IMMUNOLOGY AND rDNA TECHNOLOGY

UNIT I: Concepts, Cells, and Organs of the Immune System

Terminology and Definitions

- Antigen: A molecule capable of inducing an immune response.
- Hapten: A small molecule that, when combined with a larger carrier, can elicit an immune response.
- Antibody: A protein produced by B cells that binds specifically to an antigen. Types include IgG, IgA, IgM, IgE, and IgD.
- Antigenicity: The ability of a substance to bind specifically with the products of the immune response (antibodies or T-cell receptors).
- Immunogenicity: The ability of a substance to induce an immune response.

Types of Immunity

- Innate Immunity: The non-specific first line of defence, including physical barriers, phagocytic cells, and the complement system.
- Adaptive Immunity: The specific immune response involving lymphocytes (B cells and T cells) and memory cells.

Haematopoiesis

The process by which all blood cells are formed, originating from hematopoietic stem cells in the bone marrow.

Organs and Tissues of the Immune System

- Primary Lymphoid Organs: Bone marrow and thymus, where lymphocytes are produced and mature.
- Secondary Lymphoid Organs: Lymph nodes, spleen, and mucosa-associated lymphoid tissue (MALT), where immune responses are initiated.

Cells of the Immune System

- Lymphocytes: B cells (produce antibodies) and T cells (mediate cellular immunity).
- Cytokines: Signalling proteins that regulate immunity, inflammation, and haematopoiesis.

Complement System

A group of proteins that enhance the ability of antibodies and phagocytic cells to clear pathogens.

Major Histocompatibility Complex (MHC)

MHC molecules present antigen fragments on the cell surface, crucial for the immune system to recognize foreign molecules.

Humoral and Cell-Mediated Immune Responses

- Humoral Response: Involves B cells and the production of antibodies.

- Cell-Mediated Response: Involves T cells that directly attack infected or cancerous cells.

UNIT II: Vaccinology and Clinical Immunology

Types of Vaccines

- Live Attenuated Vaccines: Contain weakened pathogens.
- Killed/Inactivated Vaccines: Contain killed pathogens.
- Subunit Vaccines: Contain parts of the pathogen (e.g., proteins).
- Recombinant Vaccines: Use genetically engineered components.

Adjuvants

Substances added to vaccines to enhance the immune response.

Hybridoma Technology

A method to produce monoclonal antibodies by fusing an antibody-producing B cell with a myeloma cell.

Antigen and Antibody Interactions

- Precipitation: Antigens and antibodies form a visible complex.
- Agglutination: Particulate antigens clump together when bound by antibodies.
- Immune Diffusion: Antigen and antibody diffuse through a gel, forming a precipitin line.
- ELISA (Enzyme-Linked Immunosorbent Assay): A plate-based assay technique for detecting and quantifying substances.

Hypersensitivity and Autoimmunity

- Hypersensitivity: Excessive or inappropriate immune responses (e.g., allergies).
- Autoimmunity: Immune responses against self-antigens, leading to autoimmune diseases.

UNIT III: Introduction, Tools, and Techniques of rDNA Technology

Introduction to rDNA Technology

Recombinant DNA technology involves joining DNA from different sources and inserting it into a host organism to produce new genetic combinations.

Steps Involved in Cloning

1. Gene Identification and Isolation: Identifying and extracting the gene of interest.
2. Insertion into Vector: Incorporating the gene into a cloning vector.
3. Transformation: Introducing the recombinant vector into a host cell.
4. Selection and Screening: Identifying cells that contain the recombinant DNA.

Tools of Genetic Engineering

- Cloning Vectors: Plasmids and cosmids used to carry foreign DNA into host cells.
- Restriction Endonucleases: Enzymes that cut DNA at specific sequences.
- DNA Ligase: Enzyme that joins DNA fragments.
- Hosts: Organisms such as bacteria and yeast used for cloning.

Polymerase Chain Reaction (PCR)

A technique to amplify a specific DNA segment.

Blotting Techniques

- Southern Blotting: Detects specific DNA sequences.
- Northern Blotting: Detects specific RNA sequences.
- Western Blotting: Detects specific proteins.

DNA Sequencing

- Sanger Sequencing: Method of determining the nucleotide sequence of DNA.

Site-Directed Mutagenesis

A technique to introduce specific mutations into a DNA sequence.

UNIT IV: Cloning Strategies and Application of rDNA Technology

rDNA Library Construction

Creating a collection of DNA fragments that represent the entire genome of an organism.

Methods of Transformation

- Chemical Transformation: Using chemical solutions to introduce DNA into cells.
- Electroporation: Using electrical pulses to introduce DNA into cells.

Recombinant Selection and Screening Methods

Techniques to identify cells that have successfully incorporated recombinant DNA.

Applications of rDNA Technology

- Agriculture: Transgenic plants, edible vaccines, and antibodies.
- Medicine: Disease diagnosis and DNA fingerprinting.

UNIT V: Bioinformatics

Databases

- PubMed: A database of biomedical literature.
- NCBI (National Centre for Biotechnology Information): Provides access to a variety of biological databases.

- EMBL (European Molecular Biology Laboratory): Provides access to nucleotide sequences.
- ExPASy (Expert Protein Analysis System): A resource for proteomics tools.

Sequence Analysis

- BLAST (Basic Local Alignment Search Tool): Used for comparing nucleotide or protein sequences.
- Clustal W: A tool for multiple sequence alignment.
- Phylogenetic Tree Construction: Methods to infer evolutionary relationships among sequences.

Omics

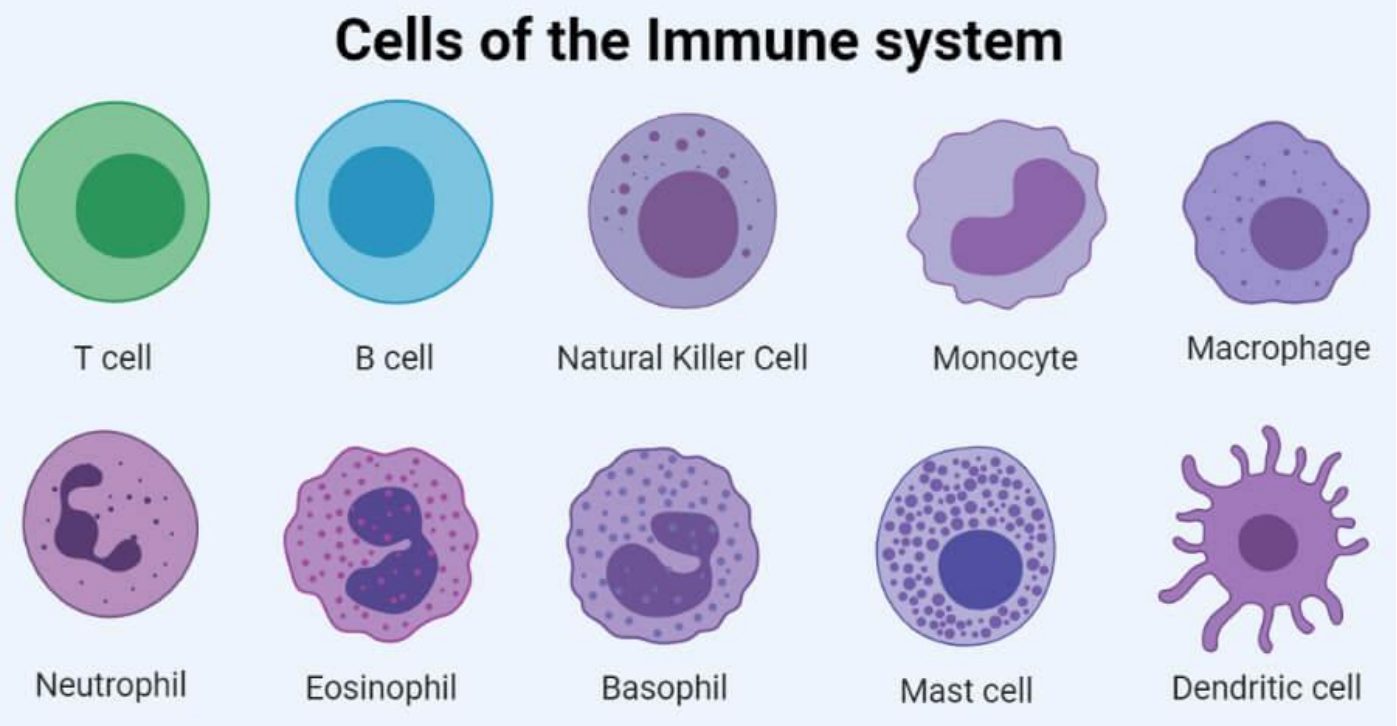
- Proteomics: Study of the entire set of proteins expressed by a genome.
- Genomics: Study of the complete set of DNA in an organism.
- Transcriptomics: Study of the complete set of RNA transcripts produced by the genome.

The immune system is made up of different immune organs and tissues located all over the body. The immune organs are categorized based on their functions and there are two main categories, which include:

1. Primary lymphoid organs provide a development and maturation site for lymphocytes, and
2. Secondary lymphoid organs whose function includes trapping antigens from the tissues, and the vascular spaces. They are also the site for lymphocyte interaction with the antigens.

All these organs are connected by the lymphatic system and the blood vessels into a functional unit. In the blood and the lymph and populating the lymphoid organs are various types of white blood cells (leukocytes) that play a key role in the body's immune responses, therefore defining the cells of the immune system. Nevertheless, white blood cells are an assemblage of different immune cells. White blood cells provide the defense mechanisms of the body fighting off foreign elements (antigens) from the body. Under the White Blood Cells group of cells, they can be categorized into lymphocytes, (including T-lymphocytes, B-lymphocytes, and Natural Killers cells), neutrophils, monocytes, and macrophages.

In all these categories, only the lymphocytes have the characteristics of diversity, specificity, memory, and self/nonself recognition, which are the hallmark features of the adaptive immune responses. All the other cells play additional roles in adaptive immunity such as activation of lymphocytes, increasing the effector mechanisms of antigen clearance by phagocytosis, or secreting various immune-effector molecules. Some white blood cells secrete protein molecules known as cytokines which are immunoregulators (regulate the immune responses). Other major proteins of the immune system include antibodies produced by B-lymphocytes, and complement proteins (activated by antibodies).



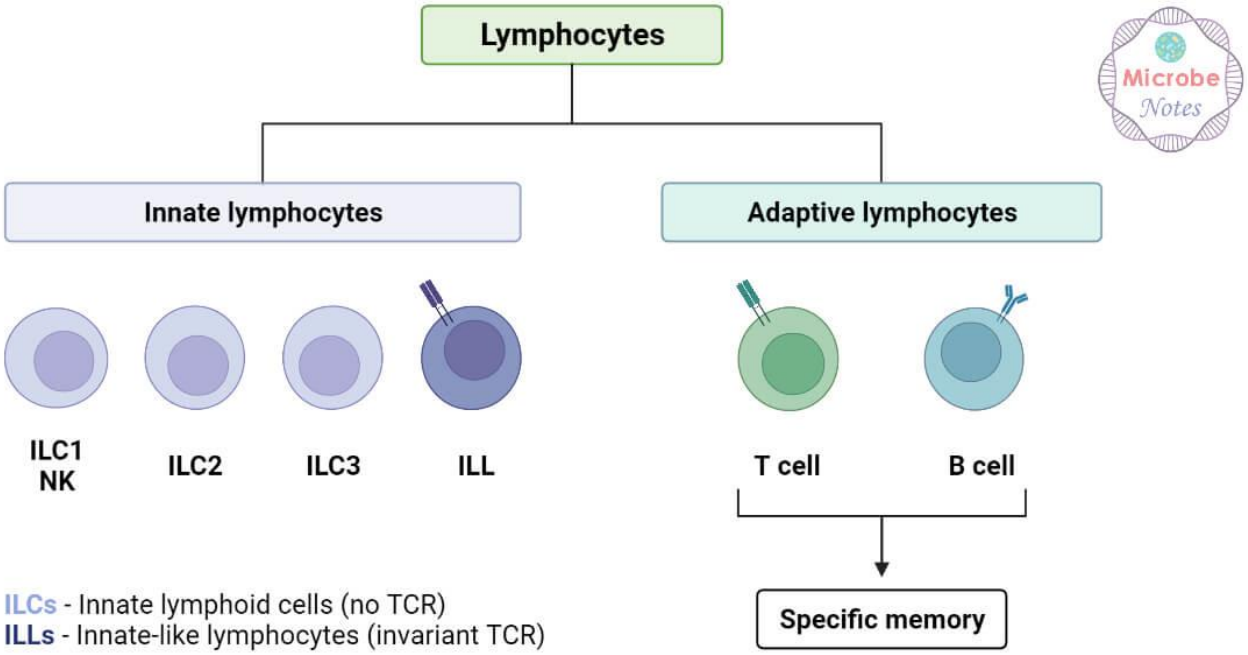
Cells of the immune system

Types of Cells of Immune System

The lymphocytes are the central cells of the immune system which are responsible for adaptive immunity and immunological features of diversity, specificity, memory, and self/non-self-recognition. The other immune cells function to engulf and destroy micro-organisms, present antigens, and secrete cytokines.

Lymphoid cells (Lymphocytes)

The lymphocytes make up 20%–40% of the body's white blood cells and 99% of the cells in the lymph. There are about 10^{11} lymphocytes in the human body. These lymphocytes circulate continuously in the blood and the lymph hence they are able to migrate into the body tissue spaces and lymphoid organs, therefore integrating the immune system to a high degree.

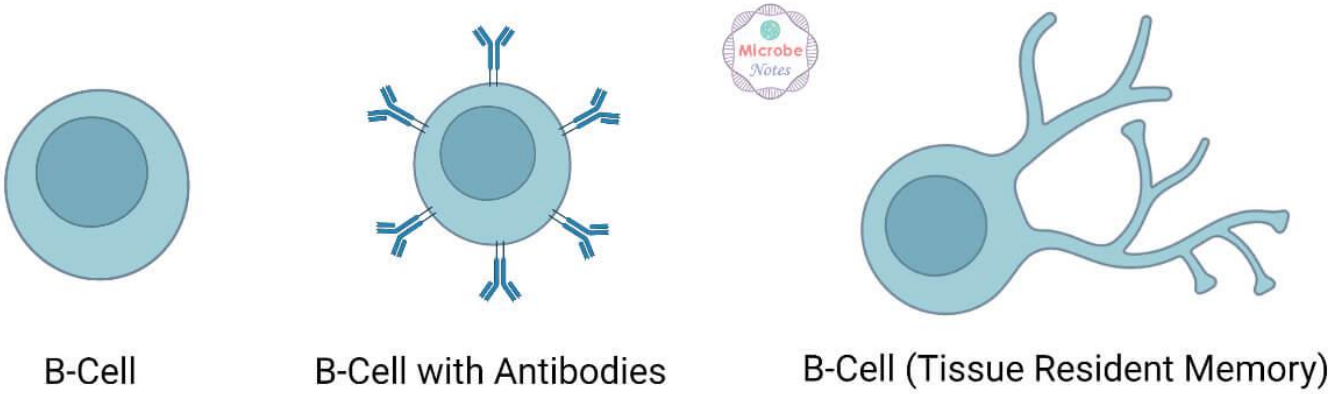


Types of Lymphocytes

The lymphocytes are broadly divided into three populations based on their functions and cell-membrane components i.e:

1. B-lymphocytes

- B-lymphocytes are also known as B-cells and on lab reports, they are known as CD19 or CD20 cells.
- They are the specialized cells of the immune system whose major function is to produce antibodies also known as immunoglobulins or gamma globulins.
- B-lymphocytes are synthesized and mature in the bone marrow from the hematopoietic stem cells, and after which they mature, migrate, and express themselves by forming unique antigen-binding receptors on their membranes, known as B-cell receptors or antibodies.
- Migration of mature B-cells moves to the bone marrow, lymph nodes, spleen, some parts of the intestines, and the bloodstream.



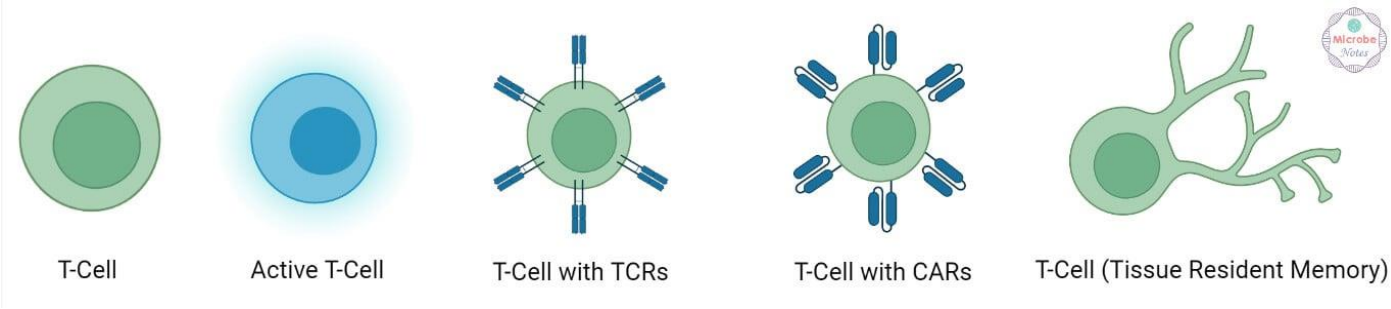
B-Cells

- When a naive B-cell interacts with an antigen for the first time and it has to match membrane-bound receptors (antibodies), the antibodies bound to the B-cell bind the antigen causing the B-cell to divide rapidly, and its progenitors to differentiate into memory B-cells and effector B-cells known as plasma cells.
- The Memory B-cells have a long life span than the naive cells, expressing the same membrane-bound antibody as the parent B-cells.
- The plasma cells are responsible for producing the antibodies that can be secreted into the bloodstream, tissues, respiratory secretions, intestinal secretions, and tears.
- Therefore, antibodies are highly specialized serum protein molecules.
- The plasma cells have a short life span of few days but they secrete large amounts of antibodies during this time, with approximately 2000 molecules of antibodies per plasma cell per second.
- The secreted antibodies play the major effector roles in the humoral immune responses.
- Note that, during maturation, B-cells are trained not to produce antibodies on healthy tissues.
- The antibody molecules are specifically designed for every foreign antigen they encounter and interact like a lock and key mechanism.
- Therefore B-cells have the ability to produce vitally a variety of antibodies for all microbes in our environment, however as stated above, each plasma cell produces only one kind of antibody.
- Antibodies' varieties are based on their specialized functions in the body with variations in their chemical structure, which ultimately determine the class of antibody.

2. T-Lymphocytes

- T-lymphocytes are also known as T-cells, often named in lab reports as CD3 cells
- They also arise in the bone marrow but migrate to the thymus gland for maturation, where they express a unique antigen-binding molecule on its membrane known as the T-cell receptor.
- The name T originated from its site of maturation, the Thymus.
- Mature T-cells leave the thymus and populate other organs of the immune system, such as the spleen, lymph nodes, bone marrow, and blood.
- Unlike the B-cell receptors that can recognize antigens alone, T-cell receptors only recognize antigens that are bound to cell membrane proteins known as Major Histocompatibility Complex (MHC) molecules.
- The MHC molecule recognizes antigens that are presented to them by antigen-processing cells (APCs) on their cell membrane.
- The two major classes of MHC molecules are Class I MHC molecules, which are expressed by nearly all nucleated cells of vertebrate species, consist of a heavy chain linked to a small invariant protein called 2-microglobulin. Class II MHC molecules, which consist of an alpha and a beta glycoprotein chain, are expressed only by antigen-presenting cells.

- When a naive T cell encounters an antigen combined with an MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells.



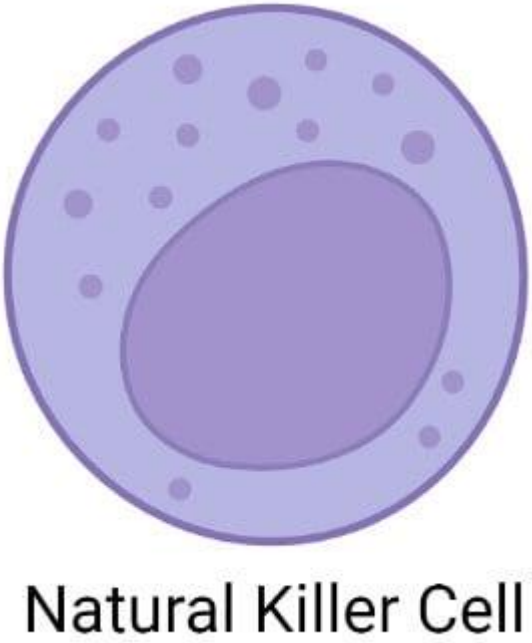
T-Cells

- The T-cells are classified into three categories: T helper (Th), T cytotoxic (Tc), and T suppressor (Ts) cells.
- The Th and Tc cells are differentiated from each other with the presence of their CD4 and CD8 membrane glycoproteins on their surfaces.
- T cells naturally displaying CD4 function as T helper (Th) cells while those displaying CD8 naturally function as T cytotoxic (Tc) cells.
- The Th cells recognize and interact with antigens that are presented on the MHC class II molecule complex, then they become activated becoming effector cells that are able to secrete various growth factors that are collectively known as cytokines.
- The cytokines that are secreted are actively involved in the activation of B-cells, T-cytotoxic cells, macrophages, and other immune cells.
- The cytokine patterns produced by the activated Th cells result in different immune responses. The Th-derived cytokines enable the recognition of an antigen-MHC class I molecule complex by the Tc cells which then proliferate and differentiate into effector cells known as Cytotoxic T-lymphocytes (CTL).
- The T-cytotoxic cells have the ability to induce cytokine secretion, unlike the Cytotoxic T-Cells which do not induce secretion of cytokines, rather they exhibit cell-killing or cytotoxic activity.
- Cytotoxic T-lymphocytes (CTL) play a key role in monitoring the body cells and eliminating any of these cells that display antigens such as tumor cells, cells infected with viruses, and cells of a foreign tissue graft.
- CTLs target foreign antigen (altered self-cells) complexes displayed by the class I MHC molecule.

3. Natural killer cells (NK cells)

- These are large granular lymphocytes, that do not express surface markers like the B and T-cell lineages
- They were first described in 1976 by indications of the presence of a small population of large granular lymphocytes that had a cytotoxic effect against a wide range of tumor cells in the absence of any previous immunization with the tumor.

- These cells also indicated that they play key roles in host defense against tumor cells and cells infected with some, not all viruses.
- They constitute 5-10% of lymphocytes in the human peripheral blood.



Natural Killer Cell

- Their ability to recognize antigens is based on two mechanisms:
    - They can employ NK cell receptors to distinguish abnormalities such as a reduction in the expression of class I MHC molecules and the abnormal profile of the surface antigens that are displayed by some tumor cells and cells infected by some viruses.
    - Secondly, the NK cells also recognize potential target cells which are tumor cells and cells that are infected by viruses. These target cells display antigens against which the immune system has already produced antibody response to as antitumor or antiviral antibodies, that bind to the surfaces of these targets.
- The NK cells express membrane receptors called CD16, which are receptors for the carboxyl-terminal end of the IgG molecule, Fc region. The NK CD16 receptors attach to these antibodies and destroy the targeted cells subsequently, by a mechanism known as the Antibody-dependent cell-mediated cytotoxicity (ADCC).
- Therefore, NK cells play an important role in host defense mechanisms against tumors.
- For example, in humans, the Chediak-Higashi syndrome, an autosomal recessive disorder is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called beige lack NK cells; these mutations are more susceptible to tumor growth than normal mice following injection with live tumor cells.
- There are some unique NK cells known as the NK1-T-cells which have been recognized to have some combined characteristics of the T-lymphocytes and the Natural Killer cells. They have T-cell receptors (TCRs) that interact with the MHC-like molecule known as a CD1, unlike the normal TCRs of the T-

cell which interact with class I or class II MHC molecules. Additionally, like the NK cells, they have variable levels of CD16 and other NK receptors which enable it to kill cells.

- A population that is triggered by NK1-T cells secretes large amounts of cytokines, rapidly. These cytokines support the antibody production by B-cells, also inflammation, and the development and expansion of cytotoxic T-cells.
- Some immunologists view this cell type as a kind of rapid response system that has evolved to provide early help while conventional TH responses are still developing.

Mononuclear Phagocytes

- These are immune cells i.e monocytes that are freely circulating in blood and macrophages that are found in the tissues.
- During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they differentiate further into mature monocytes.
- Monocytes circulate in the bloodstream for about 8 h, during which they enlarge and then migrate into the tissues and differentiate into specific tissue macrophages or into dendritic cells.
- Differentiation of monocyte into a tissue macrophage involves a number of changes
    - The cell enlarges five- to tenfold
    - Its intracellular organelles increase in both number and complexity
    - It acquires increased phagocytic ability and produces higher levels of hydrolytic enzymes
    - It begins to secrete a variety of soluble factors.
- Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages.
- Free macrophages travel by amoeboid movement throughout the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:
    - Alveolar macrophages in the lung
    - Histiocytes in connective tissues
    - Kupffer cells in the liver
    - Mesangial cells in the kidney
    - Microglial cells in the brain
    - Osteoclasts in bone
- Macrophages are normally in a resting phase but they can be activated by several immune responses.

- For example, the phagocytic mechanism of certain antigens is normally the initial stimulus for macrophages. However, macrophage activity can be further enhanced by cytokines secreted by activated TH cells, by mediators of the inflammatory response, and by components of bacterial cell walls.
- One of the most potent activators of macrophages is interferon-gamma which is secreted by activated TH cells.
- Activated macrophages effectively eliminate potential pathogens than the resting macrophages because they exhibit greater phagocytic activity, an increased ability to kill ingested microbes, increased secretion of inflammatory mediators, and an increased ability to activate T cells.
- Additionally, activated macrophages, but not resting ones, secrete various cytotoxic proteins that help them eliminate a broad range of pathogens, including virus-infected cells, tumour cells, and intracellular bacteria.
- Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. Thus, macrophages and TH cells facilitate each other's activation during the immune response.
- Some of the functions of macrophages include:
    - Phagocytosis -Phagocytosis of bacteria, viruses, and other foreign particles is the most important function of macrophages. The macrophages on their cell surfaces have Fc receptors that interact with the Fc component of the IgG, thereby facilitating the ingestion of the opsonized organisms. They also have receptors for C3b, another important opsonin. After ingestion, the phagosome containing the microbe fuses with a lysosome. The microbe within the phagolysosome is killed by reactive oxygen, reactive nitrogen compounds, and lysosomal enzymes.
    - Antimicrobial and cytotoxic activities include the oxygen-dependent and oxygen-independent cytotoxicity/killing.
    - Antigen processing – After ingestion and degradation of foreign materials, the fragments of antigen are presented on the macrophage cell surface in conjunction with class II MHC proteins for interaction with the TCR of CD4+ helper T cells. Degradation of the foreign protein is stopped following the association of antigen with the class II MHC proteins in the cytoplasm. This is followed by transportation of the complex to the cell surface by transporter proteins.
    - Secretion of growth factors important for the development of an immune response such as cytokines, such as interleukin 1 (IL-1), TNF-α, and interleukin 6 (IL-6), that promote inflammatory responses, complement proteins, hydrolytic enzymes, and a cascade of Tumor Necrotic Factors, TNF-α (GM-CSF, G-CSF, M-CSF) that induce and kill tumor cells and promote hematopoiesis.

Granulocytic Cells

- Granulocytes are white blood cells (leukocytes).

- They are classified based on their cellular morphologies and cytoplasmic staining characteristics and they include neutrophils, eosinophils, basophils, or mast cells.
- All granulocytes have multilobed nuclei that make them visually distinctive and easily distinguishable from lymphocytes, whose nuclei are round. The cytoplasm of all granulocytes is replete with granules that are released in response to contact with pathogens.
- These granules contain a variety of proteins with distinct functions: Some damage pathogens directly; some regulate trafficking and activity of other white blood cells, including lymphocytes; and some contribute to the remodelling of tissues at the site of infection.
- Neutrophils have a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorphonuclear leukocyte (PMN) for its multilobed nucleus.
- The eosinophils have a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name).
- The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue.
- Both neutrophils and eosinophils are phagocytic, whereas basophils are not.
- Neutrophils constitute the majority (50% to 70%) of circulating leukocytes and are much more numerous than eosinophils (1%–3%), basophils (≤ 1%), or mast cells (≤ 1%).

Neutrophils

- Neutrophils are produced by haematopoiesis n the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days.
- In the bone marrow, a surmountable level of neutrophils is produced in response to the types of infections and they are normally the first cells that arrive at the site of inflammation.
- The resulting transitory increases in the number of circulating neutrophils known as leucocytosis, which is an indicator of an infection, medically.
- The movement of circulating neutrophils into tissues is also known as extravasation.
- Extravasation takes place in several steps:
    - Adherence to the vascular endothelium
    - Penetration into the gap between adjacent endothelial cells lining the vessel wall
    - Penetration into the vascular basement membrane and moving out into the tissue spaces.
- Several substances can be generated during an inflammatory reaction which serves as chemotactic factors. They promote the accumulation of neutrophils at the site of inflammation. Some of these chemotactic factors include complement components, blood-clotting system components, and several cytokines secreted by activated Th Cells and macrophages.
- The functions include:

- Neutrophils are also active phagocytes just like macrophages and the mechanism of phagocytosis is similar to that of macrophages except for the lytic enzymes and bactericidal substances in neutrophils which are contained within primary and secondary granules.
- The neutrophils have larger denser primary granules which are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes, and smaller secondary granules that contain collagenase, lactoferrin, and lysozyme.
- Both the primary and the secondary granules fuse with the phagosomes and digest and eliminate the contents similar to macrophages.
- Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances.
- Neutrophils exhibit a larger respiratory burst than macrophages and they are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates.
- Neutrophils also express higher levels of defensins than macrophages.

Eosinophils

- They are motile phagocytic cells that can migrate from the blood into the tissue spaces.
- They have a phagocytic mechanism of eliminating antigens but their role as phagocytic cells is much less significant than that of neutrophils.
- They play a role in defense against multicellular parasitic organisms including worms.
- The secreted contents of eosinophilic granules may damage the parasite membrane. They can be found clustering around invading worms, whose membranes are damaged by the activity of proteins released from eosinophilic granules. Like neutrophils and basophils, eosinophils may also secrete cytokines that regulate B and T lymphocytes, thereby influencing the adaptive immune response.
- In areas where parasites are less of a health problem, eosinophils are better appreciated as contributors to asthma and allergy symptoms.

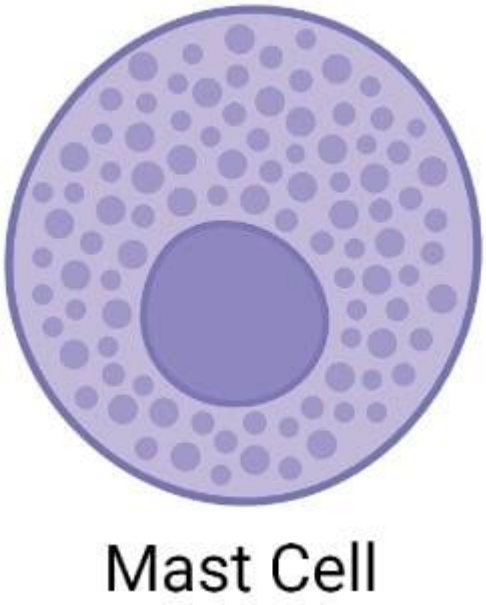
Basophils

- Basophils are nonphagocytic granulocytes containing large granules that are filled with basophilic proteins that stain blue in standard H & E staining methodologies.
- Naturally, basophils are in the body's normal circulation but they can be very potent.
- They function by binding to circulating antibodies and react by the content of their granules which are pharmacologically active substances found in their cytoplasm.
- These substances play a major role in certain allergic responses. For example, histamines are the most common and well-known protein in that basophilic granule. They play a role in increasing blood vessel permeability and smooth muscle activity.
- Additionally, just like the eosinophils, basophils are also crucial in response to parasites, and particularly the helminths (worms).

- Basophils also secrete cytokines that assist in the modulation of the adaptive immune response.

Mast Cells

- Mast cells are formed in the bone marrow.
- They are released from the bone marrow into the blood as undifferentiated cells, and when they enter the tissues, they then mature.
- Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts.



Mast Cell

- Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances.
- Mast cells also play an important role in the development of allergies.
- Basophils and mast cells share many characteristics however, their relationship is not unequivocally understood. Some speculations state that basophils are the blood-borne version of mast cells; others speculate that they have distinct origins and functions.

Dendritic Cells

- These are special cells that were discovered by Ralph Steinman in the mid-1970s, and he won a noble prize in 2011 for this discovery.
- The dendritic cells acquire their name because they are covered with long membrane extensions resembling the dendrites of the nerve cells.
- Their membranous extension extends and retracts dynamically, increasing the surface area available for browsing lymphocytes.

- They are very diverse according to research, and they seem to arise from both the myeloid and lymphoid lineages of hematopoietic cells.
- They are not easily isolated by conventional methods because the cell isolation damages their long extensions.
- Dendritic cells generally perform the distinct functions of antigen capture in one location and antigen presentation in another.
- Outside lymph nodes, immature dendritic cells monitor the body for signs of invasion by pathogens and capture intruding or foreign antigens.
- They then process these antigens, then migrate to lymph nodes, where they present the antigen to naïve T cells, initiating the adaptive immune response.
- When acting as guards in the periphery, immature dendritic cells take on their cargo of antigen in three ways.
    - engulf it by phagocytosis
    - internalize it by receptor-mediated endocytosis
    - absorb it by pinocytosis.
- During the maturation process though, they shift from an antigen-capturing phenotype to one that is specialized for the presentation of antigen to T cells. When transitioning, some attributes are lost and others are gained. Lost is the capacity for phagocytosis and large-scale pinocytosis.
- However, the ability to present antigen increases significantly, as does the expression of costimulatory molecules that are essential for the activation of naïve T cells.
- After activation, dendritic cells abandon residency in peripheral tissues, enter the blood or lymphatic circulation, and migrate to regions of the lymphoid organs, where T cells reside, and present antigen.
- There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to TH cells.
- There are four types of dendritic cells known:
    - Langerhans cells
    - interstitial dendritic cells
    - myeloid cells
    - lymphoid dendritic cells.
- Each of these types arises from hematopoietic stem cells via different pathways and in different locations.
- However different, they all constitutively express high levels of both class II MHC molecules and members of the co-stimulatory B7 family.

- Therefore, they are representatively more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs).
- Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to TH cells.
- Following microbial invasion or during inflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to TH cells that is required for the initiation of responses by those key cells.
- Another type of dendritic cell, the follicular dendritic cell, does not arise in the bone marrow and has a different function from the antigen-presenting dendritic cells.
- Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigen-presenting cells for TH-cell activation.
- These follicular dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles, which are rich in B cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody, which allows the binding of antigen-antibody complexes.
- The interaction of B cells with this bound antigen can have important effects on B cell responses.

Vaccinology and Clinical Immunology

A vaccine is a medical preparation given to provide immunity from a disease. Vaccines use a variety of different substances ranging from dead microorganisms to genetically engineered antigens to defend the body against potentially harmful microorganisms.

Effective vaccines change the immune system by promoting the development of antibodies that can quickly and effectively attack disease-causing microorganisms when it enters the body, preventing disease development.

A vaccine may contain live-attenuated or killed microorganisms or parts or products from them capable of stimulating a specific immune response comprised of protective antibodies and T cell immunity.

A vaccine should stimulate a sufficient number of memory T and B lymphocytes to yield effector T cells and antibody-producing B cells from memory cells.

The viral vaccines should also be able to stimulate high titers of neutralizing antibodies.

Injection of a vaccine into a nonimmune subject induces active immunity against the modified pathogens.

Vaccination is immunization against infectious disease through the administration of vaccines to produce active (protective) immunity in humans or other animals.

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- Injection of a vaccine into a nonimmune subject induces active immunity against the modified pathogens.
- **Vaccination** is immunization against infectious disease through the administration of vaccines to produce active (protective) immunity in humans or other animals.

Definition and History of Vaccines

- **According to the CDC, a vaccine is a preparation used to stimulate the body's immune response against diseases. Vaccines are usually administered through needle injections, but some can be administered by mouth or sprayed into the nose.**
- The history of vaccines trails down to 1877 when Loise Pasteur developed a vaccine using a weakened strain of the anthrax bacillus, *Bacillus anthracis*. He adapted a methodology of attenuating the culture of anthrax bacillus by incubation at a high temperature of 42–43°C and inoculated the attenuated bacilli in the animals, demonstrating that animals receiving inoculation of such attenuated strains developed specific protection against anthrax.
- This concept was successfully demonstrated on a farm at Pouilly-le-Fort in 1881 by vaccinating sheep, goats, and cows with the attenuated anthrax bacillus strain. The result indicated that all the vaccinated animals survived an anthrax attack which the non-vaccinated could not, hence they died of anthrax.
- In 1885, Louis Pasteur successfully prevented rabies through post-exposure vaccination. The treatment is controversial. Pasteur has unsuccessfully attempted to use the vaccine on humans twice before, and injecting a human with a disease agent is still a new and uncertain method

- Pasteur coined the term **vaccine** in commemoration of Edward Jenner who used such preparations for protection against smallpox. This led to the establishment of various institutions in several countries in the world that prepared vaccines and studied infectious diseases such as the Pasteur Institute in Paris.
- **Types of Vaccines and Their Characteristics**
- Vaccines have proved to have a strong defense against some of the most fatal diseases and if they were still unavailable, the survival of individuals would be based on their immune defenses which could either resolve the infection or lead to death from the infection.
- Therefore, the use of vaccines means, the vaccine will mimic the pathogen and cause an immune response that is similar to that that can be activated by the pathogen.
- Historically, these vaccines have eliminated fatal infections such as smallpox, almost eliminated polio, and saved many individuals from typhus, tetanus, hepatitis A and B, measles, rotavirus diseases, etc.
- However, still successful vaccines are yet to be developed for many deadly diseases that cause chronic infections such as AIDS, hepatitis C, tuberculosis, malaria, and herpes
- Successful vaccines against these chronic diseases must be able to stimulate immune responses that are similar to those resulting from most natural exposures to the pathogen but still remains a challenge.
- Various vaccines have been designed and here is a detailed approach to how these vaccines have been developed, those in use, and those still under experimentation.
- Major advances in understanding the complexities of the interaction of pathogens or microbes with the human host have revolutionized vaccine developments and advances in recent times. Coupled with advances in laboratory techniques and technologies, have aided the development of new vaccine types.
- Some more developed approaches such as vaccinomics, which is the application of genomics and bioinformatics to vaccine development, is a new approach that may solve the problem of developing vaccines against microbes and parasites.

Vaccine types can broadly be classified into three groups:

- **1. Whole-organism Vaccines**
- Inactivated (Killed) Vaccine
- Live-attenuated vaccines
- Chimeric vaccine
- **2. Subunit Vaccines**
- Polysaccharide Vaccine
- Conjugated Vaccines
- Toxoid Vaccines
- Recombinant Protein Vaccines
- Nanoparticle vaccines
- **3. Nucleic Acid Vaccines**
- DNA plasmid vaccines
- mRNA vaccines
- Recombinant vector vaccine
- **Whole-organism Vaccines**
- Many vaccines that were developed early consist of an entire pathogen that is either killed (inactivated) or weakened (attenuated) so that they cannot cause disease. They are known as the **whole-organism vaccines.** These vaccines elicit strong protective immune responses and many vaccines used today are prepared in this manner, but not all disease-causing microbes can be effectively targeted with a whole-organism vaccine.

- **1. Inactivated (Killed) Vaccine**
- These were produced by killing the pathogen (bacteria, virus, or other pathogens) with chemicals or heat, or radiation.
- The killed pathogen can not cause disease, and this means that they do not replicate in the host's body.
- **Advantage**: These vaccines are stable and safer than the live attenuated vaccines
- **Disadvantage**: The major disadvantage of this type of vaccine is that it elicits a weaker immune response and therefore, it requires more vaccine dosages and a booster dose as well, so as to confer protective immunity.
- **Examples of Inactivated Vaccines** include poliomyelitis (sulk vaccine), rabies, typhoid, cholera, pertussis, pneumococcal, rabies, hepatitis B, and influenza vaccines.
- **2. Live-attenuated vaccines**
- These vaccines were developed in the 1950s when advances in tissue culture techniques were developed.
- These vaccines are prepared from a whole organism, by weakening their pathogenicity so that they can not cause disease but can induce an immune response, hence the term **attenuation**.
- These vaccines elicit strong immune responses because they are similar to the actual disease pathogen and hence they confer a life-long immunity after only one or two doses, therefore they are very effective.
- They are also relatively easy to create for certain viruses, but difficult to produce for more complex pathogens like bacteria and parasites.
- **Disadvantages**: There is a remote chance that the weakened germ can mutate or revert back to its full strength and cause disease.
- Live attenuated vaccines should not be given to individuals with weakened or damaged immune systems.
- To maintain potency, live attenuated vaccines require refrigeration and protection from light.
- Examples include Measles/Mumps/Rubella (**MMR**) and Influenza Vaccine Live, Intranasal (**FluMist®**), Polio (Sabin vaccine), Rotavirus, Tuberculosis, Varicella, Yellow fever.
- The attenuated strain of *Mycobacterium bovis* called Bacillus Calmette- Guérin (BCG) was developed by growing *M. bovis* on a medium containing increasing concentrations of bile. After 13 years, this strain had adapted to growth in strong bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis.
- **3. Chimeric vaccine**
- The evolution of modern genetic engineering techniques has enabled the creation of chimeric viruses, which contain genetic information from one viral particle and display the biological properties of different parent viruses.
- An NIAID-developed live-attenuated **chimeric vaccine** consisting of a dengue virus backbone with Zika virus surface proteins is undergoing early-stage testing in humans.
- Whole-organism vaccines, whether alive or dead, have another big drawback. Considering that they are composed of complete pathogens, they retain molecules that are not involved in evoking immunity, including unavoidable byproducts of the manufacturing process such as contaminants that can trigger allergic or immune disruptive reactions.
- **Subunit Vaccines**
- These are vaccines that are prepared by using components or antigens of the pathogen. These components can stimulate the immune system to elicit appropriate immune responses.
- They are also known as acellular vaccines because they do not contain a whole cell, but just part of a cell of the bacteria or virus.
- These vaccines were produced to cub the inefficiencies of the live attenuated and killed vaccines prepared from whole organisms such as adverse reactions associated with the vaccines and the mutations that may lead to the virulent strains of the pathogens.

- The subunit vaccines are safe and easier to produce, however, they require the use of an adjuvant in order to produce a stronger protective immune response. This is because an antigen alone can not be able to produce sufficiently enough long-term immunity.
- One of the earliest vaccines produced against pertussis was an inactivated *Bordetella pertussis* bacteria preparation in the 1940s, but this vaccine caused minor adverse reactions such as fever and swelling at the injection site, hence the vaccine was avoided leading to a decrease in its vaccination and therefore an increase in cases of pertussis infections. This led to the development of acellular pertussis vaccines that were based on purified *B. pertussis* components. These newly prepared vaccines had no adverse reactions associated with their administration.
- Some of the subunit vaccines produced to prevent bacterial infections are based on the polysaccharides or sugars that form the outer coating of many bacteria. Therefore, there are subtypes of subunit vaccines as follows:
- **1. Polysaccharide Vaccine**
- Some microbes contain a polysaccharide (sugar) capsule which they use for protection and evading the human immune defenses, especially in infants and young children.
- Therefore, these are vaccines that are prepared using the sugar molecules, and polysaccharides from the outer layer of a bacteria or virus.
- They create a response against the molecules in the pathogen's capsule. Normally these molecules are small hence they are not immunogenic (can not induce an immune response on their own). Hence, they tend to be ineffective in infants and young children between 18-24 months, and they induce a short-term immunity associated with slow immune responses, and slow activation, and it does not increase antibody levels and it does not create an immune memory.
- Therefore, these sugar molecules are chemically linked to carrier proteins and work similarly to conjugate vaccines.
- **Examples of polysaccharide vaccines** include Meningococcal disease caused by *Neisseria meningitidis* groups A, C, W135, and Y, as well as Pneumococcal disease.
- **2. Conjugated Vaccines**
- These vaccines are prepared by linking the polysaccharides or sugar molecules on the outer layer of the bacteria to a carrier protein antigen or toxoid from the same microbe.
- The polysaccharide coating disguises a bacterium's antigens so that the immature immune systems of infants and younger children cannot recognize or respond to them.
- Conjugate vaccines get around this problem through the linkage of polysaccharides with a protein.
- This formulation greatly increased the ability of the immune systems of young children to recognize the polysaccharide and develop immunity.
- The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.
- Today, conjugate vaccines are also available to protect against pneumococcal and meningococcal infections.
- **3. Toxoid Vaccines**
- These vaccines are prepared from inactivated toxins, by treating the toxins with formalin, a solution of formaldehyde, and sterilized water.
- This process of inactivation of toxins is known as **detoxification** and the resultant inactive toxin is known as a **toxoid.**
- Detoxification makes the toxins safe to use.
- The toxins used for the preparation of toxoids are obtained from the bacteria that secrete the illness-causing toxins.
- This means that when the host body receives the harmless toxoid. the immune system adapts by learning how to fight off the natural bacterial toxin responsible for causing illness, by producing antibodies that lock onto and block the toxin.
- **Examples of toxoid vaccines** include diphtheria and tetanus toxoid vaccines.
- **4. Recombinant Protein Vaccines**

- After the start of the generic engineering era, recombinant DNA technology also evolved. This is where DNA from two or more sources is combined. This technology harnessed the development of recombinant protein vaccines.
- For recombinant vaccines to induce immunity against a pathogen, they have to be administered along with an adjuvant or expresses by a plasmid or a harmless bacterial or viral vectors.
- Production of these recombinant protein vaccines involves the insertion of DNA encoding an antigen such as a bacterial surface protein, which stimulates an immune response into bacterial or mammalian cells, expressing the antigen in these cells, and then the antigen is purified from them.
- **Advantages:**
- Recombinant protein vaccines allow the avoidance of several potential concerns raised by vaccines based on purified macromolecules. For example, the presence of contaminants in vaccines after purification may cause potential harm to the host.
- The production of recombinant vaccines also allows the production of sufficient quantities of purified antigenic components.
- The classical **example of a recombinant protein vaccine** currently in use in humans is the vaccine against hepatitis B. The vaccine antigen is a hepatitis B virus protein produced by yeast cells into which the genetic code for the viral protein has been inserted.
- Vaccines that are also used to prevent human papillomavirus (HPV) infections are also based on the recombinant protein antigens, by preparing from the proteins of the outer shell of HPV, which form particles that almost resemble the virus.
- The virus-like particles (VLPs) prompt an immune response that is similar to that elicited by the natural virus, and they are non-infectious since they do not contain the genetic materials that the virus needs to replicate inside the cells.
- An experimental recombinant protein vaccine for chikungunya fever has also been designed by the National Institute of Allergy and Infectious Disease (NIAID).
- **5. Nanoparticle vaccines**
- This vaccine development was based on a strategy to present protein subunit antigens into the immune system.
- The NIAID has also designed a universal flu vaccine, an experimental vaccine with protein ferritin which can self-assemble into microscopic pieces known as nanoparticles that display a protein antigen.
- A nanoparticle-based influenza experimental vaccine is also being evaluated in human trials (early stages).
- This new technology of vaccine delivery is also being evaluated and assessed for the development of vaccines against MERS coronavirus, respiratory syncytial virus (RSV), and Epstein-Barr virus.
- Recent advances in the subunit vaccine development and delivery systems include solving the atomic structures of proteins. For example, NIAID has been able to solve the 3-D structure of a Respiratory Syncytial Virus (RSV) surface-bound to an antibody, identifying a key part of the protein that is highly sensitive to neutralizing antibodies. They were then able to modify the RSV protein to stabilize the structural form in which it displays the neutralization-sensitive site.
- Subunit vaccines are also being developed to offer broad protection against various infections such as malaria, Zika, chikungunya, and dengue fever.
- The experimental vaccine, designed to trigger an immune response to mosquito saliva rather than a specific virus or parasite, contains four recombinant proteins from mosquito salivary glands.
- **Nucleic Acid Vaccines**
- These are vaccines designed to aim at introducing the genetic materials that code the antigen or the antigen that is aimed at inducing an immune response, enabling the host cells to use the genetic materials to produce the antigens.
- The advantages of the nucleic acid vaccine approach include:
- stimulating a broad long-term immune response
- excellent vaccine stability
- ease of large-scale vaccine manufacture

- rapid production
- reduces potential risks of working with the live pathogen
- encoding only the key antigen without including other proteins
- The advantage of the ease of production is a potential game-changer for targeting epidemic or emerging diseases where rapidly designing, constructing, and manufacturing the vaccine are crucial
- Some of the know nucleic acid vaccines models include:
- **1. DNA plasmid vaccines**
- These are vaccines that are composed of a small circular piece of DNA known as a plasmid. The plasmid carries genes that encode proteins from the pathogen of interest.
- Experimental DNA plasmid vaccines have been designed by the National Institute of Allergy and Infection Disease (NIAID) to address some viral disease threats including SARS coronavirus (SARS-CoV) in 2003, H5N1 avian influenza in 2005, H1N1 pandemic influenza in 2009, and Zika virus in 2016.
- **2. mRNA vaccines**
- mRNA is an intermediary between DNA and protein. Recent technological advances have developed mRNA vaccines overcoming the instability issues of mRNA and its delivery into the cells, with encouraging results.
- Some experimental mRNA vaccines have been designed to protect mice and monkeys against Zika virus infection, and administered in a single dose.
- **3. Recombinant vector vaccine**
- These are vaccines designed as vectors or carriers using harmless viruses or bacterium and they introduce the genetic material into cells.
- Majorly these vaccines are designed and approved for use to protect animals from infectious diseases, including rabies and distemper, but some have been developed to protect humans from viruses such as HIV, Zika virus, and Ebola virus.
- **Side Effects of Vaccines**
- The effects of vaccines are normally mild and go away within hours to days of administration. Intravenously administered vaccines can leave a sore pain on the site of administration but it goes away after a few hours or days, on their own.
- However, the effects may vary from individual most side effects of vaccination can be mild including soreness, swelling, or redness at the injection site, fever, rashes, and achiness to serious effects including seizure or life-threatening allergic reactions, but they are rare.
- Many infants and children will experience a medical event in close proximity to vaccination, which may or may not be related to vaccination. According to the Food and Drug Administration (FDA) and the Center for Disease Control (CDC), monitoring and analyzing the adverse effects of vaccination in children.
- Some of the mild effects include:
- Pain, swelling, or redness where the shot was given which may last 2-4days
- Mild fever and chills that lasts for a few hours and it occurs in 70% of all the vaccinated children
- Fatigue
- Headaches
- Muscle and joint aches
- Fainting
- Note that, some of these effects arise as a sign of the bodybuilding up immunity against a disease.
- Serious or adverse side effects are rare but may occur in 1 to 1million people, and they may include:
- Serious eye infection, or loss of vision, if the vaccine spreads to the eye eg smallpox vaccine.
- Rashes may occur on the entire body in 1 per 4,000 people.
- Severe rash on people with eczema in 1 per 26,000.
- Severe brain reaction or Encephalitis can lead to permanent brain damage occurring in 1 per 83,000.
- Severe infection begins at the site of vaccination occurring in 1 per 667,000, mostly in people with weakened immune systems.

- Death occurs in 1-2 per million, mostly in people with weakened immune systems.
- For every million people vaccinated for smallpox, between 14 and 52 could have a life-threatening reaction to the smallpox vaccine.
- Examples of vaccines and their effects
- Haemophilus influenza type B vaccine is well known for its potential side effects. Haemophilus influenza type B is a bacterium that can cause serious infections, including meningitis, pneumonia, epiglottitis, and sepsis, and it is recommended that children receive the Hib vaccination as early as 2 months old. Some of the known side effects include:
- Redness, warmth, or swelling where the shot was given (up to 1 out of 4 children)
- Fever over 101°F (up to 1 out of 20 children)
- Smallpox is a fatal infection that has a 30-40% fatality rate and it is caused by Variola major or Variola minor virus its vaccination is done mainly to military personnel and people who are first responders in the event of a bioterror attack. Some of the side effects of the smallpox vaccine include rashes, redness, and tenderness on the site of administration, fever, headaches, loss of vision, brain damage (encephalitis), and even death.

Definition and History of Vaccines

According to the CDC, a vaccine is a preparation used to stimulate the body's immune response against diseases. Vaccines are usually administered through needle injections, but some can be administered by mouth or sprayed into the nose.

- The history of vaccines trails down to 1877 when Loise Pasteur developed a vaccine using a weakened strain of the anthrax bacillus, *Bacillus anthracis*. He adapted a methodology of attenuating the culture of anthrax bacillus by incubation at a high temperature of 42–43°C and inoculated the attenuated bacilli in the animals, demonstrating that animals receiving inoculation of such attenuated strains developed specific protection against anthrax.
- This concept was successfully demonstrated on a farm at Pouilly-le-Fort in 1881 by vaccinating sheep, goats, and cows with the attenuated anthrax bacillus strain. The result indicated that all the vaccinated animals survived an anthrax attack which the non-vaccinated could not, hence they died of anthrax.
- In 1885, Louis Pasteur successfully prevented rabies through post-exposure vaccination. The treatment is controversial. Pasteur has unsuccessfully attempted to use the vaccine on humans twice before, and injecting a human with a disease agent is still a new and uncertain method
- Pasteur coined the term **vaccine** in commemoration of Edward Jenner who used such preparations for protection against smallpox. This led to the establishment of various institutions in several countries in the world that prepared vaccines and studied infectious diseases such as the Pasteur Institute in Paris.

How do vaccines work in Immune System?

- Vaccines are biological preparations that are made up of killed or attenuated pathogens (virus or bacteria) or part of the surface of the antigen.
- The preparation is made in such a way that it can not cause disease on its own, but it helps the body to develop a memory type of immunity. This means that if an individual encounter or is infected by the same pathogen (whose part has been used to prepare the vaccine), the immunity will 'remember' and induce a more vigorous immune response against the pathogen.
- Initially, the innate immune response (primary response) elicited on the first encounter with a pathogen, is normally slow and that is why one will display symptoms of the disease before the immune system can elicit a reaction to kill the pathogen, and therefore the body develops an adaptive immune response (secondary response) through specialized immune cells which counter the pathogen and create a long-lasting memory.
- Therefore, vaccination or the introduction of a vaccine into the body will have a similar kind of immune reaction (secondary response) only that it will by-pass the slow initial response but enables the body to acquire immunity (from the vaccine). In other words, the vaccine tricks the body to believe that it

has the disease, and therefore, able to fight the disease. This makes the body be able to kill the pathogen before it can have the chance to cause disease due to memory that is created from vaccination.

- Vaccination is the safest and most common way to gain immunity against bacteria or viruses that your body has yet to encounter.
- Generally, a vaccine works as follows:
  - Administration of vaccine which contains antigens for a specific disease or pathogen
  - Identification and recognition of the antigen in the vaccine as foreign, by the immune system
  - Development of antibodies by the immune system to neutralize the antigens.
  - Storage of these immune effector antibodies as memory antibodies for future response in case an individual is exposed to the live pathogen or disease.
- Significantly, vaccination is done to prevent diseases and wipe them out in eventuality. Administration of a vaccine to a significant proportion of a population.
- Vaccines are given to prevent and eventually wipe out diseases. When a vaccine is given to a significant portion of the population, it protects those who receive the vaccine as well as those who cannot receive the vaccine. This concept is called "herd immunity." When a high percentage of the population is vaccinated and immune to a disease, they do not get sick — so there is no one to spread the disease to others. This herd immunity protects the unvaccinated population from contagious (spread from person to person) diseases for which there is a vaccine.

Types of Vaccines and Their Characteristics

- Vaccines have proved to have a strong defense against some of the most fatal diseases and if they were still unavailable, the survival of individuals would be based on their immune defenses which could either resolve the infection or lead to death from the infection.
- Therefore, the use of vaccines means, the vaccine will mimic the pathogen and cause an immune response that is similar to that that can be activated by the pathogen.
- Historically, these vaccines have eliminated fatal infections such as smallpox, almost eliminated polio, and saved many individuals from typhus, tetanus, hepatitis A and B, measles, rotavirus diseases, etc.
- However, still successful vaccines are yet to be developed for many deadly diseases that cause chronic infections such as AIDS, hepatitis C, tuberculosis, malaria, and herpes
- Successful vaccines against these chronic diseases must be able to stimulate immune responses that are similar to those resulting from most natural exposures to the pathogen but still remains a challenge.
- Various vaccines have been designed and here is a detailed approach to how these vaccines have been developed, those in use, and those still under experimentation.
- Major advances in understanding the complexities of the interaction of pathogens or microbes with the human host have revolutionized vaccine developments and advances in recent times. Coupled with advances in laboratory techniques and technologies, have aided the development of new vaccine types.
- Some more developed approaches such as vaccinomics, which is the application of genomics and bioinformatics to vaccine development, is a new approach that may solve the problem of developing vaccines against microbes and parasites.

Vaccine types can broadly be classified into three groups:

1. Whole-organism Vaccines

- Inactivated (Killed) Vaccine
- Live-attenuated vaccines
- Chimeric vaccine

2. Subunit Vaccines

- Polysaccharide Vaccine
- Conjugated Vaccines
- Toxoid Vaccines

- Recombinant Protein Vaccines
- Nanoparticle vaccines

3. Nucleic Acid Vaccines

- DNA plasmid vaccines
- mRNA vaccines
- Recombinant vector vaccine

Whole-organism Vaccines

Many vaccines that were developed early consist of an entire pathogen that is either killed (inactivated) or weakened (attenuated) so that they cannot cause disease. They are known as the **whole-organism vaccines.** These vaccines elicit strong protective immune responses and many vaccines used today are prepared in this manner, but not all disease-causing microbes can be effectively targeted with a whole-organism vaccine.

1. Inactivated (Killed) Vaccine

- These were produced by killing the pathogen (bacteria, virus, or other pathogens) with chemicals or heat, or radiation.
- The killed pathogen can not cause disease, and this means that they do not replicate in the host's body.
- **Advantage**: These vaccines are stable and safer than the live attenuated vaccines
- **Disadvantage**: The major disadvantage of this type of vaccine is that it elicits a weaker immune response and therefore, it requires more vaccine dosages and a booster dose as well, so as to confer protective immunity.
- **Examples of Inactivated Vaccines** include poliomyelitis (sulk vaccine), rabies, typhoid, cholera, pertussis, pneumococcal, rabies, hepatitis B, and influenza vaccines.

2. Live-attenuated vaccines

- These vaccines were developed in the 1950s when advances in tissue culture techniques were developed.
- These vaccines are prepared from a whole organism, by weakening their pathogenicity so that they can not cause disease but can induce an immune response, hence the term **attenuation**.
- These vaccines elicit strong immune responses because they are similar to the actual disease pathogen and hence they confer a life-long immunity after only one or two doses, therefore they are very effective.
- They are also relatively easy to create for certain viruses, but difficult to produce for more complex pathogens like bacteria and parasites.
- **Disadvantages**: There is a remote chance that the weakened germ can mutate or revert back to its full strength and cause disease.
- Live attenuated vaccines should not be given to individuals with weakened or damaged immune systems.
- To maintain potency, live attenuated vaccines require refrigeration and protection from light.
- Examples include Measles/Mumps/Rubella (**MMR**) and Influenza Vaccine Live, Intranasal (**FluMist®**), Polio (Sabin vaccine), Rotavirus, Tuberculosis, Varicella, Yellow fever.
- The attenuated strain of *Mycobacterium bovis* called Bacillus Calmette- Guérin (BCG) was developed by growing *M. bovis* on a medium containing increasing concentrations of bile. After 13 years, this strain had adapted to growth in strong bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis.

3. Chimeric vaccine

- The evolution of modern genetic engineering techniques has enabled the creation of chimeric viruses, which contain genetic information from one viral particle and display the biological properties of different parent viruses.
- An NIAID-developed live-attenuated **chimeric vaccine** consisting of a dengue virus backbone with Zika virus surface proteins is undergoing early-stage testing in humans.

Whole-organism vaccines, whether alive or dead, have another big drawback. Considering that they are composed of complete pathogens, they retain molecules that are not involved in evoking immunity, including unavoidable byproducts of the manufacturing process such as contaminants that can trigger allergic or immune disruptive reactions.

Subunit Vaccines

- These are vaccines that are prepared by using components or antigens of the pathogen. These components can stimulate the immune system to elicit appropriate immune responses.
- They are also known as acellular vaccines because they do not contain a whole cell, but just part of a cell of the bacteria or virus.
- These vaccines were produced to cub the inefficiencies of the live attenuated and killed vaccines prepared from whole organisms such as adverse reactions associated with the vaccines and the mutations that may lead to the virulent strains of the pathogens.
- The subunit vaccines are safe and easier to produce, however, they require the use of an adjuvant to produce a stronger protective immune response. This is because an antigen alone cannot be able to produce sufficiently enough long-term immunity.
- One of the earliest vaccines produced against pertussis was an inactivated *Bordetella pertussis* bacteria preparation in the 1940s, but this vaccine caused minor adverse reactions such as fever and swelling at the injection site, hence the vaccine was avoided leading to a decrease in its vaccination and therefore an increase in cases of pertussis infections. This led to the development of acellular pertussis vaccines that were based on purified *B. pertussis* components. These newly prepared vaccines had no adverse reactions associated with their administration.

Some of the subunit vaccines produced to prevent bacterial infections are based on the polysaccharides or sugars that form the outer coating of many bacteria. Therefore, there are subtypes of subunit vaccines as follows:

1. Polysaccharide Vaccine

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- Therefore, these are vaccines that are prepared using the sugar molecules, and polysaccharides from the outer layer of a bacteria or virus.
- They create a response against the molecules in the pathogen's capsule. Normally these molecules are small hence they are not immunogenic (cannot induce an immune response on their own). Hence, they tend to be ineffective in infants and young children between 18-24 months, and they induce a short-term immunity associated with slow immune responses, and slow activation, and it does not increase antibody levels and it does not create an immune memory.
- Therefore, these sugar molecules are chemically linked to carrier proteins and work similarly to conjugate vaccines.
- **Examples of polysaccharide vaccines** include Meningococcal disease caused by *Neisseria meningitidis* groups A, C, W135, and Y, as well as Pneumococcal disease.

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- These vaccines are prepared by linking the polysaccharides or sugar molecules on the outer layer of the bacteria to a carrier protein antigen or toxoid from the same microbe.
- The polysaccharide coating disguises a bacterium's antigens so that the immature immune systems of infants and younger children cannot recognize or respond to them.
- Conjugate vaccines get around this problem through the linkage of polysaccharides with a protein.
- This formulation greatly increased the ability of the immune systems of young children to recognize the polysaccharide and develop immunity.
- The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.
- Today, conjugate vaccines are also available to protect against pneumococcal and meningococcal infections.

3. Toxoid Vaccines

- These vaccines are prepared from inactivated toxins, by treating the toxins with formalin, a solution of formaldehyde, and sterilized water.
- This process of inactivation of toxins is known as **detoxification** and the resultant inactive toxin is known as a **toxoid.**
- Detoxification makes the toxins safe to use.
- The toxins used for the preparation of toxoids are obtained from the bacteria that secrete the illness-causing toxins.
- This means that when the host body receives the harmless toxoid. the immune system adapts by learning how to fight off the natural bacterial toxin responsible for causing illness, by producing antibodies that lock onto and block the toxin.
- **Examples of toxoid vaccines** include diphtheria and tetanus toxoid vaccines.

4. Recombinant Protein Vaccines

- After the start of the generic engineering era, recombinant DNA technology also evolved. This is where DNA from two or more sources is combined. This technology harnessed the development of recombinant protein vaccines.
- For recombinant vaccines to induce immunity against a pathogen, they have to be administered along with an adjuvant or expresses by a plasmid or a harmless bacterial or viral vector.
- Production of these recombinant protein vaccines involves the insertion of DNA encoding an antigen such as a bacterial surface protein, which stimulates an immune response into bacterial or mammalian cells, expressing the antigen in these cells, and then the antigen is purified from them.
- **Advantages:**
    - Recombinant protein vaccines allow the avoidance of several potential concerns raised by vaccines based on purified macromolecules. For example, the presence of contaminants in vaccines after purification may cause potential harm to the host.
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- An experimental recombinant protein vaccine for chikungunya fever has also been designed by the National Institute of Allergy and Infectious Disease (NIAID).

5. Nanoparticle vaccines

- This vaccine development was based on a strategy to present protein subunit antigens into the immune system.
- The NIAID has also designed a universal flu vaccine, an experimental vaccine with protein ferritin which can self-assemble into microscopic pieces known as nanoparticles that display a protein antigen.
- A nanoparticle-based influenza experimental vaccine is also being evaluated in human trials (early stages).
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Recent advances in the subunit vaccine development and delivery systems include solving the atomic structures of proteins. For example, NIAID has been able to solve the 3-D structure of a Respiratory Syncytial

Virus (RSV) surface-bound to an antibody, identifying a key part of the protein that is highly sensitive to neutralizing antibodies. They were then able to modify the RSV protein to stabilize the structural form in which it displays the neutralization-sensitive site.

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Nucleic Acid Vaccines

- These are vaccines designed to aim at introducing the genetic materials that code the antigen or the antigen that is aimed at inducing an immune response, enabling the host cells to use the genetic materials to produce the antigens.
- The advantages of the nucleic acid vaccine approach include:
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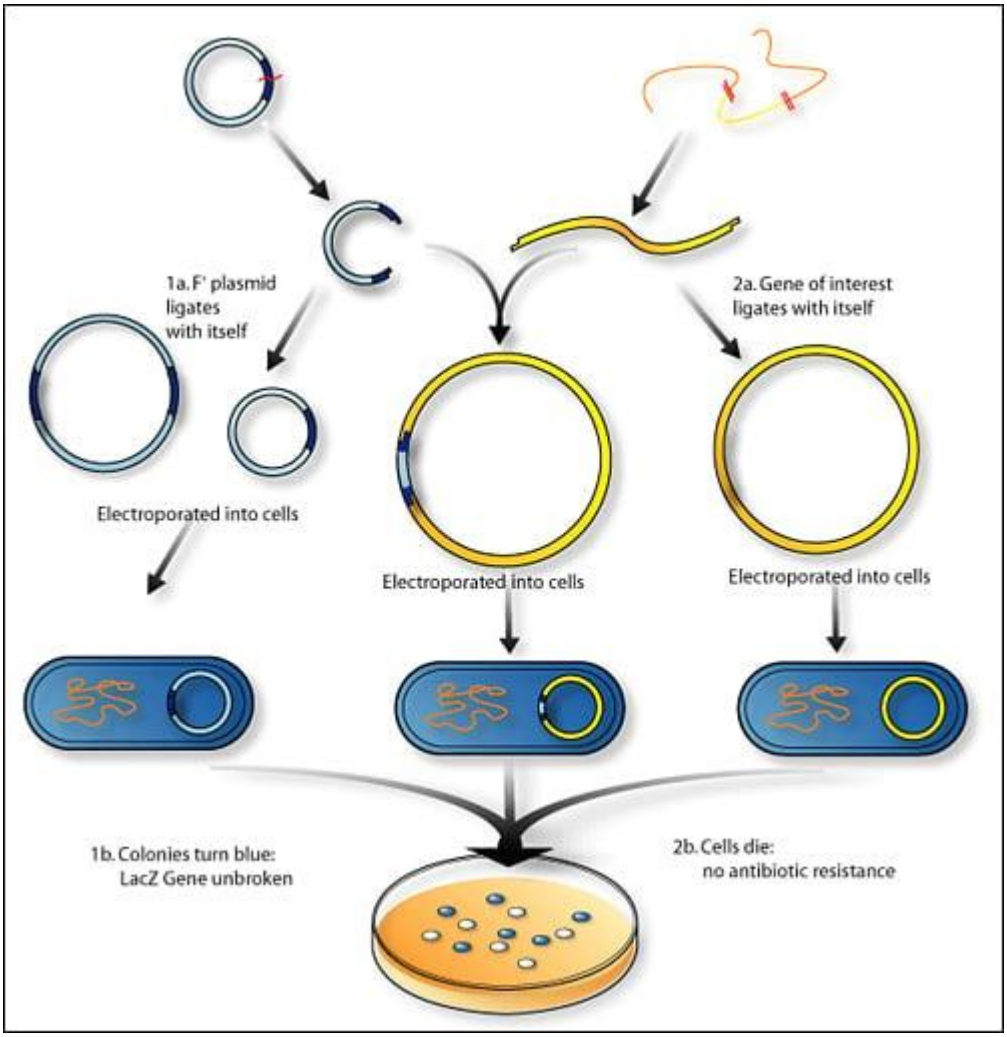
- The advantage of the ease of production is a potential game-changer for targeting epidemic or emerging diseases where rapidly designing, constructing, and manufacturing the vaccine are crucial

Some of the know nucleic acid vaccines models include:

1. DNA plasmid vaccines
    - These are vaccines that are composed of a small circular piece of DNA known as a plasmid. The plasmid carries genes that encode proteins from the pathogen of interest.
    - Experimental DNA plasmid vaccines have been designed by the National Institute of Allergy and Infection Disease (NIAID) to address some viral disease threats including SARS coronavirus (SARS-CoV) in 2003, H5N1 avian influenza in 2005, H1N1 pandemic influenza in 2009, and Zika virus in 2016.
2. mRNA vaccines
    - mRNA is an intermediary between DNA and protein. Recent technological advances have developed mRNA vaccines overcoming the instability issues of mRNA and its delivery into the cells, with encouraging results.
    - Some experimental mRNA vaccines have been designed to protect mice and monkeys against Zika virus infection, and administered in a single dose.
3. Recombinant vector vaccine
    - These are vaccines designed as vectors or carriers using harmless viruses or bacterium and they introduce the genetic material into cells.
    - Majorly these vaccines are designed and approved for use to protect animals from infectious diseases, including rabies and distemper, but some have been developed to protect humans from viruses such as HIV, Zika virus, and Ebola virus.

UNIT III

- Recombinant DNA technology refers to the joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry.
- Recombinant DNA (rDNA), on the other hand is the general name for a piece of DNA that has been created by the combination of at least two strands.
- They are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.
- Recombinant DNA in a living organism was first achieved in 1973 by Herbert Boyer, of the University of California at San Francisco, and Stanley Cohen, at Stanford University, who used *E. coli* restriction enzymes to insert foreign DNA into plasmids.



Steps of Genetic Recombination Technology

Isolation of Genetic Material

- The first step in rDNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.
- Since DNA exists within the cell membrane along with other macromolecules such as RNA, polysaccharides, proteins, and lipids, it must be separated and purified which involves enzymes such as lysozymes, cellulase, chitinase, ribonuclease, proteases etc.
- Other macromolecules are removable with other enzymes or treatments. Ultimately, the addition of ethanol causes the DNA to precipitate out as fine threads. This is then spooled out to give purified DNA.

2. Restriction Enzyme Digestion

- Restriction enzymes act as molecular scissors that cut DNA at specific locations. These reactions are called 'restriction enzyme digestions.
- They involve the incubation of the purified DNA with the selected restriction enzyme, at conditions optimal for that specific enzyme.
- The technique 'Agarose Gel Electrophoresis' reveals the progress of the restriction enzyme digestion.
- This technique involves running out the DNA on an agarose gel. On the application of current, the negatively charged DNA travels to the positive electrode and is separated out based on size. This allows separating and cutting out the digested DNA fragments.
- The vector DNA is also processed using the same procedure.

3. Amplification Using PCR

- Polymerase Chain Reaction or PCR is a method of making multiple copies of a DNA sequence using the enzyme – DNA polymerase in vitro.
- It helps to amplify a single copy or a few copies of DNA into thousands to millions of copies.
- PCR reactions are run on 'thermal cyclers' using the following components:

1. Template – DNA to be amplified
2. Primers – small, chemically synthesized oligonucleotides that are complementary to a region of the DNA.
3. Enzyme – DNA polymerase
4. Nucleotides – needed to extend the primers by the enzyme.

- The cut fragments of DNA can be amplified using PCR and then ligated with the cut vector.

4. Ligation of DNA Molecules

- The purified DNA and the vector of interest are cut with the same restriction enzyme.
- This gives us the cut fragment of DNA and the cut vector, that is now open.

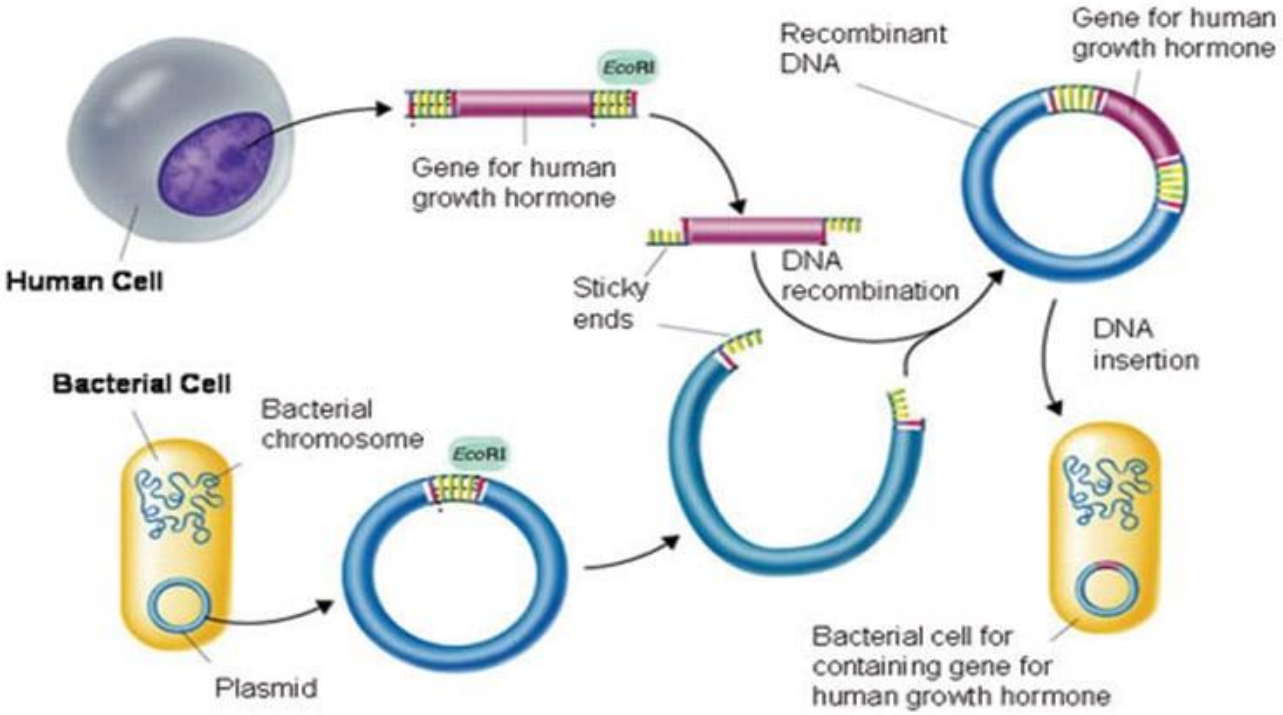
- The process of joining these two pieces together using the enzyme 'DNA ligase' is 'ligation'.
- The resulting DNA molecule is a hybrid of two DNA molecules – the interest molecule and the vector. In the terminology of genetics this intermixing of different DNA strands is called recombination.
- Hence, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the recombinant DNA technology.

5. Insertion of Recombinant DNA Into Host

- In this step, the recombinant DNA is introduced into a recipient host cell mostly, a bacterial cell. This process is 'Transformation'.
- Bacterial cells do not accept foreign DNA easily. Therefore, they are treated to make them 'competent' to accept new DNA. The processes used may be thermal shock, Ca^{++} ion treatment, electroporation etc.

6. Isolation of Recombinant Cells

- The transformation process generates a mixed population of transformed and non-trans- formed host cells.
- The selection process involves filtering the transformed host cells only.
- For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.
- For examples, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.



Application of Recombinant DNA technology

- Recombinant DNA is widely used in biotechnology, medicine, and research.
- The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences.
- Recombinant DNA is used to identify, map and sequence genes, and to determine their function.
- Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms.
- Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture, and bioengineering.

1. DNA technology is also used to detect the presence of HIV in a person.
2. Application of recombinant DNA technology in Agriculture – For example, manufacture of Bt-Cotton to protect the plant against ball worms.
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4. Gene Therapy – It is used as an attempt to correct the gene defects which give rise to heredity diseases.
5. Clinical diagnosis – ELISA is an example where the application of recombinant DNA is possible.

UNIT IV

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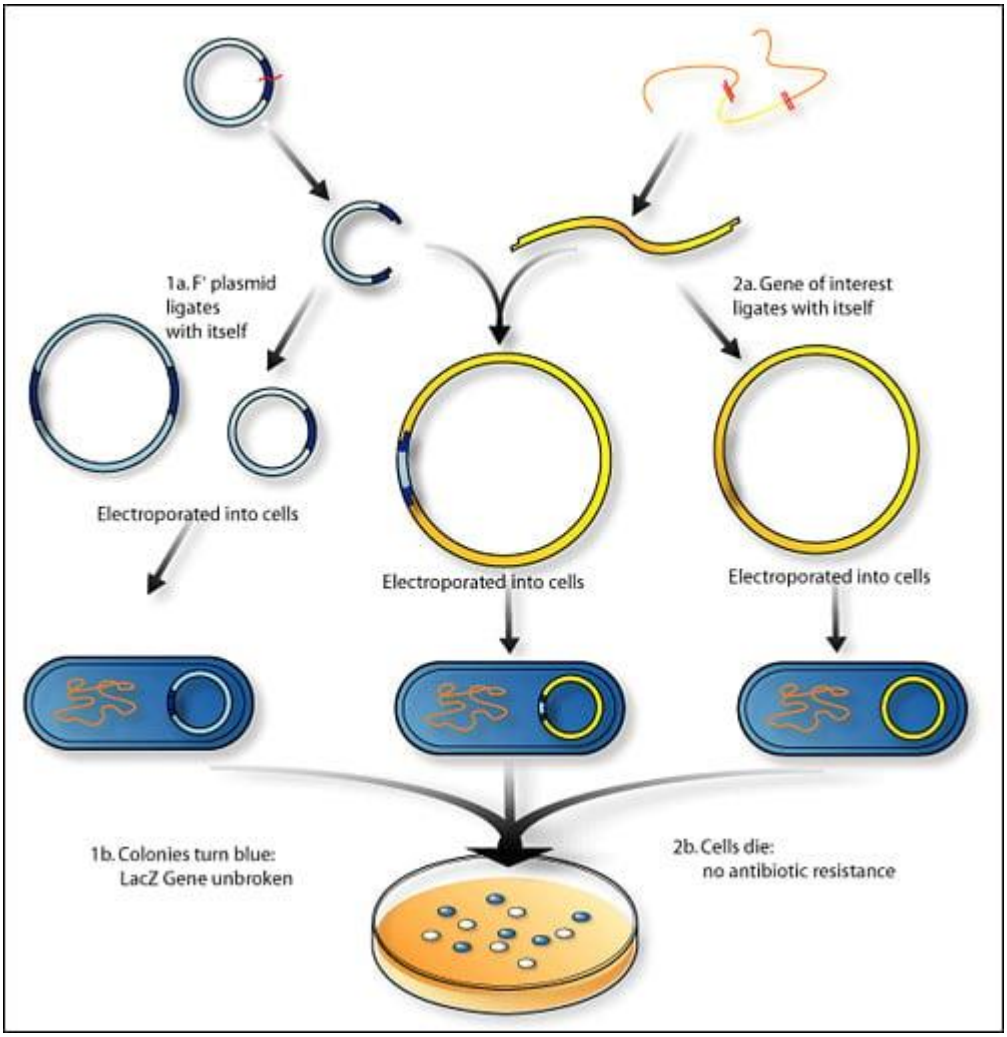

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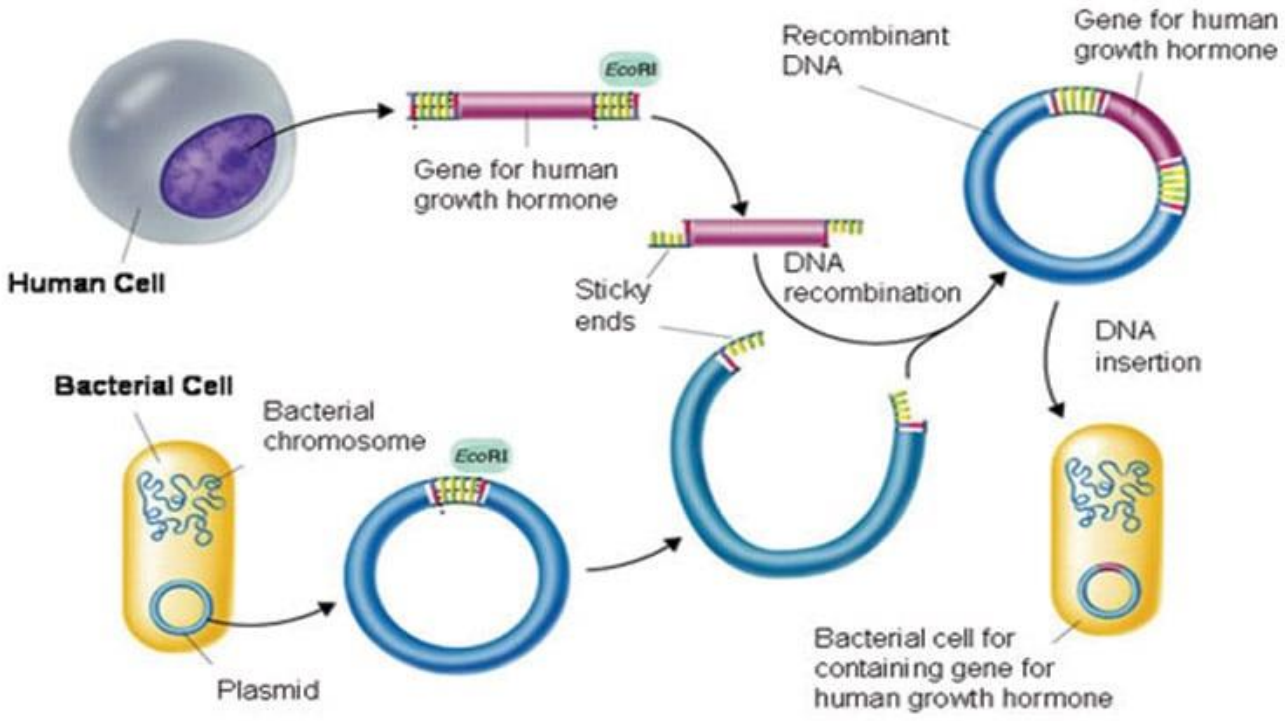

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UNIT V

Bioinformatics is an emerging field of science that deals with the application of computers to the collection, organization, analysis, manipulation, presentation, and sharing of biological data.

- Bioinformatics is an interdisciplinary field directly involving molecular biology, genetics, computer science, mathematics, and statistics.
- The central component of bioinformatics is the study of the best ways to design and operate biologic databases.
- As a large amount of nucleotide and protein sequence data are obtained via various research techniques, along with other types of information stored in primary and secondary biological databases, scientists started to use computers to obtain and analyze biological data in their daily research with bioinformatics tools.
- To help the biologists access the databases effectively and use the analysis tools efficiently, bioinformatics has eventually become a vital part of biological education.
- Bioinformatics is an evolving discipline, and complex software programs are now being used for retrieving, sorting out, analysing, predicting, and storing DNA and protein sequence data.
- One of the fundamental activities in bioinformatics is the sequence analysis of DNA and proteins using various programs and databases available on the world wide web.
- Large commercial enterprises such as pharmaceutical companies employ bioinformaticians to perform and maintain the large scale and complex bioinformatics needs of these industries.
- Apart from the analysis of genome sequence data, bioinformatics is now being used for a vast array of other vital tasks, including analysis of gene variation and expression, analysis, and prediction of gene and protein structure and function.
- Besides, bioinformatics has found its importance in tasks like prediction and detection of gene regulation networks and presentation and analysis of molecular pathways in order to understand gene-disease interactions.
- Bioinformatics even has clinical applications as the whole genome sequencing of an organism allows to produce a complete list of human gene products that may provide new drugs and gene therapy for single-gene diseases may become routine.
- Bioinformatics can be used for different other fields of the biology of different groups of living beings.