# BIOTECHNOLOGY

## UNIT 3 NOTES

- TYPES OF IMMUNITY
- STRUCTURE OF IMMUNOGLOBULINS
- STRUCTURE & FUNCTIONS OF MHC
- VACCINES
- HYBRIDOMA TECHNOLOGY
- BLOOD PRODUCTS & PLASMA SUBSTITUTES

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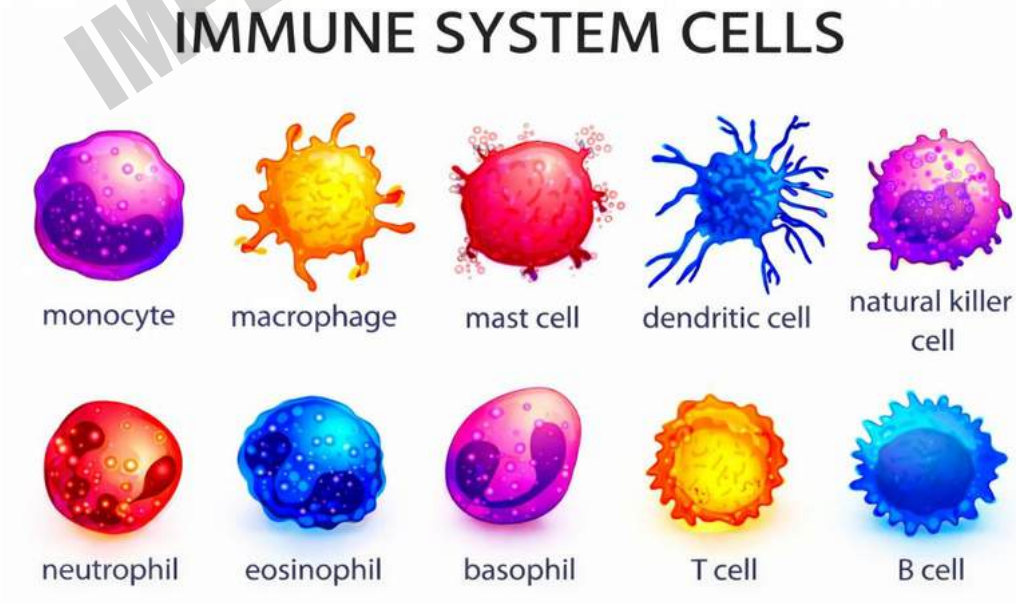
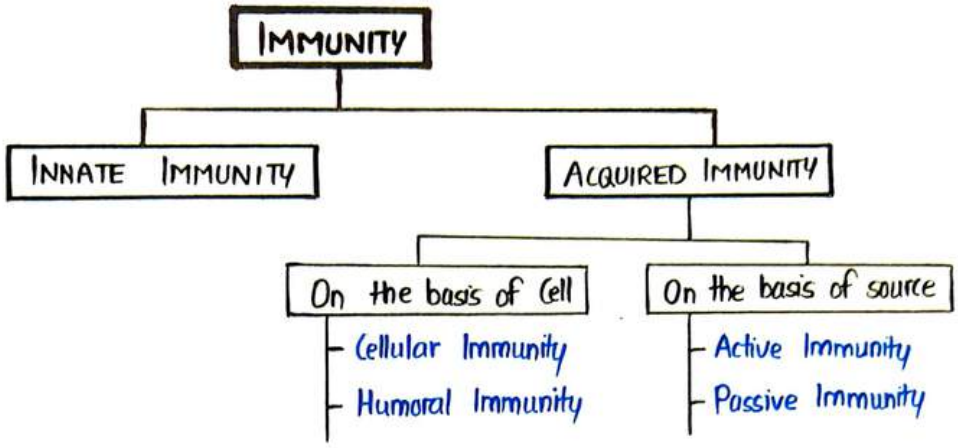
# IMMUNITY

- Immunity is the ability of the body to recognize, resist & destroy harmful substances like bacteria, viruses, toxins & parasites.
- These harmful substances are called antigens.
- The body defends itself using the immune system.
- The branch of science deals with the study of immune System is known as Immunology.
  - Organs: Bone Marrow, Thymus, spleen, lymph nodes
  - Cells: WBCs (Leukocytes)








# INNATE IMMUNITY

- Innate immunity is the first line of defense of the body.
- It is present from birth & provides immediate protection against pathogens.
- It is non-specific because it doesn't target a particular antigen.
- It doesn't have any memory (does not remmember previous infections).



## COMPONENTS OF INNATE IMMUNITY

### ① PHYSICAL BARRIERS

- These are the first protective layer that prevents entry of pathogens.
- It includes:
  - Skin: acts as a tough barrier
  - Mucus: traps microbes
  - Cilia: push microbes out (respiratory tract)
  - Tears & saliva: contain enzymes (lysozyme)
  - Stomach acid (HCl): kills pathogens

## ② Chemical Barries

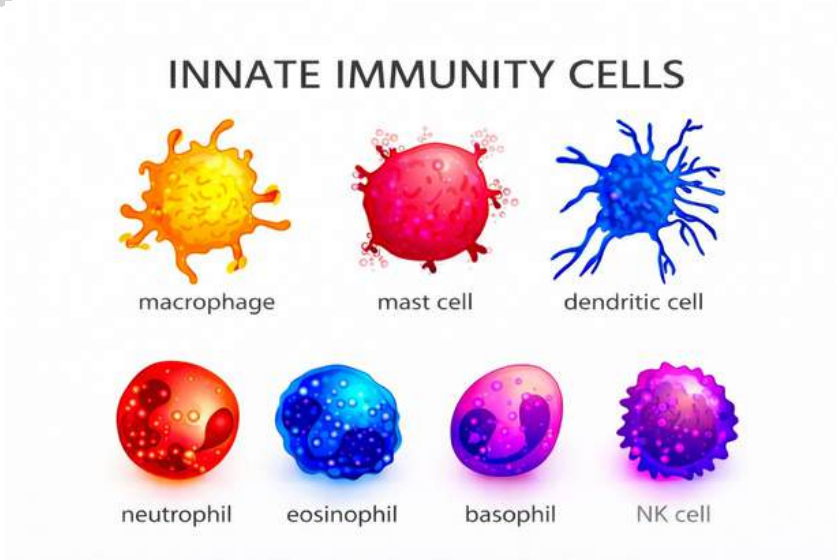
- These are chemical substances that destroy pathogens such as:
  - Lysozyme: breaks bacterial cell wall
  - Acidic pH: skin, stomach
  - Interferons: Prevent viral infection
  - Complement proteins: destroy microbes

## ③ Cellular Components

- These are the immune cells that directly attack pathogens.

| Cell | Main Function |
|---|---|
| Neutrophils | First to arrive, kill microbes (Phagocytosis) |
| Macrophages | Eat pathogens + start inflammation |
| Dendritic Cells | Show antigen to T cells |
| Nk cells | Kill virus-infected & tumor cells |
| Mast cells | Release histamine (inflammation, allergy) |
| Basophils | Help in allergy |
| Eosinophils | kill parasites |





# Mechanism Of Innate Immunity

Pathogen entry

Barrier Cells (Skin / Epithelium)

Recognition by PRRs (PAMPs detected)

Early Cellular Response
- Neutrophils - Phagocytosis
- Macrophages - Cytokiens
- Mast Cell - Histamine

Inflammation (Redness, Heat, Swelling)

Recruitment of Cells
- Nk cells → kill infected
- Eosinophils → Parasites
- Basophils → Allergy

Dendritic Cells capture Antigen

Migration to Lymph nodes

Activation of adaptive Immunity (T-cells)

# Acquired Immunity

- It is also known as Adaptive or Specific Immunity
- Acquired immunity is a type of immunity that develops after exposure to an antigen & provides a specific response against that antigen.
- It is called specific immunity because it targets a particular pathogen.
- It has memory which helps in faster response on re-exposure.
- It involves lymphocytes (B-cells & T-cells).

## Types Of Acquired Immunity

- It is further classified on two basis:

① On the basis of Cell involve
② On the Basis of Source



## ① On The Basis Of Cell Involve

- It is of two types:

ⓐ Humoral Immunity
ⓑ Cellular Immunity

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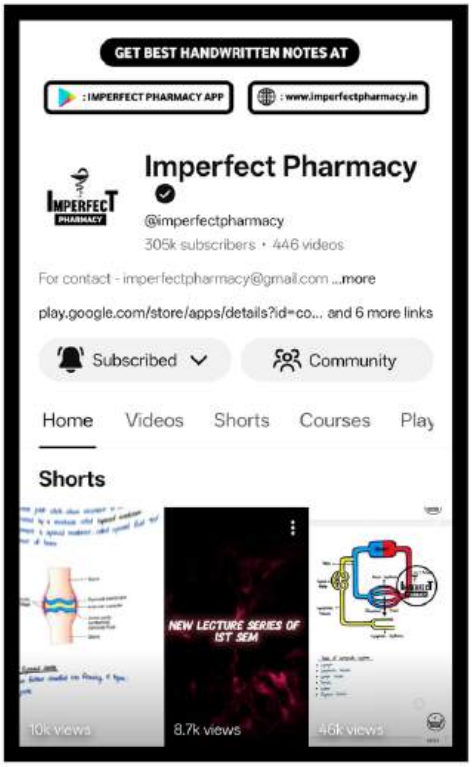


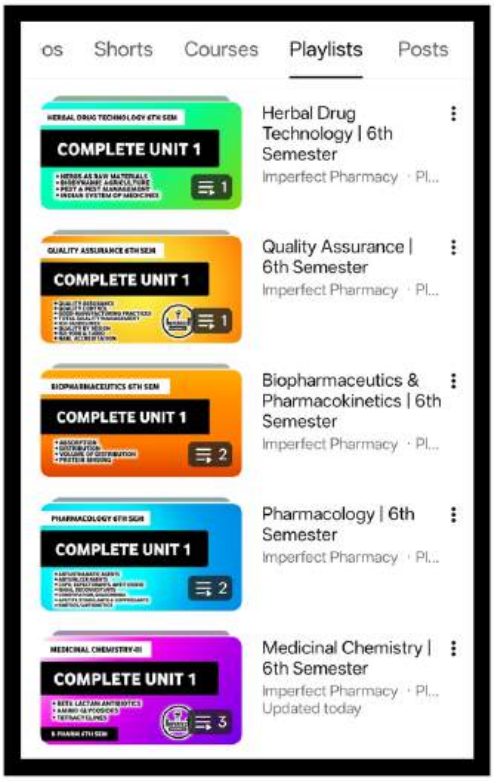


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## ④ HUMORAL IMMUNITY

- Humoral immunity is a type of adaptive immunity in which B-lymphocytes produce antibodies that circulate in body fluids/ humors (blood & lymph) to fight pathogens.
- It mainly protects against extracellular pathogens (bacteria, toxins etc).

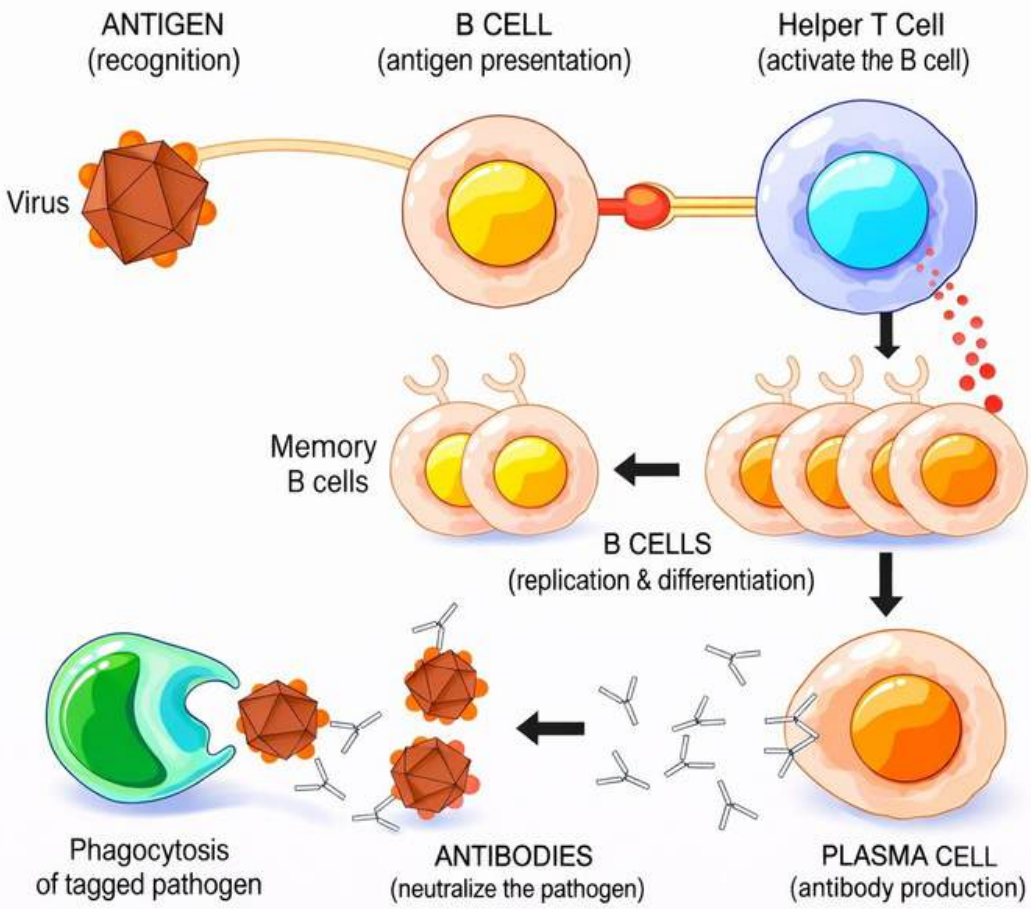
### ROLE OF B-CELLS

- Humoral immunity is mediated by B-lymphocytes.
- They're developed & matured in bone marrow.
- They recognize specific antigens via B-cells receptors (BCR).

### ACTIVATION OF B-CELLS

- B-Cells binds to antigen via BCR & engulf it.
- After engulfment it breaks antigen into peptide fragments & display it on surface through MHC-II
- A T-Helper Cell recognised the antigen - MHC II complex & release cytokines which gives the final signal for B-Cell activation.
- After activation it rapidly divided into two types of cells.

- Plasma Cells: They produce large amounts of antibodies.
- Memory B-Cells: They're long lived cells that remmember a specific antigen & provides faster action on re-exposure.


ANTIGEN
(recognition)
B CELL
(antigen presentation)
Helper T Cell
(activate the B cell)
Virus
Memory
B cells
B CELLS
(replication & differentiation)
Phagocytosis
of tagged pathogen
ANTIBODIES
(neutralize the pathogen)
PLASMA CELL
(antibody production)

## ② CELLULAR IMMUNITY

- Cellular immunity is a type of adaptive immunity in which T-lymphocytes directly attack & destroy infected, abnormal or foreign cells without using antibodies.
- It mainly protect against intracellular pathogens (viruses, some bacteria, cancer cells).

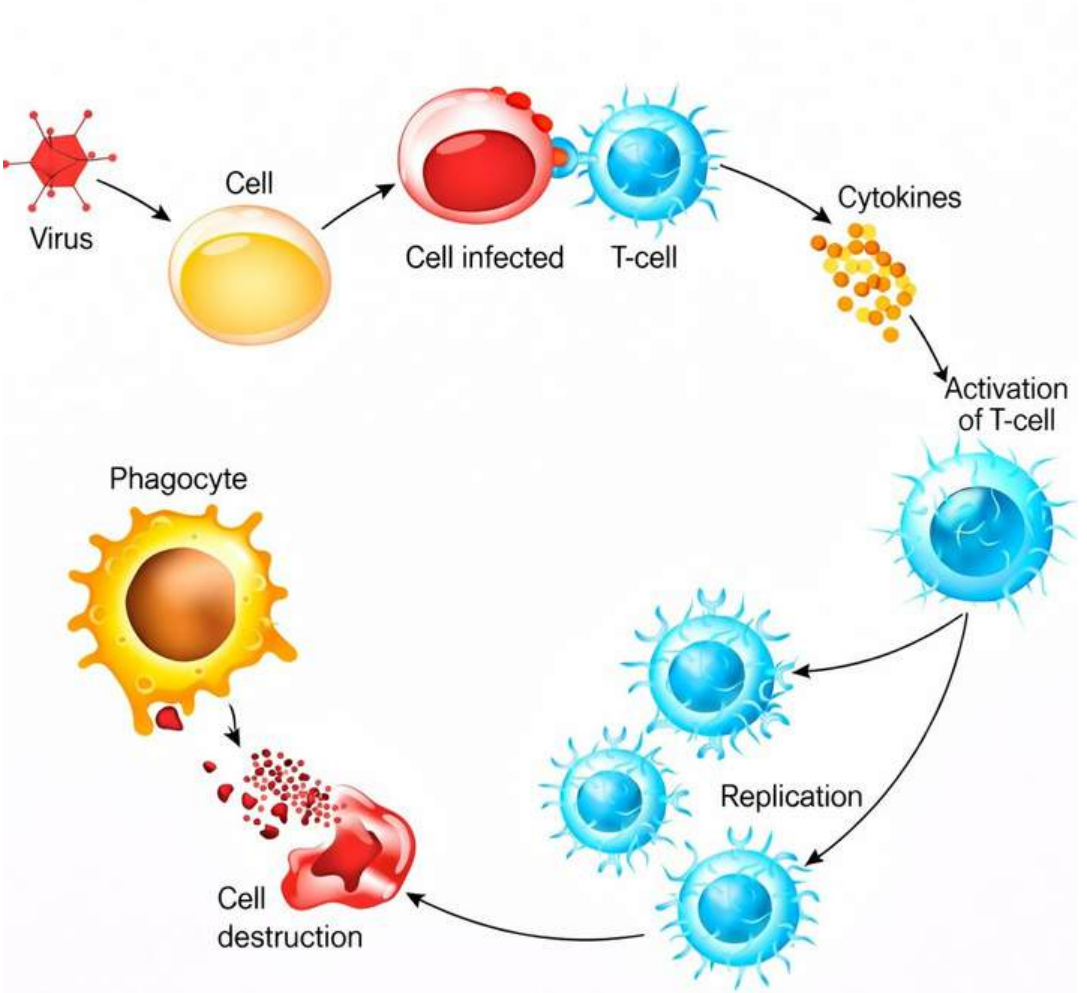
### ROLL OF T-CELLS

- Cellular immunity is mediated by T-lymphocytes
- They're developed in Bone Marrow but matured in Thymus after Positive Selection.

### ACTIVATION OF T-CELLS

- T cells can't recognize free antigen they require APCs (Antigen Presenting Cells) for Antigen recognition such as-
  - Dendritic Cells (most common)
  - Macrophages
  - B cells
- APCs presents antigen fragments on MHC molecule as MHC antigen complex, TCR binds to this complex.
  - MHC I: Cytotoxic T-cells ($CD8^+$)
  - MHC II: Helper T-cells ($CD4^+$)

# T-cell activation



## OF T-CELLS

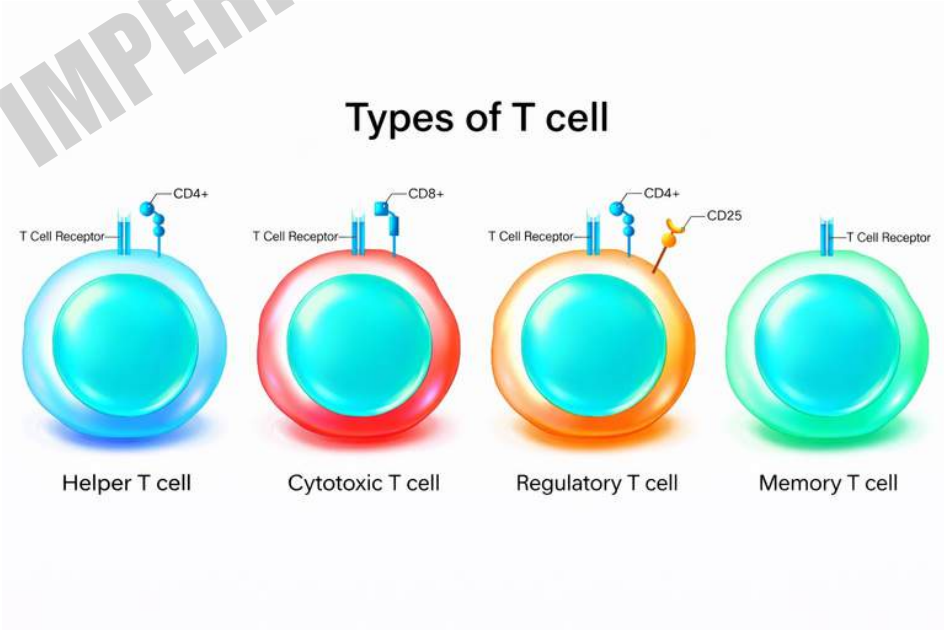
1) Cytotoxic T-cells: They directly kill infected or cancer cells & recognize antigen via MHC Class I

2) Helper T-Cells: They releases cytokines & activates other cells such as B cells, Cytotoxic T-cells, Macrophages.

3) Regulatory T-Cells: They regulates/suppress immune response to prevent autoimmunity.

4) Memory T-Cells: They're long lived cells, provides faster response on re-exposure.





## ② On The Basis Of Source

On the basis of source, it is again of two types:

ⓐ Active Immunity

ⓑ Passive Immunity

## ⓐ Active Immunity

- Active immunity is the immunity developed when body produces Antibodies & memory cells in response to an antigen.
- It is further divided into two sub-types:

① Natural AI:
- It occurs after natural infection.
- Example: Immunity after recovering from Chickepox.

② Artificial AI:
- It is developed by Vaccination.
- Example: Polio vaccine, COVID-19 Vaccine.

## ⑥ PASSIVE IMMUNITY

- Passive immunity is the immunity where ready-made antibodies are transferred to a person.
- It is again of two subtypes:

① Natural PI:
- From mother to baby
- They transferred to baby via placenta (IgG) & breast milk (IgA).

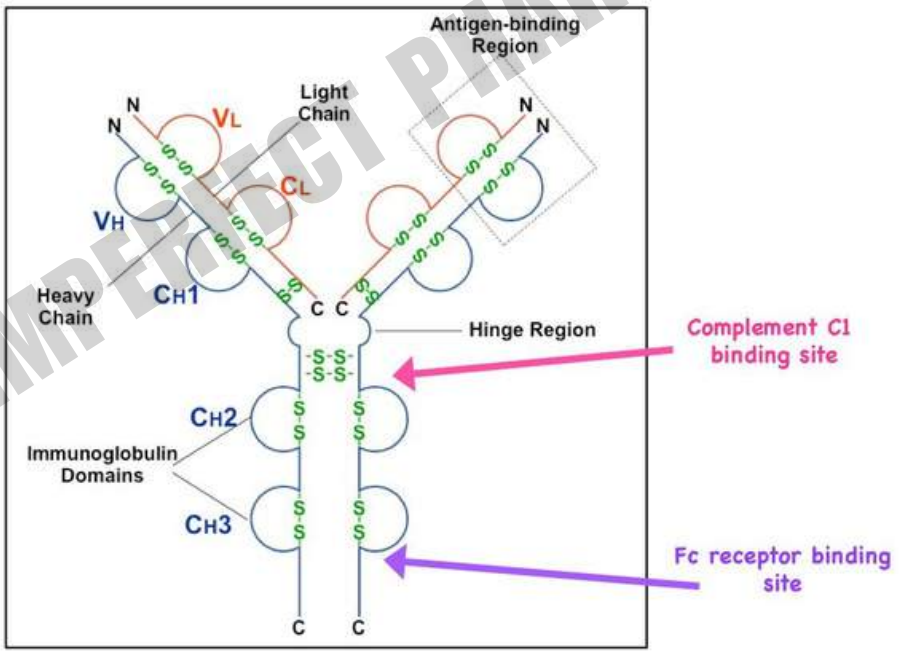
② Artificial PI:
- Injection of antibodies
- Example: Anti-rabies serum, anti-snake venom.

# IMMUNOGLOBULIN

- Immunoglobulins (Ig) are glycoproteins produced by B-lymphocytes (plasma cells) in response to an antigen.
- They're Y-shaped proteins produced by plasma cells that specifically bind to antigens to neutralize or eliminate them.
- It is highly specific to Antigen & part of Humoral Immunity.



## Basic Structure of Antibody Molecules



# Structure Of Antibody

An antibody is a Y-shaped protein & made up of 4 polypeptide chains

- 2 Heavy Chains
- 2 Light Chains

## ① Heavy Chains

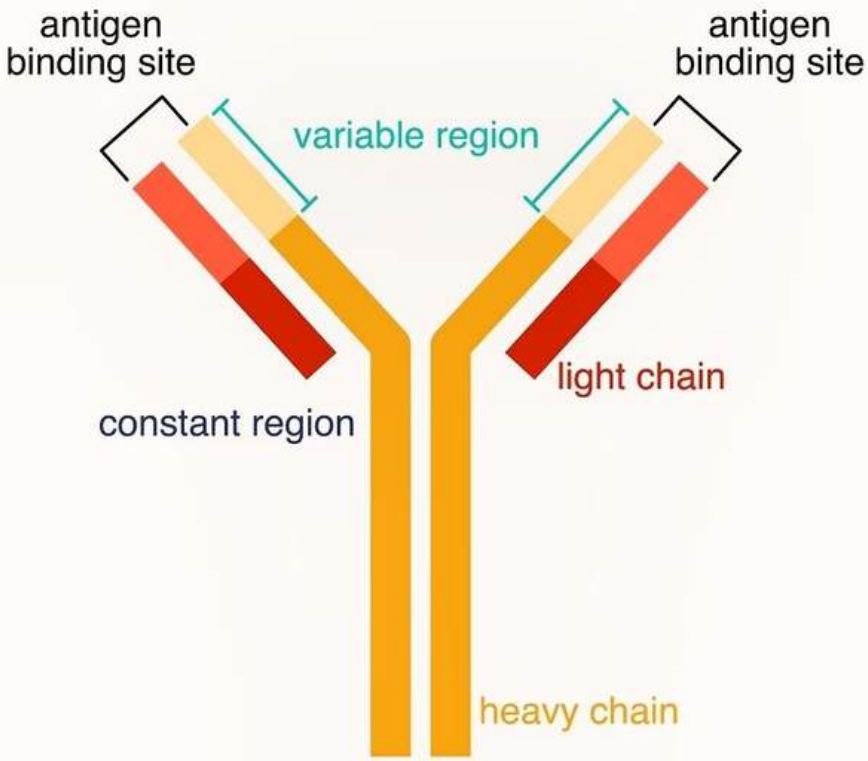
- They're two identical heavy chains.
- They're larger in size (~ 50 kDa)
- They Form the core structure of antibody.
- They determine the class of Antibody

| Heavy Chains | Antibody |
|---|---|
| γ (gamma) | IgG |
| α (alpha) | IgA |
| μ (mu) | IgM |
| ε (epsilon) | IgE |
| δ (delta) | IgD |

## ② Light Chains

- They're 2 smaller identical chains (~ 25kDa).
- They're of generally two types:
  - kappa (κ)
  - Lambda (λ)
- Each antibody has either κ or λ, not both.

# ANTIBODY STRUCTURE



# Regions Of Antibody

## ① Fab Region

- It is fragment Antigen Binding region.
- It is located at arms of Y
- It contains antigen binding site.
- Each antibody has 2 fab regions, which binds with 2 Antigens.

## ② Fc Region

- It is fragment crystallizable region.
- It is located at stem of Y.
- It activates complement system & immune system.

## ③ Variable Region

- It is present at tip of Y.
- It differs in each antibody, allowing recognisition of different antigens.
- It is the region that actually binds with antigen.

## ④ Consant Region

- It remains same in antibodies of same class.
- It is responsible for effector functions.

⑤ Hinge Region

- It is present b/w Fab & Fc
- It provides flexibility & allows arms to move freely.

## DISULFIDE BONDS

- Disulfide bonds (-S-S-) are covalent bonds present in antibody.
- They're of two types of Disulfide bonds:

① Interchain Bonds: It is present b/w heavy - heavy & heavy - light chains.

② Intrachain Bonds: It is present within the same chain.

# Types Of Immunoglobulins

On the basis of heavy chains, immunoglobulins / Antibody of 5 types:

1. IgG Antibody
2. IgA Antibody
3. IgM Antibody
4. IgE Antibody
5. IgD Antibody

## 1. IgG Antibody

- IgG is the most abundant antibody present in the blood & extracellular fluid, & it constitutes about about 70-75% of total immunoglobulins.
- It is relatively smaller in size.
- It plays a major role in long-term immunity by neutralizing toxins & viruses.
- It is capable of activating the complement system, which helps in the destruction of pathogens.
- It is the only antibody that can cross the placenta & provide passive immunity to the fetus.

## ② IgA

- IgA is mainly found in body secretions such as saliva, tears, mucus & breast milk.
- It usually exists as a dimer.
- It plays a crucial role in mucosal immunity by protecting the respiratory & GIT.
- It prevents the attachment & entry of pathogens into the body by neutralizing them at mucosal surfaces.
- It acts as the first line of defense in areas that are exposed to the external environment.

## ③ IgM

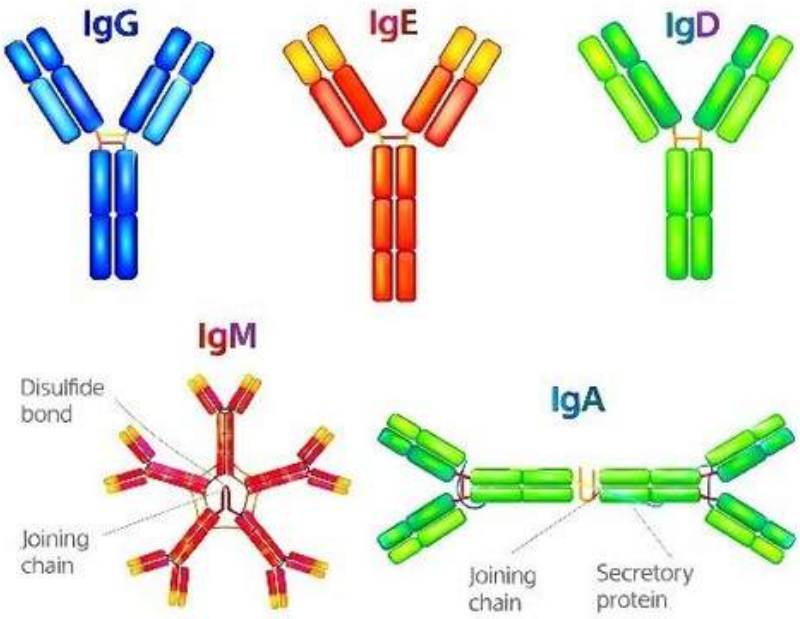
- IgM is the largest antibody & exists as a pentamer.
- It is the first antibody produced during primary immune response when an antigen enters the body.
- It is highly effective in activating the complement system & initiating immune reactions.
- Its presence in blood generally indicates a recent or acute infection.

## ④ IgE

- IgE is present in very low concentration in the blood but has a strong affinity for mast cells & basophils.
- It binds to Fc receptors on these cells & remains attached to their surface.
- It plays an important role in allergic reactions & defense against parasitic infections.
- It triggers the release of histamine & other inflammatory mediators.
- It is responsible for conditions such as asthma, hay fever, & anaphylactic reactions.

## ⑤ IgD

- IgD is the least abundant antibody & is mainly present on the surface of naïve B lymphocytes.
- It exists as a monomer & functions primarily as a receptor for antigen recognition.
- It plays a role in the activation & differentiation of B cells during the immune response.
- Its exact biological function is not fully understood, but it is important in the early stages of B cell activation.


IgG
IgE
IgD
IgM
Disulfide bond
Joining chain
IgA
Joining chain
Secretory protein

# MAJOR HISTOCOMPATIBILITY COMPLEX

- MHC is a group of genes that code for proteins or MHC molecules (glycoproteins) present on cell surfaces.
- It is also known as HLA (Humna Leukocyte Antigen) System.
- It helps the immune system to differentiate b/w self vs non-self.
- They present antigenic peptides to T-cells, which is essential for immune response.
- It is noted that B-cell can recognise antigen on their own, while T-Cell need MHC for antigen recognition.
- In humans, MHC genes are located on Chromosome 6.



## CLASSES OF MHC

MHC mainly divided into 3 classes:

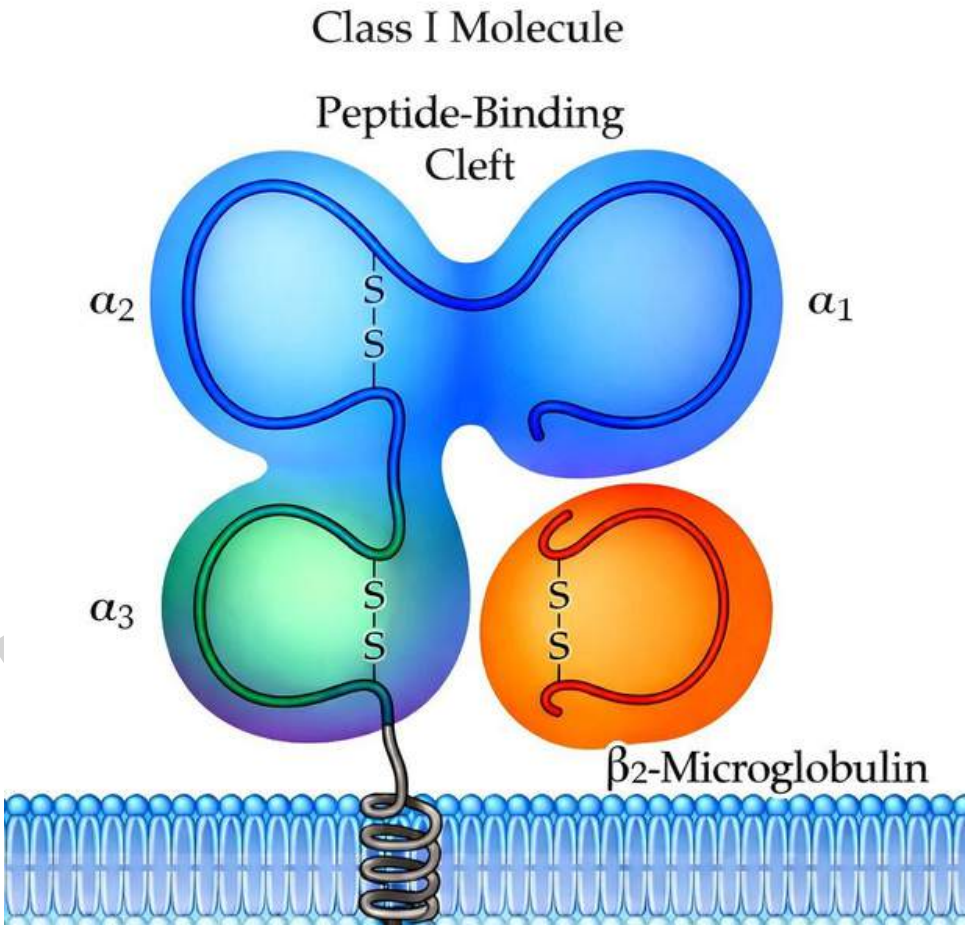
1. MHC I
2. MHC II
3. MHC III

## 1. MHC CLASS I

- It is present on all nucleated cells (Not present on RBCs)
- These molecules are encoded by HLA-A, HLA-B & HLA-C genes.
- It presents endogenous antigens (inside the cell) such as viral proteins, tumor proteins on its surface
- It consist of:
  - Heavy ($\alpha$) chain (with 3 domains $\alpha_1$, $\alpha_2$, $\alpha_3$)
  - $\beta_2$-microglobulin

## Working Mechanism

- Antigen protein → broken into peptides
- Peptides bind MHC I in ER
- Complex moves to surface
- CD8 T cell recognizes → kills infected cell.



## ② MHC CLASS II

- They're present only on Antigen Presenting Cells (APCs) such as:
  - Dendritic Cells
  - Macrophages
  - B Cells
- These molecules are encoded by HLA-DP, HLA-DQ & HLA-DR genes in humans.
- The present exogenous antigens (outside the cell) such as bacteria, toxins.
- It consist of two chains:

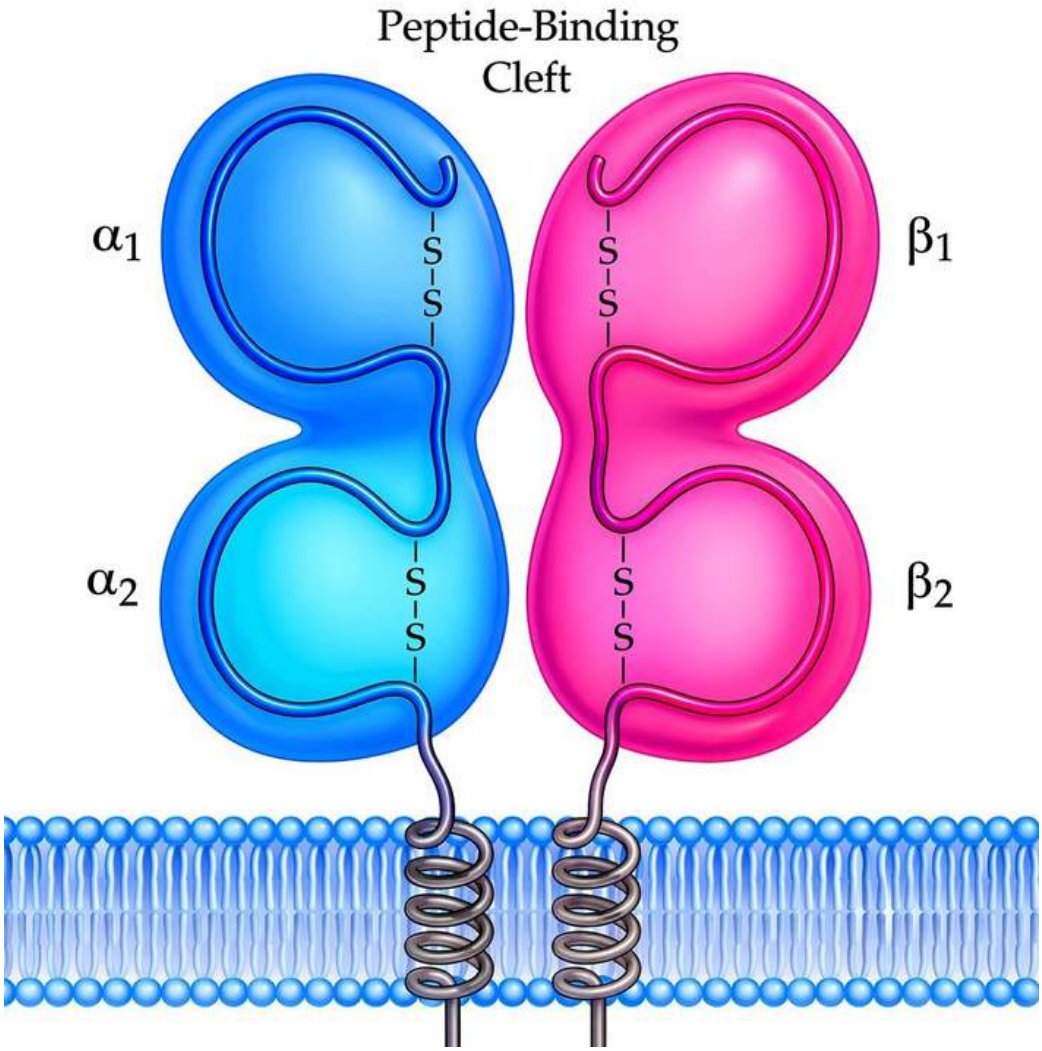
a) α chain (Two domain $\alpha_1$, $\alpha_2$)

b) β chain (Two domain $\beta_1$, $\beta_2$)

### WORKING MECHANISM

- Pathogen engufed by APCs (Phagocytosis)
- Broken into peptides lysozyme.
- MHC binds peptide
- Presented to CD4 T cell → immune activation.

# MHC Class II Molecule



## ③ MHC Class III

- MHC Class III has no role in antigen presentation.
- These genes code for several important immune system proteins, including components of the complement system such as C2, C4 & factor B.
- They also encode certain cytokines like tumor necrosis factor (TNF), which play a role in inflammation & immune regulation.
- MHC Class III products are mainly involved in enhancing immune responses rather than directly interacting with T-cells.

# HYPERSENSITIVITY REACTIONS

- Hypersensitivity reactions are exaggerated for inappropriate immune responses that result in tissue damage or discomfort rather than protection.
- These reactions occur when the immune system responds excessively to any substance which is generally harmless (known as Allergen).

## TYPES OF HYPERSENSITIVITY REACTIONS

- According to Coombs & Gell Classification, Hypersensitivity Reactions are classified into 4 types:

① Type - I (Antibody)
② TYPE - II (Cytotoxic)
③ TYPE - III (Immune Response)
④ TYPE - IV (Delayed)



## ① TYPE - I HYPERSENSITIVITY

- Type I hypersensitivity is an immidiate allergic reaction mediated by IgE antibodies.
- It occurs within minutes of exposure to the allergen in sensitized indivisuals.
- Common allergens includes pollen, dust mites, nuts, milk or certain medications.

## MECHANISM

Allergen enters body (Pollen, dust, food)

Stimulates B-Cells → IgE production

IgE binds to Fc receptors on Mast cell & basophils

On Rexposure, Allergen cross links the IgE

Cross linking triggers, degranulation & release of mediators

Hypersensitivity Reactions



## MEDIATORS

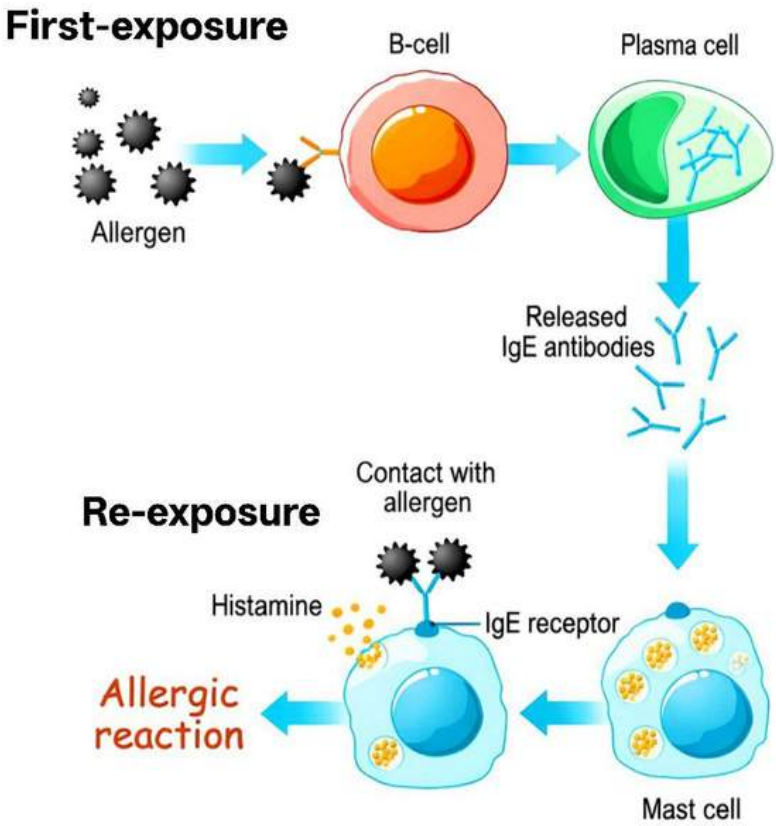
- Histamine
- Leukotriens
- Prostaglandins
- Cytokines

## EFFECTS

- Vasodilation
- Bronchoconstriction
- Increased vascular permeability
- Mucus secretion
- Redness / Swelling.

## EXAMPLES

- Asthma
- Allergic rhinitis
- Urticaria (hives)
- Anaphylaxis (severe, life threatening)



## ② TYPE -II HYPERSENSITIVITY

- Type - II Hypersensitivity reactions are immune responses in which antibodies target antigens present on the surface of cells or tissues, leading to cell damage or destruction.
- These reactions are primarily mediated by IgG or IgM Antibodies.

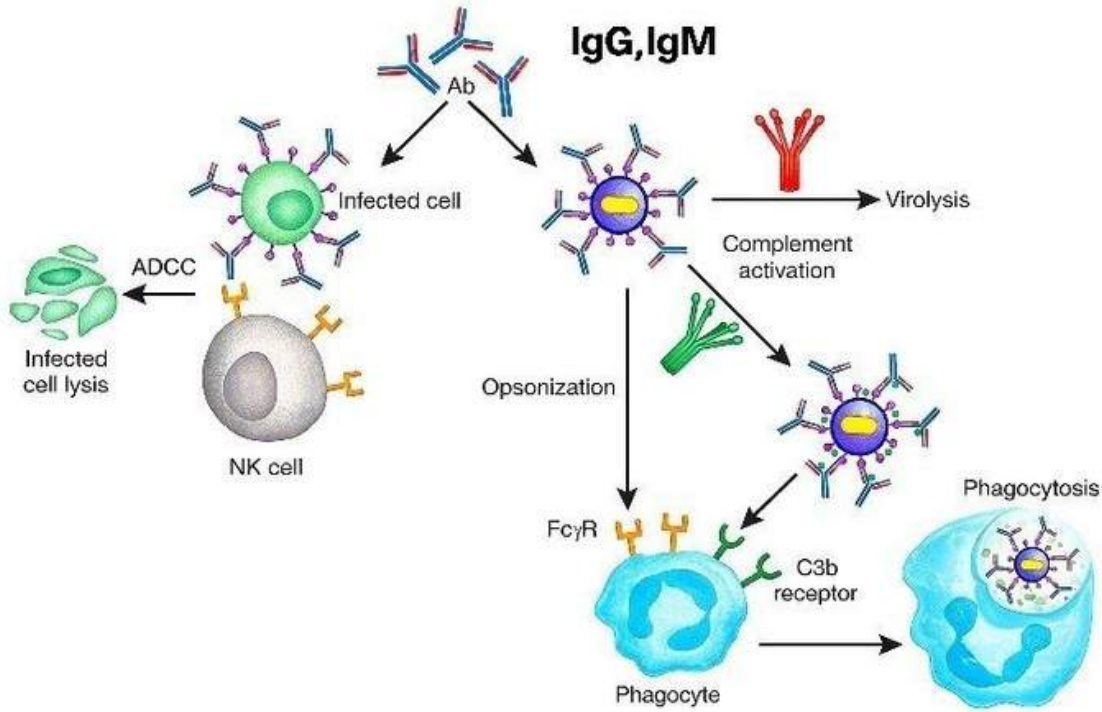
### MECHANISM

- Antigen Presence: Antigens are located on cell membrane (e.g. RBCs, Platelets) or extracellular matrix.
- Antibody binding: IgG or IgM antibodies bind to these antigens.
- Cell destruction occurs via:
  - Complement activation → leads to cell lysis.
  - Opsonization → phagocytes engulf & destroy the cells.
  - Antibody - dependent cellular cytotoxicity (ADCC) → mediated by natural killer (NK) cells.



### EXAMPLES

- Hemolytic Anemia
- Blood transfusion reaction
- Rh incompatability ( Hemolytic disease of newborn)
- Myasthenia gravis ( Ach receptor damage)


IgG,IgM
Ab
Infected cell
ADCC
Infected cell lysis
NK cell
Virolysis
Complement activation
Opsonization
FcγR
C3b receptor
Phagocyte
Phagocytosis

## ③ TYPE III HYPERSENSITIVITY

- Type III hypersensitivity reactions occur when antigen - antibody (immune) complexes form in the circulation & get deposited in tissues, leading to inflammation & tissue injury.
- These reactions are mainly mediated by IgG or IgM antibodies.

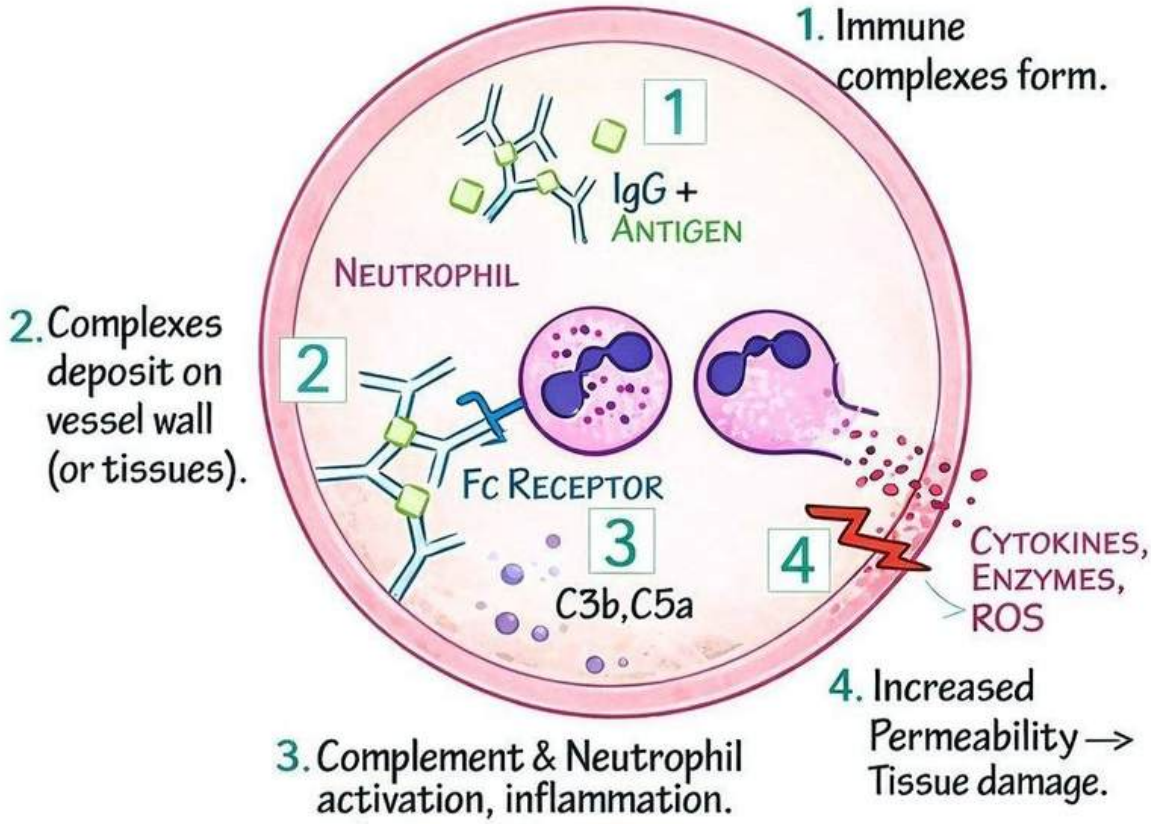
### MECHANISM

- Formation of immune complexes: Soluble antigens binds with IgG antibodies in the blood.
- Deposition in tissues: These complexes deposits in site like blood vessel walls, kidneys, joints & skin.
- Complement Activation: Triggers Inflammation.
- Recruitment of Neutrophils: These cell release enzymes & reactive substances that damage sorrounding tissues.



### EXAMPLE

- Systemic lupus erythematosus (SLE) – immune complexes deposit in multiple organs.
- Post - streptococcal glomerulonephritis – complexes deposit in kidney glomeruli.
- Serum sickness – reaction to foriegn proteins or drugs.
- Arthus reaction – localized skin reaction after antigen injection.


1. Immune complexes form.
1
IgG + ANTIGEN
NEUTROPHIL
2. Complexes deposit on vessel wall (or tissues).
2
FC RECEPTOR
3
C3b,C5a
4
CYTOKINES, ENZYMES, ROS
4. Increased Permeability → Tissue damage.
3. Complement & Neutrophil activation, inflammation.

## ④ Type IV Hypersensitivity

- Type IV Hypersensitivity is an immune reaction mediated by T lymphocytes (not antibodies).
- It is called delayed because the reaction typically appears 24-72 hours after exposure to the antigen.

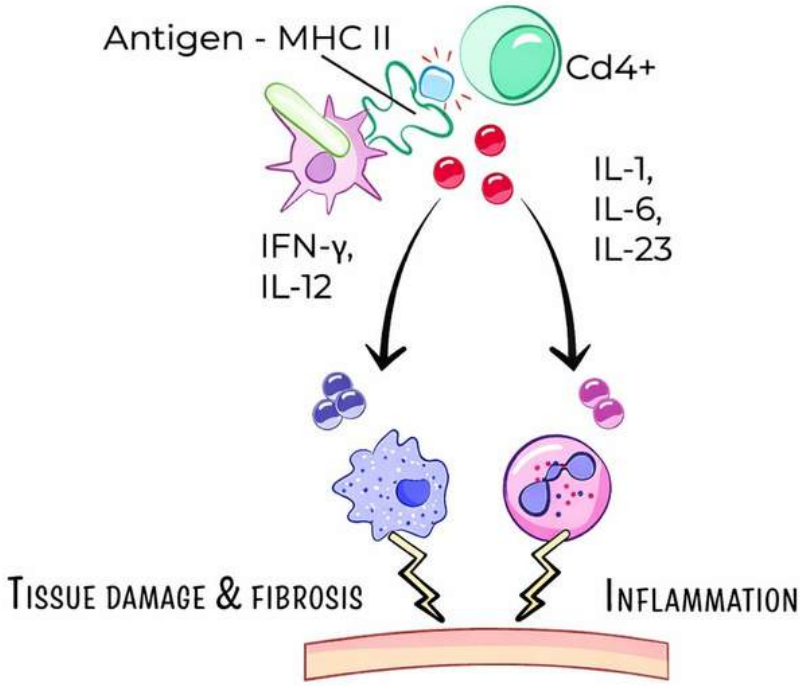
### Mechanism

- Triggered by sensitized T cells (mainly CD4+ Th1 cells & sometimes CD8+ T cells)
- On re-exposure to the antigen:
  - T-cell release cytokines (e.g. interferon-γ)
  - This activates macrophages & Cytotoxic T cells
  - Leads to inflammation & tissue damage.

### Common Examples

- Contact dermatitis (e.g. nickel, poison ivy)
- Chronic transplant rejection
- Certain autoimmune disease (e.g., Type I diabetes)

## Helper T Cell/Cytokine



# IMMUNOSUPPRESSION

- Immunosuppression refers to the reduction or inhibition of the immune response, which may occur naturally or can be induced therapeutically.
- It is mainly used when the immune system becomes overreactive or starts damaging the body's own tissues.

## MECHANISM

- Immunosuppressive agents act by inhibiting activation, proliferation, & function of immune cells such as T lymphocytes & B lymphocytes.
- They reduces cytokine production, antigen presentation, & antibody formation.



## IMMUNOSUPPRESSENT DRUGS

- **Corticosteroids:** Suppress immune response by inhibiting inflammatory mediators & cytokine production.

- **Cyclosporine:** Inhibits calcineurin, thereby preventing activation of T-cells & production of IL-2.

- **Methotrexate:** Interferes with folic acid metabolism & suppress immune cell division.

## Uses

- Immunosuppression is widely used in organ transplantation to prevent graft injection.
- It is also used in autoimmune diseases such as rheumatoid arthritis.
- To control severe allergic or inflammatory responses.

# IMMUNOSTIMULATION

- Immunostimulation refers to the enhancement or activation of the immune response to improve the body's defense mechanisms.
- It is mainly required in conditions where the immune system is weak or suppressed.

## MECHANISM

- Immunostimulants acts as increasing the activity & proliferation of immune cells such as macrophages, lymphocytes & natural killer cells.
- They enhance cytokine production & improve antigen presentation & immune signaling.



## IMMUNOSTIMULATION DRUGS

- **Interferons:** Enhance antiviral activity & activate immune cells.
- **Interleukins:** Stimulate growth, differentiation & activation of immune cells.
- **Vaccination:** Vaccines stimulate antibody & memory cell formation.

## Uses

- Immunostimulants are used in immunodeficiency conditions to strengthen immune response.
- They're used in cancer therapy to enhance the immune system's ability to destroy tumor cells.
- Prevention of infectious diseases through vaccines.

# VACCINES

- A Vaccine is a biological preparation that provides active immunity against a specific disease by stimulating the immune system.
- It contains antigens derived from pathogens in a safe form, which don't cause disease but trigger an immune response.

## MECHANISM

- The antigen is recognized by immune cells & activates B-cells & T-cells.
- Antibodies & memory cells are formed, which provide long-term protection on re-exposure.



## TYPES OF VACCINES

1. Live Attenuated Vaccines
2. Killed / Inactivated Vaccine
3. Toxoid Vaccine
4. Subunit Vaccine
5. Recombinant Vaccine
6. mRNA Vaccine

## 1. LIVE ATTENUATED VACCINES

- These contain weakened live organisms & produce strong, long-term immunity.
- They mimic natural infection but are not suitable for immunocompromised indivisuals.
- example: Measles, BCG Vaccines.

## ② Inactivated / killed Vaccines

- These vaccines contain killed pathogens & are safer but generally produce a weaker immune response.
- They usually required booster doses.
  example: Polio, Rabies.

## ③ Subunit Vaccine

- These vaccine contain specific antigenic parts of the pathogen such as proteins.
- They're safe but may need adjuvants.
  example: Hepatitis B

## ④ Toxoid Vaccines

- These contains inactivated toxins & protect against toxin-mediated diseases.
- example: Tetanus, Diptheria.

## ⑤ mRNA Vaccines

- These contain mRNA that codes for antigen production inside host cells.
- They induce strong immune response & are rapidly developed.
  example: COVID-19 Vaccines.

## ⑥ Recombinant Vaccines

- Recombinant Vaccines are produced using genetic engineering techniques in which genes coding for specific antigens are inserted into host cells to produce the antigen.
- These vaccines contains only the required antigenic protein & not the whole pathogen.
- example: Hepatitis B Vaccines

# General Method Of Vaccine Preparation

Generation of antigen/mRNA/DNA viral-vector

Isolation of antigen/mRNA/DNA viral-vector

Purification of antigen/mRNA/DNA viral-vector

Formulation of Vaccine

Filling, packaging & labeling

Inspection & quality testing

Vaccine formed

## ① Preparation Of Bacterial Vaccines

- Bacterial Vaccines are prepared either from whole microorganisms, or Bacterial toxins.
- They're mainly of 2 types:
  - Live Attenuated Vaccines
  - Inactivated (killed) Vaccines

### Steps Of Preparation

#### ⓐ Selection & Cultivation

- Suitable bacterial strains are selected.
- They're grown on culture media for 1-2 days.
- The culture is then washed with sterile normal saline.

#### ⓑ Preparation Of Suspension

- The bacteria are made into a suspension.
- It is shaken properly to get uniform distribution.

#### ⓒ Removal Of Unwanted Material

- Solid particles & impurities are removed by either:
  - Centrifugation or
  - Sedimentation

## ⓓ Sterilization / Inactivation

- The suspension is sterilized using heat, alcohol or other bactericidal agents
- For Non-sporing bacteria sterilization is done by heating at 56-60°C for about 1 hour.
- Heat treatment should kill microorganisms, but must retain antigenic properties (important for immunity).

## ⓔ Standardization & Dilution

- After sterilization:
  - Bacterial concentration is measured
  - Required dilutions are prepared
  - A preservative (bacteriostatic agent) is added

## ⓕ Filling & Packaging

- After final preparation, Vaccine is filled into:
  - Sterile containers
  - Sealed under aseptic conditions

## ② Preparation Of Viral Vaccines

- Viral vaccines are prepared by using whole viruses or their components in a form that is safe but still capable of inducing an immune response.
- The aim is to retain antigenicity while reducing or eliminating pathogenicity.

## Steps Of Preparation

### ① Selection Of Virus

- The first step is identifying the virus & the specific antigen (usually a surface protein) that can trigger a protective immune response.
- This ensures the vaccine targets the correct part of the virus.

### ② Cultivation Of Virus

- The virus is grown in a suitable host system to obtain large quantities such as:
- - Embryonated chicken eggs (used for influenza vaccines)
  - Cell cultures
  - Animal tissues

## ⓒ Attenuation Or Inactivation

- Depending on vaccine type, the virus is modified:
  - Live attenuated Vaccine: The virus is weakened so it can't cause disease but still duce immunity.
  - Inactivated Vaccines: The virus is killed using chemicals like formaldehyde or by heat.
  - Recombinant Vaccine: Only specific viral proteins are used instead of the whole virus.

## ⓓ Purification

- The viral materials are separated from host cells & impurities using filtration, centrifugation & chromatography.
- This step ensures safety & quality.

## ⓔ Formulation

- The purified product is mixed with stabilizers, preservatives & adjuvants (to enhance immune response)
- This step ensures the vaccine remains effective during storage & transport.

## (F) Quality Control & Testing

- Vaccines undergo strict evaluation for assessment of safety, potency & sterility via clinical & non-clinical trials.

## (g) Production & Distribution

- After approval, vaccines are manufactured on a large scale under strict regulatory standards & distributed with proper cold-chain maintenance.

## ③ Preparation Of Toxoid

- A toxoid is a chemically modified toxin obtained from a pathogenic micro-organisms.
- It is non-toxic, But still antigenic, hence it can be used safely as a vaccine.
- examples: Tetanus toxoid, Diptheria toxoid.

## Method Of Preparation

### ⓐ Formal Toxoid

- A suitable toxin-producing bacterial strain is selected.
- It is growth in a liquid culture medium under optimum conditions.
- The bacterial cells are removed using filtration.
- The filtrate containing toxin is obtained & sterilized.
- Formaldehyde (formalin) is added to the toxin solution.
- This mixture is incubated at ~37°C for several weeks.
- This converts toxin → toxoid (non-toxic but antigenic).



### ⓑ Alum Precipitated Toxoid (APT)

- The toxoid soln is first purified (e.g. Charcoal removal by filtration).
- Alum (aluminium salts) is added in suitable concentration.
- Alum reacts with impurities & forms a precipitate.
- The toxoid gets absorbed on this precipitate.
- The precipitate is washed & suspended in bactericide-containing saline.
- The product is obtained in the form of depot.

## © Purified Toxoid Aluminium Phosphate (PTAP)

- It is highly pure toxoid prepared using semi-synthetic medium (free from unwanted proteins).
- Toxoid is further purified using agents like Magnesium hydroxide & then further combined with Aluminium phosphate gel.
- It produces prolonged immune response & depot effect like APT.

## ④ Preparation Of Antitoxin

- Antitoxins are antibodies produced against bacterial toxins.
- They're used for passive immunity (immediate protection).
- They're usually prepared from animals like horses.
- examples: Tetanus antitoxin, Diptheria antitoxin.

## Method Of Preparation

### ⓐ Selection Of Animal

- Large animals (commonly horses) are selected because:
  - The produce large amount of antibodies.
  - The can tolerate toxin doses

### ⓑ Immunization Of Animal

- Animal is injected with small doses of toxin or toxoid.
- Dose is generally increased over time to stimulate the production.
- As animal's immune system responds, it produces high level of specific antibodies.

### ⓒ Collection Of Blood

- After sufficient antibody formation:
  - Blood is collected from the animal under sterile conditions.

## ⓓ Separation & Purification

- Blood is processed to separate the serum, which contains the antitoxin (antibodies)
- Serum is purified, to remove:
  - Unwanted Proteins
  - Impurities.

## ⓔ Standardization

- The antitoxin is tested & adjusted to proper potency.
- This ensures correct therapeutic dose.

## ⓕ Filling & Storage

- Final product is filled into sterile vials & stored under proper conditions.

## ⑤ PREPARATION OF SERUM

- Serum is the clear fluid obtained after blood clotting & contains antibodies but no clotting factors.
- It is mainly used for passive immunity & therapeutic purposes.

## METHOD OF PREPARATION

- A healthy donor (human or animal like horse), is selected & immunized with a specific antigen or toxin.
- Booster doses are given to increase antibody production in the donor's blood.
- Blood is collected under sterile conditions after sufficient antibody formation.
- The collected blood is allowed to clot, & serum is separated by centrifugation or decantation.
- The serum is purified & tested for safety, sterility & potency.
- Finally, it is preserved & storend under proper conditions for use.

# STORAGE & STABILITY OF VACCINES

- Vaccines are biological products that are highly sensitive to environmental conditions.
- Maintaining their stability (ability of a vaccine to retain its potency during storge) is critical to ensuring they remain safe & effective for the recipient.

## STORAGE CONDITIONS

### ① Cold Chain Maintenance

- Vaccines must be stored & transported at 2°C - 8°C continuosly.
- Cold chain ensures vaccines remain potent from manufacture to administration.

### ② Temperature Requirements

- For most vaccines: 2°C to 8°C
- Oral Polio Vaccine (OPV): -15°C to -25°C (for long-term storage)
- DPT, Hepatitis B, Tetanus toxoid: must not be frozen

### ③ Storage Equipment

- Ice-lined refrigerators (ILR)
- Deep freezers
- Cold boxes & vaccine carriers

### ④ Protection from light

- Vaccines like BCG & measles are light sensitive.
- They should be stored in dark conditions or original packaging.

### ⑤ Transportation

- Vaccines should be transported using cold boxes or vaccine carriers.
- Required temperature must be maintained during transport.

## STABILITY OF VACCINES

### ① Factors Affecting Stability

- Heat: decreases potency
- Freezing: damages adsorbed vaccines
- Light: inactivates live vaccines

### ② Lyophillized Vaccines

- These vaccines are more stable.
- They require reconstitution before use.
- Must be used within 4-6 hours after reconstitution.

### ③ Monitoring Of Stability

- Vaccine Vial Monitor (VVM) indicates heat exposure.
- Regular temperature monitoring is essential.

# HYBRIDOMA TECHNOLOGY

- Hybridoma Technology was first discovered by G. kohler & C. milstein in 1975.
- Hybridoma Technology is a method used to produce monoclonal antibodies (mAbs) by fusing antibody producing B-lymphocytes with immortal myeloma cells to form hybrid cells (hybridomas).
- It is a method to produce large number of identical Antibodies.

## PRINCIPLE

- The technique is based on combining two properties:
  - B lymphocytes: Produce specific antibodies but have limited lifespan.
  - Myeloma cells: Immortal but do not produce antibodies
- Fusion results in hybridoma cell that retain antibody specificity & gain immortality.



## STEPS IN HYBRIDOMA TECHNOLOGY

### ① IMMUNIZATION

- A mouse is injected with the target antigen.
- Injections are repeated multiple times at multiple sites.
- This stimulates the immune system to produce specific B-cells.

## ② Isolation of B-Lymphocytes

- The mouse is sacrificed
- Spleen is removed
- B-cells are isolated (rich source of antibody-producing cells).

## ③ Preparation Of Myeloma Cells

- Myeloma (cancerous plasma cells) are cultured.
- These cells:
  - Are HGPRT (Hypoxanthine-Guanine Phosphoribosyltransferase) deficient
- They can't survive in HAT medium.

## ④ Cell fusion

- B-cells & Myeloma cells are fused using PEG (Polyethylene glycol).
- The mixture contains:
  - Free myeloma cells
  - Free lymphocytes
  - Hybridoma Cells
  - Fused lymphocytes
  - Fused mylema cells

## ⑤ Selection Of Hybridomas

- For isolation of Hybridoma cells we use HAT Medium
- HAT = Hypoxanthine + Aminopterin + Thymidine
- In HAT, Aminopterin blocks de novo DNA synthesis, now for multiplication cells must use salvage pathway.
- Now due to this:
  - Myeloma cells: die (HGPRT absent)
  - B Cells: die (short lifespan)
  - Hybridoma: survive (have HGPRT from B cell + immortality from mylema).

## ⑥ Screening

- Hybridomas are tested for desired antibody production, methods using:
  - ELISA
  - RIA
- Only selected clones producing specific antibody are retained.

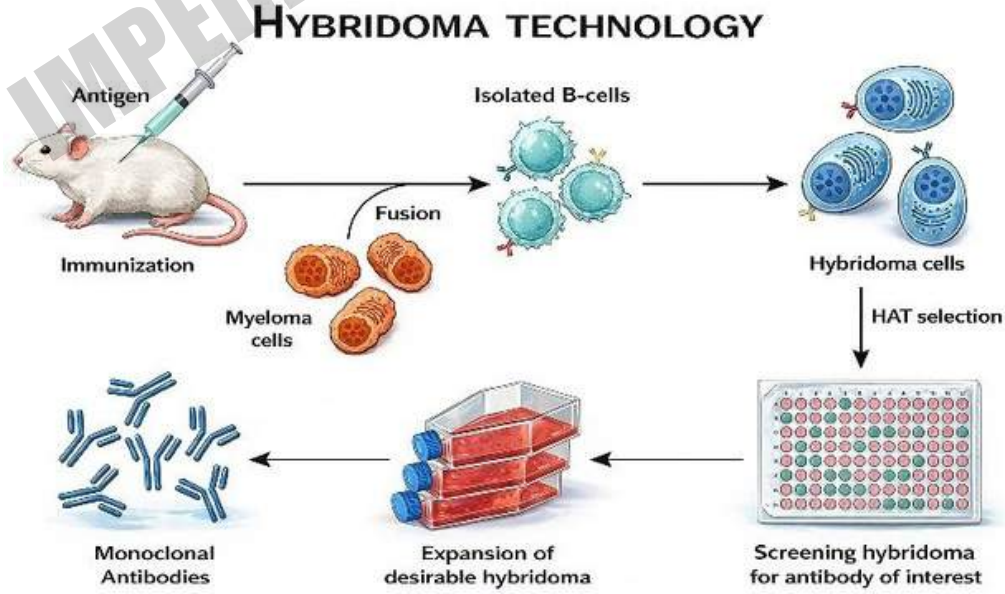
## ⑦ Clonning

- Selected hybridomas are cloned using
  - Limited dilution method
  - Soft Agar Method

## ⑧ Mass Production

- Hybridomas are cultured using bioreactors for large quantities of monoclonal antibodies production.





# PURIFICATION OF MONOCLONAL ANTIBODIES

- After mass production, monoclonal antibodies (mAbs) are obtained in a crude mixture containing:
  - Cell debris
  - Culture medium proteins
  - Serum proteins
  - DNA, lipids & other contaminents

- Purification is required to obtain:
  - High Purity
  - High specificity
  - Safe & stable antibody preparations



## STEPS OF PURIFICATION

### ① CLARIFICATION

- The culture containing Antibodies & other impurities is first collected & processed.
- The cells & debris are removed by centrifugation or filtration.

### ② PRECIPITATION

- Antibodies are precipitated using Ammonium sulfate
- This step increases antibody concentration & removes some impurities.

### ③ Affinity Chromatography

- This is the most important purification step
- Antibodies bind specifically to the column.
- Impurities are washed away first & pure antibodies are then collected.

### ④ Ion Exchange Chromatography

- In this separation occurs based on charge differences.
- Removes remaining unwanted proteins.

### ⑤ Size Exclusion Chromatography

- In this separation occurs based on molecular size
- This steps removes aggregates & small impurities.

### ⑥ Dialysis / Buffer Exchange

- This step removes salts & other chemicals.
- Maintains proper pH & stability

### ⑦ Sterilization

- It is the final filtration using 0.22 µm filter.
- It ensures the antibody preparation is sterile.

## Characteristics Of Monoclonal Antibodies

- They're highly specific & bind to a single epitope of an antigen.
- They're identical antibodies produced from a single clone of B-lymphocytes.
- They've uniform structure & consistent activity.
- They can be produced in large quantities using hybridoma technology.

## Application Of Hybridoma Technology

### ① Diagnostic Applications

- Monoclonal Antibodies are used in ELISA, RIA & rapid diagnostic tests for detection of diseases like:
  - HIV
  - Hepatitis
  - COVID-19
  - Pregnancy test



### ② Therapeutic Applications

- They're widely used as Targeted drug delivery system in the treatment of:
  - Cancer
  - Autoimmune diseases

## ③ Vaccine Development

- They helps in identifying specific antigens (epitopes)
- Used in designing better vaccines
- Supports development of subunit vaccines.

## ④ Purification Of Molecules

- They're used in affinity chromatography to purify:
  - Proteins
  - Hormones
  - Enzymes

# Blood Products

- Blood products are therapeutic components obtained from whole blood by separation & processing.
- They're used to correct specific deficiencies in blood components such as RBCs, platelets, plasma proteins, or clotting factors.

## Types Of Blood Products

### ① Whole Blood

- Whole blood contains all components - RBCs, WBCs, Platelets & Plasma.
- It is mainly used in Acute hemorrhage or Massive blood loss.
- It is rarely used now due to availability of specific components.

### ② Packed Red Blood Cells (PRBCs)

- They're concentrated erythrocytes with most plasma removed.
- They're mainly used in Anemia & acute blood loss
- They increases oxygen - carrying capacity without the risk of circulatory overload.

### ③ Fresh Frozen Plasma (FFP)

- It is Plasma separated & frozen within hours to preserve clotting factors.
- It contains all coagulation factors & plasma proteins.
- It is mainly used in coagulation disorders, Liver disease.

④ PLASMA DERIVATIVES

- They're obtained by fractionation of plasma using industrial processes.
- It includes:

ⓐ Albumin: Maintains colloidal osmotic pressure; used in burns, shock.

ⓑ Immunoglobulins: Provide passive immunity in infections & immunodeficiency.

ⓒ Clotting factor concentrates: Used in hemophilia & other bleeding disorders

# Plasma Substitutes

- Plasma Substitutes are sbl$^{n}$ that are used to restore circulating blood volume in cases of hypovolemia.
- They do not contain blood cells & are incapable of oxygen transport.

## Characteristics Of Ideal Plasma Substitutes

- They should be non-toxic, non-antigenic & free from pathogens.
- They must maintain colloidal osmotic pressure & effectively expand plasma volume.
- They should have an adequate duration of action without rapid elimination.
- They must be stable & easily available
- Should not interfere with blood grouping or coagulation.



## Types Of Plasma Substitutes

### (1) Natural Plasma Expanders

- Human albumin is considered a natural plasma expander obtained from blood plasma.
- It helps in maintaining colloidal osmotic pressure & fluid balance.

## ② Synthetic Plasma Expanders

- Dextran: It is polysaccharide that increases blood volume by retaining water in the circulation & commonly used in shock management.

- Hydroxyethy Starch (HES): It is a synthatic polymer that expands plasma volume for a longer duration & widely used in fluid resuscitation.

- Gelatin Solutions: Such as Haemaccel act as Short-term plasma expanders, They're used in cases of mild to moderate blood loss.

- Crystalloids: Such as normal saline & Ringer lactate help in fluid replacement but have a shorter duration of action.

# THANK YOU

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