Question 1

a) What is IgM antibody? Discuss the functional importance of IgM. (5 marks)

IgM (Immunoglobulin M) is one of the five major classes of antibodies (immunoglobulins) produced by the immune system. It is the first antibody to be synthesized during a primary immune response and is primarily found as a **pentamer** (five monomeric units joined by a J chain) in the blood and lymph. Each monomer consists of two heavy chains (μ chains) and two light chains, forming a Y-shaped structure.

Functional Importance of IgM:

1. **First Line of Defense**: IgM is the first antibody produced in response to an initial exposure to an antigen. It appears within a few days post-infection and plays a critical role in early pathogen neutralization.
2. **High Valency**: As a pentamer, IgM has **10 antigen-binding sites**, allowing it to bind multiple antigens simultaneously. This high avidity makes it highly effective in agglutinating pathogens (e.g., bacteria, viruses) and forming large immune complexes that are easily cleared by phagocytes.
3. **Complement Activation**: IgM is the most efficient antibody at activating the **classical complement pathway**. After binding to antigens, it undergoes a conformational change that exposes binding sites for the C1q component of complement, leading to complement-mediated lysis of pathogens, opsonization, and inflammation.
4. **Neutralization**: IgM can neutralize pathogens by blocking their attachment to host cells. Although its affinity for individual epitopes may be lower than IgG, its multivalency compensates, making it effective in preventing infection spread.
5. **B Cell Receptor (BCR)**: Monomeric IgM serves as the B cell receptor (BCR) on the surface of naïve B cells. Upon antigen binding, it initiates B cell activation, proliferation, and differentiation into plasma cells that secrete antibodies.
6. **Diagnostic Marker**: Elevated levels of IgM in serum indicate a recent or ongoing infection. Detection of pathogen-specific IgM is used in serological tests (e.g., for dengue, Zika, or COVID-19) to diagnose acute infections.
7. **Autoimmunity and Disease**: Abnormal IgM production can lead to conditions like **Waldenström's macroglobulinemia** (a cancer of B cells) or **rheumatoid factors**

(autoantibodies against IgG) in rheumatoid arthritis.

Thus, IgM is a crucial component of the humoral immune response, providing rapid, broad protection against pathogens and bridging innate and adaptive immunity.

b) Explain the concept of antigens and their classification as foreign or self-antigens. (5 marks)

Antigens (short for **antibody generators**) are substances that can be recognized and bound by the immune system, specifically by antibodies, B cell receptors (BCRs), or T cell receptors (TCRs). An antigen may elicit an immune response, leading to the production of antibodies or activation of T cells. The part of the antigen that is recognized by the immune receptor is called an **epitope** or **antigenic determinant**.

Properties of Antigens:

- **Immunogenicity**: Ability to provoke an immune response.
- **Antigenicity**: Ability to bind specifically to antibodies or TCRs.
- **Size**: Usually large molecules (proteins, polysaccharides) with molecular weight > 10 kDa.
- **Chemical Nature**: Proteins are the strongest immunogens; lipids and nucleic acids are generally poor immunogens unless conjugated to proteins.

Classification of Antigens:

1. **Foreign Antigens (Non-self Antigens):**
   These originate from outside the body and are recognized as “non-self” by the immune system. They include:
   - **Microbial Antigens**: Components of bacteria (cell wall proteins, flagellin), viruses (capsid proteins, envelope glycoproteins), fungi, and parasites.
   - **Environmental Antigens**: Pollen, dust mites, animal dander (common allergens).
   - **Alloantigens**: Antigens from individuals of the same species but genetically different (e.g., blood group antigens, HLA molecules in organ transplantation).
   - **Xenoantigens**: Antigens from different species (e.g., animal serum, snake venom).

Foreign antigens typically trigger an adaptive immune response, leading to antibody production and memory cell formation.

2. **Self-Antigens:**
   These are molecules originating from an individual's own body. Under normal conditions, the immune system is **tolerant** to self-antigens and does not attack them—a state known as **self-tolerance**. Self-antigens include:
   - **Autoantigens**: Normal cellular proteins (e.g., DNA, myelin basic protein, insulin).
   - **Tumor Antigens**: Altered self-proteins expressed by cancer cells (e.g., MART-1 in melanoma).

   Breakdown of self-tolerance leads to **autoimmunity**, where the immune system attacks self-tissues, causing autoimmune diseases (e.g., systemic lupus erythematosus, type 1 diabetes).

Additional Classifications:

- **Complete Antigens**: Can induce an immune response and bind specifically to antibodies.
- **Haptens**: Small molecules that are antigenic but not immunogenic on their own; they require conjugation to a carrier protein to elicit a response (e.g., penicillin, poison ivy urushiol).
- **T-dependent Antigens**: Require T helper cell assistance for B cell activation (usually proteins).
- **T-independent Antigens**: Can activate B cells without T cell help (e.g., polysaccharides with repetitive epitopes like bacterial capsular polysaccharides).

Understanding antigen classification is crucial for vaccine design, allergy management, transplantation, and autoimmune disease therapy.

Question 2

Differentiate between the following pairs of terms: (2½ × 4 = 10 marks)

a) Classical Pathway and Alternative Pathway of complement activation

Feature	Classical Pathway	Alternative Pathway
Trigger	Antigen-antibody complexes (mainly IgG or IgM bound to pathogens).	Direct recognition of pathogen surfaces (e.g., bacterial LPS, yeast cell walls) without antibodies.
Initiating Factor	C1 complex (C1q, C1r, C1s).	Spontaneous hydrolysis of C3 to C3(H_2O) (tickover).
Key Components	C1, C4, C2, C3.	C3, Factor B, Factor D, Properdin.
Convertase Formation	C3 convertase: C4b2a.	C3 convertase: C3bBb.
Regulation	C1 inhibitor (C1INH), C4-binding protein, Decay-accelerating factor (DAF).	Factor H, Factor I, DAF.
Biological Role	Links adaptive immunity (antibodies) to complement activation. Effective against encapsulated bacteria.	Part of innate immunity, provides rapid defense against Gram-negative bacteria, fungi, and parasites.
Evolution	More recent, found only in vertebrates.	Ancient, present in invertebrates and vertebrates.

b) B cell receptor (BCR) and T cell receptor (TCR)

Feature	BCR (B Cell Receptor)	TCR (T Cell Receptor)
Structure	Membrane-bound immunoglobulin (IgM or IgD) associated with Igα/Igβ heterodimer for signaling.	Heterodimer of α and β chains (or γ and δ in γδ T cells) associated with CD3 complex for signaling.
Antigen Recognition	Recognizes **native, conformational epitopes** on	Recognizes **processed peptide fragments**

Feature	BCR (B Cell Receptor)	TCR (T Cell Receptor)
	antigens (proteins, polysaccharides, lipids).	presented by MHC molecules.
Antigen Binding Site	Formed by variable regions of heavy and light chains.	Formed by variable regions of α and β chains.
MHC Restriction	No MHC restriction.	Yes; TCR binds peptide-MHC complex ($CD4^+$ T cells: MHC class II; $CD8^+$ T cells: MHC class I).
Effector Function	BCR activation leads to antibody production, class switching, memory B cell formation.	TCR activation leads to T cell proliferation, cytokine secretion, cytotoxicity ($CD8^+$), or help ($CD4^+$).
Somatic Hypermutation	Undergoes affinity maturation in germinal centers.	Does not undergo somatic hypermutation.

c) Active Immunization and Passive Immunization

Feature	Active Immunization	Passive Immunization
Definition	Administration of antigens to induce an adaptive immune response and memory.	Administration of pre-formed antibodies to provide immediate, short-term protection.
Immune System Involvement	Activates the recipient's own immune system.	No activation of recipient's immune system.
Onset of Protection	Slow (days to weeks) as the immune response develops.	Immediate (within hours).
Duration of Protection	Long-lasting (years to lifetime) due to immunological memory.	Short-lived (weeks to months) as antibodies are degraded.

Feature	Active Immunization	Passive Immunization
Examples	Vaccines (e.g., MMR, polio, COVID-19 mRNA vaccines).	Administration of antitoxins (e.g., tetanus antitoxin), immune globulins (e.g., rabies immunoglobulin), maternal antibodies via placenta/breast milk.
Booster Effect	Booster doses can enhance memory.	No memory formation; repeat doses needed for ongoing protection.
Use Cases	Preventive vaccination, long-term disease prevention.	Post-exposure prophylaxis (e.g., rabies, hepatitis B), treatment of toxin-mediated diseases (e.g., diphtheria), immunosuppressed individuals.

d) Primary Immunodeficiency (PID) and Secondary Immunodeficiency

Feature	Primary Immunodeficiency (PID)	Secondary Immunodeficiency (SID)
Cause	Genetic defects (inherited or de novo mutations) affecting immune system development or function.	Acquired due to external factors (infections, malnutrition, medications, cancers).
Onset	Usually congenital, presenting in infancy or childhood.	Can occur at any age, depending on the cause.
Genetic Basis	Monogenic or polygenic disorders.	Not genetic; environmental or iatrogenic.
Examples	Severe Combined Immunodeficiency (SCID), Chronic Granulomatous Disease (CGD), X-linked	HIV/AIDS (depletes $CD4^+$ T cells), malnutrition, chemotherapy, radiation, steroid therapy, aging.

Feature	Primary Immunodeficiency (PID)	Secondary Immunodeficiency (SID)
	Agammaglobulinemia (Bruton's disease).	
Treatment	Supportive care, immunoglobulin replacement, stem cell transplantation, gene therapy.	Address underlying cause (antiretroviral therapy for HIV, nutritional support, reducing immunosuppressive drugs).
Prognosis	Often chronic, may be life-threatening without intervention.	May be reversible if underlying cause is treated.

Question 3

Write short notes on the following: (2½ × 4 = 10 marks)

a) Ouchterlony Double Diffusion

Ouchterlony double diffusion (also called immunodiffusion) is a classic qualitative technique used to detect and compare antigens and antibodies based on their diffusion in a gel matrix. It involves pouring agarose gel onto a slide and cutting wells in a defined pattern (typically a central well surrounded by peripheral wells). Antigen is placed in the central well, and different antisera (antibodies) are placed in the peripheral wells. Both antigen and antibodies diffuse radially through the gel. Where they meet in optimal proportions, they form **visible precipitin lines**. The pattern of lines indicates:

- **Identity**: A continuous, fused line indicates the antigen in different wells is identical.
- **Non-identity**: Crossing lines indicate unrelated antigens.
- **Partial identity**: A spur formation indicates shared and unique epitopes.
  This method is used for antigen characterization, antibody specificity testing, and detecting cross-reactivity. Though largely replaced by ELISA and Western blot, it remains a simple, cost-effective teaching tool.

b) Superantigens

Superantigens are a class of antigens that cause non-specific, excessive activation of T cells, leading to massive cytokine release and potential toxic shock. Unlike conventional antigens that bind to the variable region of TCR and require processing and presentation, superantigens bind directly to the **Vβ region of the TCR** and to **MHC class II molecules** outside the peptide-binding groove. This cross-links TCR and MHC without regard to antigen specificity, activating up to 20% of all T cells (compared to 0.01% for conventional antigens). Examples include **staphylococcal enterotoxins** (SEA, SEB) and **toxic shock syndrome toxin-1** (TSST-1) from *Staphylococcus aureus*, and **streptococcal pyrogenic exotoxins**. Consequences include fever, rash, hypotension, multi-organ failure, and exacerbation of autoimmune diseases. Superantigens play a role in food poisoning, toxic shock syndrome, and some autoimmune disorders.

c) Anaphylaxis

Anaphylaxis is a severe, life-threatening systemic hypersensitivity reaction, typically a **type I (IgE-mediated) hypersensitivity**. It occurs rapidly after exposure to an allergen (e.g., peanuts, bee venom, drugs) in sensitized individuals. Allergen cross-links IgE bound to FcεRI receptors on mast cells and basophils, triggering degranulation and release of mediators like **histamine, leukotrienes, and prostaglandins**. This causes vasodilation, increased vascular permeability, bronchoconstriction, and smooth muscle contraction. Clinical features include urticaria, angioedema, wheezing, hypotension, gastrointestinal distress, and potentially fatal anaphylactic shock. Treatment involves immediate administration of **epinephrine** (adrenaline), antihistamines, corticosteroids, and supportive care. Prevention includes allergen avoidance and carrying epinephrine auto-injectors.

d) Immunosuppressive Therapy

Immunosuppressive therapy refers to the use of drugs or other interventions to suppress the immune system. It is used to:

1. Prevent organ transplant rejection.
2. Treat autoimmune diseases (e.g., rheumatoid arthritis, lupus).
3. Manage hypersensitivity reactions.
4. Treat certain cancers (e.g., lymphomas).

Common agents include:

- **Corticosteroids** (prednisone): Reduce inflammation and lymphocyte proliferation.
- **Calcineurin inhibitors** (cyclosporine, tacrolimus): Inhibit T cell activation.
- **Antiproliferatives** (azathioprine, mycophenolate mofetil): Inhibit DNA synthesis in lymphocytes.
- **mTOR inhibitors** (sirolimus): Block T cell proliferation.
- **Biologics** (monoclonal antibodies against TNF-α, IL-6, CD20, etc.): Target specific immune pathways.
  Risks include increased susceptibility to infections, cancer, and metabolic side effects. Therapy is tailored to balance efficacy and toxicity.

Question 4

a) Define the term “chimeric antibodies” and discuss their applications. (5 marks)

Chimeric antibodies are genetically engineered antibodies that combine regions from different species to reduce immunogenicity and enhance therapeutic utility. Typically, they are constructed by fusing the **variable regions (VH and VL)** of a mouse monoclonal antibody (which confer antigen specificity) with the **constant regions (CH and CL)** of a human immunoglobulin. This results in an antibody that is approximately 65–70% human, reducing the likelihood of a human anti-mouse antibody (HAMA) response when administered to patients.

Applications of Chimeric Antibodies:

1. **Cancer Therapy**:
   - **Rituximab**: A chimeric anti-CD20 antibody used to treat non-Hodgkin’s lymphoma and autoimmune diseases. It depletes B cells by inducing antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and apoptosis.
   - **Cetuximab**: Targets epidermal growth factor receptor (EGFR) in colorectal and head and neck cancers, blocking signaling and promoting immune-mediated tumor cell death.

2. **Autoimmune and Inflammatory Diseases**:
    - **Infliximab**: A chimeric anti-TNF-α antibody used in rheumatoid arthritis, Crohn's disease, and ulcerative colitis. It neutralizes TNF-α, a key inflammatory cytokine.
3. **Transplantation**:
    - **Basiliximab**: An anti-CD25 (IL-2 receptor) chimeric antibody used to prevent acute organ rejection by inhibiting T cell proliferation.
4. **Infectious Diseases**:
    - **Palivizumab**: A chimeric antibody against respiratory syncytial virus (RSV) used for prophylaxis in high-risk infants.
5. **Diagnostic Imaging**:
    - Chimeric antibodies labeled with radioisotopes (e.g., technetium-99m, indium-111) are used in immuno-scintigraphy to detect tumors or inflammatory sites.
6. **Research Tools**:
    - Used in flow cytometry, ELISA, and immunohistochemistry due to their specificity and reduced background staining compared to purely murine antibodies.

Advantages: Reduced immunogenicity, longer serum half-life, and ability to engage human effector functions (e.g., CDC, ADCC) better than murine antibodies.

Limitations: Still carry some murine components, which may elicit mild immune responses. This led to the development of **humanized** (90–95% human) and **fully human** antibodies.

Chimeric antibodies represent a pivotal advancement in biologic therapeutics, bridging mouse hybridoma technology with human clinical application.

b) Explain Edward Jenner's contribution to the development of vaccines. (5 marks)

Edward Jenner (1749–1823) is celebrated as the pioneer of vaccination, laying the foundation for immunology and preventive medicine. His work emerged from the observation that milkmaids who had contracted **cowpox** (a mild disease) were subsequently protected against the deadly **smallpox**. In 1796, Jenner conducted a groundbreaking experiment: he inoculated an 8-year-old boy, James Phipps, with

material from a cowpox pustule of milkmaid Sarah Nelmes. After the boy recovered from cowpox, Jenner challenged him with smallpox material—the boy remained healthy. Jenner repeated the experiment on others and published his findings in 1798, coining the term **"vaccination"** (from *vacca*, Latin for cow).

Key Contributions:

1. **Proof of Concept for Immunization**: Jenner demonstrated that deliberate infection with a milder, related pathogen could confer immunity against a more virulent one. This was the first scientific approach to disease prevention through immunization.
2. **Establishment of Vaccination as a Public Health Tool**: Despite initial skepticism, vaccination gained acceptance due to its success. By the early 1800s, vaccination replaced the risky practice of **variolation** (inoculation with smallpox itself), drastically reducing smallpox mortality.
3. **Foundation for Future Vaccinology**: Jenner's work inspired Louis Pasteur, who later developed vaccines for rabies, anthrax, and cholera, and proposed the **germ theory of disease**. The principles of using attenuated or killed pathogens stem from Jenner's original idea.
4. **Global Impact**: Smallpox, once a devastating scourge, was eventually eradicated in 1980 following a worldwide vaccination campaign led by the WHO—the only human disease eradicated to date.

Jenner's contribution transcends smallpox; it established the paradigm that the immune system can be "trained" to recognize and remember pathogens, a concept central to all modern vaccines. His legacy is a cornerstone of public health, saving countless lives and inspiring ongoing vaccine development against emerging infectious diseases.

Question 5

Describe the role of the bone marrow in the immune system, focusing on its structure and the process of hematopoiesis. (10 marks)

The bone marrow is a primary lymphoid organ and the chief site of **hematopoiesis**—the production of all blood cells, including cells of the immune system. Located

within the cavities of bones (especially flat bones like sternum, pelvis, and vertebrae), it is a spongy, highly vascular tissue that serves as the cradle for immune cell development.

Structure of Bone Marrow:

1. **Cellular Components**:
   - **Hematopoietic Stem Cells (HSCs)**: Multipotent, self-renewing cells that give rise to all blood lineages.
   - **Stromal Cells**: Include fibroblasts, adipocytes, endothelial cells, and osteoblasts that form a supportive microenvironment (niche) via cell-cell contact and secretion of growth factors (e.g., stem cell factor, IL-7).
   - **Macrophages and Dendritic Cells**: Involved in antigen presentation and removal of apoptotic cells.
2. **Vascular Network**: Sinusoids (thin-walled blood vessels) allow mature cells to enter circulation.
3. **Red vs. Yellow Marrow**:
   - **Red marrow**: Active in hematopoiesis; abundant in children and in adults in flat bones.
   - **Yellow marrow**: Mostly adipose tissue, inactive but can convert to red marrow under stress (e.g., severe blood loss).

Process of Hematopoiesis:

Hematopoiesis is a tightly regulated, multi-step differentiation process:

1. **HSC Commitment**: HSCs differentiate into either **common myeloid progenitors (CMPs)** or **common lymphoid progenitors (CLPs)**.
2. **Myeloid Lineage** (from CMP):
   - **Granulocytes**: Neutrophils, eosinophils, basophils.
   - **Monocytes**: Circulate and differentiate into tissue macrophages or dendritic cells.
   - **Megakaryocytes**: Produce platelets.
   - **Erythrocytes**: Red blood cells (though not immune cells, essential for oxygen transport).
3. **Lymphoid Lineage** (from CLP):

- **B Cells**: Complete maturation in bone marrow (central tolerance via negative selection).
- **T Cell Progenitors**: Migrate to thymus for maturation.
- **Natural Killer (NK) Cells**: Also derived from CLP.

Key Steps in B Cell Development in Bone Marrow:

- **Pro-B cell**: Rearrangement of heavy chain gene.
- **Pre-B cell**: Expression of μ heavy chain with surrogate light chain.
- **Immature B cell**: Expression of IgM on surface.
- **Negative Selection**: Self-reactive B cells are eliminated or anergized.
- **Mature B cell**: Co-expresses IgM and IgD; exits to peripheral lymphoid organs.

Role in Immune System:

1. **Source of Immune Cells**: Bone marrow continuously produces innate immune cells (neutrophils, monocytes) and adaptive immune cells (B cells, NK cells, T cell precursors).
2. **B Cell Maturation and Education**: Central tolerance ensures B cells do not react strongly to self-antigens.
3. **Memory Cell Maintenance**: Long-lived plasma cells and memory B cells may reside in bone marrow niches, providing long-term humoral immunity.
4. **Secondary Lymphopoiesis**: Under infection or stress, bone marrow can increase output of immune cells (emergency hematopoiesis).
5. **Transplantation**: Bone marrow transplants can reconstitute the entire immune system in conditions like leukemia or severe combined immunodeficiency (SCID).

Regulation: Hematopoiesis is controlled by cytokines (e.g., colony-stimulating factors, interleukins), transcription factors (e.g., PU.1, GATA-2), and stromal interactions.

Clinical Relevance: Dysregulation leads to leukemias, lymphomas, or bone marrow failure syndromes. Understanding bone marrow function is critical for stem cell therapies, cancer treatment, and immunodeficiencies.

In summary, the bone marrow is indispensable for immune cell generation, maturation, and maintenance, forming the bedrock of both innate and adaptive immunity.

Question 6

a) Define autoimmunity and discuss its role in immune system dysfunction. (5 marks)

Autoimmunity is a condition in which the immune system mistakenly attacks the body's own healthy cells, tissues, or organs, breaking down **self-tolerance**. Normally, the immune system distinguishes between self and non-self via central and peripheral tolerance mechanisms. When these fail, autoreactive lymphocytes (B and T cells) are activated, leading to inflammation, tissue damage, and autoimmune diseases.

Role in Immune System Dysfunction:

1. **Loss of Self-Tolerance**:
   - **Central Tolerance**: In bone marrow (B cells) and thymus (T cells), self-reactive lymphocytes are deleted via apoptosis or anergy. Defects in these processes (e.g., mutations in *AIRE* gene, which promotes self-antigen expression in thymus) allow escape of autoreactive cells.
   - **Peripheral Tolerance**: Mechanisms like regulatory T cells (Tregs), anergy, and immune privilege sites suppress autoreactive cells in periphery. Dysfunction (e.g., Treg deficiency) permits autoimmunity.
2. **Molecular Mimicry**: Some pathogens express antigens similar to self-proteins (e.g., streptococcal M protein and heart myosin in rheumatic fever). The immune response against the pathogen cross-reacts with self-tissues.
3. **Epitope Spreading**: Initial immune response against a self-antigen may expand to target additional self-epitopes, worsening disease (e.g., in multiple sclerosis).
4. **Inflammation and Tissue Damage**: Autoimmune responses involve:
   - **Autoantibodies**: Attack self-cells (e.g., anti-acetylcholine receptor antibodies in myasthenia gravis block neuromuscular transmission).
   - **Immune Complex Deposition**: Autoantibody-antigen complexes deposit in tissues (e.g., kidneys in lupus nephritis), activating complement and causing inflammation.
   - **T Cell-Mediated Cytotoxicity**: $CD8^+$ T cells destroy target cells (e.g., pancreatic β-cells in type 1 diabetes).

5. **Genetic and Environmental Factors**:
    - **Genes**: HLA alleles (e.g., HLA-DR4 in rheumatoid arthritis), polymorphisms in immune regulators (CTLA-4, PTPN22).
    - **Environment**: Infections, hormones, drugs, UV radiation, and stress can trigger or exacerbate autoimmunity.
6. **Systemic vs. Organ-Specific**:
    - **Systemic**: Multiple organs affected (e.g., systemic lupus erythematosus, Sjögren's syndrome).
    - **Organ-specific**: Target one organ (e.g., Hashimoto's thyroiditis, Graves' disease).

Autoimmunity represents a profound dysfunction of immune regulation, leading to chronic, debilitating conditions. Treatments aim to suppress immune activity (immunosuppressants, biologics) or restore tolerance (e.g., Treg therapy, antigen-specific tolerization).

b) Explain how Gell and Coombs classified hypersensitivity reactions. (5 marks)

In 1963, immunologists Philip Gell and Robin Coombs proposed a classification of hypersensitivity reactions into four types (I–IV) based on immune mechanisms, time course, and clinical manifestations. This framework remains essential for diagnosis and therapy.

Type I: Immediate (IgE-Mediated) Hypersensitivity

- **Mechanism**: Allergen cross-links IgE bound to FcεRI on mast cells and basophils, triggering degranulation and release of vasoactive amines (histamine), leukotrienes, and cytokines.
- **Onset**: Minutes to hours.
- **Examples**: Allergic rhinitis, asthma, anaphylaxis, urticaria.
- **Diagnosis**: Skin prick test, serum IgE levels.
- **Treatment**: Antihistamines, corticosteroids, epinephrine, allergen avoidance.

Type II: Antibody-Mediated (Cytotoxic) Hypersensitivity

- **Mechanism**: IgG or IgM antibodies bind to cell-surface or extracellular matrix antigens, leading to:
  - Complement activation (MAC lysis).
  - Opsonization and phagocytosis.
  - Antibody-dependent cellular cytotoxicity (ADCC) by NK cells.
- **Onset**: Hours to days.
- **Examples**: Autoimmune hemolytic anemia, Goodpasture's syndrome (anti-basement membrane antibodies), Rh incompatibility in newborns.
- **Treatment**: Immunosuppressants, plasmapheresis, IV immunoglobulin.

Type III: Immune Complex-Mediated Hypersensitivity

- **Mechanism**: Antigen-antibody (IgG/IgM) complexes deposit in tissues (blood vessels, kidneys, joints), activating complement and attracting neutrophils, which release enzymes causing inflammation and damage.
- **Onset**: 3–10 hours post-exposure.
- **Examples**: Systemic lupus erythematosus (SLE), rheumatoid arthritis, serum sickness, Arthus reaction.
- **Treatment**: Anti-inflammatories, immunosuppressants.

Type IV: Delayed-Type (Cell-Mediated) Hypersensitivity

- **Mechanism**: Sensitized $CD4^+$ Th1 cells (or $CD8^+$ CTLs) recognize antigen presented by macrophages, releasing cytokines (IFN-γ, TNF) that activate macrophages and cause tissue damage. No antibody involvement.
- **Onset**: 24–72 hours.
- **Examples**: Contact dermatitis (poison ivy, nickel), tuberculin skin test (PPD), granulomatous diseases (tuberculosis, leprosy), type 1 diabetes (T cell-mediated β-cell destruction).
- **Treatment**: Topical steroids, immunosuppressants.

Additional Notes: Some sources add **Type V** (stimulatory hypersensitivity, e.g., Graves' disease where antibodies stimulate receptors) and **Type VI** (antibody-dependent cellular cytotoxicity), but these are often considered subsets of Type II.

The Gell and Coombs classification guides targeted therapies: anti-IgE (omalizumab) for Type I, complement inhibitors for Type II/III, and T cell-targeted drugs for Type

IV.

Question 7

a) Describe the process of complement-induced lysis through MAC formation. (5 marks)

Complement-induced lysis is a critical effector mechanism of the immune system, culminating in the formation of the **Membrane Attack Complex (MAC)**, which creates pores in pathogen membranes, leading to osmotic lysis and death. This process is the terminal step of the complement cascade, initiated via classical, lectin, or alternative pathways.

Steps in MAC Formation:

1. **C3 Convertase Activation**:
   - Classical/Lectin pathway: C3 convertase is C4b2a.
   - Alternative pathway: C3 convertase is C3bBb.
     These enzymes cleave C3 into C3a (anaphylatoxin) and C3b.
2. **C5 Convertase Formation**:
   - C3b binds to C3 convertase to form C5 convertase (C4b2a3b in classical/lectin; C3bBbC3b in alternative).
   - C5 convertase cleaves C5 into C5a (anaphylatoxin) and C5b.
3. **Initiation of MAC Assembly**:
   - **C5b** binds to C6, forming a stable C5b-6 complex that attaches to the target membrane (e.g., bacterial outer membrane).
4. **Sequential Addition of Components**:
   - **C7** binds to C5b-6, forming C5b-7, which inserts into the lipid bilayer (hydrophobic region of C7 mediates insertion).
   - **C8** binds to C5b-7, forming C5b-8. C8 penetrates deeper into the membrane, creating a small pore that allows slow ion leakage.
   - **Polymerization of C9**: C5b-8 acts as a receptor for **C9**, inducing its conformational change and polymerization. Multiple C9 molecules (typically 12–

18) assemble into a cylindrical **pore** (~10 nm diameter) that spans the membrane.

5. **Membrane Disruption**:
    - The MAC pore allows unrestricted flow of ions and water, disrupting osmotic balance.
    - Influx of water causes cell swelling and lysis (osmotic lysis).
    - Intracellular contents leak out, leading to pathogen death.

Regulation to Prevent Host Cell Damage:

- Host cells express regulatory proteins like **CD59** (protectin) and **vitronectin (S-protein)** that bind to C5b-7 or C8, inhibiting C9 polymerization.
- **Membrane cofactor protein (MCP)** and **decay-accelerating factor (DAF)** prevent excessive C3 convertase formation on host cells.

Biological Significance:

- Effective against Gram-negative bacteria (thin peptidoglycan layer), parasites, and some enveloped viruses.
- Less effective against Gram-positive bacteria (thick peptidoglycan resists MAC insertion).
- MAC also promotes inflammation by stimulating cytokine release and tissue repair.

Deficiencies in MAC components (e.g., C5–C9) increase susceptibility to *Neisseria* infections. Understanding MAC formation aids in developing complement-targeted therapies for inflammatory diseases.

b) Briefly describe the autocrine, paracrine, and endocrine functions of cytokines. (5 marks)

Cytokines are small signaling proteins secreted by immune and other cells, mediating communication and regulating immune responses. Their actions are classified based on the distance over which they act:

1. Autocrine Action:

- The cytokine acts on the **same cell that secreted it**. The cell expresses receptors for the cytokine it produces.

- **Example**:
  - T cells produce **IL-2** and also express IL-2 receptors. IL-2 binding stimulates the same T cell to proliferate (clonal expansion).
  - Macrophages produce **TNF-α**, which can autocrinely enhance their own activation and inflammatory functions.
- **Significance**: Amplifies immune responses in a feedback loop, ensuring robust activation of specific cell populations.

2. Paracrine Action:

- The cytokine acts on **nearby cells** in the local microenvironment. Diffusion is limited to short distances.
- **Examples**:
  - Dendritic cells secrete **IL-12** to activate neighboring NK cells and T helper 1 (Th1) cells.
  - Mast cell-derived **histamine** acts on local blood vessels to increase permeability during inflammation.
  - **Chemokines** guide leukocyte migration to sites of infection (chemotaxis).
- **Significance**: Coordinates localized immune responses, cell recruitment, and cellular interactions within tissues or lymphoid organs.

3. Endocrine Action:

- The cytokine travels via the **bloodstream** to act on **distant cells or organs**. This systemic action often involves high concentrations or prolonged secretion.
- **Examples**:
  - **IL-6** produced at an infection site reaches the liver, inducing acute-phase protein synthesis (e.g., C-reactive protein).
  - **TNF-α** in severe infections can cause systemic effects like fever, cachexia, and septic shock.
  - **Erythropoietin** (a cytokine) from kidneys stimulates bone marrow red blood cell production.
- **Significance**: Integrates immune responses across the body, mediating systemic inflammation, fever, and metabolic changes.

Overlap and Redundancy: Many cytokines exhibit **pleiotropy** (multiple effects) and can act via more than one mode. For instance, TNF-α can act autocrinely on macrophages, paracrinely on endothelial cells, and endocrinely to induce fever.

Clinical Relevance: Dysregulated cytokine signaling underlies autoimmune diseases, cytokine storms (e.g., in COVID-19), and allergies. Therapeutic cytokines (e.g., interferon-α for hepatitis) or cytokine antagonists (e.g., anti-TNF antibodies) are used to modulate immune responses.

Understanding these modes of action is key to deciphering immune network communication and designing targeted immunotherapies.

Question 8

a) Discuss the pathways for processing both endogenous and exogenous antigens. (5 marks)

Antigen processing is the mechanism by which protein antigens are degraded into peptides and loaded onto MHC molecules for presentation to T cells. The pathway differs for **endogenous** (intracellular) and **exogenous** (extracellular) antigens.

1. Endogenous Antigen Processing (MHC Class I Pathway):

- **Source**: Proteins synthesized within the cell (e.g., viral proteins, tumor antigens, self-proteins).
- **Location**: Cytosol and endoplasmic reticulum (ER).
- **Steps**:
  1. **Proteasomal Degradation**: Misfolded or ubiquitinated proteins are degraded by the **proteasome** (including immunoproteasome during inflammation) into peptides (8–10 amino acids).
  2. **Transport to ER**: Peptides are transported into the ER via **TAP** (Transporter Associated with Antigen Processing).
  3. **MHC Class I Assembly**: In the ER, MHC class I α-chain and β_2-microglobulin assemble with the help of chaperones (calnexin, calreticulin, tapasin).
  4. **Peptide Loading**: Peptides bind to the peptide-binding groove of MHC class I. High-affinity peptides stabilize the complex.

5. **Transport to Cell Surface**: Loaded MHC class I travels through the Golgi to the plasma membrane for presentation to **CD8⁺ T cells** (cytotoxic T lymphocytes, CTLs).

Outcome: $CD8^+$ T cells recognize peptide-MHC I and kill infected or malignant cells.

2. Exogenous Antigen Processing (MHC Class II Pathway):

- **Source**: Extracellular proteins internalized by endocytosis or phagocytosis (e.g., bacteria, toxins, allergens).
- **Location**: Endosomes, lysosomes.
- **Steps**:
  1. **Internalization**: Antigens are taken up via phagocytosis (macrophages), receptor-mediated endocytosis (B cells), or pinocytosis (dendritic cells).
  2. **Vesicular Trafficking**: Antigens enter endosomes that mature into acidic lysosomes.
  3. **Proteolytic Degradation**: Lysosomal proteases (cathepsins) degrade antigens into peptides (13–18 amino acids).
  4. **MHC Class II Assembly**: In the ER, MHC class II α and β chains assemble with **invariant chain (Ii)**, which blocks premature peptide binding and directs MHC II to endosomes via targeting signals.
  5. **Vesicle Fusion and Peptide Loading**: Late endosomes (MHC class II compartments, MIIC) containing MHC II fuse with peptide-containing lysosomes. The invariant chain is cleaved, leaving **CLIP** (Class II-associated invariant chain peptide) in the groove. **HLA-DM** catalyzes CLIP removal and facilitates loading of antigenic peptides.
  6. **Surface Presentation**: Loaded MHC II is transported to the cell surface for presentation to **CD4⁺ T helper cells**.

Outcome: $CD4^+$ T cells recognize peptide-MHC II and provide help for B cell activation, macrophage activation, and cytokine production.

Cross-Presentation: A specialized pathway where dendritic cells present exogenous antigens on MHC class I to activate $CD8^+$ T cells. This occurs via:

- **Phagosome-to-cytosol escape**: Antigens leak into cytosol for proteasomal processing.

- **Endosomal processing**: Peptides loaded onto MHC I within endosomal compartments.
  Critical for immunity against viruses and tumors that do not infect dendritic cells directly.

These processing pathways ensure that intracellular and extracellular threats are appropriately presented to the respective T cell subsets, enabling tailored immune responses.

b) Explain the peptide-binding properties of Class I and II MHC molecules with a suitable diagram. (5 marks)

Peptide-Binding Properties of MHC Molecules:

MHC Class I:

- **Structure**: Heterodimer of α chain (three domains: α1, α2, α3) non-covalently associated with β_2-microglobulin. The peptide-binding groove is formed by α1 and α2 domains.
- **Peptide Length**: Typically binds peptides of **8–10 amino acids** (optimally 9). The ends of the groove are closed, restricting longer peptides.
- **Anchor Residues**: Peptides have conserved residues at specific positions (e.g., position 2 and C-terminus) that interact with pockets in the MHC groove.
- **Binding Stability**: Tight binding; peptides are buried deep in the groove, with both ends anchored.
- **Function**: Presents endogenous peptides to $CD8^+$ T cells.

MHC Class II:

- **Structure**: Heterodimer of α and β chains (each with two domains: α1/α2, β1/β2). The peptide-binding groove is formed by α1 and β1 domains.
- **Peptide Length**: Binds longer peptides, **13–18 amino acids** or more. The ends of the groove are open, allowing peptide extensions.
- **Anchor Residues**: Peptides interact via a **core 9-amino-acid sequence** but can have flanking regions. Multiple anchor residues along the length stabilize binding.
- **Binding Stability**: Peptide lies in an extended conformation, with hydrogen bonds along the backbone.
- **Function**: Presents exogenous peptides to $CD4^+$ T cells.

Diagram Description (verbal representation):

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text

MHC Class I Peptide Binding:
  ---------------------
  |  α1/α2 groove     |
  |  [===Peptide===]  |  <-- Peptide (8-10 aa) anchored at both ends.
  ---------------------
  β₂-microglobulin

MHC Class II Peptide Binding:
  ---------------------
  |  α1/β1 groove     |
  |  [===============] |  <-- Longer peptide (13-18 aa) with open ends.
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Key Differences Summary:

Property	MHC Class I	MHC Class II
Peptide Source	Endogenous (cytosolic)	Exogenous (extracellular)
Presented to	$CD8^+$ T cells	$CD4^+$ T cells
Peptide Length	8–10 amino acids	13–18 amino acids
Groove Ends	Closed	Open
Chain Composition	α chain + β_2m	α chain + β chain
Loading Site	Endoplasmic reticulum	Endosomal/lysosomal compartment
Typical Anchors	At peptide termini	Distributed along peptide

These distinct binding properties ensure that MHC class I and II present peptides from different compartments, enabling appropriate T cell responses against intracellular and extracellular pathogens.

Question 9

a) Discuss the flow cytometry principles and its components. (5 marks)

Flow cytometry is a powerful technology that analyzes physical and chemical characteristics of cells or particles as they flow in a fluid stream through a laser beam. It allows multiparameter analysis at single-cell level, with applications in immunology, hematology, oncology, and microbiology.

Principles:

1. **Hydrodynamic Focusing**: The sample suspension is injected into a sheath fluid (usually saline). Laminar flow focuses cells into a single file, ensuring they pass through the laser intercept one at a time.
2. **Light Scattering**:
    - **Forward Scatter (FSC)**: Measures light scattered along the laser axis, proportional to cell **size**.
    - **Side Scatter (SSC)**: Measures light scattered at 90°, proportional to cell **granularity/internal complexity**.
3. **Fluorescence Detection**: Cells labeled with fluorochrome-conjugated antibodies or dyes are excited by lasers (e.g., 488 nm blue laser). Emitted fluorescence is collected by lenses, split by dichroic mirrors, and filtered to specific detectors (photomultiplier tubes, PMTs). Each fluorochrome has a characteristic emission spectrum.
4. **Data Acquisition and Analysis**: Signals are converted to digital data. Plots (dot plots, histograms, contour plots) visualize parameters. **Gating** strategies isolate specific cell populations.

Components of a Flow Cytometer:

1. **Fluidics System**:
    - **Sheath fluid reservoir**: Maintains laminar flow.
    - **Sample injection tube**: Introduces cells.
    - **Flow cell**: Quartz chamber where cells intersect lasers.
2. **Optics System**:
    - **Lasers**: Light sources (argon ion, helium-neon, diode) of specific wavelengths.
    - **Lenses**: Collect and focus light.

- **Filters and Dichroic Mirrors**: Direct light to appropriate detectors.
- **Detectors**: PMTs convert light to electrical signals.

3. **Electronics System**:
   - **Analog-to-Digital Converters (ADCs)**: Digitize signals.
   - **Computer**: Runs acquisition software and stores data (FCS format).
4. **Sorting Option (Fluorescence-Activated Cell Sorter, FACS)**:
   - Additional component: **Droplet generator** charges and deflects cells into collection tubes based on selected parameters.

Applications in Immunology:

- Immunophenotyping (CD marker analysis).
- Cell cycle and apoptosis analysis.
- Intracellular cytokine staining.
- Detection of phosphorylated proteins.
- Monitoring HIV progression ($CD4^+$ T cell counts).

Advantages: High-speed (thousands of cells/second), multiparametric, quantitative, and capable of sorting.

Limitations: Expensive, requires technical expertise, and cannot analyze tissue architecture.

Flow cytometry is indispensable for research, diagnostics, and therapeutic monitoring in immunology.

b) Explain the DOT ELISA techniques and their working principles. (5 marks)

DOT ELISA (Enzyme-Linked Immunosorbent Assay) is a rapid, membrane-based variant of conventional ELISA, widely used for point-of-care testing, field diagnostics, and resource-limited settings due to its simplicity, low cost, and visual readout.

Working Principle:

DOT ELISA follows the same immunological principles as sandwich or indirect ELISA but uses a **nitrocellulose or PVDF membrane** as the solid phase instead of a

microtiter plate.

Procedure:

1. **Antigen Immobilization**: The antigen (or capture antibody) is directly spotted ("dotted") onto small, defined areas of the membrane. The membrane is then blocked (with BSA, skim milk) to prevent non-specific binding.
2. **Sample Incubation**: The membrane is incubated with the test sample (serum, urine, tissue extract). If target antibodies or antigens are present, they bind to the immobilized counterpart.
3. **Detection Antibody Incubation**: For indirect detection (e.g., detecting antibodies in sample):
    - A species-specific enzyme-conjugated secondary antibody (e.g., anti-human IgG-HRP) is added, which binds to the primary antibody.
      For antigen detection (sandwich format):
    - An enzyme-conjugated detection antibody specific to the antigen is added.
4. **Substrate Addition**: A chromogenic substrate (e.g., TMB, DAB for HRP; BCIP/NBT for alkaline phosphatase) is added. Enzyme activity converts the substrate into an insoluble colored precipitate at the dot site.
5. **Result Interpretation**:
    - **Positive**: Visible colored dot.
    - **Negative**: No color or faint background.
    - The intensity can be semi-quantified by comparison with standards or using a densitometer.

Types of DOT ELISA:

- **Direct DOT ELISA**: Antigen spotted; sample with antibodies binds, detected by enzyme-labeled anti-species antibody.
- **Indirect DOT ELISA**: Antigen spotted; primary antibody binds, detected by enzyme-labeled secondary antibody.
- **Sandwich DOT ELISA**: Capture antibody spotted; antigen in sample binds, detected by enzyme-labeled detection antibody.

Advantages:

- Rapid (1–2 hours).

- No need for expensive plate readers; visual assessment.
- Stable dried dots can be stored and transported.
- Multiplexing possible by spotting multiple antigens/antibodies on one membrane.

Disadvantages:

- Less quantitative than plate ELISA.
- Subjective interpretation.
- Lower sensitivity in some cases.

Applications:

- Serodiagnosis of infectious diseases (HIV, dengue, tuberculosis).
- Detection of autoantibodies (e.g., in SLE).
- Food allergen testing.
- Environmental monitoring (toxins, pesticides).

Variants: **Dot-blot** (similar but without enzymatic amplification) and **immunochromatographic rapid tests** (lateral flow) are derived from DOT ELISA principles.

DOT ELISA remains a valuable tool for screening and field diagnostics where simplicity and speed are paramount.

Question 10

Explain the importance of donor and recipient matching in transplantation immunology, focusing on the role of human leukocyte antigen (HLA) typing and its impact on reducing graft rejection. (10 marks)

Transplantation involves transferring cells, tissues, or organs from a donor to a recipient. The immune system's ability to distinguish self from non-self poses a major hurdle: **graft rejection**, where the recipient's immune system attacks the transplanted tissue as foreign. **Donor-recipient matching**, especially for **human leukocyte antigens (HLAs)**, is critical to minimize rejection and ensure graft survival.

HLA System and Its Role in Transplantation:

HLAs are the human version of the **major histocompatibility complex (MHC)** proteins, encoded by genes on chromosome 6. They are highly polymorphic, presenting peptides to T cells and triggering immune responses.

Classes of HLA:

- **HLA Class I** (HLA-A, -B, -C): Expressed on almost all nucleated cells; present endogenous peptides to $CD8^+$ cytotoxic T cells.
- **HLA Class II** (HLA-DR, -DQ, -DP): Expressed on antigen-presenting cells (APCs); present exogenous peptides to $CD4^+$ helper T cells.

HLA Typing Methods:

1. **Serological Typing**: Uses antibodies against specific HLA alleles (less precise).
2. **Molecular Typing**:
    - **PCR-SSP/SSO**: Amplifies HLA genes with sequence-specific primers or probes.
    - **Sequencing-Based Typing (SBT)**: High-resolution DNA sequencing.
    - **Next-Generation Sequencing (NGS)**: Most comprehensive, detects rare alleles.

Impact of HLA Matching on Graft Rejection:

Types of Rejection:

1. **Hyperacute Rejection**: Occurs within minutes to hours due to pre-existing antibodies against donor HLA (or ABO blood group antigens). HLA matching avoids mismatches that could lead to antibody-mediated rejection.
2. **Acute Rejection**: Within weeks to months, primarily T cell-mediated. HLA mismatches activate recipient T cells via **direct allorecognition** (donor HLA + peptide recognized as foreign) and **indirect allorecognition** (donor HLA peptides