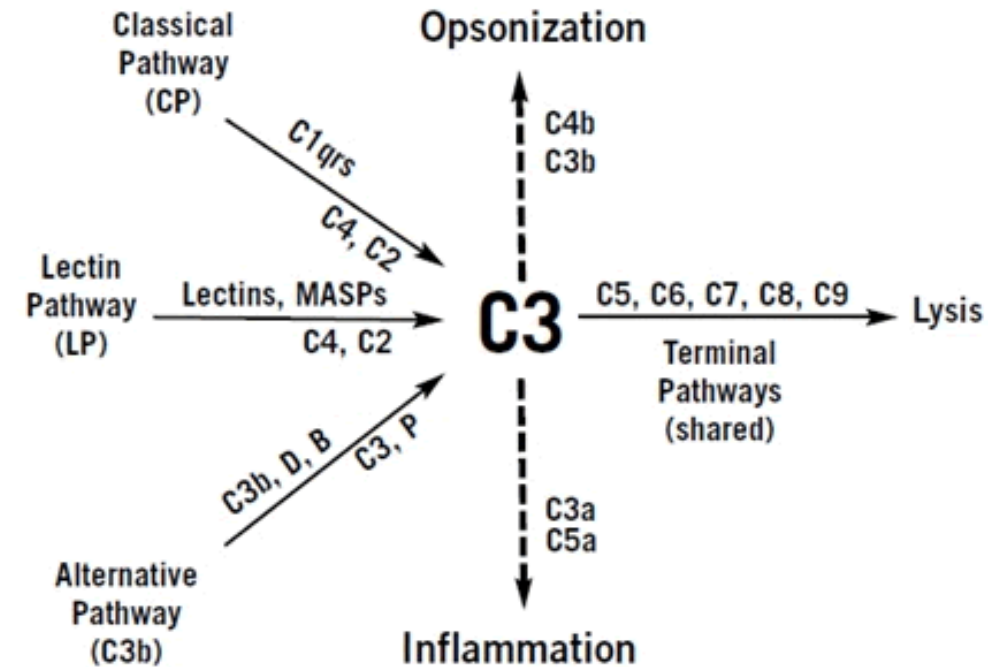
Molecules of the immune system / Acute-phase proteins

- 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)	

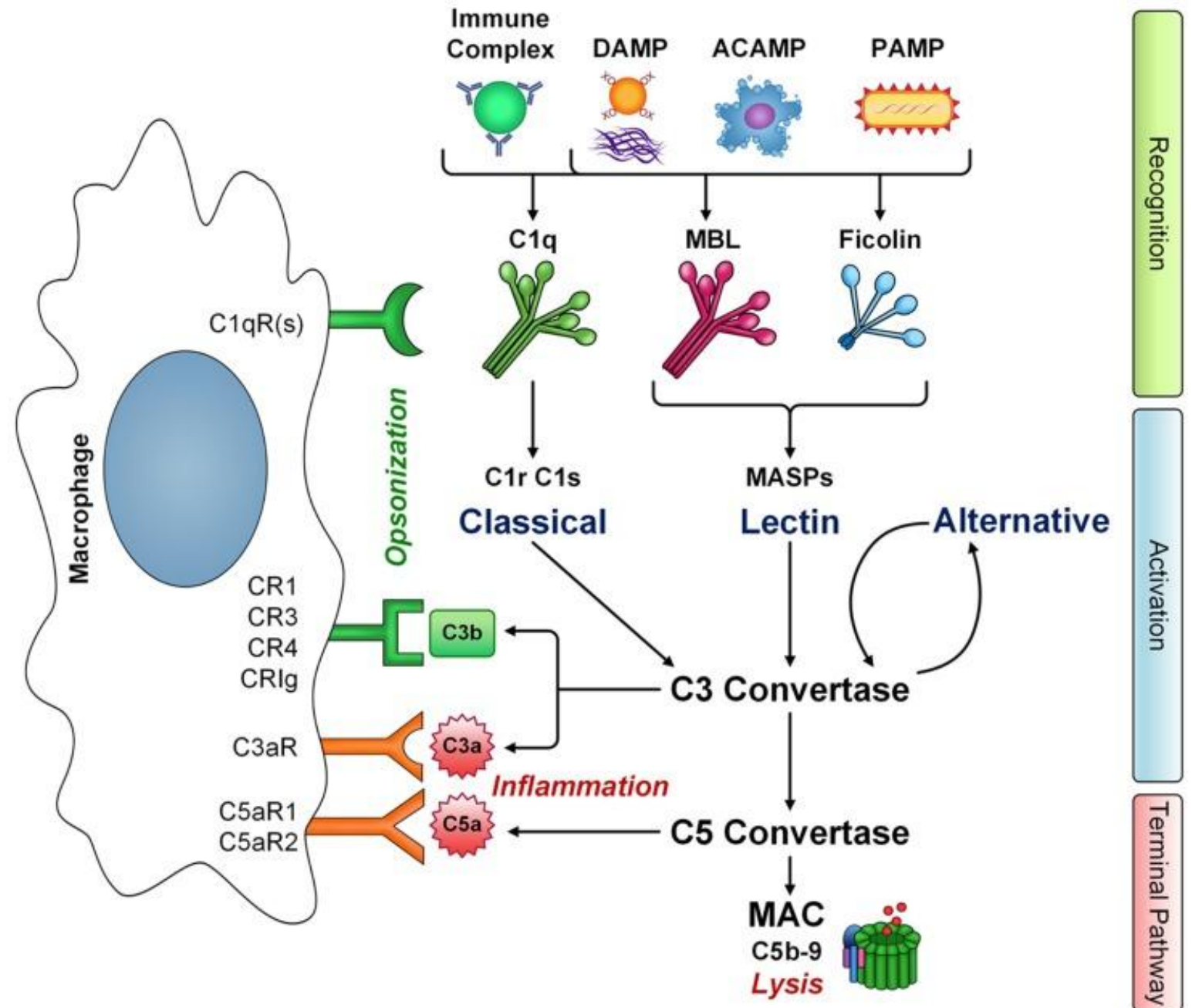
The complement system

- 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 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**.



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 soluble and cell surface proteins.





Complement: a group of serum proteins

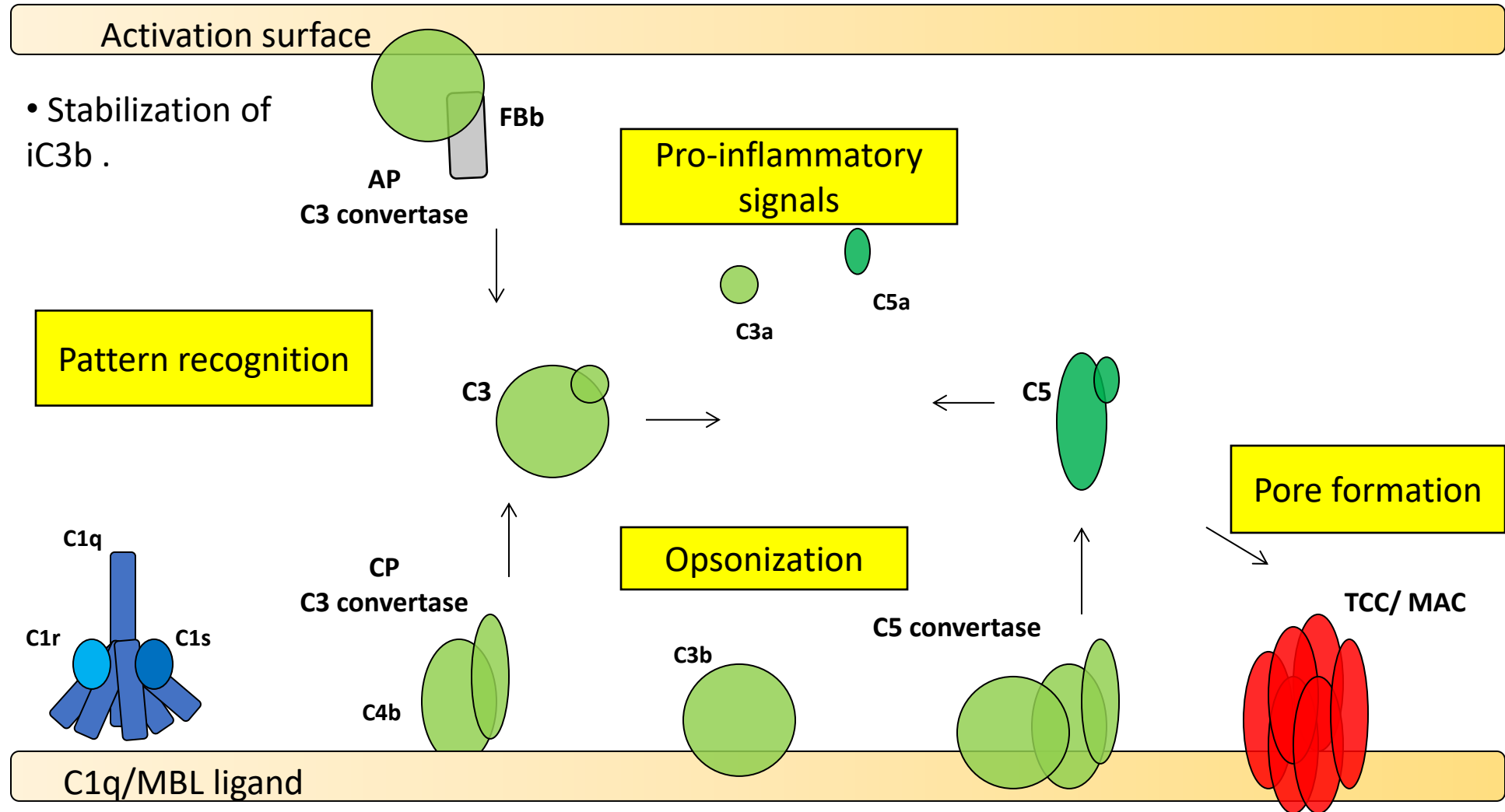
Inflammation

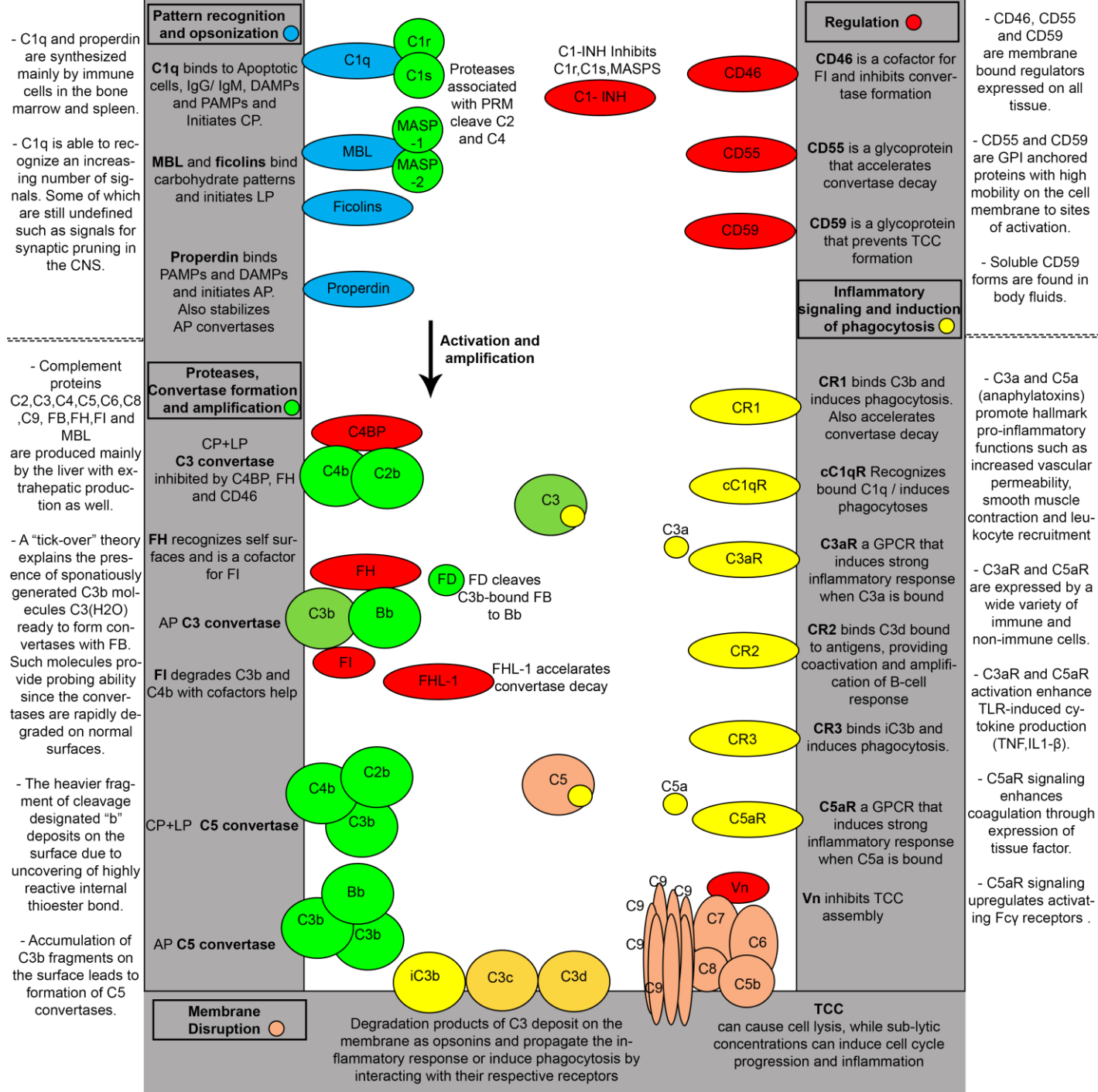
Cell lysis

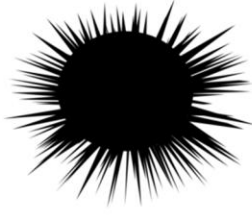
Opsonization



Complement activation







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.

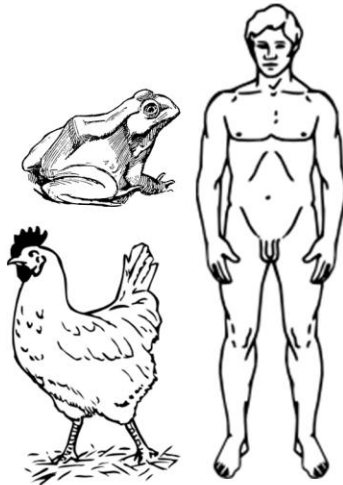
Those components found in body fluid can be induced in response to immune challenge and are thought to represent a primitive alternative pathway.



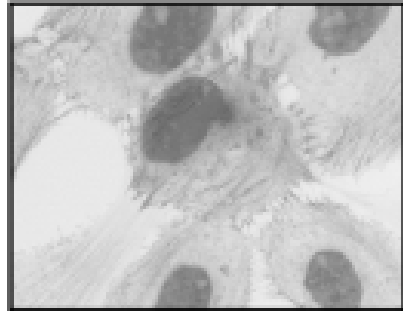
Lamprey is an early vertebrate that lacks immunoglobulins and thus a classical pathway.

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.

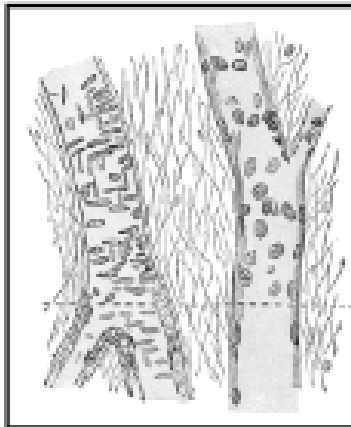
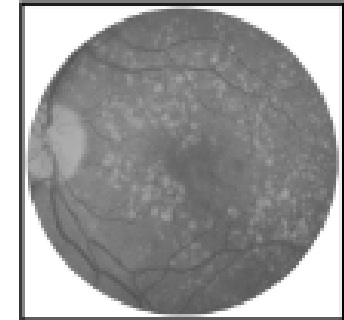


Cancer

- 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.

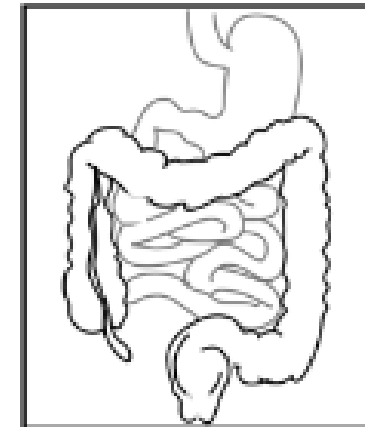


Ischemia/ reperfusion (I/R) injury

- 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.



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