Chapter 8. Antigen, Antibody and Complement

Table of Contents

CHAPTER PREVIEW

- Antigen
- Antibody
- Complement

ANTIGEN

Antigen (Ag) is defined as any substance that satisfies two distinct immunologic properties—immunogenicity and antigenicity.

1. **Immunogenicity:** It is the ability of an antigen to induce an immune response in the body

2. **Antigenicity (immunological reactivity):** It is the ability of an antigen to combine specifically with antibodies.

The substance that satisfies the first property, i.e. immunogenicity (inducing a specific immune response) is more appropriately called **“immunogen”** rather than using the word “antigen”.

Hapten

Haptens are low molecular weight molecules that **lack immunogenicity** (cannot induce an immune response) but **retain antigenicity or immunological reactivity** (i.e. can bind to their specific antibody or T cell receptor). Haptens can become immunogenic when combined with a larger protein molecule called a **carrier**.

Epitope

Epitope or antigenic determinant is the smallest unit of an antigen that is capable of reacting with the specific site of an antibody. The specific site of an antibody that reacts with the corresponding epitope of an antigen is called **paratope**.

Factors Influencing Immunogenicity of Ag

There are various factors that influence the immunogenicity of an antigen.

- **Size of the antigen:** Larger is the size more potent is the molecule as an immunogen
- **Chemical nature of the antigen:** Proteins are stronger immunogens than carbohydrates or lipids
- **Susceptibility of antigen to tissue enzymes:** Only substances that are susceptible to the action of tissue enzymes are immunogenic
- **Structural complexity:** Polymers made up of at least two or more amino acids are immunogenic
- **Foreignness to the host:** Higher is the phylogenetic distance between the antigen and the host; more is the immunogenicity
- **Genetic factor:** Different individuals of a given species show different types of immune responses towards the same antigen due to genetic differences
- **Optimal dose of antigen:** An antigen is immunologically active only in the optimal dose range. A too little dose or too large dose fails to elicit an immune response
- **Route of antigen administration:** In general, the immune response is better induced following the parenteral administration of an antigen
- **Sometimes, repeated doses of antigens** may be required to generate an adequate immune response.

Adjuvant

The term “adjuvant” refers to any substance that enhances the immunogenicity of an antigen. They are usually added to vaccines to increase the immunogenicity of the vaccine antigen.

Examples of Adjuvants

- **Alum** (aluminum hydroxide or phosphate)
- **Lipopolysaccharide** (LPS) fraction of *Bordetella pertussis* is an excellent adjuvant for diphtheria and tetanus toxoids. This explains the reason for using combined immunization for diphtheria, pertussis and tetanus in the form of DPT vaccine.

Mechanism of Adjuvant Action: Adjuvants act through the following steps:

- Delaying the release of antigen
- By activating phagocytosis
- By activating T_H cells.

ANTIBODY

Antibody or immunoglobulin is a specialized glycoprotein, produced from activated B cells (plasma cells) in response to an antigen, and is capable of combining with the antigen that triggered its production.

- Immunoglobulin (Ig) constitutes 20–25% of total serum proteins
- There are five classes of Ig recognized—IgG, IgA, IgM, IgD, and IgE.

Structure of Antibody

An antibody molecule is a 'Y-shaped' heterodimer, composed of four polypeptide chains **(Fig. 8.1)**—two light (L) chains and two heavy (H) chains.

- **Bonds:** All four H and L chains are bound to each other by disulfide bonds, and by noncovalent interactions
- **Ends:** The chains have two ends—an amino-terminal end (NH_3) and a carboxyl-terminal end (COOH)
- **H chain classes:** There are five classes of H chains. Each Ig has one type of H class. The five classes of Igs (lgG, IgA, IgM, IgD, and IgE) are classified based on the type of H chains they possess **(Table 8.1)**.

Fig. 8.1. General structure of an antibody.

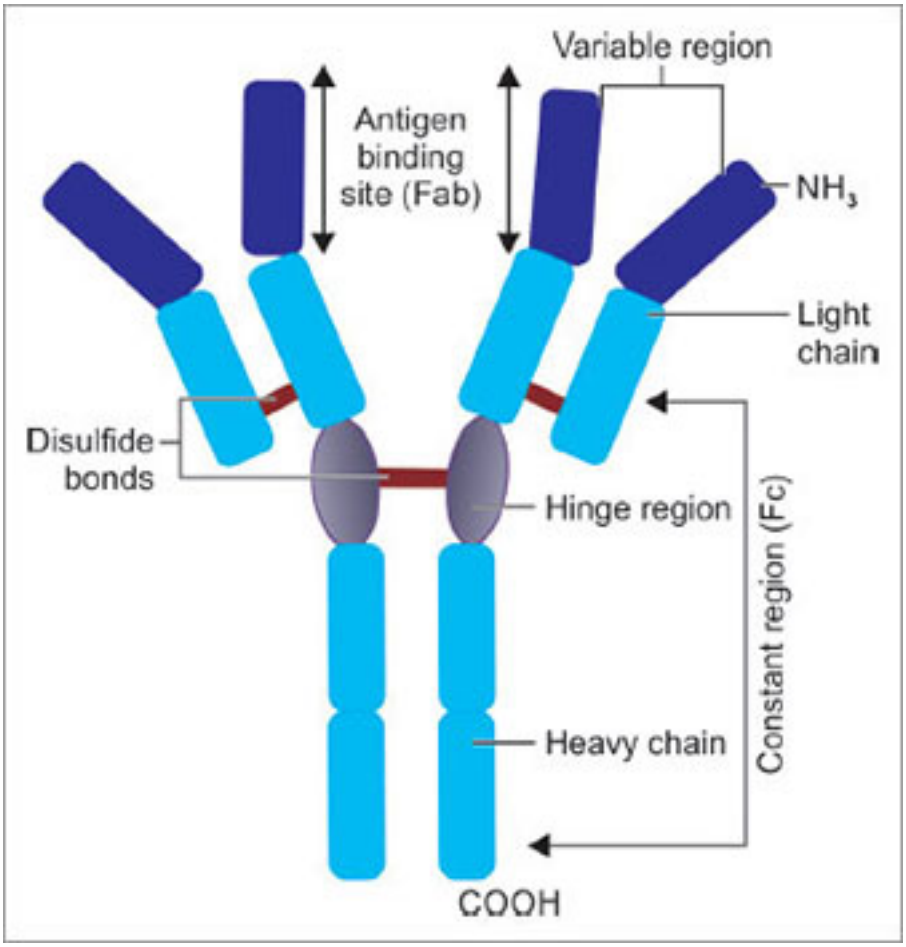


Table 8.1. Type of heavy chain in each immunoglobulin class.

Immunoglobulin class	*Heavy chain type*
IgG	γ (gamma)

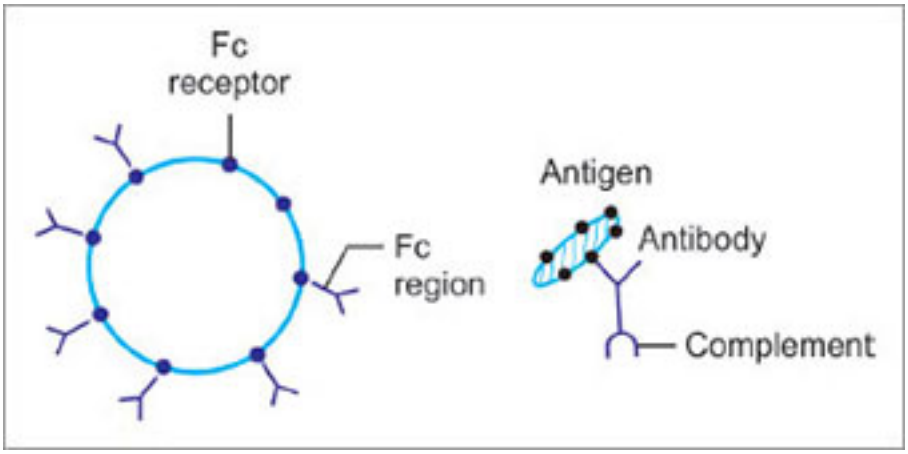
Immunoglobulin class	*Heavy chain type*
IgA	α (alpha)
IgM	μ (mu)
IgD	δ (delta)
IgE	€ (epsilon)

Variable and Constant Regions

Each H and L chain comprises two regions—variable and constant region.

1. **Variable region:** Contains a variable sequence of amino acids. It is the antigen-binding region of an antibody
   - It comprises a hypervariable region called paratope, which makes actual contact with the epitope of the antigen
   - Antibodies produced against various antigens differ from each other in the amino acid sequences of the variable region.

Fig. 8.2. Function of the constant region (Fc) of antibody.



2. **Constant region (Fc):** It constitutes the remaining part of an Ig molecule other than that of the variable region
   - The amino acid sequence of the Fc region shows a uniform pattern
   - The Fc region of the antibody mediates various effector functions such as binding to the complements, and various other cell types such as phagocytes, lymphocytes, mast cells, NK cells, eosinophils, etc. **(Fig. 8.2)**
   - These cells bear Fc receptors (FcR) that bind to the Fc region of immunoglobulins.

Immunoglobulin Classes

There are five classes of immunoglobulins.

Immunoglobulin G (IgG)

It constitutes about 70–80% of total Ig in the body. It mediates various functions.

- IgG can **cross the placenta**; hence providing immunity to the fetus and newborn

- **Complement fixing:** Fc region of IgG can bind to complement factors; thus activating the classical pathway of the complement system
- **Phagocytosis:** IgG bind to Fc receptors present on phagocytes (macrophages, neutrophils) and enhances the phagocytosis (opsonization) of antigen bound to them
- It mediates precipitation and neutralization reactions
- IgG is raised after a long time following infection and represents chronic or past infection (recovery).

Immunoglobulin M (IgM)

Among all Ig, IgM has the highest molecular weight. It is present only in the intravascular compartment, not in body fluids or secretions. It exists either in monomeric form with 2 valencies or pentameric form (5 Ig joined together with a valency of 10 **(Fig. 8.3A)**. IgM mediates various functions.

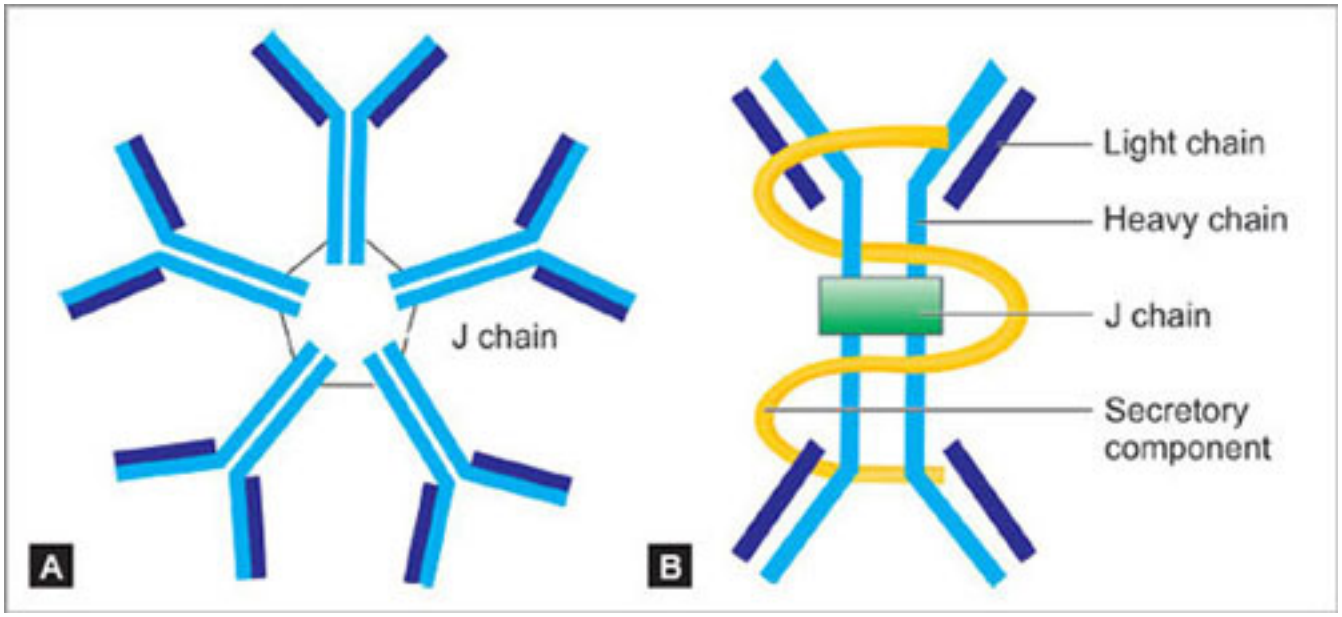
- **Acute infection:** IgM is the first antibody to be produced following an infection; represents acute or recent infection. It is also called a primary immune response antibody
- **Complement fixing:** It is the most potent activator of the classical complement pathway
- It is also present on B cell surface in monomeric form and serves as **B cell receptor** for antigen binding
- It acts as an **opsonin**; binds to an antigen which is then easily recognized and removed (opsonization)
- **Fetal immunity:** It is the first antibody to be synthesized in fetal life; thus provides immunity to the fetus
- Protection against **intravascular organisms**: IgM being intravascular, is responsible for protection against blood invasion by microorganisms
- IgM mediates **agglutination** reaction.

Immunoglobulin A (IgA)

IgA is the second most abundant class of Ig next to IgG, constituting about 10–15% of total serum Ig. It exists in both monomeric and dimeric forms.

- **Serum IgA:** IgA in serum is predominantly in monomeric form
- **Secretory IgA:** It is dimeric in nature, with a valency of four **(Fig. 8.3B)**
    - *Location:* Secretory IgA is the predominant antibody found in body secretions like milk, saliva, tears, intestinal and respiratory tract mucosal secretions
    - *Function:* The secretory IgA mediates **local or mucosal immunity**; protects against pathogens by cross-linking the antigens and preventing their entry through the mucosal surfaces.

Figs. 8.3A and B. A. Pentameric IgM; B. Dimeric IgA.



Immunoglobulin E (IgE)

Among all Ig, IgE is having the lowest serum concentration. It is also the only heat-labile antibody.

- IgE is highly potent and mediates **type I hypersensitivity** reactions by binding to the mast cells (Chapter 10)
- IgE is elevated in **helminthic infections**.

Immunoglobulin D (IgD)

IgD is found as membrane Ig on the surface of B cells and acts as a B cell receptor along with IgM.

Monoclonal Antibody

Monoclonal antibodies (mAb) are defined as the antibodies derived from a single clone of plasma cell; all having the same antigen specificity, i.e. produced against a single epitope of an antigen.

- **Production:** Monoclonal antibodies are produced by a method called as hybridoma technique
  - In the hybridoma technique, antibody-forming mouse splenic B cells are fused with myeloma cells (cancerous plasma cells) to produce hybridoma cells
  - These hybridoma cells can grow and survive long producing the desired antibody.
- **Uses:** mAb has various uses such as:
  - *Diagnostic reagents:* The widest application of mAb is the detection of antigens. The antigen detection kits employ various mAb tagged with detection molecules, such as an enzyme, which detects the specific antigens in the clinical specimens by using various formats like ELISA, rapid tests, etc.
  - *Passive immunity:* For post-exposure prophylaxis against various infections, mAb targeting specific antigens of infecting organisms can be administered. Examples include— immunoglobulins against hepatitis B, rabies, and tetanus

- **Therapeutic use:** Monoclonal antibodies are used in the treatment of various inflammatory conditions, allergic diseases, and cancers.

COMPLEMENT

The term 'complement' (C) represents a group of proteins normally found in the serum in an inactive form, but when activated by a microbial antigen, they augment the immune response and cause microbial cell lysis.

- The complement system is grouped into complement components (nine proteins, C1 to C9), and the properdin system
- The majority of the complement proteins are synthesized in the liver
- Complements are species nonspecific and heat-labile.

Complement Activation

There are three pathways of complement activation:

1. **Classical pathway:** This is an antibody-dependent pathway. The pathway is triggered by the formation of antigen-antibody complex, i.e. when the host antibody binds to antigens present on the microbial cell surface. The complements bind to the F_C region of the antibody. The nine complements (C1 to C9) bind to the antibody, one after the other in a sequential manner
2. **Alternative pathway:** This is an antibody-independent pathway. This pathway is triggered directly by the antigen (e.g. bacterial endotoxin) to which the complements and properdin proteins bind in a sequential manner
3. **Lectin pathway:** This is a recently described pathway. It resembles the classical pathway, but it is antibody independent. It is mediated through lectin proteins of the host that interact with mannose residues present on microbial surfaces. Subsequently, complement proteins bind sequentially.

Stages of Complement Activation

There are four main stages in the activation of any of the complement pathways.

1. Initiation of the pathway
2. Formation of C3 convertase
3. Formation of C5 convertase
4. Formation of membrane attack complex (MAC):

The three pathways differ from each other only in the first two stages (i.e. till the formation of C3 convertase). The steps involved in the remaining stages are exactly same in all three pathways.

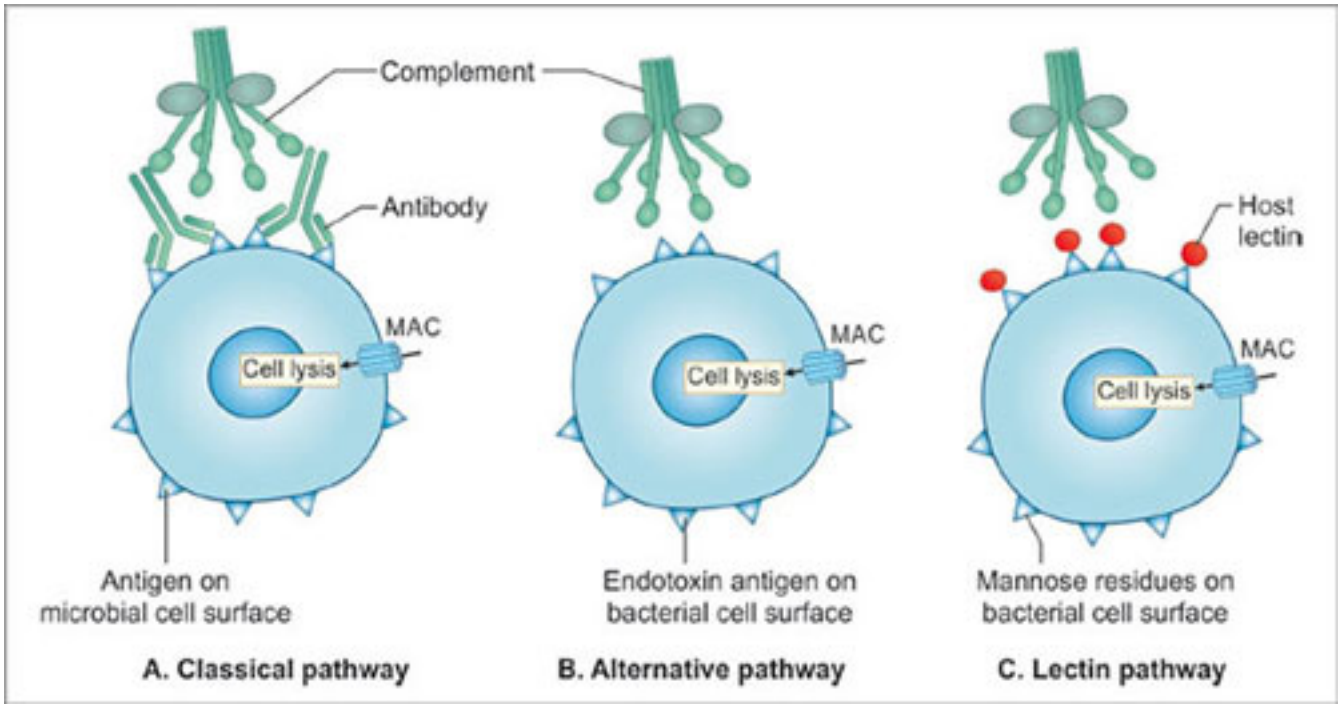
The final stage is formation of **membrane attack complex** (MAC) by binding of C5 to C9. MAC forms pores in the target cell (i.e. microbial surface), which leads to cell lysis and death **(Fig. 8.4)**.

Effector Functions of Complement

The effector functions of complement products are as follows:

- **Target cell lysis by MAC:** As already explained, bacteria, enveloped viruses, damaged cells, tumor cells, etc. are killed by complement-mediated cell lysis
- **Inflammatory response:** The complement by-products such as C3a, C4a, and C5a induce mast cell degranulation leading to vasoconstriction, and increased vascular permeability

Fig. 8.4. The complement pathways.



- **Opsonization:** Some complement by-products (C3b and C4b) act as major opsonins and facilitate phagocytosis
- Complement helps in removing the immune complexes from blood
- **Viral neutralization:** Complements play a crucial role in the neutralization of viruses.

EXPECTED QUESTIONS

1. **I. Write an essay on:**
    1. Define antibody. Describe in detail the structure and functions of various types of antibodies.
2. **II. Write a short note on:**
    1. Monoclonal antibodies and their applications.
    2. The structure and function of IgG antibody.
    3. The structure and function of IgA.
    4. The structure and function of IgM.
3. **III. Multiple Choice Questions (MCQs):**
    1. **Which antibody crosses the placenta?**
        a. IgA
        b. IgG
        c. IgE

d. IgM

2. **What is the total vacancies of IgM?**

   a. 10

   b. 5

   c. 2

   d. 1

3. **Which antibody is elevated in acute infection?**

   a. IgA

   b. IgG

   c. IgE

   d. IgM

4. **Which antibody mediates mucosal immunity?**

   a. IgA

   b. IgG

   c. IgE

   d. IgM

5. **The smallest determinant of antigenicity is called as:**

   a. Antigen

   b. Immunogen

   c. Epitope

   d. Paratope

6. **70-80% total antibody is constituted by:**

   a. IgA

   b. IgE

   c. IgG

   d. IgM

7. **The most potent activator of classical complement pathway is:**

   a. IgA

   b. IgM

c. IgG

d. IgE

8. **Complement mediated cell lysis is done by:**

a. Anaphylatoxins

b. Activation of apoptosis

c. Membrane attack complex

d. Inhibition of protein synthesis

Answers

1. b	**2.** a	**3.** d	**4.** a	**5.** c	**6.** c	**7.** b	**8.** c