HUMORAL FACTORS

INTRODUCTION - HUMORAL FACTORS

- Circulating effector blood proteins of innate immunity are
  - Complement
  - Opsonin
  - Properdin etc.

COMPLEMENT SYSTEM

- The complement system is one of the major effector mechanisms of humoral immunity and as well as of innate immunity.
- The complement system is composed of several (at least 19) heat labile (56^{o}C in 30 minutes) serum (plasma) proteins and constitutes about 10% of the globular fraction of serum.
- Molecular weight of the complement components varies from 24 KDa (factor D) to 460 KDa (C1q).
- The complement proteins are labeled numerically with the prefix C (C1, C2, C3 --- C9) or designated by letters of the alphabet (B, D, P etc).
- Peptide fragments formed by activation of a component are denoted by small letter (C3a, C3b etc).
- The complement fragments interact with one another to form functional complexes.
- Those complexes with enzymatic activity are denoted by a bar over the number or symbol.
- Complement proteins constitute nearly 10% serum proteins. Complement components are synthesized at various sites like liver macrophages.
- Complement system is normally inactive but activated under certain condition like microbial infection and generates effector mechanism to destroy the activator (i.e the microbes).
- Activation of complements involve the sequential proteolysis of proteins to generate enzymes with proteolytic activity.
- Proteins that acquire proteolytic enzymatic activity by the action of other proteases are called ***zymogens*** (proenzymes).
- Zymogens are activated sequentially i.e. the product of first reaction catalyzes a second reaction and the product of second reaction catalyzes third reaction and so on. This types of chain of enzymatic reaction are known as ***cascade reaction***.
- The products of activated complement attach covalently to microbial cell surfaces or antibody coated microbes or other antigens and cause lysis of the target cells (e.g.microbe).
- Complement activation is inhibited by regulatory proteins that are present in normal host cells in absence of microbes. Thus normal host is not affected.
- Guinea pig serum contains all the factors of the lytic complement in correct proportion. Hence, fresh guinea pig serum, preferably pooled is the best source of complement.
- While collecting blood for separation of serum for CFT, there should not be any hemolysis or tissue damage. In both these conditions anticomplement factors are released. Mouse and horse complements are incomplete and poorly lytic.
- Pathways of Complement Activation - There are three major pathways for complement activation
  - The Classical Pathway – which is activated by certain antibodies bound to antigens.
  - The Alternative Pathway – which is activated by microbial cell in the absence of antibody.
  - The Lectin Pathway - which is activated by plasma lectin bound to mannose residues on microbes.

THE CLASSICAL PATHWAY

- Free immunoglobulin molecules can not bind or activate complement components.

- When immunoglobulin binds to the antigen, the complement-binding site is exposed (because of conformational changes).
- In classical pathway, first complement component C_1 binds to CH^3 domain of IgM or CH^2 domain of IgG molecule. The C_1 molecule is composed of three separate proteins C_{1q}, C_{1r} and C_{1s} bound together by calcium (Ca++) dependent bonds.
- The C_{1q} subunit is made up of an umbrella like radial array of six chains that are connected to central stalk by a collagen like arm and each has globular head, which recognizes and binds to Fc region of immunoglobulin heavy chain.
- Each Fc region of an immunoglobulin has one C_{1q} binding site and for activation of C_1q at least two heavy chain (Fc region) must bind.
- Since IgG has one Fc region, at least two molecule of IgG must be brought close together before C_{1q} can bind and this is possible when they bind to a multivalent antigen.
- IgM being a pentamer, one single molecule can activate C_1.Thus IgM is more efficient complement fixing antibody than IgG.
- C_1r and C_1s are serine esterases and they form a tetramer complex containing two molecule of each and located between C_1q strands. Binding of C_{1q} to two or more Fc regions leads to enzymatic activation of C_1r that cleaves and activates C_1s.
- The activation is normally prevented by a protein C_1 inhibitor (C_1 – INH), it also removes C_{1r} and C_{1s} from the complex but this inhibition is overcome when immunoglobulin is bound to antigen.

☐ Activated C_1s cleaves the next protein in the cascade, C_4 to generate C_{4b} (the small fragment C_{4a} leaves the major fragment C_{4b} and the removal of C_{4a} activates and expose a thioester bond on the C_4b molecule that generates a reactive carbonyl (=C=O) group and binds C_4b to target cell surface (i.e. antigen).

☐ The C_2 glycoprotein binds C_{4b} to form C_{4b2} in presence of Mg^{++} ions. Activated C_{1s} splits bound C_2 into C_{2a} (larger fragment) and C_{2b} (smaller, soluble fragment). The C_2 must be bound to C_4 before it is cleaved and this is called ***substrate modulation***. The C_{4b2a} complex is the *classical pathway C_3 convertase*. The C_{4b2a} protease breaks down C_3into C_{3a} and C_{3b}. The small fragment C_{3a} is removed and C_{3b} form covalent bonds with target cell surface or with the antibody where complement activation was initiated. Once C_{3b} is deposited, it can bind to factor B and generate more C_3 convertase by the alternative pathway. Thus, a single molecule of C_{4b2a} complex can lead to the deposition of hundreds or thousands of molecules of C_{3b} on the cell surface where complement is activated. C_{4b2a3b} complex function as the *classical pathway C_5 convertase* and cleaves $C_5$and initiate the terminal steps of complement of activation.

☐ The classical pathway was first identified and characterized.

THE ALTERNATIVE (INDIRECT) COMPLEMENT PATHWAY

- In alternative pathway C3 is activated and form a stable attachment of C3b to microbial cell surface without the involvement of antibody.
- Normally C_3 in plasma breaks down spontaneously into C_3a and C_3b. The newly formed C_3b binds covalently through thioester bonds to the surface of cells including microbes.
- Under normal condition cell bound C_3b binds to H factor.
- H factor binds with sialic acid or other neutral and anionic polysaccharides present in cell surfaces.
- Binding of H factor activate I factor (protease) and C_{3b} is destroyed thus complement activation stops.
- Since mammalian cell surface glycoproteins are heavily sialylated, it does not trigger the alternative complement pathway.
- Bacterial cell walls, bacterial lipopolysaccharides, viruses, aggregated immunoglobulin (IgA), cobra venom etc. permit activation of C_{3b}. Thus, activation can occur by both immunologically and non-immunologically.
- The bound C_{3b} binds to a plasma protein called B factor and once bound, factor B is cleaved by a plasma serine protease (called factor D) to generate a bound fragment called B_b(also a soluble fragment Ba).
- Factor D acts only on B factor after it is bound to C_{3b} (another example of substrate modulation).
- The *complex $C_{3b}B_b$ is the alternative pathway C_3 convertase* and cleaves C_3 to C_{3b} and C_{3a}. C_{3a} is released and C_{3b} remain attached to cells. Half-life of C_{3b} is only 5 minutes.

- Another protein called *properdin (factor P)* binds to the complex to form $C_{3b}B_bP$ and increase the half-life to 30 minutes. Microbial cells favour the attachment of Properdin.
- C_{3b} may also be generated by other protease from activated phagocytic cells and there is generation of C_{3b} at the site of inflammation.
- Some C_{3b} molecules generated by alternative pathway bind to C3 convertase itself and form C3bBb3b, which function as the *alternative pathway C5 convertase* and cleave C5 to initiate the terminal steps of complement activation.

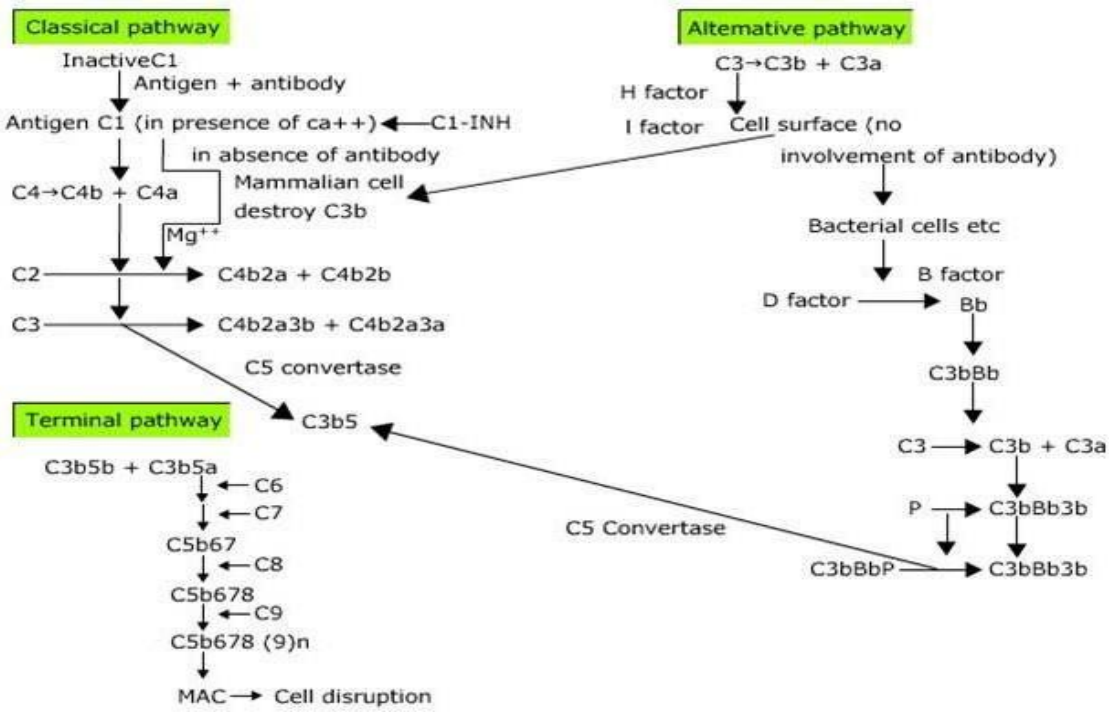
THE LECTIN PATHWAY/MANNOSE - BINDING PATHWAY

- When macrophages ingest bacteria or other foreign materials, they are stimulated to secrete IL-1, IL-6 and TNF- a .
- These three cytokines act on hepatocytes and stimulate them to secrete acute phase proteins, one such protein is Lectin (mannose binding protein).
- Mannose is a major component of bacterial cell wall glycoproteins.
- The mannose binding protein (MBP or MBL) binds to bacteria in blood stream and acts as *opsonin*.
- MBP is structurally similar to C_{1q}, and activate classical pathway of complement by activating C_{1r}-C_{1s} complex or MBP associated serine esterase.
- In cattle, buffalo and other bovidae, there are at least three other mannose-binding proteins and one such is *conglutinin*.
- Conglutinin can bind to cell bound C_{3b} (C_{3b} has mannose rich oligosaccharide side chains) and clump or conglutinate C_{3b} coated particles.

Terminal pathway of complement activation

- Once C_5 binds to C_{3b}, C_5convertase generated by classical pathway (C_{4b2a}), alternative pathway ($C_{3b}B_b$) or mannose binding pathway cleaves C_5 to small peptide C_{5a} (released) and C_{5b}, which attach to C_{3b}.
- This cleavage exposed a site on C_{5b} and binds C_6 and C_7 to form C_{5b67}.
- The C_{5b67} can detach itself from C_{3b} and insert into the lipid bilayer of nearby cell or microbial membrane.
- Once it is inserted into lipid bilayer, it binds to one C_8 molecule and multiple C_9 molecules (about 12 to 18) to form a complex [$C_{5b678\ (9)\ n}$] of tubular tranmembrane pore called the *membrane attack complex (MAC)*.
- The MAC form a large doughnut shaped structure that inserts itself into a cell membrane and forms a tranmembrane channel and cause osmotic lysis of the target cell.

SUMMARY



REGULATION OF COMPLEMENT SYSTEM

- The regulation is accomplished through several regulatory proteins.
- C_1– INH (C_1 inactivator) - It is serine protease inhibitor and present in plasma. It binds to C_1r and C_1s and dissociate them from C_1q thus control assembly of C_{4b2b}(Classic pathway)
- *Factor - I:* It is a serine protease and present in plasma. It cleaves C_{3b} and C_{4b} by using factor H and MCP (membrane cofactor for protein), C_4BP or CR_1 (Type 1 complement receptor) as co factors.
- *Factor – H:* It as a plasma protein binds C3b and displaces BP. It is a cofactor for factor I- mediated cleave of C_{3b}
- *C_4 binding protein (C_4BP):* Binds C_{4b} and displaces C_2. It acts as cofactor for factor I mediated cleaves of C_{4b}.
- *Membrane cofactor for protein (MCP):* It is present in leukocytes, epithelial cells and endothelial cells. It acts as cofactor for factor I mediated cleavage of C_{3b} and C_{4b}
- *Decay accelerating factor (DAF):* present in blood cells, endothelial cells and epithelial cells. It displaces C2b from C4b and Bb from C3b.
- *Vitronectin, clusterin or Protectin:* Present in Blood cells, endothelial cells and epithelial cells. They block C_9 binding and prevent formation of MAC (membrane attack complex).

FUNCTIONS OF COMPLEMENT

- During activation of complements, several components are produced and each has distinct roles.
  - *Opsonization and phagocytosis:* Complement coated (C_{3b} or C_{4b}) microbe are phagocytosed by binding to specific receptors on macrophages and neutrophils.
  - *Complement mediated cytolysis:* Cause lysis of foreign organisms mediated by the MAC and osmotic lysis .
  - Responsible for removal of immune complexes
  - Activates the B cells and provides a signal for initiating humoral immune responses.
  - *Anaphylaxis:* Complement fragments C_{3a}, C_{4a}, and C_{5a} bind to mast cells and induce degranulation with the release of vasoreactive substances like histamine. These three peptides are also called anaphylatoxins as they trigger the mast cell reactions and cause anaphylaxis.

GENERAL BARRIERS OF INNATE IMMUNITY

- General barrier is the non-susceptibility of an individual against a particular disease.
- Examples: Genetic factors, physiologic factors, nutritional factors etc.
- Genetic factors
    - *Species specificity* - Rinderpest is a disease of animals (cattle, sheep and goats) but not for human beings. FMD virus does not infect horses and dogs.
    - *Genetically resistant groups* - For example, African blacks are more susceptible to tuberculosis in America.White coloured (B_1 line) broiler chickens are more susceptible to Hydro pericardium syndrome disease etc.
- Physiologic factors
    - Cold blooded animals are not susceptible to tetanus toxins.
    - Body temperature at 42 ^{o} c (birds) does not allow many organisms to grow even Fever is beneficial to the body.
    - Age: Very young and old age groups are susceptible to infection with many organisms.