Role of complement in health and disease

Presented by: Dr. Mandeep kaur
Moderated by: Dr. Varsha Gupta
Complement

• **Definition:** Term ‘complement’ refers to a system of factors that occur in normal serum and are activated characteristically by antigen-antibody interaction and subsequently mediate a number of biologically significant consequences.
Complement system

- Major effector of humoral branch of immune system.
- **Complement system** - biochemical cascade that helps or “complements” the ability of antibodies to clear pathogens from an organism.
- Part of innate immune system.
- However, can be recruited and brought into action by the adaptive immune system.
Complement System

**Innate immunity**
- Opsonization
- Lysis of pathogens
- Chemotaxis
- Inflammation
- Cell activation

**Disposal system**
- Clearance of immune complexes and apoptotic cells

**Adaptive immunity**
- Augmentation of antibody response
- Promotion of T-cell response
- Elimination of self-reactive B cells
- Enhancement of immunologic memory
History

• 1889- **Hans Ernst Buchner**, first observed that bactericidal effect of the serum was destroyed by heating at 55° C for one hour.

• 1894- **Pfieffer** discovered that cholera vibrios were lysed when injected intraperitoneally into specifically immunized guinea pigs (bacteriolysis in vivo or *Pfieffer’s phenomenon*).

• 1895- **Joules Bordet** established that immune bacteriolysis and hemolysis required two factors- heat stable antibody and a heat labile factor, k/a alexine.
History

• Later on, **Ehrlich** coined the term complement because this factor complemented the action of the antibody.

• **1901- Bordet and Gengou**, described the complement fixation test, using the hemolytic indicator system, as a sensitive serological reaction.
Functions of the complement

1. **Lysis** of cells, bacteria and viruses.
2. **Opsonization**, which promotes phagocytosis of particulate antigens.
3. Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, **inflammation**, and secretion of immunoregulatory molecules.
4. **Immune Clearance**, which removes immune complexes from immune system and deposits them in the spleen and liver.
Antibodies attach to antigens (this prevents the pathogen from entering a host cell)

Antibodies cause pathogens to clump together

Inactive complement protein attaches

Antibody

Antigen

Pathogen

Complement protein activated

Complement causes pathogen cell to burst

Antibody activates complement protein
General properties

• Present in sera of all mammals and also in most other animals, including birds, amphibians and fish.
• Nonspecific serological reagent- complement from one species can react with antibodies of other species.
• Soluble proteins and glycoproteins
• Synthesized mainly by liver hepatocytes, also by blood monocytes, tissue macrophages and epithelial cells of GIT and GUT.
• 5 % of normal serum proteins, not increased by immunization.
• Heat labile; destroyed in 30 mins at 56° C.
• Inactivated serum- serum deprived of complement activity.
• Present as ‘Zymogens’ or inactive forms (denoted by ‘i’) in circulation and are activated by proteolytic cleavage.
## Complement components

<table>
<thead>
<tr>
<th>Classic pathway component</th>
<th>Molecular weight</th>
<th>Serum concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>410,000</td>
<td>70</td>
</tr>
<tr>
<td>C1r</td>
<td>85,000</td>
<td>34</td>
</tr>
<tr>
<td>C1s</td>
<td>85,000</td>
<td>31</td>
</tr>
<tr>
<td>C2</td>
<td>102,000</td>
<td>25</td>
</tr>
<tr>
<td>C3</td>
<td>190,000</td>
<td>1200</td>
</tr>
<tr>
<td>C4</td>
<td>206,000</td>
<td>600</td>
</tr>
<tr>
<td>C5</td>
<td>190,000</td>
<td>85</td>
</tr>
<tr>
<td>C6</td>
<td>128,000</td>
<td>60</td>
</tr>
<tr>
<td>C7</td>
<td>120,000</td>
<td>55</td>
</tr>
<tr>
<td>C8</td>
<td>150,000</td>
<td>55</td>
</tr>
<tr>
<td>C9</td>
<td>71,000</td>
<td>60</td>
</tr>
</tbody>
</table>
## Complement-components

<table>
<thead>
<tr>
<th></th>
<th>Molecular weight</th>
<th>Serum concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alternative pathway component</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Properdin</td>
<td>53,000</td>
<td>25</td>
</tr>
<tr>
<td>Factor B</td>
<td>90,000</td>
<td>225</td>
</tr>
<tr>
<td>Factor D</td>
<td>25,000</td>
<td>1</td>
</tr>
<tr>
<td><strong>Inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td>105,000</td>
<td>275</td>
</tr>
<tr>
<td>Factor I</td>
<td>88,000</td>
<td>34</td>
</tr>
<tr>
<td><strong>Regulatory proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4-binding protein</td>
<td>560,000</td>
<td>8</td>
</tr>
<tr>
<td>Factor H</td>
<td>150,000</td>
<td>500</td>
</tr>
<tr>
<td>S protein (vitronectin)</td>
<td>80,000</td>
<td>500</td>
</tr>
</tbody>
</table>
Complement- components

• Components- designated by numerals (C1-C9), letter symbols (e.g. factor D) or trivial names (homologous restriction factor).
• Larger fragment is designated as ‘b’ and smaller fragment as ‘a’ except C2a (larger fragment).
• Larger fragment participates in cascade while smaller fragment diffuses away.
• Activated form is denoted by a bar over the number or symbol e.g. $\bar{C}4b$
Complement Pathways

• Early steps culminating in formation of C5 convertase, can occur by:
  a) Classical pathway,
  b) Alternative pathway, or
  c) Lectin pathway.

• Final steps leading to formation of MAC are identical in all three pathways.
Structure of C1

- C1 is the initiator of cascade in classical complement pathway.
- C1 is a macromolecular complex composed of 3 different proteins (C1q, C1r and C1s) held together by Calcium ions.
- Each C1 complex composed of one C1q, 2 C1r and 2 C1s chains.
- Enzymatic potential resides in C1r and C1s.
- C1q complex is itself composed of 6 identical subunits each carrying 3 different polypeptide chains forming triple helical structure.
Structure of C1

• 6 subunits are arranged to form a globular central core from which 6 arms radiate outward.

• At the end of each arm is a podlike hand that is formed by carboxy termini of all 3 chains and mediates binding to Fc piece (\(C_H^2\) domains of IgG and \(C_H^4\) of IgM) of immunoglobulins of appropriate subclasses.

• C1q binds to IgM, IgG3, IgG1 and IgG2 but not IgG4, IgA and IgE.
Classical Pathway

• Pathway triggered by formation of Ag-Ab complex.
• Nonimmunologic pathway activators-
  - certain bacteria and viruses,
  - surface of urate crystals,
  - myelin basic protein,
  - denatured DNA,
  - bacterial endotoxin and
  - polyanions such as heparin.
Classical Pathway

• C1q binds antigen bound antibody. C1r activates auto catalytically and activates the second C1r; both activate C1s.
• C1s cleaves C4 and C2. Cleaving C4 exposes the binding site for C2.
• C4b binds the surface near C1 and C2 binds C4, forming C3 convertase.
Classical Pathway

• C3 convertase hydrolyzes many C3 molecules. Some combine with C3 convertase to form C5 convertase.
• C3b component of C5 convertase binds C5, permitting C4b2a to cleave C5.
• C5b binds C6, initiating the formation of the membrane attack complex.
Activated C1s cleaves C4 to C4a and C4b, which binds to the microbial surface.

C4b then binds C2, which is cleaved by C1s, to C2a and C2b, forming the C4b2b complex.

C4b2b is an active C3 convertase cleaving C3 to C3a and C3b, which binds to the microbial surface or to the convertase itself.

One molecule of C4b2b can cleave up to 1000 molecules of C3 to C3b. Many C3b molecules bind to the microbial surface.
Alternative Pathway

• No antigen antibody complexes required for initiation.
• Component of innate immune system.
• Four serum proteins are required:
  i. C3
  ii. Factor B
  iii. Factor D
  iv. Properdin
## Initiators of alternative pathway

<table>
<thead>
<tr>
<th>Pathogens and particles of microbial origin</th>
<th>Non pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many strains of gram negative bacteria</td>
<td>Human IgG, IgA, and IgE complexes</td>
</tr>
<tr>
<td>LPS from gram negative bacteria</td>
<td>Rabbit and guinea pig IgG in complexes</td>
</tr>
<tr>
<td>Many strains of gram positive bacteria</td>
<td>Cobra venom factor</td>
</tr>
<tr>
<td>Teichoic acid from gram positive cell walls</td>
<td>Heterologous erythrocytes (rabbit, mouse, chicken)</td>
</tr>
<tr>
<td>Fungal and yeast cells (zymosan)</td>
<td>Anionic polymers (dextran sulfate)</td>
</tr>
<tr>
<td>Some viruses and virus infected cells</td>
<td>Pure carbohydrates (agarose, inulin)</td>
</tr>
<tr>
<td>Some tumor cells</td>
<td></td>
</tr>
<tr>
<td>Parasites (trypanosomes)</td>
<td></td>
</tr>
</tbody>
</table>
Alternative Pathway

• C3 hydrolyzes spontaneously to form C3(H₂O) which in presence of Mg ions bind Factor B, and further acted on by Factor D to cleave Factor B forming a complex having C3 convertase (C3bBb) activity.

• Binding of Properdin stabilizes C3 convertase, slowing its decay.
Alternative Pathway

• C3 convertase further cleave additional C3 to form C3b and form larger complex C3bBb3b k/a C5 convertase.

• C5b binds to antigenic surface commencing final phase of lytic cycle.
Lectin pathway (MBL - MASP)

- Lectins are proteins that bind to specific carbohydrate targets.
- Lectin activating complement binds to mannose residues; present on surface of various bacterial cells, fungi and viruses, so also called as MBL (mannan-binding lectin pathway)
- Human cells have sialic acid residues covering sugar groups hence are not the target for binding.
- Lectin pathway is homologous to classical pathway but not activated by antibodies like alternate pathway.
Lectin pathway (MBL - MASP)

- MBL- acute phase protein; conc. increases during inflammation.
- Similar in structure and function to C1q.
- After MBL bind to carbohydrate, MBL associated serine proteases, MASP-1 and MASP-2 bind to MBL.
- This active complex causes cleavage of C4 and C2 forming C3 and then C5 convertase.
Formation of MAC

- Terminal sequence of complement activation involves C5b, C6, C7, C8 and C9, which interact sequentially to form a macromolecular structure called the membrane attack complex.

- It forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane.
Formation of MAC

• C5b formed by C5 convertase is rapidly inactivated unless stabilized by C6 next component in the cascade.
• C5b6 complex binds C7 to form strongly hydrophobic molecule i.e. C5b67 which is capable of inserting itself into the lipid bilayer of cell membranes.
• This complex accepts one molecule of C8 and multiple molecules of C9 to ultimately form a ‘cylindrical transmembrane channel’ termed as membrane attack complex (MAC).
Formation of MAC

- MAC has outer hydrophobic surface and inner hydrophilic core through which small ions and water can pass.
- Water enters cell because of high osmotic pressure inside the cell and the cell swells and bursts.
Regulation of complement pathway

• The complement system has the potential to be extremely damaging to host tissues, hence regulatory mechanisms are required to restrict the complement pathway.

• **Passive mechanism**: highly labile components that undergo spontaneous inactivation if not stabilized by other components.

• **Active mechanism**: series of regulatory proteins that inactivate various complement components.
Regulation of complement pathway

- Present at a higher concentration in the blood plasma than the complement proteins and also on the membranes of self-cells preventing them from being targeted by complement e.g. CD59
- Reaction catalyzed by C3 convertase is the major amplification step in 3 pathways generating hundreds of C3b molecules.
- Many regulatory proteins check the activity of C3 convertase.
Regulation of complement pathway

• These regulatory proteins (DAF, Factor H, C4BP, CR1 and CR2) contain repeating amino acid sequences of about 60 residues termed short consensus repeats (SCRs) and are encoded at a single location on Chr. 1 in humans k/a regulators of complement activation (RCA) gene cluster.

• Genes for C4, C2 and Factor B are located on short arm of Chr. 6 in humans and are termed as class III histocompatibility genes.
## Regulatory Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Type of protein</th>
<th>Pathway affected</th>
<th>Immunologic function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor (C1Inh)</td>
<td>Soluble</td>
<td>Classical</td>
<td>Serine protease inhibitor; causes C1r₂s₂ to dissociate from C1q; Also an inhibitor of activated Hageman factor</td>
</tr>
<tr>
<td>C4b-binding protein (C4Bp)</td>
<td>Soluble</td>
<td>Classical and lectin</td>
<td>Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I</td>
</tr>
<tr>
<td>Factor H</td>
<td>Soluble</td>
<td>Alternative</td>
<td>Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I</td>
</tr>
<tr>
<td>Protein</td>
<td>Type of protein</td>
<td>Pathway affected</td>
<td>Immunologic function</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Factor I</td>
<td>Soluble</td>
<td>Classical, alternative and lectin</td>
<td>Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor</td>
</tr>
<tr>
<td>S protein/ Vitronectin</td>
<td>Soluble</td>
<td>Terminal</td>
<td>Binds soluble C5b67 and prevents its insertion into cell membrane</td>
</tr>
<tr>
<td>Anaphylatoxin inactivator</td>
<td>Soluble</td>
<td>Effector</td>
<td>Inactivates anaphylatoxin activity of C3a and C5a by carboxypeptidase N-catalyzed removal of C-terminal Arginine.</td>
</tr>
<tr>
<td>Protein</td>
<td>Type of protein</td>
<td>Pathway affected</td>
<td>Immunologic function</td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>-----------------</td>
<td>-----------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Decay-accelerating factor (DAF or CD35)</td>
<td>Membrane bound</td>
<td>Classical, alternative and lectin</td>
<td>Accelerates dissociation of $\mathrm{C}_4\mathrm{b}2\mathrm{a}$ and $\mathrm{C}_3\mathrm{b}B\mathrm{b}$ (classical and alternative C3 convertases)</td>
</tr>
<tr>
<td>Homologous restriction factor (HRF)</td>
<td>Membrane bound</td>
<td>Terminal</td>
<td>Bind to C5b678 on autologous cells, blocking binding of C9</td>
</tr>
<tr>
<td>CD59</td>
<td>Membrane bound</td>
<td>Terminal</td>
<td>Bind to C5b678 on autologous cells, blocking binding of C9</td>
</tr>
<tr>
<td>Complement receptor type 1 (CR1 or CD35) Membrane-cofactor protein (MCP or CD46)</td>
<td>Membrane bound</td>
<td>Classical, alternative and lectin</td>
<td>Blocks formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b</td>
</tr>
</tbody>
</table>
Complement Receptors (CR)

• Many of the biological activities of the complement system depend on binding of complement fragments to complement receptors, expressed by various cells.

• Some complement also play an important role in regulating complement activity by binding biologically active complement components.
# Complement Binding Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Major ligands</th>
<th>Activity</th>
<th>Cellular distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1 (CD35)</td>
<td>C3b, C4b</td>
<td>Blocks formation of C3 convertase; binds immune complexes to cells</td>
<td>Erthrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells</td>
</tr>
<tr>
<td>CR2 (CD21)</td>
<td>C3d, C3dg,* iC3b</td>
<td>Part of B-cell coreceptor; binds Epstein-Barr virus</td>
<td>B cells, some T cells</td>
</tr>
<tr>
<td>CR3 (CD11b/18)</td>
<td>iC3b</td>
<td>Bind cell-adhesion molecules on neutrophils, facilitating their extravasation; blind immune complexes, enhancing their phagocytosis</td>
<td>Monocytes, macrophages, neutrophils, natural killer cells, some T cells</td>
</tr>
<tr>
<td>CR4 (CD11c/18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3a/C4a receptor</td>
<td>C3a, C4a</td>
<td>Induces degranulation of mast cells and basophils</td>
<td>Mast cells, basophils, granulocytes</td>
</tr>
<tr>
<td>C5a receptor</td>
<td>C5a</td>
<td>Induces degranulation of mast cells and basophils</td>
<td>Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells</td>
</tr>
</tbody>
</table>

*Cleavage of C3dg by serum proteases generates C3d and C3g.*
Biological Consequences of complement activation

• Complement serves as an important mediator of humoral response by amplifying the response and converting it into an effective defence mechanism to destroy invading microorganisms.
### Biological effects of components of complement system

<table>
<thead>
<tr>
<th>Effect</th>
<th>Complement product mediating*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell lysis</strong></td>
<td>C5b–9, the membrane-attack complex (MAC)</td>
</tr>
<tr>
<td><strong>Inflammatory response</strong></td>
<td></td>
</tr>
<tr>
<td>Degranulation of mast cells and basophils†</td>
<td>C3a, C4a, and C5a (anaphylatoxins)</td>
</tr>
<tr>
<td>Degranulation of eosinophils</td>
<td>C3a, C5a</td>
</tr>
<tr>
<td>Extravasation and chemotaxis of leukocytes at inflammatory site</td>
<td>C3a, C5a, C5b67</td>
</tr>
<tr>
<td>Aggregation of platelets</td>
<td>C3a, C5a</td>
</tr>
<tr>
<td>Inhibition of monocyte/macrophage migration and induction of their spreading</td>
<td>Bb</td>
</tr>
<tr>
<td>Release of neutrophils from bone marrow</td>
<td>C3c</td>
</tr>
<tr>
<td>Release of hydrolytic enzymes from neutrophils</td>
<td>C5a</td>
</tr>
<tr>
<td>Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils</td>
<td>C5a</td>
</tr>
<tr>
<td>Opsonization of particulate antigens, increasing their phagocytosis</td>
<td>C3b, C4b, iC3b</td>
</tr>
<tr>
<td>Viral neutralization</td>
<td>C3b, C5b–9 (MAC)</td>
</tr>
<tr>
<td>Solubilization and clearance of immune complexes</td>
<td>C3b</td>
</tr>
</tbody>
</table>
OPSONIZATION BY C3b

- Bacterium
- IgG
- Complement activation
- C3b
- CR1
- Fc receptor
- Phagocyte
- Phagocytosis
- Nucleus
CLEARING OF IMMUNE COMPLEXES
Evasion of Complement system by microorganism

• MAC causes lyses of various cells like most gram negative bacteria, some gram positive bacteria, enveloped viruses etc.

• But some gram negative and most of gram positive bacteria have developed mechanisms for evading complement-mediated damage.
Microbial Evasion of complement mediated damage

<table>
<thead>
<tr>
<th>Microbial component</th>
<th>Mechanism of evasion</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long polysaccharide chains in cell-wall LPS</td>
<td>Side chains prevent insertion of MAC into bacterial membrane</td>
<td>Resistant strains of <em>E. coli</em> and <em>Salmonella</em></td>
</tr>
<tr>
<td>Outer membrane protein</td>
<td>MAC interacts with membrane protein and fails to insert into bacterial membrane</td>
<td>Resistant strains of <em>Neisseria gonorrhoeae</em></td>
</tr>
<tr>
<td>Elastase</td>
<td>Anaphylatoxins C3a and C5a are inactivated by microbial elastase</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptidoglycan layer of cell wall</td>
<td>Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan</td>
<td><em>Streptococcus</em></td>
</tr>
<tr>
<td>Bacterial capsule</td>
<td>Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1</td>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>on phagocytic cells</td>
<td></td>
</tr>
<tr>
<td><strong>Other microbes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteins that mimic complement regulatory proteins</td>
<td>Proteins present in various bacteria, viruses, fungi, and protozoans inhibit the complement cascade</td>
<td>Vaccinia virus, herpes simplex, <em>Epstein-Barr virus</em>, <em>Trypanosoma cruzi</em>, <em>Candida albicans</em></td>
</tr>
</tbody>
</table>
Evasion mechanisms by viruses

- Interference with the binding of complement to antigen-antibody complexes e.g. Herpes virus
- Viral mimicry of mammalian complement regulators e.g. Vaccinia virus
- Incorporation of cellular complement regulators in the virion e.g. HTLV-1
Evasion mechanism by helminths

- Helminths usually evade the effects of complement by inhibiting complement action or increased local consumption of complement factors.
- In Schistosomes, following mechanisms are there-
  1. Secretion of muscle protein ‘paramyosin’- bind C1q and inhibit binding of C4; prevent complement activation.
  2. Lipid anchored protease on surface of larva- cleave C3 and C9; inhibit complement mediated and neutrophil dependent killing.
iii. Protease inhibitor (similar to CD59)- blocks assembly of MAC.

iv. Incorporation of DAF in outer surface membrane of cyst wall*.

• In *Echinococcus granulosus*, Factor H gets incorporated into cyst wall*.

*-- DAF and Factor H inhibit the activation of complement cascade downstream of C3bBb complex.

• In *Taenia taeniaeformis*, early complement factors get stick to the mucopolysaccharide in cystic bladder fluids.
Complement system associated diseases

• 3 groups of diseases that result from abnormality of complement system:

1. Deficiency of some component of complement system
2. Abnormalities of regulation of complement system
3. Stimulation of complement system by abnormal stimuli.
Disorders of Complement system

- Infectious diseases, sepsis, anaphylaxis
- I/R injury
- Paroxysmal nocturnal hemoglobinuria
- Psoriasis
- Myasthenia gravis
- Systemic lupus erythematosus
- Multiple sclerosis
- Transplant rejection
- Cancer
- Stroke
- Alzheimer’s disease
- Age-related macular degeneration
- Asthma
- Myocardial infarction
- Atypical hemolytic uremic syndrome
- Crohn’s disease
- Rheumatoid arthritis
Complement deficiencies

- Deficiency of early components like C3, C2 and C4.
- Terminal complex component deficiency i.e. C5-C9 resulting in lack of MAC complex.
- Abnormalities of regulatory proteins
## COMPLEMENT DEFICIENCY DISEASES

<table>
<thead>
<tr>
<th>Complement deficiency</th>
<th>Disease/pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> Classical pathway deficiency</td>
<td></td>
</tr>
<tr>
<td>C1q, C1r, C1s</td>
<td>SLE with pyogenic infections</td>
</tr>
<tr>
<td>C4</td>
<td>SLE with glomerulonephritis</td>
</tr>
<tr>
<td>C2</td>
<td>SLE, vasculitis, glomerulonephritis and pyogenic infection</td>
</tr>
<tr>
<td><strong>B</strong> Alternative pathway deficiency</td>
<td></td>
</tr>
<tr>
<td>Properdin</td>
<td>Neisserial infections and pyogenic infections</td>
</tr>
<tr>
<td>Factor D</td>
<td></td>
</tr>
<tr>
<td><strong>C</strong> Common deficiencies in both pathways</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>Immune complex disease, pyogenic infections and glomerulonephritis</td>
</tr>
<tr>
<td><strong>D</strong> Terminal component deficiency</td>
<td></td>
</tr>
<tr>
<td>C5, C6, C7, C8</td>
<td>Disseminated Neisserial infections</td>
</tr>
<tr>
<td>C9</td>
<td>None</td>
</tr>
</tbody>
</table>
# Complement regulatory protein diseases

<table>
<thead>
<tr>
<th>Protein</th>
<th>Complement abnormality</th>
<th>Disease/pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor (C1 inh)</td>
<td>Overactive classical pathway</td>
<td>Hereditary angioneurotic oedema</td>
</tr>
<tr>
<td>DAF and CD 59</td>
<td>Deregulated C3 convertase activity- increased RBC lysis</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
</tr>
<tr>
<td>Factor I</td>
<td>Deregulated classical pathway with overconsumption of C3</td>
<td>Immune complex disease; recurrent pyogenic infections</td>
</tr>
<tr>
<td>Factor H</td>
<td>Deregulated alternative pathway with increased C3 convertase</td>
<td>Immune complex disease and pyogenic infections</td>
</tr>
</tbody>
</table>
Abnormalities arising from normal complement system

- Bystander damage of the normal cells: damage of cells in the immediate vicinity of the released inflammatory mediators e.g. free radicals, histamine etc.

- Intravascular thrombosis leading to ischemic effects due to following possible reasons:
  
  i. Damaged endothelial surface following complement activation favours thrombosis.
  
  ii. Pre-cytolytic MAC complexes- cause activation of prothrombinases.
  
  iii. C5a may alter endothelial surface heparan sulphate promoting coagulation.
Role of complement in hypersensitivity reactions

• Complement system plays role in type II and type III hypersensitivity reactions.

• **Type II Reaction**: mediated by IgG or IgM to foreign antigens which are bound to cell surfaces or other molecules.

• Opsonised antigen stimulates various mechanisms aimed at elimination such as phagocytosis and complement activation leading to MAC formation and ADCC.

• IgG also stimulates the complement pathway by binding to it through the complement binding sites on Fc fragment.

• Example: blood transfusion reactions and drug induced hemolytic anaemia.
• **Type III reaction**: this reaction is due to excessive formation of immune complex (Ag-Ab complex) which initiate an inflammatory reaction through the activation of complement system leading to tissue damage.

• **Localized reaction**: in case of antibody excess or antigen antibody equivalence, large insoluble complexes are formed which tend to localize at the site of antigen administration, e.g. Arthus reaction.

• **Generalized reaction**: in case of extreme antigen excess particularly monovalent antigen, small soluble complexes are formed which tend to distribute widely in the body, e.g. Serum sickness.
Complement therapies

• Breakthrough came in early 1990s, when it was demonstrated that an engineered recombinant soluble form of a natural C regulator, CR1, was powerful inhibitor of C activation both in vitro and vivo.

• Considerations:
  i. Side effects of long term systemic inhibition of C synthesis.
  ii. Choice of most efficient point at which to inhibit the complement.
  iii. Rapid clearance of reagents in vivo.
  iv. High cost of biological therapies.
# Complement therapies

<table>
<thead>
<tr>
<th>Agent</th>
<th>History and status</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCR1 (TP10)</td>
<td>The first of the new generation of anti-C therapies; used in many models; first in clinical trials. Now superseeded?</td>
<td>Proof of concept; works across species</td>
<td>Expensive Systemic</td>
</tr>
<tr>
<td>Sle-sCR1 (TP20)</td>
<td>First of the modified sCR1 agents; binds endothelium at sites of inflammation, tested in many models, no clinical trial.</td>
<td>May be more ‘site specific’ than sCR1</td>
<td>Expensive Systemic</td>
</tr>
<tr>
<td>APT-070</td>
<td>Truncated, membrane-targeted sCR1 derivative, tested in several models, early stages of clinical trials.</td>
<td>‘Site specific’ ?, retained at injection site? Made in bacteria so cheaper?</td>
<td>Unproven Systemic</td>
</tr>
</tbody>
</table>
# Complement therapies

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<tr>
<td>h5G1.1 and derivatives</td>
<td>Recombinant scFv of anti-C5 monoclonal antibody (mAb); permits opsonization; effective in several models; well advanced in clinical trials</td>
<td>Long term half life compared with sCR1; relatively cheap; nearest to the clinic. The mAb-based therapies well accepted</td>
<td>Systemic</td>
</tr>
<tr>
<td>C5aR antagonists</td>
<td>Several agents vying for this niche; attractive drug target; many positive results in models.</td>
<td>Small molecule agents (peptides and others); may be inexpensive, may work orally or topically</td>
<td>Unproven</td>
</tr>
<tr>
<td>Small molecule antagonists of components</td>
<td>Numerous agents, best explored is the C3 inhibitor Compstatin; good results in models, no trials</td>
<td>May be inexpensive, may be active orally or topically</td>
<td>Unproven</td>
</tr>
</tbody>
</table>
Complement fixation test

• Complement takes part in many immunological reactions and is absorbed during the combination of antigens with their antibodies.

• The ability of antigen-antibody complexes to ‘fix’ complement is made use of in the complement fixation test (CFT).

• Very versatile and sensitive test, capable of detecting as little as 0.04mg of antibody and 0.1ml of antigen.
Complement fixation test

• CFT- complex procedure consisting of two steps and five reagents- Antigen, Antibody, Complement, Sheep erythrocytes and Amboceptor (Rabbit antibody to sheep red cells).

• Each of these agents have to be separately standardized.
Complement Fixation System

Complement is used in the Indicator system therefore lysis of the RBC
CFT

- Antigen: soluble/particulate
- Antibody: heat inactivated serum used to destroy complement activity and nonspecific inhibitors of complement
- Source of complement: guinea pig serum
- Indicator system: sheep erythrocytes with amboceptor
- Diluent used for titrations and for CFT-physiological saline with added calcium and magnesium ions
**CFT**

- Guinea pig serum should be titrated for activity.
- One unit or MHD of complement is defined as the highest dilution of the guinea pig serum that lyses one unit volume of washed sheep erythrocytes in the presence of excess hemolysin (amboceptor) within fixed time (usually 30 or 60 minutes) at a fixed temperature (37° C).
CFT

• Amboceptor should be titrated for hemolytic activity.
• One MHD of amboceptor is defined as the least amount (or highest dilution) of the inactivated amboceptor that lyses one unit volume of washed sheep erythrocytes in the presence of excess complement within a fixed time (usually 30 or 60 minutes) at a fixed temperature (37° C).
CFT

• Adequate controls should be used; like
  1. Antigen and serum controls to ensure that they are not anticomplementary
  2. Complement control to ensure that the desired amount of complement is added
  3. Cell control to see that sensitised erythrocytes do not undergo lysis in the absence of complement.
37°C \rightarrow \text{stage I} \rightarrow \text{37°C stage II} \rightarrow \text{readings}

\text{indicator system:}
\text{sheep red cells sensitized with anti-sheep antibody now added}

\text{controls}
\begin{align*}
A & \quad \text{complement} \rightarrow \text{sensitized cells} \rightarrow \text{hemolysis} \\
B & \quad \text{antigen + complement} \rightarrow \text{sensitized cells} \rightarrow \text{hemolysis} \\
C & \quad \text{antibody + complement} \rightarrow \text{sensitized cells} \rightarrow \text{hemolysis}
\end{align*}

\text{test system}
\begin{align*}
\text{antigen + antibody + complement} & \rightarrow \text{sensitized cells} \rightarrow \text{no hemolysis - positive test} \\
\text{complement becomes bound or "fixed" here} & \rightarrow \text{hemolysis - negative test}
\end{align*}
CFT

• Classical example of CFT: Wassermann reaction
• Indirect complement fixation test: used for certain avian and mammalian sera not fixed by guinea pig complement
• Conglutinating complement absorption test: alternative method for the sera not fixing guinea pig complement
Complement Fixation Test

- Serum with antibodies
- Antigen binds with antibodies
- Complement binds with Ag/Ab complex
- Hemolysin Sensitized red blood cells serve as an indicator
- RBCs settle into a pellet
- Reactive

- Serum without antibodies
- Unbound Antigen
- Unbound complement
- Hemolysin Sensitized RBCs serve as an indicator
- RBCs lysed by unbound complement
- Lysis
- Nonreactive
Other complement dependent tests

• Immune adherence: When bacteria react with the specific antibody in presence of complement and particulate materials such as erythrocytes and platelets, bacteria are aggregated and adhere to the cells.
• Immobilisation test e.g. *Treponema pallidum immobilisation test*
• Cytolytic or cytocidal tests: also complement dependent; e.g. measure anticholera antibodies
• Immunoflourescence test: can also use labelled complements for detection of Ag or Ab.
Thank you!