1. (i) Define the following terms (Any four):

- **(a) Extravasation:**
    - Extravasation is the process by which cells, particularly leukocytes (white blood cells), move from the bloodstream through the intact walls of blood vessels into surrounding tissues. This is a critical step in immune responses, allowing immune cells to reach sites of infection or inflammation.
- **(b) Paratope:**
    - A paratope is the specific region on an antibody molecule that directly binds to an antigen. It is also known as the antigen-binding site and is formed by the variable regions of both the heavy and light chains of the antibody.
- **(c) Avidity:**
    - Avidity refers to the overall strength of interaction between a multivalent antibody and a multivalent antigen. It takes into account both the affinity (strength of a single binding site) of individual antibody-antigen bonds and the number of binding sites, leading to a much stronger overall bond compared to individual binding events.
- **(d) Opsonization:**
    - Opsonization is the process by which pathogens or other foreign particles are coated with molecules, such as antibodies (e.g., IgG) or complement proteins (e.g., C3b), that make them more easily recognized and engulfed by phagocytic cells (e.g., macrophages, neutrophils). These coating molecules are called opsonins.
- **(e) Anaphylatoxin:**
    - Anaphylatoxins are peptide fragments, primarily C3a, C4a, and C5a, generated during complement activation. They act as potent mediators of inflammation, inducing smooth muscle contraction, increasing vascular permeability, and causing the degranulation of mast cells and

basophils, leading to the release of histamine and other inflammatory mediators.

1**. (ii) Differentiate between the following (Any three):**

- **(a) Primary and Secondary Immune Response:**
    - **Primary Immune Response:**
        - Occurs upon the first exposure to a specific antigen.
        - Characterized by a lag phase (several days) before antibodies are detected.
        - Produces a relatively low antibody titer, primarily IgM, followed by IgG.
        - Antibody levels decline relatively rapidly.
        - Generation of memory cells occurs.
    - **Secondary Immune Response:**
        - Occurs upon subsequent exposure to the same antigen.
        - Characterized by a shorter lag phase or no lag phase.
        - Produces a much higher antibody titer, predominantly IgG.
        - Antibody levels are sustained for a longer duration.
        - Faster, stronger, and more prolonged response due to the presence of memory B and T cells.
- **(b) Innate and Adaptive Immunity:**
    - **Innate Immunity:**
        - Non-specific defense mechanisms present from birth.
        - Provides immediate, rapid protection against a wide range of pathogens.

- Does not exhibit immunological memory.
- Components include physical barriers (skin, mucous membranes), phagocytic cells (macrophages, neutrophils), natural killer (NK) cells, and complement proteins.

- **Adaptive Immunity:**
  - Specific, acquired defense mechanisms that develop in response to exposure to specific antigens.
  - Provides a slower, more tailored response, but with high specificity and efficiency.
  - Exhibits immunological memory, leading to faster and stronger responses upon re-exposure.
  - Components include lymphocytes (B cells and T cells) and antibodies.

- **(c) Exogenous and Endogenous Antigens:**
  - **Exogenous Antigens:**
    - Antigens that originate from outside the cell and are taken into the cell (e.g., by phagocytosis).
    - Examples include bacteria, bacterial toxins, viruses (extracellular), pollen, and dust.
    - Typically processed by antigen-presenting cells (APCs) and presented on Class II MHC molecules to helper T cells ($CD4^+$ T cells).
  - **Endogenous Antigens:**
    - Antigens that are generated within the cell, often due to viral infection or abnormal protein synthesis (e.g., tumor antigens).
    - Examples include viral proteins synthesized inside an infected cell, or abnormal proteins produced by cancer cells.

        - Typically processed and presented on Class I MHC molecules to cytotoxic T lymphocytes (CTLs or $CD8^+$ T cells).

- **(d) Salk and Sabin Vaccine:**
    - **Salk Vaccine (Inactivated Polio Vaccine - IPV):**
        - Type: Inactivated (killed) vaccine.
        - Administration: Injected.
        - Immunity: Induces systemic immunity (IgG antibodies in the blood) to prevent paralytic poliomyelitis, but does not prevent gut infection or shedding of the virus.
        - Safety: Cannot revert to virulence, very safe.
        - History: Developed by Jonas Salk, introduced in 1955.
    - **Sabin Vaccine (Oral Polio Vaccine - OPV):**
        - Type: Live-attenuated vaccine.
        - Administration: Oral drops.
        - Immunity: Induces both systemic immunity (IgG) and local mucosal immunity (IgA in the gut), preventing infection and shedding, leading to herd immunity through passive immunization via vaccinated individuals.
        - Safety: Very rarely (1 in 2.7 million doses) can revert to a virulent form causing vaccine-associated paralytic poliomyelitis (VAPP).
        - History: Developed by Albert Sabin, introduced in 1961.

1. (iii) Expand the following (Any six):

- **(a) PAMPs:** Pathogen-Associated Molecular Patterns
- **(b) MASP:** MBL-Associated Serine Protease

- **(c) MAC:** Membrane Attack Complex
- **(d) CLIP:** Class II-associated Invariant Chain Peptide
- **(e) DTH:** Delayed-Type Hypersensitivity
- **(f) ADCC:** Antibody-Dependent Cell-mediated Cytotoxicity
- **(g) ISCOM:** Immune Stimulating Complex

1. (iv) Write the contribution of the following:

- **(a) Elie Metchnikoff:**
    - Elie Metchnikoff (1845-1916) is renowned for his groundbreaking work on phagocytosis. He observed and described the process by which certain cells (phagocytes) engulf and digest foreign particles, including bacteria. His discovery of phagocytosis in starfish larvae and later in human white blood cells laid the foundation for understanding cellular immunity and earned him a share of the Nobel Prize in Physiology or Medicine in 1908. He is considered one of the fathers of immunology.

- **(b) Jules Bordet:**
    - Jules Bordet (1870-1961) was a Belgian immunologist and microbiologist who made significant contributions to the understanding of the complement system. He discovered complement (initially called "alexine") and showed that it is a heat-labile component of serum essential for antibody-mediated lysis of bacteria and red blood cells. His work elucidated a crucial non-cellular component of innate and adaptive immunity, for which he received the Nobel Prize in Physiology or Medicine in 1919.

2. (i) Draw the basic structure of Immunoglobulin. Compare the structure and functions of IgA and IgM.

The request to "Draw the basic structure of Immunoglobulin" asks for a diagram. As per the instructions, I cannot create any diagrams.

- **Comparison of IgA and IgM Structure and Functions:**
    - **Immunoglobulin A (IgA):**
        - **Structure:**
            - Can exist as a monomer in serum, but is primarily found as a **dimer** in secretions (e.g., mucus, tears, saliva, breast milk, gastrointestinal fluid).
            - The dimeric form consists of two IgA monomer units linked by a **J chain** (joining chain).
            - Secretory IgA (sIgA) also includes a **secretory component (SC)**, which is a portion of the poly-Ig receptor that facilitates its transport across epithelial cells and protects it from degradation in harsh environments.
            - Each monomer has two heavy chains (alpha, α) and two light chains (kappa, κ or lambda, λ).
        - **Functions:**
            - **Mucosal Immunity:** Primary role in protecting mucous membranes from pathogens (respiratory, gastrointestinal, genitourinary tracts). It prevents pathogen adherence to epithelial surfaces.
            - **Passive Immunity (Breast Milk):** Transferred from mother to infant via breast milk, providing passive immunity to the newborn's digestive and respiratory tracts.
            - **Neutralization:** Can neutralize toxins and viruses.
            - **Agglutination:** Can agglutinate antigens, preventing their spread.
            - Does not typically activate the classical complement pathway, and is generally a weak opsonin.

- **Immunoglobulin M (IgM):**
  - **Structure:**
    - Exists as a **pentamer** in serum, formed by five IgM monomer units linked by a **J chain**.
    - Can also exist as a monomer on the surface of B cells, where it acts as a B cell receptor (BCR).
    - Each monomer has two heavy chains (mu, μ) and two light chains (kappa, κ or lambda, λ).
    - The pentameric form has 10 antigen-binding sites, giving it high avidity.
  - **Functions:**
    - **Primary Immune Response:** It is the first antibody class produced during a primary immune response, providing immediate, short-term protection.
    - **B Cell Receptor:** Monomeric IgM serves as the antigen-binding receptor on the surface of naive B lymphocytes, playing a crucial role in B cell activation.
    - **Potent Complement Activator:** Due to its pentameric structure and multiple binding sites, IgM is the most efficient activator of the classical complement pathway, leading to robust lysis of pathogens.
    - **Agglutination:** Highly effective at agglutinating (clumping) antigens due to its multivalency, which facilitates clearance by phagocytes.
    - **Neutralization:** Can neutralize toxins and viruses.
    - Does not cross the placenta.

2. (ii) Explain the experiments on the basis of which Immunoglobulin structure was deduced.

- The basic structure of immunoglobulin (antibody) was primarily deduced through a series of elegant biochemical and biophysical experiments conducted by several scientists in the 1950s and 1960s, notably Gerald Edelman and Rodney Porter, who shared the Nobel Prize in 1972 for their discoveries concerning the chemical structure of antibodies.
    - **1. Rodney Porter's Proteolytic Cleavage Experiments (Papain and Pepsin Digestion):**
        - **Papain Digestion:** Porter treated rabbit IgG with the enzyme papain.
            - **Observation:** Papain cleaved IgG into three fragments of approximately equal size.
            - **Analysis:**
                - Two identical fragments retained antigen-binding ability (Fab - Fragment antigen-binding). These fragments could bind antigen but could not precipitate it.
                - One fragment was found to crystallize easily (Fc - Fragment crystallizable) and lacked antigen-binding ability. This Fc fragment was responsible for mediating effector functions, such as binding to complement or to Fc receptors on cells.
            - **Conclusion:** This experiment demonstrated that the antibody molecule has distinct regions for antigen binding and effector functions, suggesting a symmetric Y-shaped structure.
        - **Pepsin Digestion:** Porter also treated IgG with the enzyme pepsin.

- **Observation:** Pepsin cleaved IgG into one large fragment and several smaller fragments.
- **Analysis:** The large fragment, designated $F(ab')_2$, consisted of two Fab-like portions linked together. This fragment retained both antigen-binding capacity and the ability to cross-link and precipitate antigens. The Fc portion was degraded into smaller peptides.
- **Conclusion:** This indicated that the two antigen-binding arms are connected and suggested the presence of a hinge region susceptible to enzymatic cleavage. The $F(ab')_2$ fragment showed that the two Fab regions are covalently linked.

- **2. Gerald Edelman's Reduction and Alkylation Experiments:**
  - Edelman performed experiments on human IgG using reducing agents (like β-mercaptoethanol) and alkylating agents (like iodoacetamide).
    - **Observation:** When IgG was treated with reducing agents, it dissociated into four polypeptide chains. When these chains were then treated with an alkylating agent to prevent re-formation of disulfide bonds, they remained separate.
    - **Analysis:** Two of these chains were larger (heavy chains, ~50 kDa), and two were smaller (light chains, ~25 kDa). The heavy and light chains were present in equal molar ratios (2:2).
    - **Conclusion:** This demonstrated that the immunoglobulin molecule is composed of four polypeptide chains held together by disulfide bonds. It suggested a basic structure of two identical heavy chains and two identical light chains.

- **3. Studies on Myeloma Proteins (Bence-Jones Proteins):**
    - The availability of purified monoclonal antibodies from patients with multiple myeloma (a cancer of plasma cells) and the detection of Bence-Jones proteins (free light chains) in the urine of these patients provided pure, homogeneous preparations of immunoglobulin chains for detailed analysis.
    - **Analysis:** Chemical sequencing of these proteins confirmed the existence of distinct heavy and light chains and revealed **variable (V)** and **constant (C)** regions within these chains. The variable regions were found at the amino-terminal ends and were responsible for antigen specificity, while the constant regions mediated effector functions.
- **4. Disulfide Bond Mapping:**
    - Further biochemical analysis involved mapping the disulfide bonds within and between the polypeptide chains. This confirmed that disulfide bonds link the heavy chains to each other and heavy chains to light chains, forming the characteristic Y-shaped structure with distinct domains.

- **Overall Conclusion:** These collective experiments, particularly the elegant enzymatic digestion and reduction/alkylation studies, precisely defined the four-chain, Y-shaped structure of the immunoglobulin molecule, with its distinct domains for antigen binding (Fab) and effector functions (Fc), and established the presence of variable and constant regions crucial for antibody diversity and function.

3. (i) Explain the cytosolic pathway for processing of endogenous antigens.

- The cytosolic pathway (also known as the MHC Class I pathway or Endogenous Pathway) is a crucial mechanism for presenting intracellular antigens, such as those derived from viral infections or tumor cells, to cytotoxic T lymphocytes ($CD8^+$ T cells). This pathway allows the immune system to detect and eliminate infected or cancerous cells.

- **1. Synthesis of Endogenous Antigens:**
  - Proteins that are synthesized within the cell's cytoplasm, including viral proteins (in infected cells) or mutated/abnormal proteins (in tumor cells), are targeted for degradation.
  - These proteins are often tagged with **ubiquitin** molecules, marking them for destruction.
- **2. Proteasomal Degradation:**
  - The ubiquitinated proteins are delivered to the **proteasome**, a large, multi-subunit protease complex located in the cytoplasm.
  - The proteasome degrades these proteins into small peptide fragments, typically 8-10 amino acids in length, which are the optimal size to fit into the groove of MHC Class I molecules.
- **3. Transport of Peptides into the Endoplasmic Reticulum (ER):**
  - The newly generated peptide fragments are transported from the cytoplasm into the lumen of the **endoplasmic reticulum (ER)**.
  - This transport is mediated by a specialized transporter protein called **Transporter Associated with Antigen Processing (TAP)**. TAP is a heterodimer composed of TAP1 and TAP2, and it uses ATP to actively pump peptides into the ER. TAP shows a preference for peptides of 8-16 amino acids with hydrophobic or basic C-terminal residues.
- **4. MHC Class I Molecule Assembly and Peptide Loading:**
  - Inside the ER, nascent **MHC Class I molecules** are being synthesized. An MHC Class I molecule consists of two main components: an α (heavy) chain and a β_2-microglobulin chain.
  - Proper folding and assembly of the MHC Class I molecule are facilitated by chaperone proteins, including **calnexin** and **calreticulin**.

- Initially, the MHC Class I heavy chain associates with calnexin. Once β_2-microglobulin binds, calnexin dissociates, and the complex associates with calreticulin and **tapasin**.
- **Tapasin** is a crucial bridging protein that links the MHC Class I molecule to TAP, bringing the peptide-loading complex closer to the source of peptides.
- As peptides are pumped into the ER by TAP, they bind to the empty peptide-binding groove of the waiting MHC Class I molecule.
- A peptide-editing process can occur, where low-affinity peptides are released and replaced by higher-affinity peptides.

- **5. Export to the Cell Surface:**
  - Once a stable MHC Class I-peptide complex is formed, it disengages from the chaperone proteins and the TAP complex.
  - The MHC Class I-peptide complex then exits the ER, travels through the Golgi apparatus, and is ultimately transported to the **cell surface**, where it is displayed on the plasma membrane.
- **6. T Cell Recognition:**
  - On the cell surface, the MHC Class I-peptide complex is recognized by the T cell receptor (TCR) of **cytotoxic T lymphocytes ($CD8^+$ T cells)**.
  - The $CD8$ co-receptor on the CTL simultaneously binds to the $\alpha 3$ domain of the MHC Class I molecule.
  - If the $CD8^+$ T cell recognizes the peptide presented in the MHC Class I molecule as foreign (e.g., viral or tumor-derived), it becomes activated and can then differentiate into an effector CTL, which is capable of killing the antigen-presenting cell.

3. (ii) What is complement system. Explain Classical pathway of complement activation.

- **Complement System:**
    - The complement system is a crucial part of the innate immune system, comprising over 30 soluble proteins (proteases and regulatory proteins) that circulate in the blood and tissue fluids in an inactive pro-enzyme (zymogen) form. When activated, these proteins undergo a cascade of proteolytic cleavages, leading to the formation of effector molecules that play a vital role in host defense against pathogens and in inflammatory responses. The name "complement" refers to its ability to "complement" the action of antibodies in destroying microbes.
- **Classical Pathway of Complement Activation:**
    - The Classical Pathway is typically initiated by the binding of antibodies (primarily IgM or IgG) to an antigen on the surface of a pathogen or foreign cell, though it can also be activated by certain pathogen surfaces directly.
    - **1. Initiation (C1 Activation):**
        - The pathway begins with the binding of the **C1 complex** to an antigen-antibody complex. The C1 complex consists of three subcomponents: C1q, C1r, and C1s (C1qrs).
        - **C1q** is the recognition subcomponent. It has six globular heads that bind to the Fc (Fragment crystallizable) regions of antibody molecules that have bound to an antigen.
        - **IgM:** Due to its pentameric structure, one IgM molecule bound to an antigen is usually sufficient to activate C1.
        - **IgG:** At least two IgG molecules bound in close proximity on the antigen surface are required to activate C1.
        - Upon binding to the antibody-antigen complex, C1q undergoes a conformational change, which activates the associated **C1r** molecules. Activated C1r (C1r esterase) then cleaves and activates **C1s**. The activated C1s is a serine protease.

- **2. C4 and C2 Cleavage (Formation of C3 Convertase):**
  - Activated C1s (C1s esterase) then acts on two other complement components: **C4** and **C2**.
  - C1s cleaves **C4** into two fragments: a large fragment, **C4b**, and a small fragment, C4a. C4b has a reactive thioester bond and quickly binds covalently to the pathogen surface.
  - C1s also cleaves **C2** into two fragments: a large fragment, **C2a**, and a small fragment, C2b.
  - **C2a** then binds to the C4b fragment already attached to the pathogen surface. This complex, **C4b2a**, is the **C3 convertase** of the classical pathway.
- **3. C3 Cleavage and Amplification:**
  - The C3 convertase (C4b2a) is highly enzymatic and cleaves numerous molecules of **C3** into two fragments: a large fragment, **C3b**, and a small fragment, **C3a**.
  - **C3b** has a highly reactive thioester bond and can either:
    - Covalently bind to the pathogen surface (acting as an opsonin to promote phagocytosis).
    - Bind to the existing C4b2a complex to form the C5 convertase.
  - **C3a** is released into the fluid phase and acts as an anaphylatoxin, promoting inflammation.
  - This step is a major amplification point in the complement cascade, as one C3 convertase can generate many C3b molecules.
- **4. C5 Convertase Formation:**

- When C3b binds to the C4b2a complex on the pathogen surface, it forms the **C4b2a3b complex**, which is the **C5 convertase** of the classical pathway.

- **5. Membrane Attack Complex (MAC) Formation:**
  - The C5 convertase (C4b2a3b) cleaves **C5** into **C5b** and C5a.
  - **C5b** then initiates the formation of the Membrane Attack Complex (MAC). C5b sequentially binds C6, C7, C8, and multiple molecules of C9 (typically C9n, where n is 10-16).
  - C6 and C7 bind to C5b and facilitate its insertion into the lipid bilayer of the pathogen membrane.
  - C8 then inserts into the membrane, followed by the polymerization of multiple C9 molecules around C5b678.
  - This creates a transmembrane pore (a donut-shaped channel) in the pathogen's cell membrane.

- **6. Cell Lysis:**
  - The formation of the MAC pore disrupts the osmotic balance of the pathogen cell, leading to an influx of water and ions, swelling, and ultimately **lysis** (rupture) of the target cell.

4. (i) Describe Gell and Coomb's classification of hypersensitivity.

- Gell and Coombs' classification categorizes hypersensitivity reactions into four main types (Type I, II, III, and IV) based on the immunological mechanisms involved, the time course of the reaction, and the effector molecules and cells. This classification helps in understanding and diagnosing various allergic and autoimmune disorders.

- **Type I: Immediate Hypersensitivity (Anaphylactic Type)**
  - **Mechanism:** Mediated by **IgE antibodies** and the release of mediators from mast cells and basophils.

- **Process:** Upon first exposure to an allergen, B cells are activated to produce IgE. IgE then binds to high-affinity Fc receptors ($Fc\epsilon RI$) on the surface of mast cells and basophils. Upon subsequent exposure to the same allergen, the allergen cross-links adjacent IgE molecules on the sensitized mast cells/basophils, triggering their degranulation.
- **Mediators:** Histamine, leukotrienes, prostaglandins, tryptase, cytokines, etc.
- **Onset:** Rapid, within minutes of exposure (immediate).
- **Examples:** Allergic rhinitis (hay fever), allergic asthma, atopic dermatitis, food allergies, systemic anaphylaxis (e.g., to bee stings, penicillin).

- **Type II: Antibody-Mediated (Cytotoxic) Hypersensitivity**
  - **Mechanism:** Mediated by **IgG or IgM antibodies** directed against antigens on the surface of cells or in extracellular matrix components. The binding of these antibodies leads to cell lysis or dysfunction.
  - **Processes:**
    - **Complement-mediated lysis:** Antibody binding activates the classical complement pathway, leading to MAC formation and cell lysis.
    - **Opsonization and Phagocytosis:** Antibody and/or complement (C3b) coating leads to opsonization and subsequent phagocytosis by macrophages and neutrophils.
    - **Antibody-Dependent Cell-mediated Cytotoxicity (ADCC):** Antibodies coat target cells, and the Fc portion is recognized by Fc receptors on NK cells or other effector cells, leading to target cell lysis.

- **Receptor modulation:** Antibodies bind to and either stimulate or block normal cellular receptor function (without cell destruction).

- **Onset:** Hours to days.
- **Examples:**
  - Transfusion reactions (e.g., ABO incompatibility).
  - Hemolytic disease of the newborn (Rh incompatibility).
  - Autoimmune hemolytic anemia.
  - Goodpasture's syndrome (antibodies against basement membrane in kidney and lung).
  - Graves' disease (stimulating antibodies against TSH receptor).
  - Myasthenia gravis (blocking antibodies against acetylcholine receptors).

- **Type III: Immune Complex-Mediated Hypersensitivity**
  - **Mechanism:** Mediated by the formation of **soluble antigen-antibody (IgG or IgM) immune complexes** that deposit in tissues, leading to inflammation and tissue damage.
  - **Process:** When there is an excess of antigen relative to antibody, small-to-medium-sized immune complexes are formed. These complexes are not efficiently cleared by phagocytes and become deposited in various tissues (e.g., blood vessel walls, glomeruli of kidneys, joint capsules). The deposited immune complexes activate complement (classical pathway) and recruit inflammatory cells (neutrophils), leading to the release of lysosomal enzymes and reactive oxygen species, causing tissue damage.
  - **Onset:** Hours to days.

- **Examples:**
    - Systemic lupus erythematosus (SLE).
    - Rheumatoid arthritis.
    - Serum sickness (reaction to foreign serum proteins).
    - Arthus reaction (localized cutaneous reaction).
    - Post-streptococcal glomerulonephritis.

- **Type IV: Delayed-Type Hypersensitivity (Cell-Mediated Hypersensitivity)**
    - **Mechanism:** Mediated by **T lymphocytes** (especially $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells), not antibodies.
    - **Process:**
        - **Sensitization Phase:** Initial exposure to an antigen leads to the activation and proliferation of antigen-specific T cells (e.g., T_H1 cells, T_C cells).
        - **Effector Phase:** Upon re-exposure, the sensitized T_H1 cells recognize the antigen presented by APCs, become activated, and release cytokines (e.g., IFN-γ, TNF-β). These cytokines recruit and activate macrophages and other inflammatory cells, leading to a localized inflammatory response and tissue damage. $CD8^+$ T cells can also directly kill target cells presenting the antigen.
    - **Onset:** Delayed, typically 24-72 hours after antigen exposure.
    - **Examples:**
        - Contact dermatitis (e.g., to poison ivy, nickel).
        - Tuberculin skin test (PPD test for tuberculosis exposure).
        - Granuloma formation (e.g., in tuberculosis, sarcoidosis).

- Graft rejection.

4. (ii) What are the cardinal features of adaptive immunity.

- Adaptive (acquired or specific) immunity is a highly specialized and powerful arm of the immune system that develops in response to exposure to specific pathogens or antigens. It possesses several key characteristics that distinguish it from innate immunity and enable it to provide highly effective and long-lasting protection. The cardinal features include:
    - **1. Specificity:**
        - Adaptive immunity is highly specific, meaning that individual lymphocytes (B cells and T cells) and the antibodies they produce recognize and respond to unique, distinct antigenic determinants (epitopes).
        - For example, an antibody generated against the measles virus will not effectively bind to the influenza virus. This ensures a precise and targeted response against the invading pathogen.
    - **2. Diversity:**
        - The adaptive immune system has the remarkable ability to recognize an enormous repertoire of diverse antigens (estimated to be 10^7 to 10^9 different specificities) that have never been encountered before.
        - This vast diversity is generated through genetic recombination mechanisms (V(D)J recombination) in the genes encoding T cell receptors (TCRs) and immunoglobulin (antibody) molecules, leading to a unique antigen-binding site on each lymphocyte clone.
    - **3. Memory (Immunological Memory):**
        - One of the most defining features of adaptive immunity is its ability to "remember" previous encounters with specific antigens.

- After the initial (primary) exposure and response, specialized **memory cells** (memory B cells and memory T cells) are generated and persist in the body for long periods, sometimes for life.
- Upon subsequent (secondary) exposure to the same antigen, these memory cells enable a much faster, stronger, and more prolonged immune response, leading to rapid elimination of the pathogen and often preventing disease symptoms (immunity).

- **4. Clonal Expansion:**
  - When a specific antigen is encountered, only those lymphocytes (T cells or B cells) that have receptors capable of recognizing that antigen are activated.
  - These activated lymphocytes then undergo rapid proliferation, generating a large number of genetically identical progeny (clones) that are all specific for the same antigen. This process, called **clonal expansion**, ensures that there are enough effector cells to combat the infection effectively.

- **5. Self-Limitation/Contraction:**
  - Once the infection is cleared and the antigen is eliminated, the majority of the effector lymphocytes (those that expanded during the response) undergo apoptosis (programmed cell death).
  - This contraction phase prevents the immune response from becoming excessive and causing damage to host tissues. A small population of memory cells persists, ensuring long-term protection.
- **6. Self-Tolerance (Discrimination of Self from Non-Self):**
  - The adaptive immune system is normally able to distinguish between "self" components (host's own molecules and cells) and "non-self" components (pathogens, foreign molecules).

- Mechanisms like central tolerance (deletion or inactivation of self-reactive lymphocytes during development in primary lymphoid organs) and peripheral tolerance (mechanisms in secondary lymphoid organs and tissues) ensure that the immune system does not mount destructive responses against its own healthy tissues, thereby preventing autoimmune diseases. Failure of self-tolerance leads to autoimmunity.

5. (i) Give an account of different kinds of vaccines.

- Vaccines are biological preparations that provide active acquired immunity to a particular infectious disease. They work by introducing an antigen into the body in a way that stimulates an immune response without causing the actual disease, thus preparing the immune system to recognize and rapidly combat future encounters with the pathogen. Different types of vaccines are developed based on the nature of the antigen used and the method of presentation to the immune system.

  - **1. Live-Attenuated Vaccines:**
    - **Description:** Contain a weakened (attenuated) form of the live pathogen (virus or bacteria) that has lost its virulence but retains its ability to replicate within the host.
    - **Mechanism:** The attenuated pathogen replicates in the vaccinated individual, mimicking a natural infection and inducing a strong, long-lasting immune response, including both humoral (antibody) and cell-mediated (T cell) immunity.
    - **Advantages:** Strong, long-lasting immunity, often requiring only one or two doses. Induces mucosal immunity if given orally.
    - **Disadvantages:** Potential for reversion to virulence (rare), not suitable for immunocompromised individuals or pregnant women. Requires cold chain storage.

- **Examples:** Measles, Mumps, Rubella (MMR), Oral Polio Vaccine (OPV), Varicella (chickenpox), Yellow Fever.

- **2. Inactivated (Killed) Vaccines:**
  - **Description:** Contain whole pathogens (viruses or bacteria) that have been inactivated or killed by heat, chemicals (e.g., formalin), or radiation, rendering them unable to replicate or cause disease.
  - **Mechanism:** The inactivated pathogen presents its antigens to the immune system, primarily stimulating a humoral (antibody) response.
  - **Advantages:** Cannot revert to virulence, generally safer for immunocompromised individuals.
  - **Disadvantages:** Weaker immune response compared to live vaccines, often requiring multiple doses (boosters) to achieve and maintain protective immunity. Primarily induces humoral immunity, less effective at stimulating cell-mediated immunity.
  - **Examples:** Inactivated Polio Vaccine (IPV - Salk vaccine), Hepatitis A, Rabies, most influenza vaccines.
- **3. Subunit Vaccines:**
  - **Description:** Contain only specific purified antigenic components (e.g., proteins, polysaccharides) of the pathogen that are essential for inducing a protective immune response, rather than the whole pathogen.
  - **Mechanism:** The purified antigens directly stimulate a humoral immune response.
  - **Advantages:** Very safe as they contain no genetic material from the pathogen and cannot cause disease.

- **Disadvantages:** Often require adjuvants (substances that enhance the immune response) and multiple booster doses. Primarily humoral immunity.
- **Types of Subunit Vaccines:**
    - **Protein Subunit Vaccines:** Contain purified proteins from the pathogen.
        - *Examples:* Hepatitis B (recombinant HBsAg), Acellular Pertussis (aP), HPV (VLP - Virus-like particle).
    - **Polysaccharide Vaccines:** Contain purified capsular polysaccharides from bacteria.
        - *Examples:* Pneumococcal polysaccharide vaccine (PPSV23 - for adults), Meningococcal polysaccharide vaccine.
    - **Conjugate Vaccines:** Polysaccharide antigens are conjugated (covalently linked) to a protein carrier. This makes the polysaccharide antigen T-dependent, allowing for a stronger immune response, T cell help, and immunological memory, especially in infants.
        - *Examples:* Haemophilus influenzae type b (Hib), Pneumococcal conjugate vaccine (PCV13), Meningococcal conjugate vaccine.
    - **Toxoid Vaccines:** Contain inactivated bacterial toxins (toxoids) that have been modified to be non-toxic but retain their antigenicity. They target diseases where bacterial toxins are the primary cause of pathology.
        - *Examples:* Diphtheria, Tetanus.

- **4. Viral Vector Vaccines:**

- **Description:** Use a harmless virus (the vector) to deliver genetic material (DNA or RNA) that codes for a specific antigen from the target pathogen into host cells.
- **Mechanism:** The host cells express the antigen, which then stimulates an immune response (both humoral and cell-mediated). The vector itself does not cause disease.
- **Advantages:** Can mimic natural infection more closely, inducing strong and long-lasting immunity.
- **Disadvantages:** Pre-existing immunity to the vector can reduce vaccine efficacy.
- **Examples:** AstraZeneca and Johnson & Johnson COVID-19 vaccines (using adenovirus vectors), experimental Ebola vaccines.

- **5. Nucleic Acid Vaccines (DNA and mRNA Vaccines):**
  - **Description:** Contain genetic material (DNA plasmid or mRNA) that encodes for a specific antigen of the pathogen.
  - **Mechanism:** The genetic material is delivered into host cells, which then use their own cellular machinery to produce the antigen protein. This protein is then displayed to the immune system, stimulating both antibody and T cell responses.
  - **Advantages:** Rapid development and manufacturing, do not contain live virus, no risk of integration into host genome (mRNA), can induce strong cell-mediated immunity.
  - **Disadvantages:** Newer technology, long-term safety data is still accumulating for some.
  - **Examples:** Pfizer-BioNTech and Moderna COVID-19 vaccines (mRNA).

5. (ii) Describe the structure and function of Class I and Class II MHC.

- Major Histocompatibility Complex (MHC) molecules are a group of genes that encode proteins on the surface of cells that are crucial for antigen presentation to T lymphocytes. They play a central role in both adaptive immunity and self-tolerance. There are two main classes: Class I MHC and Class II MHC.
    - **Class I MHC Molecules:**
        - **Structure:**
            - Composed of two non-covalently associated polypeptide chains:
                - A polymorphic α **(heavy) chain** (~45 kDa) with three extracellular domains (α_1, α_2, α_3), a transmembrane region, and a cytoplasmic tail.
                - A non-polymorphic (invariant) β_2**-microglobulin** chain (~12 kDa) that is non-covalently associated with the α_3 domain and is crucial for the proper folding and surface expression of the α chain.
            - The peptide-binding groove (cleft) is formed by the α_1 and α_2 domains of the heavy chain. This groove is relatively narrow and can typically accommodate peptides of 8-10 amino acids (optimal 9-mer).
            - The α_3 domain is conserved and serves as the binding site for the $CD8$ co-receptor of cytotoxic T lymphocytes (CTLs).
        - **Expression:**
            - Expressed on the surface of **almost all nucleated cells** in the body (except red blood cells, which lack a nucleus). They are also expressed at low levels on some non-nucleated cells and can be induced.
        - **Function:**

- **Presentation of Endogenous Antigens:** Primarily presents peptides derived from **intracellular (endogenous) proteins**, such as viral proteins synthesized during infection or abnormal proteins produced by tumor cells.
- **Activation of Cytotoxic T Lymphocytes ($CD8^+$ T cells):** Presents these peptides to $CD8^+$ T cells. When a $CD8^+$ T cell recognizes a foreign peptide presented by Class I MHC, it becomes activated to become a cytotoxic T lymphocyte, which can then kill the antigen-presenting cell (e.g., infected cell, cancer cell).
- **Immune Surveillance:** Enables the immune system to continuously monitor the internal environment of cells for signs of infection or malignancy.

- **Class II MHC Molecules:**
  - **Structure:**
    - Composed of two non-covalently associated polypeptide chains of approximately equal size:
      - An α **chain** (~33 kDa) with two extracellular domains (α_1, α_2).
      - A β **chain** (~28 kDa) with two extracellular domains (β_1, β_2).
    - Both chains traverse the cell membrane.
    - The peptide-binding groove is formed by the α_1 and β_1 domains. This groove is more open-ended compared to Class I and can typically accommodate longer peptides, ranging from 13-18 amino acids.
    - The β_2 domain is conserved and serves as the binding site for the $CD4$ co-receptor of helper T lymphocytes.

- **Expression:**
  - Primarily expressed on the surface of professional **antigen-presenting cells (APCs)**, including:
    - Macrophages
    - Dendritic cells (the most potent APCs)
    - B lymphocytes
  - Can also be induced on other cell types under inflammatory conditions (e.g., by IFN-γ).
- **Function:**
  - **Presentation of Exogenous Antigens:** Primarily presents peptides derived from **extracellular (exogenous) proteins** that have been internalized by APCs through phagocytosis or endocytosis.
  - **Activation of Helper T Lymphocytes ($CD4^+$ T cells):** Presents these peptides to $CD4^+$ T cells. When a $CD4^+$ T cell recognizes a foreign peptide presented by Class II MHC, it becomes activated and differentiates into a helper T cell.
  - **Coordination of Immune Response:** Activated helper T cells then provide essential "help" (via cytokine secretion) to B cells (for antibody production) and $CD8^+$ T cells (for full activation and differentiation into CTLs), thus orchestrating and amplifying adaptive immune responses.

6. Write short notes on (Any three):

- **(i) Clonal Selection Theory:**
  - The Clonal Selection Theory, proposed by Macfarlane Burnet in 1957, is a fundamental principle in immunology that explains how the

adaptive immune system generates specific and diverse responses to a vast array of antigens, while maintaining self-tolerance and immunological memory.

- **Core Principles:**
  - **Pre-existing Diversity:** Before exposure to any antigen, the body contains a vast repertoire of lymphocytes (B and T cells), each with a unique antigen-specific receptor (BCR or TCR) on its surface. This diversity is generated randomly through genetic recombination events.
  - **Antigen-Specific Activation:** When an antigen enters the body, it "selects" and binds to the small number of lymphocytes that have pre-existing receptors with complementary specificity for that antigen. This binding event, along with co-stimulation, activates the specific lymphocyte clone.
  - **Clonal Expansion:** The activated lymphocyte then undergoes rapid proliferation (mitosis), creating a large population of genetically identical cells, all bearing the same antigen-specific receptor. This "clonal expansion" ensures that there are enough effector cells to combat the pathogen.
  - **Differentiation:** The expanded clone differentiates into effector cells (e.g., plasma cells producing antibodies, cytotoxic T lymphocytes) that directly fight the infection, and long-lived **memory cells**.
  - **Immunological Memory:** The memory cells persist in the body and allow for a faster, stronger, and more efficient secondary immune response upon subsequent encounters with the same antigen.
  - **Self-Tolerance:** Lymphocytes that react strongly to self-antigens during their development are either deleted (clonal deletion) or inactivated (clonal anergy), ensuring that the immune system does not attack the host's own tissues.

- **Significance:** Clonal selection elegantly explains the specificity, diversity, memory, and self-tolerance of adaptive immunity and is a cornerstone of modern immunological understanding, underpinning vaccination strategies.

- **(ii) Properties of Cytokines:**
  - Cytokines are a diverse group of small, soluble protein messengers (non-antibody proteins) produced by virtually all cells involved in innate and adaptive immune responses. They act as intercellular mediators, regulating the intensity and duration of immune responses and inflammation. Their key properties include:
  - **1. Pleiotropy:**
    - A single cytokine can have multiple different effects on various target cell types, depending on the cell's receptors and state of differentiation. For example, IL-4 stimulates B cell proliferation, promotes IgE class switching, and induces differentiation of T_H2 cells.
  - **2. Redundancy:**
    - Different cytokines can have similar or overlapping effects on the same target cell. This provides a backup system and ensures that essential immune functions are maintained even if one cytokine is deficient. For example, IL-2, IL-4, and IL-5 can all induce B cell proliferation.
  - **3. Synergy:**
    - The combined effect of two or more cytokines is greater than the sum of their individual effects. For example, IFN-γ and TNF-α together are more effective at inducing MHC Class I expression than either cytokine alone.
  - **4. Antagonism:**

- The effects of one cytokine can inhibit or oppose the effects of another cytokine. For example, IFN-γ inhibits the proliferation of T_H2 cells, and IL-10 inhibits the production of T_H1 cytokines.

- **5. Cascade Induction:**
  - The action of one cytokine on a target cell can stimulate that cell to produce other cytokines, leading to a cascade amplification of the immune response. For example, activated macrophages produce IL-1, which then stimulates T cells to produce IL-2, and so on.
- **6. Autocrine, Paracrine, and Endocrine Action:**
  - **Autocrine:** Cytokine acts on the cell that secreted it (e.g., IL-2 on activated T cells).
  - **Paracrine:** Cytokine acts on nearby cells (most common mode).
  - **Endocrine:** Cytokine enters the bloodstream and acts on distant target cells (e.g., IL-1 and TNF-α causing fever).
- **7. High Affinity Binding:**
  - Cytokines bind to specific, high-affinity receptors on target cells, ensuring their effectiveness even at very low concentrations.

- **(iii) B cell and T Cell Epitopes:**
  - Epitopes (also called antigenic determinants) are the specific molecular regions on an antigen that are recognized by antigen-specific receptors on lymphocytes (B cells and T cells) or by antibodies. The nature of epitopes differs for B cells and T cells due to the way they recognize antigens.
  - **B Cell Epitopes:**

- **Recognition:** B cells recognize antigens directly via their B cell receptors (BCRs), which are membrane-bound antibodies.
- **Structure:** B cell epitopes are typically located on the surface of the antigen and are usually accessible to the BCR. They are often **conformational epitopes**, meaning their recognition depends on the three-dimensional shape of the folded protein. They can also be linear (sequential) epitopes if the sequence is exposed.
- **Chemical Nature:** Can be proteins, polysaccharides, lipids, or nucleic acids.
- **Size:** Can vary in size but typically involve 6-20 amino acid residues or monosaccharide units.
- **Solubility:** Often present on soluble antigens or on the surface of pathogens.
- **Accessibility:** Must be accessible on the native antigen.

- **T Cell Epitopes:**
  - **Recognition:** T cells do not recognize free antigens directly. Instead, they recognize processed fragments of antigens (peptides) presented within the groove of MHC molecules on the surface of antigen-presenting cells (APCs). This is known as MHC restriction.
  - **Structure:** T cell epitopes are almost always **linear (sequential) peptides**. The antigen must first be degraded (processed) into peptide fragments, and then these peptides are presented by MHC molecules. Therefore, T cell epitopes can be derived from internal parts of a protein that are not accessible on the surface of the native antigen.
  - **Chemical Nature:** Primarily **proteins** (peptides). T cells do not directly recognize polysaccharides or lipids.

- **Size:**
  - For MHC Class I (recognized by $CD8^+$ T cells): Peptides are typically 8-10 amino acids long.
  - For MHC Class II (recognized by $CD4^+$ T cells): Peptides are typically 13-18 amino acids long.
- **Location:** Can be derived from any part of the protein, including buried regions, as long as they can be processed and presented.

- **Summary:** The key difference lies in the form of antigen recognized: B cells recognize intact, often conformational, antigens, while T cells recognize processed linear peptide fragments presented by MHC molecules.

- **(iv) Autoimmunity:**

  - Autoimmunity is a condition in which the immune system, which is normally responsible for protecting the body from foreign invaders, mistakenly attacks the body's own healthy tissues, cells, or organs. This breakdown of **self-tolerance** leads to the development of autoimmune diseases.
  - **Mechanisms of Breakdown in Self-Tolerance:**
    - **Molecular Mimicry:** A pathogen antigen may share structural similarities with a self-antigen. An immune response against the pathogen might then inadvertently cross-react with the self-antigen (e.g., rheumatic fever, where antibodies against streptococcal antigens cross-react with heart tissue).
    - **Bystander Activation:** Tissue damage (e.g., from infection, trauma, toxins) can release normally sequestered self-antigens or alter self-antigens, making them immunogenic. This can lead to the activation of self-reactive lymphocytes that escaped central tolerance.

- **Abnormal MHC Expression:** Upregulation of MHC molecules or expression of MHC molecules on cells that normally do not express them can present self-antigens inappropriately, leading to T cell activation.
- **Defects in Regulatory T cells (T_{reg} cells):** These cells normally suppress self-reactive immune responses. Dysfunction or deficiency of T_{reg} cells can lead to autoimmune disease.
- **Genetic Predisposition:** Many autoimmune diseases have a genetic component, often involving specific MHC (HLA) alleles that influence susceptibility.
- **Environmental Factors:** Infections, toxins, and certain drugs can trigger or exacerbate autoimmune conditions in genetically susceptible individuals.

o **Types of Autoimmune Diseases:**

- **Organ-Specific:** Immune response is directed against antigens unique to a single organ or tissue.
  - *Examples:* Type 1 diabetes (pancreatic β-cells), Grave's disease (thyroid), Hashimoto's thyroiditis (thyroid), Myasthenia gravis (neuromuscular junction), Multiple Sclerosis (myelin sheath).
- **Systemic:** Immune response is directed against widely distributed antigens, affecting multiple organs and tissues.
  - *Examples:* Systemic Lupus Erythematosus (SLE - affects joints, skin, kidneys, brain), Rheumatoid Arthritis (joints), Sjogren's Syndrome (lacrimal and salivary glands).

o **Treatment:** Aims to suppress the overactive immune response and manage symptoms, often involving immunosuppressants,

corticosteroids, or targeted biological therapies that block specific immune mediators or cells.

- **(v) Monoclonal Antibodies and their applications:**
    - **Monoclonal Antibodies (mAbs):**
        - Monoclonal antibodies are highly specific antibodies produced by a single clone of B lymphocytes. Unlike polyclonal antibodies (which are a mixture of antibodies recognizing multiple epitopes on an antigen), mAbs recognize and bind to only a single, specific epitope on an antigen. They are produced using hybridoma technology (Kohler and Milstein, 1975).
        - **Hybridoma Technology:** Involves fusing antibody-producing B lymphocytes (from an immunized animal, typically a mouse) with immortal myeloma (cancer) cells. The resulting hybridoma cells have the desired properties: they produce a single type of antibody (like the B cell) and can grow indefinitely in culture (like the myeloma cell). These hybridoma cells are then cloned, and the best-producing clones are selected to grow large quantities of the specific mAb.
    - **Key Characteristics:**
        - **Specificity:** Bind to a single epitope, ensuring high precision.
        - **Homogeneity:** All antibody molecules are identical in structure and binding characteristics.
        - **Reproducibility:** Can be produced in unlimited quantities.
    - **Applications of Monoclonal Antibodies:**
        - **1. Diagnostics:**
            - **ELISA (Enzyme-Linked Immunosorbent Assay):** Used for highly specific detection and quantification of antigens (e.g., hormones, viral proteins, tumor markers)

or antibodies in patient samples (e.g., HIV testing, pregnancy tests).

- **Immunohistochemistry/Immunofluorescence:** Used to detect specific antigens in tissue sections or cells, aiding in disease diagnosis (e.g., identifying cancer cell markers, infectious agents).
- **Rapid Diagnostic Tests:** Pregnancy tests, rapid strep tests, COVID-19 antigen tests.

- **2. Therapeutics (Biologics):**
  - **Cancer Treatment:**
    - **Naked mAbs:** Block growth factor receptors (e.g., Trastuzumab for HER2+ breast cancer), inhibit angiogenesis (e.g., Bevacizumab), or block immune checkpoints (e.g., Pembrolizumab, Nivolumab for PD-1/PD-L1 in various cancers), allowing T cells to attack cancer.
    - **Conjugated mAbs:** Antibodies linked to toxins (immunotoxins), chemotherapy drugs (antibody-drug conjugates, ADCs), or radioactive isotopes, delivering destructive agents directly to cancer cells.
  - **Autoimmune Diseases & Inflammatory Disorders:** Block pro-inflammatory cytokines (e.g., Infliximab, Adalimumab for TNF-α in rheumatoid arthritis, Crohn's disease) or deplete specific immune cells (e.g., Rituximab for CD20+ B cells in rheumatoid arthritis, lymphoma).
  - **Infectious Diseases:** Neutralize viruses (e.g., Palivizumab for RSV, some COVID-19 mAbs) or bacterial toxins. Used for passive immunization.

- **Transplant Rejection:** Deplete T cells or block T cell activation to prevent rejection of transplanted organs (e.g., Basiliximab).

- **3. Research Tools:**
  - **Western Blotting/Immunoprecipitation:** For specific protein detection and isolation.
  - **Flow Cytometry:** To identify and quantify specific cell populations based on surface markers.
  - **Cell Sorting:** To isolate specific cell types for further study.
  - **Drug Discovery:** As tools to validate drug targets or as lead compounds.