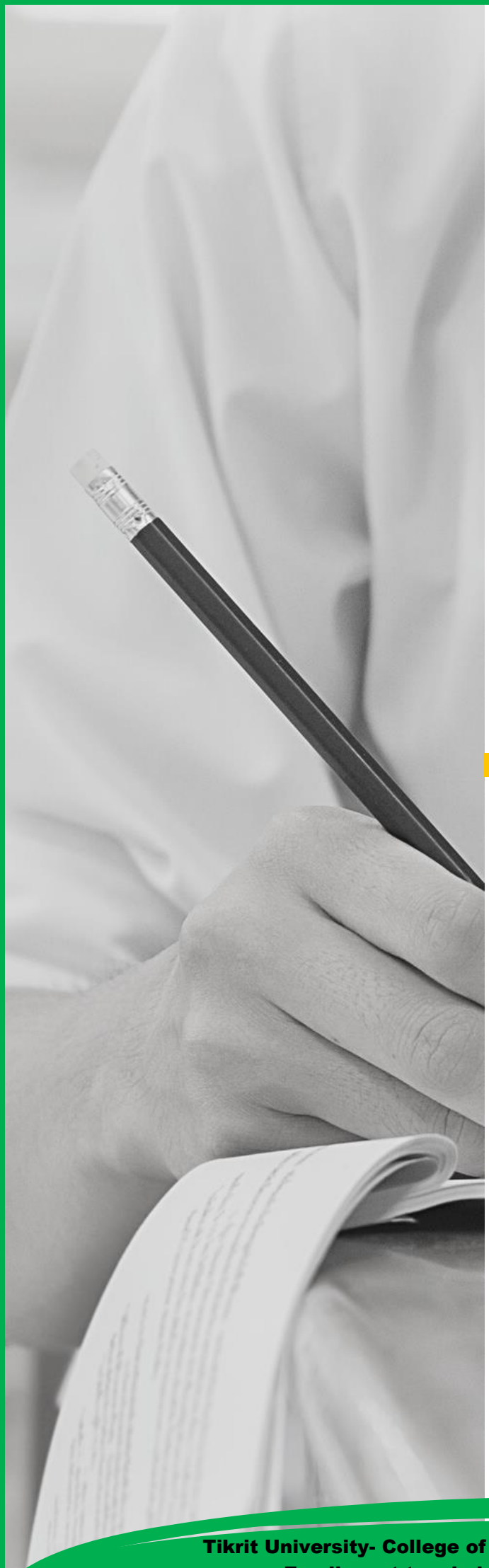

Tikrit University
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# Lecture 8: Antibodies (Immunoglobulins)

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# Antibodies (Immunoglobulins)

## Antibodies

**Antibodies**, also known as **immunoglobulins (Ig)**, are specialized glycoproteins found in serum and tissue fluids that bind specifically to antigens. They belong to the gamma globulin fraction of plasma proteins and play a critical role in the adaptive immune response. They represent roughly **20–25% of total serum proteins** and are produced primarily by **plasma cells** following stimulation of B lymphocytes by foreign antigens.

## Historical Background

The concept of antibodies emerged in **1890**, when **von Behring and Kitasato** discovered that serum from immunized animals protected against diphtheria and tetanus toxins. The protective substance was first called *antitoxin* and later recognized as *antibody*. In **1939**, **Tiselius and Kabat** demonstrated through electrophoresis that hyperimmunization enhanced the gamma globulin fraction responsible for antibody activity. In **1959**, **Porter** used papain to digest IgG, revealing its heavy and light chains and defining its molecular structure. In **1964**, the **World Health Organization (WHO)** officially adopted the term **immunoglobulin**.

## Structure of Immunoglobulins

All immunoglobulins share a basic **Y-shaped** structure composed of:

- **Two heavy chains (H)**
- **Two light chains (L)**
- **Variable regions (V)** that bind antigen
- **Constant regions (C)** that mediate biological functions
- A flexible **hinge region** (in some classes)

The two arms of the Y form the **Fab regions**, responsible for antigen binding, while the stem forms the **Fc region**, responsible for effector functions such as complement activation and receptor binding.

**Immunoglobulin Domains**

**1. What are Immunoglobulin Domains?**

Immunoglobulin (Ig) domains are **small, repeated protein units** that make up the structure of every antibody.

They give the antibody **strength, stability, and flexibility**.

There are **two main types** of domains:

- **Variable domains (V domains)**
- **Constant domains (C domains)**

**Heavy and Light Chains**

**Heavy Chains**

Each Ig molecule contains **two identical heavy chains**, each consisting of:

- **One variable domain (VH)**
- **Three or four constant domains (CH1, CH2, CH3, CH4)**

**Types of Heavy Chain Constant Regions**

Each antibody class is defined by its heavy chain:

| Ig Class | Heavy Chain | Constant Region | # Constant Domains |
|---|---|---|---|
| **IgG** | γ (gamma) | Cγ | 3 domains |
| **IgA** | α (alpha) | Cα | 3 domains |
| **IgM** | μ (mu) | Cμ | 4 domains |
| **IgD** | δ (delta) | Cδ | 3 domains |
| **IgE** | ε (epsilon) | Cε | 4 domains |

- **IgG, IgA, and IgD** include a true hinge region.
- **IgM and IgE** lack a classic hinge, instead possessing an extended constant region.

**Light Chains**

Each Ig molecule contains **two identical light chains**, each with:

- **One variable domain (VL)**
- **One constant domain (CL)**

**Types of Light Chain Constant Regions**

| Light Chain | Constant Region |
|---|---|
| **Kappa (κ)** | Cκ |
| **Lambda (λ)** | Cλ |

A single antibody uses **either κ or λ**, never both.

In humans, the **κ:λ ratio is approximately 2:1**.

**Variable and Constant Regions**

**Variable (V) Regions**

Found at the N-terminal ends of heavy and light chains.

Contain **hypervariable regions (complementarity-determining regions, CDRs)** that determine antigen specificity.

**Constant (C) Regions**

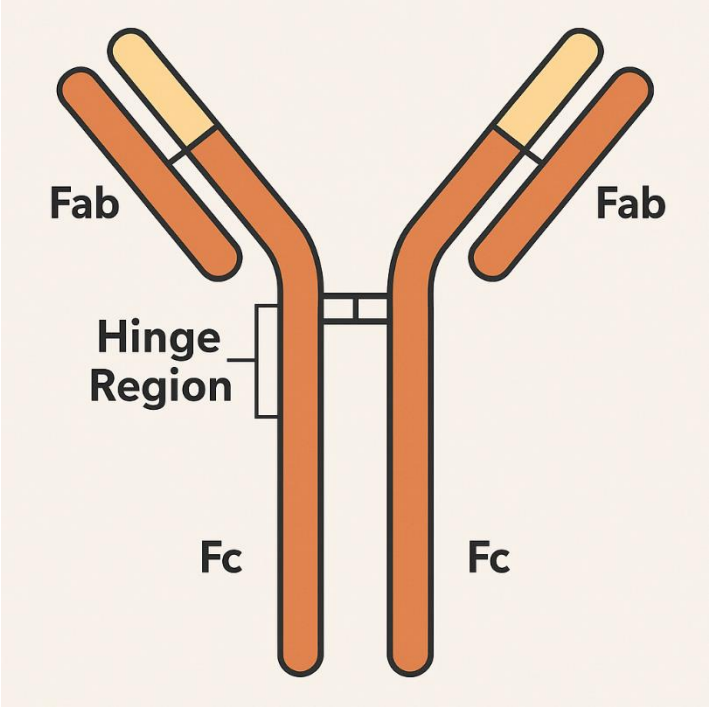
Responsible for:

- Complement activation
- Binding to Fc receptors
- Placental transfer (IgG)
- Determining the class and effector function

**Hinge Region**

The hinge region provides flexibility, allowing antibodies to adjust the angle between Fab arms to better bind antigens.

Present in **IgG, IgA, and IgD**, absent in **IgM and IgE**.




**Enzymatic Digestion of Immunoglobulins (IgG)**

**1. Papain Digestion**

**Papain** is a proteolytic enzyme that cuts the antibody **above the inter-chain disulfide bonds**, specifically in the **hinge region**.

**Papain produces:**

- **Two Fab fragments**
  (each Fab can bind antigen individually)
- **One Fc fragment**
  (contains the constant region but cannot bind antigen)

**Important Points**

- Fab fragments remain **monovalent** (one antigen-binding site each).
- Fc fragment loses most effector functions.
- Papain completely separates the arms.

## 2. Pepsin Digestion

**Pepsin** cuts **below the inter-chain disulfide bonds**, closer to the Fc region.

**Pepsin produces:**

- **One $F(ab)_2$ fragment**

  (contains **two Fab arms still connected**, so it is **bivalent** → stronger antigen binding)

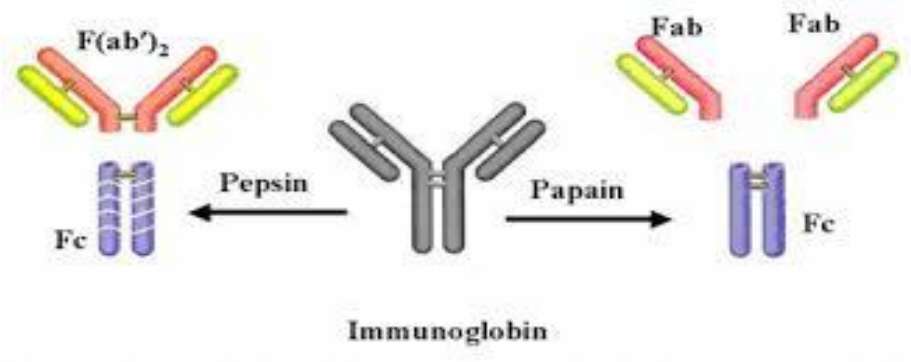
- **Degraded Fc fragments**

  (Fc region is digested into small peptides)

### Important Points

- $F(ab)_2$ fragment can still cause **agglutination** and cross-linking.
- Fc region is mostly destroyed.
- Pepsin preserves the **link between the two Fab arms**, unlike papain.

| Feature | Papain | Pepsin |
|---|---|---|
| Cut location | Above hinge disulfide bonds | Below hinge disulfide bonds |
| Main product | **2 Fab + 1 Fc** | **1 $F(ab)_2$ + degraded Fc** |
| Antigen binding | Monovalent | Bivalent |
| Fc functions | Preserved but separated | Destroyed |



## Biosynthesis of Immunoglobulins

Immunoglobulins are synthesized by:

### 1. B Lymphocytes

Produce membrane-bound Ig (B-cell receptors, BCRs).

### 2. Plasma Cells

Secrete large quantities of antibodies.

### Biosynthesis Steps

- Heavy and light chains synthesized separately
- Assembled in the rough ER
- Undergo **glycosylation**
- Transported to the cell surface or secreted

### Polymeric Forms

- **IgM** exists as a **pentamer**
- **IgA** exists as a **dimer**

  Both require a **J chain**, present only in polymeric antibodies.

## Classes of Immunoglobulins (Detailed Explanation)

### 1. IgG

The most abundant immunoglobulin in serum.

### Key Features

- Monomer
- 80% of serum antibodies
- Crosses the placenta
- Activates classical complement pathway
- Long half-life (23 days)

### Functions

- Long-term immunity

- Neutralization of toxins and viruses
- Opsonization
- Passive immunity to the fetus

**2. IgA**

The major immunoglobulin of mucosal surfaces.

**Key Features**

- Monomer in blood
- Dimer in secretions (saliva, tears, milk)
- Contains a **secretory component**
- J chain present in dimeric form

**Functions**

- Prevents microbial adhesion
- Protects mucosal surfaces
- Provides immunity to newborns through breast milk

**3. IgM**

First antibody produced after antigen exposure.

**Key Features**

- Pentamer (largest antibody)
- Strong complement activation
- Indicator of **recent infection**
- Does not cross placenta

**Functions**

- Primary immune response
- High avidity binding
- Efficient at agglutination

**4. IgD**

Found mainly on the surface of B cells.

**Key Features**

- Monomer
- Very low concentration in serum
- Does not activate complement

**Functions**

- B-cell receptor (BCR) for antigen recognition
- Helps initiate B-cell activation

**5. IgE**

Responsible for allergic reactions and parasite immunity.

**Key Features**

- Monomer
- Binds tightly to mast cells and basophils
- Very low serum levels
- Short half-life

**Functions**

- Mediates Type I hypersensitivity
- Triggers histamine release
- Defense against helminths

**Antigenic Determinants of Immunoglobulins**

**1. Isotypes**

Differences in constant regions that define Ig classes and subclasses (e.g., IgG1, IgA2).

**2. Allotypes**

Genetic variations (allelic differences) between individuals.

**3. Idiotypes**

Unique antigenic determinants located in the hypervariable regions of an antibody.

## Monoclonal Antibodies

Monoclonal antibodies are identical antibodies derived from a **single clone** of plasma cells, produced using **hybridoma technology** (Köhler and Milstein, 1975).

Monoclonal antibodies (mAbs) are laboratory-made proteins engineered to mimic the immune system's ability to fight off specific targets, such as antigens on cancer cells. They are identical, derived from a single parent cell, and bind to only one specific antigen. These targeted proteins are used in treating a range of diseases, including cancer, inflammatory, and infectious diseases.

### Applications

- Cancer therapy
- Autoimmune disease treatment
- Prevention of infections
- Diagnostic tests: ELISA, immunofluorescence

### Antibodies vs Immunoglobulins

- **Immunoglobulins** = the entire protein family
- **Antibodies** = immunoglobulins that specifically bind and neutralize antigens

**Comparison Table of Immunoglobulin Classes**

| Property | IgG | IgA | IgM | IgD | IgE |
|---|---|---|---|---|---|
| Structure | Monomer | Dimer (secretions) | Pentamer | Monomer | Monomer |
| Serum % | 80% | 10–13% | 5–8% | 0.2% | 0.002% |
| Location | Blood, lymph | Mucosal secretions | Blood | B-cell surface | Mast cells/basophils |
| MW (kDa) | 150 | 160 | 900 | 180 | 190 |
| Serum Level | 12 mg/mL | 2 mg/mL | 1.2 mg/mL | 0.03 mg/mL | 0.00004 mg/mL |
| Half-life | 23 days | 6–8 days | 2 days | 2–8 days | 1–5 days |
| Complement | Yes | Alternate | Yes | No | No |
| Placenta | Yes | No | No | No | No |
| Present in Milk | No | Yes | No | No | No |
| Heat Stability | Stable | Stable | Unstable | Unstable | Unstable |
| Opsonization | Yes | No | Yes | No | No |