


IMMUNOLOGY MODULE III



Complement
Tolerance
Vaccines



Complement



“Complement enhances, aids, support or ‘complements’ the action of Abs in killing target cells during infection”

Principal means by which Abs defend vertebrates against most bacterial infections

Hence, major effector of the humoral immune response

Complement

Definition : series of heat-labile serum proteins

Site : serum and all tissue fluids except urine and CSF

Synthesis : in liver and also by macrophages – appear in fetal circulation during 13 W

Function : Responsible for certain aspects of immune and inflammatory responses

Activation : antigen-antibody complex or endotoxin, capsule series of proteins activated sequentially

Inactivation: inhibitors in plasma (short lived)

Biological effects: either beneficial or harmful to host

Are soluble serum globulins, but not Igs.
(*how can you ascertain this?*)

Circulate in blood and other extra-cellular fluids

Constitute 15% serum globulins

Mainly synthesized by liver hepatocytes; also blood macrophages (appear in fetal circulation during 13 W)

Most circulate in serum functionally inactive unless triggered by immune response

Categories of C system

Fall into 3 broad groups:

- a) C serum proteins- which react with foreign bodies in either Ab-dependent or Ab-independent manner (i.e C1 – C9, Factors B and D).
- b) Regulatory proteins- present in serum or membranes of host cells. They protect host's own cells from accidental self-inflicted damage

- c) Cell surface receptors- that bind to products of C activation and signal host cells to participate in inflammatory and immune reactions

The interaction of the various components of the C system ensures that minimal destruction occurs to the host tissue during C activation



Composition.

Composed of 11 major proteins and several protein fragments,

Major proteins: C1q, C1r, C1s, C2 – C9
numbered in order of their discovery

C1 – C4, Factors B and D called early
Complement components.

Most are inactive unless activated by an
immune response



C5 – C9 are late Complement components

form the Membrane Attack Complex (MAC)

leading to lysis of an invading microorganism.

Functions of Complement:

- **Plays many roles in both the innate and acquired immune responses:**
- Responsible for the effective killing of foreign cells by Abs, i.e. humoral response
- Also plays many regulatory roles:
 - Inflammatory responses
 - Opsonization of Ags
 - Viral neutralization

Complement Activation and Cell Lysis

- C activation involves the sequential proteolysis of proteins to generate enzymes with proteolytic activity
- **Activation occurs by 3 pathways:**
Classical Alternative Lectin
- Each gives rise to the formation of C5b

Complement pathway

A) Classical pathway:

- Complement is activated by antigen –antibody complex (IgM or IgG)
- Fc portion of the antibody form a binding site for C1q
- The numerical sequence of the complement factors in the classic pathway is:
C1q,r,s , C4, C2, C3, C5, C6, C7, C8, C9

A) Classical Pathway

The reaction sequence divided into three stages:

1) Recognition stage:

- C1q act as the recognition element
- It binds to Fc portion of IgM or IgG
- The activated C1 molecule can cleave many C4 molec.

2) Activation stage:

The complement components C4, C2, C3, C5, C6, C7, C8, C9 participate in that order

3) Membrane attack stage:

Complement components C5, C6, C7, C8, C9 participate where cell membrane damage and cell lysis occur

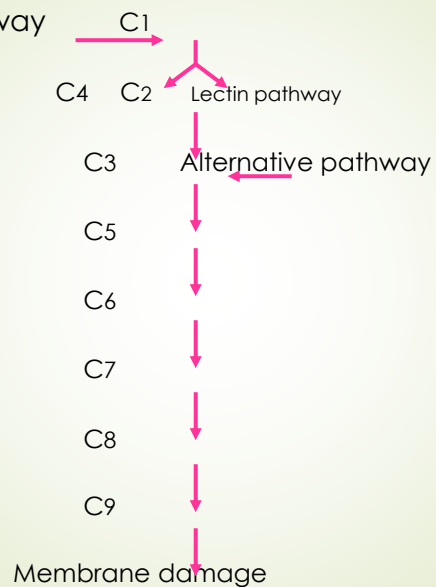
B) Alternative pathway

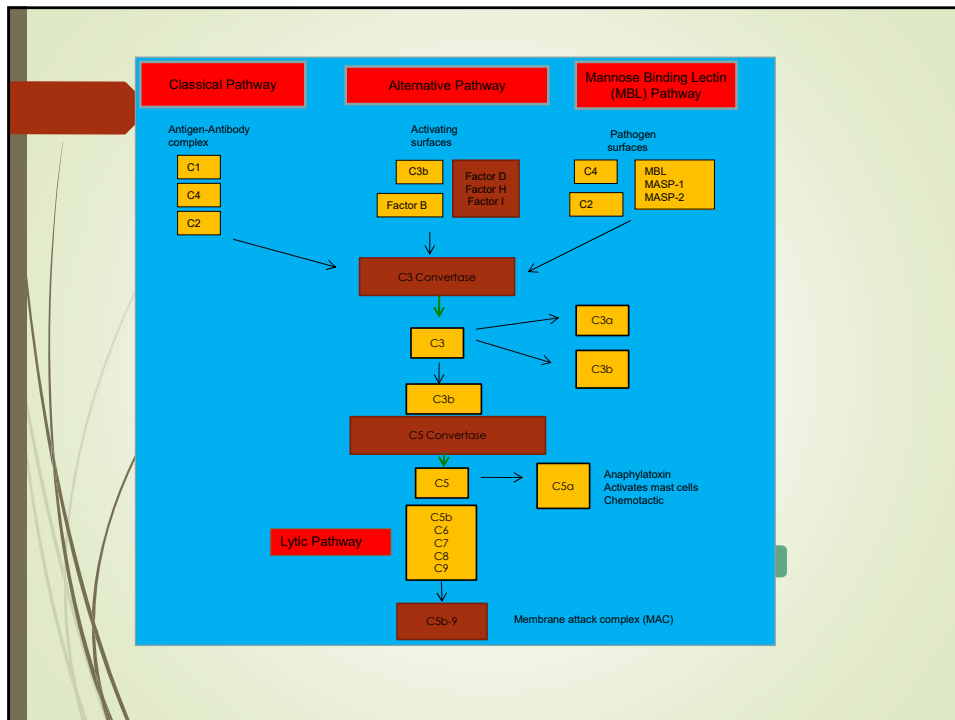
This pathway is initiated by:

- * Bacterial endotoxin, polysaccharide capsule, aggregates of IgE and properdin
- * It starts at C3 then C5, C6, C7, C8, C9
- * The complement compon. C1, C4, C2 are by-passed
- * Antibodies are not required to initiate activation of this pathway
- * This pathway provides a means of non-specific resistance

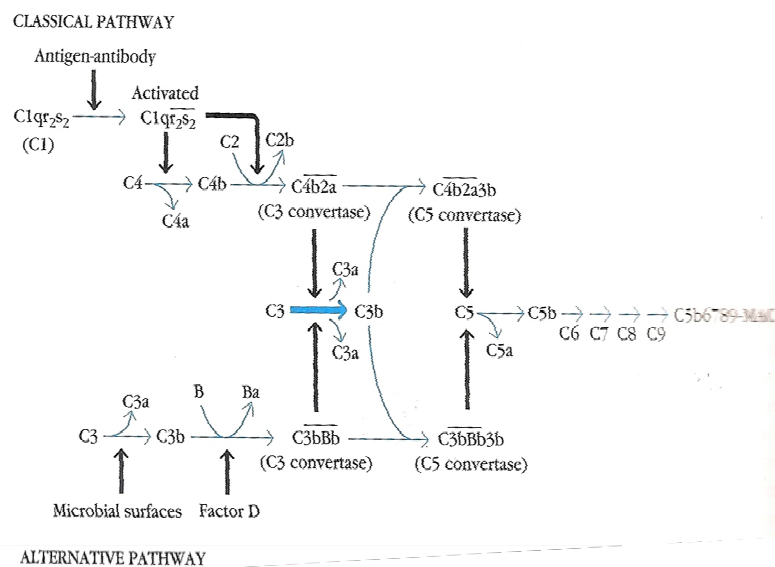
Complement Activation

Classical Pathway





• Fig. 3: Classical and Alternative Pathways



Classic And Alternative pathways

Classic Pathway	Alternative pathway
<ul style="list-style-type: none"> * Specific acquired immunity * Initiated by antibody * Interaction of all components * Properdin system not involved 	<ul style="list-style-type: none"> * Non-specific innate immunity * Bacterial endotoxin, capsule * C1, C4, C2 are by-passed * Properdin system is involved

Properdin is a positive regulator of complement activation that stabilizes the alternative pathway convertases (C3bBb).

Properdin and factor H are two key regulatory proteins having opposite functions in the alternative complement **pathway**

Lectin pathway

- This initiated by binding of the protein called Mannan-binding-lectin (MBL) or mannose binding protein (MBP) to mannose of N-acetylglucosamine residues on microbial surfaces
- This eventually leads to formation of C5b as in the other pathways

C3 and C5 Convertases

- ▶ The C3 and C5 Convertases formed by the Classical and Alternative pathways are different enzymes structurally in each case.
- ▶ Hydrolysis of C3 is the major amplification step in all pathways for the reaction cascade to occur

- ▶ All reactions take place on cell surface since all activated components must bind to cell surfaces to be active
- ▶ C3 is the pivot around which reactions occur.
- ▶ Since each activated enzyme can cleave many molecules of the next inactive enzyme in the chain, the lysis reaction is greatly amplified & effective

Limitations of C activation:

- ▶ Activation is normally on cell surfaces leading to lysis
- ▶ If activation on only immune complexes (Ag-Ab) then the hydrophobic binding sites cannot anchor the complex and it is released. These released complexes can bind to any nearby cells & mediate lysis.
- ▶ This referred to as “Innocent bystander” lysis

Regulation of C Activation


Activation is controlled to prevent host cell lysis, viz:

1. Spontaneous decay after target cell lysis since cannot survive independently of cell
2. Termination of the activation cascade by inhibitor proteins in blood: RCA, MCP, both prevent assembly of C3 Convertase
3. DAF dissociates assembled C3 Convertase



Glossary

- ▀ RCA – Regulators of C Activity
- ▀ MCP – Membrane Cofactor Protein
- ▀ DAF – Decay Accelerating factor



Complement Activation – biological consequences

- ▀ Cell lysis is the major function of C for an effective humoral immune response
- ▀ MAC formed can lyse a broad spectrum of microbes, viruses, RBC and nucleated cells.
- ▀ Since the Alternative pathway does not require prior Ab-Ag reaction, C serves as an important innate system of non-specific immune response.

Biological consequences contd.

- Most viruses, especially the enveloped ones are susceptible to C lysis; e.g. herpes virus, retroviruses, etc.
- C system also effective in lysing G-ve bacteria, though some are resistant to it; e.g. *E. coli* and *Salmonella* sp. are resistant due to long cell-wall lipopolysaccharide (LPS) component which prevents the insertion of MAC

Consequences contd.

- G +ve bacteria are resistant to C lysis due to the thick peptidoglycan layer which prevents insertion of C into the inner cell membrane (look for structure)

Also various bacteria, viruses, protozoans and fungi contain various proteins which can disrupt the cascade reaction, thus mimicking some of the regulatory components of C such as DAF etc. and thus preventing lysis

Other effects of C activation:

- Most of C activities mediated by binding of C fragments to membrane receptors on various cell types:
- Type 1 C receptors – function mainly to promote phagocytosis of C3b and C4b coated particles
- Type 2 receptors – stimulate humoral immune responses by enhancing B cell activation
- There are also types 3 and 4 C receptors CR3 and CR4 respectively, that play other roles

Other effects of C activation

1. Inflammatory responses:

- Various peptides generated during the formation of MAC play roles in development of an acute inflammatory response by activating mast cells and neutrophils, e.g. C3a, C4a, C5a, called anaphylatoxins typically induce these responses.
- They also bind to receptors on mast cells to induce degranulation with release of pharmacologically active mediators which can induce smooth-muscle contraction and increased vascular permeability; typical anaphylactic responses, hence called anaphylatoxins

Opsonization effects

- C3b is the major opsonin of the complement system.
- Complement cascade amplification reaction results in C3b coating surfaces of immune complexes and particulate Ags.
- This enhances their phagocytosis
- *Note: Opsonization refers to enhancement of phagocytosis as a result of coating of foreign bodies by host proteins*

Viral neutralisation

C defends hosts by neutralising viral infectivity by several mechanisms

Some viruses e.g. retroviruses, Epstein-Bar virus, Newcastle disease virus, rubella virus etc. can activate C thro' the alternative pathway

1. Neutralisation occurs as result of larger viral aggregate formation which reduces number of infectious virions (viral particles)

Neutralisation contd.

2. Binding of Ab/C to surface of viral particle forms a thick protein coating on the virus

This coating therefore neutralises viral infectivity by blocking its attachment to susceptible host cell

3. C can also lyse most enveloped viruses resulting in their fragmentation

Consequences of C deficiencies

1. Genetic deficiencies of early C components result in increased immune complex diseases such as vasculitis, glomerulonephritis etc
2. Deficiencies in factor D and properdin predisposes individuals to *Neisseria* infection

Deficiencies contd.

3. C3 deficiency most severe clinical manifestation. Individuals experience severe re-current bacterial infections

WHY??

Clinical Application of C

Used for diagnosis of infections, though no longer popular.

- Test called Complement Fixation Test or Assay (CFT or CFA)
- **Test system consists of: Ag; Ab; Fresh guinea pig serum as source of C; SRBC coated with Ab as indicator system**
- **Above called Immunological zoo**

Principle behind CFA

1. In presence of particulate Ag – Ab complexes, C will be fixed by this complex and hence not available to lyse a cell system when added to it.
2. If immune complex not formed in 1 above, C will be available to lyse a cell when added to it

Biological Effects of Complement

Beneficial effects:

1) Cytolysis:

- activated complement proteins polymerize on cell

surfaces of bacteria or erythrocyte to form pores

Biological Effects of Complement

2) Opsonization:

- binding of complement proteins opsonin (C3b) to surfaces of foreign organisms or particles
- Phagocytic cells express specific receptors for opsonins, so promote phagocytosis

Biological Effects of Complement

3) Inflammatory response :

Small fragments released during complement activation have several inflammatory actions:

- a) C5a is chemotactic and attract neutrophils and macrophages
- b) C5a activate phagocytes and neutrophils
- c) C3, C4 and C5 are anaphylatoxins
Cause degranulation of mast cells and release of histamine and other inflammatory mediators

Biological Effects of Complement

4) Immune complex clearance:

- C3b facilitate binding of immune complex to several surfaces (erythrocytes) and enhance removal by liver and spleen
- binds erythrocytes to blood vessels , make them as easy prey for phagocytosis
- C3 deficiency associated with Immunocomplex disease and susceptibility to recurrent infections

Biological Effects of Complement

5-Enhancement of antibody production:

- Binding of C3b to its receptors on activated B cells
(CR2) greatly enhances antibody production
- Patient who are deficient in C3b produce much less antibody than normal individuals and more susceptible to pyogenic infection

Biological Effects of Complement

Harmful effects:

- If complement activate systematically on a large scale
- If activated by an autoimmune response to host cells

Thanks