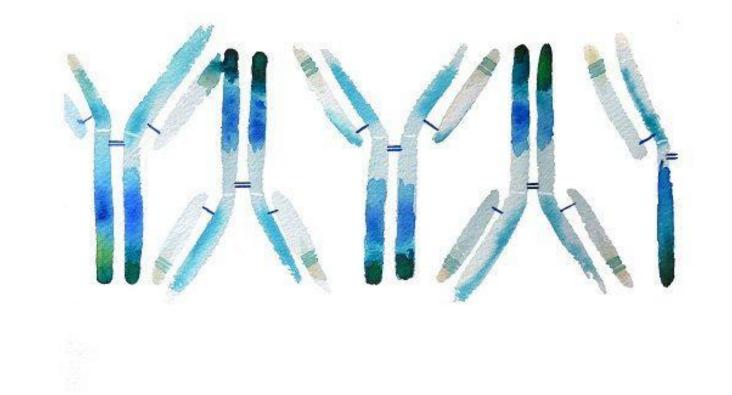
Medical Immunology



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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

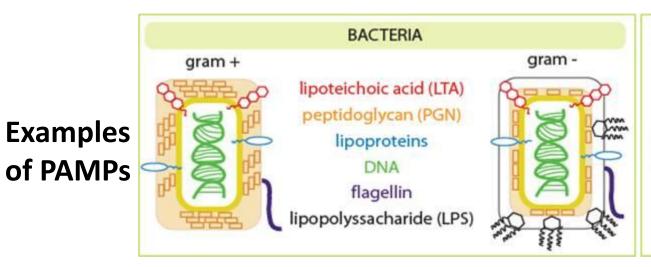
The complement system

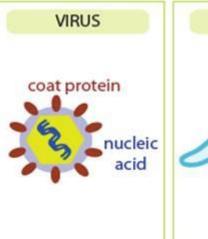
- The cells and soluble molecules of innate immunity either exist in a fully functional state before encounter with microbes or are rapidly activated by microbes
- The innate immune system recognizes molecular structures that are characteristic of microbial pathogens but not mammalian cells.
- The innate 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.

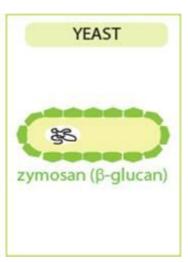
TABLE 4–2 Examples of PAMPs and DAMPs					
Pathogen-Associated Molecular Patterns Microbe Type					
Nucleic acids	ssRNA dsRNA CpG	Virus Virus Virus, bacteria			
Proteins	Pilin Flagellin	Bacteria Bacteria			
Cell wall lipids	LPS Lipoteichoic acid	Gram-negative bacteria Gram-positive bacteria			
Carbohydrates	Mannan Dectin glucans	Fungi, bacteria 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)

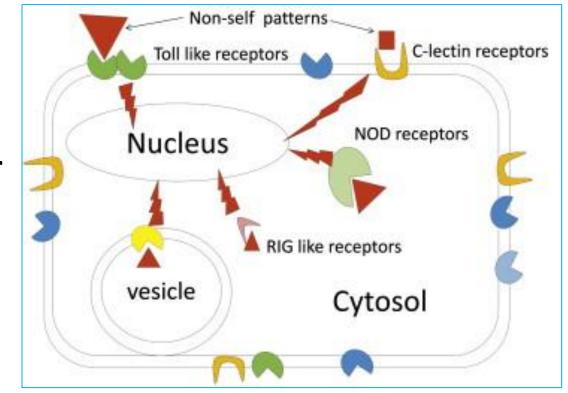








Examples of cellular PRR



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	Microbial surface carbohydrates with terminal mannose and fructose	
8-8		Dectin	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	

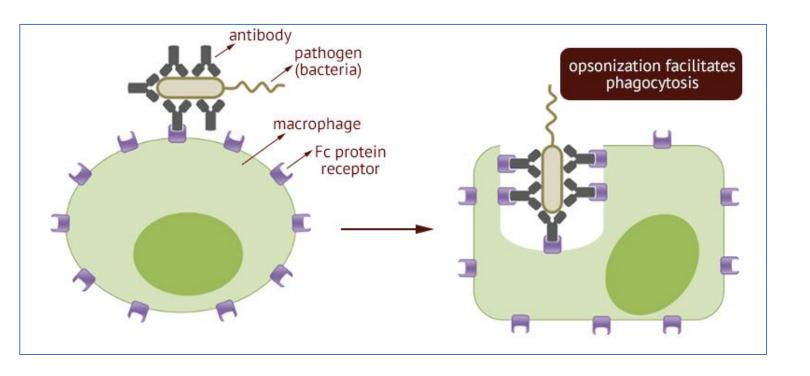
Molecules of the immune system / Soluble PRR

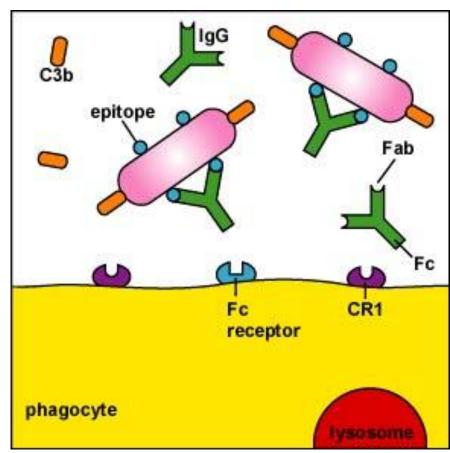
Soluble Recognition Molecules	Location	Specific Examples	PAMP Ligands
Pentraxins	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins	Plasma	Mannose-binding lectin	Carbohydrates with terminal mannose and fructose
	Alveoli	Surfactant proteins SP-A and SP-D	Various 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

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

- 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.

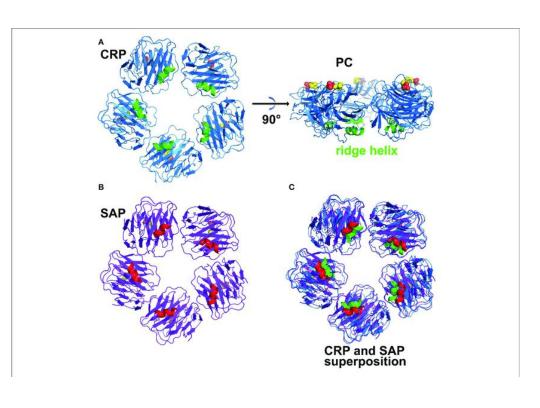




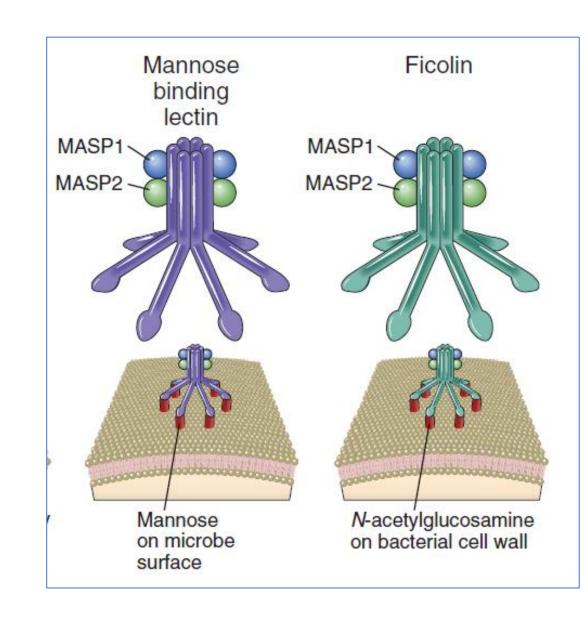
- There are subsets of B cells that produce antibodies with only a limited number of specificities without overt exposure to foreign antigens, and these are called natural antibodies. (different from adaptive immunity antibodies).
- They recognize common molecular patterns on microbes or stressed and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

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.

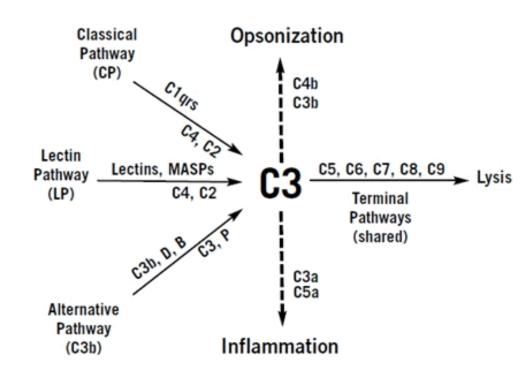


- Acute-phase proteins (APPs) are a class of proteins whose plasma concentrations increase in response to inflammation. This response is called the acutephase reaction.
- In response to injury or infection, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL1, and IL6, and TNFα. The liver responds by producing a large number of acutephase reactants.
- Measurement of acute-phase proteins, especially
 C-reactive protein, is a useful marker of inflammation in medical clinical pathology.

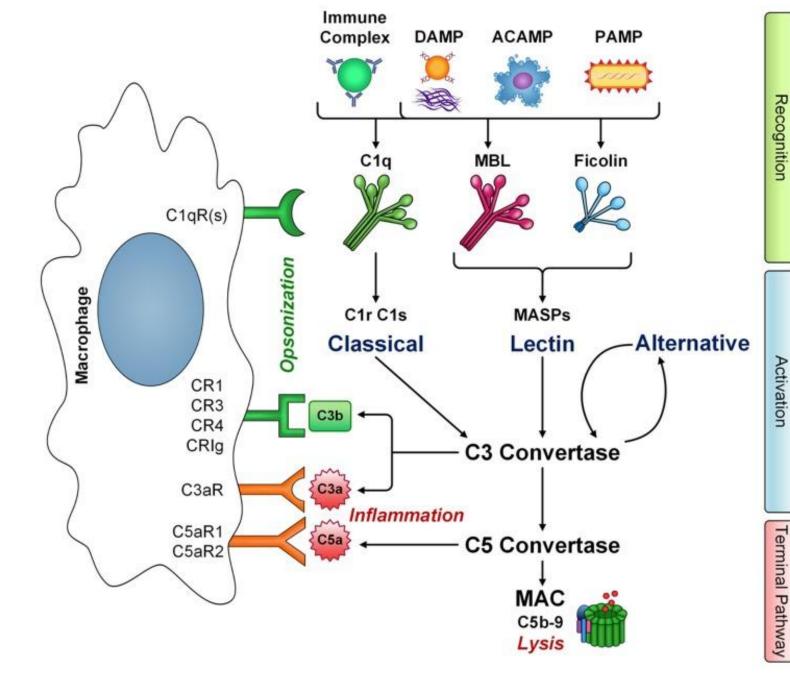
Positive APPs	Negative APPs	
C-reactive protein (CRP)	Albumin 7	
Serum Amyloid A (SAA)	Transferrin	
Haptoglobin (Hp)	Transthyretin	
Ceruloplasmin	Retinol-binding protein	
α2-Macroglobulin	Note: Negative Acute Phase Protein "decreases" in inflammation	
α1-Acid glycoprotein (AGP)		
Fibrinogen		
Complement (C3, C4)		

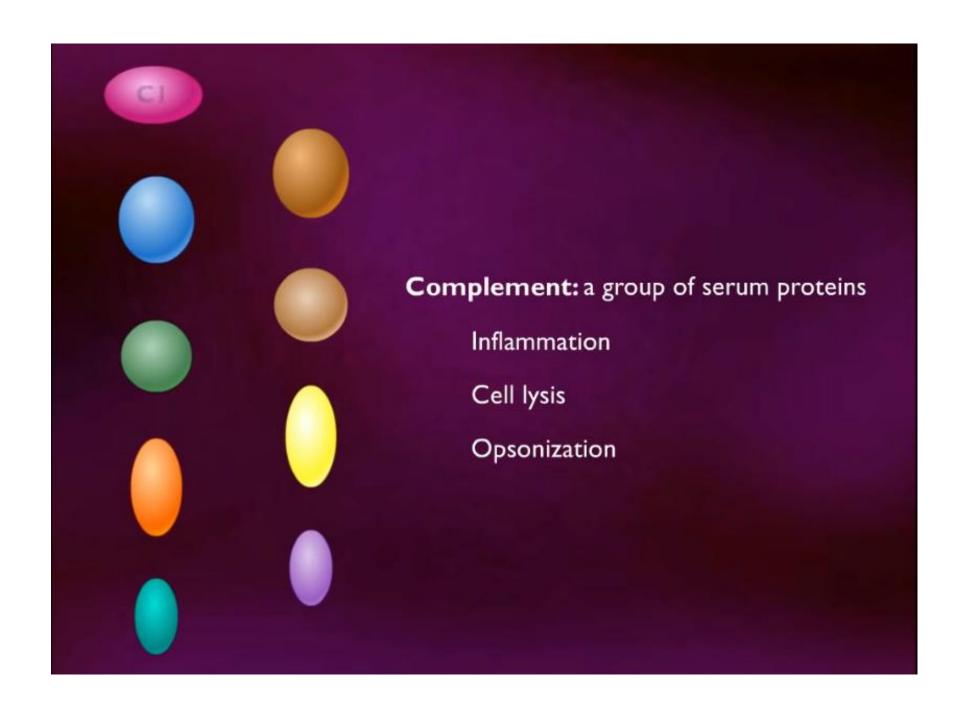
The complement system

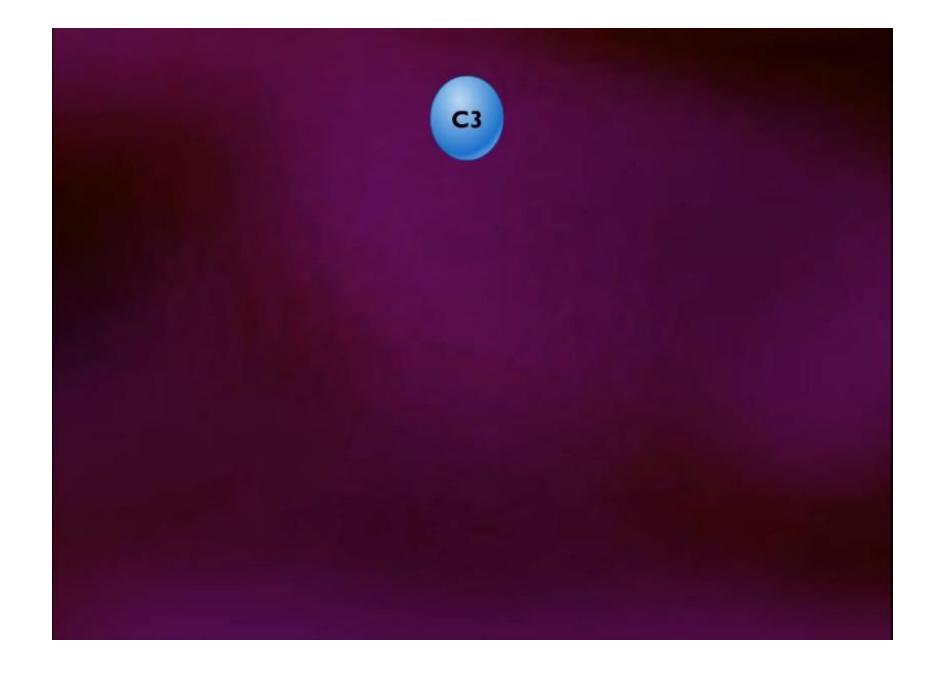
- The complement system is a group of proteins that circulate the blood in inactive form, until a pattern is sensed with proteins like (C1q, Lectins) which leads to a series of reactions of protein cleavage and activation.
- Complement has the following functions:
- > Opsonization of the pathogen (or 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.



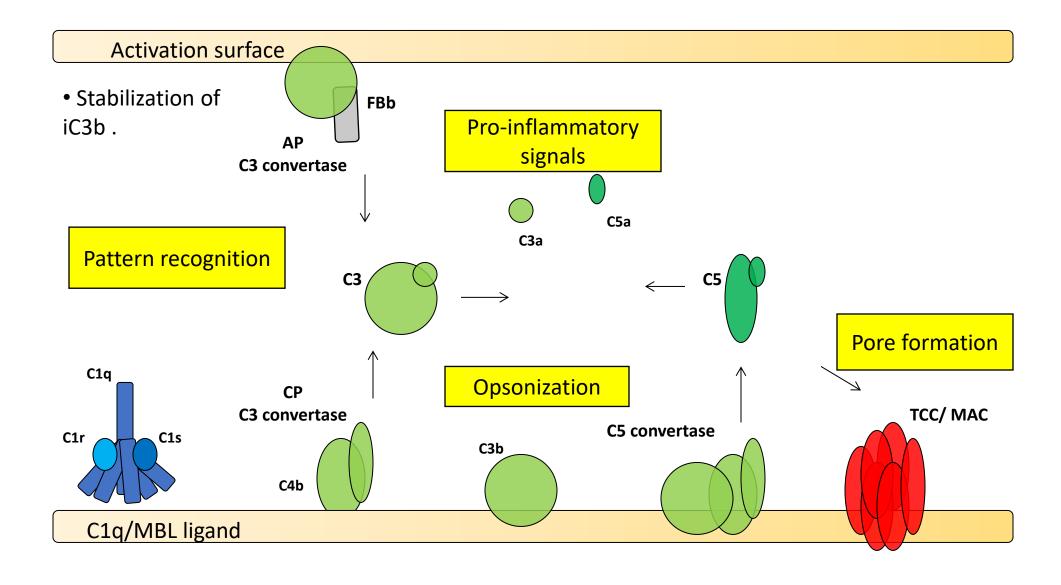
- 3 pathways of complement activation depend on different PRR but converge at C3 activation.
- A C3 convertase is formed from activated complement proteins, In the classical and lectin pathways, C3 convertase is made from C2bC4b, while in the alternative pathway, it's made from C3bFb.
- Each step of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







Complement activation



Pattern recognition - CD46, CD55 Regulation | and opsonization and CD59 - C1q and properdi C1-INH Inhibits are membrane are synthesized C1q CD46 is a cofactor for C1q binds to Apoptotic Proteases C1r,C1s,MASPS CD46 bound regulators mainly by immune FI and inhibits convercells, IgG/ IgM, DAMPs associated expressed on all cells in the bone C1- INH tase formation and PAMPs and with PRM tissue. marrow and spleen. Initiates CP. cleave C2 and C4 CD55 and CD59 - C1q is able to rec-CD55 is a glycoprotein MBL are GPI anchored ognize an increas-MBL and ficolins bind that accelerates proteins with high ing number of sigcarbohydrate patterns convertase decay mobility on the cell nals. Some of which and initiates LP Ficolins membrane to sites are still undefined CD59 is a glycoprotein of activation. such as signals for CD59 that prevents TCC synaptic pruning in Properdin binds formation - Soluble CD59 the CNS. PAMPs and DAMPs forms are found in Properdin and initiates AP. Inflammatory body fluids. signaling and induction Also stabilizes AP convertases of phagocytosis _____ ______ **Activation and** amplification - Complement Proteases. - C3a and C5a proteins CR1 binds C3b and Convertase formation (anaphylatoxins) C2.C3,C4,C5,C6,C8 induces phagocytosis and amplification CR1 promote hallmark ,C9, FB,FH,FI and Also accelerates pro-inflammatory convertase decay CP+LP functions such as are produced mainly C3 convertase increased vascular by the liver with ex-C4b/ C2b cC1qR Recognizes inhibited by C4BP, FH cC1qR permeability, trahepatic producbound C1q / induces C3 and CD46 smooth muscle tion as well. phagocytoses contraction and leukocyte recruitment - A "tick-over" theory FH recognizes self sur-C3aR a GPCR that C3aR explains the presfaces and is a cofactor induces strong FH - C3aR and C5aR for FI ence of sponatiously FD FD cleaves inflammatory response are expressed by a generated C3b mol-C3b-bound FB when C3a is bound wide variety of ecules C3(H2O) C3b Bb to Bb immune and AP C3 convertase ready to form con-CR2 binds C3d bound non-immune cells. vertases with FB. to antigens, providing CR2 Such molecules pro-FI coactivation and amplifi FHL-1 accelarates FI degrades C3b and C3aR and C5aR vide probing ability cation of B-cell convertase decay activation enhance C4b with cofactors help since the converresponse TLR-induced cytases are rapidly detokine production CR3 binds iC3b and graded on normal CR3 (TNF,IL1-β). induces phagocytosis. surfaces. C2b - C5aR signaling - The heavier frag-C4b enhances ment of cleavage C5aR a GPCR that coagulation through C5aR designated "b" CP+LP C5 convertase induces strong expression of deposits on the inflammatory response tissue factor. surface due to when C5a is bound uncovering of highly - C5aR signaling reactive internal Bb Vn inhibits TCC upregulates activatthioester bond. assembly ing Fcy receptors - Accumulation of AP C5 convertase C6 C3b fragments on the surface leads to iC3b C3d С3с C5b formation of C5 TCC convertases. Membrane Degradation products of C3 deposit on the can cause cell lysis, while sub-lytic Disruption (membrane as opsonins and propagate the inconcentrations can induce cell cycle flammatory response or induce phagocytosis by progression and inflammation interacting with their respective receptors



Sea urchins present around 500 million years ago have 2 components with significant homology to vertebrate C3 and factor B (Bf), calledSpC3 and SpBf, respectively.

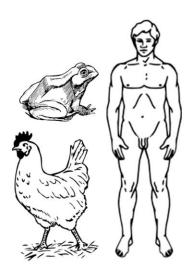
Those components found in body fluid can be induced in response to immune challenge and are thought to represent a primitive alternative pathway.



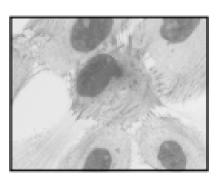
Lamprey is an early vertebrate that lacks immunoglobulins and thus a classical pathway.

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

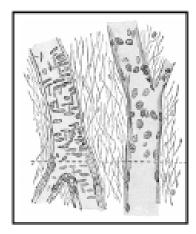
- Deposition of activation fragments including TCC in breast cancer tissue, but not benign lesions.
- Upregulation of complement regulatory proteins in several solid tumors.
- Increased levels of activation fragments including TCC in serum of oral carcinoma patients compared to healthy subjects.



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.



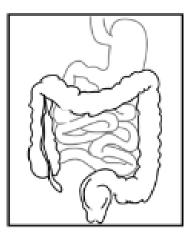


Ischemia/ reperfusion (I/R) injury

- 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