The Complement System

Lec. 7

Introduction

The complement system is a part of the innate immune system and consists of a series of proteins that interact with one another in a highly regulated manner, in order to eliminate pathogens. It helps antibodies and phagocytic cells to clear pathogens and damaged cells; promote inflammation and attack the pathogen's plasma membrane. Proteins that take part in the complement system are called complements that collectively work as a biological cascade; the sequence of reactions, each being the catalyst for the next.

- The complement system refers to an extremely complex group of proteins present in normal human and animal serum.
- It is an integral part of the body's immune system that has ability to:

1. Lyse red blood cells (hemolytic activity)
2. Destroy Gram-negative bacteria (bacteriolytic activity)
3. Kill Gram-positive bacteria without lysis (bactericidal activity)
4. Inactivate viruses (virus neutralization)
5. Damage tumor cells

- Ag-Ab complexes absorb it nonspecifically and mediate a number of immunological and biological activities.
- Paul Ehrlich named this lytic agent as complement as it complemented the action of antibody. Earlier it was known as Alexine.

The properties of complement

- Complement is a glycoprotein-constituting approximately 5% to 10% of human serum proteins
- Complement as a whole is heat labile.
- It undergoes spontaneous denaturation slowly at room temperature and in 30 minutes at 56°C (inactivation)
- It does not combine with Ag or Ab alone
- • It is destroyed by trypsin

The components of complements

- Electrophoretic separation shows nine distinct proteins, C 1 to C9
- • C1-heat labile, forms the main bulk of the complement. It is made up of three protein subunits, viz. C1q, C1r and C1s bound together by one molecule of calcium
- • C2-heat labile component
- • C3-heat stable component, a relatively larger protein which has the ability to combine with yeast cell wall (Zymosan)
- • C4-heat stable, susceptible to ammonia and hydrazine
- • C5 to C9- These are the terminal components of membrane attack pathway required for the final show of immune cytolysis

The complement activation

- • Complement is normally present in an inactive form
- • It is activated sequentially in an enzyme cascade manner by:
- Ag-Ab complexes (classical pathway)

- Other stimuli such as bacterial endotoxin, Aggregated immunoglobulins,

cobra venom factor, nephritic factor, initiate (alternate pathway)

- Mannose-binding lectin (MBL) is an acute phase protein, which binds mannose, found on microbial surface (e.g. Salmonella species). MBL level can rise rapidly in response to infection and interact with MBL-activated serum protease (MASP). Activation of MASP leads to subsequent activation of components C2, C4 and C3 (Mannan-binding Lectin Pathway)

- The initial steps are different in different pathways, the final steps that lead to a membrane attack which produce damage to target cell or lysis causing cell death are same in both pathways

The Classical Pathway

- • This pathway involves operation of all nine components (C1 to C9) acting in sequence

- It begins with the activation of Cl q (first component) initiated by binding with CH2 domain on the Fc portion of Ab forming complex with Ag

- • Fc structures are different in different immunoglobulins and

only IgG and IgM possess complement-binding sites

- When the C 1q is activated, it acquires the ability to activate several molecules of the next component in a series.

- Each of these then acts on next component and so on,

producing a cascade effect leading to immune cytolysis

The activation of classical pathway

- The activation of complement starts when IgM or IgG combines with the surface membrane of the cell in the presence of complement
- • C1q then binds to Fc portion of lg molecule and activates C1r, which is a protease that subsequently cleaves C 1 s and forms an enzymatically active component C 1s esterase
- The activated C1s acts on the next component-C4 splitting it into C4a and C4b.
- The C4a has anaphylatoxin activity while C4b, which has an active site, binds to cell membrane along with C1
- The next component C2 with the help of Mg2+ ions binds to the cell bound C4b and is cleaved into C2a and C2b
- C2b is released into fluid medium while C2a binds to the C4b forming C4b2a protease enzyme, which has 'C3 convertase' activity, which cleaves C3 into C3a and C3b

- C3a released into the fluid medium possesses anaphylatoxin activity and is chemotactic for polymorphonuclear leucocytes and C3b joins the cascade and binds to the surface of the target cell along with C4b2a forming a trimolecular complex C4b2a3b, which has C5 convertase activity " C3b fragments not joining the cascade are scattered over the cell membrane, participate in immune adherence and increase susceptibility to phagocytosis (opsonization)
- C5 convertase acts on C5, splits it into C5a and C5b. C5a has anaphylatoxin and chemotactic activity and larger fragment C5b joins the cascade and binds to C6 and C7 forming a trimolecular complex C5b67 which binds to the cell membrane of target cell and prepares it for lysis by C5 and C9
- The addition of C5 and C9 accelerates membrane damage and lysis of the cell

- The mechanism of lysis is by punching holes approximately 100 A in diameter on the cell membrane which disrupts the osmotic integrity of the membrane leading to release of the cell contents
- The complex CSb67 also renders unsensitized 'bystander' cells susceptible to lysis by C5 andC9.
- The complex also has chemotactic and leucocyte activating properties

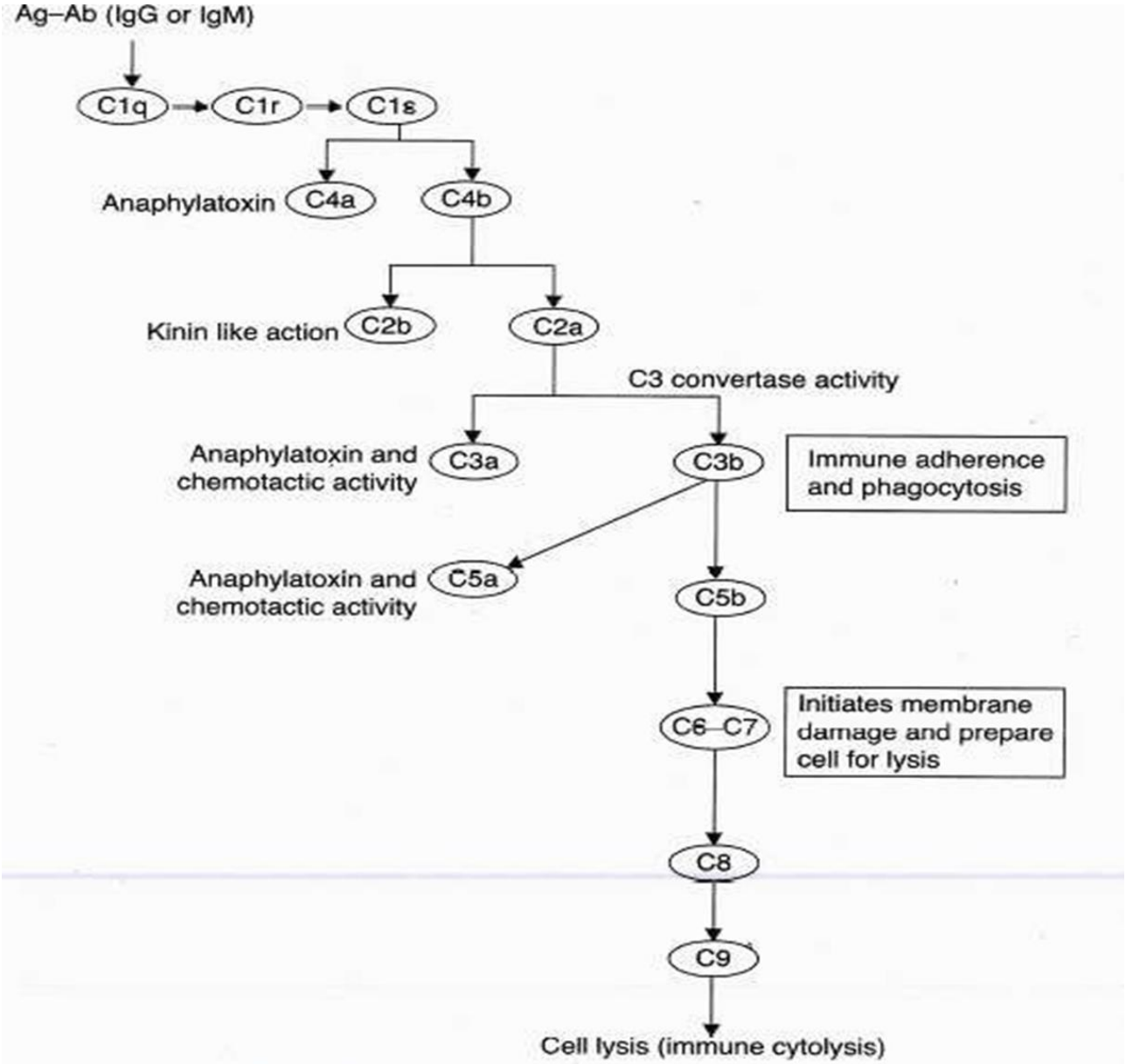
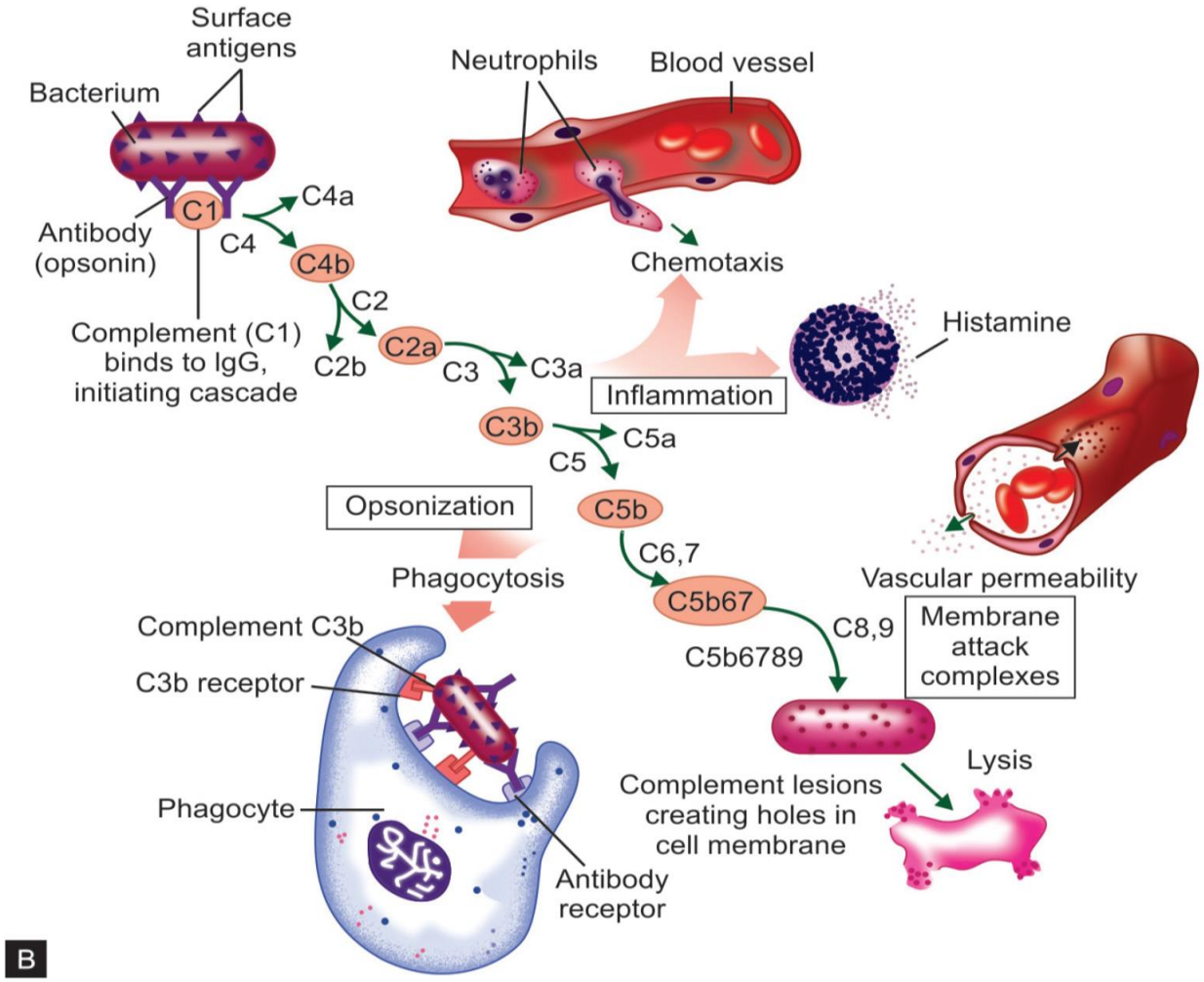


Fig. 20.1 Classical complement pathway.

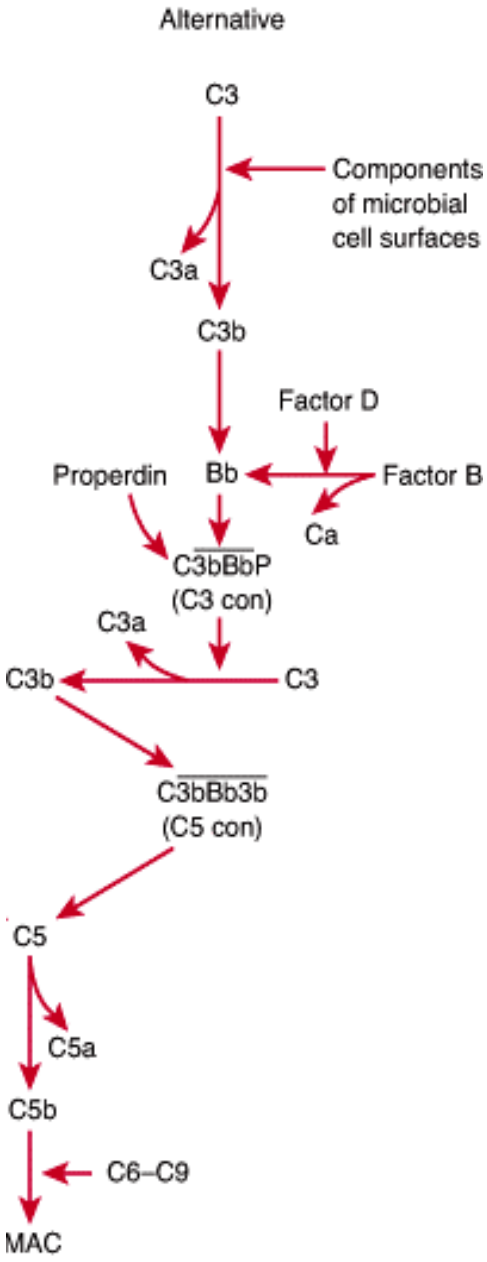


The alternate pathway

- In classical pathway the C3 activated by C142
- In alternate pathway the C3 activated without C1,4,2
- Pillemer in 1954 describe the alternate pathway
- He demonstrate Mg and properdin and zymosan forms P-Z complex which activate C3 directly

The components of alternate pathway

- Properdin (P): glycoprotein normally present in serum, requires Mg2+ ions and other factors for activation of C3
- Factor-B: thermolabile normal serum protein, it is C3 proactivator (C3PA) also known as glycine rich betaglobulin (GBG)
- Factor-D: C3 proactivator convertase (C3 pase, GBGase), it acts on C3b and factor-B forming convertase, which breaks down the C3
- C3b: normally present in serum in trace amount

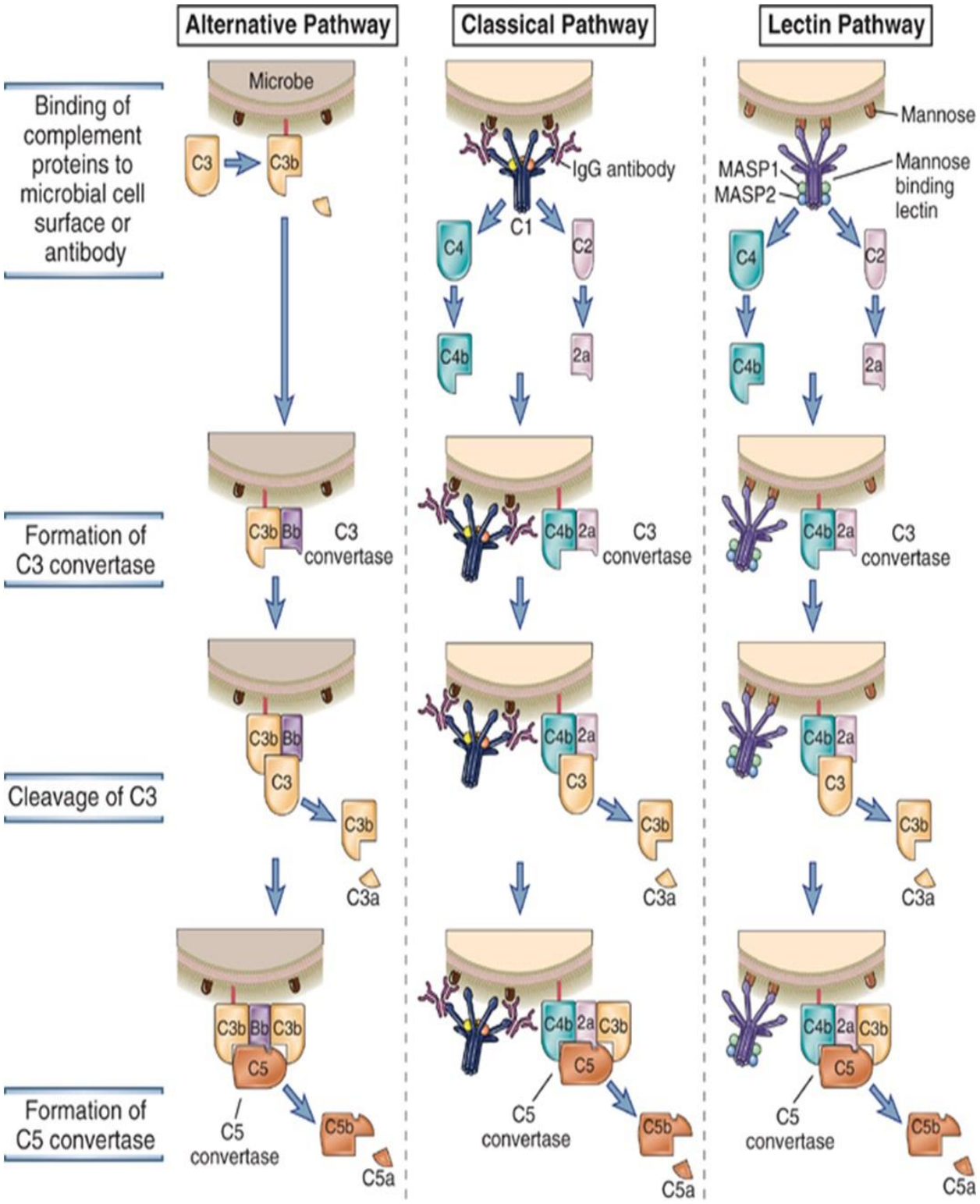


Activation of C3 in alternate pathway

- The factor-B binds to C3b that forms a magnesium dependent C3bB complex, which is cleaved by factor-D into C3bBb and Ba
- C3bBb complex has C3 convertase activity but it is extremely labile and looses its activity; but by binding with properdin it forms PC3bBb complex and becomes stable PC3bBb acts on C3 cleaving it into C3a and Disease Research Laboratory activating C6, C7, C8, C9
- The steps are same as in classical pathway

The biological functions of complement

1. Lysis of cells, bacteria, and viruses – the major effector of the humoral branch of the immune system
2. Opsonization, which promotes phagocytosis of particulate Ags
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 the circulation and deposits them in the spleen and liver


Alternative Pathway
Classical Pathway
Lectin Pathway
Binding of complement proteins to microbial cell surface or antibody
Formation of C3 convertase
Cleavage of C3
Formation of C5 convertase
Microbe
C3
C3b
IgG antibody
C1
C4
C2
C4b
2a
Mannose
MASP1
MASP2
Mannose binding lectin
C3b Bb
C3 convertase
C4b 2a
C3
C3a
C3b Bb C3b
C5
C5 convertase
C5b
C5a