Effector Mechanisms of Humoral Immunity

Learning Objectives of lecture:

- Describe the mechanism of antibody mediated opsonization of an antigen
- Explain the different mechanisms of complement activation
- Describe the effector mechanisms of complement action
- Discuss some of the mechanisms that stop complement from damaging our own cells
- Describe how ‘passive immunity of the newborn’ is achieved
Antibody Structure

Domains at ends of heavy chain and light chain are highly variable and responsible for binding antigen

- the gene segments encoding these domains are mutated in GC B cells during antibody affinity maturation

From Immunity: The Immune Response in Infectious and Inflammatory Disease by DeFranco, Locksley and Robertson
5 Different Antibody Classes

- When B cells switch heavy chain, they keep the same Variable domain (and same light chain) and thus the same antigen binding specificity

- the different heavy chains confer different effector functions
Features of primary and secondary antibody responses

(A) Primary antibody response

- First infection
  - Antibody-secreting cells in peripheral lymphoid tissues
  - Activated B cells
  - Naive B cell

- Repeat infection
  - Low-level antibody production
  - Plasma cells in bone marrow
  - Memory B cell

(B) Secondary antibody response

- Antibody-secreting cells
- IgG
- IgM
- Memory B cell
- Plasma cells in bone marrow

Days after antigen exposure:
- 0 to 5
- >30

Amount of antibody:
- Increase

Legend:
- Purple cells: B cells
- Brown cells: Antibody-secreting cells
- IgG: Immunoglobulin G
- IgM: Immunoglobulin M
The effector functions of antibodies

- Neutralization of microbe and toxins
- Opsonization and phagocytosis of microbes
- Antibody-dependent cellular cytotoxicity
- Lysis of microbes
- Phagocytosis of microbes opsonized with complement fragments (e.g., C3b)
- Inflammation
-> antibody responses can be long-lasting – can exist for years after the initial encounter or vaccination (e.g. ~10 years for tetanus toxoid vaccination)
Antibody-mediated opsonization and phagocytosis of microbes

Phagocytic cells (macrophages, neutrophils) express activating Fcγ receptors - when crosslinked they transmit signals that promote engulfment and increased bactericidal activity.

Therapeutic antibodies such as Rituxan (against B cell surface molecule CD20) also utilize this pathway to promote clearance of targeted cells.
Antibody-dependent cellular cytotoxicity (ADCC)

**A** Surface antigen IgG Antibody-coated cell

Surface antigen binds to IgG, which then binds to antibody-coated cell. This complex is recognized by NK cells expressing low-affinity FcγRIII receptors, leading to the killing of the antibody-coated cell.

**B** IgE Helminth Eosinophil

IgE binds to eosinophils, which express high-affinity FceRI receptors. This binding leads to the killing of the helminth.
The Complement (C’) System

- Complement system opsonizes antigens for phagocytosis and can promote direct lysis of some bacteria

- All the C’ components pre-exist in an inactive form in the blood (mostly made in the liver); C3 is the most abundant

- Enzymatic (proteolytic) cascade - initial signal strongly amplified

- When a C’ component is cleaved, the larger ‘b’ fragment is chemically labile and becomes covalently bound to nearby surfaces; the smaller ‘a’ fragment is soluble and can diffuse away
Activation of Complement (C’)

**Lectin Pathway**
- Mannose polysaccharide & MBL

**Classical Pathway**
- Antibody/Antigen Complex & C1

**Alternative Pathway**
- Microbial surface-bound C3b

MBL = Mannose Binding Lectin

C4bC2b

C3bBb

“C3 convertase”

C3 → C3b + C3a
Early steps of complement activation

- **Alternative Pathway**
  - Binding of complement proteins to microbial cell surface or antibody
  - Formation of C3 convertase
  - Cleavage of C3
  - Covalent binding of C3b to microbial surface

- **Classical Pathway**
  - IgM or IgG antibody

Note: because C3 is very abundant, once a C3 convertase forms, lots of C3b is generated and becomes covalently bound to nearby surfaces.
Early steps of complement activation

• **Classical Pathway**: activation of the endogenous protease activity of C1 following binding to antigen-bound IgM or IgG leads to generation of a protease that cleaves C3 (a C3 convertase)

• **Alternative pathway**: a low level of C3 is spontaneously activated; when this occurs near a microbial surface the active C3 (C3b) can bind and then interact with components of the alternative pathway, leading again to formation of a C3 convertase
The late steps of complement activation

Key point: C5b catalyzes formation of the Membrane Attack Complex

-> Gram negative organisms have a thin peptidoglycan layer and are the most sensitive to the MAC (e.g. *Neisseria*)
**Activation of Complement (C’)**

**Lectin Pathway**
- Mannose polysaccharide & MBL

**Classical Pathway**
- Antibody/Antigen Complex & C1

**Alternative Pathway**
- Microbial surface-bound C3b

MBL is C1-like but activated by binding Mannose-rich polysaccharides

Various complement components activated to generate

“C3 convertase”

C3 $\rightarrow$ C3b + C3a

C3b decorates surface; some C3b forms a C5 convertase, generating C5a and C5b; C5b causes formation of the Membrane Attack Complex (C5-C9)
C5a, C3a and C4a also known as Anaphylatoxins
-> induce mast cell and basophil mediator release (when occurring systemically, can cause anaphylactic shock)
Regulation of complement (C’) activation

- Host cells express membrane anchored C’ regulatory proteins that inactivate complement when it deposits on a host cell
  - Some of these proteins are linked to the membrane via a glycosylphosphatidylinositol (GPI) anchor

- Plasma contains soluble C1-Inhibitor protein that limit the extent of C’ activation
Complement in disease

• Complement overactivity:
  1) Immune complex glomerulonephritis
     – damage caused by Ag-Ab complexes deposited in glomerular basement membrane activating C’ and recruiting and activating neutrophils
     – *Strep pyogenes* can cause acute glomerulonephritis (AGN)
  2) Hereditary angioedema (deficiency of C1-INH)
     – severe attacks of edema because the cascade is more easily activated and a lot of C3a, C5a are made
  3) Paroxysmal nocturnal hemoglobulinuria (deficiency of GPI anchored membrane proteins - includes several C’ regulatory proteins)
     – increased autologous red cell lysis

• Complement deficiency
  C3 - increased risk of infection by many types of bacteria
  C5, C6, C7, or C8 - increased risk of *Neisseria* infections
The poly-Ig receptor is a special Fc receptor that binds dimeric IgA.

The process of transporting IgA across the cell is known as **transcytosis**.

The IgA released into the gut lumen remains associated with part of the poly-Ig receptor (known as the *secretory component*) and this provides protection against proteolysis by gut proteases.
Neonatal Immunity

- Maternal IgG is transported by the neonatal Fc receptor (FcRn)
  - across the placenta to the fetus
  - from colostrum across the newborn gut epithelium
    - Colostrum is the protein-rich fluid secreted by the early postnatal mammary gland
- Confers passive immunity in the newborn
- The duration of protection is 3-4 months (or ~5 IgG half-lives)
  - explains the high incidence of disease after this period by bacteria such as *Haemophilus influenzae*
- Human milk contains IgA
  - provides some protection against gut pathogens
- Neonatal protection is only as good as the titer of IgG (and IgA) in the mother against the specific organism

![Fraction of adult level of serum immunoglobulins](image-url)
IgG half-life

- FcRn is also present in the adult and involved in protecting IgG from degradation
- Accounts for the long (3 week) half-life of IgG compared to other Ig isotypes
- Therapeutic agents that are fused to IgG Fc regions take advantage of this property e.g. Enbrel (TNFR-Fc)
Evasion of humoral immunity by microbes

- Many viruses and bacteria mutate their antigen surface molecules such that they are no longer recognized by the existing antibody
  - basis for existence of multiple serotypes of some pathogens (e.g. rhinoviruses, *Salmonella enterica*, *Strep. pneumoniae*)
  - population builds up immunity to some serotypes and remains susceptible to the others
- Some viruses have only a single serotype (e.g. measles, mumps) and this is the basis for the success of the vaccine
Ig Heavy chain class (isotype) switching

Helper T cells: CD40L, cytokines

- IFN-γ
- IL-4

Mucosal tissues; cytokines, e.g., TGF-β

<table>
<thead>
<tr>
<th>Isotype switching</th>
<th>IgM</th>
<th>IgG subclasses (IgG1, IgG3)</th>
<th>IgE</th>
<th>IgA</th>
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Principal effector functions

- Principal effector functions
Vaccines

- Most vaccines work by inducing neutralizing Abs
  - attenuated forms of microbes (treated to abolish infectivity and pathogenicity, but retain antigenicity) are most effective
  - route of administration important e.g. oral administration of Polio vaccine ensures generation of neutralizing IgA
  - immunization with inactivated microbial toxins generates toxin neutralizing antibodies
- Led to world-wide eradication of Small Pox
- May soon (?) achieve eradication of Polio
- Many vaccines still needed - HIV, Malaria, Schistosomes etc
### Classification of Licensed Vaccines

<table>
<thead>
<tr>
<th>Types of Vaccine</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Live attenuated viral</td>
<td>Poliomyelitis (OPV Sabin), measles, mumps, rubella, rabies, varicella, vaccinia, yellow fever, rotavirus, influenza (intranasal)</td>
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<tr>
<td>Live attenuated bacterial</td>
<td>BCG for tuberculosis or leprosy, Ty21a for typhoid fever</td>
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<tr>
<td>Killed whole virus</td>
<td>Poliomyelitis (IPV-Salk), influenza, hepatitis A, rabies</td>
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<tr>
<td>Killed whole-cell bacterial</td>
<td>Pertussis, cholera, anthrax, plague</td>
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<tr>
<td>Toxoids</td>
<td>Diphtheria, tetanus</td>
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<tr>
<td>Molecular vaccines —proteins</td>
<td>Acellular pertussis, subunit influenza, hepatitis B</td>
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<tr>
<td>Molecular vaccines —carbohydrate</td>
<td>Hib, Vi typhoid, meningococci, pneumococci</td>
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<tr>
<td>Molecular vaccines —carbohydrate-protein conjugate</td>
<td>Hib, meningococci, pneumococci</td>
</tr>
<tr>
<td>Combination vaccines</td>
<td>DPT, MMRV, DPT-Hib, DPT-Hib-IPV-hep B</td>
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