Complement System (Day 15)

Complement system can be described as set of serum proteins that act in a cascade fashion to increase immune response.

Sheep antiserum + Vibrio cholerea → lysis
↳ heat → no lysis → fresh serum added without antibodies
↳ lysis

Bordet → specific antibodies which survive heating process + heat sensitive serum component
↳ required for lysis

- complement system of higher vertebrates — 60 soluble and membrane proteins
  - part of proteolytic cascades - lysis
  - protect host from microbes — opsonisation
  - remove debris — immune clearance
  - promote cell survival — secretion of immunoregulato molecules
- affect both innate and acquired immunity

Components of Complement System

- synthesised mainly by hepatocytes, monocytes, macrophages, epithelial cells of GI & GU tracts
- 5% of serum globulin
- mostly circulate as inactive proenzymes (zymogens) — during proteolytic cleavage, inhibitory fragment removed → activation
- components designated by numerals (C_1–C_9), letter symbols (factor D) or by trivial names (homologous restriction factor)

  ⟶ cleavage → peptide fragments

  smaller (a)
  - diffuses, binds to specific receptors → localised inflammatory response

  larger (b)
  - binds to target site near site of activation

fragments interact with one another → functional enzymatic complexes (e.g., $\overline{C4b2a}$)

Functions of complement system

1. Attraction of neutrophils to site of microbial attack (chemotaxis)

2. Enhancement of attachment of phagocyte to microbe (opsonisation)
3. Killing of microbe activating membrane attack complex (lysis)
4. Initiation of (acute) inflammation by direct activation of mast cells

- C-activation: alteration of C proteins such that they interact with the next component
- C-fixation: utilization of C by Ag-Ab complexes
- C-inactivation: denaturation (usually by heat) of an early C-component resulting in loss of hemolytic activity
- Convertase/esterase: altered C-protein which acts as a proteolytic enzyme for another C-component

Complement Activation

- Classical pathway — antibody dependent, triggered by soluble Ag-Ab complex, or by binding of antibody on target cell surface
- Lectin pathway — similar to classical pathway but antibody independent
- Alternative pathway — stimulated by antigen directly

Classical pathway

Formation of soluble antigen-antibody complexes or binding of antibody to target cell

↓

Conformational changes in F_c of IgM & some IgG

↓

exposes binding site for C_1 component

↓

each C_1 molecule must bind to at least 2 F_c sites by its C_1q globular heads for stable interaction

↓

C_1r converted to active protease enzyme (serine) $[\overline{C_1r}]$

↓

cleaves C_1s to a similar active enzyme $\overline{C_1s}$

↓

$\overline{C_1s}$ has two substrates C_4 & C_2

↓

$C_4 \xrightarrow{\overline{C_1s}} C_4a$ hydrolysed from C_4 amino terminus, exposing binding site of C_4b (larger)

↓

C_4b attaches to target surface in vicinity of C_1

↓

C_2 proenzyme attaches to C_4b binding site

↓

C_2 cleaved by neighbouring $\overline{C_1s}$

↓

smaller C_2b diffuses away

↓

$\overline{C4b2a}$ complex (C_3 convertase formed)

↓

smaller components of C4, C3, C5 – anaphylatoxin (mediates inflammation)

↓

C3 converted into C3a (small) → C_3b (by C3 convert

Single C_3 convertase → 200 C_3b (amplification)

↓

Some C3b binds to $\overline{C4b2a}$ → $\overline{C4b2a3b}$ (C_5 convertase)

↓

C3b component of complex binds C_5 and alters conformation

↓

$\overline{C4b2a}$ component can cleave C5 into C5a (which diffuses awa

↓

C_5b attaches to C_6 → initiates formation of membrane attack complex.

(C5b extremely labile – half-life of 2 mins, unless C6 binds and stabilises)

↓

C5b6 + C7 → complex undergoes hydrophilic → amphiphilic transition

↓

exposes binding sites – for target-cell memb. phospholipids for insertion into lipid bilayer.

↓

C8 + memb. bound C5b67 → conformational change in C8

↓

hydrophilic - amphiphilic Transition → interacts with plasma membrane

↓

C5b678 creates small pore in memb → 10Å → can lead to RBC lysis but not nucleated cells (no internal repair mechanism)

↓

final step – binding and polymerisation of C9 (perforin-like) to C5b678 complex – as many as 10-17 molecules of C9 can bind to a single C5b678 molecule

↓

During polymerisation, C9 molecules undergo hydrophilic → amphiphilic ⇒ insert into membrane.

Completed MAC (tubular, functional pore size of 70-100 Å)

= C5b678 complex surrounded by poly-C9 complex

→ ions & small molecules diffuse freely → osmotic balance disrupted

↓

cell killed by influx of H_2O & loss of electrolytes

Alternate Pathway

- antibody independent
- part of innate immune system
- involved C3, factor B, D and properdin
- initiated by foreign cell surface constituents

C3 subjected to slow spontaneous hydrolysis of unstable thioester bond

↓

C3b binds to foreign antigens on surface or host's own cells

↓

↑ sialic acid in memb of mammalian cells deactivates it, otherwise

↓

C3b + B → complex stabilised by Mg^{2+}

↓

Binding exposes site on B for factor D

↓

D cleaves C3bB → releases Ba & generates $\overline{C3bBb}$ (C3 convertase) + (properdin)

↓

$\overline{C3bBb}$ activates unhydrolysed C3 (amplification)

↓

$\overline{C3bBb3b}$ complex (C5 convertase)

↓

C5b, C6, C7, C8, C9 → MAC

Lectin pathway

- lectins bind to sp. carbohydrate targets
- antibody independent but more like classical pathway

Mannose binding Lectin (MBL) binds to mannose residues on foreign glycoproteins (acute phase protein produced in inflammatory responses

↓

MBL associated serine proteases (MASP-1 & MASP-2) bind to MBL

↓

cleavage & activation of C4 & C2

↓

Classical pathway.

Regulation

- The complement system is a powerful mediator of inflammation and destruction and could cause extensive damage to host cells if uncontrolled.
- However, complement components rapidly lose binding capacity after activation, limiting their membrane-damaging ability to the immediate vicinity of the activation site.
- The complement system is also tightly regulated by inhibitory/regulatory proteins.

Protein	Function
C1 inhibitor	Binds to C1r and C1s; prevents further activation of C4 and C2
Factor I	Enzymatically inactivates C4b and C3b
C4b-binding protein	Binds to C4b displacing C2b
Factor H	Displaces C2b and C3b by binding C4b
DAF	Inactivates C3b and C4b
MCP	Promotes C3b and C4b inactivation
CD59	Prevents binding of C5b,6,7 complexes to host cells

C1 inhibitor, factor I, C4b-binding protein, factor H, decay-accelerating factor (DAF), membrane cofactor protein (MCP), and CD59 (protectin)