Unit-3

Pharmaceutical Biotechnology

B.Pharma 6th Sem Notes

Unit: 3

- **Types of immunity-** humoral immunity, cellular immunity.
- Structure of Immunoglobulins.
- Structure and Function of MHC.
  Hypersensitivity reactions, Immune stimulation and Immune suppressions.
  General method of the preparation of bacterial infections, toxoids, viral vaccine, antitoxins,
  serum-immune blood derivatives and other products relative to immunity.
  Storage conditions and stability of official vaccines.
  Hybridoma technology- Production, Purification and Applications, Blood products and
  Plasma Substitutes.

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Immunity:

Immunity is a defence mechanism of the body provided by the immune system that helps fight disease-causing organisms. It allows the body to recognize and eliminate pathogens, preventing their harmful effects and promoting overall health. In simple words, it is the body's defence system that fights germs like bacteria, viruses, and other harmful substances.

Types of Immunity

Immunity is the body's ability to resist or eliminate potentially harmful foreign materials or abnormal cells. There are two main types of immunity:

1. **Innate immunity** is the body's first line of defence and is present from birth. It includes physical barriers such as the skin and mucous membranes, as well as specialised cells such as macrophages, Natural Killer cells, or neutrophils, which act rapidly to eliminate invading pathogens.
2. **Adaptive immunity** develops throughout life and is highly specific for particular pathogens. It is subdivided into two main categories:
   A. **Humoral immunity**: involves the production of antibodies by B cells (B lymphocytes) that circulate in the blood and body fluids, and are essential for neutralising pathogens and toxins.
   B. **Cell-mediated immunity**: involves the action of T cells (T lymphocytes) that detect and destroy cells infected by intracellular pathogens, such as viruses and some bacteria.

A. Humoral Immunity (Antibody-Mediated Immunity)

Definition: This type of immunity is mediated by antibodies produced by B lymphocytes (B cells). It protects against extracellular pathogens (bacteria, viruses in blood).

Features:

- Involves B lymphocytes that produce antibodies
- Antibodies circulate in blood and body fluids
- Effective against bacteria, viruses, and toxins in extracellular spaces
- Provides immunological memory for faster response on re-exposure

Mechanism:

1. Antigen enters the body
2. B cells recognize the antigen
3. B cells differentiate into plasma cells and memory cells
4. Plasma cells produce antibodies specific to the antigen
5. Antibodies neutralize, agglutinate, or precipitate antigens

B. Cellular Immunity (Cell-Mediated Immunity)

Definition: This immunity is mediated by T lymphocytes (T cells) and does not involve antibodies. It protects against intracellular pathogens (viruses inside cells, fungi, cancer cells).

Features:

- Involves T lymphocytes (T cells)
- Directly attacks infected cells, cancer cells, and transplanted tissues
- Does not involve antibodies
- Important for defense against intracellular pathogens

Types of T Cells:

- **Helper T cells (CD4+):** Activate other immune cells
- **Cytotoxic T cells (CD8+):** Directly kill infected cells
- **Memory T cells:** Provide long-term immunity

Structure of Immunoglobulins (Antibodies)

Immunoglobulins are glycoproteins produced by plasma cells that function as antibodies. They are Y-shaped molecules with specific binding sites for antigens.

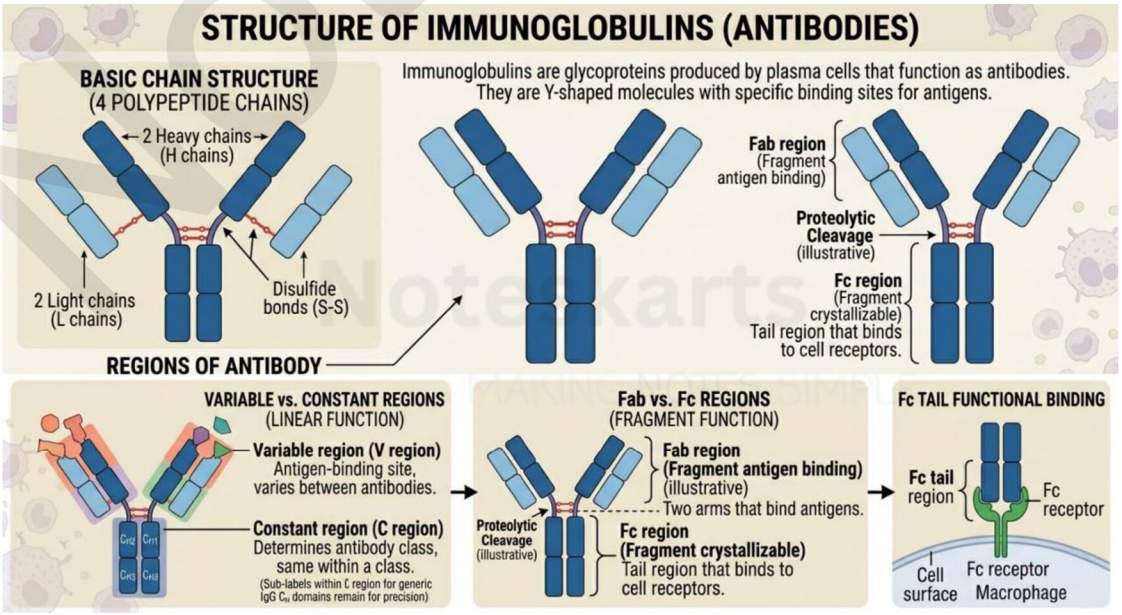
2.1 Basic Structure

An antibody consists of:

- **4 polypeptide chains:**
  - 2 Heavy chains (H chains) - longer chains
  - 2 Light chains (L chains) - shorter chains
- Chains are held together by disulfide bonds

Regions of Antibody:

- **Variable region (V region):** Antigen-binding site, varies between antibodies
- **Constant region (C region):** Determines antibody class, same within a class
- **Fab region (Fragment antigen binding):** Two arms that bind antigens
- **Fc region (Fragment crystallizable):** Tail region that binds to cell receptors



Classes of Immunoglobulins

There are five main classes based on the type of heavy chain:

IgG (Immunoglobulin G)

- Most abundant antibody in blood (75-80%)
- Can cross the placenta (provides immunity to fetus)
- Provides long-term immunity
- Activates complement system

IgM (Immunoglobulin M)

- First antibody produced in immune response
- Pentamer structure (5 Y-shaped units)
- Cannot cross placenta
- Very effective at agglutination

IgA (Immunoglobulin A)

- Found in secretions (saliva, tears, breast milk, mucus)
- Protects mucosal surfaces
- Prevents pathogen attachment to epithelial cells

IgE (Immunoglobulin E)

- Involved in allergic reactions
- Binds to mast cells and basophils
- Protects against parasitic infections

IgD (Immunoglobulin D)

- Found on surface of B cells
- Acts as antigen receptor during B cell maturation

Structure and Function of MHC

MHC (Major Histocompatibility Complex) are cell surface proteins that present antigens to T cells. They are crucial for immune recognition and transplant rejection.

Types of MHC Molecules

MHC Class I

- **Structure:** Single heavy chain + β2-microglobulin
- **Location:** Found on all nucleated cells
- **Function:** Present intracellular antigens (viral proteins) to CD8+ T cells
- **Clinical importance:** Involved in viral immunity and transplant rejection

MHC Class II

- **Structure:** Two chains (α and β)

- **Location:** Found on antigen-presenting cells (macrophages, dendritic cells, B cells)
- **Function:** Present extracellular antigens to CD4+ T cells
- **Clinical importance:** Essential for initiating immune response

Functions of MHC

- Antigen presentation to T cells
- Self vs non-self recognition
- Transplant compatibility determination
- Disease susceptibility (some MHC types linked to autoimmune diseases)

Hypersensitivity Reactions

Hypersensitivity is an exaggerated or inappropriate immune response that causes tissue damage. There are four types:

Type I: Immediate Hypersensitivity (Allergic Reactions)

- **Mechanism:** IgE-mediated, mast cell degranulation
- **Time:** Immediate (within minutes)
- **Examples:** Asthma, hay fever, food allergies, anaphylaxis
- **Treatment:** Antihistamines, epinephrine (for anaphylaxis)

Type II: Cytotoxic Hypersensitivity

- **Mechanism:** IgG or IgM antibodies against cell surface antigens
- **Time:** Minutes to hours
- **Examples:** Blood transfusion reactions, hemolytic disease of newborn, drug-induced hemolytic anemia
- **Treatment:** Remove triggering agent, immunosuppressive drugs

Type III: Immune Complex Hypersensitivity

- **Mechanism:** Antigen-antibody complexes deposit in tissues
- **Time:** 3-8 hours
- **Examples:** Serum sickness, farmer's lung, systemic lupus erythematosus (SLE)
- **Treatment:** Anti-inflammatory drugs, corticosteroids

Type IV: Delayed Hypersensitivity (Cell-Mediated)

- **Mechanism:** T cell-mediated, no antibodies involved
- **Time:** 24-72 hours
- **Examples:** Contact dermatitis, tuberculin test, transplant rejection
- **Treatment:** Topical corticosteroids, avoid allergen

Immune Stimulation and Immune Suppression

Immune Stimulation (Immunostimulants)

Substances that enhance immune response. Used when immune system is weak.

Types:

- **Vaccines:** Stimulate specific immunity
- **Adjuvants:** Enhance vaccine effectiveness (e.g., Alum)
- **Cytokines:** Interferons, interleukins (boost immune cells)
- **Bacterial products:** BCG (Bacillus Calmette-Guérin)

Uses:

- Cancer treatment (activate immune cells against tumor)
- Immunodeficiency disorders
- Chronic infections

Immune Suppression (Immunosuppressants)

Substances that reduce or prevent immune response. Used when immune system is overactive.

Types:

- **Corticosteroids:** Prednisolone, dexamethasone (reduce inflammation)
- **Cytotoxic drugs:** Azathioprine, cyclophosphamide (kill dividing cells)
- **Calcineurin inhibitors:** Cyclosporine, tacrolimus (block T cell activation)
- **Monoclonal antibodies:** Target specific immune cells

Uses:

- Organ transplantation (prevent rejection)
- Autoimmune diseases (rheumatoid arthritis, lupus)
- Allergic conditions

Preparation of Biological Products

Bacterial Vaccines

General Method:

6. **Culture bacteria:** Grow in nutrient medium
7. **Harvest:** Collect bacterial cells
8. **Inactivation or Attenuation:**
    - Killed vaccine: Heat or chemical treatment (formalin, phenol)
    - Live attenuated: Weaken bacteria by repeated culture passages
9. **Purification:** Remove impurities
10. **Formulation:** Add stabilizers, preservatives, adjuvants
11. **Testing:** Sterility, safety, potency tests

Examples:

- BCG vaccine (tuberculosis) - live attenuated
- Typhoid vaccine - killed or live attenuated
- Pertussis vaccine - killed

Toxoids

Definition: Inactivated bacterial toxins that retain immunogenic properties but lack toxicity.

Preparation Method:

1. Culture toxin-producing bacteria
2. Extract and purify the toxin
3. Detoxification: Treat with formaldehyde (0.3-0.4%) at 37°C for 3-4 weeks
4. Testing for toxicity and immunogenicity
5. Adsorption on adjuvant (alum) for better immune response
6. Standardization and packaging

Examples:

- Tetanus toxoid
- Diphtheria toxoid

Viral Vaccines

General Method:

1. **Virus Cultivation:**
   - Embryonated eggs (influenza)
   - Cell culture (Vero cells, MRC-5 cells)
2. Harvest virus particles
3. **Attenuation or Inactivation:**
   - Live attenuated: Serial passages in non-human hosts or cell cultures
   - Inactivated: Chemical treatment (formaldehyde, β-propiolactone) or heat
4. Purification and concentration
5. Formulation with stabilizers
6. Safety and potency testing

Examples:

- Live attenuated: MMR (measles, mumps, rubella), polio (oral)
- Inactivated: Polio (injectable), hepatitis A, rabies

Antitoxins

Definition: Antibodies against specific toxins, obtained from immunized animals or humans.

Preparation Method:

- Immunization: Inject animals (horses, rabbits) with toxoid
- Multiple doses given over weeks to boost antibody production
- Blood collection from immunized animal
- Serum separation

- Purification: Remove unwanted proteins, concentrate antibodies
- Digestion (optional): Pepsin treatment to remove Fc portion (reduces allergic reactions)
- Standardization: Test potency against standard antitoxin

Examples:

- Tetanus antitoxin
- Diphtheria antitoxin
- Snake venom antitoxin (antivenin)

Serum and Immune Blood Derivatives

Types:

- **Immune Serum Globulin (ISG):** Pooled antibodies from healthy donors
- **Specific Immune Globulins:** Antibodies against specific pathogens

Preparation:

12. Collection: Blood from immunized or convalescent donors
13. Plasma separation
14. Fractionation: Separate immunoglobulins (Cohn fractionation method)
15. Viral inactivation: Heat treatment or solvent-detergent method
16. Testing for safety and potency

Examples:

- Hepatitis B immune globulin (HBIG)
- Rabies immune globulin (RIG)
- Tetanus immune globulin (TIG)

Storage Conditions and Stability of Vaccines

Proper storage is critical to maintain vaccine potency and safety.

General Storage Requirements

- **Temperature:** Most vaccines stored at 2-8°C (refrigerator)
- Some vaccines require -20°C or -70°C (freezer)
- **Light protection:** Store in dark or amber containers
- **Monitoring:** Use temperature logs, digital thermometers

Vaccine-Specific Storage

Refrigerated Vaccines (2-8°C)

- Most inactivated vaccines (hepatitis B, IPV, tetanus, diphtheria)
- Do NOT freeze - freezing destroys vaccine
- Shelf life: Typically 2-3 years when properly stored

Frozen Vaccines (-20°C or below)

- Some live attenuated vaccines
- Varicella (chickenpox) vaccine

Ultra-Cold Storage (-70°C)

- Some modern vaccines (e.g., certain mRNA vaccines)

Cold Chain Management

Cold chain is the system of transporting and storing vaccines at recommended temperatures from manufacturing to administration.

Components:

- Refrigerated vehicles for transport
- Cold boxes and vaccine carriers with ice packs
- Temperature monitoring devices (VVM - Vaccine Vial Monitors)
- Trained personnel for handling

Stability Factors

Factors affecting vaccine stability:

- **Temperature fluctuations:** Repeated freeze-thaw cycles damage vaccines
- **Light exposure:** UV light degrades some vaccines
- **Time:** Check expiry dates regularly
- **Contamination:** Use aseptic technique when reconstituting

Hybridoma Technology

Hybridoma technology is used to produce monoclonal antibodies (identical antibodies from a single B cell clone). Developed by Köhler and Milstein in 1975.

Production of Hybridomas

Step-by-Step Process:

Immunization:
- Inject antigen into mouse or rat
- Multiple injections over weeks to boost immune response

Isolation of B cells:
- Remove spleen from immunized animal
- Extract antibody-producing B cells

Cell Fusion:
- Mix B cells with myeloma cells (immortal cancer cells)
- Use polyethylene glycol (PEG) or electrofusion to fuse cells
- Creates hybridoma cells (combine antibody production + immortality)

Selection (HAT Medium):
- Grow cells in HAT medium (Hypoxanthine, Aminopterin, Thymidine)

- Only hybridoma cells survive (unfused B cells die, unfused myeloma cells cannot grow)

Screening and Cloning:

- Test culture supernatants for desired antibodies (ELISA, RIA)
- Select positive clones
- Perform limiting dilution to ensure single-cell clones

Propagation:

- In vitro: Grow in cell culture flasks (small-scale)
- In vivo: Inject into mouse peritoneum to produce ascites fluid (large-scale)

Purification of Monoclonal Antibodies

Common Methods:

- **Protein A/G Affinity Chromatography:**
  - Most common method
  - Protein A/G binds to Fc region of antibodies
  - High purity (>95%)
- **Ion Exchange Chromatography:**
  - Separates based on charge
- **Ammonium Sulfate Precipitation:**
  - Initial purification step
- **Dialysis and Concentration:**
  - Final purification and buffer exchange

Applications of Monoclonal Antibodies

Diagnostic Applications

- Pregnancy tests (detect hCG hormone)
- Blood typing and tissue matching
- Detection of infectious diseases (HIV, hepatitis)
- Cancer markers detection (PSA, CEA)

Therapeutic Applications

- **Cancer therapy:**
  - Rituximab (lymphoma)
  - Trastuzumab/Herceptin (breast cancer)
- **Autoimmune diseases:**
  - Infliximab (rheumatoid arthritis, Crohn's disease)
- **Transplant rejection:**
  - Basiliximab (prevent organ rejection)
- **Infectious diseases:**
  - Palivizumab (RSV in infants)

Research Applications

- Immunofluorescence microscopy
- Western blotting
- Flow cytometry
- Protein purification

Blood Products and Plasma Substitutes Blood Products

Blood products are therapeutic substances derived from human blood.

Whole Blood

- **Contains:** All blood components (RBCs, WBCs, platelets, plasma)
- **Use:** Massive blood loss, exchange transfusion
- **Storage:** 1-6°C for 35-42 days (with anticoagulant preservatives)

Packed Red Blood Cells (PRBC)

- **Contains:** Concentrated red blood cells (plasma removed)
- **Use:** Anemia, surgical blood loss
- **Advantage:** Reduces volume overload

Fresh Frozen Plasma (FFP)

- **Contains:** All clotting factors, proteins
- **Use:** Clotting factor deficiencies, warfarin reversal
- **Storage:** -18°C or colder for up to 1 year

Platelet Concentrate

- **Contains:** Concentrated platelets
- **Use:** Thrombocytopenia, platelet dysfunction
- **Storage:** 20-24°C with gentle agitation for 5 days

Cryoprecipitate

- **Contains:** Factor VIII, fibrinogen, von Willebrand factor
- **Use:** Hemophilia A, von Willebrand disease, fibrinogen deficiency

Plasma Substitutes (Volume Expanders)

Plasma substitutes are fluids used to replace blood volume without using actual blood components. They do not carry oxygen.

Crystalloids

Definition: Solutions of small molecules that can easily cross capillary membranes.

Types:

- **Normal Saline (0.9% NaCl):** Isotonic, used for fluid resuscitation
- **Ringer's Lactate:** Contains electrolytes, better for large volume replacement
- **Dextrose solutions:** 5% dextrose in water, provides calories

Advantages:

- Inexpensive, readily available
- No risk of disease transmission

Disadvantages:

- Short intravascular duration, require large volumes

Colloids

Definition: Solutions containing large molecules that stay in blood vessels longer.

Types:

- **Albumin (5% or 25%):**
  - Natural protein from human plasma
  - Maintains oncotic pressure
- **Dextrans (Dextran 40, Dextran 70):**
  - Synthetic polysaccharides
  - Improve blood flow, prevent clotting
- **Hydroxyethyl Starch (HES):**
  - Synthetic starch derivative
  - Various molecular weights available
- **Gelatin solutions:**
  - Derived from collagen

Advantages:

- Stay in bloodstream longer than crystalloids
- Require smaller volumes

Disadvantages:

- More expensive
- Risk of allergic reactions

Clinical Uses of Plasma Substitutes

- Hypovolemic shock (blood loss)
- Burns (fluid loss)
- Surgery (maintain blood pressure)
- Trauma
- Dehydration

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