COURSE INTRODUCTION

The objective of this course deals basic introduction to immune system in concerned to immunological reactions. The aim is to provide brief introduction of immunity and its types. The course is organized into following blocks:

Block 1 It covers the immune system

Block 2 It deals the elements of immune system

Block 3 It describes the infectious diseases and immunology.

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Immunology

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Block- I

Immune System

Unit-1
Elements of Immune System

Unit-2
Immunity

Introduction

This is the first block on Immune System. It consists of following two units:

Unit 1: This unit covers the immune system and its types. The immune system refers to a collection of cells and proteins that function to protect the skin, respiratory passages, intestinal tract and other areas from foreign antigens, such as microbes (organisms such as bacteria, fungi, and parasites), viruses, cancer cells, and toxins. The immune system can be simplistically viewed as having two "lines of defense": innate immunity and adaptive immunity. Innate immunity represents the first line of defense to an intruding pathogen. It is an antigen-independent (non-specific) defense mechanism that is used by the host immediately or within hours of encountering an antigen. The innate immune response has no

immunologic memory and, therefore, it is unable to recognize or "memorize" the same pathogen should the body be exposed to it in the future. Adaptive immunity, on the other hand, is antigen-dependent and antigen-specific and, therefore, involves a lag time between exposure to the antigen and maximal response. The hallmark of adaptive immunity is the capacity for memory which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen. Innate and adaptive immunity are not mutually exclusive mechanisms of host defense, but rather are complementary, with defects in either system resulting in host vulnerability.

Unit 2: Immunity refers to the body's ability to prevent the invasion of pathogens. Pathogens are foreign disease-causing substances, such as bacteria and viruses, and people are exposed to them every day. Antigens are attached to the surface of pathogens and stimulate an immune response in the body. An immune response is the body's defense system to fight against antigens and protect the body. There are several types of immunity, including innate immunity, passive immunity, and acquired/active immunity. The active immunity as a process of exposing the body to an antigen to produce an adaptive immune response, while passive immunity "borrows" antibodies from another person.

Unit- 1: Elements of Immune System

Structure

1.1 Introduction

Immunology has its origins in the study of how the body protects itself against infectious diseases caused by microorganisms, such as bacteria, viruses, protozoa, and fungi, and also parasitic organisms, such as helminth worms. Important initial barriers to infection are physical (e.g. the **skin**), enhanced by substances secreted by the body, such as saliva and tears, that contain molecules that can neutralise bacteria. The internal **mucosal tissues** (e.g. **lungs & airways**, and the **gut**) are coated with mucus that is able to trap potential infectants. In the airways, mobile ciliate hairs work together to transport contaminants away from vulnerable areas. Tissues such as the skin, mucosal surfaces and airways also contain populations of immune cells that can respond to infectants that breach these physical defences.

In its most complex forms, the immune system consists of two branches: the **innate immune system** that utilises certain 'hard-wired' strategies to provide a rapid, general, response when alerted by certain typical signals of infection (essentially forming a first-line of defence); and the **adaptive immune system** that is able to develop highly specific responses (and a persistent 'immune memory') to target infection with extraordinary accuracy. Both systems work in close cooperation and, to an important extent, the adaptive immune system relies upon the innate immune system to alert it to potential targets, and shape its response to them.

Objectives

This is the first block on Immunology. It consists of following two units. Under first unit (Elements of Immune System) we have following objectives. These are as under:

- To know about purpose and principles of immunology.
- To know about immune system and its properties.
- To know about immunity and its types.
- What are the important aspects of the adaptive immune response?
- To know immunoglobulins and their types.
- What are primary and secondary lymphoid Organs?

1.2 Immunology

Immunology can be defined as the study of the immune system and is a very important branch of the medical and biological sciences. The immune system protects us from infection through various lines of defence. If the immune system does not work properly it can result in disease, such as autoimmunity, allergy and cancer. It has been also now become clear that immune responses contribute to the development of many common disorders such as immunologic, including metabolic, cardiovascular, and neurodegenerative conditions eg. Alzheimer's. In broader sense Immunology can also be defined as the scientific study of how the body an individual protects itself against infectious diseases which are caused by different pathogenic microorganisms such as bacteria, viruses, protozoa, and fungi, and also parasitic organisms, such as helminthes worms.

1.2 History of Immunology

1.2.1 Origin of Immunology

The word "*immune*" derived from Lartin-"immunus" which means "*free of or exempt*". This term was originally used in the context of being free of the burden of taxes or military conscription. Earlier immunology was started as a branch of microbiology, which led to the study of infectious diseases and then the body's response to it.

The concept of immunity from disease can be traced back at least to Greece in the 5th century BC. During this time, Thucydides observed and wrote about individuals who recovered from the plague, which was raging in Athens. However, the earliest recognized attempt to intentionally "induce" immunity to an infectious disease was in the 10th century in China, where smallpox was endemic or regularly found.

In 1546 Girolamo Fracastro, a colleague of Copernicus, wrote about contagion, which may be a cause of a disease. In 1798, Edward Jenner gave the concept of immunity in response to contagion. His co-worker had a cowpox infection (cow means Vacca) and consequently, she was become resistant to smallpox. Edward Jenner noticed that cowpox infection induced immunity against smallpox. This discovery started the process of vaccination. The term "*Vaccination*" was coined by Edward Jenner for his treatment (from the Latin, *vacca* means a cow) and later on it was adopted by Louis Pasteur for immunization against any disease.

Some major breakthrough in the history of immunology includes:

- 1798 Edward Jenner initiates smallpox vaccination.
- 1877 Paul Erlich recognizes mast cells.
- 1879 Louis Pasteur develops an attenuated chicken cholera vaccine.
- 1883 Elie Metchnikoff develops cellular theory of vaccination.
- 1885 Louis Pasteur develops rabies vaccine.
- 1891 Robert Koch explored delayed type hypersensitivity.
- 1900 Paul Erlich theorizes specific antibody formation.
- 1906 Clemens von Pirquet coined the word allergy.
- 1938 John Marrack formulates antigen-antibody binding hypothesis.
- 1942 Jules Freund and Katherine McDermott research adjuvants.
- 1949 Macfarlane Burnet & Frank Fenner formulate immunological tolerance hypothesis.
- 1959 Niels Jerne, David Talmage, Macfarlane Burnet develop clonal **selection** theory.

- 1957 Alick Isaacs & Jean Lindemann discover interferon (cytokine).
- 1962 Rodney Porter and team discovery the structure of antibodies.
- 1962 Jaques Miller and team discover thymus involvement in cellular immunity.
- 1962 Noel Warner and team distinguish between cellular and humoral immune responses.
- 1968 Anthony Davis and team discover T cell and B cell cooperation in immune response.
- 1974 Rolf Zinkernagel and Peter Doherty explore **major histocompatibility complex** restriction.
- 1985 Susumu Tonegawa, Leroy Hood, and team identify immunoglobulin genes.
- 1987 Leroy Hood and team identify genes for the T cell receptor.
- 1985 Scientists begin the rapid identification of genes for immune cells that continues to the present.

1.3 Primary and Secondary Lymphoid Organs

The cells involved in the immune response are effectively organized into tissues and organs. Based on different roles they perform, lymphoid organs can be classified into central (primary) and peripheral (secondary) lymphoid organs.

1.3.1 Primary Lymphoid Organs

Primary lymphoid organs are the major sites of lymphocyte development (lymphopoiesis). They provide an environment for stem cells to divide and mature into B- and T- cells: There are two primary lymphatic organs: the red bone marrow and the thymus gland.

1.3.1.1 Bone marrow

Both T-cell and B-cells are 'originate' in the bone marrow. B cells mature in the bone marrow. After maturation in bone marrow, B cells immediately enter the circulatory system and travel to secondary lymphoid organs in search of pathogens. Whereas T cells from the bone marrow enter the thymus for their development and maturation. Mature T cells then join B cells in search of pathogens.

1.3.1.2 Thymus

The thymus is a primary lymphoid organ of the immune system. The thymus is located just behind the upper part of the heart. It has two lobes surrounded by a fibrous capsule. Thymus cell lymphocytes or *T cells* mature in thymus. T cells play important role in adaptive immune system. The thymus provides an inductive environment for the development of T cells from

hematopoietic progenitor cells. The loss or lack of the thymus results in severe immunodeficiency diseases. Usually the thymus consists of lobules which are divided by septa.

1.3.2 Secondary Lymphoid Organs

Secondary lymphoid organs provide the inductive environment for the proliferation and maturation of cells involved in the adaptive immune response, for filtering and trapping antigens. It is in these organs where the cells of the immune system do their actual job of fighting off germs and foreign substances. Secondary lymphoid organs include: lymph nodes, tonsils, spleen**,** Peyer's patches and mucosa associated lymphoid tissue (MALT**).**

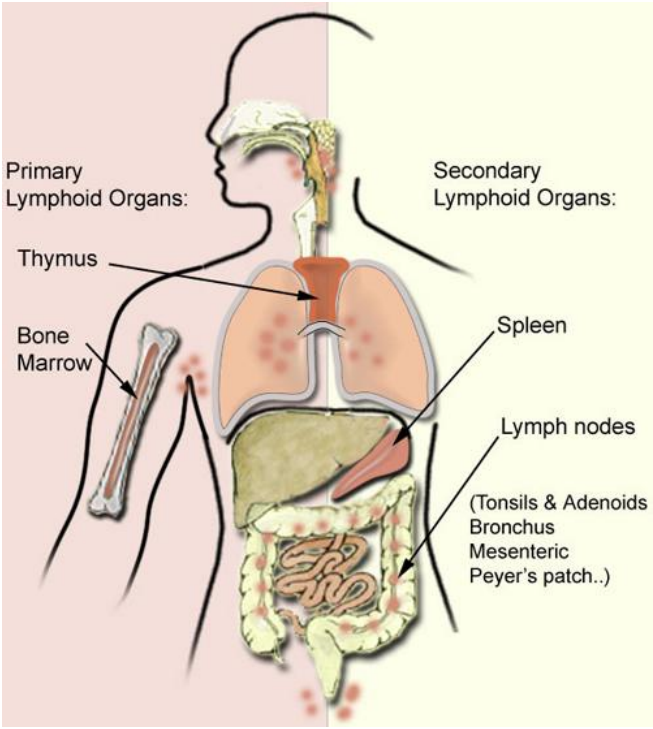

Fig. 1. Primary and Secondary Lymphoid Organs

1.3.2.1 Lymph Nodes

These are small rounded or bean-shaped masses of lymphatic tissue called lymph nodes. Lymph nodes are also called as lymph glands. There are hundreds of lymph nodes found throughout the body. Lymph nodes are found in the neck, chest, abdomen, axilla (underarm), and groin. Lymph nodes are connected to one another by lymph vessels. The substance of a lymph node consists of lymphoid follicles in an outer portion called the cortex. Anatomically

lymph nodes consist of two regions. The inner portion of the node is called the medulla, which is surrounded by the cortex on all sides except for a portion known as the hilum.

Lymph nodes filter the lymphatic fluid and store special cells that can trap cancer cells or bacteria that are traveling through the body in the lymph fluid. The lymph nodes are critical for the body's immune response and are principal sites where many immune reactions are initiated Lymph nodes contains lymphocytes (white blood cells) that help the body fight infection and disease.

1.3.2.2 Spleen

The spleen is located in the left upper abdomen, beneath the diaphragm, and is responsible for different kinds of functions:

- Spleen stores various immune system cells. When needed, they move through the blood to other organs. Scavenger cells (phagocytes) in the spleen act as a filter for germs that get into the bloodstream.
- Spleen breaks down red blood cells (erythrocytes).Hence also called Graveyard of RBCs.
- It is also the site of storage and breaks down of platelets (thrombocytes), which are responsible for the clotting of blood, among other things.

1.3.2.3 Tonsils

These are located in the throat and palate. They can stop germs entering the body through the mouth or the nose. The tonsils also contain a lot of white blood cells, which are responsible for killing germs. There are different types of tonsils: palatine tonsils, adenoids and the lingual tonsil. All of these tonsillar structures together are sometimes called Waldeyer's ring since they form a ring around the opening to the throat from the mouth and nose.

1.3.2.4 Mucosa Associated Lymphoid Tissues

Mucosa-associated lymphoid tissue (MALT) is located within the mucosal linings and constitutes the most extensive component of human lymphoid tissue. These surfaces protect the body from an enormous quantity and variety of antigens. For examples tonsils, the Peyer’s

patches within the small intestine, and the vermiform appendix. The nomenclature uitilises location; therefore, MALT is understood to include gut-associated lymphoid tissue (GALT), bronchial/tracheal-associated lymphoid tissue (BALT), nose-associated lymphoid tissue (NALT), and vulvovaginal-associated lymphoid tissue (VALT). MALT contains lymphocytes (Tcells and B cells), plasma cells and macrophages, each of which to encounters antigens passing through the mucosal epithelium. MALT constitutes about 50% of the lymphoid tissue in human body.

1.3.3 Maturation and Selection of T Cells

1.3.3.1 T Cells

T cells belong to lymphocytes and play a central role in the cell-mediated branch of the adaptive immune system. T cells differ from B cells and natural killer cells (NK cells) by the presence of a T cell receptor (TCR) on their cell surface. T cells are produced in the bone marrow but transfer to the thymus for their maturation.

1.3.3.2 Classes of T Cells

T cells can be categorized into two classes: helper T cells and cytotoxic T cells. These classes are based on whether they express CD4 (helper) or CD8 (cytotoxic) glycoprotein, their mode of activation and their functional roles in adaptive immunity.

T cells produce a variety of CD molecules among these CD4and CD8 are the two most commonly used for differentiation of the classes. Helper T cells are characterized by the expression of CD4 on their surface, whereas cytotoxic T cells are characterized by the expression of CD8.

1.3.3.3 Types of T Cells

Class	Surface CD Molecules	Activation	Function

Helper T Cells	**CD4**	APCs presenting antigens associated with MHC II	Orchestrate humoral and cellular immunity
Cytotoxic T Cells	**CD8**	APCs or infected nucleated cells presenting antigens associated with MHC I	Destroy cells infected with intracellular pathogens

1.3.4 Maturation of T Cells

T cells are originated from hematopoietic stem cells in the bone marrow and undergo positive and negative selection in the thymus for their maturation.

1.3.4.1 Generation of T Cells

Development of T cells occurs in the thymus. The thymic microenvironment directs the differentiation of T cells as well as positive and negative selection of T cells. Lymphoid progenitors cells (developed from hematopoietic stem cells in the bone marrow) migrate to the thymus to complete their antigen-independent maturation into functional T cells. Immature T cells that migrate to the thymus are called thymocytes. In the thymus, T cells develop their specific T cell markers, including TCR, CD3, CD4 or CD8, and CD2.

The **thymus** is a multi-lobed organ composed of **cortical** and **medullary** areas surrounded by a capsule. T cell progenitors enter the **subcapsular** cortical areas, where they encounter networks of **cortical epithelial cells** (the **thymic stroma**) and undergo a period of proliferation. As they differentiate, they move from the cortex towards the medulla of the thymus; different microenvironments within the thymus direct T cell development. Most cells that enter the thymus die by **apoptosis** without successfully completing the steps required for becoming a mature naive T cell. When the thymus becomes inactive in older stages of life, existing immature T cells will proliferate through clonal expansion.

The primary thymocytes express neither CD4 nor CD8, and are therefore categorised as double-negative (CD4-CD8-) cells. As they progress through their development they become double-positive thymocytes (CD4+CD8+) and finally mature to single-positive (CD4+CD8- or CD4-CD8+) thymocytes and are released from the thymus to peripheral tissues. These mature thymocytes are still considered as either “immature” or “naive” because they have not

been presented with an antigen. They now migrate to sites that contain secondary lymphoid tissues, such as the lymph nodes and tonsils, where antigen presentation. This facilitates the development of antigen-specific adaptive immunity.

1.3.4.2 Selection of T Cells

About 98% of thymocytes die during the development processes in the thymus by failing either positive selection or negative selection, while the other 2% survive and leave the thymus to become mature immunocompetent T cells. **Thymic selection** is a three-step process of negative and positive selection that determines which T cells will mature and exit the thymus into the peripheral bloodstream. The most characteristic property of mature T-cells is that they identify only foreign antigen combined with self MHC molecules. For this purpose, the thymocyte undergoes two selection processes in thymus.

1.3.4.3 Positive Selection of T Cells

Positive selection occurs in the cortical region of the thymus. Positive selection designates T cells capable of interacting with MHC. Double-positive thymocytes (CD4+/CD8+) move deep into the thymic cortex tissue where they are presented with self-antigens. These are expressed by thymic cortical epithelial cells that express both MHC I and MHC II molecules on the surface of cortical cells. Only those thymocytes that interact with MHC I or MHC II will receive a vital "survival signal." Those that can't interact will undergo apoptosis (cell death). This ensures T cell functionality since T cells with non-functional receptors cannot receive antigens and are thus useless to the immune system.

A thymocyte's differentiation into either a helper or cytotoxic type is also determined during positive selection. Double-positive cells (CD4+/CD8+) that are positively selected on MHC class II molecules will eventually become CD4+ helper T cells, while cells positively selected on MHC class I molecules mature into CD8+ cytotoxic T cells. A T cell is then signalled by the thymus to become a CD4+ cell by reducing expression of its CD8 cell surface receptors. If the cell does not lose its signal, it will continue reducing CD8 and become a CD4+, single positive cell. But if there is a signal interruption, it will instead reduce CD4 molecules, eventually becoming a CD8+, single positive cell. This process does not remove thymocytes

that may become sensitized against self-antigens, which causes autoimmunity. The potentially autoimmune cells are removed by the process of negative selection.

1.3.4.4 Negative selection:

T cells that survive positive selection migrate further into the cortico-medullary junction of the thymus where they encounter macrophages and dendritic cells, bone-marrow derived APC with high expression of MHC-self peptide complexes. T cells which bind self peptide-MHC with high affinity at this stage undergo **negative selection** and die by apoptosis. Negative selection removes thymocytes that are capable of strongly binding with self-antigens presented by MHC. Thymocytes that survive positive selection migrate towards the boundary of the thymic cortex and thymic medulla (the part of the thymus where T cells enter circulation). While in the medulla, they are again presented with self-antigen in complex with MHC molecules on thymic epithelial cells. Thymocytes that interact too strongly with the antigen receive an apoptotic signal that leads to cell death. Tolerance to self-antigens encountered in the thymus achieved by eliminating T-cells that are reactive to these antigens.

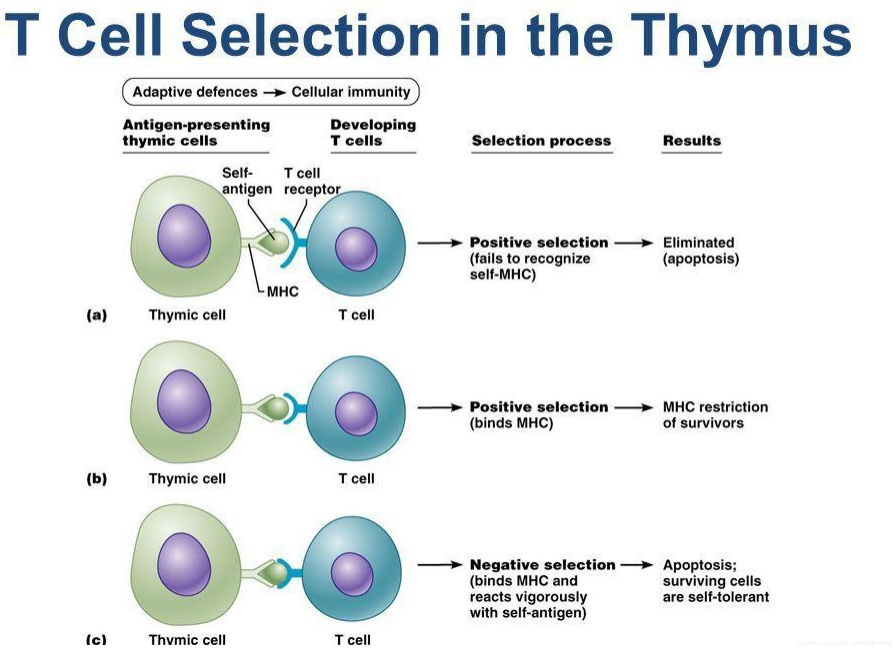

Fig. 2. T Cell selection in Thymus

1.3.5 Immunoglobulins

Antibodies are often referred to as **immunoglobulins** (immune proteins). Antibodies, or Y-shaped immunoglobulins, are proteins found in the blood that help to fight against foreign substances called antigens. Antigens, which are usually proteins or polysaccharides, stimulate the immune system to produce antibodies. The antibodies inactivate the antigen and help to remove it from the body. While antigens can be the source of infections from pathogenic bacteria and viruses, organic molecules detrimental to the body from internal or environmental sources also act as antigens. Genetic engineering and the use of various mutational mechanisms allow the construction of a vast array of antibodies (each with a unique genetic sequence).

Antibodies are all in a Y-shape with differences in the upper branch of the Y. These structural differences of amino acids in each of the antibodies enable the individual antibody to recognize an antigen. An antigen has on its surface a combining site that the antibody recognizes from the combining sites on the arms of its Y-shaped structure. In response to the antigen that has called it forth, the antibody wraps its two combining sites like a "lock" around the "key" of the antigen combining sites to destroy it.

An antibody's mode of action varies with different types of antigens. With its two-armed Y-shaped structure, the antibody can attack two antigens at the same time with each arm. If the antigen is a toxin produced by pathogenic bacteria that cause an infection like diphtheria or tetanus, the binding process of the antibody will nullify the antigen's toxin. When an antibody surrounds a virus, such as one that causes influenza, it prevents it from entering other body cells. Another mode of action by the antibodies is to call forth the assistance of a group of immune agents that operate in what is known as the plasma complement system. First, the antibodies will coat infectious bacteria and then white blood cells will complete the job by engulfing the bacteria, destroying them, and then removing them from the body. There are five different antibody types, each one having a different Y-shaped configuration and function. They are the Ig G, A, M, D, and E antibodies.

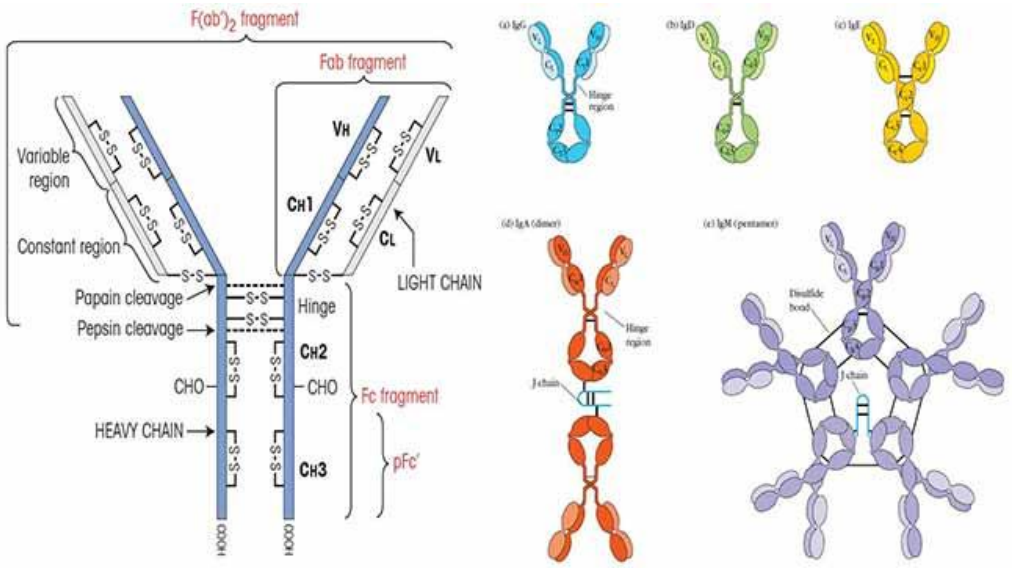

Fig. 3. Structure and types of Antibodies

1.3.5.1 Structure of Immunoglobulins (Antibody)

All antibody molecules share the same basic structural characteristics but display remarkable variability in the regions that bind antigens. Both heavy chains and light chains consist of aminoterminal **variable (V) regions** that participate in antigen recognition and carboxy-terminal **constant (C) regions**; the C regions of the heavy chains mediate effector functions.

1.3.5.2 Heavy Chain and Light Chain

An antibody molecule has a symmetric core structure composed of two identical **light chains** and two identical **heavy chains**. Both the light chains and heavy chains contain a series of repeating homologous units, each about 110 amino acid residues in length, that fold independently in a globular motif that is called an Ig domain. In the **heavy chains**, the **V region** is composed of one Ig domain, and the **C region** is composed of three or four Ig domains.

Each **light chain** is composed of one **V region** Ig domain and one **C region** Ig domain. The N-terminal half of light chains is thus referred to as the variable, or VL, region of the light chain, and the less variable part of the sequence is termed the constant, or CL, region. The two major light chain constant region sequences are referred to as **k (kappa) or λ (lambda) chains**. As more light-chain sequences were generated, it became apparent that the lambda chain constant region sequences could be further subdivided into four subtypes- λ 1, λ 2, λ 3, and λ 4- based on amino acid substitutions at a few positions.

In humans, the light chains are fairly evenly divided between the two light-chain classes; 60% of human light chains are kappa whereas only 40% are lambda. In mice, the situation is quite different: Only 5% of mouse light chains are of the lambda light-chain type. All light chains have a molecular weight of approximately 22 kDa. Variable regions are so named because their amino acid sequences vary among antibodies made by different B cell clones. The **V region** of one **heavy chain (VH)** and the adjoining **V region** of one **light chain (VL)** form an antigen-binding site. Because the core structural unit of each antibody molecule contains two heavy chains and two light chains, every antibody molecule has at least two antigen-binding sites. The C region Ig domains are distant from the antigen-binding site and do not participate in antigen recognition.

The heavy chain C regions interact with other effector molecules and cells of the immune system and therefore mediate most of the biologic functions of antibodies. In addition, heavy chains exist in two forms that differ at their carboxy-terminal ends: one form of the heavy chain anchors membrane-bound antibodies in the plasma membranes of B lymphocytes, and the other form is found only in secreted antibodies. The C regions of light chains do not participate in effector functions and are not directly attached to cell membranes.

1.3.5.3 Disulfide Bond

Heavy and light chains are covalently linked by **disulfide bonds** formed between cysteine residues in the carboxy terminus of the light chain and the CH1 domain of the heavy chain. Non-covalent interactions between the VL and VH domains and between the CL and CH1 domains may also contribute to the association of heavy and light chains. The two heavy chains of each antibody molecule are covalently linked by disulfide bonds. In IgG antibodies, these bonds are formed between cysteine residues in the CH2 domains, close to the region known as the hinge. In other isotypes, the disulfide bonds may be in different locations. Non-covalent interactions (e.g., between the third CH domains [CH3]) may also contribute to heavy chain pairing.

1.3.5.4 Hypervariable Region

Most of the sequence differences and variability among different antibodies are confined to three short stretches in the V region of the heavy chain and to three stretches in the V region

of the light chain. These segments of the greatest diversity are known as **hypervariable regions**.

1.3.5.5 Complementarity Determining Regions (CDRs).

The hypervariable loops can be thought to resemble fingers protruding from each variable domain, with three fingers from the heavy chain and three fingers from the light chain coming together to form the antigen-binding site. Because these sequences form a surface that is complementary to the three-dimensional shape of the bound antigen, the hypervariable regions are also called **complementaritydetermining regions (CDRs).**

1.3.5.6 Hinge Region

Antibody molecules are flexible, permitting them to bind to different arrays of antigens. Every antibody contains at least two antigen-binding sites, each formed by a pair of VH and VL domains. Many Ig molecules can orient these binding sites so that two antigen molecules on a planar (e.g., cell) surface may be engaged at once. This flexibility is conferred, in large part, by a **hinge region** located between CH1 and CH2 in certain isotypes. The hinge region varies in length from 10 to more than 60 amino acid residues in different isotypes.

1.3.5.7 Fab regions and Fc Region

The hinge region is particularly susceptible to proteolytic cleavage by the enzyme **papain**. Papain cleavage resolves the antibody molecule into two identical fragments that retain the antigen-binding specificity of the original antibody (Fab regions), and the remaining region of the molecule, which consists of the non antigen-binding portion. This latter region, which is identical for all antibodies of a given class, crystallizes easily and was thus called the **Fc region** (fragment crystallizable). Each Fab region and Fc region of antibodies mediates its own particular functions during an antibody response to an antigen. The Fab regions bind to the antigen, and the Fc region of the antigen-coupled antibody binds to Fc receptors on phagocytic or cytolytic cells, or to immune effector molecules.

1.3.6 Types of Immunoglobulins

Using antibodies directed toward the constant region of immunoglobulins and amino acid sequencing of immunoglobulins derived from plasmacytoma tumor cells, investigators discovered that the sequences of the heavy-chain constant regions fall into five basic patterns. These five basic sequences have been named with Greek letters: μ (mu), δ (delta), γ (gamma),

ε (epsilon), and α (alpha). Each different heavy-chain constant region is referred to as an isotype, and the isotype of the heavy chains of a given antibody molecule determines its class. Thus, antibodies with a heavy chain of the μ isotype are of the IgM class; those with a δ heavy chain are IgD; those with γ, IgG; those with ε, IgE; and those with α, IgA. Th e length of the constant region of the heavy chains is either 330 amino acid residues (for γ, δ, and α chains) or 440 amino acids (for μ and ε chains). Correspondingly, the molecular weights of the heavy chains vary according to their class. IgA, IgD, and IgG heavy chains weigh approximately 55 kDa, whereas IgM and IgE antibodies are approximately 20% heavier.

Antibodies are glycoproteins produced in membrane-bound or secreted form by B lymphocytes in response to exposure to foreign structures known as antigens. The human immunoglobulins are a family of proteins that confer humoral immunity and perform vital roles in promoting cellular immunity. There are five classes of antibodies or immunoglobulins termed immunoglobulin G (IgG), IgM, IgA, IgD and IgE. All these classes have the basic four – chain antibody structure but they differ in their heavy chains termed γ, μ, α, δ and ε respectively.

The antibodies are the gamma globulins. Antibodies are often referred to as "first line of defense" against infection. The most important function of antibodies is to confer protection against microbial pathogens. Antibodies confer protection in the following ways:

- They prevent attachment of microbes to mucosal surfaces of the host.
- They reduce virulence of microbes by neutralizing toxins and viruses.
- They facilitate phagocytosis by opsonization of microbes.
- They activate complement, leading to complement-mediated activities against microbes.

1.3.6.1 Immunoglobulin A(IgA)

- Molecular weight: 320,000 Da
- H- chain type: Alpha (55000 Da)
- IgA constitute 10-15% of total serum immunoglobulin.

- It is the predominant Immunoglobulin n external secretions such as breast milk, saliva, tears and mucus of bronchial, genitourinary and digestive tracts.
- IgA primarily exists as monomeric form but dimeric, trimeric and some tetrameric form are also present.
- IgA in blood occurs in monomeric form whereas those in body secretion occurs in dimeric or multimeric forms.
- Dimeric form of IgA contains J-chain and secretory chain. Secretory chains helps in transcytosis.
- IgA can cross epithelial layer and enter into body secretion. The process of crossing epithelial layer by IgA is known as transcytosis.
- There are two sub-class of IgA ie. IgA1 and IgA2.

1.3.6.1.1 Biological functions;

- IgA can cross the epithelial layer and enter into body secretion and provides local immunity in GI tracts, respiratory tract, genital tract etc
- In body secretion IgA neutralize viruses and prevent attachment on host surface.

1.3.6.2 Immunoglobulins G (IgG)

- Molecular weight: 150,000 Da
- H-chain type: gamma (53,000 Da)
- IgG is the most abundant class of Immunoglobulin in serum and constitute of about 80% of total serum immunoglobulin.
- IgG molecule consists of two gamma (γ) heavy chains and two kappa (k) or two lambda (λ) light chains.
- There are four sub class of IgG (IgG1, IgG2, IgG3 and IgG4) on the basis of decreasing serum concentration.
- It has longest half-life among other antibodies. Half-life is about 23 days.
- IgG is the only antibody that can cross placenta. It cross placenta and provide immunity to fetus upto 6 month of age. The immunity is known as natural passive immunity.

- It can also activate complement.

1.3.6.2.1 Biological functions

- IgG is the major antibody produced in secondary immune response.
- Ig, IgG3 and IgG4 readily cross the placenta and play important role in protecting the fetus.
- IgG3 is the most effective complement activator followed by IgG1 and IgG2. IgG4 is not able to activate complement at all.
- IgG1 and IgG3 binds with high affinity to Fc receptor on phagocytic cell and thus mediate opsonization.
- IgG helps in bacterial immobilization.
- IgG neutralize toxin and viruses.

1.3.6.3 Immunoglobulins M (IgM)

- Molecular weight: 900,000 Da
- H-chain type: mu (65,000 Da)
- IgM accounts for 5-10% of total serum Immunoglobulin with an average serum concentration of 1.5mg/dl.
- IgM is secreted by plasma cell and it exists in pentameric form in which five IgM mononers are linked together by disulphide bond (J-chain).
- Due to large size, IgM is also known as millionare molecule.
- There are 10 antigen binding site (Fab) in pentameric IgM molecule but it cannot bind to 10 complete antigen due to steric hindrance.
- It is the major antibody produced during primary immune response.
- Monomeric form IgM (180000 Da) is also expressed as membrane bound receptor on B-cell.

1.3.6.3.1 Biological functions

- IgM is the first antibody produced in primary immune response and it is also the first Ig produced by neonate.
- IgM has higher valency (antigen binding ste) due to its pentameric form.
- Due to pentameric form, IgG is very effective in agglutination reaction.
- IgM is more efficient than IgG in complement activation.
- IgM plays important accessory role as secretory immunoglobulin due to J-chain.

1.3.6.4 Immunoglobulins D (IgD)

- Molecular weight: 180,000 Da
- H-chain type: Delta (70000 Da)
- IgD is present in extremely low concentration and it constitute 0.2% of total serum immunoglobulin.
- IgD together with IgM is the major membrane bound immunoglobulin expressed on mature B-cell.
- There are two sub-classes of IgD (IgD1 and IgD2)
- IgD plays important role in maturation and proliferation of B-cell.

1.3.6.5 Immunoglobulins E (IgE)

- Molecular weight: 200,000 Da
- H-chain type: epsilon (73,000Da)
- IgE accounts for 0.3% of total serum Immunoglobulin.
- IgE is also known as reagenic antibody due to its involvement in allergic reaction. IgE mediate immediate hypersensitivity reaction and responsible for symptoms like hey fever, asthma, anaphylactic shocks, etc.
- Fc region of IgE binds on blood basophils and tissue mast cells. The cross reaction with antigen to Fc region bound IgE causes degranulation of mast cell and basophils releasing histamine. Histamine is responsible for symptoms of allergy.

1.3.6.5.1 Biological functions

- IgE provides immunity against parasite by Antibody dependent cell mediated cytotoxicity (ADCC).
- Level of IgE antibody in blood of normal individual is very low and its level increases during parasitic infection and in allergic reactions.

1.3.7 Immunization

Immunization is the process by which a person naturally acquires or is induced to acquire immunity or resistance to an infectious disease.

Immunization is a proven highly effective process for controlling and eliminating life-threatening infectious diseases.

An individual can acquire such immunity either passively or actively and thus immunization may be active or passive immunization.

1.3.7.1 Active Immunization

- In active immunization, the immune system is induced to produce antibodies against a particular infectious agent and thus the immune system of the individual to which immunity is to be conferred is actively involved in the process.
- It may arise naturally, such as when an individual is exposed to an antigen or pathogen.
- For example, an individual who recovers from a first case of the measles is immune to further infection by the measles-causing virus, because the virus stimulates the immune system to produce antibodies that specifically recognize and neutralize the pathogen the next time it is encountered.
- Active immunization can also be conferred artificially by means of vaccines. Vaccines consist of a nontoxic antigen preparation that infers protective immunity by inducing a memory response to an infectious microorganism. This results in immunity which may either be antibody mediated immunity and/or cellular immunity.
- The purpose of vaccination is to ensure that a large number of antibodies and lymphocytes capable of reacting against a specific pathogen or toxin are available before exposure to it occurs.
- Artificial active immunization can be induced by two different routes :

1.3.7.2 Systemic immunization

It involves injecting the vaccine subcutaneously or intramuscularly into the deltoid muscle. Examples: systemic vaccines for measles, mumps and rubella, and against *Pneumococcus, Haemophilus and Meningococcus* infections etc.

1.3.7.3 Mucosal immunization

It involves on the mucosal route as the site of choice for immunization either orally or through the nasal associated immune tissue (NALT) such as the oral immunization against Polio.

- Active immunization is mostly performed as a prophylaxis measure.
- Some infections in which active immunization is performed include Hepatitis A infection, Influenza, Measles, Mumps, Rubella, Yellow fever etc.

1.3.7.3.1 Advantages

- The protection provided by active immunization is long-lived since it leads to the formation of long-lasting memory immune cells.
- Active immunization may be reactivated quickly by a recurrence of the infection or by revaccination.
- It is less costly in preparation and to administer than passive immunization techniques.

1.3.7.3.1.1 Drawbacks

- The protective response takes time to establish ranging from few days to weeks which makes it inefficient as a post exposure remedy.
- Since active immunization is dependent on the individuals' immune responses, it may not be suitable for protection of immuno-compromised or immuno-deficient individuals.

1.3.7.4 Passive immunization

- In passive immunization**,** a person receives antibodies or lymphocytes that have been produced by another individual's immune system while in active immunization the individual's own immune system is stimulated to produce antibodies and lymphocytes.
- **Passive immunization**
- It is hence the administration of preformed antibodies, usually IgG.
- It may arise naturally, such as when a foetus receives antibodies from the mother across the placenta or when a breast-feeding infant ingests antibodies in the mother's milk.

- However, passive immunization also can be conferred artificially by means of preformed antibodies administered through intravenous or intramuscular routes.
- These antibodies may be derived from individuals who have high titres to particular microbes and are used to provide rapid protection.
- Antibodies given to immune deficient patients are usually IgG-derived from pooled normal plasma or purified blood products of immune people.
- Antibodies preformed in animals has also been used against some diseases, the most common being that of the horses. However, the danger of immune complex formation and conditions like serum sickness with repeated administration must be checked for.
- Passive immunization is also used to provide protection in immune compromised individuals who are unable to make the appropriate antibody response.
- Pre-formed antibodies have to be given on a continuous basis, ideally every three weeks, since they are continuously catabolized and only effective for a short period.
- Infections in which passive immunization is important include diptheria, tetanus, rabies etc., in events of accidental exposure to certain pathogens such as hepatitis B or at other instances such as snake bites.

1.3.7.4.1 Advantages

- Passive immunization with preformed antibodies leads to prompt availability of large amounts of antibody. It is thus quick acting, producing an immune response within hours or days, faster than a vaccine.
- It helps to prevent or slow down the course of disease.
- It is beneficial to high-risk individuals, such as people with immune system deficiencies.

1.3.7.4.2 Drawbacks

- The protection provided by passive immunization is short-lived, usually lasting only a few weeks or months since it do not lead to the formation of long-lasting memory immune cells.
- In passive immunity it is possible to initiate hypersensitivity reactions if the antibody is from another species.
- Antibody treatment cannot be used for routine cases of diseases.
- Antibodies can be difficult and costly to produce.
- Many antibody treatments must be given via intravenous injection, which is a more time-consuming and potentially complicated procedure than the injection of a vaccine.

1.3.7.4.3 Stem Cells

Stem cells are unique cells present in the body that have the potential to differentiate into various cell types or divide indefinitely to produce other stem cells. In future stem cells may be used to replace cells and tissues that have been damaged or lost due to disease.

- These cells are the earliest cells of the cell lineage in all tissues and are found in both embryonic and adult organisms.

1.3.7.4.3.1 Properties of Stem Cells

All the stem cells of all living systems have three important properties:

1. Stem cells are capable of dividing and renewing themselves for long periods of time. These cells undergo a period of cell proliferation while preserving the undifferentiated state.
2. Stem cells are undifferentiated cells that can turn into specific cells. These provide a continuous supply of new cells that make up the tissues and organs of organisms.
3. All stem cells are unspecialized or undifferentiated. These are present as a mass of cells that differentiate later during their period of division.

1.3.7.4.3.2 Sources of Stem Cells

Stem cells are originated from two main sources: embryos and adult body tissues. Stem cells from other cells can also be developed in laboratory by using genetic "reprogramming" techniques. The stem cells in the embryonic organism are present in the inner cell mass of the **blastocyte**, which then differentiates into all other cells in the body. The stem cells in adults are localized to specific areas within the body such as in the bone marrow and the gonads.

1.3.7.4.4 Types of Stem Cell

Depending on the source of the stem cells or where they are present, stem cells are divided into various types;

1. Embryonic stem cells

- Embryonic stem cells are a group of cells that are present in the inner cell mass of the embryo at a very early stage of development, called a blastocyst.
- Cells in the early embryonic stage are totipotent and can differentiate to become any type of body cell.

- The blastocyst stage in embryonic development is reached within 4-5 days after fertilization, and the number of cells at that point is about 50-150.
- These cells are pluripotent, meaning they can develop and differentiate into various cell types (approx 250 types) during their proliferation. These do not, however, contribute to the extraembryonic cells like the placenta.
- The embryonic germ cells in the gonadal region in animals also act like embryonic stem cells. These cells, also called primordial cells, later differentiate and divide to form male and female gametes.

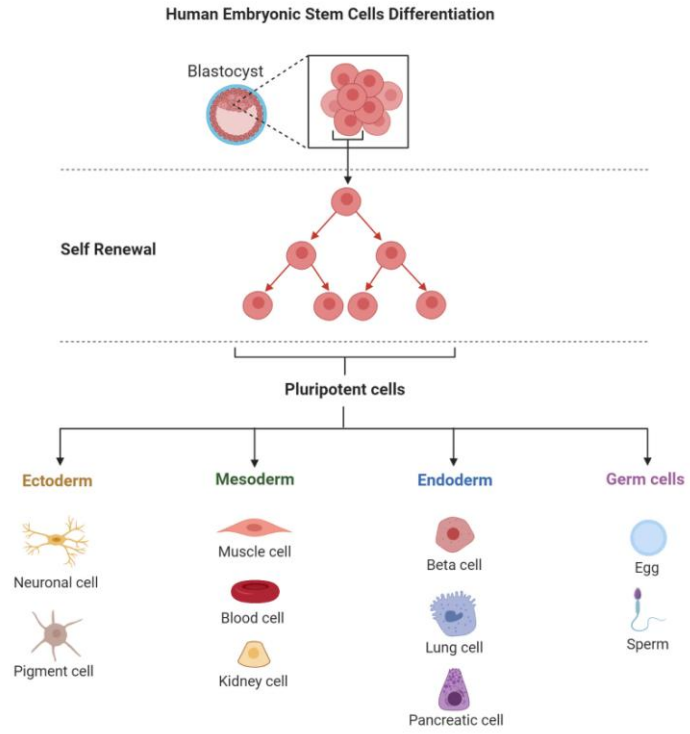

Fig. 4 Human Embryonic Stem Cells Differentiation

1.3.7.4.4.1 Adult stem cells

Adult stem cells also called somatic stem cells. These cells are found in specific tissues that function to repair.

- These cells are considered less potent than embryonic stem cells as they cannot differentiate to different cell types.

- These cells are found in certain tissues that undergo continuous cellular turn over. The cells in the epidermis layer divide continuously to form new cells as the keratinocytes are shed off. Some tissue like the liver tissue, however, undergoes minimal division only when the tissue is damaged.
- Adult stem cells are found in both children and adults and mostly localized in tissue like the epidermis, bone marrow, and lining of the intestine.
- Adult stem cells present in the bone marrow are the hematopoietic cells that differentiate to form three different types of blood cells and immune cells.

1.3.7.4.4.2 Induced Pluripotent Stem Cells (iPSCs)

- These stem cells are created in a lab by using skin cells and other tissue-specific cells cells by the process of reprogramming the genes.
- Induced pluripotent stem cells are similar to embryonic stem cells in that they can also be stimulated to differentiate into different cell types.
- These cells are of significant importance as they can be used in therapeutic medicine where doctors will be able to generate cells of practically all organs of the body for each patient.
- Induced stem cells of the heart and the eyes can be used in the transplantation of the cells during severe heart and eye-related diseases.

1.3.7.4.4.3 Perinatal stem cells

- Perinatal stem cells are a type of intermediate cells carrying the characteristics of both embryonic stem cells and adult stem cells. They are derived from extra-embryonic cells of the foetal membrane, umbilical cord, and amniotic fluid.
- These cells are active, non-tumorigenic, and are multipotent that can differentiate into cells of the endothelium, hepatic, adipose, and even neural tissues.
- Perinatal stem cells also have research and therapeutic applications in the treatment of renal disease, cardiac disease, inflammatory disease, bone regeneration, and the treatment of spinal cord injury.

1.3.7.4.4.4 Mesenchymal stem cells (MSCs)

- Mesenchymal stem cells are a type of adult stem cell or somatic stem cell mostly found in the tissues of muscles, liver, and bone marrow.

- Human MSCs (hMSCs) are the multipotent stem cells with the capacity to differentiate into mesodermal cell lines such as osteocytes, adipocytes, and chondrocytes as well ectodermal (neurocytes) and endodermal cell lines (hepatocytes).
- Mesenchymal stem cells are found not only in foetal tissues but also in many adult tissues.
- The potential to produce cells of different cell lines, immunomodulation, and secretion of anti-inflammatory molecules makes this stem cell a useful tool in the treatment of chronic diseases.

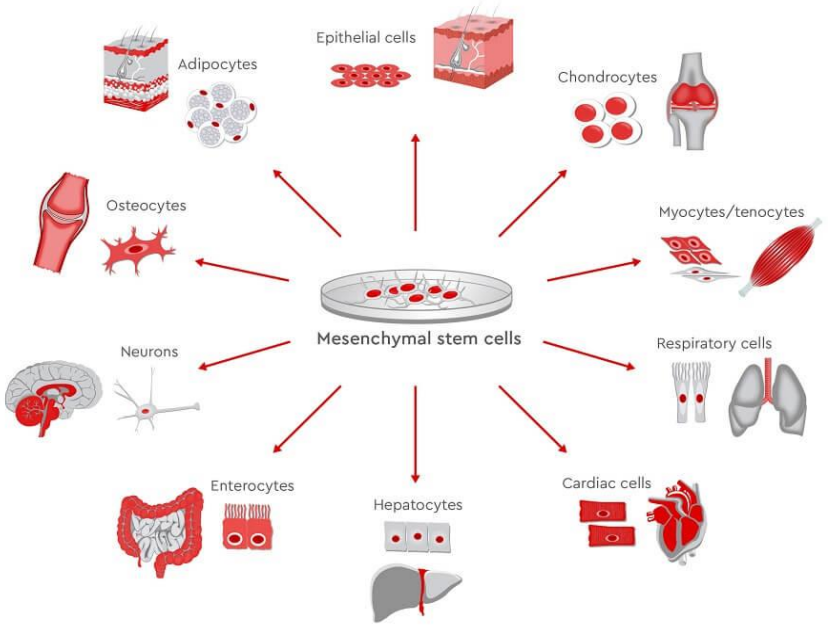

Fig. 5. Mesenchymal stem cells (MSCs).

1.3.8 Stem Cell Therapy

Stem cell therapy is an exciting and active field of biomedical research. Scientists and physicians are investigating the use of stem cells in therapies to treat a wide variety of diseases and injuries. For a stem cell therapy to be successful, a number of factors must be considered. The appropriate type of stem cell must be chosen, and the stem cells must be matched to the recipient so that they are not destroyed by the recipient's immune system. It is also critical to develop a system for effective delivery of the stem cells to the desired location in the body. Finally, devising methods to "switch on" and control the differentiation of stem cells and ensure that they develop into the desired tissue type is critical for the success of any stem cell therapy.

Researchers are currently examining the use of stem cells to regenerate damaged or diseased tissue in many conditions, including heart disease,Parkinson's disease,Spinal cord injury,Diabetes mellitus,Amyotrophic lateral sclerosis,Arthritis,Burns etc.

1.3.9 Immune Technology or Immunotechnology

Immunotechnology is a technology based on applications of cells and molecules of the immune system. The exquisite specificity of antigen –antibody interactions has led to the development of a variety of immunological techniques. These techniques have played a vital role in diagnosing disease, monitoring the level of the humoral immune response, and identifying molecules of biological or medical interest.

Advances in immunodiagnostic technologies provide the basis for developing antigen-detection platforms capable of meeting stringent requirements for sensitivity, specificity, assay speed, robustness, and simplicity. Antibody-based microarray is setting a novel proteomic technology setting a new standard for molecular profiling of non-fractionated complex proteomes.

A number of techniques are used extensively by researchers investigating humoral and cellular immune responses to oral organisms both in local oral tissues and fluids and systemically in peripheral blood.

1.4.0 Immunochemical techniques

Immunochemical techniques are based on a reaction of antigen with antibody, or more

exactly, on a reaction of an antigenic determinants with the binding site of the antibody. The antibodies used are produced by various ways.

1.4.1 Monoclonal antibodies

They are products of a single clone of plasma cells derived from B-lymphocytes, prepared in the laboratory by hybridoma technology, based on cellular fusion of tumour (myeloma) cells with splenic lymphocytes of immunised mice. Monoclonal antibodies are directed against single epitope; and are all identical copies of immunoglobulin molecule with the same primary structure and specificity of antigen binding site. They typically display excellent specificity, but poor ability to precipitate antigen.

1.4.2 Polyclonal antibodies

They are (conventional antibodies) are prepared by immunisation of animals (rabbits, goats, sheep) with the antigen. Blood serum of the immunised animal that contains antibodies against the antigen used, is called an **antiserum**. If one antigen (e.g. one protein) is used for immunisation, **monospecific antibodies** (antiserum) result. However, as every epitope stimulates different clone of B cells, and complex antigens bear several epitopes, the antiserum contains mixture of monoclonal antibodies, differing in their affinity and specificity towards particular epitopes on the antigen used for immunisation.

Immunisation of an animal with mixture of antigens results in production of **polyspecific antibodies**[1], containing immunoglobulins against many antigens (e.g. antiserum against human serum proteins used in immunoelectrophoresis).

1.4.3 Quantitative precipitation Curve

Measurement of antigen-antibody complex formation has proved extremely useful for analysing many constituents of body fluids. A wide variety of immunochemical methods has been developed based on the fundamental principle of quantitative precipitin curve described by **Heidelberger** and **Kendall** in 1935.

The precipitin test is an example of a clinical test based on antigen-antibody reaction. Antibodies are able to precipitate antigens through multivalent binding, in which 2 Fab fragments in a single antibody can simultaneously bind to 2 antigens. A matrix of antigen:antibody complexes in a solution will then lead to a formation of a visible

precipitate. In the precipitin test, a soluble antigen and antibody diffuse toward each other, and a visible precipitate forms when the 2 solutes meet at an optimal concentration. The precipitin curve describes the relationship between the antigen concentration and the amount of precipitate for a constant quantity of antibody. Three zones can be distinguished on the precipitin curve in the given figure.

1.4.3.1 The antibody excess zone

The amount of precipitate increases proportionally as the concentration of antigen increases. If the antibody is present in an excess, all the antigen binding sites are covered with the antibody and only small soluble antigen-antibody complexes are formed. No free antigen is detected in the supernatant, but free (unbound) antibodies can be found. Such conditions are useful for immunoturbidimetry, immunonephelometry and non-competitive immunoassays.

1.4.3.2 The equivalence zone

Molecules of antigen and antibody are cross-linked forming large, insoluble complexes. The complexes further aggregate and precipitate. Neither free antigen nor free antibody can be detected in the supernatant. Equivalence is reached in ***immunodiffusion techniques***.

The antigen excess zone: The amount of precipitate decreases due to the high antigen concentration. Large aggregated immunocomplexes decay. As all the antibody sites are saturated by antigen, small soluble complexes prevail. No free antibody but an increasing amount of free antigen may be found in the liquid phase. Excess of free antigen is required for ***competitive immunoassays***.

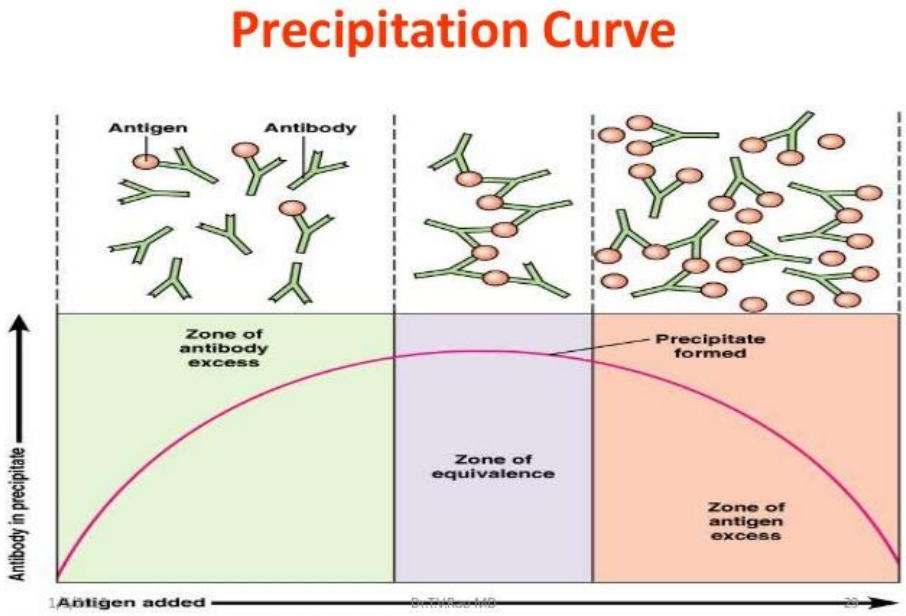

Fig. 6. precipitation Curve

This immunoprecipitation curve forms the basis of many immunochemical assays that can be performed in a solution as well as in a gel.

1.4.3.3 Precipitation methods in gel

Immuno-diffusion is a technique for the detection or measurement of antibodies and antigens by their precipitation which involves diffusion through a substance such as agar or gel agarose. Simply, it denotes precipitation in gel. **Based on the method employed, immuno-diffusion may be:**

1. Radial immunodiffusion
2. Ouchterlony Double Diffusion

1.4.3.3.1 Radial immunodiffusion (RID) or Mancini method is also known as Mancini immunodiffusion or single radial immunodiffusion assay. It is a single diffusion technique whereby a solution containing the antigen is placed into wells in a gel or agar surface evenly impregnated with antibody. The diameter of the ring that precipitates around the well as a result of antigen antibody reaction corresponds to the amount of antigen in the solution.

Objectives of Radial Immunodiffusion

The Mancini immunodiffusion test may be carried out with one or more of the following objectives:

1. To detect antigen-antibody complexes.
2. Describe the circumstances under which antigen-antibody complexes precipitate out.
3. Determine relative concentration of antigens.

1.4.3.3.2 Principle of Radial Immunodiffusion

Radial immuno-diffusion is a type of precipitation reaction. It is thus based on the principles of the precipitin curve which states that antigen-antibody interact forming visible cross-linked precipitate when the proper ratio of antigen to antibody is present. In the test, antibody is incorporated into agar and poured into a glass plate to form a uniform layer. Circular wells are cut into the agar and antigen is introduced into the wells. Specific antigens to the impregnated antibodies diffuse through the agar in all directions from the well and react with the antibody present forming visible precipitate or a precipitin ring. Ring shaped bands of precipitates from concentrically around the well indicating reaction. The diameter of the precipitate ring formed, corresponds to the amount of antigen in the solution.

1.4.3.3.3 Procedure of Radial Immunodiffusion

1. An agar containing an appropriate antiserum (antibody) is poured in plates.
2. Carefully circular wells are cut and removed from the plates.
3. A single or series of standards containing known concentration of antigen are placed in separate wells, while control and “unknown” samples are placed in other remaining wells.
4. As the antigen diffuses radially, a ring of precipitate will form in the area of optimal antigen – antibody concentration.
5. The ring diameters are measured and noted.
6. A standard curve is prepared using the ring diameters of the standards versus their concentrations. This curve is then used to determine the concentration of the control and unknown samples.

1.4.3.3.4 Result of Radial Immunodiffusion

1. The presence of a precipitin ring around the antigen wells indicate specific antigen-antibody interaction.
2. Absence of precipitin ring suggest absence of reaction.

3. The greater the amount of antigen in the well, the farther the ring will form from the well.

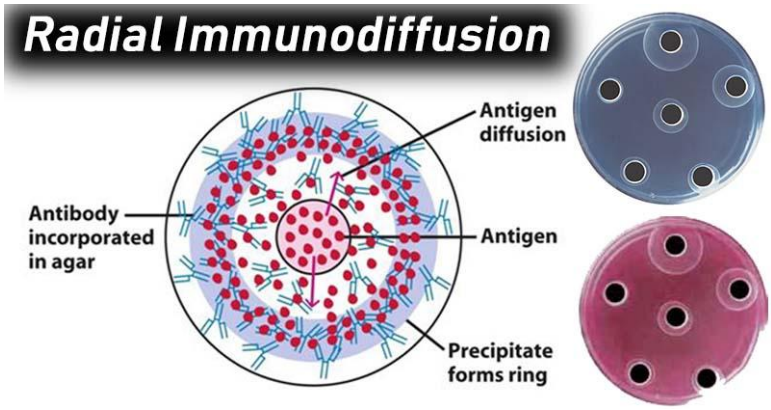

Fig. 7. Radial Immunology

1.4.3.3.5 Applications of Radial Immunodiffusion

- Immuno-diffusion techniques are mostly used in immunology to determine the quantity or concentration of an antigen in a sample.
- Estimation of the immunoglobulin classes in sera.
- Estimation of IgG, IgM antibodies in sera to influenza viruses.

1.4.3.3.6 Advantages of Radial Immunodiffusion

1. Precipitation in gels is believed to provide more specific and sensitive results than other methods available.
2. The reaction is in the form of bands of precipitation and can be stained for better viewing as well as preservation.
3. If a large number of antigens are present, each antigen-antibody reaction will give rise to a separate line of precipitation.
4. This technique also indicates identity, cross reaction and non identity between different antigens.

1.4.3.3.7 Limitations of Radial Immunodiffusion

1. Long reaction time (18-48 hours)
2. It has also been proposed that the results of Mancini's test is influenced by the presence bound metal cations in the test samples (protein).

3. Single diffusion menthod of precipitation is considered relatively wasteful than other methods.
4. The test has been recently replaced by more sensitive and automated methods, such as nephelometry and enzyme-linked immunosorbent assays.

1.4.3.3.8 Ouchterlony Double Diffusion

- It is a special precipitation reaction on gels where antibodies react with specific antigens forming large antigen-antibody complexes which can be observed as a line of the precipitate.
- In double immunodiffusion, both the antibody and antigen are allowed to diffuse into the gel.
- After application of the reactants in their respective compartments, the antigen and the antibody diffuse toward each other in the common gel and a precipitate is formed at the place of equivalence.

1.4.3.3.9 Double diffusion in one dimension

The method also called Oakley–Fulthrope procedure involves the incorporation of the antibody in agar gel in a test tube, above which a layer of plain agar is placed. The antigen is then layered on top of this plain agar. During incubation, the antigen and antibody move toward each other through the intervening layer of plain agar. In this zone of plain agar, both antigen and antibody react with each other to form a band of precipitation at their optimum concentration.

1.4.3.3.10 Double diffusion in two dimension

It is more commonly known as Ouchterlony double diffusion or passive double immunodiffusion. In this method, both the antigen and antibody diffuse independently through agar gel in two dimensions, horizontally and vertically.

1.4.3.3.1.1 Objectives

The Ouchterlony double immunodiffusion test may be carried out with one or more of the following objectives:

1. To detect antigen-antibody complexes.
2. Describe the circumstances under which antigen-antibody complexes precipitate out.
3. Detect the presence of an antigen-specific antibody.

4. To test the similarity between antigens.

1.4.3.3.1.2 Principle

In the test, an antigen solution or a sample extract of interest is placed in wells bore on gel plates while sera or purified antibodies are placed in other remaining wells (Mostly, an antibody well is placed centrally). On incubation, both the antigens in the solution and the antibodies each diffuse out of their respective wells. In case of the antibodies recognizing the antigens, they interact together to form visible immune complexes which precipitate in the gel to give a thin white line (precipitin line) indicating a reaction.

In case multiple wells are filled with different antigen mixtures and antibodies, the precipitate developed between two specific wells indicate the corresponding pair of antigen-antibodies.

1.4.3.3.1.3 Procedure

1. Dissolve 100 mg of agarose in 10 ml of the buffer by boiling to completely dissolve the agarose.
2. Cool solution to 55 °C and pour agarose solution to a depth of 1 – 2 mm on a clean glass plate (petri dish or rectangular plate) placed on a horizontal surface.
3. Allow the gel to set for 30 minutes.
4. Wells are punched into the gel using a gel borer corresponding to the marks on the template if used.
5. Fill wells with solutions of antigen and antiserum (of same or different dilutions) until the meniscus just disappears. Antiserum is usually placed in the central well and different antigens are added to the wells surrounding the center well.
6. Incubate the glass plate in a moist chamber overnight at 37 °C.

1.4.3.3.1.4 Results

- The presence of an opaque precipitant line between the antiserum and antigen wells indicates antigen-antibody interaction.
- Absence of precipitant line suggests the absence of reaction.
- When more than one well is used there are many possible outcomes based on the reactivity of the antigen and antibody selected.

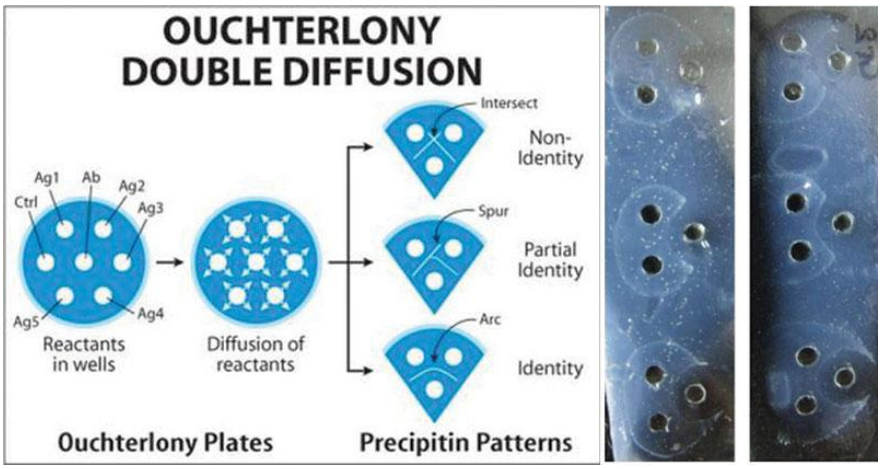

Fig. 8. Ouchterlony double immunodiffusion

- The results may be either of the following:
- **A full identity (i.e. a continuous line):** Line of precipitation at their junction forming an arc represents serologic identity or the presence of a common epitope in antigens.
- **Non-identity (i.e. the two lines cross completely):** A pattern of crossed lines demonstrates two separate reactions and indicates that the compared antigens are unrelated and share no common epitopes.
- **Partial identity (i.e. a continuous line with a spur at one end):** The two antigens share a common epitope, but some antibody molecules are not captured by the antigen and traverse through the initial precipitin line to combine with additional epitopes found in the more complex antigen.
- The pattern of the lines that form can determine whether the antigens are the same.

1.4.3.3.1.5 Applications

1. It is useful for the analysis of antigens and antibodies.
2. It is used in the detection, identification, and quantification of antibodies and antigens, such as immunoglobulins and extractable nuclear antigens.
3. Agar gel immunodiffusions are used as serologic tests that historically have been reported to identify antibodies to various pathogenic organisms such as *Blastomyces*.
4. Demonstration of antibodies in serodiagnosis of smallpox.
5. Identification of fungal antigens.
6. Elek's precipitation test in the gel is a special test used for demonstration of toxigenicity of *Corynebacterium diphtheriae.*

1.4.4 Immunoelectrophoresis

Immunoelectrophoresis is a qualitative method that combines protein electrophoresis with immunodiffusion.

- Immunoelectrophoresis refers to precipitation in agar under an electric field.
- The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953.

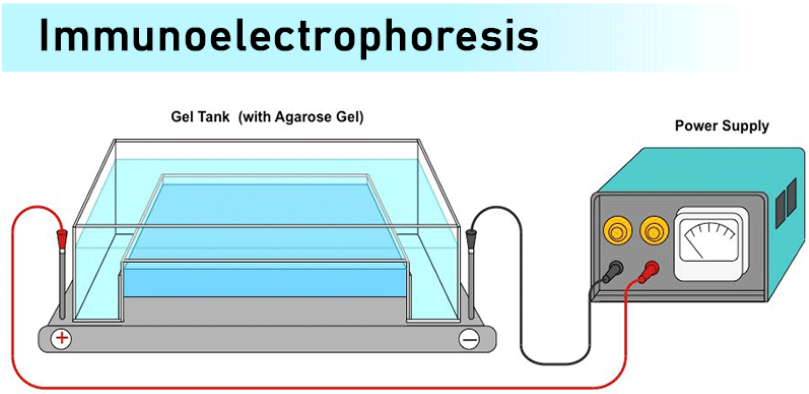

Fig. 9. Immunoelectrophoresis

1.4.4.1 Principle

When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size. Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration and diffusion is allowed to occur. Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with its antibody.

1.4.4.2 Procedure

1. Agarose gel is prepared on a glass slide put in a horizontal position.
2. Using the sample template, wells are borne on the application zone carefully.
3. The sample is diluted 2:3 with protein diluent solution (20µl antigen solution +10 µl diluent).
4. Using a 5 µl pipette, 5 µl of control and sample is applied across each corresponding slit (Control slit and Sample slit).
5. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts.

6. After electrophoresis completes, 20 µl of the corresponding antiserum is added to troughs in a moist chamber and incubated for 18- 20 hours at room temperature in a horizontal position.
7. The agarose gel is placed on a horizontal position and dried with blotter sheets.
8. The gel in saline solution is soaked for 10 minutes and the drying and washing repeated twice again.
9. The gel is dried at a temperature less than 70°C and may be stained with protein staining solution for about 3 minutes followed by decolorizing the gel for 5 minutes in distaining solution baths.
10. The gel is dried and results evaluated.

1.4.4.3 Results

1. The presence of elliptical precipitin arcs represents antigen-antibody interaction.
2. The absence of the formation of precipitate suggests no reaction.
3. Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

1.4.4.4 Applications

1. The test helps in the identification and approximate quantization of various proteins present in the serum. Immunoelectrophoresis created a breakthrough in protein identification and in immunology.
2. Immunoelectrophoresis is used in patients with suspected monoclonal and polyclonal gammopathies.
3. The method is used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum.
4. Used to analyze complex protein mixtures containing different antigens.
5. The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).
6. This method is useful to monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens.
7. Immunoelectrophoresis is an older method for qualitative analysis of M-proteins in serum and urine.
8. Immunoelectrophoresis aids in the diagnosis and evaluation of the therapeutic response in many disease states affecting the immune system.

1.4.4.5Advantages

1. Immunoelectrophoresis is a powerful analytical technique with high resolving power as it combines the separation of antigens by electrophoresis with immunodiffusion against an antiserum.
2. The main advantage of immunoelectrophoresis is that a number of antigens can be identified in serum.

1.4.4.6 Limitations

1. Immunoelectrophoresis is slower, less sensitive, and more difficult to interpret than Immunofixation electrophoresis.
2. IEP fails to detect some small monoclonal M-proteins because the most rapidly migrating immunoglobulins present in the highest concentrations may obscure the presence of small M-proteins.
3. The use of immunoelectrophoresis in food analysis is limited by the availability of specific antibodies.

1.4.4.7 **Counter Current Immunoelectrophoresis**

- Counter Current Immunoelectrophoresis is a modification of immunoelectrophoresis in which antigen and antibody move in opposite directions and form precipitates in the area where they meet in concentrations of optimal proportions.

1.4.4.8 Objectives

Countercurrent immunoelectrophoresis is mostly carried out with one or more of the following objectives:

1. To rapidly check any antisera for the presence and specificity of antibodies for a particular antigen.
2. To detect antigens and/or antibodies in serum for diagnosis of a particular disease

1.4.4.9 Principle

Counter-current immunoelectrophoresis depends on the movement of antigen towards the anode and of antibody towards the cathode through the agar under the electric field. The test is performed on a glass slide in agarose gel of high electro-endosmotic flow. A pair of wells is punched out where one well is filled with antigen and the other with the antibody. Electric current is then passed through the gel. The migration of antigen and antibody is greatly

facilitated under the electric field, and the line of precipitation as precipitin arcs is made visible in 30–60 minutes, which indicates a positive reaction.

1.4.5.0 Procedure

1. 10 ml of 1.0% Agarose (0.1 g/10 ml) in 1X Assay Buffer is prepared by heating slowly until agarose dissolves completely.
2. The ends of a glass slide are marked as +ve and -ve so that when placed in the electrophoresis apparatus, the +ve mark is faced towards the anode and the negative mark faced towards the cathode.
3. The glass plate or slide is placed on a horizontal surface. 5 ml of agarose is pipetted and spread onto the glass slide. It is allowed to solidify for 15 minutes.
4. Wells are cut in the gel according to the template using gel puncher. The distance between the two wells is not kept more than 0.5 cm.
5. The slide is placed in the electrophoresis tank and the tank filled with 1X electrophoresis buffer till the buffer just covers the gel surface.
6. 10µl of antigen is added in each of the two wells towards the cathode (Negative electrode) and 10µl of positive control antiserum and test antisera in wells towards the anode (Positive Electrode).
7. The power cord is connected to the electrophoretic power supply according to the convention.
8. 50 V is applied and the electrophoresis is allowed to continue for about 45 minutes after the completion of which results are interpreted.

1.4.5.1 Results

Precipitin line between the antigen and antisera wells indicate positive reaction or specific antigen-antibody reaction due to the presence of antibody specific to the antigen.

The absence of the precipitin line indicates no reaction or the absence of any corresponding antibody-antigen.

The presence of more than one precipitin line indicates the heterogeneity of the antibody for the antigen.

1.4.5.2 Applications

The counter-current immuno-electrophoresis has many uses:

1. It is a rapid and a highly specific method for detection of both antigen and antibodies in the serum, cerebrospinal fluid, and other body fluids in the diagnosis of many infectious diseases including bacterial, viral, fungal, and parasitic.
2. The test was very popular in the past for detecting various antigens such as alpha-fetoprotein in serum and capsular antigens of *Cryptococcus* and *Meningococcus* in cerebrospinal fluid.
3. Still today, it is commonly used for Hepatitis B surface antigen (HBsAg), fetoprotein, hydatid and amoebic antigens in the serum, and cryptococcal antigen in the CSF.
4. It is a rapid sensitive method for detecting pneumococcal capsular antigens in sputum.

1.4.5.3 Advantages

- A fast method of antigen-antibody detection (takes 30 minutes).
- More sensitive than electro-immunodiffusion (EID) because it involves simultaneous electrophoresis of the antigen and the antibody in gel in opposite directions resulting in band formation.

1.4.5.4 Limitations

- Much faster and more sensitive than the double diffusion technique.
- It is more expensive than agglutination based tests.
- It is believed to have decreased sensitivity, speed, and simplicity, then latex agglutination tests.

1.4.6 Radioimmunoassay

When radioisotopes instead of enzymes are used as labels to be conjugated with antigens or antibodies, the technique of detection of the antigen-antibody complex is called radioimmunoassay (RIA). Radioimmunoassay (RIA) is an *in vitro* assay that measures the presence of an antigen with very high sensitivity. RIA was first described in 1960 for the measurement of endogenous plasma insulin by **Solomon Berson and Rosalyn Yalow** of the Veterans Administration Hospital in New York.

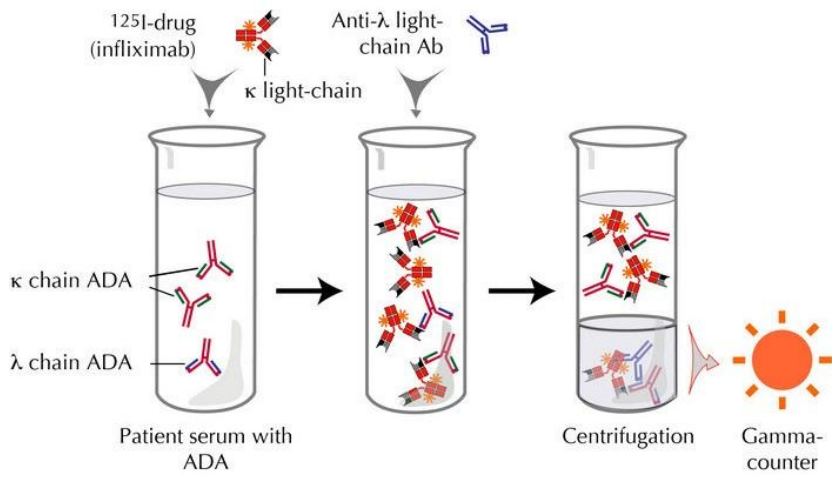

Fig. 10. Radioimmunoassay

The classical RIA methods are based on the principle of competitive binding. In this method, an unlabeled antigen competes with a radiolabeled antigen for binding to an antibody with the appropriate specificity. Thus, when mixtures of radiolabeled and unlabeled antigen are incubated with the corresponding antibody, the amount of free (not bound to antibody) radiolabeled antigen is directly proportional to the quantity of unlabeled antigen in the mixture.

1.4.6.1 Principle

It involves a combination of three principles.

1. An immune reaction i.e. antigen, antibody binding.
2. A competitive binding or competitive displacement reaction. (It gives specificity)
3. Measurement of radio emission. (It gives sensitivity)

When a foreign biological substance enters into the body bloodstream through a non-oral route, the body recognizes the specific chemistry on the surface of foreign substance as antigen and produces specific antibodies against the antigen so as nullify the effects and keep the body safe. The antibodies are produced by the body's immune system so, it is an immune reaction. Here the antibodies or antigens bind move due to chemical influence. This is different from principle of electrophoresis where proteins are separated due to charge.

1.4.6.2 Measurement of radio emission

Once the incubation is over, then washings are done to remove any unbound antigens. Then radio emission of the antigen-antibody complex is taken, the gamma rays from radiolabeled

antigen are measured. The target antigen is labeled radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added). A sample, for e.g. blood-serum, is added in order to initiate a competitive reaction of the labeled antigens from the preparation, and the unlabeled antigens from the serum-sample, with the specific antibodies. The competition for the antibodies will release a certain amount of labeled antigen. This amount is proportional to the ratio of labeled to an unlabeled antigen. A binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived. That means as the concentration of unlabeled antigen is increased, more of it binds to the antibody, displacing the labeled variant. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigens remaining in the supernatant is measured.

Antigen-antibody complexes are precipitated either by crosslinking with a second antibody or by means of the addition of reagents that promote the precipitation of antigen-antibody complexes. Counting radioactivity in the precipitates allows the determination of the amount of radiolabeled antigen precipitated with the antibody. A standard curve is constructed by plotting the percentage of antibody-bound radiolabeled antigen against known concentrations of a standardized unlabeled antigen, and the concentrations of antigen in patient samples are extrapolated from that curve.

1.4.6.3 Applications

The test can be used to determine very small quantities (e.g. nanogram) of antigens and antibodies in the serum.

1. The test is used for quantitation of hormones, drugs, HBsAg, and other viral antigens.
2. Analyze nanomolar and picomolar concentrations of hormones in biological fluids.

1.4.6.4 Limitations

1. The cost of equipment and reagents
2. Short shelf-life of radiolabeled compounds
3. The problems associated with the disposal of radioactive waste.

1.4.6.5 Immunofluorescence

Immunofluorescence is an assay which is used primarily on biological samples and is classically defined as a procedure to detect antigens in cellular contexts using antibodies. The specificity of antibodies to their antigen is the base for immunofluorescence. The property of

certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as **fluorescence.** In immunofluorescence test, fluorescent dye which illuminates in UV light are used to detect/show the specific combination of an antigen and antibody. The dye usually used is **fluorescein isothiocynate**, which gives yellow-green fluorescence. Immunofluorescence tests are also termed as **fluorescent antibody test (FAT).**

Fluorescent dyes, such as fluorescein isothiocyanate and lissamine rhodamine, can be tagged with antibody molecules. They emit blue-green and orange-red fluorescence, respectively under ultraviolet (UV) rays in the fluorescence microscope. This forms the basis of the immunological test. Immunofluorescence tests have wide applications in research and diagnostics. These tests are broadly of two types:

1. Direct immunofluorescence test
2. Indirect immunofluorescence test

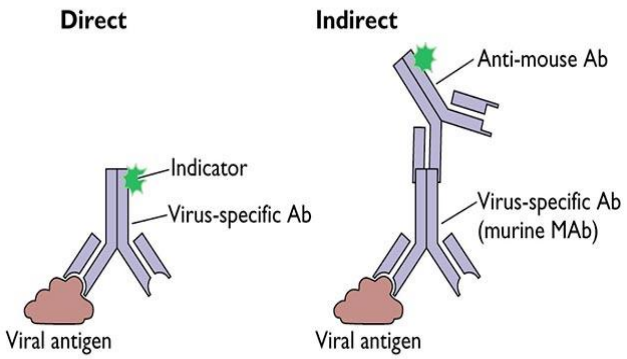

Fig 11 Immunoflorescence Technique

1.4.6.6 Direct Immunofluorescence Test

It is used to detect unknown antigen in a cell or tissue by employing a known labeled antibody that interacts directly with unknown antigen. If antigen is present, it reacts with labeled antibody and the antibody coated antigen is observed under UV light of the fluorescence. It involves use of labeled antiviral antibody.

1.4.6.7 Method

The specimen is placed on slide; fluorescent labeled antibody is then added to it and allowed for some time for Antigen-Antibody reaction. The preparation is then washed which will

allow the removal of other components except the complex of antigen and fluorescent labeled antibody. On microscopy (Fluorescence Microscopy), Antigen- Antibody complex are observed fluorescing due to the dye attached to antibody. The need for preparation of separate labeled antibody for each pathogen is the major disadvantage of the direct immunofluorescence test.

1.4.6.8 Indirect immunofluorescence Test

Indirect fluorescence is a double antibody technique. The unlabeled antibodies which have bound to the antigens are visualized by a fluorescent antiglobulin reagent directed at the unlabeled antibodies. The indirect immunofluorescence test is used for detection of specific antibodies in the serum and other body fluids for sero-diagnosis of many infectious diseases.

Indirect immunofluorescence is a two-stage process.

1.4.6.9 First stage

A known antigen is fixed on a slide. Then the patient's serum to be tested is applied to the slide, followed by careful washing. If the patient's serum contains antibody against the antigen, it will combine with antigen on the slide.

1.4.6.1.0 Second stage

The combination of antibody with antigen can be detected by addition of a fluorescent dye-labeled antibody to human IgG, which is examined by a fluorescence microscope.

The first step in the indirect immunofluorescence test is the incubation of a fixed antigen (e.g., in a cell or tissue) with unlabeled antibody, which becomes associated with the antigen. After careful washing, a fluorescent antibody (e.g. fluorescent labeled anti-IgG) is added to the smear. This second antibody will become associated to the first, and the antigen–antibody complex can be visualized on the fluorescence microscope.

The indirect method has the advantage of using a single labeled antiglobulin (antibody to IgG) as a "universal reagent" to detect many different specific antigen–antibody reactions. The test is often more sensitive than the direct immunofluorescence test.

1.4.6.1.1 Applications

1. Detect specific antibodies for serodiagnosis of syphilis, leptospirosis, amoebiasis, toxoplasmosis, and many other infectious diseases;
2. Identify the class of a given antibody by using fluorescent antibodies specific for different immunoglobulin isotypes;

3. Identify and enumerate lymphocyte subpopulations by employing monoclonal antibodies and cytofluorographs; and
4. Detect autoantibodies, such as antinuclear antibodies in autoimmune diseases.

Immunofluorescence may also be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. Immunofluorescence has been widely used in biological research and medical research.

1.4.6.1.2 Agglutination

Agglutination (from latin 'agglutino' – to glue, to attach) is an immunochemical technique in which a specific antibody reacts with the ***corpuscular antigen***. Agglutination reaction is based on the formation of bridges between bivalent (IgG) or multivalent (IgM) antibodies and antigenic particles with multiple epitopes. Bivalency or multivalency of the used antibody and multiple antigenic determinants on the surface of particles are necessary for the creation of cross-linking and the formation of a high-molecular-weight lattice that is observable macroscopically. IgM antibodies with ten antigenic-combining sites permit a more effective bridging than IgG. Some antibodies react with the corpuscular antigen, but may not produce agglutination. In this case, the agglutination may be achieved if an anti-immunoglobulin is added into the reaction mixture (the Coombs test).

1.4.7 Hemagglutination

It is a variant of agglutination technique in which red blood cells are used as the antigen-bearing particles. Agglutination reactions are performed on slides, in test tubes or microtiter plates. They are more sensitive in comparison with immunoprecipitation methods. The agglutination methods produce qualitative or semiquantitative results. Agglutination assays may be classified as ***direct*** or ***indirect tests***.

1.4.7.1 Direct agglutination

In a direct agglutination test, the antigen is an integral part of the cell surface (red blood cells, bacteria). A suspension of particles is directly agglutinated by specific antibodies present in the examined sample. This assay is frequently used in the hematology for the ***determination of blood group*** or in the immunological diagnostics for detection of specific antibodies directed against naturally occurring antigens on the surface of some microbes (for example against *Salmonella typhi* – the ***Widal test***). For the examination of antibodies,

the test is usually performed with serial dilutions of the sample. The highest dilution of serum that still causes agglutination is denoted as a ***titer of the antibody***.

1.4.7.2 Indirect agglutination

Indirect agglutination assay utilises particles with the antigens that have been passively attached to their surface. Originally, red blood cells were used as carriers for antigens; lately, inert particles such as latex, colloid gold and other substances have been shown to be more versatile for the agglutination technique. Many proteins, bacterial and viral antigens are easily adsorbed onto the particle, while other substances require modification by tannic acid or chromium chloride.

The particles coated with the specific antigens are used ***for the detection of antibodies*** against surface antigens in some pathogens and some autoantibodies (e.g. rheumatoid factor[3]) (Fig. 9). Instead of the antigens, particles can also be coated by specific antibodies. This technique is called ***reverse agglutination*** and can be utilised ***for the detection of soluble antigens*** (for example C-reactive protein or human chorionic gonadotrophin).

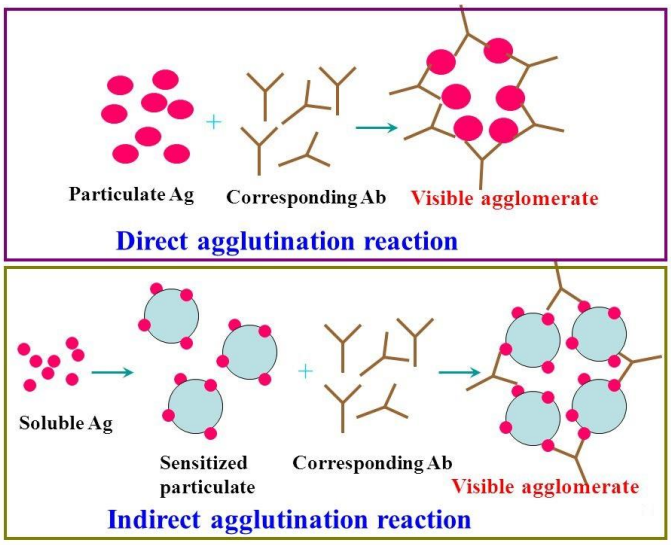

Fig. 12 Agglitination

1.4.8 Enzyme Immunoassay

ELISA is an antigen antibody reaction. In 1971, ELISA was introduced by Peter Perlmann and Eva Engvall at Stockholm University in Sweden. It is a common laboratory technique

which is usually used to measure the concentration of antibodies or antigens in blood. ELISA is a plate based assay technique which is used for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. An enzyme conjugated with an antibody reacts with colorless substrate to generate a colored product. Such substrate is called chromogenic substrate. A number of enzymes have been used for ELISA such as alkaline phosphatase, horse radish peroxidase and beta galactosidase. Specific substrate such as ortho-phenyldiamine dihydrochloride (for peroxidase), paranitrophenyl phosphate (for alkaline phosphatase) are used which are hydrolysed by above enzymes to give colored end product.

1.4.8.1 Principle

ELISAs are typically performed in 96-well polystyrene plates. The serum is incubated in a well, and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface. After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer.

To detect the bound antibodies or antigens, a secondary antibodies that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well. After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a color. This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of color/ optical density is measured at 450nm. The intensity of the color gives an indication of the amount of antigen or antibody.

1.4.8.2 Types of ELISA

Frequently there are 3 types of ELISA on the basis of binding structure between the Antibody and Antigen.

- Indirect ELISA
- Sandwich ELISA
- Competitive ELISA

1.4.8.2.1 Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form colored products. The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.

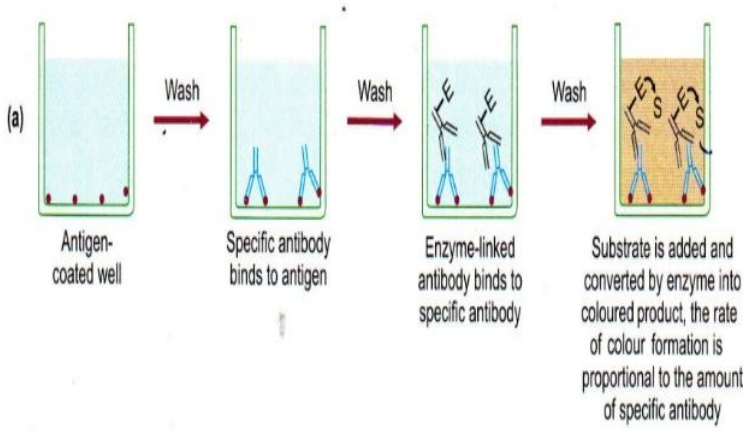

Fig. 13. Indirect ELISA

1.4.8.2.2 Procedure of Indirect ELISA

1. Coat the micro titer plate wells with antigen.
2. Block all unbound sites to prevent false positive results.
3. Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.
4. Wash the plate, so that unbound antibody is removed.
5. Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
6. Wash the plate, so that unbound enzyme-linked antibodies are removed.
7. Add substrate which is converted by the enzyme to produce a colored product.
8. Reaction of a substrate with the enzyme to produce a colored product.

1.4.8.2.2 Advantages

- Increased sensitivity, since more than one labeled antibody is bound per primary antibody.

- A wide variety of labeled secondary antibodies are available commercially.
- Maximum immunoreactivity of the primary antibody is retained because it is not labeled.
- Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
- Flexibility, since different primary detection antibodies can be used with a single labeled secondary antibody.
- Cost savings, since fewer labeled antibodies are required.
- Different visualization markers can be used with the same primary antibody.

1.4.8.2.3 Disadvantages

- Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
- An extra incubation step is required in the procedure.

1.4.8.2.4 Sandwich ELISA

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.

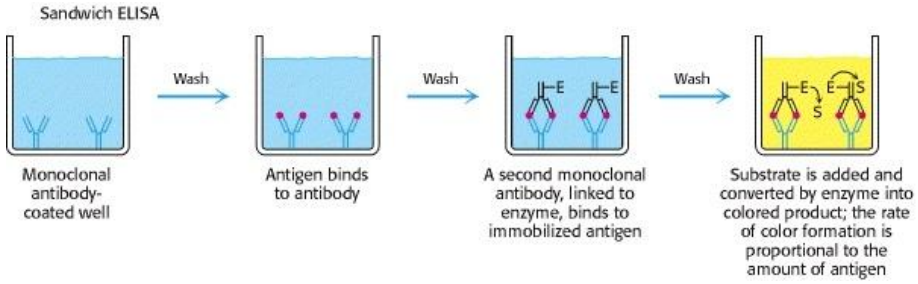

Fig. 14. Sandwitch ELISA

1.4.8.2.5 Procedure of Sandwich ELISA

1. Prepare a surface to which a known quantity of antibody is bound.
2. Add the antigen-containing sample to the plate and incubate the plate at 37°c.
3. Wash the plate, so that unbound antigen is removed.

4. Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme to produce a colored product.
7. Reaction of a substrate with the enzyme to produce a colored product.

1.4.8.2.6 Advantages

- High specificity, since two antibodies are used the antigen is specifically captured and detected.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.
- Flexibility and sensitivity, since both direct and indirect detection methods can be used.

14.8.2.7 Competitive ELISA

This test is used to measure the concentration of an antigen in a sample. In this test, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to the microtitre well which is coated with antigen. The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well. The higher the concentration of antigen in the sample, the lower the absorbance.

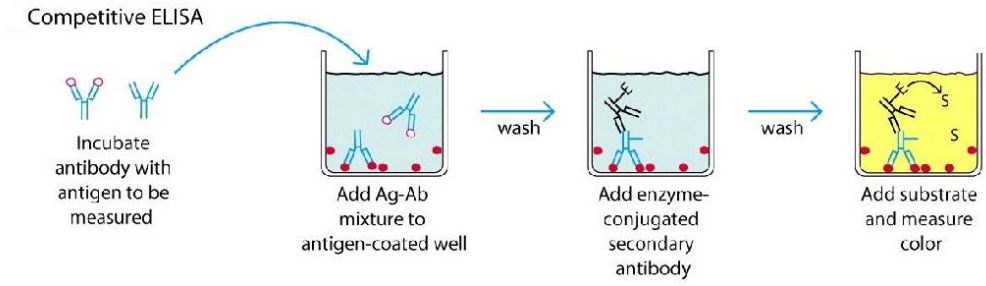

Fig. 15 Competitive ELISA

1.4.8.2.8 Procedure

1. Antibody is incubated with sample containing antigen.
2. Antigen-antibody complex are added to the microtitre well which are pre-coated with the antigen.

3. Wash the plate to remove unbound antibody.
4. Enzyme linked secondary antibody which is specific to the primary antibody is added.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme into a fluorescent signal.

1.4.8.2.9 Advantages

- High specificity, since two antibodies are used.
- High sensitivity, since both direct and indirect detection methods can be used.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.

1.4.8.3.0 Application of ELISA

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody concentrations in a virus test.
3. Used in food industry when detecting potential food allergens.
4. Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc.

1.4.8.3 Clonal Selection Theory

The clonal selection theory explains the mechanism by which the body's immune system responds to the appearance of antigens present in or released from an agent of infection.

The clonal selection theory was proposed by Frank Macfarlane Burnet in 1957, in an attempt to explain the great diversity of antibodies formed during initiation of the immune response. The theory has become the widely accepted model for how the human immune system responds to infection and how certain types of B and T lymphocytes are selected for destruction of specific antigens.

According to this theory, an individual B or T lymphocyte expresses many copies of a membrane receptor that is specific for a single, distinct antigen. This unique receptor specificity is determined in the lymphocyte before it is exposed to the antigen. Binding of antigen to its specific receptor activates the cell, causing it to proliferate into a clone of daughter cells that have the same receptor specificity as the parent cell. In the case of B cells,

some of the progeny become plasma cells that synthesize and secrete quantities of antibody that will react with additional antigen; other progeny become memory cells that are reserved for a response to a subsequent exposure to the same antigen. Thus the clones that respond to the presence of the antigen are, in effect, selected by the antigen, and this is what is meant by "clonal selection."

The **clonal selection theory** helps explain how lymphocytes recognize antigenic determinants and respond. According to this theory, small populations (clones) of lymphocytes bear receptors on their cell membranes. Production of these receptors is genetically determined. On B-lymphocytes, the receptors consist of antibody molecules, while on T-lymphocytes, they are clusters of amino acids. When lymphocytes encounter an antigenic determinant on the surface of a macrophage, their receptors match with the antigenic determinant and a stimulation follows. A match is also made between a set of molecules called **major histocompatibility (MHC) molecules** and their receptors.

The clonal selection theory suggests that B-lymphocytes and T-lymphocytes exist for all antigenic determinants even before contact with an antigen is made. The theory also says that antigenic determinants stimulate the lymphocytes to endow their progeny with identical specificity. The B-lymphocytes and T-lymphocytes that might potentially react with the body's own cells are deleted or in some way inactivated to ensure that an immune response to the host organism does not develop.

Clonal selection leads to the eventual production of:

- a pool of antibody-secreting plasma cells. Plasma cells are B-cells that have tooled up (e.g., forming a large endoplasmic reticulum) for massive synthesis and secretion of an antibody
- a pool of **"memory" cells**. These are B lymphocytes with receptors of the same specificity as those on the original activated B cell.

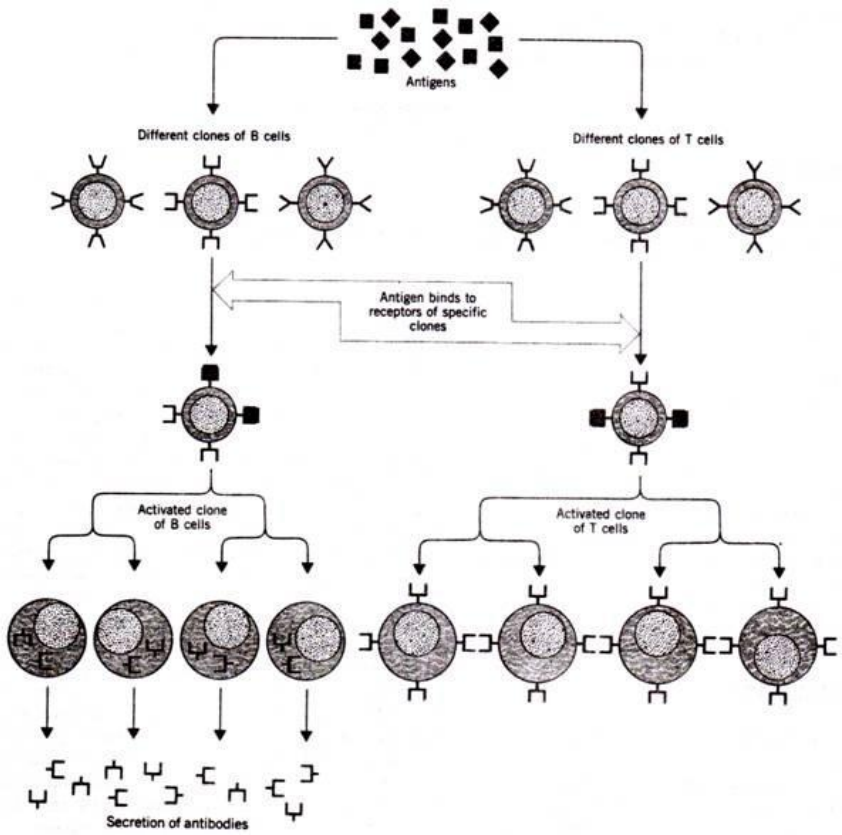

Fig. 16. Clonal Selection Theory

In short, the theory is an explanation of the mechanism for the generation of diversity of antibody specificity. The first experimental evidence came in 1958, when Gustav Nossal and Joshua Lederberg showed that one B cell always produces only one antibody. The idea turned out to be the foundation of molecular immunology, especially in adaptive immunity.

14.8.4 Summary

The human defense system consists of factors that provide innate and acquired immunity against microorganisms. The system evolved from primitive but effective defenses found in more ancient animal species. The innate defenses include 1) structural barriers, 2) acids, bases, and other chemical agents produced at various sites, such as mucosal surfaces, and 3) highly phagocytic, motile scavenger cells that have well-developed killing and digestive powers. As a result of the evolutionary process, the mammalian immune system has become more specific, efficient, regulated, and complex. The development of specialized innate and acquired recognition/regulatory proteins (antibodies, cell receptors, and cytokines) expanded the repertoire, and control the magnitude of the protective responses. One of the most

important consequences of this evolution is the ability of the immune system to discriminate between self and non-self antigens and maintain a memory of previous encounters with antigens, including those from microorganisms.

The evolutionary changes allowed development of B and T cells which express antigen-specific receptors on their cell surface. These changes permit humans to survive in an environment laden with microbial pathogens and environmental toxins. The pathogenic features of those microorganisms include the ability to 1) enter the body through portals such as the skin, respiratory system, and the alimentary tract; 2) utilize nutrients from those sites; 3) adhere to epithelium; 4) produce virulence factors and toxins; 5) commandeer the replicative machinery of the host's cells; 6) evade the immunologic system; 7) cripple the defenses of the host; and 8) cause autoimmune responses by acting as cross-reactive antigens.

14.8.5 Terminal questions

Q.1. Explain historical background of immunology.

Answer:----------

Q.2. Describe immunity and its types.

Answer:----------

Q.3. Describe immunoglobulins and their types.

Answer:----------

Q.4. What do you mean by immunization? Describe it.

Answer:----------

Q.5. Write a short note on clonal selection theory.

Answer:----------

Q.6. Write a short note on Hemagglutination.

Answer:----------

Q.7. Explain ELISA and its types.

Answer:----------

Q.8. What do you mean by stem cell therapy?

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-2: Immunity

Structure

2.2.1Types of immunity:

2.2.1.1 Innate or Natural immunity or Non Specific (L. innatus = inborn)

2.2.1.2 Acquired Immunity (= Adaptive or Specific Immunity):

2.2.1.2.1 Characteristics of Acquired Immunity:

2.2.1.2.2 Components of Acquired Immunity:

2.3 Types of Acquired Immunity:

Acquired (= Adaptive) Immunity is of two types: active immunity and passive immunity.

2.3.1 Active Immunity:

2.3.2 Passive Immunity:

2.4 Immune Response

2.5 Hormonal Influence on Immune Response

2.6 Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins complex.

2.7 HLA Complex

2.8 Major Histocompatibility Complex (MHC) Class I

2.8.1 Structure of Major Histocompatibility Complex (MHC) Class I

2.8.2 Mechanism of MHC I

2.8.3 Functions of MHC class I:

2.8.4 Major Histocompatibility Complex (MHC) Class II

2.8.5 Structure of Major Histocompatibility Complex (MHC) Class II

2.8.6 Mechanism of MHC II

2.8.6.1 Functions of MHC class II:

2.8.7 MHC Class I Vs. MHC Class II

2.8.8 Peptide binding by class I and class II MHC molecules

2.8.9 Summary

2.9.0 Terminal questions

2.1 Introduction

Immunity is a state of specific resistance to infection. Specific resistance is directed against a particular type of microorganism and is the single most important characteristic of immunity. The immune system enables the body to recognize a foreign agent as nonself, which is something other than a person's own substances (self). The immune system takes a specific

action for neutralizing, killing, and eliminating that agent. The action involves nonspecific resistance as well. On occasion, the immune system activity may lead to tissue damage as seen in allergic disorders and other states of hypersensitivity.

The immune system's activity is based on its ability to distinguish characteristic proteins or protein-linked components associated with alien substances. Once this distinction has been made, certain lymphocytes are provoked to produce antibodies directed against the foreign matter, while other lymphocytes are sensitized to the invading agent and react with it directly. Thus, there are two major branches of the immune system: antibody-mediated immunity (also known as humoral immunity) and cell-mediated immunity.

The human defense system consists of factors that provide innate and acquired immunity against microorganisms. The system evolved from primitive but effective defenses found in more ancient animal species. The innate defenses include 1) structural barriers, 2) acids, bases, and other chemical agents produced at various sites, such as mucosal surfaces, and 3) highly phagocytic, motile scavenger cells that have well-developed killing and digestive powers. As a result of the evolutionary process, the mammalian immune system has become more specific, efficient, regulated, and complex. The development of specialized innate and acquired recognition/regulatory proteins (antibodies, cell receptors, and cytokines) expanded the repertoire, and control the magnitude of the protective responses. One of the most important consequences of this evolution is the ability of the immune system to discriminate between self and non-self antigens and maintain a memory of previous encounters with antigens, including those from microorganisms.

The evolutionary changes allowed development of B and T cells which express antigen-specific receptors on their cell surface. These changes permit humans to survive in an environment laden with microbial pathogens and environmental toxins. The pathogenic features of those microorganisms include the ability to 1) enter the body through portals such as the skin, respiratory system, and the alimentary tract; 2) utilize nutrients from those sites; 3) adhere to epithelium; 4) produce virulence factors and toxins; 5) commandeer the replicative machinery of the host's cells; 6) evade the immunologic system; 7) cripple the defenses of the host; and 8) cause autoimmune responses by acting as cross-reactive antigens.

2.2 Immunity

Immunity is derived from Latin word *"immunis"* which means free from burden. In this case burden refers to disease caused by microorganisms or their toxic products. Therefore **Immunity** is defined as the state of resistance or in susceptibility to disease caused by particular microorganisms or their toxic products. For example some individuals having genetic deficiency of glucose-6-phosphate dehydrogenase are resistant to malaria. Such individuals are said to be immune to *Plasmodium*. Immunity is our natural defense mechanism and it is comparable to our real-life defense mechanisms.

2.2.1Types of immunity:

There are two major types of immunity: innate or natural or nonspecific and acquired or adaptive.

2.2.1.1 Innate or Natural immunity or Non Specific (L. innatus = inborn)

Innate immunity is inherited by the organism from the parents and protects it from birth throughout life. For example humans have innate immunity against distemper, a fatal disease of dogs. Innate immunity acts as first line of defense to particular microorganisms. Innate immunity is provided by various components such as Skin, mucus membrane, Phagocytic cells etc. As its name nonspecific suggests that it lacks specific responses to specific invaders. Innate immunity or nonspecific immunity is well done by providing different barriers to the entry of the foreign agents into our body. Innate immunity consists of four types of barriers—physical, physiological, cellular and cytokine barriers.

1. Physical Barriers

They are mechanical barriers to many microbial pathogens. These are of two types. Skin and mucous membrane.

(a) Skin

The skin is physical barrier of body. Its outer tough layer, the stratum corneum prevents the entry of bacteria and viruses.

(b) Mucous Membranes

Mucus secreted by mucous membrane traps the microorganisms and immobilises them. Microorganisms and dust particles can enter the respiratory tract with air during breathing

which are trapped in the mucus. The cilia sweep the mucus loaded with microorganisms and dust particles into the pharynx (throat). From the pharynx it is thrown out or swallowed for elimination with the faeces.

2. Physiological Barriers

The skin and mucous membranes secrete certain chemicals which dispose off the pathogens from the body. Body temperature, pH of the body fluids and various body secretions prevent growth of many disease causing microorganisms. Some of the important examples of physiological barriers are as follows:

- Acid of the stomach kills most ingested microorganisms,
- Bile does not allow growth of microorganisms.
- Cerumen (ear wax) traps dust particles, kills bacteria and repels insects,
- Lysozyme is present in tissue fluids and in almost all secretions except in cerebrospinal fluid, sweat and urine. Lysozyme is in good quantity in tears from eyes. Lysozyme attacks bacteria and dissolves their cell walls. Lysoenzyme is also found in saliva.
- Nasal Hair. They filter out microbes and dust in nose.
- Urine. It washes microbes from urethra.
- Vaginal Secretions. It is slightly acidic which discourages bacterial growth and flush microbes out of vagina.
- (h) Sebum (sweat). It forms a protective acid film over the skin surface that inhibits growth of many microbes.

3. Cellular Barriers

These are certain white blood corpuscles (leucocytes), macrophages, natural killer cells, complement system, inflammation, fever, antimicrobial substances, etc.

(i) Certain Leucocytes

Neutrophils and monocytes are major phagocytic leucocytes.

(a) Polymorpho-nuclear Leucocytes (PMNL- neutrophils)

As they have multilobed nucleus they are normally called polymorphonuclear leucocytes (PMNL-neu- trophils). Neutrophils are short lived and are highly motile phagocytic killers. Neutrophils are formed from stem cells in the bone marrow. Neutrophils are the most numerous of all leucocytes. They die after a few days and must therefore, be constantly replaced. Neutrophils constitute about 40% to 75% of the blood leucocytes in humans.

(b) Monocytes

They are the largest of all types of leucocytes and somewhat amoeboid in shape. They have clear cytoplasm (without cytoplasmic granules). The nucleus is bean-shaped. Monocytes constitute about 2-10% of the blood leucocytes. They are motile and phagocytic in nature and engulf bacteria and cellular debris. Their life span is about 10 to 20 hours. Generally they change into macrophages after entering tissue spaces.

(ii) Macrophages

Monocytes circulate in the bloodstream for about 8 hours, during which time they enlarge and then migrate into the tissues and differentiate into specific tissue macrophages. Macrophages are long lived and are highly motile phagocytic. Macrophages contain more cell organelles especially lysosomes. Macrophages are of two types.

(a) Some take up residence in particular tissues becoming fixed macroph- ages.

(b) Whereas other remain motile and are called wandering macrophages. Wandering macrophages move by amoeboid movement throughout the tissues. Fixed macrophages serve different functions in different tissues and are named to reflect their tissue location. Some examples are given below:

- ✓ Pulmonary alveolar macrophages in the lung
- ✓ Histiocytes in connective tissues
- ✓ Kupffer cells in the liver
- ✓ Glomerular Mesangial cells in the kidney
- ✓ Microglial cells in the brain
- ✓ Osteoclasts in bone

(iii) Natural Killer Cells (NK Cells):

NK cells constitute 5%-10% of the peripheral blood lymphocytes in humans.Besides the phagocytes, there are natural killer cells in the body which are a type of lymphocytes and are present in the spleen, lymph nodes and red bone marrow. NK cells do not have antigen receptors like T cells and B cells. NK cells cause cellular destruction in at least two ways:

(a) NK cells produce perforins which are chemicals that when inserted into the plasma membrane of a microbe make so weak that cytolysis (breakdown of cells particularly their outer membrane) occurs and creates pores in the plasma membrane of the target cells. These pores allow entry of water into the target cells, which then swell and burst. Cellular remains are eaten by phagocytes.

(b) Another function of NK cells is apoptosis which means natural cell death. It occurs naturally as part of the normal development, maintenance and renewal of cells, tissues and organs.

(iv) Complement:

Complement is a group of 20 proteins, many of which are enzyme precursors and are produced by the liver. These proteins are present in the serum of the blood (the fluid portion of the blood excluding cells and clotting factors) and on plasma membranes. They are found circulating in the blood plasma and within tissues throughout the body. They were named complement by Ehrlich because they complement the actions of other components of the immune system (e.g., action of antibody on antigen) in the fight against infection. Jules Bordet is the discoverer of complement.

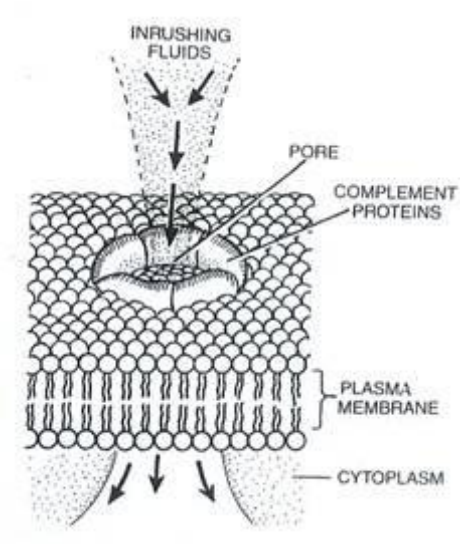

Fig.1 Complement proteins creating a hole in the plasma membrane

Complement proteins create pores in the plasma membrane of the microbes. Water enters the microbes. The latter burst and die. The proteins of complement system destroy microbes by

- Cytolysis
- Inflammation and
- Phagocytosis.

These proteins also prevent excessive damage of the host tissues.

(v) Inflammation:

Inflammation is a defensive response of the body to tissue damage. The conditions that may produce inflammation are pathogens, abrasions (scraping off) chemical irritations, distortion or disturbances of cells, and extreme temperatures. The signs and symptoms of inflammation are redness, pain, heat and swelling. Inflammation can also cause the loss of function in the injured area, depending on the site and extent of the injury. Inflammation is an attempt to dispose of microbes, toxins, or foreign material at the site of injury to prevent their spread to other tissues, and to prepare the site for tissue repair. Thus, it helps restore tissue homeostasis.

Broken mast cells release histamine. Histamine causes dilation of capillaries and small blood vessels. As a result more blood flows to that area making it red and warm and fluid (plasma) takes out into the tissue spaces causing its swelling. This reaction of the body is called inflammatory response.

(vi) Fever:

Fever may be brought about by toxins produced by pathogens and a protein called endogenous pyrogen (fever producing substance), released by macrophages. When enough pyrogens reach the brain, the body's thermostat is reset to a higher temperature, allowing the temperature of the entire body to rise. Mild fever strengthens the defence mechanism by activating the phagocytes and by inhibiting the growth of microbes. A very high temperature may prove dangerous. It must be quickly brought down by giving antipyretics.

4. Cytokine Barriers:

Cytokines (Chemical messengers of immune cells) are low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells. They are involved in the cell to cell communication. Kinds of cytokines include interleukins produced by leucocytes, lymphocytes produced by lymphocytes, tumour necrosis factor and interferon's (IFNs). Interferon's protect against viral infection of cells.

2.2.1.2 Acquired Immunity (= Adaptive or Specific Immunity):

The immunity that an individual acquires after the birth is called acquired or adaptive or specific immunity. It is specific and mediated by antibodies or lymphocytes or both which make the antigen harmless. It not only relieves the victim of the infectious disease but also prevents its further attack in future. The memory cells formed by B cells and T cells are the basis of acquired immunity. Thus acquired immunity consists of specialized B and T lymphocytes and Antibodies.

2.2.1.2.1 Characteristics of Acquired Immunity:

(i) Specificity:

It is the ability to differentiate between various foreign molecules (foreign antigens).

(ii) Diversity:

It can recognise a vast variety of foreign molecules (foreign antigens).

(iii) Discrimination between Self and Non-self:

It can recognise and respond to foreign molecules (non-self) and can avoid response to those molecules that are present within the body (self) of the animal.

(iv) Memory:

When the immune system encounters a specific foreign agent, (e.g., a microbe) for the first time, it generates immune response and eliminates the invader. This is called first encounter. The immune system retains the memory of the first encounter. As a result, a second encounter occurs more quickly and abundantly than the first encounter. The cells of the immune system are derived from the pluripotent stem cells in the bone marrow. Pluripotent means a cell that can differentiate into many different types of tissue cells. The pluripotent stem cells can form either myeloid stem cells or lymphoid stem cells.

Myeloid stem cells give rise to monocytes, macrophages and granulocytes (neutrophils eosinophil's, and basophils). RBCs and blood platelets (lymphoid stem cells) form B lymphocytes (B cells), T lymphocytes (T-cells) and natural killer (NK) cells.

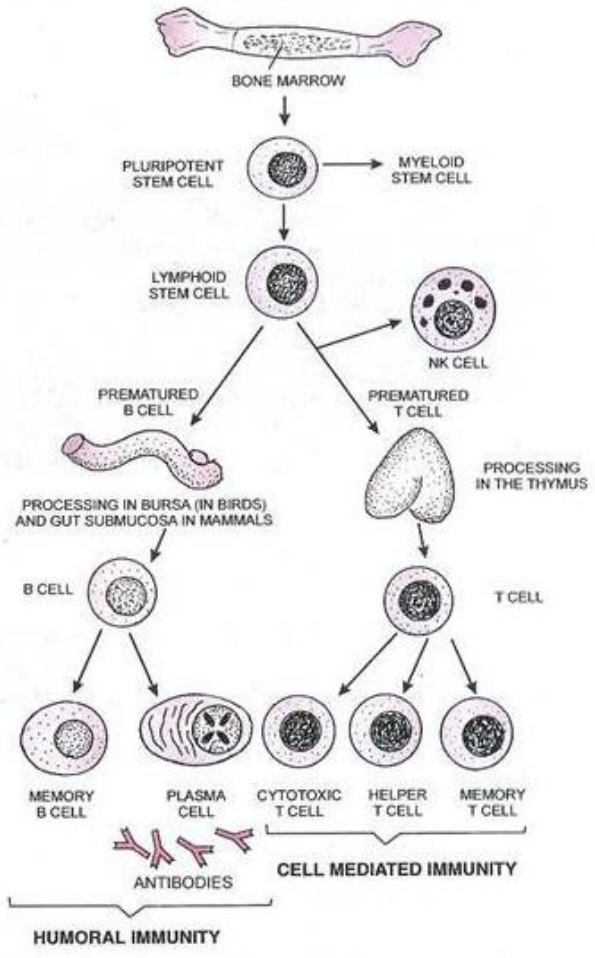

Fig. 2 Development of B and T lymphocytes

2.2.1.2.2 Components of Acquired Immunity:

Acquired immunity has two components: humeral immunity or Antibody mediated immune system (AMIS) and cellular immunity or cell mediated immune system (CMIS).

I. **Antibody Mediated Immune System (AMIS) or Humoral Immunity:**

It consists of antibodies (specialised proteins produced in the body in response to antigen) that circulate in the body fluids like blood plasma and lymph. The word 'humor' pertains to fluid. B lymphocytes (B cells) produce antibodies that regulate humoral immunity. The T-lymphocytes themselves do not secrete anti-bodies but help B lymphocytes produce them.

Certain cells of the bone marrow produce B lymphocytes and mature there. Since B lymphocytes produce antibodies, therefore, this immunity is called antibody mediated or humoral immunity. Humoral immunity or antibody-mediated immune system (AMIS) provides defence against most extracellular bacterial pathogens and viruses that infect through the respiratory and intestinal tract.

Formation of Plasma B cells and Memory B cells:

When antibodies on B cell's surface bind antigens (any substances that cause antibodies formation) the B cell is activated and divides, producing a clone (descendants of a single cell) of daughter B cells. These clones give rise to plasma B cells and memory B cells. This phenomenon is called clonal selection.

(a) Plasma B Cells (Effector B cells):

Some of the activated B cells enlarge, divide and differentiate into a clone of plasma cells. Although plasma cells live for only a few days, they secrete enormous amounts of antibody during this period.

(b) Memory B Cells:

Some activated B cells do not differentiate into plasma cells but rather remain as memory cells (Primed cells). They have a longer life span. The memory cells remain dormant until activated once again by a new quantity of the same antigen.

Role of AMIS:

The AMIS protects the body from (i) viruses (ii) some bacteria and (iii) toxins that enter the body fluids like blood and lymph.

II. Cell-Mediated Immune System (CMIS) or T-Cell Immunity:

A healthy person has about a trillion lymphocytes. Lymphocytes are of two types: T lymphocytes or T cells and B lymphocytes or B cells. As we know both types of lymphocytes and other cells of the immune system are produced in the bone marrow. The process of production of cells of immune system in the bone marrow is called haematopoiesis. Because T lymphocytes (T cells) mature in the thymus, this immunity is also called T- cell immunity. The T-cells play two important functions—effector and regulatory. The effector function includes cytolysis (destruction of cells by immune processes) of cells infected with microbes and tumour cells and lymphokine production. The regulatory functions are either to increase or to suppress other lymphocytes and accessory cells.

Types of T-cells and their Functions:

1. Helper T cells (T_H):

T_H cells are most numerous of the T cells. They help in the functions of immune system. They produce a growth factor that stimulates B-cell proliferation and differentiation and also stimulates antibody production by plasma cells; enhance activity of cytotoxic T cells.

2. Cytotoxic T cells (T_c) or Killer cells:

These cells are capable of killing microorganisms and even some of the body's own cells directly hence they are called killer cells. The antigen receptors on the surfaces of the cytotoxic cells cause specific binding with antigens present on the surface of foreign cell. Cell after binding, the cytotoxic T cell secretes hole-forming proteins, called perforins, that punch large round holes in the membrane of the foreign cell. Then fluid flows quickly into the cell from the interstinal space. In addition, the cytotoxic T cell releases cytotoxic substances directly into the foreign cell. Almost immediately, the foreign cell becomes greatly swollen and it usually dissolves shortly thereafter. Thus they destroy body cells infected by viruses and attack and kill bacteria, fungi, parasites and cancer cells.

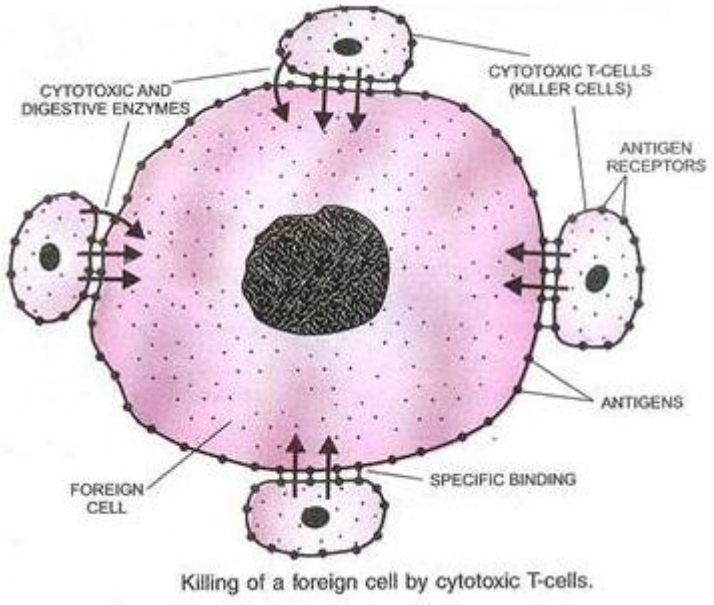

Fig. 3 Killing of a foreign body by cytotoxic T cells

3. Memory T Cells (Primed Cells):

These cells are also formed by T-lymphocytes as a result of exposure to antigen and remain in the lymphatic tissue (e.g., spleen, lymph nodes). They recognize original invading antigens even years after the first encounter. These cells keep ready to attack as soon as the same pathogens infect the body again. They proliferate and differentiate into cytotoxic T cells, helper T cells, suppressor T cells, and additional memory cells.

4. Suppressor Cells (Regulatory T cells (T_R)):

These cells are capable of suppressing the functions of cytotoxic and helper T cells. They also inhibit the immune system from attacking the body's own cells. It is believed that suppressor cells regulate the activities of the other cells. For this reason, the suppressor cells are classified as regulatory T cells.

Natural Killer (NK) Cells:

NK cells attack and destroy target cells, participate in antibody dependent cell mediated cytotoxicity. They can also attack parasites which are much larger than bacteria.

2.3 Types of Acquired Immunity:

Acquired (= Adaptive) Immunity is of two types: active immunity and passive immunity.

2.3.1 . Active Immunity:

In this immunity person's own cells produce antibodies in response to infection or vaccination. It is slow and takes time in the formation of antibodies. It is long lasting and is harmless. Active immunity may be natural or artificial.

(a) A person who has recovered from an attack of small pox or measles or mumps develops natural active immunity.

(b) Artificial active immunity is the resistance induced by vaccines. Examples of vaccines are as follows: Bacterial vaccines, (a) Live- BCG vaccine for tuberculosis, (b) Killed vaccines- TAB vaccine for enteric fever. Viral vaccines, (a) Live – sabin vaccine for poliomyelitis, MMR vaccine for measles, mumps, rubella, (b) Killed vaccines- salk vaccine for poliomyelitis, neural and non-neural vaccines for rabies. Bacterial products. Toxoids for Diphtheria and Tetanus.

2.3.2 Passive Immunity:

When ready-made antibodies are directly injected into a person to protect the body against foreign agents, it is called passive immunity. It provides immediate relief. It is not long lasting. It may create problems. Passive immunity may be natural or artificial.

(a) Natural passive immunity is the resistance passively transferred from the mother to the foetus through placenta. IgG antibodies can cross placental barrier to reach the foetus. After birth, immunoglobulin's are passed to the new-born through the breast milk. Human colostrum (mother's first milk) is rich in IgA antibodies. Mother's milk contains antibodies which protect the infant properly by the age of three months.

(b) Artificial passive immunity is the resistance passively transferred to a recipient by administration of antibodies. This is done by administration of hyper-immune sera of man or animals. Serum (pi. sera) contains antibodies. For example, anti-tetanus serum (ATS) is prepared in horses by active immunisation of horses with tetanus toxoid, bleeding them and separating the serum. ATS is used for passive immunisation against tetanus. Similarly anti-diphtheric serum (ADS) and anti-gas gangrene serum (AGS) are also prepared.

2.4 Immune Response

The immune response involves primary immune response and secondary immune response.

(a) The primary immune response:

After an initial contact with an antigen, no antibodies are present for a period of several days. Then, a slow rise in the antibody titer o(arbitrary units) occurs, first IgM and then IgG followed by a gradual decline in antibody titer. This is called the primary immune response.

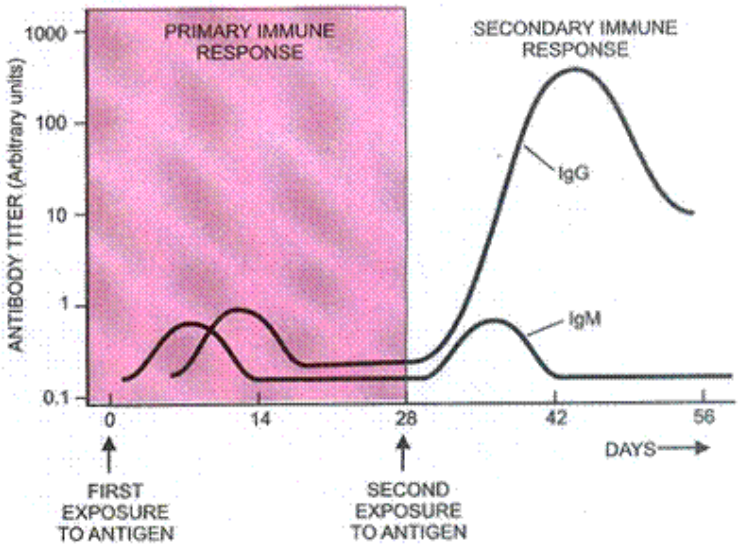

Fig. 4 Production of antibodies in primary and secondary responses

(b) The secondary immune response:

Memory cells may remain in the body for decades. Every new encounter with the same antigen results in a rapid proliferation of memory cells. This is also called "booster response". The antibody titer after subsequent encounters is far greater than during a primary response and consists mainly of IgG antibodies. This accelerated, more intense response is called the secondary immune response. Antibodies produced during a secondary response have an even higher affinity for the antigen.

A person who had been suffering from diseases like measles, small pox or chicken pox becomes immune to subsequent attacks of these diseases. It includes spleen, lymph nodes, tonsils, Peyer's patches of small intestine and appendix. The increased power and duration of the secondary immune response explain why immunization (method of providing immunity

artificially, it is called vaccination) is usually accomplished by injecting antigen in multiple doses.

2.5 Hormonal Influence on Immune Response

Immune system does not work in isolation as immune, endocrine, and central nervous systems are integrated through a network of signal molecules (cytokines, hormones, and neurotransmitters) that act on a common set of receptors. It is influenced by many other factors that may be neuroendocrine peptides, sex hormones, or other metabolites. The interaction of these systems is shown in Fig.

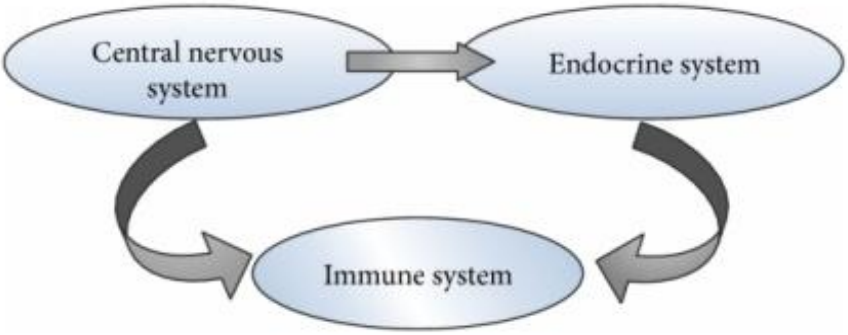

Fig. 5 This figure shows the influence of different systems on the immune system.

There is a bidirectional influence between the immune and CNS and immune and endocrine system (particularly gonadal) and endocrine (gonadal) and CNS. Sex hormones act on the CNS, macrophage/monocyte system, or the immune system itself, to affect immune responses.

Effect of hormones on the immune response has been shown due to their effect on differentiation and maturation of immunocytes. Sex hormones affect the immune system by increasing the number of circulating immune cells. Sex hormones either affect proliferation/apoptosis of the cells or induce production of new cells from the bone marrow. The studies in early 1940s ascertained that females have enhanced capability of producing antibodies. This enhanced immune reactivity in females helps mount an effective resistance to infection and therefore females are less susceptible to viral infections, but can develop immune-pathogenic effects and predisposition to autoimmunity due to hyper immune responses. Sex hormones can also control the immune response via circadian rhythm.

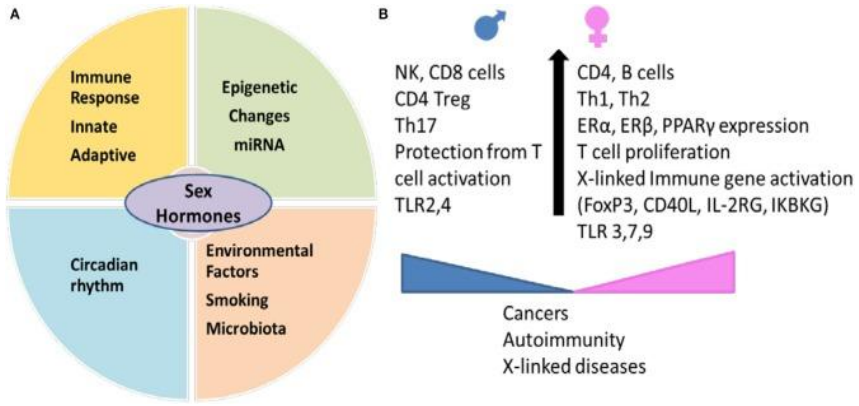

Fig. 6 Sex hormones interact with genetic and environmental factors and determine immunity in an individual.

(A) Environmental factors like smoking and gut microbiome generate sex-hormones dependent immunity leading to differences in circadian rhythm, innate and adaptive immune response and epigenetic changes between males and females. Sexual dimorphism between miRNA expression contributes to sex-specific regulation of function in various tissues.

(B) Sex determines expression of cell markers involved in innate and adaptive immunity. Females have higher expression of genes on X chromosome which include immune markers like regulatory marker FoxP3, CD40L. Females produce higher Th2 response and antibodies and better protection from infections but the hyperimmune response makes them susceptible to autoimmune diseases. Males generate more of Th17 response and are less likely to develop autoimmunity but have higher percent of non-reproductive cancers. miRNA, microRNA; TLR, Toll like receptor; ER, estrogen receptor; PPAR, peroxisome proliferator-activated receptor; IKBKG, inhibitor of nuclear factor kappa B kinase; IL2RG, interleukin receptor subunit gamma.

Immune cells synthesize, store and secrete hormones, which are identical with the hormones of the endocrine glands. These are: the POMC hormones (ACTH, endorphin), the thyroid system hormones (TRH, TSH, T3), growth hormone (GH), prolactin, melatonin, histamine, serotonin, catecholamines, GnRH, LHRH, hCG, renin, VIP, ANG II. Hormones can act as immunomodulators which alternate the sensitivity of the immune system. For example, female sex hormones are known immunostimulators of both adaptive and innate immune responses. Some autoimmune diseases such as lupus erythematosus strike women preferentially, and their onset often coincides with puberty. By contrast, male sex

hormones such as testosterone seem to be immunosuppressive. Other hormones can also regulate the immune systeme for example prolactin, growth hormone and vitamin D.

2.6 Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins

T-cell receptors only recognize pieces of antigen that are positioned on the surface of other cells. These antigen pieces are held within the binding groove of a cell surface protein called the **Major histocompatibility complex (MHC) molecule** encoded by a cluster of genes collectively called the MHC locus. These fragments are generated inside the cell following antigen digestion, and the complex of the antigenic peptide plus MHC molecule then appears on the cell surface. MHC molecules thus act as a cell surface vessel for holding and displaying fragments of antigen so that approaching T cells can engage with this molecular complex via their T-cell receptors. The MHC in humans is known as **human leukocyte antigens (HLA) complex**.

- Major histocompatibility complex (MHC) is the cluster of gene arranged within a long continuous stretch of DNA on chromosome number 6 in Human which encodes MHC molecules.
- MHC molecule is a cell surface glycoprotein receptor present in APCs and acts as antigen presenting structure It plays vital role in immune recognition, including interaction between T cells and other cell types.

2.7 HLA Complex

In humans, the HLA complex of genes is located on short arm of chromosome 6 containing several genes that are critical to immune function. The HLA complex of genes is classified into three classes as follows:

1. **Class I:** HLA-A, HLA-B, and HLA-C.
2. **Class II:** HLA-DR, HLA-DQ, and HLA-DP. All of these are present within HLA-D region of HLA complex.
3. **Class III:** Complement loci that encode for C2, C4, and factor B of complement system and TNFs alpha and beta.

Gene product of HLA Complex

1. **Class I MHC genes** encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of endogenous peptide antigens to $CD8^+$ T cells.

2. **Class II MHC genes** encode glycoproteins expressed predominantly on APCs (macrophages, dendritic cells, and B cells), where they primarily present exogenous antigenic peptides to $CD4^+$ T cells.
3. **Class III MHC genes** encode several different proteins, some with immune functions, including components of the complement system and molecules involved in inflammation.

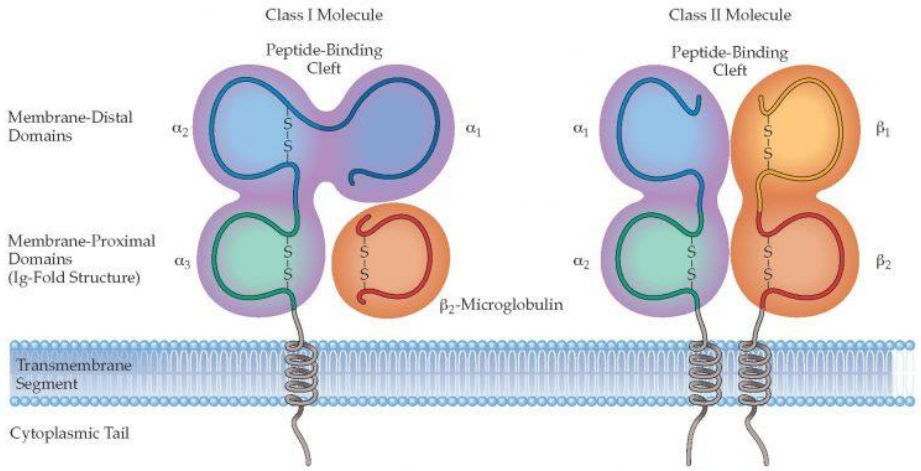

Fig. 7 MHC Class I and II Molecules

2.8 Major Histocompatibility Complex (MHC) Class I

- MHC class I proteins are encoded by the HLA-A, HLA-B, and HLA-C genes encoding HLA-A, HLA-B, and HLA-C molecules respectively.
- Class I molecules are found on virtually all nucleated cells in the body including platelets. Key exceptions are observed on cells in the retina and brain and the non-nucleated red blood cells.
- They are recognized by CD8 co-receptors through the MHC Class I β2 subunit.
- These MHC Class I molecules sample peptides generated within the cell and signal the cell's physiological state to effector cells of the immune system, particularly CD8+ T lymphocyte.

2.8.1 Structure of Major Histocompatibility Complex (MHC) Class I

- MHC Class I molecules in both human and mouse consist of two polypeptide chains that dramatically differ in size. Class-I MHC is a glycoprotein molecule containing a 45KDa α-chain associated non-covalentely with a 12KDa β2 microglobulin molecule.

- The larger (α) chain has a molecular weight of 44 kDa in humans and 47 kDa in the mouse, and is encoded by an MHC Class I gene.
- The smaller chain, called β-2 microglobulin, has a molecular weight of 12 kDa in both species, and is encoded by a nonpolymorphic gene that is mapped outside of the MHC complex.
- There are no known differences in the structure of the human MHC Class I antigen a chains encoded by the HLA-A locus compared to those encoded by the HLA-B or the HLA-C loci, or in the structure of the murine MHC Class I antigen a chains encoded by the H-2K locus compared to those encoded by the H-2D or H-2L loci.

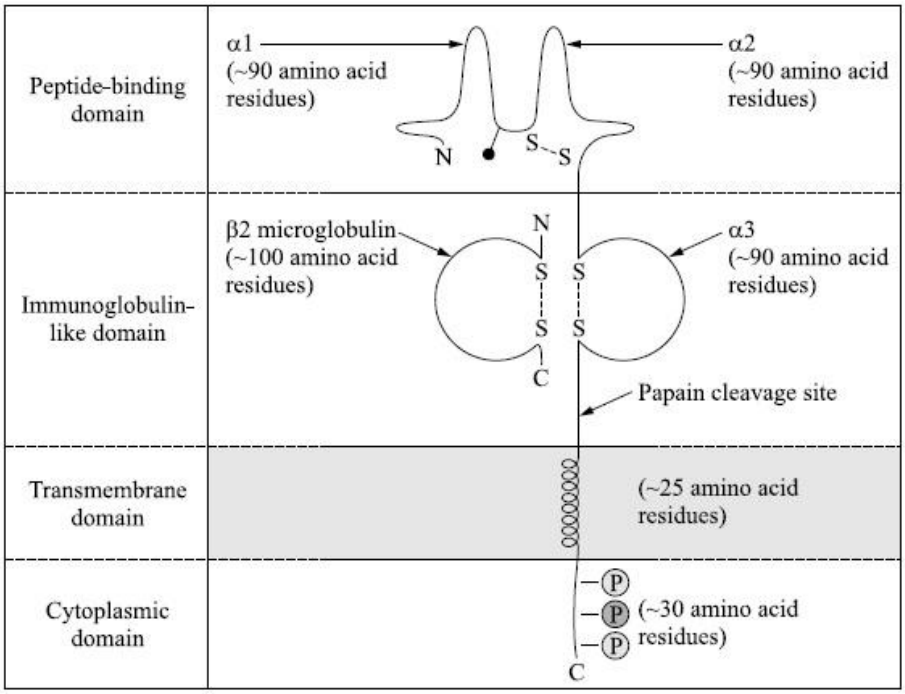

Fig. 8 Structure of MHC Class I antigens

Regardless of which of these loci codes it, the a chain can be subdivided into the following regions, or domains:

1. The peptide-binding domain;
2. The immunoglobulin-like domain;
3. The transmembrane domain; and
4. The cytoplasmic domain.

- The peptide-binding domain is the most N-terminal; it is the only region of the molecule where allelic differences in the amino acid sequence can be localized.
- As seen from its name, the peptide-binding domain of the molecule includes the site to which antigenic peptides bind.
- It makes much sense to have this site exactly where the allelic differences are, because different MHC alleles accommodate peptides better or worse, thus influencing on the magnitude of the T-cell response.
- X-ray crystallography showed that the peptide-binding site in the MHC Class I molecules looks like a cleft that has a ‘‘floor’’ and two ‘‘walls’’ formed by spiral shaped portions of the alpha chain, called alpha 1 and alpha 2.
- Since the ‘‘floor’’ of the peptide-accommodating cleft is closed, only relatively small peptides, consisting of 9 to 11 amino acid residues, can be ‘‘stuffed’’ there.
- The immunoglobulin-like domain is structurally conserved, and resembles a domain of an antibody C-region.
- It contains the binding site for the T-cell accessory molecule CD8.
- The transmembrane and the cytoplasmic domains ensure that the alpha chain spans the membrane and is properly expressed by the cell.
- The β-2-microglobulin chain is also vitally important for the proper expression of the alpha chain.

2.8.2 Mechanism of MHC I

- MHC class I glycoproteins present antigens of endogenous origin to TCRs of CD8+ T cells.Endogenous peptides derive from degradation of intracellular proteins, including viral or tumor antigens in infected or transformed cells, through the proteasome.
- Degradation products translocate from the cytoplasm to the endoplasmatic reticulum (ER) where they are loaded on MHC class I molecules via the peptide-loading complex that includes the ER transporter associated with antigen processing (TAP1/2), tapasin, the oxidoreductase ERp57, and the chaperone protein calreticulin.
- Cellular components involved in the presentation of endogenous antigens, from proteasome subunits to the peptide-loading complex, are collectively referred to as antigen-processing machinery (APM).

- CD8+ T lymphocytes express CD8 receptors, in addition to the T-cell receptors (TCR).When a Cytotoxic T cell CD8 receptor docks to a MHC class I molecule and the TCR fits the epitope within the MHC class I molecule, the CD8+ T lymphocytes triggers the cell to undergo programmed cell death by apoptosis.
- This helps mediate cellular immunity which is the primary means to address intracellular pathogens, such as viruses and some bacteria.

2.8.3 Functions of MHC class I

1. Antigen presentation and processing

Major function of MHC-I is to bind peptide antigens and present to CD8+ T cells (T helper cells). Nucleated cell normally present peptides, mostly self peptides derived from protein turnover and defective ribosomal products. Also, during viral infection, intracellular microorganism infection, or cancerous transformation, such proteins degraded inside the cell by proteasomes are also loaded onto MHC class I molecules and displayed on the cell surface.

2. Transplant Rejection

During transplant of an organ or stem cells, MHC molecules themselves act as antigens and can provoke immune response in the recipient causing transplant rejection. Since, the MHC variation in the human population is high and no two individuals except identical twins express the same MHC molecules, they can mediate transplant rejection.

2.8.4 Major Histocompatibility Complex (MHC) Class II

Class-II MHC is the glycoprotein molecule expressed primarily on antigen presenting cells such as macrophages, dendritic cells and B-cells.

- MHC class II molecules are a class of major histocompatibility complex (MHC) molecules normally found only on antigen-presenting cells which are important in initiating immune responses.
- MHC Class II proteins are encoded by the genes of HLA-D region of the genome in humans.
- Unlike class I proteins, they have a restricted tissue distribution and are chiefly found on macrophages, dendritic cells, B cells, and other Antigen Presenting Cells (APCs) only.
- The antigens presented by class II peptides are derived from extracellular proteins and not endogenous antigens as in MHC class I.

- Class II MHC molecules have β1 and β2 subunits and thus can be recognized by CD4 co-receptors.
- These MHC Class II molecules sample extracellular peptides mainly extracellular pathogens and by interacting with immune cells like the T helper cell (TCD4+) regulate how T cells respond to an infection.

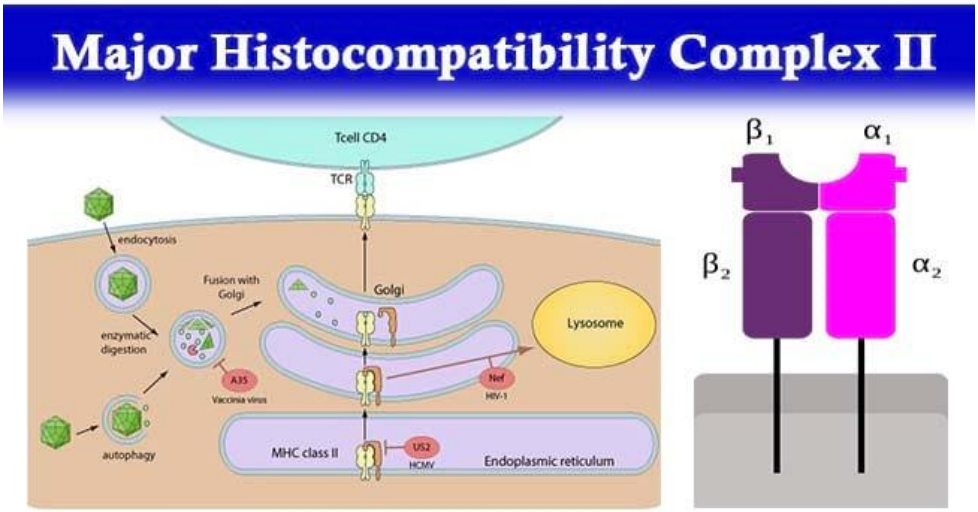

Fig. 9 Major Histocompatibility Complex (MHC) Class II

2.8.5 Structure of Major Histocompatibility Complex (MHC) Class II

- Class II MHC molecules in both human and mouse consist of two polypeptide chains that have a similar, albeit not identical size.
- One of them is called alpha (α) and the other beta (β).
- The molecular weight of the a chain is 32–34 kDa, and of the b chain 29–32 kDa.
- A separate gene controls each of the chains.
- Thus, the murine I-A locus actually consists of the Iα and Iβ genes, the human HLA-DR locus of the HLA-DRα and HLA-DRβ, etc. Both the α and the β genes are polymorphic.
- The β genes of some of the MHC Class II loci can be tandemly duplicated, so, instead of one gene per homologous chromosome, a cell can have two or three.
- Because of that, one cell can simultaneously express more than two allelic products of each of the MHC Class II loci.

- For example, a cell can express allelic products of its HLA-DR molecule that can be identified as HLADRα1– HLA-DRβ1; HLA-DRα2 – HLA-DRβ2; HLA-DRα1 – HLA-DRβ2; HLA-DRα2 – HLA-DRβ1; etc.
- Overall, one cell can simultaneously express as many as 20 different MHC Class II gene products because of this tandem duplication phenomenon.

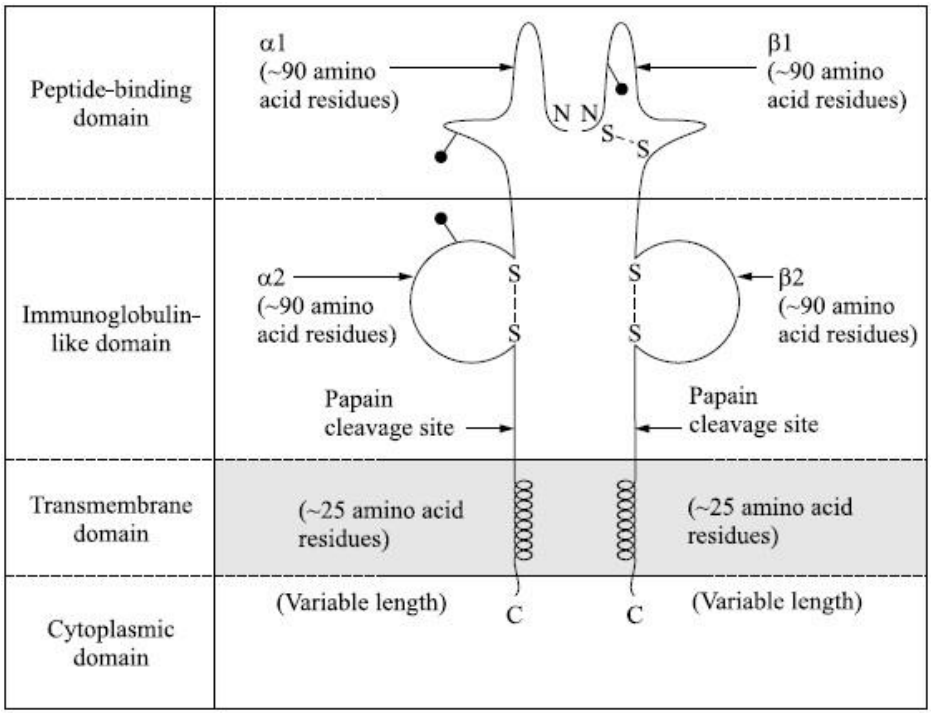

Fig. 10 Structure of MHC Class II antigens

2.8.6 Mechanism of MHC II

- MHC class II molecules present antigens of exogenous origin to CD4+ T cells.
- Phagocytes such as macrophages and immature dendritic cells take up entities by phagocytosis into phagosomes which fuse with lysosomes and the acidic enzymes cleave the uptaken protein into many different peptides.
- During synthesis of class II MHC, the molecules are transported from the endoplasmic reticulum (ER) via the Golgi to endosomal compartments. The α and β chains produced are complexed with a special polypeptide known as the invariant chain (Ii). The Ii prevents endogenous peptides from binding to the groove of MHC class II molecules.
- After removal of Ii in the acidic endosomal compartments, peptides are able to bind to the MHC groove.
- A particular peptide exhibiting immunodominance loads onto MHC class II molecules.

- Peptide-loaded MHC class II molecules are then transported to the membrane surface for antigen presentation.
- The peptide:MHC class II complex is then recognized by the cognate T cell receptor (TCR) of helper T cells.

2.8.6.1 Functions of MHC class II:

- The TCR–peptide: MHC class II engagement is crucial to the induction and regulation of adaptive immunity by selecting the mature CD4+ T cell repertoire in the thymus and activating these lymphocytes in the periphery.
- The secure attachment to the MHC molecule with the presented peptide ensure stable peptide binding which enhance T cell recognition of the antigen, T cell recruitment, and a proper immune response.
- Since they sample and present antigens from exogenous sources, MHC class II molecules are critical for the initiation of the antigen-specific immune response.

2.8.7 MHC Class I Vs. MHC Class II

The major differences between MHC I and MHC II are as given below.

S.N.	Characteristics	MHC-I molecule	MHC -II molecule
1.	Distribution	Present on almost all nucleated cells including platelets.	Have a restricted tissue distribution and are chiefly found on macrophages, dendritic cells, B cells, and other antigen-presenting cells only.
2.	Encoding genes	MHC class I proteins are encoded by the HLA-A, HLA-B, and HLA-C genes.	MHC Class II proteins are encoded by the genes of the HLA-D region.
3.	Nature of antigen presented	Antigens presented by MHC class I molecules are of endogenous origin.	Antigens presented by MHC class II molecules are derived from extracellular proteins.
4.	Antigen	Cytosolic proteins; they sample peptides generated within the cell or those that may enter cytosol from phagosomes.	Class II molecules sample peptides outside the cell such as lysosomal proteins mostly internalized from extracellular environment.
5.	Enzymes involved in	Cytosolic proteasome	Endosomal and lysosomal proteases

	peptide generation		
6.	Peptide loading of MHC	Endoplasmic reticulum	Specialized vesicular compartment
7.	Peptide-loading complex	Includes the ER transporter associated with antigen processing (TAP1/2), tapasin, the oxidoreductase ERp57, and the chaperone protein calreticulin.	Chaperones in ER; invariant chain in ER, Golgi and MHC Class II compartment/Class II vesicle
8.	Recognizing co-receptor	They are recognized by CD8 co-receptors through the MHC Class I β2 subunit.	They are recognized by CD4 co-receptors through β1 and β2 subunits.
9.	Receptor T cell	Present antigens to CD8+ T cells.	Present antigens to CD4+ T cells.
10.	Structure	MHC class I molecules consist of one membrane-spanning α chain produced by MHC genes, and one β chain produced by the β2-microglobulin gene.	MHC class II molecules consist of two membrane-spanning chains, α and β both produced by MHC genes.
11.	Building amino acids	Possess 8-10 amino acids.	Possess 13-18 amino acids.
12.	Peptide binding domains	α1 and α2 are peptide binding domains.	α1 and β1 are peptide binding domains.
13.	Invariant chain	Has no invariant chain.	Has an invariant chain.
14.	Functional effect	Presence of abundant antigens target cell for destruction.	Presences of foreign antigens induce antibody production.
15.	Detection Method	Serology	Serology and mixed lymphocyte reaction

2.8.8 Peptide binding by class I and class II MHC molecules

Major histocompatibility complex (MHC) antigens bind peptides of diverse sequences with high affinity. They do this in order to generate maximal immunological protection by covering the spectrum of peptides that may be seen by a host over the course of its lifetime. However, in many circumstances the immune system does not recognize a particular peptide that it should for maximum advantage over the pathogen. In other situations, the immune system goes awry and incorrectly recognizes a self-peptide that it should not. This results in

disease characterized by recognition and attack of self. Rheumatoid arthritis is an example of just such a disease. In either of these situations, peptide-based modalities for immune therapy would be an advantage. However, peptide-based therapies require a thorough understanding of the forces involved in peptide binding. Great strides have been made in elucidating the mechanisms by which these MHC proteins may bind peptides with diverse sequences and high affinity.

Major histocompatibility complex (MHC) class I and II proteins have been in the focus of interest for immunologists, biochemists, cell biologists, and structural biologists for decades. With dozens of entries in the Protein Data Bank, their crystal structures are now sufficiently well understood, while their dynamic properties such as peptide binding and intracellular trafficking and their immunological (as well as non-immunological) functions are still being intensely investigated. In recent years, new methods and technologies have emerged to detect and characterize the conformational changes and intermediate states that accompany peptide binding and exchange by MHC proteins. These techniques have delivered more detailed information and allowed us to compare the molecular mechanisms of peptide selection between MHC class I and II proteins, suggesting both similarities and differences.

2.8.9 Summary

From a functional perspective, the immune system consists of innate immunity and adaptive immunity, two separate, but interacting and overlapping defensive systems that provide an additional array of defensive weapons. In addition, innate immunity and adaptive immunity are activated by recognition of molecular shapes that are "foreign" to our body. By distinguishing between "self" and "non-self" these systems are (normally) able to identify, destroy, and remove foreign cells, infectious agents, and large foreign molecules without directly attacking our own cells and tissues.

Many bacteria, viruses, and protozoa have glycoproteins and glycolipids on their surface that have distinctive shapes (referred to as "pathogen-associated molecular patterns" or PAMPS) on their surface that enable them to be recognized in a non-specific way as "non-self" by the innate immune system. There are perhaps 100-200 of these PAMPs that have remained unchanged over the course of evolution, and they are molecular shapes that are not present in

our tissues. The innate immune system has certain "sentinel cells (monocytes, macrophages, and specialized macrophages called a dendritic cells) that have so-called toll-like receptors that bind to PAMPs, triggering rapid cellular responses directed against the pathogens.

It is possible for a given PAMP to be present on a number of different types of pathogen, and the innate system will respond to them in the same way without distinguishing among them. Consequently, the innate system is non-specific in how it recognizes and responds to pathogens. And this system is referred to as innate or natural immunity, because the sentinel cells in the innate system will recognize a PAMP and respond to it on the first encounter.

Cells of the lymphatic (or lymphoid) system provide adaptive immunity, which, unlike innate immunity, is highly specific in its ability to recognize and defend against specific foreign agents using both cellular weapons (e.g., cytotoxic T-lymphocytes) and humoral weapons (antibodies manufactured by plasma cells). The lymphatic system is distinct from the arterial and venous systems, but like them, it consists of a complex network of vessels (lymphatic ducts), and the distribution of the lymphatic network often runs in parallel with the arterial and venous systems. Along the lymphatic vessels, there are intermittent lymph nodes, which filter lymph and also house many defensive cells (leukocytes or "white blood cells") and provide a site where the various leukocytes can communicate with one another. When fighting an infection, nearby lymph nodes often become enlarged due to aggregation and increased production of leukocytes and and removal of foreign material. Filtered lymph eventually is emptied into the subclavian vein where it mixes with blood and contributes to the plasma fraction of blood. The thymus and the spleen are also important components of the lymphatic system. The lymphatic system, thymus, and spleen play important roles in immune function, but cellular elements of the immune system are the real "soldiers" in the battle against foreign agents.

2.9.0 Terminal questions

Q.1. What do you mean by immunity? Describe with types.

Answer:----------

Q.2. Describe major histocompatibility complex (MHC) with their types.

Answer:----------

Q.3. Describe the mechanism of MHC I.

Answer:----------

Q.4. What are the components of acquired immunity?

Answer:----------

Q.5. What are the differences between active and passive immunity?

Answer:----------

Q.6. Write a short note on HLA complex.

Answer:----------

Q.7. Explain hormonal influence on immune response.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Block- II

Elements of Immune System

Unit-3
Cells and Organs of immune System

Unit-4
Antigens Processing and Presentation

Introduction

This is the second block on Elements of Immune System. It consists of following two units:

Unit 3: The key primary lymphoid organs of the immune system include the thymus and bone marrow, as well as secondary lymphatic tissues including spleen, tonsils, lymph vessels, lymph nodes, adenoids, skin, and liver. The thymus "educates" T cells and provides an inductive environment for the development of T cells from hematopoietic progenitor cells. The thymus is largest and most active during the neonatal and pre-adolescent periods of development. By the early teens, the thymus begins to atrophy and thymic stroma is replaced by adipose tissue. Nevertheless, residual T-lymphopoiesis continues throughout adult life.

Bone marrow is the flexible tissue found in the interior of bones. In humans, red blood cells are produced in the heads of long bones. The red bone marrow is a key element of the lymphatic system, being one of the primary lymphoid organs that generate lymphocytes from immature hematopoietic progenitor cells. Bone marrow and thymus constitute the primary lymphoid tissues involved in the production and early selection of lymphocytes.

The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels that carry a clear fluid, called lymph, unidirectionally towards the heart. The lymphatic system has multiple interrelated functions including the transportation of white blood cells to and from the lymph nodes into the bones, and the transportation of antigen -presenting cells (such as dendritic cells) to the lymph nodes where an immune response is stimulated. Lymphoid tissue is found in many organs, particularly the lymph nodes.

Unit 4: T cells can only recognise antigens when they are displayed on cell surfaces. This is carried out by Antigen-presenting cells (APCs)**,** the most important of which are dendritic cells, B cells and macrophages. APCs can digest proteins they encounter and display peptide

fragments from them on their surfaces for another immune cell to recognise. This process of antigen presentation allows T cells to “see” what proteins are present in the body and to form an adaptive immune response against them. In this article we shall discuss antigen processing, presentation and recognition by T cells.

Unit- 3: Cells and Organs of Immune System

Structure

3.1 Introduction

Leukocytes (white blood cells) are immune system cells involved in defending the body against infectious disease and foreign materials. Five different types of leukocytes exist, all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. The innate leukocytes include the phagocytes, mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are important mediators in the activation of the adaptive immune system.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte. During the acute phase of inflammation neutrophils migrate toward the site of inflammation and are usually the first cells to arrive at the scene of infection. Macrophages reside within tissues and produce a wide array of chemicals. They

also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system. Dendritic cells are phagocytes in tissues that are in contact with the external environment, and are located mainly in the skin, nose, lungs, stomach, and intestines. These cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T-cells, one of the key cell types of the adaptive immune system.

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis. Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites, and play a role in allergic reactions, such as asthma.Natural killer cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses. The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.

Objectives

This is the second block on elements of immune system. It consists of following two units. Under third unit (Cells and Organs of immune System) we have following objectives. These are as under:

- To know various cells of immune system.
- To know macrophages and their presence in various body tissues.
- Definition of antigen presenting cells (APCs).
- Definition of lymphocytes.
- To know different steps in B lymphocyte development pathway.

3.2 Cells of the Immune System

Cells of immune system are dispersed throughout the body to provide rapid responses to infection. Cells travel through the bloodstream or in specialized vessels called lymphatics. Blood contain three major categories of formed elements: red blood cells (RBCs), also

called **erythrocytes**; **platelets**, also called **thrombocytes**; and white blood cells (WBCs), also called **leukocytes**.

Red blood cells are primarily responsible for carrying oxygen to tissues. Platelets are cellular fragments that participate in blood clot formation and tissue repair. Several different types of WBCs participate in various nonspecific mechanisms of innate and adaptive immunity. In this section, we will focus primarily on the innate mechanisms of various types of WBCs. The cells of the immune system can be categorized as lymphocytes (T-cells, B-cells and NK cells), neutrophils, and monocytes/macrophages. These are all types of white blood cells. These cells that serve specialized roles in innate and adaptive immune responses.

3.3 Leukocytes can be further subdivided into **granulocytes**, which are characterized by numerous granules visible in the cytoplasm, and **agranulocytes**, which lack granules.

1. Granulocytes

The various types of **granulocytes** can be distinguished from one another in a blood smear by the appearance of their nuclei and the contents of their granules, which confer different traits, functions, and staining properties. Granulocytes are further classified as neutrophils**,** Eosinohils and basophils.

(i) Neutrophils

Neutrophils, also called **polymorphonuclear leukocytes**, are the most abundant population of circulating white blood cells and mediate the earliest phases of inflammatory reactions. Neutrophils circulate as spherical cells about 12 to 15 μm in diameter with numerous membranous projections. The cytoplasm contains granules of two types.

Specific granules filled with enzymes such as lysozyme, collagenase, and elastase. These granules do not stain strongly with either basic or acidic dyes (hematoxylin and eosin, respectively), which distinguishes neutrophil granules from those of two other types of circulating granulocytes, called basophils and eosinophils. The remainder of the granules of neutrophils, called **azurophilic granules**, are lysosomes that contain enzymes and other microbicidal substances, including defensins and cathelicidins.

They are found in the bloodstream and can migrate into sites of infection within a matter of minutes. These cells, like the other cells in the immune system, develop from hematopoietic stem cells in the bone marrow. Neutrophils increase in number in the bloodstream during infection and are in large part responsible for the elevated white blood cell count seen with some infections. They are the cells that leave the bloodstream and accumulate in the tissues during the first few hours of an infection and are responsible for the formation of "pus." Their major role is to ingest bacteria or fungi and kill them. Their killing strategy relies on ingesting the infecting organisms in specialized packets of cell membrane that then fuse with other parts of the neutrophil that contain toxic chemicals that kill the microorganisms. They have little role in the defense against viruses.

(ii) Basophils

The **basophils** have a two-lobed nucleus and large granules that stain dark blue or purple.Basophils have cytoplasmic granules of varied size and are named for their granules' ability to absorb the basic dye methylene blue. Their stimulation and degranulation can result from multiple triggering events.Although they are normally not present in tissues, basophils may be recruited to some inflammatory sites.Basophils express IgE receptors, bind IgE, and can be triggered by antigen binding to the IgE.

This cell type is important in allergic reactions and other responses that involve inflammation. One of the most abundant components of basophil granules is **histamine**, which is released along with other chemical factors when the basophil is stimulated. These chemicals can be chemotactic and can help to open the gaps between cells in the blood vessels. Other mechanisms for basophil triggering require the assistance of antibodies.

(iii) Eosinophils

The **eosinophils** have fewer lobes in the nucleus (typically 2–3) and larger granules that stain reddish-orange.Eosinophils are blood granulocytes that express cytoplasmic granules containing enzymes that are harmful to the cell walls of parasites but can also damage host tissues. Some eosinophils are normally present in peripheral tissues, especially in mucosal linings of the respiratory, gastrointestinal, and genitourinary tracts, and their numbers can increase by recruitment from the blood in the setting of inflammation.

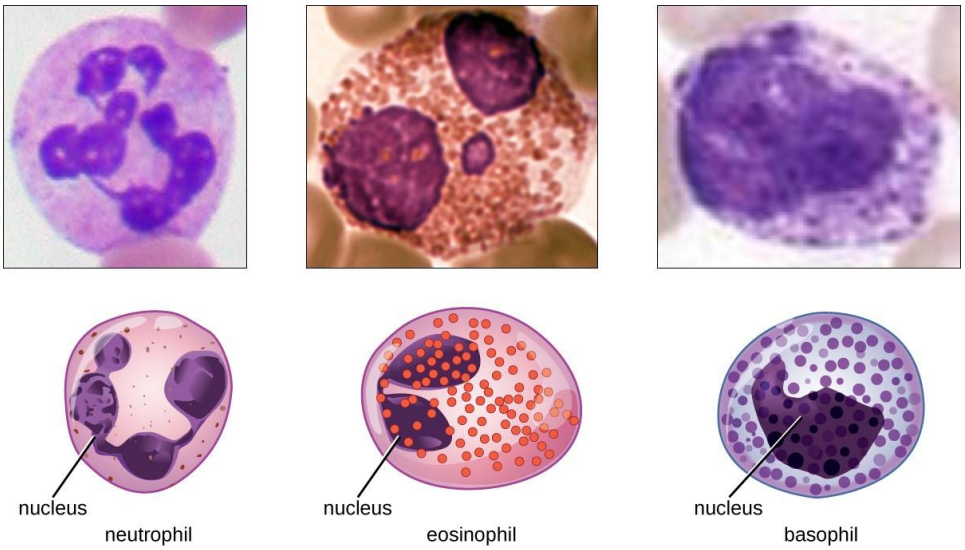

Fig. 1 Types of Granulocytes

3.3.1 Mast Cells

Mast cells are bone marrow–derived cells present in the skin and mucosal epithelia. Functionally, mast cells are very similar to basophils, containing many of the same components in their granules (e.g., **histamine**) and playing a similar role in allergic responses and other inflammatory reactions. mast cells leave the circulating blood and are most frequently found residing in tissues. They are often associated with blood vessels and nerves or found close to surfaces that interface with the external environment, such as the skin and mucous membranes in various regions of the body.

Mast cells express high affinity plasma membrane receptors for a type of antibody called **IgE** and are usually coated with these antibodies. When the antibodies on the mast cell surface bind antigen, signaling events are induced that lead to release of the cytoplasmic granule contents into the extracellular space. The released granule contents, including histamine, promote changes in the blood vessels that cause inflammation. Mast cells function as sentinels in tissues, where they recognize microbial products and respond by producing cytokines and other mediators that induce inflammation.

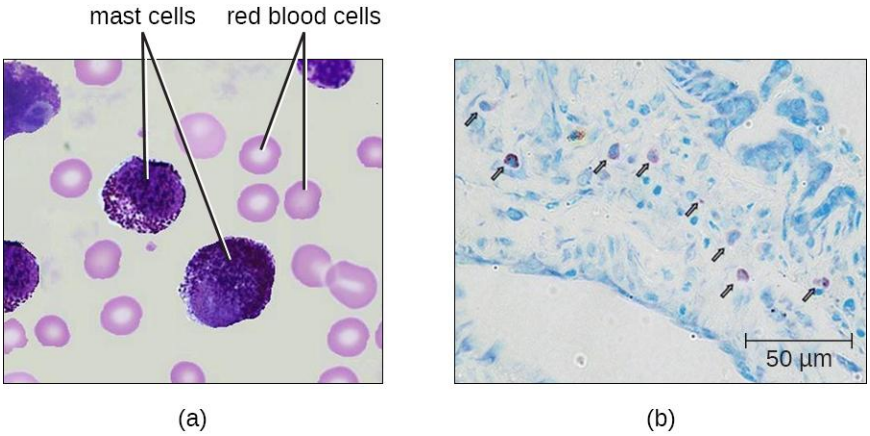

Fig. 2 Mast Cells

3.3.2 Agranulocytes

As their name suggests, **agranulocytes** lack visible granules in the cytoplasm. Agranulocytes can be categorized as lymphocytes or monocytes (Figure 2). Among the lymphocytes are natural killer cells, which play an important role in nonspecific innate immune defenses. Lymphocytes also include the B cells and T cells, which are discussed in the next chapter because they are central players in the specific adaptive immune defenses. The monocytes differentiate into **macrophages** and **dendritic cells**, which are collectively referred to as the mononuclear phagocyte system.

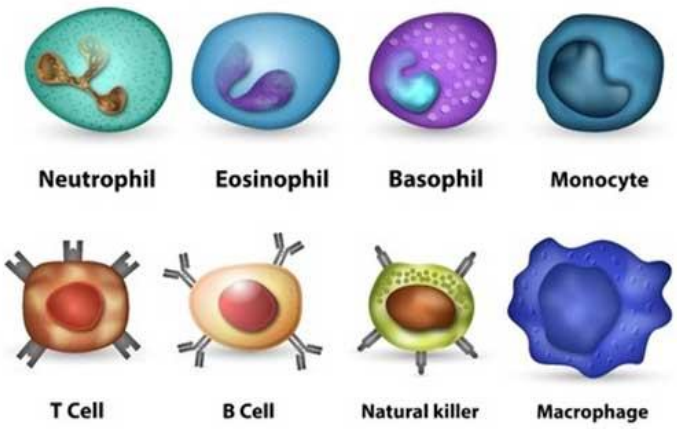

Fig. 3 Types of Immune Cells

3.3.3 Phagocytes

Phagocytes, including neutrophils and macrophages, are cells whose primary function is to ingest and destroy microbes and get rid of damaged tissues. The functional responses of phagocytes in host defense consist of sequential steps: recruitment of the cells to the sites of infection, recognition of and activation by microbes, ingestion of the microbes by the process of phagocytosis, and destruction of ingested microbes.

3.3.4 Monocytes

The largest of the white blood cells, **monocytes** have a nucleus that lacks lobes, and they also lack granules in the cytoplasm. Nevertheless, they are effective phagocytes, engulfing pathogens and apoptotic cells to help fight infection. Monocytes are 10 to 15 μm in diameter, and they have bean-shaped nuclei and finely granular cytoplasm containing lysosomes, phagocytic vacuoles, and cytoskeletal filaments. When monocytes leave the bloodstream and enter a specific body tissue, they differentiate into tissue-specific phagocytes called **macrophages** and **dendritic cells**. Some macrophages are long-term residents in tissues and play an important role in regulating their repair and regeneration. Monocytes, macrophages, and dendritic cells are all highly phagocytic and important promoters of the immune response through their production and release of cytokines.

3.3.5 Macrophages

Macrophages in specific body tissues develop characteristics suited to the particular tissue. Not only do they provide immune protection for the tissue in which they reside but they also support normal function of their neighboring tissue cells through the production of cytokines. Other macrophages participate in the innate immune response and undergo a number of key changes when they are stimulated by encounters with pathogens or tissue damage. These are referred to as inflammatory macrophages and play a dual role in the immune system as effective phagocytes that can contribute to the clearance of pathogens from a tissue, as well as antigen-presenting cells that can activate T lymphocytes. Macrophages are given tissue-specific names, and a few examples of tissue-specific macrophages are listed as below.

3.3.6 Macrophages Found in Various Body Tissues

Tissue	Macrophage
• Brain and central nervous system	• Microglial cells
• Liver	• Kupffer cells
• Lungs	• Alveolar macrophages (dust cells)
• Peritoneal cavity	• Peritoneal macrophages

3.4 Antigen-presenting cells (APCs)

Antigen-presenting cells (APCs) are cells that capture microbial and other antigens, display them to lymphocytes, and provide signals that stimulate the proliferation and differentiation of the lymphocytes. The major type of APC that is involved in initiating T cell responses is the **dendritic cell. Macrophages and B cells** present antigens to T lymphocytes in cell mediated and humoral immune responses.

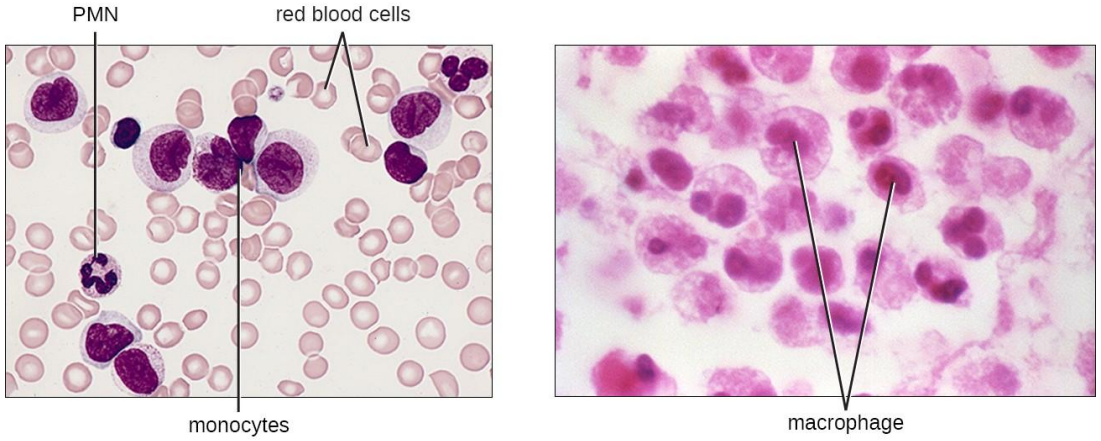

Fig. 4 Monocytes and Macrophages

3.4.1 Dendritic Cells

Dendritic cells are the most important APCs for activating naive T cells, and they play major roles in innate responses to infections and in linking innate and adaptive immune responses. They have long membranous projections and phagocytic capabilities and are widely distributed in lymphoid tissues, mucosal epithelium, and organ parenchyma. Most dendritic

cells are part of the myeloid lineage of hematopoietic cells and arise from a precursor that can also differentiate into monocytes but not granulocytes.

3.4.2 Lymphocytes

Lymphocytes help the body to remember previous invaders and recognize them if they come back to attack again. Lymphocytes begin their life in bone marrow. Some stay in the marrow and develop into B lymphocytes (B cells), others head to the thymus and become T lymphocytes (T cells). Lymphocytes are the principal cell players in the adaptive immune response. They represent 20% to 40% of circulating white blood cells and 99% of cells in the lymph. Lymphocytes can be broadly subdivided into three major populations on the basis of functional and phenotypic differences:

1. B lymphocytes (B cells)
2. T lymphocytes (T cells)
3. Natural killer (NK) cells.

3.4.2.1 B- Lymphocytes

B lymphocyte (B cell) derived its letter designation from its site of maturation, in the bursa of Fabricius in birds. B-cells develop in the bone marrow from hematopoietic stem cells. As part of their maturation in the bone marrow, B-cells are trained or educated so that they do not produce antibodies to healthy tissues. When mature, B-cells can be found in the bone marrow, lymph nodes, spleen, some areas of the intestine, and the bloodstream. When B-cells encounter foreign material (antigens), they respond by maturing into another cell type called plasma cells. B-cells can also mature into memory cells, which allows a rapid response if the same infection is encountered again. Plasma cells are the mature cells that actually produce the antibodies.

3.4.2.2 T- Lymphocytes

T lymphocytes (T cells) derive their letter designation from their site of maturation in the thymus. T-cells develop from hematopoietic stem cells in the bone marrow but complete their development in the thymus. The thymus is a specialized organ of the immune system in the chest. Within the thymus, immature lymphocytes develop into mature T-cells (the “T” stands for the thymus) and T-cells with the potential to attack normal tissues are eliminated. The thymus is essential for this process, and T-cells cannot develop if the fetus does not have a

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.

Each T-cell reacts with a specific antigen, just as each antibody molecule reacts with a specific antigen. In fact, T-cells have molecules on their surfaces that are similar to antibodies. The variety of different T-cells is so extensive that the body has T-cells that can react against virtually any antigen. T-cells have different abilities to recognize antigen and are varied in their function. There are "killer" or cytotoxic T-cells (often denoted in lab reports as CD8 T-cells), helper T-cells (often denoted in lab reports as CD4 T-cells), and regulatory T-cells.

Killer, or cytotoxic, T-cells perform the actual destruction of infected cells. Killer T-cells protect the body from certain bacteria and viruses that have the ability to survive and even reproduce within the body's own cells. Killer T-cells also respond to foreign tissues in the body, such as a transplanted kidney. The killer cell must migrate to the site of infection and directly bind to its target to ensure its destruction. Helper T-cells assist B-cells to produce antibodies and assist killer T-cells in their attack on foreign substances.

Regulatory T-cells suppress or turn off other T-lymphocytes. Without regulatory cells, the immune system would keep working even after an infection has been cured. Without regulatory T-cells, there is the potential for the body to "overreact" to the infection. Regulatory T-cells act as the thermostat of the lymphocyte system to keep it turned on just enough—not too much and not too little. T-cell receptors only recognize processed pieces of antigen (typically peptides) bound to cell membrane proteins called major histocompatibility complex (MHC) molecules.

They become activated, proliferate, and differentiate into an effector cell called a cytotoxic T lymphocyte (CTL). The CTL has a vital function in monitoring the cells of the body and eliminating any cells that display foreign antigen complexed with class I MHC,

3.4.3 Natural Killer Cells

Natural killer (NK) cells are so named because they easily kill cells infected with viruses. They are said to be "natural killer" cells as they do not require the same thymic education that T-cells require. NK cells are derived from the bone marrow and are present in relatively low

numbers in the bloodstream and in tissues. They are important in defending against viruses and possibly preventing cancer as well.

Natural killer (NK) cells are lymphoid cells that are closely related to B and T cells. However, they do not express antigen specific receptors. NK cells constitute 5% to 10% of lymphocytes in human peripheral blood. They are efficient cell killers and attack a variety of abnormal cells, including some tumor cells and some cells infected with virus.

Cancer cells and cells infected with viruses are two examples of cellular abnormalities that are targeted by NK cells. Recognition of such cells involves a complex process of identifying inhibitory and activating molecular markers on the surface of the target cell. Molecular markers that make up the **major histocompatibility complex (MHC)** are expressed by healthy cells as an indication of "self."

NK cells are able to recognize normal MHC markers on the surface of healthy cells, and these MHC markers serve as an inhibitory signal preventing NK cell activation. However, cancer cells and virus-infected cells actively diminish or eliminate expression of MHC markers on their surface. When these MHC markers are diminished or absent, the NK cell interprets this as an abnormality and a cell in distress. This is one part of the NK cell activation process (Figure 5). NK cells are also activated by binding to activating molecular molecules on the target cell. These activating molecular molecules include "altered self" or "nonself" molecules. When a NK cell recognizes a decrease in inhibitory normal MHC molecules and an increase in activating molecules on the surface of a cell, the NK cell will be activated to eliminate the cell in distress.

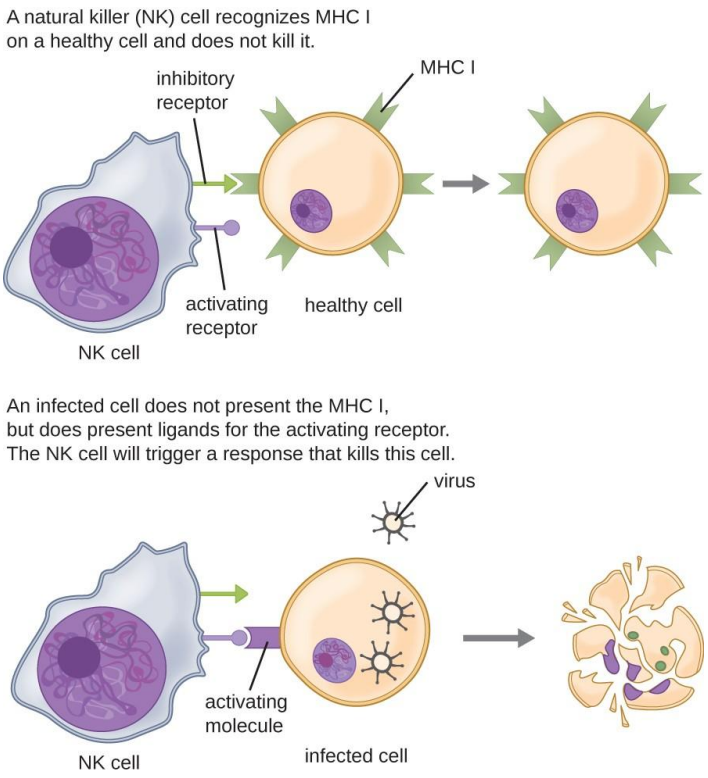

Fig. 5 Organs of the Immune System

Our immune system is made up of both individual cells and proteins as well as entire organs and organ systems. The organs of the immune system include skin and mucous membranes, and the organs of the lymphatic system too.

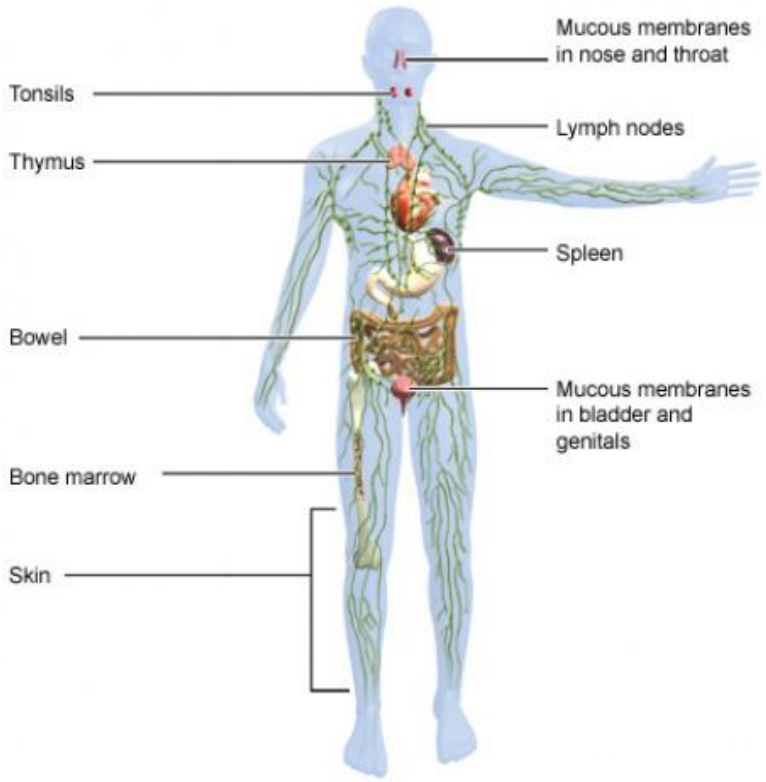

Fig. 6 Lymphoid Organs

3.4.4 Lymphoid Organs

The lymphatic system is composed of:

1. Primary lymphoid organs: These organs include the bone marrow and the thymus. They create special immune system cells called lymphocytes.

2. Secondary lymphoid organs: Secondary lymphoid organs include: lymph nodes, tonsils, spleen**,** Peyer's patches **and** mucosa associated lymphoid tissue (MALT**).**It is in these organs where the cells of the immune system do their actual job of fighting off germs and foreign substances.

3.4.5 Bone marrow

Bone marrow is a sponge-like tissue found inside the bones. That is where most immune system cells are produced and then also multiply. These cells move to other organs and tissues through the blood. At birth, many bones contain red bone marrow, which actively creates

immune system cells. Over the course of our life, more and more red bone marrow turns into fatty tissue. In adulthood, only a few of our bones still contain red bone marrow, including the ribs, breastbone and the pelvis.

3.4.5.1 Thymus

The thymus is located behind the breastbone above the heart. This gland-like organ reaches full maturity only in children, and is then slowly transformed to fatty tissue. Special types of immune system cells called thymus cell lymphocytes (T cells) mature in the thymus. Among other tasks, these cells coordinate the processes of the innate and adaptive immune systems. T cells move through the body and constantly monitor the surfaces of all cells for changes.

3.4.5.2 Lymph nodes

Lymph nodes are small bean-shaped tissues found along the lymphatic vessels. The lymph nodes act as filters. Various immune system cells trap germs in the lymph nodes and activate the creation of special antibodies in the blood. Swollen or painful lymph nodes are a sign that the immune system is active, for example to fight an infection.

3.4.5.3 Spleen

The spleen is located in the left upper abdomen, beneath the diaphragm, and is responsible for different kinds of jobs:

- It stores various immune system cells. When needed, they move through the blood to other organs. Scavenger cells (phagocytes) in the spleen act as a filter for germs that get into the bloodstream.
- It breaks down red blood cells (erythrocytes).
- It stores and breaks down platelets (thrombocytes), which are responsible for the clotting of blood, among other things.

There is always a lot of blood flowing through the spleen tissue. At the same time this tissue is very soft. In the event of severe injury, for example in an accident, the spleen may rupture easily. Surgery is then usually necessary because otherwise there is a danger of bleeding to death. If the spleen needs to be removed completely, other immune system organs can carry out its roles.

3.4.5.4 Tonsils

The tonsils are also part of the immune system. Because of their location at the throat and palate, they can stop germs entering the body through the mouth or the nose. The tonsils also contain a lot of white blood cells, which are responsible for killing germs. There are different types of tonsils: palatine tonsils, adenoids and the lingual tonsil. All of these tonsillar structures together are sometimes called Waldeyer's ring since they form a ring around the opening to the throat from the mouth and nose. There is also lymphatic tissue on the side of the throat, which can perform the functions of the palatine tonsils if they are removed.

3.4.5.5 Mucosa Associated Lymphoid Tissues

Mucosa-associated lymphoid tissue (MALT) is located within the mucosal linings and constitutes the most extensive component of human lymphoid tissue. These surfaces protect the body from an enormous quantity and variety of antigens. For examples tonsils, the Peyer's patches within the small intestine, and the vermiform appendix. The nomenclature uitilises location; therefore, MALT is understood to include
gut-associated lymphoid tissue (GALT), bronchial/tracheal-associated lymphoid tissue (BALT), nose-associated lymphoid tissue (NALT), and vulvovaginal-associated lymphoid tissue (VALT).

MALT contains lymphocytes (T cells and B cells), plasma cells and macrophages, each of which to encounters antigens passing through the mucosal epithelium. MALT constitutes about 50% of the lymphoid tissue in human body.

3.4.6 B Cell Production and Maturation

Before birth, the yolk sac, foetal liver and foetal bone marrow are the major sites of B cell maturation. After birth, the generation of mature B-cells occur in the bone marrow from hematopoietic stem cells (HSC). B cells are formed from multipotent **hematopoietic stem cells** (HSCs) in the bone marrow and follow a pathway through lymphoid stem cell and lymphoblast.Lymphoblasts destined to become B cells do not leave the **bone marrow** and continue to mature in the bone marrow.

3.4.7 Steps in B lymphocyte developmental pathway

3.4.7.1 Bone marrow-dependent stages

Most of the stages of B lymphocyte development take place in this primary lymphoid organ. The pluripotent HSCs gradually differentiate into progenitors, which have increasingly lower potency. Initially, they form a population of cells that are known as multipotent progenitors (MPPs). These progenitors, in turn, give rise to two main progenitor populations: common granulocyte/megakaryocyte/granulocyte progenitor (CFU-GEMM) and early lymphoid progenitor (ELP). CFU-GEMMs subsequently develop into cells that have either myeloid or erythroid potential. On the other hand, cells with lymphoid potential arise from ELPs.

Thus, CFU-GEMMs are the primary source of those elements of blood that are non-lymphoid in nature, whereas the lymphoid elements originate from ELPs. Two major precursors arise from the ELPs, common lymphocyte progenitor (CLP) and early T-lineage precursor (ETP). Both Pre-NK cells and Pre-B cells develop from CLPs, which eventually give rise to NK cells and B cells, respectively.

The CLPs give rise to early Pro-B cells first. They mature to form the late Pro-B cells, which eventually develop into Pre-B cells. Immature B cells arise from these Pre-B cells and they leave the bone marrow to enter into the secondary lymphoid organs. Subsequent stages of B cell development primarily continue in the spleen.

3.4.7.2 Spleen-dependent stages

The immature B cell undergoes final stages of development in the spleen to form mature B cells. The spleen primarily consists of red pulp, white pulp and marginal zone. The red pulp is made up of large, blood-filled sinuses and serves as the blood-filtering system of the spleen. The white pulp is organised in line with the lymph nodes and consists of lymphoid sheaths having distinct B-cell and T-cell compartments.

The marginal zone is a layer of highly specialised cells that surrounds the white pulp. It plays a very important role in immunity because those haematopoietic cells that remain in circulation (as part of the surveillance mechanism) need to be able to migrate through blood and lymphatic systems continuously. The specialised cells that constitute the marginal zone include two subsets of macrophages, the marginal-zone macrophages and the marginal-zone metallophilic macrophages. The first subset is present as an outer ring and the second subset is present as an inner ring, lies closer to the white pulp.

A specialised B-cell population, known as **marginal zone B cell**, and DCs are located in between these two rings of macrophages. Figure 1 shows the major cell populations that are generated in the bone marrow and peripheral lymphoid organs during the process of B cell development.

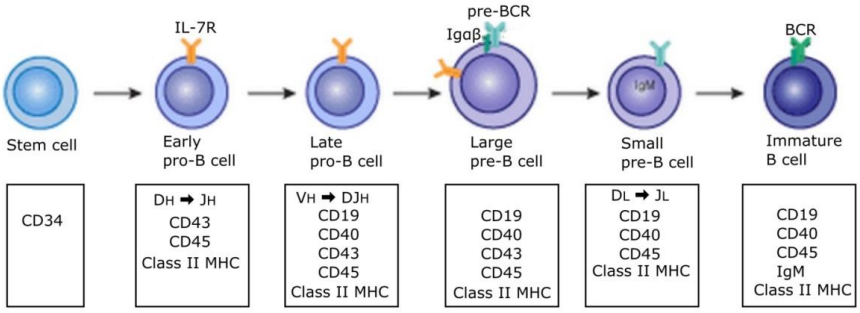

Fig. 7 B cell development

3.4.8 Selection of B Cells

B cells undergo two types of selection while developing in the bone marrow to ensure proper development, both involving B cell receptors (BCR) on the surface of the cell.

(i) Positive selection

Positive selection requires signaling through the antigen receptor for the cell to survive. Developing B cells are positively selected when the pre-B receptor binds its ligand. It occurs through antigen-independent signaling involving both the pre-BCR and the BCR. If these receptors do not bind to their ligand, B cells do not receive the proper signals and cease to develop.

(ii) Negative selection

It occurs through the binding of self-antigen with the BCR; If the BCR can bind strongly to self-antigen then negative selection process leads to a state of central tolerance, in which the mature B cells do not bind self antigens present in the bone marrow. N**egative selection** is used to eliminate **self-reacting B cells** and minimize the risk of **autoimmunity**. Negative selection of self-reacting B cells can involve elimination by **apoptosis**, editing or modification of the receptors so they are no longer self-reactive, or induction of **energy** in the B cell.

To complete development, immature B cells migrate from the bone marrow into the spleen as transitional B cells, passing through two transitional stages: T1 and T2. Throughout their migration to the spleen and after spleen entry, they are considered T1 B cells. Within the spleen, T1 B cells transition to T2 B cells. T2 B cells differentiate into either follicular (FO) B cells or marginal zone (MZ) B cells depending on signals received through the BCR and other receptors. Once differentiated, they are now considered mature B cells, or naive B cells.

3.4.9 Regulation of B Cell Development

Progenitor cells receive signals from bone marrow stromal cells via cell-cell contacts and secreted signals. This bone marrow **microenvironment** is responsible for B cell development. One set of CAMs involved in both B and T cell development is **SCF** (**stem cell factor**) on the stromal cell membrane and **kit** (CD117) on the lymphocyte membrane. A secreted cytokine important for both B and T cell development is **IL-7**, secreted by the stromal cell and bound to IL-7R on the developing lymphocyte. Signals from these binding events initiate cytoplasmic cascades resulting in altered expression of proteins required for development. As the B cells develop in the marrow, they migrate from the outer part of the marrow towards the core.

3.4.9.1 Activation of B Cells

Activation of B cells occurs in the secondary lymphoid organs (SLOs), such as the spleen and lymph nodes. After B cells mature in the bone marrow, they migrate to SLOs, where B cell activation begins when the B cell binds to an antigen via its BCR. Activation of B cells occurs through different mechanisms depending on the molecular class of the antigen. Activation of a B cell by a protein antigen requires the B cell to function as an APC, presenting the protein epitopes with MHC II to helper T cells.

Because of their dependence on T cells for **activation of B cells**, protein antigens are classified as **T-dependent antigens**. In contrast, polysaccharides, lipopolysaccharides, and other nonprotein antigens are considered **T-independent antigens** because they can activate B cells without antigen processing and presentation to T cells.

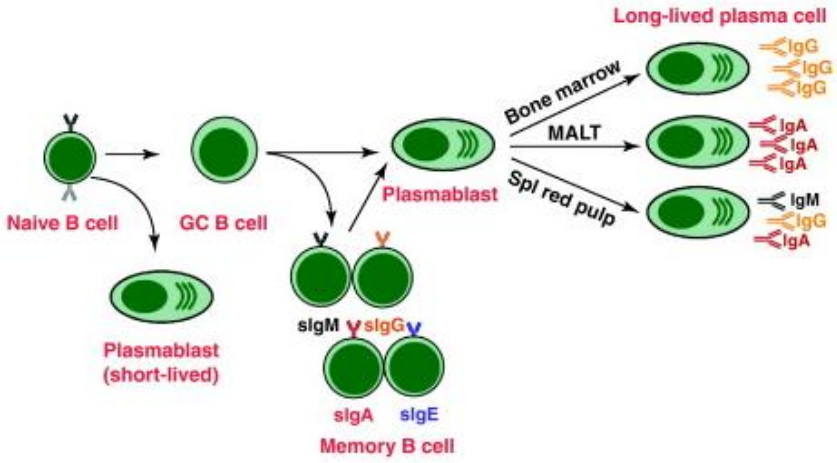

Fig. 8 B cell activation: from immature B cell to plasma cell or memory B cell

3.4.9.2 T Cell-Independent Activation of B cells

Activation of B cells without the cooperation of helper T cells is referred to as **T cell-independent activation** and occurs when BCRs interact with T-independent antigens. T-independent antigens (e.g., polysaccharide capsules, lipopolysaccharide) have **repetitive epitope units** within their structure, and this repetition allows for the **cross-linkage** of multiple BCRs, providing the first signal for activation (Figure 2). Because T cells are not involved, the second signal has to come from other sources, such as interactions of **toll-like receptors** with **PAMPs** or interactions with factors from the **complement system**.

Once a B cell is activated, it undergoes **clonal proliferation** and daughter cells differentiate into plasma cells. **Plasma cells** are antibody factories that secrete large quantities of antibodies. After differentiation, the surface BCRs disappear and the plasma cell secretes **pentameric IgM** molecules that have the same antigen specificity as the BCRs (Figure 2).

The T cell-independent response is short-lived and does not result in the production of **memory B cells**. Thus it will not result in a secondary response to subsequent exposures to T-independent antigens.

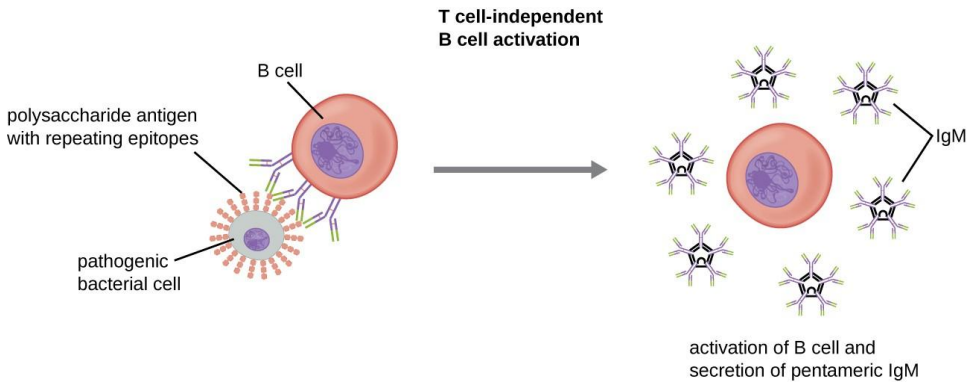

Fig. 9 T cell independent Activation of B cells

3.4.9.3 T Cell-Dependent Activation of B cells

T cell-dependent activation of B cells results in stronger immune response and develops memory. T cell-dependent activation can occur either in response to **free protein antigens** or to protein antigens associated with an intact pathogen. Interaction between the BCRs on a naïve mature B cell and a free protein antigen stimulate **internalization** of the antigen, whereas interaction with antigens associated with an intact pathogen initiates the extraction of the antigen from the pathogen before internalization. Once internalized inside the B cell, the protein antigen is processed and presented with **MHC II**. The presented antigen is then recognized by **helper T cells** specific to the same antigen. The TCR of the helper T cell recognizes the foreign antigen, and the T cell's **CD4** molecule interacts with MHC II on the B cell. The coordination between B cells and helper T cells that are specific to the same antigen is referred to as **linked recognition**.

Once activated by linked recognition, **T_H2 cells** produce and secrete **cytokines** that activate the B cell and cause proliferation into clonal daughter cells. After several rounds of proliferation, additional cytokines provided by the T_H2 cells stimulate the differentiation of activated B cell clones into **memory B cells**, which will quickly respond to subsequent exposures to the same protein epitope, and plasma cells that lose their membrane BCRs and initially secrete pentameric IgM.

After initial secretion of IgM, **cytokines** secreted by T_H2 cells stimulate the plasma cells to switch from IgM production to production of **IgG**, **IgA**, or **IgE**. This process, called **class switching** or **isotype switching**, allows **plasma cells** cloned from the same activated B cell to

produce a variety of antibody classes with the same epitope specificity. Class switching is accomplished by **genetic rearrangement** of gene segments encoding the **constant region**, which determines an antibody's class. The **variable region** is not changed, so the new class of antibody retains the original epitope specificity.

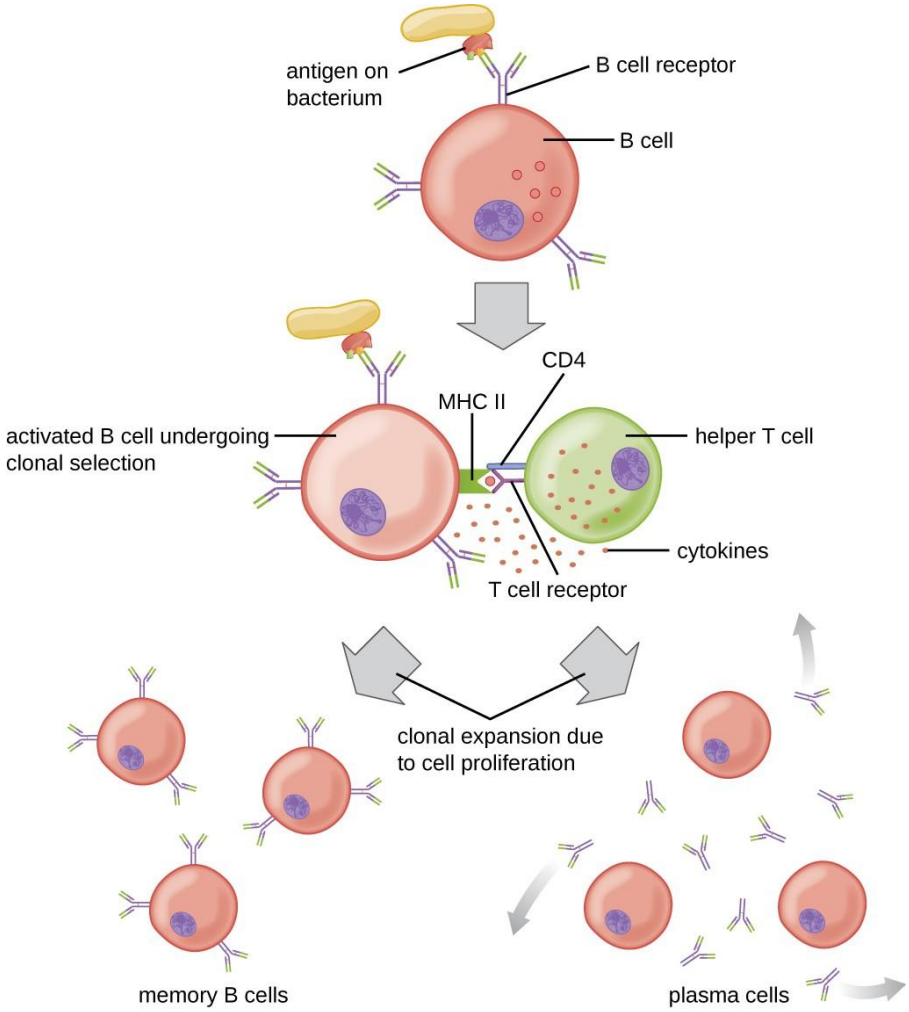

Fig. 10 T Cell Dependent Activation of B Cells

Subsets of B Cells

In general, three subsets of B cells derive from naive B cells, B-1 B cells, follicular B cells and marginal zone (MZ) B cells. Furthermore, B-1B cells form two subsets, B-1a and B-1 b B cells. All the subsets can be clearly identified on the basis of their surface markers. These surface markers can also be used to identify their progenitor populations. All these subsets of B cells produce functionally important antibodies. However, they vary in huge terms in reference to their origin and function.

3.5.0 B-1 B cells

These cells have not been successfully studied in mammals, including humans. Thus, most of the findings are based on studies performed in mice. The most interesting finding from these studies is that although the progenitors of B-1 a and B-1 b cells are distinct, they are found to occupy the same locations, namely the pleural and peritoneal cavities.

2. Follicular B cells

Follicular B cells reside in two main niches during their circulation/recirculation through the bone marrow. Out of these, the "follicular niche" presents in the spleen/lymph nodes/Peyer's patches is the main site that is occupied by these cells. These "follicular sites" are thought to play important role in those immune responses against protein antigens, which are T cell-dependent. Most common type of B cell and, when not circulating through the blood, is found mainly in the lymphoid follicles of secondary lymphoid organs (SLOs). They are responsible for generating the majority of high-affinity antibodies during an infection. Interestingly, the follicular B cells residing in the bone marrow are involved in T cell-independent immune responses against microbial pathogens harboured by blood.

3. Marginal zone (MZ) B cells

MZ B cells mainly home near the marginal sinus of the spleen. These cells are mainly involved in T cell-independent immune responses against blood-borne microbes. It has also been reported that these MZ B cells can transport pathogens from the marginal sinus to the splenic follicles, sites where the follicular B cells reside.

3.5.1 T Cell Production and Maturation

T cells, like all other white blood cells involved in innate and adaptive immunity, are formed from multipotent **hematopoietic stem cells** (HSCs) in the bone marrow. However, T cells differentiate first into lymphoid stem cells that then become small, immature lymphocytes, sometimes called **lymphoblasts**. The first steps of differentiation occur in the red marrow of bones, after which immature T lymphocytes enter the bloodstream and travel to the **thymus** for the final steps of maturation. Once in the thymus, the immature T lymphocytes are referred to as **thymocytes**.

The maturation of thymocytes within the thymus can be divided into tree critical steps of positive and negative selection, collectively referred to as **thymic selection**. The first step of thymic selection occurs in the cortex of the thymus and involves the development of a functional **T-cell receptor (TCR)** that is required for activation by APCs. Thymocytes with defective TCRs are removed by negative selection through the induction of **apoptosis** (programmed controlled cell death). The second step of thymic selection also occurs in the cortex and involves the positive selection of thymocytes that will interact appropriately with MHC molecules.

Thymocytes that can interact appropriately with MHC molecules receive a positive stimulation that moves them further through the process of maturation, whereas thymocytes that do not interact appropriately are not stimulated and are eliminated by **apoptosis**. The third and final step of thymic selection occurs in both the cortex and medulla and involves **negative selection** to remove **self-reacting thymocytes**, those that react to self-antigens, by apoptosis. This final step is sometimes referred to as **central tolerance** because it prevents self-reacting T cells from reaching the bloodstream and potentially causing **autoimmune disease**, which occurs when the immune system attacks healthy "self" cells.

Despite central tolerance, some self-reactive T cells generally escape the thymus and enter the peripheral bloodstream. Therefore, a second line of defense called **peripheral tolerance** is needed to protect against autoimmune disease. Peripheral tolerance involves mechanisms of **energy** and inhibition of self-reactive T cells by **regulatory T cells**. Anergy refers to a state of nonresponsiveness to antigen stimulation. In the case of self-reactive T cells that escape the thymus, lack of an essential **co-stimulatory signal** required for activation causes anergy and prevents autoimmune activation. Regulatory T cells participate in peripheral tolerance by inhibiting the activation and function of self-reactive T cells and by secreting anti-inflammatory cytokines.

It is not completely understood what events specifically direct maturation of thymocytes into regulatory T cells. Current theories suggest the critical events may occur during the third step of thymic selection, when most self-reactive T cells are eliminated. Regulatory T cells may receive a unique signal that is below the threshold required to target them for negative

selection and apoptosis. Consequently, these cells continue to mature and then exit the thymus, armed to inhibit the activation of self-reactive T cells.

It has been estimated that the three steps of thymic selection eliminate 98% of thymocytes. The remaining 2% that exit the thymus migrate through the bloodstream and **lymphatic system** to sites of secondary lymphoid organs/tissues, such as the **lymph nodes**, **spleen**, and **tonsils** (Figure 3), where they await activation through the presentation of specific antigens by APCs. Until they are activated, they are known as **mature naïve T cells**.

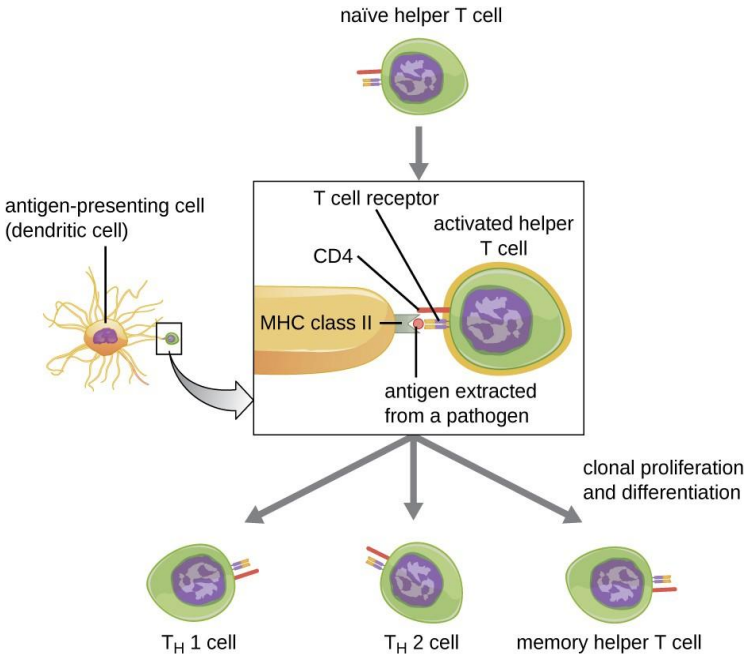

Fig. 11 Activation of T helper Cell

Activated helper T cells can differentiate into one of four distinct subtypes, summarized in Table 2. The differentiation process is directed by APC-secreted **cytokines**. Depending on which APC-secreted cytokines interact with an activated helper T cell, the cell may differentiate into a T helper 1 (T_H1) cell, a T helper 2 (T_H2) cell, or a memory helper T cell. The two types of helper T cells are relatively short-lived **effector cells**, meaning that they perform various functions of the immediate immune response. In contrast, **memory helper T cells** are relatively long lived; they are programmed to "remember" a specific antigen or epitope in order to mount a rapid, strong, **secondary response** to subsequent exposures.

1. T_H1 cells secrete their own cytokines that are involved in stimulating and orchestrating other cells involved in adaptive and innate immunity. For example, they stimulate cytotoxic T cells, enhancing their killing of infected cells and promoting differentiation into **memory cytotoxic T cells**. T_H1 cells also stimulate **macrophages** and **neutrophils** to become more effective in their killing of intracellular bacteria. They can also stimulate **NK cells** to become more effective at killing *target* cells.

2. T_H2 cells play an important role in orchestrating the humoral immune response through their secretion of **cytokines** that activate **B cells** and direct B cell differentiation and **antibody production**. Various cytokines produced by T_H2 cells orchestrate **antibody class switching**, which allows B cells to switch between the production of IgM, IgG, IgA, and IgE as needed to carry out specific antibody functions and to provide pathogen-specific humoral immune responses.

3. A third subtype of **helper T cells** called **T_H17 cells** was discovered through observations that immunity to some infections is not associated with T_H1 or T_H2 cells. T_H17 cells and the cytokines they produce appear to be specifically responsible for the body's defense against chronic mucocutaneous infections. Patients who lack sufficient T_H17 cells in the mucosa (e.g., HIV patients) may be more susceptible to bacteremia and gastrointestinal infections.

3.5.3 Activation and Differentiation of Cytotoxic T Cells

Cytotoxic T cells (also referred to as **cytotoxic T lymphocytes**, or CTLs) are activated by APCs in a three-step process similar to that of helper T cells. The key difference is that the **activation of cytotoxic T cells** involves recognition of an antigen presented with MHC I (as opposed to MHC II) and interaction of CD8 (as opposed to CD4) with the receptor complex. After the successful co-recognition of foreign epitope and self-antigen, the production of **cytokines** by the APC and the cytotoxic T cell activate **clonal proliferation** and differentiation. Activated cytotoxic T cells can differentiate into effector cytotoxic T cells that target pathogens for destruction or **memory cells** that are ready to respond to subsequent exposures.

Once activated, cytotoxic T cells recognize infected cells through antigen presentation of pathogen-specific epitopes associated with **MHC I**. Once an infected cell is recognized, the TCR of the cytotoxic T cell binds to the epitope and releases **perforin** and **granzymes** that destroy the infected cell Perforin is a protein that creates pores in the target cell, and **granzymes** are proteases that enter the pores and induce **apoptosis**. This mechanism of **programmed cell death** is a controlled and efficient means of destroying and removing infected cells without releasing the pathogens inside to infect neighboring cells, as might occur if the infected cells were simply lysed.

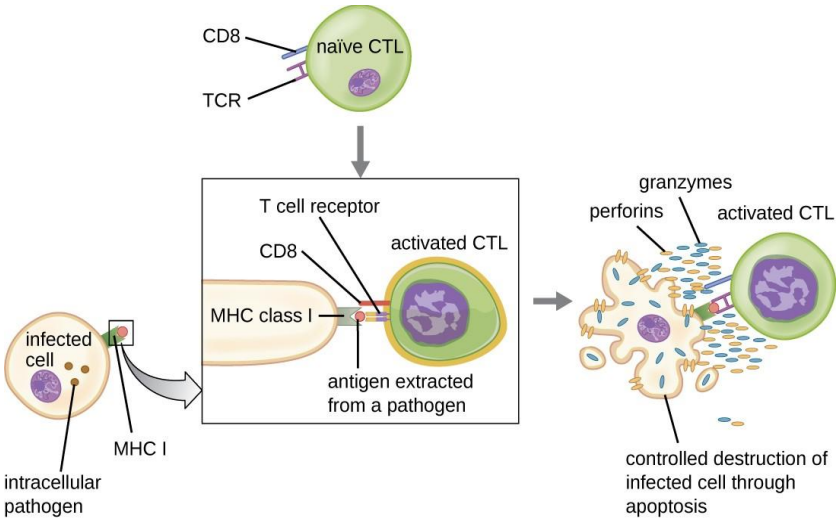

Fig. 12 Activation of Cytotoxic T cell

3.5.4 Organization and Expression of Immunoglobulin Genes

B-lymphocytes of the immune system produce antibodies in the presence of antigen. The antibodies are formed from the assembly of some protein chains. For this, there must be millions of genes for each antibody. But how can it be possible because a mammalian genome does not contain more than about a million of genes, out of which only a fraction of genome directs the synthesis of antibodies. This clearly shows that neither the germ cells nor embryonic cells contain a complete set of all genes but have the basic genes which are shuffled during developmental stages of B-lymphocytes.

3.5.5 B Cell Development and Immunoglobulin Gene Rearrangement

During the development of B cells, the immunoglobulin gene undergoes sequences of rearrangements that lead to formation of the antibody repertoire. For example, in the lymphoid cell, a partial rearrangement of the heavy-chain gene occurs which is followed by complete rearrangement of heavy-chain gene.

In germ line DNA, multiple gene segments encode a single immunoglobulin heavy or light chain. These gene segments are carried in the germ cells that cannot be transcribed and translated into heavy and light chains until these are arranged into functional genes. During the differentiation of B-cells in bone marrow these gene segments are randomly shuffled about 10^8 specificities by a dynamic genetic system.

This is maintained by germ line theory. Differentiation of B-cells from a progenitor B-cell to a mature cell involves an ordered progress in rearrangement of immunoglobin genes. When the process of B-cell division is over, a mature immuno-competent B-cell contains a single functional variable region DNA sequence for its heavy chain and a single functional variable region DNA sequence for its light chain.

This has been confirmed through the recent evidences that a single variable region sequence specific for a particular antigen can be associated with the multiple sequences of C-region of heavy chains. It means that the different isotypes of antibodies (i.e. IgG, IgM) can be expressed having identical sequences of V-region.

(i) Dryer and Bennett's Two Gene Model:

In 1965, W.Dryer and J. Bennett in their classical theoretical paper suggested for encoding of immunoglobulin chains. The two separate genes encode two different chains, one the light chain and the other heavy chain. They hypothesized that the two genes must come together and form a complete set of genes that can transcribe and translate the full message and can yield a single heavy or light protein chain.

Experimental evidence of gene rearrangement:

For the first time, **Hozumi** and **Tonegawa** (1976) provided the experimental evidence for the rearrangement of two separate genes encoding the V and C-regions of immunoglobulin during

the course of differentiation of B-lymphocytes, and produce millions of antibodies. For this novel work, Tonegawa was awarded Nobel Prize in 1987 in medicine and physiology.

They used the newly developed Southern Blotting Technique. They took myeloma cells because they are like Plasma cells and produce large amount of single antibodies, and prepared radiolabelled RNA i.e. ^{32}p-mRNA for K-light and heavy chains, and also for constant chain. ^{32}P- mRNA was used as probe to test two kinds of cells, embryonic cells (that do not produce antibodies) and B-cells (produces antibodies) (Fig. 13)

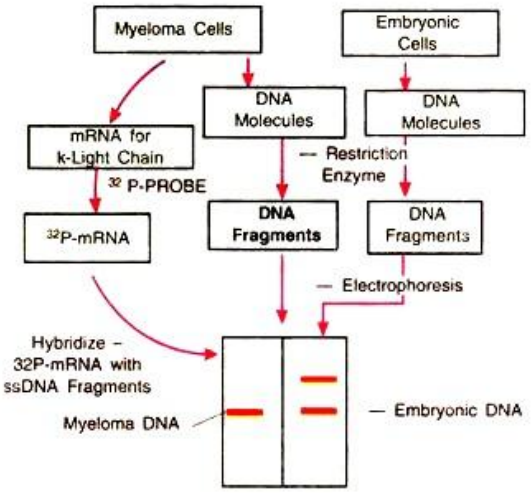

Fig. 22.15: Experimental demonstration for rearrangement of genes encoding k-light chain.

Fig. 13

The DNA of both myeloma cells and embryonic cells was treated with restriction enzymes and subjected to gel electrophoresis. The gel was then sliced; DNA fragments were eluted from the slice, denatured into single stranded DNA and finally incubated with ^{32}p-mRNA encoding K- light chain. The ^{32}p-mRNA probe hybridized with two bands from the germ line embryonic DNA, but with only a single band from the differentiated myeloma DNA. This clearly reveals that in the fully differentiated plasma cells, which is represented by the myeloma cells, the genes for V and C regions had gone rearrangement. Now they are present together on a single restriction DNA fragment, that is why the ^{32}P-mRNA probe hybridized with a single band only.

(ii) Multi-Gene Organization of Immunoglobulin Gene:

The result of Hozumi and Tonegawa (1976) are analogous to the theoretical two gene model of Dryer and Bennett (1965). This provides evidence for organization of multi-gene family into the immunoglobulin gene. In the embryonic cells the DNA encoding C-regions is far away from the DNA that encodes for V-region.

In plasma cells (i.e. cells producing antibodies, also B-cells) and C and V-regions are together (Fig. 22.16). The k and λ light chains and the heavy chains are encoded by separate multi-gene families situated on different chromosome, that contain a series of coding sequences which are known as gene segments. The k and λ light chain families contain L, V, J and C gene segments, whereas the heavy chain family contains L, V, D, S and C gene segments.

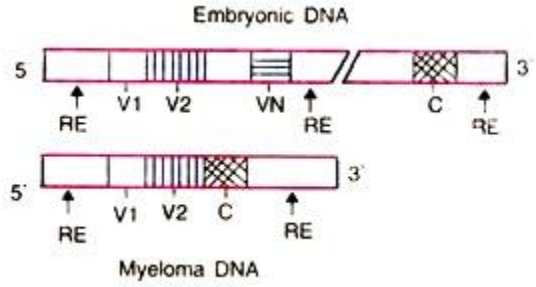

Fig. 14 Arrangement of gene segments of λ-light chain in embryonic and myeloma DNA molecules. RE, restriction enzyme.

During the process of differentiation of B-cells, a long segment of DNA is deleted resulting in close rearrangement of V (i.e. V_2 segment to J-segment). The RNA transcript of immunoglobulin gene that contains intron (non-coding segment within the gene) is processed and correct transcript of mRNA is formed which is translated into a polypeptide light chain. The rearranged VJ-gene segments encode the V-region of the light chain, whereas VDI-gene segments encode the V-region of the heavy chain. The C-segments encode the C-region of the light or heavy chain of the gene segment encodes a short signal sequence.

The signal sequence guides the light or heavy chain through endoplasmic reticulum but is broken before assembly of the immunoglobulin molecule. Therefore, the amino acids that correspond to L-gene segment do not appear in light or heavy chains.

3.5.6 Multi-gene families of λ-chain, K-chain and heavy chain:

For the first time Tonegawa (1983) gave the evidence that V-region of light chain is encoded by two gene segments (V_1 and V_2) by closing the germ-line gene encoding V-region of mouse λ light chain. The complete sequence of nucleotide was determined. When it was compared with known sequences of the λ-chain V-region, a discrepancy was found. In mouse λ-chain multi-gene family contains two V gene segment (V_1 and V_2), four J-gene segments (J_1, J_2, J_3 and J_4) and four C gene segment (C_1, C_2, C_3, C_4). The arrangement of the gene segments is shown in given figure.

The gene segments, J_4 and C_4, are defective, therefore, called pseudogenes. A functional V-region of λ-chain gene consists of two coding segments i.e. exons (a V-gene segment and a J-segment) which are separated by a non-coding sequence (i.e. intron) in un-rearranged germ line DNA. In humans, there are an estimated 100 V-gene segments, 6 J-segment and 6 C-segments. In mouse, the k-chain gene family consists of about 300 V gene segments, five J-segment (one segment is pseudo gene) and a single C-gene segment. Unlike λ chain there are no sub classes of k-light chain as only one C-gene segment is found (Fig. 22.17 B). In humans, the k-chain gene family consists of about 100 V-gene segments, 5J segments and a single C-segment.

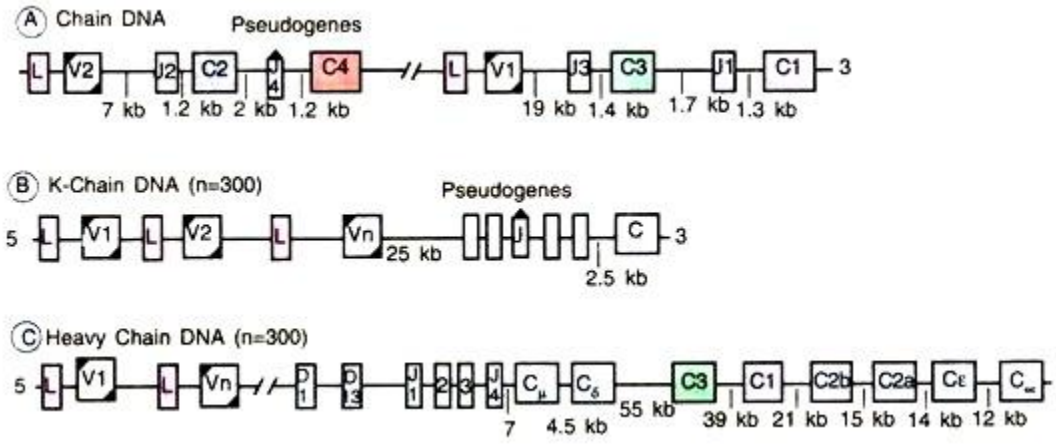

Fig. 15. Germ line organization of λ-light chain (A) , κ-light chain (B), and heavy chain (C) gene segment in the mouse.

Number of Antibodies possible through the Combinatorial Joining of Mouse Germ Line Genes

l light chains	V regions = 2 J regions = 3 Combinations = 2 × 3 = 6
κ light chains	V_κ regions = 250 – 350 Combinations = 250 × 4 = 1,000 = 350 × 4 = 1,400
Heavy chains	V_H = 250 – 1,000 D = 10 – 30 Combinations = 250 × 10 × 4 = 10,000 = 1,000 × 30 × 4 = 120,000
Diversity of antibodies	k-containing : 1,000 × 10,000 = 10^7 1,400 × 120,000 = 2 × 10^8 l-containing 6 × 10,000 = 6 × 10^4 6 × 120,000 = 7 × 10^5

In mouse the heavy chain multi-gene family of immunoglobulin is like λ and k chain but a little complex. It is located on chromosome 12. The heavy chain multi-gene family consists of about 200-1000 V-gene segments, 13 D-gene segments, four J-gene segments and a series of C-gene segments.

Each V-gene segment has a leader sequence a short distance upstream from it. The J-gene segments are located downstream from the D-gene segment. Each C-gene segment encodes the C-region of an immunoglobulin heavy chain iso-type. Similarly each C-gene segment encodes separate domain of the heavy chain C-region. In mouse C-gene segments are arranged in the order Cμ – Cδ – CY3 – CY1 – CY2b – CY2a – Cε – Cα.

3.5.7 Summary

The immune system is composed of a variety of different cell types and proteins. Each element performs a specific task aimed at recognizing and/or reacting against foreign material. The immune system is a wonderful collaboration between cells and proteins that work together to provide defense against infection. These cells and proteins do not form a single organ like the heart or liver. Instead, the immune system is dispersed throughout the body to provide rapid responses to infection. Cells travel through the bloodstream or in specialized vessels called lymphatics. Lymph nodes and the spleen provide structures that facilitate cell-to-cell communication.

The bone marrow and thymus represent training grounds for two cells of the immune system (B-cells and T-cells, respectively). The development of all cells of the immune system begins in the bone marrow with a hematopoietic (blood-forming) stem cell (*Figure 2*). This cell is called a "stem" cell because all the other specialized cells arise from it. Because of its ability to generate an entire immune system, this is the cell that is most important in a bone marrow or hematopoietic stem cell transplant. It is related to embryonic stem cells, but is a distinct cell type. In most cases, development of one cell type is independent of the other cell types.

Antigen processing, or the cytosolic pathway, is an immunological process that prepares antigens for presentation to special cells of the immune system called T lymphocytes. It is considered to be a stage of antigen presentation pathways. This process involves two distinct pathways for processing of antigens from an organism's own (self) proteins or intracellular pathogens (e.g. viruses), or from phagocytosed pathogens (e.g. bacteria); subsequent presentation of these antigens on class I or class II major histocompatibility complex (MHC) molecules is dependent on which pathway is used.

Both MHC class I and II are required to bind antigen before they are stably expressed on a cell surface. MHC I antigen presentation typically (considering cross-presentation) involves the endogenous pathway of antigen processing, and MHC II antigen presentation involves the exogenous pathway of antigen processing. Cross-presentation involves parts of the exogenous and the endogenous pathways but ultimately involves the latter portion of the endogenous pathway (e.g. proteolysis of antigens for binding to MHC I molecules).

3.5.8 Terminal questions

Q.1. Explain different cells of the immune system.

Answer:----------

Q.2. Write a short note on phagocytes, monocytes and macrophages.

Answer:----------

Q.3. Describe macrophages found in various body tissues.

Answer:----------

Q.4. Describe lymphocyte and their types.

Answer:----------

Q.5. Explain thymus and tonsils in brief.

Answer:----------

Q.6. Explain spleen and lymph nodes.

Answer:----------

Q.7. Explain antigen presenting cells (APCs) and dendritic cells.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-4: Antigen processing and presentation

Structure

4.1. Introduction

In order to be capable of engaging the key elements of adaptive immunity (specificity, memory, diversity, self/nonself discrimination), antigens have to be processed and presented to immune cells. Foreign antigens must be specially processed before they can be presented to the antigen-sensitive cells of immune system. Before discussing about the antigen processing and presentation we need to understand about the nature and types of antigens which are involved in generating immune responses.

4.2 Antigen

An antigen is defined as an organism, a molecule, or part of a molecule or substance which may be self or non-self, can evoke noticeable immune response and can bound distinctively with antibodies. Antigens are macromolecules that elicit an immune response in the body Antigens, which are able to induce adaptive immunity, are called immunogens. All immunogens are antigens unless their ability to stimulate an immune response is significant.

4.2.1 Nature of Antigens

Antigen may be a chemical substance like a protein or a polysaccharide. It may be a biological entity like, Bactria, bacterial products, fungi, parasites, viruses, different microbes, or larger parasites.. Antigens can be -proteins, polysaccharides, conjugates of lipids with proteins (lipoproteins) and polysaccharides (glycolipids). Besides these, different biological products, milk, egg albumin, bee venom, snake venom, pollen grains may be a good source of antigen. Different parts of bacterial cells like flagella, pili, lipopolysaccharides of outer membrane of Gram- negative bacteria, the capsular polysaccharides of the cell membrane, cytoplasmic proteins, exotoxins, endotoxins etc. can have antigenic property and can evoke immune response against them. Sometimes (normally very rare); self-protein can be recognized as

non-self by body and can be treated as foreign substances against which body will take necessary steps to control the annomeles (called as auto-immune disease).

4.2.2 Types of Antigens

The antigens that trigger an immune response are of two types.

(1) Exogenous and

(2) Endogenous antigens.

4.2.2.1 Exogenous antigens

Exogenous antigens are those antigens which enter within the host body from their surroundings or external environments. These are basically of pollutants, microorganisms, pollens, drugs etc. Different infectious diseases, are caused by these type of introduced or foreign external agents are normally called communicable diseases, e.g., influenza virus, malarial protozoa etc.

4.2.2.2 Endogenous antigens

These types of antigens are located within the individual itself. These are actually made within the body of the host.Thus if a virus invades a cell and takes over its biosynthetic rocesses,then new viral riteins are formed within the infected cells. These antigens are further classified into three sub-categories named as:

- Xeno-genic or Heterogenic antigens
- Allogenic or Idiotypic antigens
- Autologous antigens.

4.2.2.2.1 Xeno-genic Antigens

Usually these are also called heterogenic antigens as they are related with phylogenetically unrelated species. These are related with tissue transplantation and serology.

4.2.2.2.2 Allogenic antigens

These are those antigens which are genetically determined, polymorphic in nature and help to differentiate one individual of one species from another individual belonging to the same species. When an individual (recipient) receives a blood transfusion or undergoes transplantation operation (like plastic surgery, kidney etc.). These phenomena lead to

incompatibility, agglutination and graft rejection. In case of human beings these types of antigenic determinants are located on erythrocytes, leukocytes, platelets, cell surface markers, serum proteins and histocompatibility antigens.

4.2.2.2.3 Autologous Antigens

This group of antigens is very rare and unnatural. In normal condition, self-components are non-immunogenic in nature, but in an abnormal condition self-body components are started to be considered as non-self or antigenic component.

4.3 Antigen Processing

Antigen processing, or the cytosolic pathway, is an immunological process that prepares antigens for presentation to special cells of the immune system called T lymphocytes. It is considered to be a stage of antigen presentation pathways. A foreign protein (antigen) to be recognized by a T-cell must be degraded into small antigenic peptides that form physical complex with Class I or Class II Major Histocompatibility Complex (MHC) molecules. This conversion of proteins into MHC associated peptide fragments is called antigen processing and presentation.

4.3.1 Antigen-Presenting Cells (APCs)

All nucleated cells in the body have mechanisms for processing and presenting antigens in association with MHC molecules. This signals the immune system, indicating whether the cell is normal and healthy or infected with an intracellular pathogen. However, only macrophages, dendritic cells, and B cells have the ability to present antigens specifically for the purpose of activating T cells; for this reason, these types of cells are sometimes referred to as antigen-presenting cells (APCs)**.**

While all APCs play a similar role in adaptive immunity, there are some important differences to consider. Macrophages and dendritic cells are **phagocytes** that ingest and kill pathogens that penetrate the first-line barriers (i.e., skin and mucous membranes). B cells, on the other hand, do not function as phagocytes but play a primary role in the production and secretion of antibodies. In addition, whereas macrophages and dendritic cells recognize pathogens through nonspecific receptor interactions (e.g., **PAMPs**, **toll-like receptors**, and receptors for opsonizing **complement** or antibody), B cells interact with foreign pathogens or their free antigens using antigen-specific immunoglobulin as receptors (monomeric **IgD** and **IgM**).

When the immunoglobulin receptors bind to an antigen, the B cell internalizes the antigen by endocytosis before processing and presenting the antigen to T cells.

Antigen presentation is mediated by **MHC class I molecules**, and the **class II molecules** found on the surface of **antigen-presenting cells** (APCs) and certain other cells. MHC class I and class II molecules are similar in function: they deliver short peptides to the cell surface allowing these peptides to be recognised by **CD8+** (cytotoxic) and **CD4+** (helper) T cells, respectively. The difference is that the peptides originate from different sources – endogenous, or intracellular**,** for MHC class I; and exogenous, or extracellular for MHC class II. There is also so called cross-presentation in which exogenous antigens can be presented by MHC class I molecules. Endogenous antigens can also be presented by MHC class II when they are degraded through autophagy.

4.3.2 Endogenous Pathway or MHC Class I Antigen Presentation Pathway

MHC I molecules, found on all normal, healthy, **nucleated cells**, signal to the immune system that the cell is a normal "self" cell. In a healthy cell, proteins normally found in the cytoplasm are degraded by **proteasomes** (enzyme complexes responsible for degradation and processing of proteins) and processed into **self-antigen epitopes**; these self-antigen epitopes bind within the MHC I antigen-binding cleft and are then presented on the cell surface. Immune cells, such as NK cells, recognize these self-antigens and do not target the cell for destruction. However, if a cell becomes infected with an intracellular pathogen (e.g., a virus), protein antigens specific to the pathogen are processed in the proteasomes and bind with **MHC I** molecules for presentation on the cell surface. This presentation of pathogen-specific antigens with MHC I signals that the infected cell must be targeted for destruction along with the pathogen.

Before elimination of infected cells can begin, APCs must first activate the T cells involved in cellular immunity. If an intracellular pathogen directly infects the cytoplasm of an APC, then the processing and presentation of antigens can occur as described (in proteasomes and on the cell surface with MHC I).

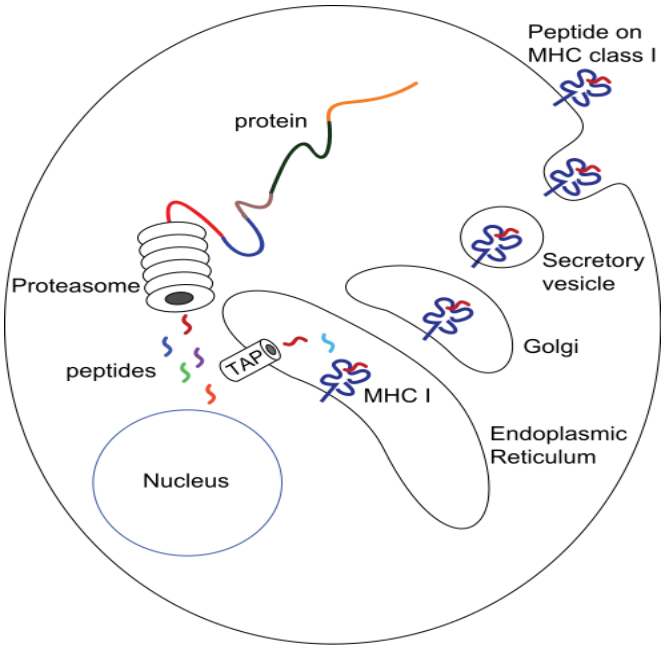

Fig.1 MHC Class I Antigen **P**resentation **P**athway

4.3.3 Exogenous Pathway or MHC Class II Antigen Presentation Pathway

MHC II molecules are only found on the surface of APCs. Macrophages and dendritic cells use similar mechanisms for processing and presentation of antigens and their epitopes in association with MHC II; B cells use somewhat different mechanisms that will be described further in **B Lymphocytes and Humoral Immunity.** For now, we will focus on the steps of the process as they pertain to dendritic cells.

After a dendritic cell recognizes and attaches to a pathogen cell, the pathogen is internalized by phagocytosis and is initially contained within a **phagosome**. Lysosomes containing antimicrobial enzymes and chemicals fuse with the phagosome to create a phagolysosome, where degradation of the pathogen for antigen processing begins. Proteases (protein-degrading) are especially important in antigen processing because only protein antigen epitopes are presented to T cells by MHC II. APCs do not present all possible epitopes to T cells; only a selection

APCs do not present all possible epitopes to T cells; only a selection of the most antigenic or **immunodominant** epitopes are presented. The mechanism by which epitopes are selected

for processing and presentation by an APC is complicated and not well understood; however, once the most antigenic, immunodominant epitopes have been processed, they associate within the antigen-binding cleft of MHC II molecules and are translocated to the cell surface of the dendritic cell for presentation to T cells.

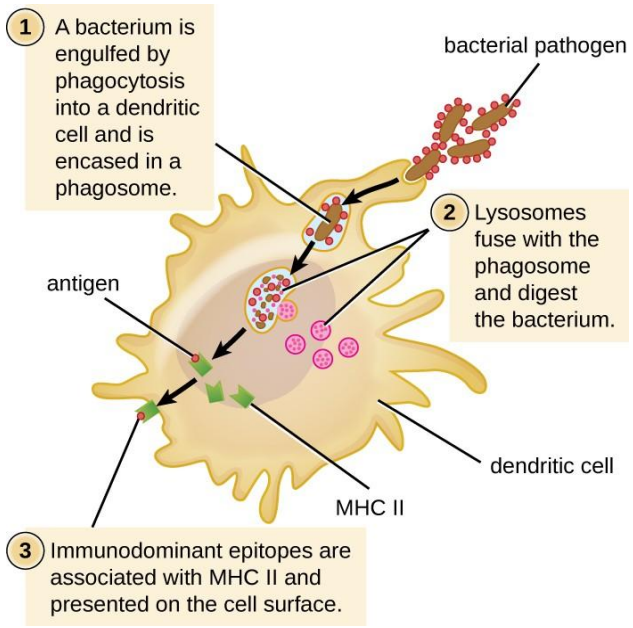

Fig. 2 MHC Class II Antigen **P**resentation **P**athway

4.3.4 Cross Presentation Pathway

If the intracellular pathogen does not directly infect APCs, an alternative strategy called **cross-presentation** is utilized. In cross-presentation, antigens are brought into the APC by mechanisms normally leading to presentation with MHC II (i.e., through phagocytosis), but the antigen is presented on an MHC I molecule for CD8 T cells. The exact mechanisms by which cross-presentation occur are not yet well understood, but it appears that cross-presentation is primarily a function of **dendritic cells** and not macrophages or B cells. Not all antigen-presenting cells utilize cross-presentation.

4.3.5 Effector Responses of Cell Mediated and Humoral Immunity

4.3.5.1 Cell Mediated Immune Responses

The cell-mediated immune system is the host's primary response against invasive bacteria and viruses that cause intracellular infections. It is also essential for fighting against and destroying cancer cells. Furthermore, the cell-mediated immune system plays a role in the

rejection of organ transplants or graft tissue. Cell mediated immune response is carried out by the T-cells or T lymphocytes. So, it is also called T-cell immunity. This type of immune response is to defend against pathogens that may invade host cells. The surface of the T-cell has receptor molecule that can bind with antigens. These receptor molecules are made of a variable unit similar to the variable portion of the humoral antibody. A single T-cell has about 100,000 receptor sites.

The precursors of T-lymphocytes produced by stem cells of bone marrow pass through liver and spleen before reaching the thymus where they are processed, hence called thymus-dependent (T) lymphocytes. These lymphocytes come under the influence of the hormone "thymosin" and become immunologically competent and are called lymplioblasts. When stimulated by an appropriate antigen, the lymphoblasts divide and differentiate into cytotoxic T-lymphocyte (killer T-Iymphocytes), helper T-cells, and suppressor T-cells.

Cell-mediated immunity is an immune response that does not involve antibodies, but rather involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. The principal role of cell-mediated immunity is to detect and eliminate cells that harbour intracellular pathogens. The antigen-specific cells contribute to the cell-mediated immunity include CD8+ cytotoxic T lymphocytes (CTLs), and cytokine secreting CD4+ TH (T helper cells)cells that mediate delayed type hypersensitivity. Effector T cells generated in response to antigen are responsible for cell-mediated immunity. Both activated T helper cells and cytotoxic T lymphocytes (CTLs) play effector cells in cell-mediated immune reactions.

Cytokines secreted by T helper cells can activate phagocytic cells and enabling them to phagocytose and kill microorganisms more effectively. The antigen-nonspcific cells that contribute to the cell-mediated immunity include NK cells, macrophages, neutrophills and eosinophils. T cells, NK cells and macrophages are the most important sources of the cytokines that organize and support cell-mediated immunity. Cell-mediated immunity can recognize and eliminate tumour cells that have undergone genetic modifications so that they express antigens not typical of normal cells. Though humoral and cell-mediated immunity have many distinctive features, they are not completely independent. Macrophages, NK cells, neutrophils and eosinophils can use antibodies as receptors for killing target cells. Activation

of complement system by antigen-antibody complexes gives rise to chemotactic peptides which help in assembling the cell types required for a cell-mediated response.

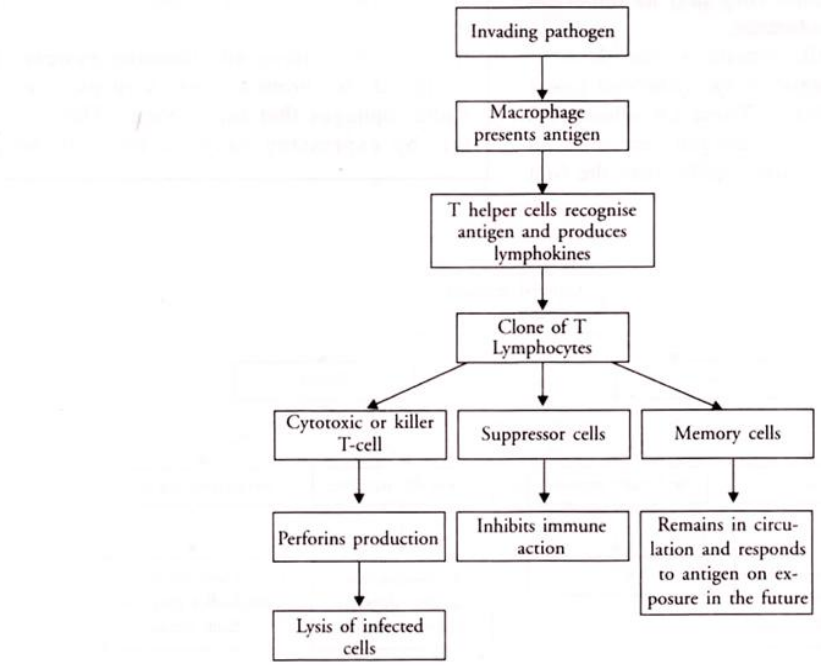

Fig. 3 Flow chart of Cell Mediated Immune Response

When an antigen enters the body, the macrophages first attack the antigen and fragment it into pieces. It then presents a piece of antigen to the T-helper cells. The T helper cells recognize the antigen and trigger off a series of cell mediated response. A clone of T-lymphocytes is first formed after being activated by the T-helper cells. There are different kinds of T-cells, which are morphologically similar but differ functionally. The cytotoxic T-lymphocytes possess specific cell surface proteins, called T-cell receptors, on their surface and respond to only major histocompatibility complex antigens (MHC-antigens) bound to the surface of other cells. After the interaction between T-cell receptor and MHC-antigen is established and the cytotoxic T-lymphocyte cells binds the MHC-antigen containing cell, the latter undergoes lysis and is phagocytised.

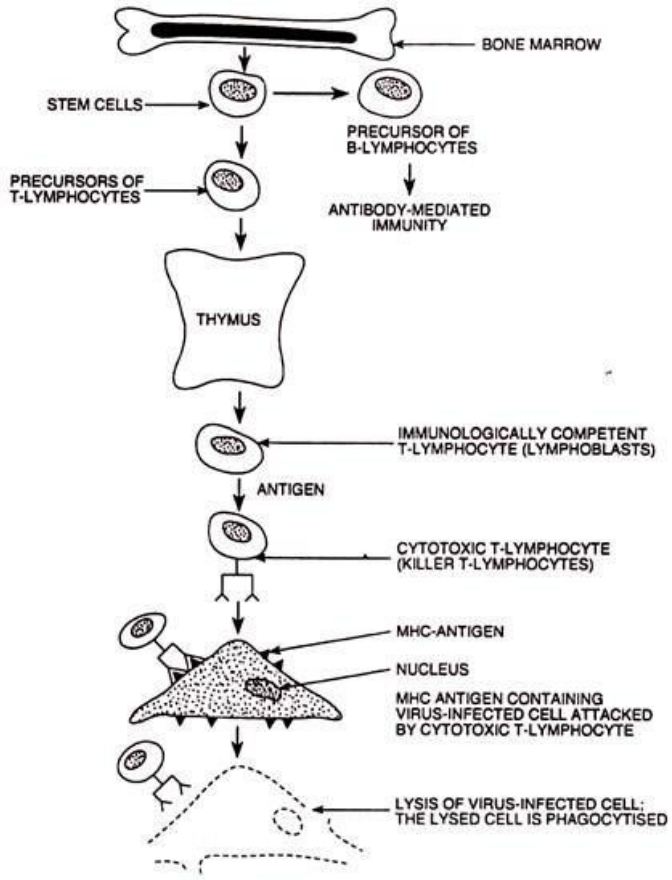

Fig. 4 Cell Mediated Immune Response

The cell-mediated immunity is important in controlling those infections where the pathogens are intracellular and reproduce within the infected cells (e.g., viruses, rickettsia, chlamydia, some protozoans like Trypanosomes, etc.)

4.3.5.1.1 The actions of the different types of T-cell are summarized below

a. Helper cells react by producing small peptide molecules called lymphokines. The lymphokines promote proliferation of more T-cells, stimulate B cells to produce antibodies and also help in accumulating macrophages in the inflamed tissues and by promoting phagocytosis.
b. Cytotoxic cells or Killer cells kill cells infected by viruses, cancerous cells and transplants.
c. Suppressor cells, the third type of T-cells produce lymphokines that suppress the action of the phagocytes and the different types of WBC cells. They play an important role in immuno tolerance.

d. Some of the cells remain as the memory cells that get lodged in the lymphoid tissue throughout the body. These on subsequent exposure to the same antigen can cause an immune response more rapidly than the first exposure.

4.3.6 Humoral Immune Response

The humoral immune response, also known as the antibody-mediated immune response, targets pathogens circulating in "humors," or extracellular fluids, such as blood and lymph. Antibodies target invading pathogens for destruction via multiple defense mechanisms, including neutralization, opsonization, and activation of the complement system. Patients that are impaired in the production of antibodies suffer from severe and frequent infections by common pathogens and unusual pathogens.

4.3.6.1 B Cells Are Produced by the Bone Marrow and Circulate through Body Fluids

B lymphocytes, also called B cells, detect pathogens in the blood or lymph system. Although B cells originate in the bone marrow, their name is derived from a specialized organ in birds in which B cells were first discovered, the bursa of Fabricius. After release from the bone marrow, B cells mature in secondary lymphoid tissues, such as the spleen, lymph nodes, tonsils and mucosa-associated lymphoid tissue throughout the body.

4.3.6.2 B Cells Differentiate into Antibody Releasing Plasma Cells and Memory B Cells

B cells bind to specific parts of a pathogen, called antigens, via their B cell receptors. In addition to antigen binding, B cells require a second signal for activation. This signal can be provided by helper T cells or, in some cases, by the antigen itself. When both stimuli are present, B cells form germinal centers, where they proliferate into plasma cells and memory B cells. All cells that are derived from a common ancestral B cell (monoclonal) respond to the same antigen. Each plasma cell secretes genetically identical antibodies that circulate in the bloodstream. Memory B cells produce antibodies that are bound to the cell's surface and are highly specific against the antigen that initially led to the production of the memory B cell. Memory B cells are long-lived and enable the organism to react much faster and stronger upon secondary exposure to the same pathogen.

4.3.7 Antibodies Kill Pathogens in Diverse Ways

Antibodies bind to antigens that they encounter in body fluids. The resulting antibody-antigen complex activates three major defense mechanisms: neutralization, opsonization and the complement system.

4.3.7.1 Neutralization: Antibodies "neutralize" a pathogen by interfering with its ability to infect host cells. For example, when an antibody binds to the surface of a virus, it may impair the ability of the virus to attach to or gain entry into target cells, effectively inhibiting the infection.

4.3.7.2 Opsonization: Antibodies function as opsonins, which "tag" pathogens for destruction. Specifically, the formation of the antigen-antibody complex attracts and stimulates phagocytic cells that engulf and destroy the pathogen.

4.3.7.3 Complement: Antibodies can activate the complement system, which plays a role in both innate and adaptive immunity. The complement system is a sequential cascade of more than 30 proteins. With the help of antibodies, these proteins opsonize pathogens for destruction by macrophages and neutrophils, induce an inflammatory response with the recruitment of additional immune cells, and promote lysis (destruction) of the pathogen. Most defenses that are mediated by antibody present in the plasma, lymph and tissue fluids are called humoral immune responses. It protects against extra-cellular bacteria and foreign macromolecules. Transfer of antibodies confers this type of immunity on the recipient. Humoral immune responses have an activation phase and an effector phase.

These phases occur as follows

1. The antigen is taken up by phagocytosis and degraded in a lysosome in an APC, such as a macrophage.

2. A T-cell receptor recognizes processed antigen bound to a class II MHC protein on the macrophage.

3. Cytokines released by the T_H cell and IL-1 released by macrophage stimulate the T_H cell to produce a clone of differentiated cells capable of interacting with B-cells. Activation phase occurs in lymphatic tissue.

4. B-cells are also antigen presenting cells. Binding of antigen to a specific IgM receptor triggers receptor mediated endocytosis, degradation and display of the processed antigen on class II MHC proteins.

5. When a T_H cell receptor binds to the displayed antigen—MHC II complex on the B cell, it releases cytokines.

6. These cytokines cause the B-cell to produce a clone of B-cells.

7. Now, these B-cells produce antibody secreting plasma cells.

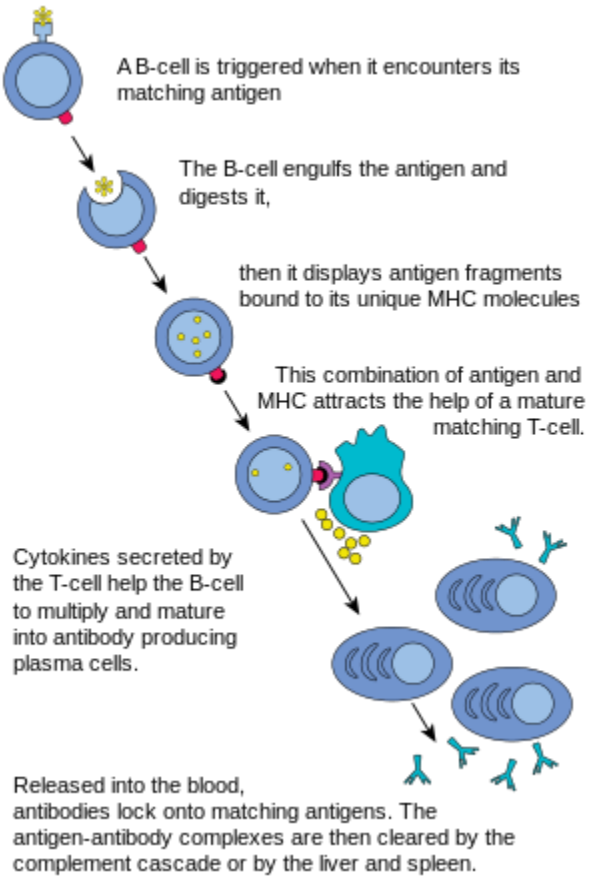

Fig. 5 Humoral Immune Responses

4.3.8 Cytokines

Cytokines are low molecular weight regulatory proteins secreted by cells of innate and adaptive immunity in response to microbes and other antigens. Cytokines regulate the intensity and duration of the immune response by stimulating or inhibiting the activation, proliferation and/or differentiation of various cells and by regulating the secretion of antibodies and other cytokines. Thus they act as messengers of the immune system just as hormones serve as messengers of endocrine system.

4.3.8.1 Nomenclature of Cytokines

The nomenclature of cytokines is often based on their cellular sources. Cytokines that are secreted by mononuclear phagocytes were formerly known as monokines, and those produced by lymphocytes were called lymphokines. With the development of anti-cytokine antibodies and molecular probes, it became clear that secretion of many lymphokines and monokines is not limited to lymphocytes and monocytes as these terms imply, but extends to a broad spectrum of cells and types. For this reason, the more inclusive term cytokine is preferred for all such molecules.

Many of the cytokines are referred to as interleukins because these are secreted by leucocytes and act on other leucocytes. This term is also imperfect because many cytokines that are synthesized only by leucocytes and that act only on leucocytes are not called interleukines. For historical reasons, many cytokines are called interleukines but these are made by and act on cells other than leukocytes. Nevertheless, the term has been useful because as new cytokines are molecularly characterized. They are assigned as interleukins (IL) number (e.g., IL-1, IL-2, IL-3 and so on) to maintain a standard nomenclature.

4.3.8.2 General Characteristics of Cytokines

4.3.8.2.1 Cytokines bind to specific receptors on target cell membrane

All cytokines initiate their action by binding to specific membrane receptors on target cells. Binding with receptors triggers the signal transduction pathways that ultimately alter gene expression in the target cells. Cytokines and their receptors show very high affinity for each other, with dissociation constant ranging from 10^{-10} to 10^{-12}M. As a consequence, only very little quantities of cytokine are required to elicit a biological effect.

4.3.8.2.2 Cytokine secretion is a brief, self- limited event

The synthesis of cytokines is transient, i.e., their synthesis is initiated by new gene transcription as a result of cellular activation. Such transcriptional activation is transient as the mRNA encoding most cytokines are unstable. Once synthesised, cytokines are rapidly secreted but never remain stored as preformed molecules.

4.3.8.2.3 The action of cytokines is pleiotropic and redundant

A given cytokine may show different biological effects on different target cells – it is the pleiotropic action of cytokines. On the other hand, redundancy refers to the property of multiple cytokines having the same functional effects. Because of this property, mutation of one cytokine gene may not have functional consequences, as other cytokine may compensate its effect.

4.3.8.2.4 Cytokines show synergy, antagonism and cascade induction

Cytokine synergism occurs when the combined effect of two cytokines on cellular activity is greater than that of the additive effects of the individual cytokine. In some cases, cytokines show antagonism that is, the effect of one cytokine inhibits or offset the effects of another cytokine. Cascade induction occurs when the action of one cytokine on a target cell induces that cell to produce one or more other cytokines which in turn may induce other target cells to produce other cytokines.

4.3.8.2.5 Cytokines exert autocrine, paracrine and endocrine action

A particular cytokine may act on the same cell that secreted it, exerting autocrine action; it may act on a target cell in close proximity to the producer cell, exerting its paracrine action. In some cases, it may bind to target cells in distant part of the body, exerting its endocrine effect.

4.3.9 Structure of Cytokines

1. Cytokines are proteins or glycoproteins which normally have a molecular mass of less than 30 KDa.

2. Structural predictions suggest that cytokines belong to haematopoietins, included in the haematopoietin family of interleukins (2-7, 9, 11-13 and 15) GM-CSF, G-GSF, leukomia-inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) [confirmed by X-ray crystallographic sequence analysis].

3. The amino acid sequences of the various haematopoietins differ accordingly—all of them have a high-degree of α-helical structure and little or no β-sheet structure.

4.3.9.1 Cytokine Receptors

The cytokine receptors are trans membrane proteins with extracellular portions that bind with cytokines and cytoplasmic portions that are responsible for initiating intracellular signaling pathways. On the basis of homologies among the extracellular cytokine binding domains, cytokine receptors are classified into five families:

(i) Immunoglobulin super family receptors
(ii) Class I cytokine receptor family (also known as haematoprotein receptor family)
(iii) Class II cytokine receptor family (also known as the interferon receptor family)
(iv) TNF-receptor family
(v) Chemokine receptor family.

4.3.9.2 Functional Categories of Cytokines:

Cytokines may be classified into three main functional categories based on their principal biologic actions.

4.3.9.2.1 Cytokines acting as mediators and regulators of innate immunity

Different cytokines that regulate and mediate innate immunity are produced by mononuclear phagocytes in response to microbial agents such as lipopolysaccharide of bacteria, double stranded-RNA of virus etc. These cytokines act mainly on endothelial cells and leucocytes to stimulate early inflammatory reactions to microbes, and some functions to control these responses. Examples of such cytokines, their sources and biological actions are tabulated in the given table.

Table 6.16 : Cytokines of innate immunity

Cytokine	Size	Principal cell sources	Principal cell targets and biological effects
Tumor necrosis factor (TNF)	17 kD; 51 kD homotrimer	Macrophages, T cells	Endothelial cells : activation (inflammation, coagulation) Neutrophils : activation Hypothalamus : fever Liver : synthesis of acute-phase proteins Muscle fat : catabolism (cachexia) Many cell types : apoptosis
Interleukin-1 (IL-1)	17 kD mature form, 33 kD precursors	Macrophages, endothelial cells, some epithelial cells	Endothelial cells : activation (inflammation, coagulation) Hypothalamus : fever Liver : synthesis of acute-phase proteins
Chemokines	8-12 kD	Macrophages, endothelial cells, T cells, fibroblasts, platelets	Leukocytes : chemotaxis, activation; migration into tissues
Interleukin-12 (IL-12)	Heterodimer of 35 kD + 40 kD subunits	Macrophages, dendritic cells	T cells : T_H1 differentiation NK cells and T cells : IFN-γ synthesis, increased cytolytic activity
Type I IFNs (IFN-α, IFN-β)	IFN-α : 15-21 kD IFN-β : 20-25 kD	IFN-α : macrophages IFN-β : fibroblasts	All cells : antiviral state, increased class I MHC expression NK cells : activation
Interleukin-10 (IL-10)	Homodimer of 34-40 kD; 18 kD subunits	Macrophages, T cells (mainly T_H2)	Macrophages, dendritic cells : inhibition of IL-12 production and expression of co-stimulators and class II MHC molecules
Interleukin-6 (IL-6)	19-26 kD	Macrophages, endothelial cells (mainly T_H2)	Liver : synthesis of acute-phase proteins B cells : proliferation of antibody-producing cells
Interleukin-15 (IL-15)	13 kD	Macrophages, others	NK cells : proliferation T cells : proliferation (memory CD^+ cells)
Interleukin-18 (IL-18)	17 kD	Macrophages	NK cells and T cells : IFN-γ synthesis

4.3.9.2.2 Cytokines acting as mediators and regulators of adaptive immunity:

These cytokines are mainly produced by T lymphocytes in response to specific recognition of foreign antigens. Many of such cytokines regulate the growth and differentiation of various lymphocytes. Thus, they stimulate the activation phase of T-cell dependent immune responses. Other cytokines may recruit, activate and regulate specialized effector cells like mononuclear phagocytes, neutrophils and eosinophils to eliminate antigens in the effector phase of adaptive immune responses. For examples of such cytokines as given table.

4.4.0 Cytokines of adaptive immunity

Cytokine	Size	Principal cell sources	Principal cell targets and biological effects
Interleukin-2 (IL-2)	14-17 kD	T cells	T cells : proliferation, increased cytokine synthesis; potentiates Fas-mediated apoptosis NK cells : proliferation, activation B cells : proliferation, antibody synthesis (*in vitro*)
Interleukin-4 (IL-4)	18 kD	$CD4^+$ T cells (T_H2), mast cells	B cells : isotype switching to IgE T cells : T_H2 differentiation, proliferation Macrophages : inhibition of IFN-γ mediated activation Mast cells : proliferation (*in vitro*)
Interleukin-5 (IL-5)	45-50 kD; homodimer of 20 kD subunits	$CD4^+$ T cells (T_H2)	Eosinophils : activation increased production B cells : proliferation, IgA production
Interferon-γ (IFN-γ)	50 kD (glycosylated); homodimer of 21 to 24 kD subunits	T cells (T_H1,$CD8^+$ T cells), NK cells	Macrophages : activation (increased microbial functions) B cells : isotype switching to opsonizing and complement-fixing IgG subclasses T cells : T_H1 differentiation Various cells : increased expression of class I and class II MHC molecules, increased antigen processing and presentation to T cells
Transforming growth factor-β (TGF-β)	25 kD homodimer of 12.5 kD subunits	T cells, macrophages, other cell types	T cells : inhibition of proliferation and effector functions B cells : inhibition of proliferation; IgA production Macrophages : inhibition
Lymphotoxin (LT)	21-24 kD secreted as homotrimer or associated with $LT\beta_2$ on the cell membrane	T cells	Recruitment and activation of neutrophils lymphoid organogenesis
Interleukin-13 (IL-13)	15 kD	$CD4^+$ T cells (T_H2)	B cells : isotype switching to IgE Epithelial cells : increased mucus production Macrophages : inhibition

4.4.0.1 Cytokines acting as stimulators of haematopoiesis

Cytokines stimulating haemotopoiesis are produced by bone marrow stromal cells, leucocytes and other cells. These cause stimulation of growth and differentiation of immature leucocytes.

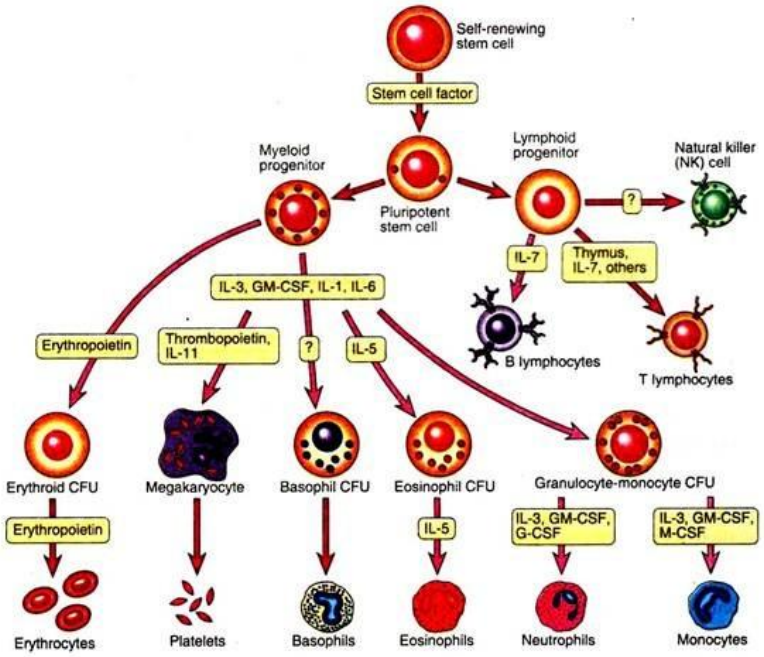

Fig. 6 Role of Cytokines in haematopoiesis

In general, the cytokines of innate and adaptive immunities are produced by different cell populations and act on different target cells. A comparative account of these cytokines is represented in the above table. However, these distinctions are not absolute because same cytokines may be produced during both innate and adaptive immune reactions. Moreover, different cytokines produced during such reactions may have overlapping actions.

4.4.0.2 Cytokine-Related Diseases

Defects in the complex regulatory networks controlling the expression of cytokines and cytokine receptors may lead to various abnormal conditions. Both over-expression and under-expression of cytokines and their receptors may result in several diseases.

4.4.0.3 Bacterial septic shock

When bacterial cell-wall cytotoxins stimulate macrophages to overproduce IL-1 and TNF-α, bacterial septic shock may develop. The symptoms of bacterial septic shock, which is very often fatal, include a drop in blood pressure, fever, diarrhoea and blood clotting in various organs. Higher levels of TNF-α were found in patients who died of meningitis than in those who recovered.

4.4.0.4 Bacterial toxic shock:

Many microorganisms are known to produce toxins that act as super-antigens which, unlike the antigens, are not internalized, processed and presented by antigen presenting cells. The super-antigens can activate large number of T cells to produce excessive amount of cytokines. These elevated cytokines, in turn, can induce systemic reactions that include fever, widespread blood clotting and shock.

4.4.0.5 Lymphoid and myeloid cancers

Development of some types of cancer is associated with the abnormal production of cytokines. For example, excessive high levels of IL-6 are secreted by cardiac myxoma cells, myeloma and plasma cytoma cells and cervical and bladder cancer cells. In myeloma cells, IL-6 acts in an autocrine manner to stimulate cell proliferation. Monoclonal antibodies to IL-6 if added to in vitro cultures of myeloma cells, their growth was found to be inhibited.

4.4.0.6 Chagas' disease

Chagas' disease is caused by the protozoan parasite Trypanosoma cruzi and is characterised by severe immuno suppression. It has been found that in presence of T. cruzi, T cells show a dramatic reduction in the expression of a subunit of IL-2 receptors, resulting in their inactivation of T cells with many antigens. These, in turn, lead to immunosuppression in patients with Chagas' disease.

4.4.0.7 Therapeutic Uses of Cytokines and their Receptors

From the above discussion it appeared that most cytokines are powerful mediators of innate and adaptive immunities. Now a days, a number of cytokines and soluble cytokine receptors have been purified and cloned. Many of such cytokines notably, Interferons and colony-stimulating factors, such as GM-CSF have proven to be therapeutically very useful.

4.4.1 Interferons

Different types of interferons have antiviral activity and various other effects including the capacity to induce cell differentiation, to inhibit proliferation by some cell types, to inhibit angiogenesis and to function in various immunoregulatory roles.

Some examples include:

(i) INF-α (trade name Roferon and Intron- A) has been used for the treatment of hepatitis C, hepatitis B and many types of cancer. Chronic myelogenous leukemia, Kaposi's sarcoma, non-Hodgkins lymphoma, cutaneous T-cell lymphoma and multiple myeloma etc. respond well to treatment with IFN-α.

(ii) INF-β. In the autoimmune neurologic disease multiple sclerosis (MS), a progressive neurologic dysfunction occurs. Treatment of that patient with IFN-p provides longer period of remission and reduces the severity of relapses.

(iii) IFN-y has found to be effective for the treatment of a rare hereditary disease, chronic granulomatous disease (CGD) where the patients' phagocytic cells are seriously impaired to kill the ingested microbes. Therapy of CGD patients with IFN-y significantly reduces the incident of infections. The use of interferons in clinical practice is likely to expand, particularly as more is learned about their effects in combination with other therapeutic agents. Although, a number of factors are likely to raise difficulties in adapting cytokines for safe and effective routine medical use.

These include the need to maintain appropriate dose levels, to formulate effective combination of cytokines with other drugs or cytokines, and in some cases, to control cytokines side effects or toxicity. However, some cytokine-related therapies that either decrease or increase the immune response still offer promise of reducing graft rejection, treating certain cancers and immunodeficiency diseases, and reducing allergic reactions.

4.4.2 Complement System

The complement system is a part of the immune system which consists of a series of serum proteins that interact with one another in order to eliminate pathogens. It helps antibodies and phagocytic cells to clear pathogens and damaged cells; promote inflammation and attack pathogen's plasma membrane. Proteins that take part in the complement system are called complements that collectively work as a **biological cascade**; the sequence of reactions, each being the catalyst for the next. The complement system refers to a group of plasma proteins called the complement proteins, which are produced in the liver, and act collectively to help

destroy pathogens. The complement system is so named because it is complementary to the antibody response of the adaptive immune system.

4.4.2.1 Components of Complement System

An array of approximately 20 types of soluble proteins, called a **complement**, functions to destroy extracellular pathogens. Complement was discovered by **Jules Bordet** (1895) as a heat-labile component of normal plasma that causes the **opsonisation** and **killing of bacteria**. Cells of the liver and macrophages synthesize complement proteins continuously; these proteins are abundant in the blood serum and are capable of responding immediately to infecting microorganisms. Complement is a chain of enzymes whose activation eventually results in the disruption of cell membranes and the destruction of cells or invading microorganisms. Complement is an essential part of the body defense system. Most of the proteins are normally inactive, but in response to the recognition of molecular components of microorganisms they become sequentially activated in an enzyme cascade – the activation of one protein enzymatically cleaves and activates the next protein in the cascade.

Complement can be activated via three different pathways, which can each cause the activation of **C3**, cleaving it into a large fragment, **C3b**, that acts as an **opsonin**, and a small fragment **C3a** (anaphylatoxin) that promotes inflammation. Activated C3 can trigger the **lytic pathway**, which can damage the plasma membranes of cells and some bacteria. C5a, produced by this process, attracts **macrophages** and **neutrophils** and also activates **mast cells**. Different pathways of complement finally generate a macro-molecular membrane-attack complex (MAC) which helps to lyse a variety of cells, bacteria and viruses.

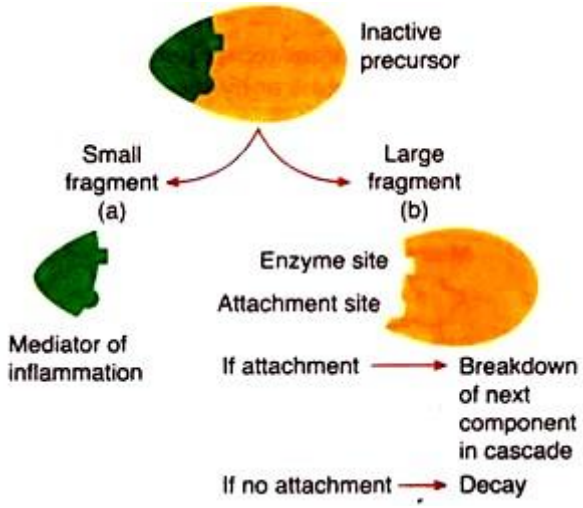

Fig. 7 Cleavage of Complement system

The complement products amplify the initial antigen-antibody reaction and convert that reaction into a more effective defense mechanism. Continuous proteolytic cleavage and activation of successive complement proteins lead to the covalent bonding or fixing of complement fragments to the pathogen surface. Each precursor of complement is cleaved into two major fragments- named as larger fragment (designated as 'b') and smaller fragment (designated as 'a'). The major or larger 'b' fragment has two biologically active sites—one binds to cell membranes to the target cell towards the site of activation and the other for enzymatic cleavage of the next complement component. The smaller 'a' fragments diffuse from the site and play a role in initiating a localized inflammatory response (chemotactic activity).

1. The proteins and glycoproteins forming the complement system are synthesized largely by liver hepatocytes, some by blood monocytes, tissue macrophages and epithelial cells of the gastro-intestinal and genitourinary tracts.

2. The proteins that form the complement system are labelled numerically with the prefix C (e.g., C_1 –C_9).

3. Some complement components are designated by letter symbols (e.g., factor B, D, P) or by trivial names (e.g., homologous factor).

4. There are at least 19 of these components; they are all serum proteins and together they make up about 10% globulin fraction of serum.

5. The molecular weights of the complement components vary between 24 kDa for factor D and 460 kDa for C_{19}.

6. Serum concentration in humans varies between 20 μg/ml of C_2 and 1300 μg/ml of C_3.

7. Complement components are synthesized at various sites like C2, C3, C4, C5; B, D, P and I are from macrophages, C3, C6, C8 and B from liver in the given table.

4.4.2.2 Complement Components

Name	MW (kDa)	Serum concentration (μg/ml)
Classical pathway		
C1q	460	80
C1r	83	50
C1s	83	50
C4	200	600
C2	102	20
C3	185	1300
Alternative pathway		
D	24	1
B	90	210
Terminal components		
C5	204	70
C6	120	65
C7	120	55
C8	160	55
C9	70	60
Control proteins		
C1-INH	105	200
C4-bp	550	250
H	150	480
I	88	35
P	4 × 56	20
Vitronectin	83	500

4.4.2.3 Components of Complements system and their functions

Functionally distinct classes of complement protein	
Function	**Protein**
Binding to antigen; antibody complexes	C1q
Activating enzymes	C1r C1s C2b Bb D
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a
Membrane attack proteins	C5b C6 C7 C8 C9

4.4.3 Complement activation and cell lysis

The early step of complement system varies in different pathways. However, all the pathways form enzyme complexes; C3 convertase, which cleaves C3 into C3a and C3b; and the C5 convertase, which cleaves C5 into C5a and C5b.C3b, thus formed, binds C3 convertase to form C5 convertase. C5 convertase, generated by the alternative, classical, or lectin pathway,

initiates the activation of late components of the complement system to form membrane attack complex (MAC) and ultimately kills the pathogen.

This occurs through three pathways; Classical pathway, activated by antigen-antibody reaction, Alternative pathway**,** activated on microbial cell surfaces, and **Mannose** binding Lectin pathway**,** activated by a plasma lectin that binds to mannose residues on microbes.

4.4.3.1 Classical Pathway

The classical pathway begins with the formation of antigen-antibody complex (immune complex). When an antigen enters the body, the antibody (IgM/IgG) binds to it. This induces conformational changes in the Fc portion of the antibody which exposes a binding site for C1 protein. Hence, the antibody activates the complement system only when bound to an antigen. C1 is a large, multimeric, protein complex composed of one molecule of C1q and two molecules each of C1r and C1s subunits. C1q binds to the antigen bound antibody (Fc portion). C1r and C1s are proteases which help to cleave C4 and C2.

The immune complex bound to C1 calls another protein C4 which is cleaved into C4a and C4b. C4a goes away whereas activated C4b attaches to the target surface near C1q. Now, C4b attracts C2 which is also cleaved into C2a and C2b. C2a binds C4b forming the C4b2a complex whereas C2b goes away. The active C4bC2a activates C3. The C4b2a complex is also known as C3 convertase as this converts C3 into an active form by separating C3a and C3b. One molecule of C4b2a can cleave a large number of C3 molecules. C3b binds to the microbial surface or to the convertase itself.

C3b when binds to C3 convertase forms C4bC2aC3b (**C5 convertase**) which activates C5. C5 convertase cleaves C5 into C5a and C5b. C5a diffuses away but C5b is stabilized by binding C6. Then C5bC6 binds to C7. C5bC6C7 complex is then inserted into the phospholipid bilayer of the cell membrane which further binds C8. These all (C5b678) activate C9 to form a macromolecular structure called the membrane attack complex (MAC)**.** This makes hole in the bacterium, as a result, the intracellular contents leak out and unwanted substances get in.

Thus, the cell cannot maintain its osmotic stability and the lysis occurs by an influx of water and loss of electrolytes.

This is more effective in Gram negative bacteria than in Gram positive bacteria because MAC formation is easy in the outer membrane in Gram negatives whereas it is difficult in the rigid thick layer of peptidoglycan in Gram positives. Some of the C3b molecules do not associate with C4b2a; instead these molecules coat immune complexes or microbial cell surfaces and work as opsonins. This process is called opsonization in which opsonin molecule binds one side to the particulate matter i.e. in bacteria, tumor cell, RBC and on the other side they bind to the receptor of phagocytic cell(like, neutrophils and macrophages) which enhance the process of phagocytosis. Smaller complement subunits diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors.

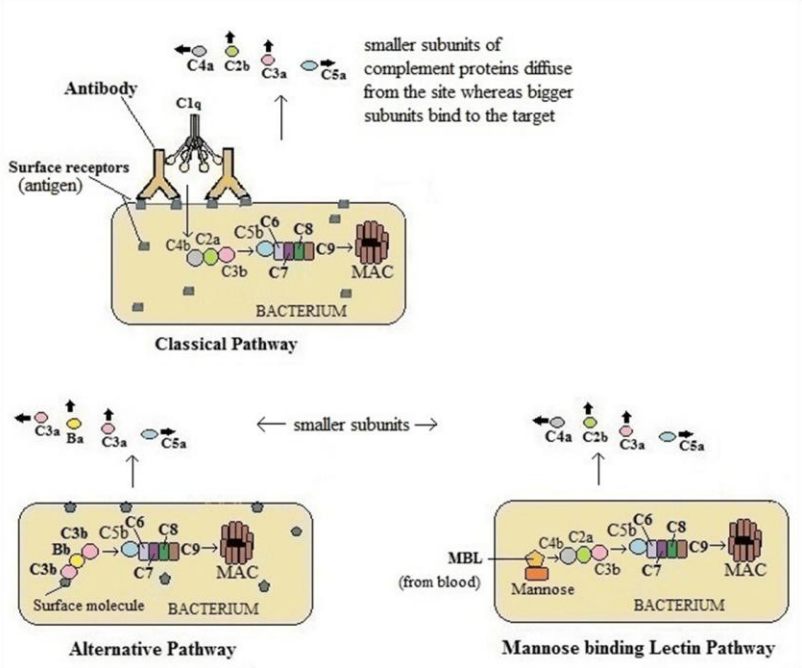

Fig. 8 Complement Pathways

4.4.3.2 Alternative Pathway

Unlike classical pathway, alternative pathway does not require Ag-Ab complex for the initiation of complement pathway. It is initiated by cell surface constituents that are foreign to the host. These surface molecules may be **lip**opolysaccharide etc. When a bacterium enters the

host body, as a result of inflammation, complements reach towards the site, where C3 molecules directly touch antigen and become active. In this pathway, serum C3 containing an unstable thioester bond undergoes slow spontaneous hydrolysis to yield C3a and C3b. C3b binds the surface of foreign cell and then binds to another serum protein called factor B. Now the factor B exposes the site which serves as the substrate for enzymatically active serum protein D. Then factor D cleaves B into Ba and Bb forming C3 convertase (C3bBb). C3 convertase then forms C5 convertase which ultimately forms a MAC as in classical pathway.

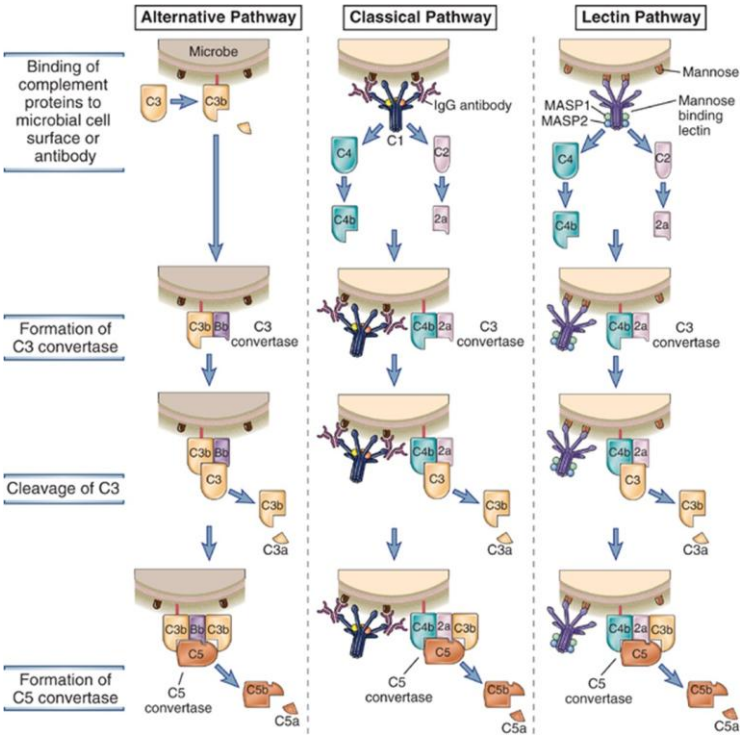

Fig. **9** Comparative represenataion of Complementation Pathways

4.4.3.3 Mannose binding Lectin (MBL) Pathway

Some bacteria can activate complement system without having antibody and endotoxin. This occurs through MBL pathway which is activated when circulating lectin (MBL) binds to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms. Microorganisms inducing MBL pathway are bacteria, such as Salmonella, Listeria, and Neisseria strains, some fungi and some viruses including HIV-1. MBL is an acute phase

protein and its concentration increases during inflammation. The lectin recognizes and binds the carbohydrate of the target cell which then activates complements.

MBL pathway resembles classical pathway as it proceeds through the action of C4 and C2 to produce activated proteins of the complement system. MBL works same as C1q which it resembles in structure.

After the MBL binds to carbohydrate residues on the surface of a cell or pathogen, two components, MASP-1 and MASP-2 bind to MBL. MASP stands for MBL-associated serine proteases. Two proteases form a tetrameric complex similar to the one formed by C1r and C1s and cleaves C4 and C2 forming C3 convertase. The process now continues to form of C5 convertase and the MAC as in classical pathway.

- Functions of Complements
- Function of Complement Pathway

Some major functions of complements are:

4.4.4 Opsonization and phagocytosis

C3b, bound to immune complex or coated on the surface of pathogen, activate phagocytic cells. These proteins bind to specific receptors on the phagocytic cells to get engulfed.

4.4.4.1 Cell lysis

Membrane attack complex formed by C5b6789 components ruptures the microbial cell surface which kills the cell.

4.4.4.2 Chemotaxis

Complement fragments attract neutrophils and macrophages to the area where the antigen is present. These cell surfaces have receptors for complements, like C5a, C3a, thus, run towards the site of inflammation, i.e. chemotaxis.

4.4.4.3 Activation of mast cells and basophils and enhancement of inflammation

The proteolytic complement fragments, C5a, C4a, and C3a induce acute inflammation by activating mast cells and neutrophils. All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine. These peptides are also called anaphylatoxins because the mast cell reactions they trigger are characteristic of

anaphylaxis. Binding to specific complement receptors on cells of the immune system, they trigger specific cell functions, inflammation, and secretion of immunoregulatory molecules.

4.4.4.4 Production of antibodies

B cells have receptor for C3b. When C3b binds to B-cell, it secretes more antibodies. Thus C3b is also an antibody producing amplifiers which converts it into an effective defense mechanism to destroy invading microorganism.

4.4.4.5 Immune clearance

The complement system removes immune complexes from the circulation and deposits them in the spleen and liver. Thus it acts as anti-inflammatory function. Complement proteins promote the solubilization of these complexes and their clearance by phagocytes.

4.4.4.6 Role of Complement in Disease

The complement system plays a critical role in inflammation and defence against some bacterial infections. Complement may also be activated during reactions against incompatible blood transfusions, and during the damaging immune responses that accompany autoimmune disease. Deficiencies of individual complement components or inhibitors of the system can lead to a variety of diseasesas, which gives some indication of their role in protection against disease.

Diseases associated with complement deficiencies

Complement Deficiency	Disease
C3 and Factor B	Severe Bacterial Infections
C3b-INA, C6 and C8	Severe Neisseria Infections
Deficiencies of early C components C1, C4, C2.	Systemic Lupus Erythematosus (SLE), Glomerulonephritis and Polymyositis
C1-inhibitor	Hereditary Angioedema

4.4.5 Common vaccine for humans

4.4.5.1 Vaccine

Vaccine (L. vacca = cow) is a preparation/suspension or extract of dead/attenuated (weakened) germs of a disease which on inoculation (injection) into a healthy person provides temporary/permanent active/passive immunity by inducing antibodies formation. Thus antibody provoking agents are called vaccines. 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.

4.4.5.2 Vaccination

The process of introduction of vaccine into an individual to provide protection against a disease is called vaccination. In vaccination, a preparation of antigenic proteins of pathogens or inactivated/weakened pathogens (vaccine), is introduced into the body. These antigens generate the primary immune response, and the memory B and T cells. When the vaccinated person is attacked by the same pathogen, the existing memory T or B cells recognise the antigen quickly and attack the invaders with a massive production of lymphocytes and antibodies.

4.4.5.3 Immunization

The principle of immunisation or vaccination is based on the property of 'memory' of the immune systems. Vaccines also generate memory-B and T cells that recognize the pathogen quickly. In snake bites the injection which is given to the patients contains preformed antibodies against the snake venom. This type of immunisation is called passive immunisation.

4.4.6 History

Edward Jenner the "Father of Immunology" (1749-1823), conceived the idea of vaccination when he observed that the milkmaids did not suffer from small pox, a dreaded disease, as they were exposed to a milder form of this disease called cowpox. So he proposed that an induced mild form of a disease would protect a person from a virulent form (which can cause more damage to the host).

He termed it as vaccine (Latin-Vacca means “Cow”) and vaccination for protective inoculation. Jenner, the county doctor, experimented this concept successfully on a healthy body named James Philips in 1796 and could save thousands of people from his discovery. Nearly after a century, Lewis Pasteur (1881) succeeded in producing vaccines for anthrax, rabies (hydrophobia). Victims of bites by rabid dogs are now immunized by a series of injections called pasteur treatment.

4.4.7 Types of Vaccines

For a vaccine to provide protection against a disease, it must expose an individual to pathogen-specific antigens that will stimulate a protective adaptive immune response. By its very nature, this entails some risk. As with any pharmaceutical drug, vaccines have the potential to cause adverse effects. However, the ideal vaccine causes no severe adverse effects and poses no risk of contracting the disease that it is intended to prevent. Various types of vaccines have been developed with these goals in mind.

4.4.7.1 Live Attenuated Vaccines

Live attenuated vaccines expose an individual to a weakened strain of a pathogen with the goal of establishing a subclinical infection that will activate the adaptive immune defenses. Pathogens are attenuated to decrease their virulence using methods such as genetic manipulation (to eliminate key virulence factors) or long-term culturing in an unnatural host or environment (to promote mutations and decrease virulence).

By establishing an active infection, live attenuated vaccines stimulate a more comprehensive immune response than some other types of vaccines. Live attenuated vaccines activate both cellular and humoral immunity and stimulate the development of memory for long-lasting immunity. In some cases, vaccination of one individual with a live attenuated pathogen can even lead to natural transmission of the attenuated pathogen to other individuals. This can cause the other individuals to also develop an active, subclinical infection that activates their adaptive immune defenses. Disadvantages associated with live attenuated vaccines include the challenges associated with long-term storage and transport as well as the potential for a patient to develop signs and symptoms of disease during the active infection (particularly in immuno-compromised patients). There is also a risk of the attenuated pathogen reverting back to full virulence.

4.4.7.2 Inactivated Vaccines

Inactivated vaccines contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of key antigens on the pathogen. Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. Typically the response involves only humoral immunity, and the pathogen cannot be transmitted to other individuals. In addition, inactivated vaccines usually require higher doses and multiple boosters, possibly causing inflammatory reactions at the site of injection.

Despite these disadvantages, inactivated vaccines do have the advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. However, inactivated vaccines are not without their side effects.

4.4.7.3 Subunit Vaccines

Whereas live attenuated and inactive vaccines expose an individual to a weakened or dead pathogen, **subunit vaccines** only expose the patient to the key antigens of a pathogen—not whole cells or viruses. Subunit vaccines can be produced either by chemically degrading a pathogen and isolating its key antigens or by producing the antigens through genetic engineering. Because these vaccines contain only the essential antigens of a pathogen, the risk of side effects is relatively low. Table 1 lists examples of subunit vaccines.

4.4.7.4 Toxoid Vaccines

Like subunit vaccines, **toxoid vaccines** do not introduce a whole pathogen to the patient; they contain inactivated **bacterial toxins**, called toxoids. Toxoid vaccines are used to prevent diseases in which bacterial toxins play an important role in pathogenesis. These vaccines activate humoral immunity that neutralizes the toxins.

4.4.7.5 Conjugate Vaccines

A **conjugate vaccine** is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. Conjugate vaccines have been developed to enhance the efficacy of subunit vaccines against pathogens that have protective polysaccharide capsules that help them evade **phagocytosis**, causing invasive infections that can lead to **meningitis** and other serious conditions. The subunit vaccines against these pathogens introduce T-independent

capsular polysaccharide antigens that result in the production of antibodies that can opsonize the **capsule** and thus combat the infection; however, children under the age of two years do not respond effectively to these vaccines. Children do respond effectively when vaccinated with the conjugate vaccine, in which a protein with **T-dependent antigens** is conjugated to the capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide. These different classes of vaccines are summarized in the table.

Class	**Description**	**Examples**
Live attenuated	Weakened strain of whole pathogen	Chickenpox, German measles, measles, mumps, tuberculosis, typhoid fever, yellow fever
Inactivated	Whole pathogen killed or inactivate with heat,chemicals or radiation	Cholera,Hepetitis A,Influenza, Plaques,Rables, Polio (IPV)
Subunit	Immunogenic antigen	Anthrax,Hepatitis B, Hepatitis B Influenza (injection) *Haemophilus influenza* type b (Hib) Pertussis,meningitis,pneumococcal pneumoni
Toxoid	Inactivated Bacterial toxins	Botulism,Diphtheria,pertusis,tetanus
Conjugate	Capsule polysaccharide conjugated to proteins	Haemophilus influenza,Streptococcus pneunoninae,Neisseria meningitides

4.4.7.6 Hepatitis Vaccine

4.4.7.6.1 Hepatitis B

Hepatitis B is a widespread disease in man. It primarily affects liver causing chronic hepatitis, cirrhosis and liver cancer. Hepatitis B virus is a 42 nm particle, called Dane particle. It consists of a core containing a viral genome (DNA) surrounded by a phospholipid envelope carrying surface antigens. Infection with hepatitis B virus produced Dane particles and 22 nm sized particles. The latter contain surface antigens which are more immunogenic. It is however, very difficult to grow hepatitis B virus in mammalian cell culture and produce surface antigens.

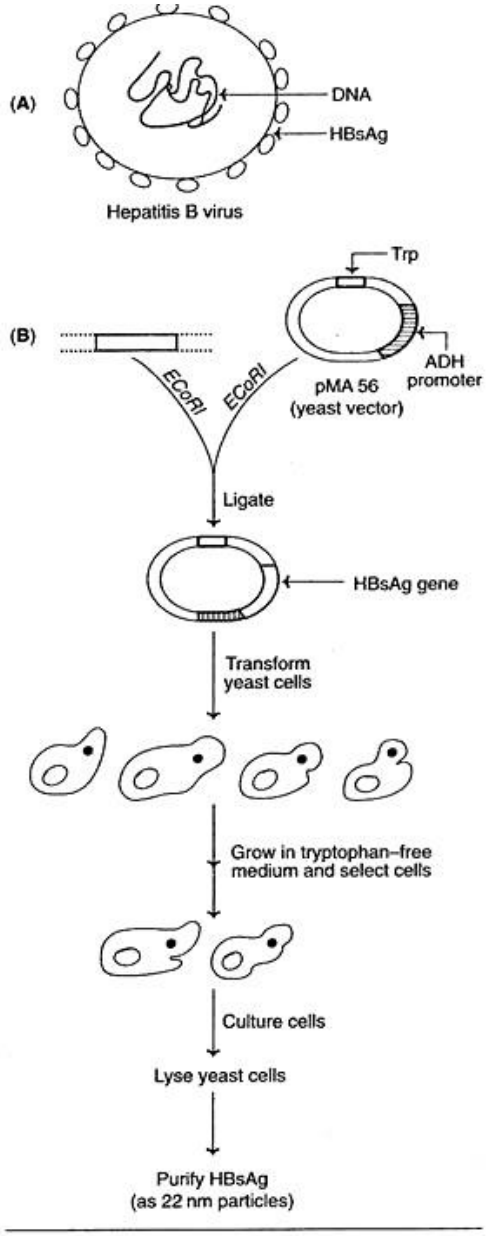

Fig. 10 (A) Hepatitis B virus-Dane particle(42nm particle), (B) Production of Hepatitis B surface antigen (HBsAg) in yeast cells (Trp-Tryptophan,ADH-Alcohol dehydrogenase).

The gene encoding for hepatitis B surface antigen (HBsAg) has been identified. Recombinant hepatitis B vaccine as a subunit vaccine, is produced by cloning HbsAg gene in yeast cells. Saccharomyces cerevisiae, a harmless baking and brewing yeast, is used for this purpose (Fig. 16.1B). The gene for HBsAg is inserted (pMA 56) which is linked to the alcohol dehydrogenase promoter. These plasmids are then transferred and cultured.

The cells grown in tryptophan, free medium are selected and cloned. The yeast cells are cultured. The HBsAg gene is expressed to produce 2nm sized particles similar to those found in patients infected with hepatitis B. (These particles are immunoreactive with anti-HBsAg antibodies). The subunit HBsAg as 22 nm particles can be isolated and used to immunize individuals against hepatitis B.

4.4.7.6.2 Hepatitis B vaccine-the first synthetic vaccine:

In 1987, the recombinant vaccine for hepatitis B (i.e. HBsAg) became the first synthetic vaccine for public use. It was marketed by trade names Recombivax and Engerix-B. Hepatitis B vaccine is safe to use, very effective and produces no allergic reactions. For these reasons, this recombinant vaccine has been in use since 1987. The individuals must be administered three doses over a period of six months. Immunization against hepatitis B is strongly recommended to anyone coming in contact with blood or body secretions. All the health professionals—physicians, surgeons, medical laboratory technicians, nurses, dentists, besides police officers, firefighters etc., must get vaccinated against hepatitis B.

4.4.7.6.3 Hepatitis B vaccine in India:

India is the fourth country (after USA, France and Belgium) in the world to develop an indigenous hepatitis B vaccine. It was launched in 1997, and is now being used.

4.4.7.6.4 Hepatitis B vaccine tomato?

Biotechnologists have been successful in inserting hepatitis B gene into the cells of the tomato plant. These genetically engineered plants produce hepatitis B antigens. The day may not be far off to get immunized against hepatitis B by having a tomato with lunch.

4.4.7.6.5 Influenza Vaccine

Influenza, also called **flu** or **grippe**, an acute viral infection of the upper or lower respiratory tract that is marked by fever, chills, and a generalized feeling of weakness and pain in the muscles, together with varying degrees of soreness in the head and abdomen. Smith et al (1933) first isolated influenza type A from throat washing of a patient and in 1940 Type B virus was isolated along with type A in cell culture; Influenza virus type C, the third serotype, was isolated by Taylor (1949).

4.4.7.6.6 Classification of Influenza Viruses

Influenza is caused by any of several closely related viruses in the family Orthomyxoviridae (a group of RNA viruses). Influenza viruses are categorized as types A, B, C, and D. These major types generally produce similar symptoms but are completely unrelated antigenically, so that infection with one type confers no immunity against the others. The A viruses cause the great influenza epidemics, and the B viruses cause smaller localized outbreaks. The C viruses cause only mild respiratory illness in humans. Influenza D viruses are not known to infect humans and have been observed only in pigs and cattle.

Influenza A viruses are classified into subtypes, and both influenza B and subtypes of influenza A are further divided into strains. Subtypes of influenza A are differentiated mainly on the basis of two surface antigens (foreign proteins)—hemagglutinin (H) and neuraminidase (N). Examples of influenza A subtypes include H1N1, H5N1, and H3N2. Influenza B viruses are subdivided into two major lineages, B/Yamagata and B/Victoria. Strains of influenza B and strains of influenza A subtypes are further distinguished by variations in genetic sequence.

4.4.7.6.7 Morphology of Influenza Viruses:

Types A and B are morphologically identical but Type C differs from them in some aspects.

4.4.7.6.7.1 They are pleomorphic

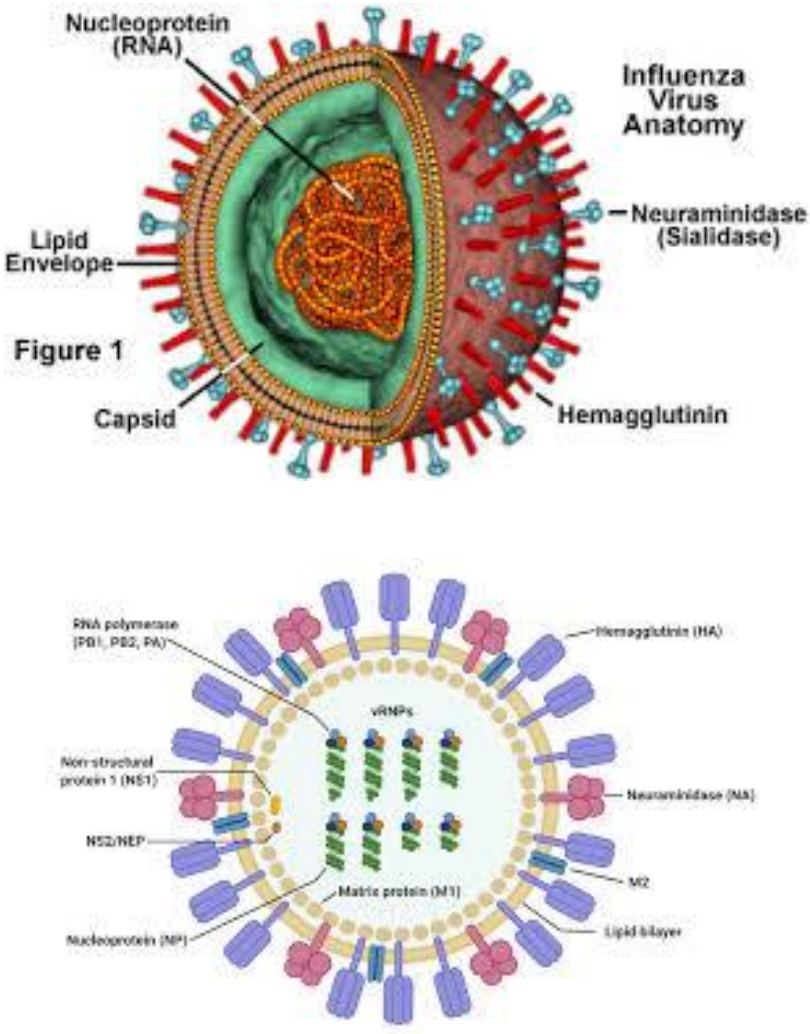

Fig. 11 Structure of Influenza Virus

4.4.7.6.7.2 Spherical Particle

1. Diameter 80-100 nm.

2. A lipid envelope derived from the host cell membrane separates the spikes from the inner core.

3. Attached to lipid layer of the envelope are densely arranged radial projections of two types of peplomers-haemagglutinin (HA) spikes and neuraminidase (NA)-mushroom shaped. Distal tip of HA contains sites for binding to host cell.

4. Single stranded RNA genome is segmented.

4.4.8 Antigenic Classification of Influenza Virus

4.4.8.1 Influenza virus possesses four antigens (structural proteins)

(1) The internal or nucleocapsid (nucleo-protein-NP) is closely associated with ss-RNA protein core);

(2) Matrix protein of the viral envelope (M); and (3) two surface glycoproteins (HA, haemaggludnin; NA, neuroaminidase). Three polymerase polypeptides, PA, PB and PB_2 are also present. Influenza viruses are divided into three types,; A, B, C—on the basis of antigenic structure differences of NP and M proteins. Again, the types are divided into subtypes on the basis of antigenic variation of HA and NA.

Till now, 15 types of HA (H1-H13) and 9 types of NA (N1-N9) in different combination have been isolated from influenza viruses of birds, animals or humans. Human viruses have 3 HA (H_1, H_2, H_3) and 2 NA (N_1 N_2) types. Type A human virus has 2 main subtypes; A_1 (H_1 N_1) and A_2 (H_2 N_2). Non-human influenza viruses belong to type A whereas human influenza viruses belong to type A, B and C. Non-human viruses do not infect man but play an important role in the emergence of pandemic influenza by genetic re-assortment with human viruses.

4.4.8.2 Pathogenesis of Influenza Viruses:

The portal of entry of influenza virus is respiratory tract. Ciliated cells of respiratory tract are mainly susceptible, because its mucous membrane contains the specific mucoprotein receptors, for influenza virus. After entry they encounter respiratory secretions with mucoprotein. Viral neuraminidase lowers viscosity of the mucus. Then a large number of cells are infected and killed.

4.4.8.3 Clinical Features of Influenza Viruses:

Usually the incubation period is 1 to 4 days; subclinical infection are common. Influenza is an acute respiratory illness and clinical manifestations vary from a mild coryza to fulminating pneumonia. During acute illness, due to necrosis of virally infected cells, there is extensive desquamation of respiratory epithelium. Influenza is characterised by fever, chills, headache, dry cough, and generalized myalgia. Fever lasts for about 3 days and respiratory symptoms last for 3 to 4 days. An uncomplicated case usually resolves within 7 days. It is self-limiting disease secondary bacterial pneumonia due to staphylococcus aureus and Haemophilus influenzae is usually observed in 10% cases.

4.4.8.4 Epidemiology of Influenza Viruses:

Its patterns are variable in three types of influenza virus. **Type A** causes severe and wide spread epidemics; **B** causes sporadic and sometimes epidemics, but **C** does not cause epidemics, but does cause in-apparent infections. Epidemics and pandemics of influenza are associated with antigenic shift which is exhibited by only Type A virus. Periodical epidemics can be caused by antigenic drift also, which is exhibited by all three types. Type B, and C are confined to humans, while Type A viruses circulate in ecosystem (birds, swine, horses). These animal viruses infect man. Since antiquity epidemics occurred at regular intervals. Influenza epidemic starts abruptly, spreads rapidly and often distributed worldwide.

4.4.8.5 Complications

Pneumonia is the most commonly seen complication of influenza infection. Typically, it is caused by a secondary bacterial infection such as *Haemophilus influenzae* or *Streptococcus pneumoniae*. The flu can also lead to sinus and ear infections, worsen existing medical conditions such as chronic pulmonary diseases, or cause inflammation of the heart.

Although any flu patient can experience complications from the disease, certain groups are at a higher risk for flu complications than others: older individuals, young children, people with asthma, and pregnant women are some of those whose risk for complications is elevated. In a typical flu season, people 65 or older account for 90% of deaths from the flu. (Some pandemic influenzas behave quite differently than expected in this regard; in the 2009 H1N1 pandemic, almost 90% of deaths from H1N1 influenza were among people *younger* than 65)

4.4.8.6 Treatment and Care

Generally, flu patients are encouraged to stay home and rest, both to recover and to avoid infecting others. In mild cases, treatment is limited to addressing the symptoms of the disease: over-the-counter medicines such as acetaminophen or ibuprofen may be used to reduce fever and/or relieve aches and pains, and cough medicines or drops may be used for sore throats and to reduce coughing. Drinking extra fluids may be encouraged to prevent dehydration.

The antiviral drugs amantadine and rimantadine have beneficial effects on cases of influenza involving the type A virus. However, viral resistance to these agents has been observed,

thereby reducing their effectiveness. A newer category of drugs, the neuraminidase inhibitors, which includes oseltamivir (Tamiflu) and zanamivir (Relenza), was introduced in the late 1990s; these drugs inhibit both the influenza A and B viruses. Other than this, the standard treatment remains bed rest, ingestion of fluids, and the use of analgesics to control fever. It is recommended that children and teenagers with the flu not be given aspirin, as treatment of viral infections with aspirin is associated with Reye syndrome, a very serious illness.

4.4.8.7 Available Vaccines and Vaccination Campaigns

Because new strains of influenza appear frequently, the seasonal flu vaccine usually changes each year. Each season vaccine is generally designed to protect against three strains of influenza: two "A" strains, and one "B" strain. From start to finish—the selection of which three strains to target with the vaccine, to the production of the final product—the development process for the seasonal flu vaccine can take up to eight months.

Influenza surveillance centers around the world monitor the circulating influenza strains for trends year-round. Genetic data is collected and new mutations are identified. The World Health Organization is then responsible for selecting three strains most likely to genetically resemble strains circulating in the coming winter flu season. For the northern hemisphere winter, this decision is made in the February prior. In some cases, one of the strains used in the previous year's vaccine may be chosen again, if that strain continues to circulate. From this point, the development and production of the vaccine can begin. Four to five months after the three vaccine strains have been selected (in June or July), the three vaccine strains that have been developed are separately tested for purity and potency. Only after individual testing is completed are the three strains combined into a single seasonal vaccine.

4.4.8.8 Currently Licensed Seasonal Influenza Vaccines

Vaccination is the best method for the prevention and control of influenza. Vaccination can reduce illness and lessen severity of infection. Currently licensed influenza vaccines focus on the production of antibodies against the viral HA protein, which binds host receptors to mediate viral entry. Strain-specific antibodies produced against the HA neutralize the virus and prevent infection (Figure 1). The current seasonal vaccines require annual evaluation and

reformulation to keep pace with the antigenic drift of circulating strains. This process is completed twice a year, once each for the northern and southern hemispheres.

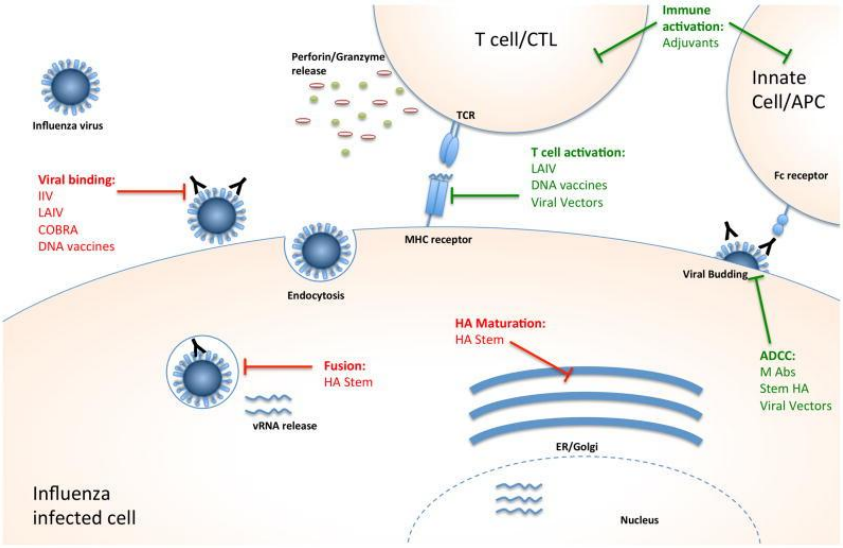

Fig. 12 Stages of the viral life cycle and vaccine targets

Antigenic drift results from mutations that occur because the error-prone viral RNA-dependent RNA polymerase lacks proofreading function, resulting in mutations in the HA and other viral proteins. Additionally, the HA is under positive selection for antigenic escape from neutralization by pre-existing antibodies. Selection of the vaccine composition for the upcoming season's vaccine must take place 7 to 8 months in advance of "flu season" to accommodate the steps of vaccine production.

In the case of a pandemic, an additional vaccine may be created to protect against a particularly virulent or widespread strain of influenza. The need for a 2009 H1N1 influenza vaccine became apparent after the strains for the seasonal flu vaccine had already been selected, so that a separate vaccine was created. There are three classes of licensed seasonal vaccines including inactivated, live attenuated, and recombinant HA vaccines. All three vaccines are multivalent, with components representing influenza A and B viruses anticipated to circulate in the next influenza season.

4.4.8.9 The inactivated influenza vaccine (IIV)

It is a split virion or subunit vaccine that contains 15μg of each purified HA protein administered intramuscularly, or 9μg of each purified HA protein administered intradermally. There is also a higher dose of antigen available for the elderly population aged 65 years and older, in which 60 μg of each HA is administered in order to increase the immunogenicity of the vaccine. The trivalent inactivated vaccine (TIV) contains H1N1 and H3N2 subtypes of influenza A along with the predicted dominant lineage of influenza B. A recently licensed quadrivalent influenza vaccine (QIV) includes two lineages of influenza B along with the H1N1 and H3N2 subtypes of influenza A. The IIV induce a strain-specific serum IgG antibody response and are licensed for individuals aged 6 months and older.

4.3.8.10 Live attenuated influenza vaccine (LAIV)

This vaccine also contains a mixture of the same four influenza strains as the QIV, but is administered intranasally as a spray. The LAIV contains live viruses with temperature-sensitive and attenuating mutations. As a result of these mutations, the vaccine virus is restricted in replication at the temperature of the lower respiratory tract, but can replicate at the cooler temperature of the nasal cavity. Vaccination with LAIV results in the production of strain-specific serum IgG as well as mucosal IgA and T cell responses.

4.4.8.11 The third licensed product is FluBlok

It is a recombinant HA vaccine with HA proteins that are expressed in insect cells from baculovirus vectors. FluBlok is currently licensed for adults aged 18 to 49 years and can be used in individuals who are allergic to eggs. The manufacturing process for this vaccine has a shorter timeframe, which would be valuable during a pandemic response.

4.4.8.12 Challenges in Optimizing Influenza Vaccines

The currently licensed influenza vaccines are effective in healthy young adults. They include the dependence on embryonated eggs for vaccine production, the lengthy timeline for vaccine production, the need for annual vaccination, the emergence of antigenically novel viruses, the need for improved immunogenicity in the elderly, and the need for an improved correlate of protection. Several approaches have been developed to overcome these challenges and improve the immunogenicity and efficacy of influenza vaccines.

4.4.9 Measles Vaccine

Measles is an extremely contagious disease caused by a virus from the paramyxovirus family and spread by air. Its symptoms include fever and coughing as well as its infamous rash. Typically, fever occurs before the measles rash; however, with the appearance of the rash, the existing fever may rise to temperatures of 104°F or higher. These symptoms usually begin one to two weeks after infection with the measles virus; most people recover within two to three weeks. Measles is also called "rubeola," but should not be confused with *rubella*, or the so-called German measles.

4.4.9.1 Measles Vaccine

4.4.9.1.1 Transmission

Measles is a highly contagious disease. The virus is found in the nose and throat of infected patients and is sprayed via coughs and sneezes into the air, where it can remain active and infectious for up two hours. As a result, a person can become infected simply by breathing the air in a room that had been occupied by a measles patient as much as two hours earlier.

4.4.9.1.2 Treatment and Care

There is no direct treatment for measles. Supportive care may be provided, including efforts to keep the patient hydrated and to lower the fever associated with the disease.

4.4.9.1.3 Complications

Measles can lead to complications ranging in severity from diarrhea to encephalitis (swelling of the brain), with adult patients typically being subject to more severe complications. Although the disease is rarely fatal in developed countries, mortality can be high in low-income nations. Case-fatality rates have been recorded as high as 28%, and tend to rise during wars or widespread food shortages. As recently as 2000, measles caused 1.1 million deaths globally among young children in a year's time.

4.4.9.1.4 Available Vaccines

Measles is a serious disease that used to be very common in the United States. But thanks to the measles vaccine, the number of measles cases in Americans has dropped by over 99%. There are 2 vaccines that can prevent measles:

- The **MMR vaccine** protects children and adults from measles, mumps, and rubella
- The **MMRV vaccine** protects children from measles, mumps, rubella, and chickenpox

A vaccine to protect against measles was developed in the 1960s and was quickly adopted. Widespread vaccination programs, including the Measles Initiative launched in 2001 by the American Red Cross, the United Nations Foundation, the U.S. Centers for Disease Control and Prevention, UNICEF and the World Health Organization, contributed to global decreases in measles cases until the case count among children fell as low as 118, 000 by 2008.

4.4.9.1.5 Vaccine development

In 1954, John F. Enders and Dr. Thomas C. Peebles collected blood samples from several ill students during a measles outbreak in Boston, Massachusetts. They wanted to isolate the measles virus in the student's blood and create a measles vaccine. They succeeded in isolating measles in 13-year-old David Edmonston's blood.

In 1963, John Enders and colleagues transformed their Edmonston-B strain of measles virus into a vaccine and licensed it in the United States. In 1968, an improved and even weaker measles vaccine, developed by Maurice Hilleman and colleagues, began to be distributed. This vaccine, called the Edmonston-Enders (formerly "Moraten") strain has been the only measles vaccine used in the United States since 1968. Measles vaccine is usually combined with mumps and rubella (MMR), or combined with mumps, rubella and varicella (MMRV). Learn more about measles vaccine.

4.4.9.1.6 Measles Elimination

In 1978, CDC set a goal to eliminate measles from the United States by 1982. Although this goal was not met, widespread use of measles vaccine drastically reduced the disease rates. By 1981, the number of reported measles cases was 80% less compared with the previous year. However, a 1989 measles outbreaks among vaccinated school-aged children prompted the Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics (AAP), and the American Academy of Family Physicians (AAFP) to recommend a second dose of MMR vaccine for all children. Following widespread implementation of this recommendation and improvements in first-dose MMR vaccine coverage, reported measles cases declined even more.

Measles was declared eliminated (absence of continuous disease transmission for greater than 12 months) from the United States in 2000. This was thanks to a highly effective vaccination program in the United States, as well as better measles control in the Americas region

4.4.10 Blood Groups System

The term “blood group” refers to the entire blood group system comprising red blood cell (RBC) antigens whose specificity is controlled by a series of genes which can be allelic or linked very closely on the same chromosome. “Blood type” refers to a specific pattern of reaction to testing antisera within a given system. Over a period of time, our understanding on blood groups has evolved to encompass not only transfusion-related problems but also specific disease association with RBC surface antigens.

4.4.10.1 Blood Groups

International Society of Blood Transfusion has recently recognized 33 blood group systems. Apart from ABO and Rhesus system, many other types of antigens have been noticed on the red cell membranes. The antigens can be integral proteins where polymorphisms lie in the variation of amino acid sequence (e.g., rhesus [Rh], Kell), glycoproteins or glycolipids (e.g., ABO). Some of the important groups are mentioned below.

4.4.10.2 Blood group systems

Name	Symbol	Number of Antigens	Gene Name	Chromosome
ABO	ABO	4	ABO	9
MNS	MNS	43	GYPA,GYPB,GYPE	4
P	P1	1	P1	22
Rhesus	Rh	49	RhD, RhCE	`1
Lutheran	LU	20	LU	19
Kell	KEL	25	KEL	7
Lewis	LE	6	FUT3	19
Duffy	FY	6	FY	1
Kidd	JK	3	SLC14A1	18

4.4.10.3 ABO system

Among the 33 systems, ABO remains the most important in transfusion and transplantation since any person above the age of 6 months possess clinically significant anti-A and/or anti-B antibodies in their serum. Blood group A contains antibody against blood group B in serum and vice-versa, while blood group O contains no A/B antigen but both their antibodies in serum.

4.4.10.4 Rhesus System

Rhesus-system is the second most important blood group system after ABO.Currently, the Rh-system consists of 50 defined blood group antigens out of which only five are important. RBC surface of an individual may or may not have a Rh factor or immunogenic D-antigen. Accordingly, the status is indicated as either Rh-positive (D-antigen present) or Rh-negative (D-antigen absent). In contrast to the ABO system, anti-Rh antibodies are, normally, not present in the blood of individuals with D-negative RBCs, unless the circulatory system of these individuals has been exposed to D-positive RBCs. These immune antibodies are immunoglobulin G (IgG) in nature and hence, can cross the placenta. Prophylaxis is given against Rh immunization using anti-D Ig for pregnant Rh-negative mothers who have given birth to Rh-positive child.

4.4.10.5 MNS Antigen System

MNS antigen system, first described by **Landsteiner** and **Levine** in 1927 is based on two genes: Glycophorin A and Glycophorin B. The blood group is under control of an autosomal locus on chromosome 4 and also under control of a pair of co-dominant alleles LM and LN. Anti-M and anti-N antibodies are usually IgM types and rarely, associated with transfusion reactions.

4.4.10.6 H-antigen

H-antigen is the precursor to the ABO blood group antigens. It is present in all RBCs irrespective of the ABO system. Persons with the rare Bombay phenotype are homozygous for the H gene (HH), do not express H-antigen on their RBCs. As H-antigen acts as precursor, its

absence means the absence of antigen A and B. However, the individuals produce isoantibodies to H-antigen as well as to antigens A and B.

4.4.10.7 Lutheran System

Lutheran system comprised of four pairs of allelic antigens representing single amino acid substitution in the Lutheran glycoprotein at chromosome 19. Antibodies against this blood group are rare and generally not considered clinically significant.

4.4.10.8 Kell System

These erythrocyte antigens are the third most potent immunogenic antigen after ABO and Rh system, and are defined by an immune antibody, anti-K. It was first noticed in the serum of Mrs. Kellacher. She reacted to the erythrocytes of her newborn infant resulting in hemolytic reactions. Since then 25 Kell antigens have been discovered. Anti-K antibody causes severe hemolytic disease of the fetus and newborn (HDFN) and haemolytic transfusion reactions (HTR).

4.4.10.9 Duffy System

Duffy-antigen was first isolated in a patient called Duffy who had haemophilia. It is also known as Fy glycoprotein and is present in the surface of RBCs. It is a nonspecific receptor for several chemokines and acts as a receptor for human malarial parasite, *Plamodium vivax*. Antigens Fya and Fyb on the Duffy glycoprotein can result in four possible phenotypes, namely Fy(a+b−), Fy(a+b+), Fy(a−b+), and Fy(a−b−). The antibodies are IgG subtypes and can cause HTR.

4.4.10.10 Kidd System

Kidd antigen (known as Jk antigen) is a glycoprotein, present on the membrane of RBCs and acts as a urea transporter in RBCs and renal endothelial cells. Kidd antibodies are rare but can cause severe transfusion reactions. These antigens are defined by reactions to an antibody designated as anti-Jk^a, discovered in the serum of Mrs. Kidd who delivered a baby with HDFN. Jk^a was the first antigen to be discovered by Kidd blood group system, subsequently, two other antigens Jk^b and Jk3 were found. Two types of blood groups — ABO Blood Group and Rh Blood Group (Rh Factor) are widely used all over the world.

4.4.11 ABO Blood Group System

The **ABO blood group system** is used to denote the presence of one, both, or neither of the A and B antigens on erythrocytes. ABO blood types are also present in other primates such as apes and Old World monkeys. There are more than 30 antigens on the surface of blood cells that give rise to different blood groups. In a blood transfusion, certain blood groups, e.g., ABO blood group, of the recipient and donor must be matched, otherwise the recipient's immune system will produce antibodies that cause agglutination of the transfused cells and block blood circulation through capillaries.

4.4.11.1 History

Karl Landsteiner has been credited for the discovery of ABO blood group system in 1900. Karl Landsteiner has been credited for the discovery of ABO blood group system in 1900. His extensive research on serology based on simple but strong scientific reasoning led to identification of major blood groups such as O, A, and B types, compatibility testing, and subsequent transfusion practices. He was awarded Noble Prize in 1930 for this discovery.

4.4.11.2 ABO Antigens and Antibodies

The ABO blood group is the most important of all the blood group systems. The **ABO blood group** name consists of three letters, ABO blood typing designates the presence or absence of just two antigens, A and B. Both are glycoproteins. People whose erythrocytes have A antigens on their erythrocyte membrane surfaces are designated blood type A, and those whose erythrocytes have B antigens are blood type B. People can also have both A and B antigens on their erythrocytes, in which case they are blood type AB. People with neither A nor B antigens are designated blood type O. ABO blood types are genetically determined. The basis of ABO grouping is of two antigens- Antigen A and Antigen B. The ABO grouping system is classified into four types based on the presence or absence of antigens on the red blood cells surface and plasma antibodies.

Normal healthy individuals, from early in childhood, make red cell antibodies against A or B antigens that are not expressed on their own cells. These naturally occurring antibodies are mainly IgM immunoglobulins. They attack and rapidly destroy red cells carrying the corresponding antigen. For example, anti-A attacks red cells of Group A or AB. Anti-B attacks red cells of Group B or AB.

Name of Blood Group	Antigens present on the red cell surface	ABO antibodies present in the plasma
Type O	Nil	anti-A and anti-B
Type A	A antigen	anti-B
Type B	B antigen	anti-A
Type AB	A and B antigens	nil

If ABO incompatible red cells are transfused, red cell haemolysis can occur. For example if group A red cells are infused into a recipient who is group O, the recipient's anti-A antibodies bind to the transfused cells. An ABO incompatible transfusion reaction may result in overwhelming haemostatic and complement activation, resulting in shock, renal failure & death.

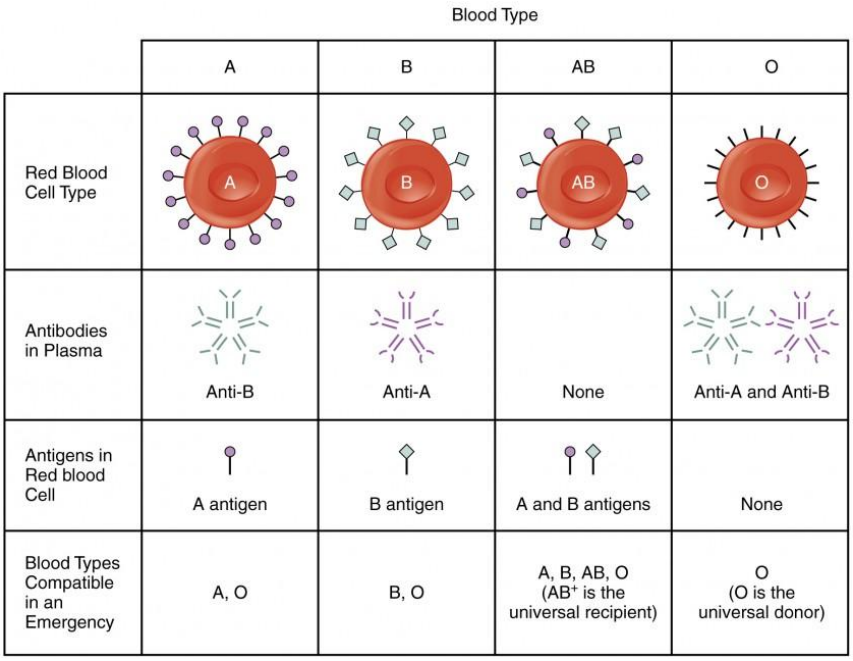

Fig. 13 Characteristics of blood type in ABO blood Group

4.4.11.3 Genetics of ABO Blood Groups

Bernstein in 1924 found that the blood group antigens are controlled by an autosomal gene designated I (iso-haemaglutinin) which has 3 alleles I^A, I^B and I^O. Allele I^A produces type A

blood and is dominant over the allele I^O which in the homozygous state (I^OI^O) produces type O blood.

Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABO gene) with three types of alleles inferred from classical genetics: *i*, I^A, and I^B. The gene encodes a glycosyltransferase—that is, an enzyme that modifies the carbohydrate content of the red blood cell antigens. The gene is located on the long arm of the ninth chromosome (9q34). AB type persons have the genotype I^AI^B where both alleles are co-dominant and equally expressed.

4.4.11.4 Phenotype and Genotype and Compatibility of ABO Blood types

Phenotype	Genotype	Antigen	Antibody	Can Give Blood(RBCs) to	Can receive Blood(RBCs) from
A	AA or AI	A	B	A and AB	A and O
B	BB or BI	B	A	B and AB	B and O
AB	AB	A and B	None	AB	AB,A,B,O
O	I	None	A and B	AB,A,B,O	O

4.4.11.5 Rh (Rhesus) Blood Group System:

The Rh blood group system was discovered in 1940 by **Karl Landsteiner** and **A.S. Weiner**.The **Rh blood group system** is a human blood group system. It contains proteins on the surface of red blood cells. It is the second most important blood group system, after the ABO blood group system. The Rh blood group system consists of 49 defined blood group antigens, among which the five antigens D, C, c, E, and e are the most important. There is no d antigen. Rh(D) status of an individual is normally described with a positive or negative suffix after the ABO type (e.g., someone who is A Positive has the A antigen and the Rh(D) antigen, whereas someone who is A Negative lacks the Rh(D) antigen).

The terms Rh factor, Rh positive, and Rh negative refer to the Rh(D) antigen only. Antibodies to Rh antigens can be involved in hemolytic transfusion reactions and antibodies to the Rh(D) and Rh antigens confer significant risk of hemolytic disease of the fetus and newborn. Depending on the race, 85 to 99 percent of the white population have this rhesus antigen (also called Rh factor) and are called Rh positive (Rh^+).

Others who do not have this factor arc known as Rh negative (Rhr). Rh^+ is dominant to Rh^-. Whites Rh^+ 85%, Rh^- 15%, American Blacks Rh^+ 95%, Rh^- 5%, African Blacks Rh^+ 100%. Formation of Rh protein is controlled by a dominant gene which may be called as R. Thus, RR (homozygous) and Rr (heterozygous) persons are dominant and are Rh positive and rr (homozygous) are recessive and are Rh^- negative. Both Rh^+ and Rh^- individuals are phenotypically normal. The problem arises during blood transfusion and pregnancy.

4.4.11.6 Rh Antigen

The Rh antigens are expressed as part of a protein complex in the RBC membrane. This complex is only expressed in cells of the erythroid line, and therefore Rh antigens are only expressed in RBCs. The composition of the complex is unknown, but it is thought to be a tetramer, consisting of two molecules of Rh-associated glycoprotein (RhAG) and two molecules of Rh proteins. The Rh proteins may be RhD (carrying the D antigen) or RhCE (carrying the C or c antigen and the E or e antigen). It is unknown whether both RhCE and RhD can be in a single complex, but in D-negative individuals the complex would only contain RhCE.

RhAG must be present to direct the Rh antigens to the RBC membrane. If it is missing, none of the Rh antigens are expressed. RHAG is related to the Rh proteins, sharing about 35% of their primary sequence and is the same type of transmembrane protein. However, it is not polymorphic and does not carry Rh antigens itself.

4.4.11.7 Function of Rh proteins

The Rh antigens are thought to play a role in maintaining the integrity of the RBC membrane—RBCs which lack Rh antigens have an abnormal shape. Individuals with the rare Rh_{null} phenotype caused by the deletion of RHAG have RBCs that do not express any of the Rh antigens because they cannot be targeted to the RBC membrane. The absence of the Rh

complex alters the RBC shape, increases its osmotic fragility, and shortens its lifespan, resulting in a hemolytic anemia that is usually mild in nature. These patients are at risk of adverse transfusion reactions because they may produce antibodies against several of the Rh antigens. Rh antigens may also be involved in the transport of ammonium across the RBC membrane.

4.4.11.8 Rh Antibodies

Rh antibodies are IgG antibodies which are acquired through exposure to Rh-positive blood (generally either through pregnancy or transfusion of blood products). The D antigen is the most immunogenic of all the non-ABO antigens. Approximately 80% of individuals who are D-negative and exposed to a single D-positive unit will produce an anti-D antibody. The percentage of alloimmunization is significantly reduced in patients who are actively exsanguinating.

All Rh antibodies *except* D display dosage (antibody reacts more strongly with red cells homozygous for an antigen than cells heterozygous for the antigen (EE stronger reaction vs Ee). If anti-E is detected, the presence of anti-c should be strongly suspected (due to combined genetic inheritance). It is therefore common to select c-negative and E-negative blood for transfusion patients who have an anti-E. Anti-c is a common cause of delayed hemolytic transfusion reactions.

4.4.11.9 Clinical significance of Rh Antibodies

The Rh antigens are highly immunogenic, and most of the Rh antibodies should be considered as potential causes of hemolytic transfusion reactions and HDN. The Rh antigen poses a danger for the Rh-negative person, who lacks the antigen, if Rh-positive blood is given in transfusion.

4.4.11.10 Incompatibility during Blood Transfusion

The first blood transfusion of Rh^+ blood to the person with Rhr blood causes no harm because the Rh^- person develops anti Rh factors or antibodies in his/her blood. In second blood transfusion of Rh^+ blood to the Rh^- person, the latter's anti Rh factors attack and destroy the red blood corpuscles of the donor.

4.3.11.11 Incompatibility during Pregnancy

If father's blood is Rh^+ and mother's blood is Rh^-, the foetus' (baby in the uterus) blood is Rh^+. This is a serious problem. If the Rh^- blood of mother has not earlier come in contact with Rh^+ blood through transfusion, her first child does not suffer (although the Rh^+ blood of the foetus stimulates the formation of anti Rh factors or antibodies in the mother's blood yet enough anti Rh factors are not produced in the mother's blood to harm the foetus).

But in the subsequent Rh^+ foetuses, the anti Rh factors (antibodies) of the mother' blood destroy the foetal red blood corpuscles. This results in haemolytic disease of the newborn (HDN). It is called erythroblastosis foetalis (destruction of the erythrocytes of foetus). nNewborn may survive but it is often anaemic. In order to prevent HDN, Rh^- mothers are injected with a defective anti Rh-antibody during all pregnancies carrying Rh^+ foetus. Marriage between Rh^- woman and Rh^+ man is not recommended. Rh^+ is dominant.

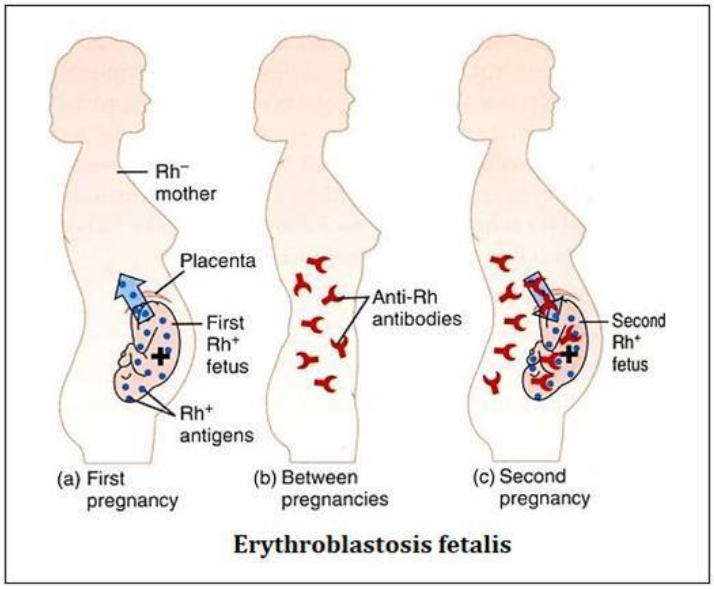

Fig. 14 Erythroblastosis Foetalis

Terminal questions

Q.8. Describe antigens with their nature and types.

Answer:----------

Q.9. Describe endogenous pathway or MHC class I antigen presentation pathway.

Answer:----------

Q.10. Describe exogenous pathway or MHC class II antigen presentation pathway.

Answer:----------

Q.11. Explain cell mediated immune response.

Answer:----------

Q.12. Explain humoral immune response.

Answer:----------

Q.13. Describe cytokine and its structure.

Answer:----------

Q.14. Write a short note on the followings.

(a) Interferon

(b) Vaccines.

Answer:----------

Q.15. Write a short note on the followings.

(c) Influenza virus

(d) Measles vaccine

Further readings

6. Biochemistry- Lehninger A.L.

7. Biochemistry –J.H.Weil.

8. Biochemistry fourth edition-David Hames and Nigel Hooper.

9. Textbook of Biochemistry for Undergraduates - Rafi, M.D.

10. Biochemistry and molecular biology- Wilson Walker.

Block- III

Infectious diseases & Immunology

Unit-5
Immune response to infectious diseases

Unit-6
Transplantation immunology

Introduction

This is the third block on Infectious diseases and immunology. It consists of following two units as given below.

Unit: 5: All living things are subject to attack from disease-causing agents. Even bacteria, so small that more than a million could fit on the head of a pin, have systems to defend against infection by viruses. This kind of protection gets more sophisticated as organisms become more complex. Multicellular animals have dedicated cells or tissues to deal with the threat of infection. Some of these responses happen immediately so that an infecting agent can be quickly contained. Other responses are slower but are more tailored to the infecting agent. Collectively, these protections are known as the **immune system**. The human immune system is essential for our survival in a world full of potentially dangerous microbes, and serious impairment of even one arm of this system can predispose to severe, even life-threatening, infections.

Unit: 6 Transplantation is the process of moving cells, tissues or organs from one site to another for the purpose of replacing or repairing damaged or diseased organs and tissues. It saves thousands of lives each year. However, the immune system poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another. Rejection is caused by the immune system identifying the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue. Long term survival of the transplant can be maintained by manipulating the immune system to reduce the risk of rejection. Donor and recipient are carefully matched prior to transplantation to minimise the risk of rejection. They are matched based on their blood group, tissue typing, and how the recipient's blood serum reacts to donor cells. Immunosuppressive drugs are used to prevent and to treat transplant rejection by dampening the overall immune response.

However, immunosuppressive drugs are non-specific and leave patients more susceptible to disease as well as being associated with numerous unwanted side effects. Further research on the immunological mechanisms of rejection will help improve cross matching, diagnosis and treatment, as well as facilitating the discovery of novel strategies for preventing. The immune system plays a critical role in transplantation. The complex mechanisms of immunity, which under normal circumstances work to

identify foreign microbes and direct the immune system to destroy them, pose a significant barrier to successful transplantation. Rejection of a transplant occurs in instances where the immune system identifies the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue.

Unit-5: Immune response to infectious diseases

Structure

5.1 Introduction

Objectives

This is the first block on Immunology. It consists of following two units. Under first unit (Elements of Immune System) we have following objectives. These are as under:

- To know about purpose and principles of immunology.
- To know about immune system and its properties
- To know about natural immunity, acquired immunity and immunization.
- To know about mechanism of hypersensitivity and immediate (type I) hypersensitivity
- To know about transplant immunology and the immunology of transplant rejection

5.2 Infectious diseases

Infectious diseases are disorders caused by organisms, such as bacteria, viruses, fungi or parasites. Many organisms live in and on our bodies. They're normally harmless or even helpful. But under certain conditions, some organisms may cause disease. Some infectious diseases can be passed from person to person. Some are transmitted by insects or other animals. And you may get others by consuming contaminated food or water or being exposed to organisms in the environment. Signs and symptoms vary depending on the organism causing the infection, but often include fever and fatigue. Mild infections may respond to rest and home remedies, while some life-threatening infections may need hospitalization. Many infectious diseases, such as measles and chickenpox, can be prevented by vaccines. Frequent and thorough hand-washing also helps protect you from most infectious diseases.

5.2.1 Symptoms

Each infectious disease has its own specific signs and symptoms. General signs and symptoms common to a number of infectious diseases include:

- Fever
- Diarrhea
- Fatigue
- Muscle aches
- Coughing

5.2.2 Causes

Infectious diseases can be caused by:

- **Bacteria.** These one-cell organisms are responsible for illnesses such as strep throat, urinary tract infections and tuberculosis.
- **Viruses.** Even smaller than bacteria, viruses cause a multitude of diseases ranging from the common cold to AIDS.
- **Fungi.** Many skin diseases, such as ringworm and athlete's foot, are caused by fungi. Other types of fungi can infect your lungs or nervous system.
- **Parasites.** Malaria is caused by a tiny parasite that is transmitted by a mosquito bite. Other parasites may be transmitted to humans from animal feces.

5.2.3 Direct contact

An easy way to catch most infectious diseases is by coming in contact with a person or an animal with the infection. Infectious diseases can be spread through direct contact such as:

- **Person to person.** Infectious diseases commonly spread through the direct transfer of bacteria, viruses or other germs from one person to another. This can happen when an individual with the bacterium or virus touches, kisses, or coughs or sneezes on someone who isn't infected.

  These germs can also spread through the exchange of body fluids from sexual contact. The person who passes the germ may have no symptoms of the disease, but may simply be a carrier.

- **Animal to person.** Being bitten or scratched by an infected animal — even a pet — can make you sick and, in extreme circumstances, can be fatal. Handling animal waste can be hazardous, too. For example, you can get a toxoplasmosis infection by scooping your cat's litter box.

- **Mother to unborn child.** A pregnant woman may pass germs that cause infectious diseases to her unborn baby. Some germs can pass through the placenta or through breast milk. Germs in the vagina can also be transmitted to the baby during birth.

5.2.4 Indirect contact

Disease-causing organisms also can be passed by indirect contact. Many germs can linger on an inanimate object, such as a tabletop, doorknob or faucet handle. When you touch a doorknob handled by someone ill with the flu or a cold, for example, you can pick up the germs he or she left behind. If you then touch your eyes, mouth or nose before washing your hands, you may become infected.

5.2.5 Insect bites

Some germs rely on insect carriers, such as mosquitoes, fleas, lice or ticks — to move from host to host. These carriers are known as vectors. Mosquitoes can carry the

malaria parasite or West Nile virus. Deer ticks may carry the bacterium that causes Lyme disease.

5.2.6 Food contamination

Disease-causing germs can also infect you through contaminated food and water. This mechanism of transmission allows germs to be spread to many people through a single source. Escherichia coli (E. coli), for example, is a bacterium present in or on certain foods — such as undercooked hamburger or unpasteurized fruit juice.

5.2.7 Complications

Most infectious diseases have only minor complications. But some infections — such as pneumonia, AIDS and meningitis — can become life-threatening. A few types of infections have been linked to a long-term increased risk of cancer:

- Human papillomavirus is linked to cervical cancer.
- Helicobacter pylori is linked to stomach cancer and peptic ulcers.
- Hepatitis B and C have been linked to liver cancer.

In addition, some infectious diseases may become silent, only to appear again in the future — sometimes even decades later. For example, someone who's had chickenpox may develop shingles much later in life.

5.2.8 Prevention

Follow these tips to decrease the risk of infection:

- **Wash your hands.** This is especially important before and after preparing food, before eating, and after using the toilet. And try not to touch your eyes, nose or mouth with your hands, as that's a common way germs enter the body.
- **Get vaccinated.** Vaccination can drastically reduce your chances of contracting many diseases. Make sure to keep up to date on your recommended vaccinations, as well as your children's.
- **Stay home when ill.** Don't go to work if you are vomiting, have diarrhea or have a fever. Don't send your child to school if he or she has these signs, either.

- **Prepare food safely.** Keep counters and other kitchen surfaces clean when preparing meals. Cook foods to the proper temperature, using a food thermometer to check for doneness. For ground meats, that means at least 160 F (71 C); for poultry, 165 F (74 C); and for most other meats, at least 145 F (63 C).

  Also promptly refrigerate leftovers — don't let cooked foods remain at room temperature for long periods of time.

- **Practice safe sex.** Always use condoms if you or your partner has a history of sexually transmitted infections or high-risk behavior.

- **Don't share personal items.** Use your own toothbrush, comb and razor. Avoid sharing drinking glasses or dining utensils.

- **Travel wisely.** If you're traveling out of the country, talk to your doctor about any special vaccinations, such as yellow fever, cholera, hepatitis A or B, or typhoid fever — you may need.

5.3 Immune response to infection

When a pathogenic (disease-causing) microorganism invades the body for the first time, the clinical (observable) response may range from nothing at all, through various degrees of nonspecific reactions, to specific infectious disease. Immunologically, however, there is always a response, the purpose of which is defense. If the defense is completely successful, there is no obvious bodily reaction; if it is partially successful, the affected person exhibits symptoms but recovers from an infectious disease; if unsuccessful, the person may be overwhelmed by the infectious process and die.

The two responses; the clinical and the immunologic, can be illustrated by the natural history of the disease poliomyelitis. When the virus of this disease enters the body for the first time, it multiplies in the throat and in the intestinal tract. In some people, it gets no farther; virus is shed to the outside from the throat and the bowel for a few weeks, and then the shedding ceases and the infection is over. The host, however, has responded and has developed circulating antibodies to a type of poliovirus. These

antibodies are specific antipoliovirus proteins in the blood and body fluids that subsequently prevent disease should the poliovirus again be encountered. In addition, the infected individual develops an antipoliovirus response in a subset of white blood cells known as T cells. Antipoliovirus T cells persist throughout the individual's lifetime.

In other people, the same process occurs but some virus also gets into the bloodstream, where it circulates for a short time before being eliminated. In a few individuals, the virus passes from the bloodstream into the central nervous system, where it circulates for a short time before being eliminated. Finally, in some individuals, the virus passes from the bloodstream into the central nervous system, where it may enter and destroy some of the nerve cells that control movement in the body and so cause paralysis. Such paralysis is the least-common result of infection with poliomyelitis virus; most infected persons have no symptoms at all. Those whose bloodstream contains virus often have a mild illness, consisting of no more than malaise, slight headache, and possibly a sore throat. This so-called minor illness of poliomyelitis is unlikely to be recognized except by those in close contact with someone later paralyzed by the disease.

If the nervous system becomes invaded by the virus, the infected person has a severe headache and other symptoms suggesting meningitis. Such persons are acutely ill, but most recover their normal health after about one week. Only a few of those with this type of infection have paralysis. Of all the people infected with poliomyelitis virus, not more than 1 in 100, possibly as few as 1 in 1,000, has paralysis, though paralysis is the dominant feature of the fully developed clinical picture of poliomyelitis. This poliomyelitis is actually an uncommon complication of poliovirus infection.

This wide range of response to the poliomyelitis virus is characteristic of most infections, though the proportions may vary. Influenza virus, for example, may cause symptoms ranging from a mild cold to a feverish illness, severe laryngitis (inflammation of the larynx, or voice box) or bronchitis, or an overwhelming and fatal pneumonia. The proportions of a population with these differing outcomes may vary from one epidemic to another. There is perhaps more

uniformity of pattern in the operation of the defense mechanisms of the body, called the immune response.

5.4 Natural and acquired immunity

Every animal species possesses some natural resistance to disease. Humans have a high degree of resistance to foot-and-mouth disease, for example, while the cattle and sheep with which they may be in close contact suffer in the thousands from it. Rats are highly resistant to diphtheria, whereas unimmunized children readily contract the disease. What such resistance depends on is not always well understood. In the case of many viruses, resistance is related to the presence on the cell surface of protein receptors that bind to the virus, allowing it to gain entry into the cell and thus cause infection. Presumably, most causes of absolute resistance are genetically determined; it is possible, for example, to produce by selective breeding two strains of rabbits, one highly susceptible to tuberculosis, the other highly resistant. In humans there may be apparent racial differences, but it is always important to disentangle such factors as climate, nutrition, and economics from those that might be genetically determined.

In some tropical and subtropical countries, for example, poliomyelitis is a rare clinical disease, though a common infection, but unimmunized visitors to such countries often contract serious clinical forms of the disease. The absence of serious disease in the residents is due not to natural resistance, however, but to resistance acquired after repeated exposure to poliovirus from infancy onward. Unimmunized visitors from other countries, with perhaps stricter standards of hygiene, are protected from such immunizing exposures and have no acquired resistance to the virus when they encounter it as adults.

Natural resistance, in contrast to acquired immunity, does not depend upon such exposures. The human skin obviously has great inherent powers of resistance to infection, for most cuts and abrasions heal quickly, though often they are smothered with potentially pathogenic microorganisms. If an equal number of typhoid bacteria are spread on a person’s skin and on a glass plate, those on the skin die much more quickly than do those on the plate, suggesting that the skin has some bactericidal property against typhoid germs. The skin also varies in its resistance to infectious organisms at

different ages: impetigo is a common bacterial infection of children's skin but is rarer in adults, and acne is a common infection of the skin of adolescents but is uncommon in childhood or in older adults. The phenomenon of natural immunity can be illustrated equally well with examples from the respiratory, intestinal, or genital tracts, where large surface areas are exposed to potentially infective agents and yet infection does not occur.

If an organism causes local infection or gains entry into the bloodstream, a complicated series of events ensues. These events are described in detail in the article immune system, but they can be summarized as follows: special types of white blood cells called polymorpho nuclear leukocytes or granulocytes, which are normally manufactured in the bone marrow and which circulate in the blood, move to the site of the infection. Some of these cells reach the site by chance, in a process called random migration, since almost everybody site is supplied constantly with the blood in which these cells circulate. Additional granulocytes are attracted and directed to the sites of infection in a process called directed migration, or chemotaxis. When a granulocyte reaches the invading organism, it attempts to ingest the invader. Ingestion of bacteria may require the help of still other components of the blood, called opsonins, which act to coat the bacterial cell wall and prepare it for ingestion. An opsonin generally is a protein substance, such as one of the circulating immunoglobulins or complement components.

Once a prepared bacterium has been taken inside the white blood cell, a complex series of biochemical events occurs. A bacterium-containing vacuole (phagosome) may combine with another vacuole that contains bacterial-degrading proteins (lysozymes). The bacterium may be killed, but its products pass into the bloodstream, where they come in contact with other circulating white blood cells called lymphocytes. Two general types of lymphocytes—T cells and B cells, are of great importance in protecting the human host. When a T cell encounters bacterial products, either directly or via presentation by a special antigen-presenting cell, it is sensitized to recognize the material as foreign, and, once sensitized, it possesses an immunologic memory. If the T cell encounters the same bacterial product again, it immediately recognizes it and sets

up an appropriate defense more rapidly than it did on the first encounter. The ability of a T cell to function normally, providing what is generally referred to as cellular immunity, is dependent on the thymus gland. The lack of a thymus, therefore, impairs the body's ability to defend itself against various types of infections.

After a T cell has encountered and responded to a foreign bacterium, it interacts with B cells, which are responsible for producing circulating proteins called immunoglobulins or antibodies. There are various types of B cells, each of which can produce only one of the five known forms of immunoglobulin (Ig). The first immunoglobulin to be produced is IgM. Later, during recovery from infection, the immunoglobulin IgG, which can specifically kill the invading microorganism, is produced. If the same microorganism invades the host again, the B cell immediately responds with a dramatic production of IgG specific for that organism, rapidly killing it and preventing disease.

In many cases, acquired immunity is life long, as with measles or rubella. In other instances, it can be short-lived, lasting not more than a few months. The persistence of acquired immunity is related not only to the level of circulating antibody but also to sensitized T cells (cell-mediated immunity). Although both cell-mediated immunity and humoral (B-cell) immunity are important, their relative significance in protecting a person against disease varies with particular microorganisms. For example, antibody is of great importance in protection against common bacterial infections such as pneumococcal pneumonia or streptococcal disease and against bacterial toxins, whereas cell-mediated immunity is of greater importance in protection against viruses such as measles or against the bacteria that cause tuberculosis.

5.5 Immunization

Antibodies are produced in the body in response to either infection with an organism or, through vaccination, the administration of a live or inactivated organism or its toxin by mouth or by injection. When given alive, the organisms are weakened, or attenuated, by some laboratory means so that they still stimulate antibodies but do not produce their characteristic disease. However stimulated, the antibody-producing cells of the body remain sensitized to the infectious agent and can respond to it again, pouring out

more antibody. One attack of a disease, therefore, often renders a person immune to a second attack, providing the theoretical basis for active immunization by vaccines.

Antibody can be passed from one person to another, conferring protection on the antibody recipient. In such a case, however, the antibody has not been produced in the body of the second person, nor have his antibody-producing cells been stimulated. The antibody is then a foreign substance and is eventually eliminated from the body, and protection is short-lived. The most common form of this type of passive immunity is the transference of antibodies from a mother through the placenta to her unborn child. This is why a disease such as measles is uncommon in babies younger than one year. After that age, the infant has lost its entire maternal antibody and becomes susceptible to the disease unless protective measures, such as measles vaccination, are taken. Sometimes antibody is extracted in the form of immunoglobulin from blood taken from immune persons and is injected into susceptible persons to give them temporary protection against a disease, such as measles or hepatitis A.

Generally, active immunization is offered before the anticipated time of exposure to an infectious disease. When unvaccinated people are exposed to an infectious disease, two alternatives are available: active immunization may be initiated immediately in the expectation that immunity can be developed during the incubation period of the disease, or passive immunity can be provided for the interim period and then active immunization given at an appropriate time. The antigens (foreign substances in the body that stimulate the immune defense system) introduced in the process of active immunization can be live attenuated viruses or bacteria, killed microorganisms or inactivated toxins (toxoids), or purified cell wall products (polysaccharide capsules, protein antigens).

There are five basic requirements for an ideal vaccine. The agents used for immunization should not in themselves produce disease. The immunizing agent should induce long-lasting, ideally permanent, immunity. The agent used for immunization should not be transmissible to susceptible contacts of the person being vaccinated. The vaccine should be easy to produce, its potency easy to assess, and the antibody response to it measurable with common and inexpensive techniques. Finally, the agent in the

vaccine should be free of contaminating substances. It is also recognized, however, that vaccine transmissibility can be helpful—e.g., in the case of live polio vaccine, which can be spread from vaccinated children to others who have not been vaccinated.

The route by which an antigen is administered frequently determines the type and duration of antibody response. For example, intramuscular injection of inactivated poliomyelitis virus (Salk vaccine) generates less production of serum antibody and induces only a temporary systemic immunity; it may not produce substantial local gastrointestinal immunity and, therefore, may not prevent the carrying of the virus in the gastrointestinal tract. Live, attenuated, oral poliomyelitis virus (Sabin vaccine) induces both local gastrointestinal and systemic antibody production; thus, immunization by mouth is preferred.

The schedule by which a vaccine is given depends upon the epidemiology of the naturally occurring disease, the duration of immunity that can be induced, the immunologic status of the host, and, in some cases, the availability of the patient. Measles, for example, is present in many communities and poses a potential threat to many children over 5 months of age. A substantial number of infants, however, are born with measles antibody from their mothers, and this maternal antibody interferes with an adequate antibody response until they are between 12 and 15 months of age. Generally, the immunization of infants after the age of 15 months benefits the community at large. In measles outbreaks, however, it may be advisable to alter this schedule and immunize all infants between 6 and 15 months of age.

Lymphocytes are white blood cells that are also one of the body's main types of immune cells. They are made in the bone marrow and found in the blood and lymph tissue. The immune system is a complex network of cells known as immune cells that include lymphocytes. These cells work together to defend the body against foreign substances, such as bacteria, viruses, and cancer cells that can threaten its functioning.

5.6 Types of Lymphocytes

There are two categories of lymphocytes known as B lymphocytes and T lymphocytes. These are commonly referred to as B cells and T cells. Both types originate from stem cells in the bone marrow. From there, some cells travel to the thymus, where they become T cells. Others remain in the bone marrow, where they become B cells.

The job of B cells is to make antibodies, which are proteins produced by the immune system to fight foreign substances known as antigens. Each B cell is set to make one specific antibody. Each antibody matches an antigen in the same way that a key matches a lock, and when this happens, the antigen is marked for destruction. The job of T cells is to help the body kill cancer cells and control the immune response to foreign substances. They do this by destroying cells in the body that have been taken over by viruses or become cancerous. A third type of lymphocyte, known as a natural killer or NK cell, comes from the same place as B and T cells. NK cells respond quickly to several foreign substances and are specialized in killing cancer cells and virus-infected cells.

5.7 Roles and functions

There are different types of B cells and T cells that have specific roles in the body and the immune system.

B cells

Memory B cells

Memory B cells circulate in the body to start a fast antibody response when they find a foreign substance. They remain in the body for decades and become memory cells, which remember previously found antigens and help the immune system respond faster to future attacks.

Regulatory B cells

Regulatory B cells or Bregs make up around 0.5 percent of all B cells in healthy people. Although few in number, they have a vital role to play. Bregs have protective anti-inflammatory effects in the body and stop lymphocytes that cause inflammation. They also interact with several other immune cells and promote the production of regulatory T cells or Tregs.

T cells

Killer T cells

Killer or cytotoxic T cells scan the surface of cells in the body to see if they have become infected with germs, or if they have turned cancerous. If so, they kill these cells.

Helper T cells

Helper T cells “help” other cells in the immune system to start and control the immune response against foreign substances. There are different types of helper T cells, and some are more effective than others against different types of germs. For instance, a Th1 cell is more effective against germs that cause infection inside other cells, such as bacteria and viruses, while a Th2 cell is more effective against germs that cause infection outside of cells, such as certain bacteria and parasites.

Regulatory T cells or Tregs

Tregs control or suppress other cells in the immune system. They have both helpful and harmful effects. They maintain tolerance to germs, prevent autoimmune diseases, and limit inflammatory diseases. But they can also suppress the immune system from doing its job against certain antigens and tumors.

Memory T cells

Memory T cells protect the body against previously found antigens. They live for a long time after an infection is over, helping the immune system to remember previous infections. If the same germ enters the body a second time, memory T cells remember it and quickly multiply, helping the body to fight it more quickly.

Natural killer T cells

Natural killer T cells are a mixed group of T cells that share characteristics of both T cells and natural killer cells. They can influence other immune cells and control immune responses against substances in the body that trigger an immune response.

5.8 Normal ranges and levels

Lymphocyte levels can change according to a person's race, gender, location, and lifestyle habits. The normal lymphocyte range in adults is between 1,000 and 4,800 lymphocytes in 1 microliter (μL) of blood. In children, the normal range is between 3,000 and 9,500 lymphocytes in 1 μL of blood. Unusually high or low lymphocyte counts can be a sign of disease.

5.9 Primary and Secondary Immune Response

Humans and other animals live in an environment that is heavily populated by microorganisms. Some microbes are pathogenic and cause various types of infections. The immune system is the natural defense system of our body and the first line of defense designed to fight off against all potential risks that make us sick. It is made up of a network of cells, tissues and organs working together for the protective function. White blood cells are the most important defense cells found in the blood stream and lymphoid. There are different types of white blood cells such as T cells, B cells, macrophages, and neutrophils.

When an antigen (bacteria, virus, parasites, fungi, toxin, etc.) enters our body, the immune system reacts against the foreign particle and prevents the initiation of an infection. The reaction of the cells and fluids of the immune system against the foreign invading particle or pathogen is known as an immune response. There are two types of immune responses named primary immune response and secondary immune response. Primary immune response occurs when an antigen contacts immune system for the first time. Secondary immune response occurs when the immune system is exposed to the same antigen for the second and subsequent times. This is the key difference between primary and secondary immune response.

5.9.1 What is Primary Immune Response?

The immune system is evolved to combat various types of infections using diverse mechanisms. These mechanisms work together to respond to the invading pathogen or the antigen. When the antigen meets the immune system for the first time, the reaction that results from the immune cells and fluids is the primary immune response. Here, the immune system is exposed to the threat for the first time. Hence, it takes a longer time

to recognise the antigen and react against it. In general, the lag phase of the primary immune response goes several days to weeks without producing antibodies against the pathogen.

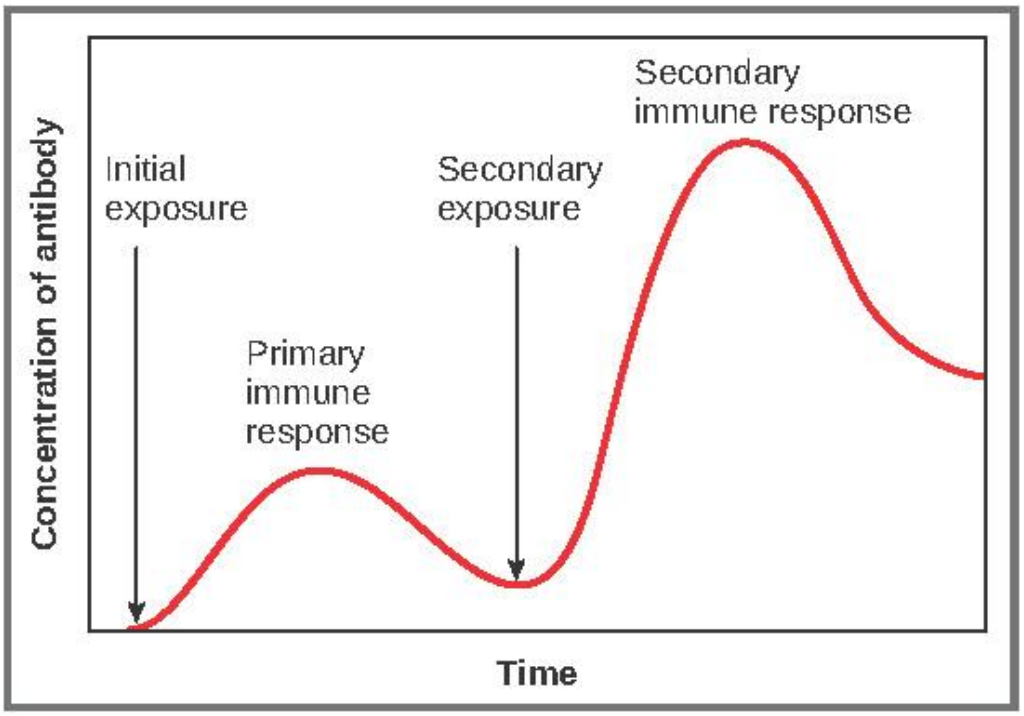

Fig. 1. Primary and Secondary Immune Responses

The duration of the lag phase depends on the nature of the antigen it encounters and the site of antigen entry. A low amount of antibodies is produced during the primary immune response by the naive B cells and T cells. The primary immune response appears mainly in lymph nodes and spleen. First antibodies produced are IgMs. Compared to IgG, IgM antibodies are produced more, and these antibodies drastically decline with time.

5.9.2 What is Secondary Immune Response?

The secondary immune response is the reaction of the immune system when an antigen contacts with it for the second and subsequent times. Since the immune cells have been exposed to the antigen previously, the establishment of immunity against the antigen is quick and strong. With the previous immunological memory, the immune response occurs immediately and starts making antibodies. Hence, the lag phase is very short in secondary immune response due to the presence of memory cells produced by B cells.

The amount of produced antibodies is high in secondary immune response, and they remain for a longer time, providing a good protection to the body. Within a short time, the level of the antibody rises to the peak. The main type of antibody produced is IgG. However, a small amount of IgM is also produced during the secondary immune response.

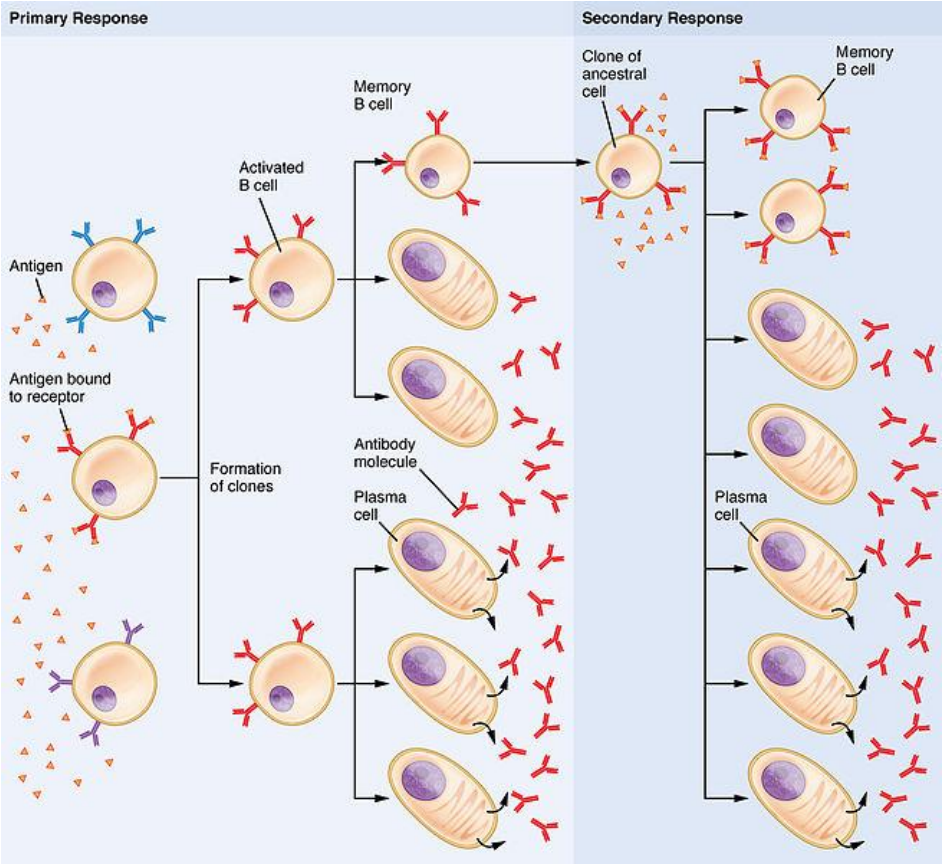

Fig. 2. Memory cells involved in Immune Response

Secondary immune response is mainly carried out by memory cells. Hence, the specificity is high, and the antibody affinities with antigens are also high in secondary immune response. Therefore, secondary immune response is considered to be more effective and stronger than primary immune response.

5.9.3 Primary vs Secondary Immune Responses

Immune responses can be categorized as primary and secondary immune responses. Primary immunity response occurs when an antigen contacts the immune system for the first time. Primary immune response takes a longer time to establish immunity over the antigen. Secondary immune response occurs when the same antigen contacts the immune system for the second and subsequent occasions. Due to immunological memory, secondary response rapidly establishes the immunity over those antigens. Primary immune response is done by naïve B cells and T cells. Secondary immune response is done by memory cells. This is the difference between primary and secondary immune response.

5.9.4 Signal transduction

Signal transduction is the process by which a chemical or physical signal is transmitted through a cell as a series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in a cellular response. Proteins responsible for detecting stimuli are generally termed receptors, although in some cases the term sensor is used. The changes elicited by ligand binding (or signal sensing) in a receptor give rise to a biochemical cascade, which is a chain of biochemical events known as a signaling pathway.

It is the process of transferring a signal throughout an organism, especially across or through a cell. Signal transduction relies on proteins known as *receptors*, which wait for a chemical, physical, or electrical signal. Chemical signals are called *ligands*, and can be produced by organisms to control their body or received from the environment. Regardless of which type of signal, it must be transferred throughout the body and across cell membranes. This process is known as signal transduction. A generalized image of signal transduction can be seen below.

Receptor proteins are specialized by the type of cell they are attached to. Each type of cell receives different signals from the body and environment, and must be specialized so that the body can produce a specific and coordinated response. Each of these specialized proteins has a special method of transferring a signal into the cell. Some proteins activate other molecules, called *second messengers*, which carry the message

to the nucleus or other organelles. Other proteins use the energy from ATP to activate enzymes, which carry out metabolic reactions. The different routes which signal transduction takes to carry a signal are known as *signal transduction pathways*.

5.9.5 Signal Transduction Pathway

During signal transduction, a signal may have many components. There is the *primary messenger*, which may be a chemical signal, electrical pulse, or even physical stimulation. Then, the receptor protein embedded in the cellular membrane must accept the signal. Upon receiving the signal, this protein goes through a *conformational change*. This changes its shape and thus, how it interacts with the molecules around it.

The many different receptor proteins act in different ways. Above is a simple representation of the many different signal transduction pathways in mammals. Do not be overwhelmed by the complexity of the drawing. The important thing to realize is that all of these signal transduction pathways contain the same elements. A signal is received by a receptor protein, and the protein transfers the signal through the cell membrane and into the cell. The kinds of receptors and the second messengers they create can be very different. This is based on the action which the signal must stimulate. There are some examples in the next section which will help shed light on the many differences and similarities between pathways.

5.9.5.1 Examples of Signal Transduction

Touch and Vision

The signal transduction pathway of touch and vision works in the same way that many nerve signals do. Instead of creating a second messenger or processing a signal internally, the stimulation of the receptor protein causes an influx of ions into the cell. This causes the cell membrane to *depolarize*. A normal cell membrane is *polarized*, or has a voltage potential across it. This voltage potential is created by the cell actively pumping ions out of the cell. Because ions are charged, by building them up in placed, a voltage can be created. When only one receptor protein is stimulated, only a small section of the membrane depolarizes. But when you receive a strong signal, such as

pressing your finger against a surface or seeing a bright light, the entire membrane of many cells is depolarized at the same time. This event triggers an *action potential*, which is how the signal travels down a nerve. This is caused by a series of other receptor proteins which are sensitive to the change in voltage. Upon feeling the voltage change, they too allow the ions to even out, which sends the signal down the cell.

Upon reaching the end of the first cell, the signal must cross a *synapse* to another nerve cell. To do this, another signal transduction pathway is used. As the action potential reaches the end of the first cell, specialized receptor proteins receive the signal, and trigger the release of *neurotransmitters*. These small ligands travel across the space between cells by diffusing through the fluid, and arrive at receptor proteins on the next cell. These receptors are also *gated ion channels*, and upon activation cause another action potential in the next nerve. In this way, a signal can travel from your finger or eye to your brain in a matter of microseconds.

Hormones

Unlike touch and vision, hormones are signals that your body creates to regulate itself. Hormones can cause the body to do many different things, and they themselves are often triggered by a separate signal transduction pathway. Typically, a hormone is release from an endocrine gland, such as the thyroid or pancreas. These hormones control everything from metabolism to growth. The signals they transmit are almost always transmitted through a ligand-receptor signal transduction pathway. Reproductive organs also release hormones, which function to prepare the body for reproduction.

Upon being triggered to release hormone, the cells in endocrine glands will release their stored hormone, which they have spent time building up. They do this by forcing vesicles full of the hormone to merge with the cell membrane, spilling the hormone into the intracellular space. *Capillaries*, or tiny blood vessels, run through this space. The hormone dissolves into the bloodstream, where it can be carried around the body.

Certain cells have specific receptors, which can activate different pathways upon receiving a signal. For instance, the hormone insulin can cause muscle cells to uptake

and store glucose, whereas it will cause liver cells to stop producing glucose. This helps regulate the total amount of glucose in the blood. The receptors in these different tissues both accept insulin as a ligand, but the signal transduction pathway is different. One pathway stimulates a cellular process in the muscle cells which increases the number of glucose transporters in their cell membrane. The other signal transduction pathway in the liver turns off a key enzyme which is required to produce glucose.

When signaling pathways interact with one another they form networks, which allow cellular responses to be coordinated, often by combinatorial signaling events. At the molecular level, such responses include changes in the transcription or translation of genes, and post-translational and conformational changes in proteins, as well as changes in their location. These molecular events are the basic mechanisms controlling cell growth, proliferation, metabolism and many other processes. In multicellular organisms, signal transduction pathways regulate cell communication in a wide variety of ways.

Once a signaling molecule (ligand) from one cell has bound to a receptor on another cell, is the signaling process complete? If we're talking about intracellular receptors, which bind their ligand inside of the cell and directly activate genes, the answer may be yes. In most cases, though, the answer is no, not by a long shot! For receptors located on the cell membrane, the signal must be passed on through other molecules in the cell, in a sort of cellular game of telephone.

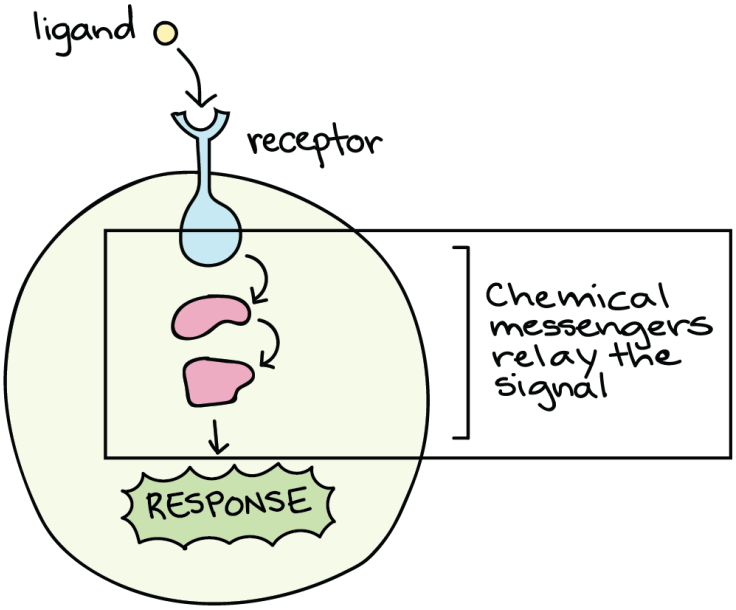

Fig. 3 Signaling through receptors

The chains of molecules that relay signals inside a cell are known as **intracellular signal transduction pathways**. Here, we'll look at the general characteristics of intracellular signal transduction pathways, as well as some relay mechanisms commonly used in these pathways.

5.1.0 Binding initiates a signaling pathway

When a ligand binds to a cell-surface receptor, the receptor's intracellular domain (part inside the cell) changes in some way. Generally, it takes on a new shape, which may make it active as an enzyme or let it bind other molecules. The change in the receptor sets off a series of signaling events. For instance, the receptor may turn on another signaling molecule inside of the cell, which in turn activates its own target. This chain reaction can eventually lead to a change in the cell's behavior or characteristics, as shown in the cartoon below.

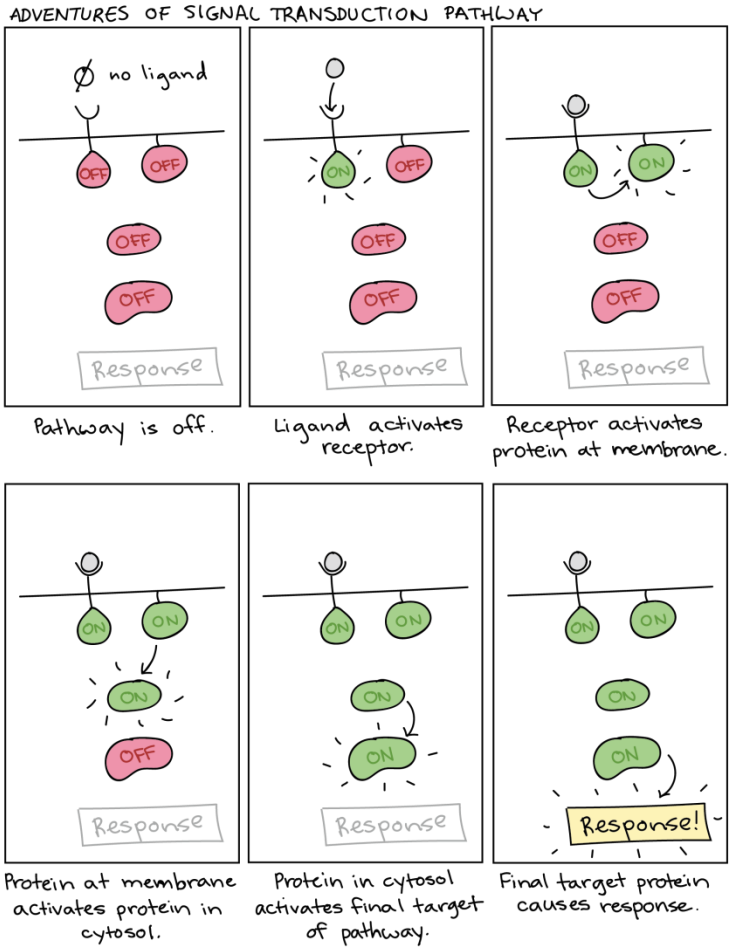

Fig. 4. Adventourous of signal transduction pathway

Because of the directional flow of information, the term **upstream** is often used to describe molecules and events that come earlier in the relay chain, while **downstream** may be used to describe those that come later (relative to a particular molecule of interest). For instance, in the diagram, the receptor is downstream of the ligand but upstream of the the proteins in the cytosol. Many signal transduction pathways amplify the initial signal, so that one molecule of ligand can lead to the activation of many molecules of a downstream target. The molecules that relay a signal are often proteins. However, non-protein molecules like ions and phospholipids can also play important roles.

5.1.1 Phosphorylation

The cartoon above features a bunch of blobs (signaling molecules) labeled as “on” or “off.” What does it actually mean for a blob to be on or off? Proteins can be activated or inactivated in a variety of ways. However, one of the most common tricks for altering protein activity is the addition of a phosphate group to one or more sites on the protein, a process called **phosphorylation**.

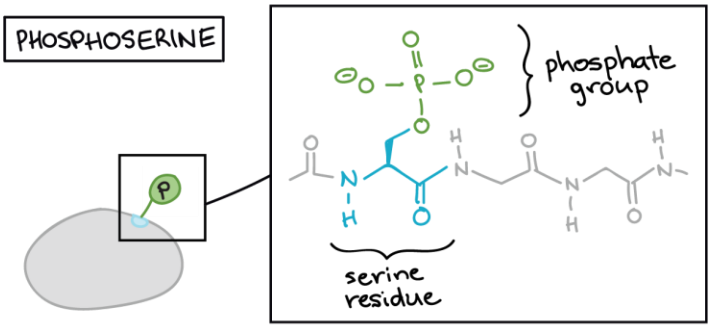

Fig. 5.

Phosphate groups can’t be attached to just any part of a protein. Instead, they are typically linked to one of the three amino acids that have hydroxyl (-OH) groups in their side chains: tyrosine, threonine, and serine. The transfer of the phosphate group is catalyzed by an enzyme called a **kinase**, and cells contain many different kinases that phosphorylate different targets.

Phosphorylation often acts as a switch, but its effects vary among proteins. Sometimes, phosphorylation will make a protein more active (for instance, increasing catalysis or letting it bind to a partner). In other cases, phosphorylation may inactivate the protein or cause it to be broken down. In general, phosphorylation isn’t permanent. To flip proteins back into their non-phosphorylated state, cells have enzymes called **phosphatases**, which remove a phosphate group from their targets.

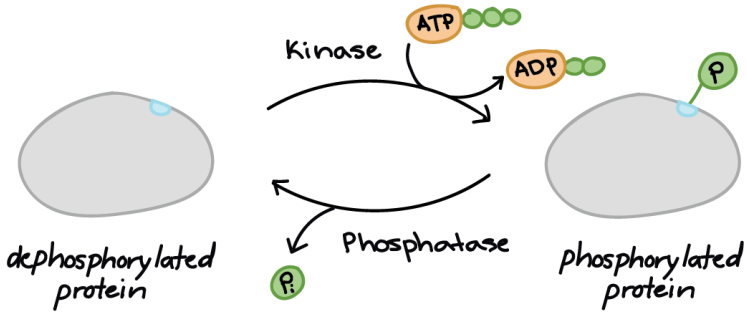

Fig. 6.

Phosphorylation example: MAPK signaling cascade

To get a better sense of how phosphorylation works, let's examine a real-life example of a signaling pathway that uses this technique: growth factor signaling. Specifically, we'll look at part of the epidermal growth factor (EGF) pathway that acts through a series of kinases to produce a cellular response. This diagram shows part of the epidermal growth factor signaling pathway:

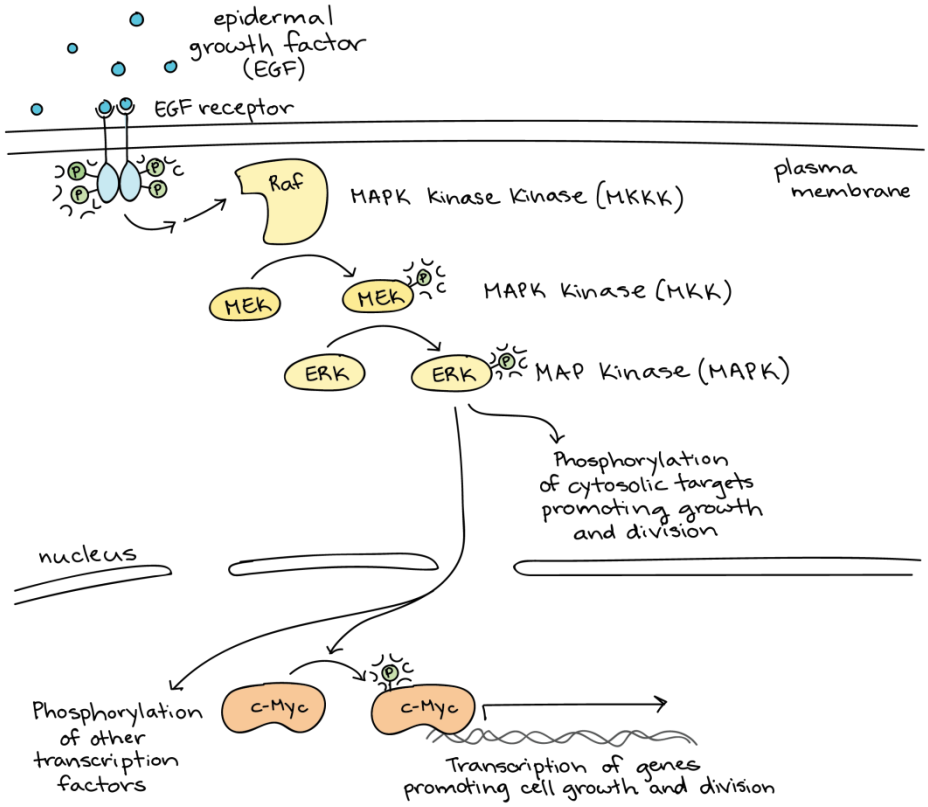

Fig. 7. Phosphorylation (marked as a P) is important at many stages of this pathway.

- When growth factor ligands bind to their receptors, the receptors pair up and act as kinases, attaching phosphate groups to one another's intracellular tails. Read more in the article on receptors and ligands.
- The activated receptors trigger a series of events (skipped here because they don't involve phosphorylation). These events activate the kinase Raf.
- Active Raf phosphorylates and activates MEK, which phosphorylates and activates the ERKs.
- The ERKs phosphorylate and activate a variety of target molecules. These include transcription factors, like c-Myc, as well as cytoplasmic targets. The activated targets promote cell growth and division.

  Together, Raf, MEK, and the ERKs make up a three-tiered kinase signaling pathway called a **mitogen-activated protein kinase** (**MAPK**) cascade. (A *mitogen* is a signal that causes cells to undergo *mitosis*, or divide.) Because they play a central role in promoting cell division, the genes encoding the growth factor receptor, Raf, and c-Myc are all proto-oncogenes, meaning that overactive forms of these proteins are associated with cancer. MAP kinase signaling pathways are widespread in biology: they are found in a wide range of organisms, from humans to yeast to plants. The similarity of MAPK cascades in diverse organisms suggests that this pathway emerged early in the evolutionary history of life and was already present in a common ancestor of modern-day animals, plants, and fungi squared.

Second messengers

Although proteins are important in signal transduction pathways, other types of molecules can participate as well. Many pathways involve **second messengers**, small, non-protein molecules that pass along a signal initiated by the binding of a ligand (the "first messenger") to its receptor. Second messengers include Ca^{2+} ions; cyclic AMP (cAMP), a derivative of ATP; and inositol phosphates, which are made from phospholipids.

Calcium ions

Calcium ions are a widely used type of second messenger. In most cells, the concentration of calcium ions Ca2+ in the cytosol is very low, as ion pumps in the

plasma membrane continually work to remove it. For signaling purposes, Ca^{2+} may be stored in compartments such as the endoplasmic reticulum. In pathways that use calcium ions as a second messenger, upstream signaling events release a ligand that binds to and opens ligand-gated calcium ion channels. These channels open and allow the higher levels of Ca^{2+} that are present outside the cell (or in intracellular storage compartments) to flow into the cytoplasm, raising the concentration of cytoplasmic Ca^{2+}.

How does the released Ca^{2+} help pass along the signal? Some proteins in the cell have binding sites for Ca^{2+} ions, and the released ions attach to these proteins and change their shape (and thus, their activity). The proteins present and the response produced are different in different types of cells. For instance, Ca^{2+} signaling in the β-cells of the pancreas leads to the release of insulin, while Ca^{2+} signaling in muscle cells leads to muscle contraction.

5.1.2 Cyclic AMP (cAMP)

Another second messenger used in many different cell types is **cyclic adenosine monophosphate** (**cyclic AMP** or **cAMP**), a small molecule made from ATP. In response to signals, an enzyme called **adenylyl cyclase** converts ATP into cAMP, removing two phosphates and linking the remaining phosphate to the sugar in a ring shape. Once generated, cAMP can activate an enzyme called **protein kinase A** (**PKA**), enabling it to phosphorylate its targets and pass along the signal. Protein kinase A is found in a variety of types of cells, and it has different target proteins in each. This allows the same cAMP second messenger to produce different responses in different contexts.

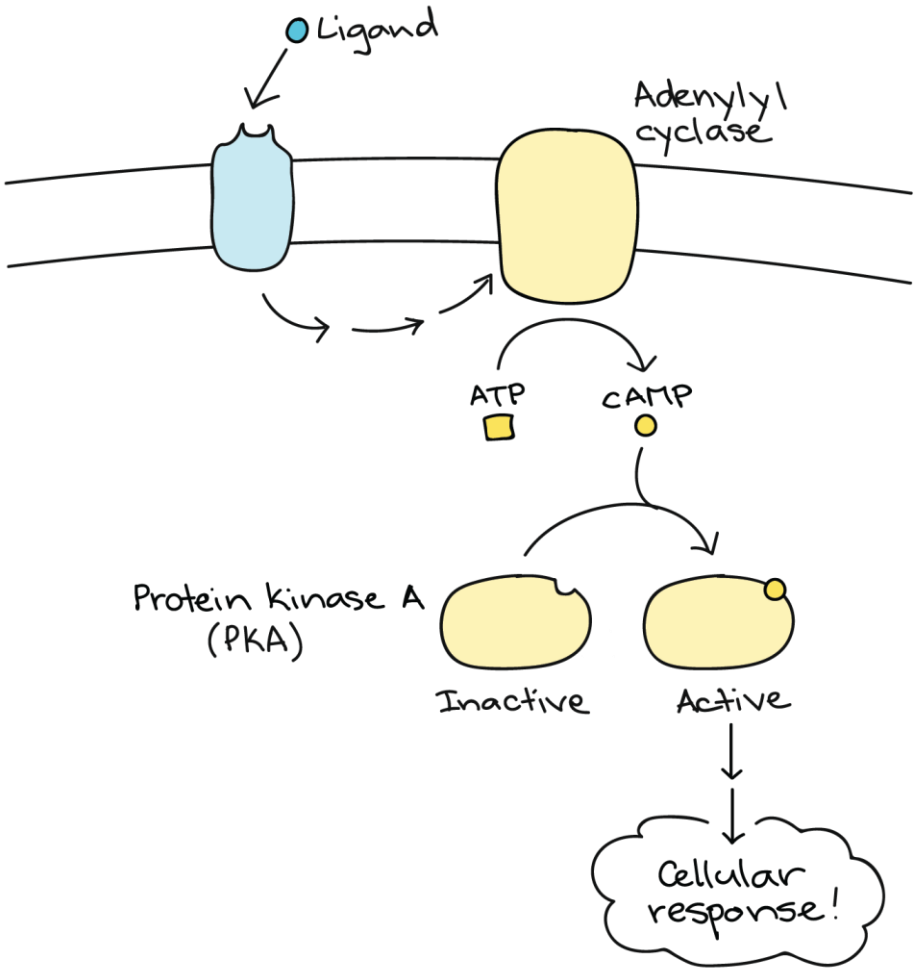

Fig. 8.

cAMP signaling is turned off by enzymes called **phosphodiesterases**, which break the ring of cAMP and turn it into adenosine monophosphate (AMP).

5.1.3 Inositol phosphates

Although we usually think of plasma membrane phospholipids as structural components of the cell, they can also be important participants in signaling. Phospholipids called **phosphatidylinositols** can be phosphorylated and snipped in half, releasing two fragments that both act as second messengers. One lipid in this group that's particularly important in signaling is called PIP2. In response to a signal, an enzyme called phospholipase C cleaves PIP2 into two fragments, DAG and IP3. These fragments made can both act as second messengers.

DAG stays in the plasma membrane and can activate a target called protein kinase C (PKC), allowing it to phosphorylate its own targets. IP3 diffuses into the cytoplasm and can bind to ligand-gated calcium channels in the endoplasmic reticulum, releasing Ca^{2+} that continues the signal cascade.

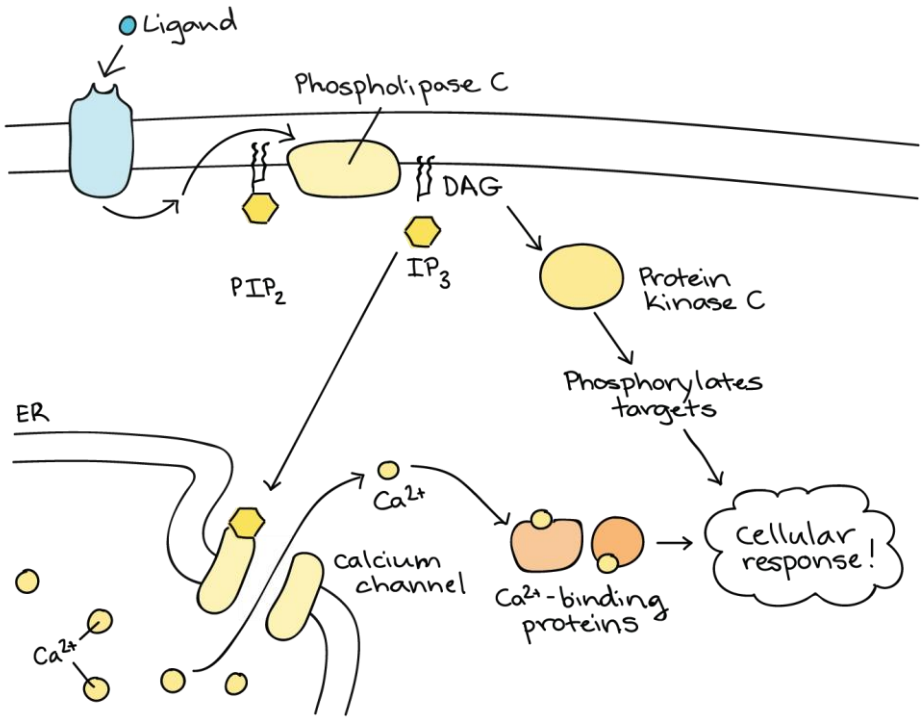

Fig. 9.

Antibody, also called **immunoglobulin**, a protective protein produced by the immune system in response to the presence of a foreign substance, called an antigen. Antibodies recognize and latch onto antigens in order to remove them from the body. A wide range of substances are regarded by the body as antigens, including disease-causing organisms and toxic materials such as insect venom.

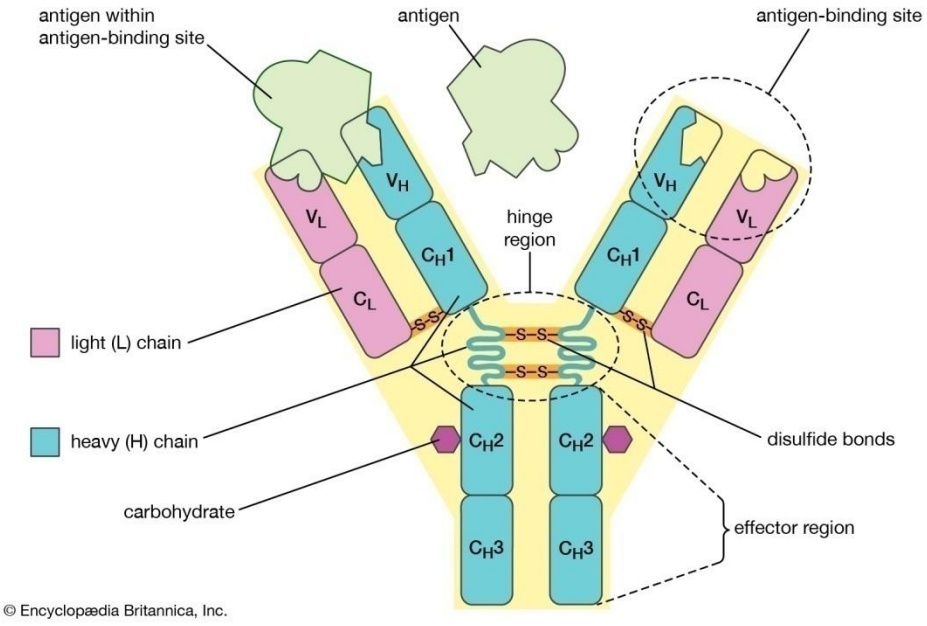

Fig. 10. Antibody structure

The four-chain structure of an antibody, or immunoglobulin, molecule. The basic unit is composed of two identical light (L) chains and two identical heavy (H) chains, which are held together by disulfide bonds to form a flexible Y shape. Each chain is composed of a variable (V) region and a constant (C) region.

When an alien substance enters the body, the immune system is able to recognize it as foreign because molecules on the surface of the antigen differ from those found in the body. To eliminate the invader, the immune system calls on a number of mechanisms, including one of the most important—antibody production. Antibodies are produced by specialized white blood cells called B lymphocytes (or B cells). When an antigen binds to the B-cell surface, it stimulates the B cell to divide and mature into a group of identical cells called a clone. The mature B cells, called plasma cells, secrete millions of antibodies into the bloodstream and lymphatic system.

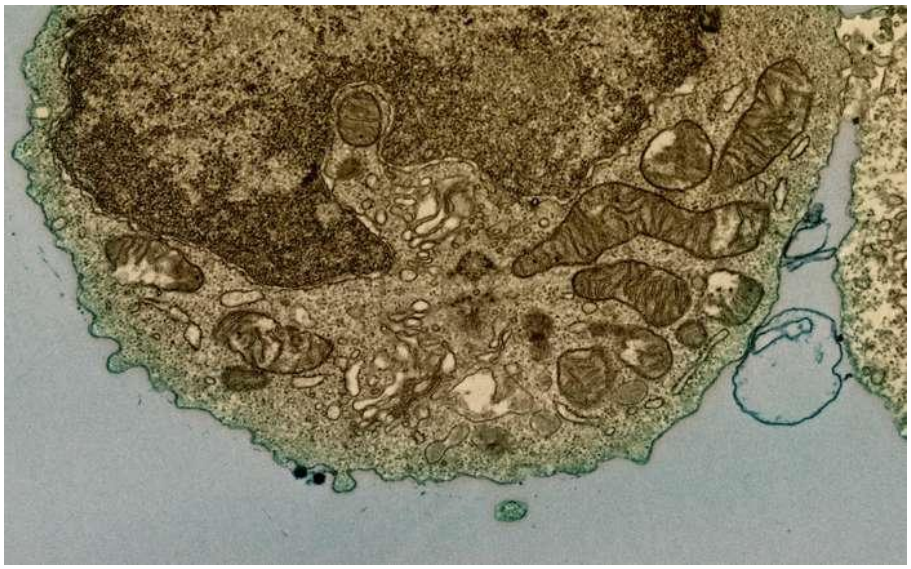

Fig. 11. Transmission electron micrograph
of a human B cell, or B lymphocyte. National Institute of Health, NIAID

As antibodies circulate, they attack and neutralize antigens that are identical to the one that triggered the immune response. Antibodies attack antigens by binding to them. The binding of an antibody to a toxin, for example, can neutralize the poison simply by changing its chemical composition; such antibodies are called antitoxins. By attaching themselves to some invading microbes, other antibodies can render such microorganisms immobile or prevent them from penetrating body cells. In other cases the antibody-coated antigen is subject to a chemical chain reaction with complement, which is a series of proteins found in the blood. The complement reaction either can trigger the lysis (bursting) of the invading microbe or can attract microbe-killing scavenger cells that ingest, or phagocytose, the invader. Once begun, antibody production continues for several days until all antigen molecules are removed. Antibodies remain in circulation for several months, providing extended immunity against that particular antigen.

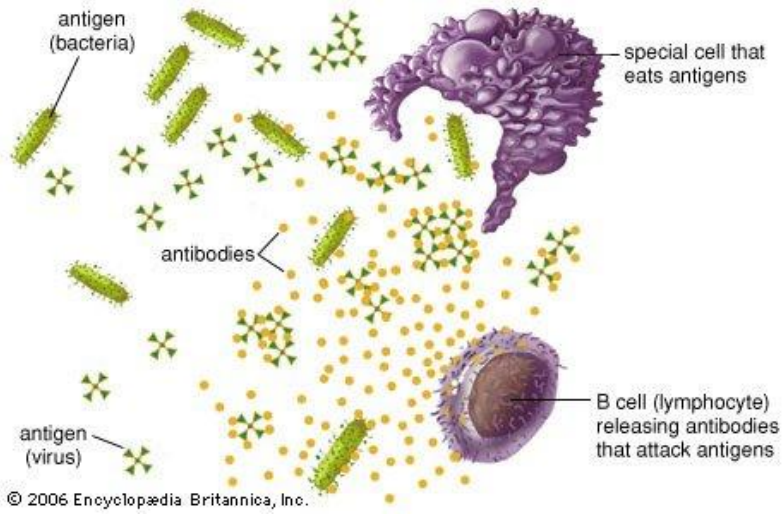

Fig. 12. Antigen; antibody; lymphocyte
Phagocytic cells destroy viral and bacterial antigens by eating them, while B cells produce antibodies that bind to and inactivate antigens.

B cells and antibodies together provide one of the most important functions of immunity, which is to recognize an invading antigen and to produce a tremendous number of protective proteins that scour the body to remove all traces of that antigen. Collectively B cells recognize an almost limitless number of antigens; however, individually each B cell can bind to only one type of antigen. B cells distinguish antigens through proteins, called antigen receptors, found on their surfaces. An antigen receptor is basically an antibody protein that is not secreted but is anchored to the B-cell membrane. All antigen receptors found on a particular B cell are identical, but receptors located on other B cells differ.

Although their general structure is similar, the variation lies in the area that interacts with the antigen—the antigen-binding, or antibody-combining, site. This structural variation among antigen-binding sites allows different B cells to recognize different antigens. The antigen receptor does not actually recognize the entire antigen; instead it binds to only a portion of the antigen's surface, an area called the antigenic determinant or epitope. Binding between the receptor and epitope occurs only if their structures are complementary. If they are, epitope and receptor fit together like two pieces

of a puzzle, an event that is necessary to activate B-cell production of antibodies.

Each antibody molecule is essentially identical to the antigen receptor of the B cell that produced it. The basic structure of these proteins consists of two pairs of polypeptide chains (lengths of amino acids linked by peptide bonds) that form a flexible Y shape. The stem of the Y consists of one end of each of two identical heavy chains, while each arm is composed of the remaining portion of a heavy chain plus a smaller protein called the light chain. The two light chains also are identical. Within particular classes of antibodies the stem and the bottom of the arms are fairly similar and thus are called the constant region. The tips of the arms, however, are highly variable in sequence. It is these tips that bind antigen. Thus each antibody has two identical antigen-binding sites, one at the end of each arm, and the antigen-binding sites vary greatly among antibodies.

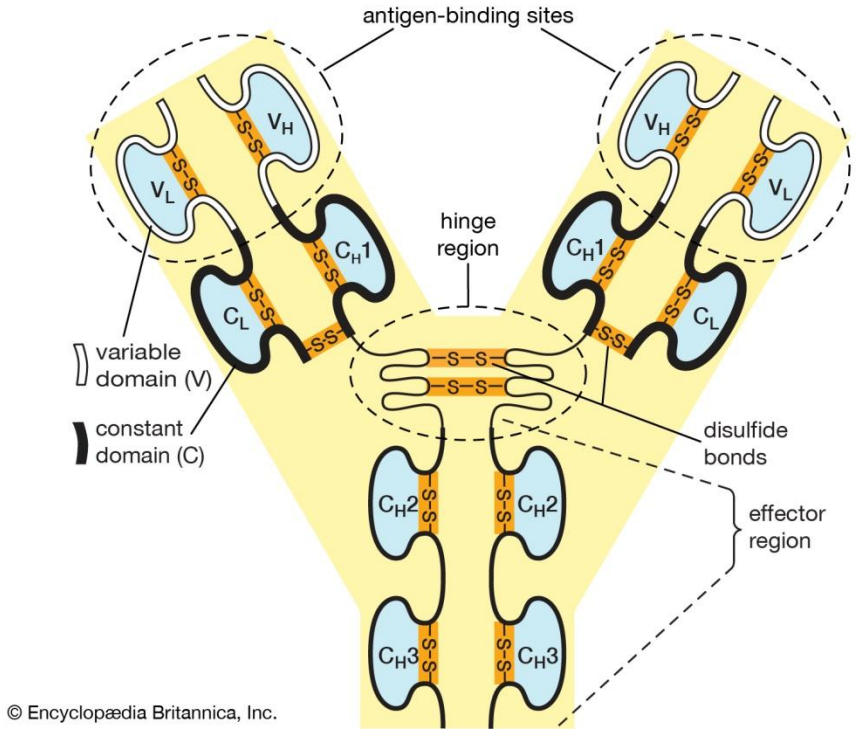

Fig. 13. Variable and constant domains of an antibody

Variable (V) and constant (C) domains within the light (L) and heavy (H) chains of an antibody, or immunoglobulin, molecule. The folded shapes of the domains are maintained by disulfide bonds (−S−S−).

5.1.4 Hypersensitivity

- **Hypersensitivity** is increased reactivity or increased sensitivity by the animal body to an antigen to which it has been previously exposed.

- The term is often used as a synonym for allergy, which describes a state of altered reactivity to an antigen.
- Hypersensitivity has been divided into categories based upon whether it can be passively transferred by antibodies or by specifically immune lymphoid cells.
- The most widely adopted current classification is that of Coombs and Gell that designates immunoglobulin-mediated (immediate) hypersensitivity reactions as types I, II, and III, and lymphoid cell-mediated (delayed-type) hypersensitivity/cell-mediated immunity as a type IV reaction.
- "Hypersensitivity" generally represents the "dark side," signifying the undesirable aspects of an immune reaction, whereas the term "immunity" implies a desirable effect.
- A hypersensitive response (HR) is an anti-pathogen response in plants produced by avr-R system activation that leads to alterations in Ca+ flux, MAPK activation, and NO and ROI formation.
- There is rapid necrosis of plant cells in contact with the pathogen.
- This process prevents spread of the pathogen and releases hydrolytic enzymes that facilitate injury to the pathogen's structural integrity.

5.1.4.1 Causes of Hypersensitivity

Immune responses that are the cause of hypersensitivity diseases may be specific for antigens from different sources:

- Autoimmunity: reactions against self antigens.
- Reactions against microbes.
- Reactions against non-microbial environmental antigens.

5.1.4.2 Mechanism of Hypersensitivity

Hypersensitivity diseases are commonly classified according to the type of immune response and the effector. mechanism responsible for cell and tissue injury. These mechanisms include some that are predominantly dependent on antibodies and others predominantly dependent on T cells, although a role for both humoral and cell-mediated immunity is often found in many hypersensitivity diseases.

5.1.4.2.1 Immediate (type I) hypersensitivity

It is caused by IgE antibodies specific for environmental antigens and is the most prevalent type of hypersensitivity disease. Immediate hypersensitivity diseases, commonly grouped under allergy or atopy, are often caused by activation of interleukin-4 (IL-4), IL-5, and IL-13 producing Th2 cells and the production of IgE antibodies, which activate mast cells and eosinophils and induce inflammation.

5.1.4.2.2 Antibody-mediated (type II) hypersensitivity

IgG and IgM antibodies specific for cell surface or extracellular matrix antigens can cause tissue injury by activating the complement system, by recruiting inflammatory cells, and by interfering with normal cellular functions. Immune complex-mediated (type III) hypersensitivity IgM and IgG antibodies specific for soluble antigens in the blood form complexes with the antigens, and the immune complexes may deposit in blood vessel walls in various tissues, causing inflammation, thrombosis, and tissue injury.

5.1.4.2.3 T cell-mediated (type IV) hypersensitivity

In these disorders, tissue injury may be due to T lymphocytes that induce inflammation or directly kill target cells. In most of these diseases, the major mechanism involves the activation of CD4+ helper T cells, which secrete cytokines that promote inflammation and activate leukocytes, mainly neutrophils and macrophages. CTLs contribute to tissue injury in some diseases.

5.1.4.2.4 Types of Hypersensitivity Reactions

The Gell's and Coombs' classification of hypersensitivity reactions considers four types of reactions. Type I, II, and III reactions are basically mediated by antibodies with or without participation of the complement system; type IV reactions are cell-mediated.

While in many pathological processes mechanisms classified in more than one of these types of hypersensitivity reactions may be operative, the subdivision of hypersensitivity states into four broad types aids considerably in the understanding of their pathogenesis.

Type I: Immediate reaction

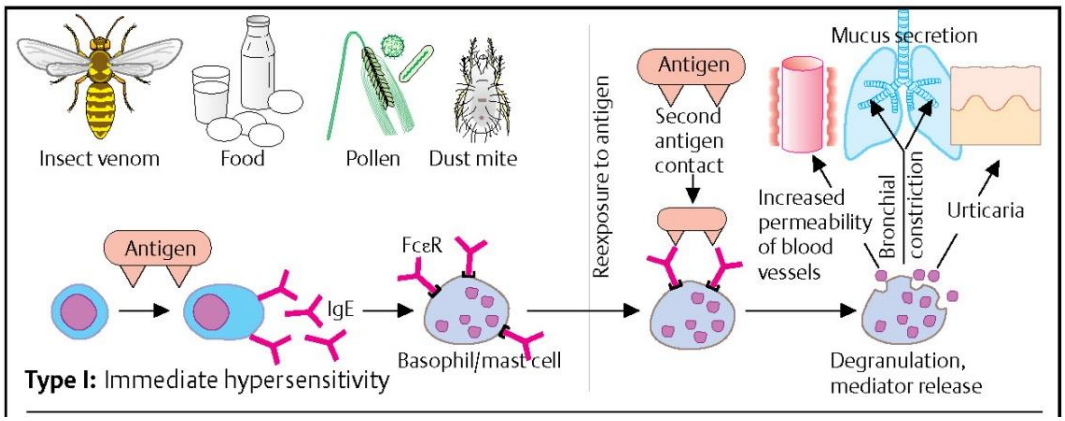

Fig. 14.

Some antigens (allergens), such as insect venom, foods, pollen, and dust mite, can induce the formation of IgE antibodies in individuals with a corresponding predisposition. The IgE antibodies bind via Fc receptors to mast cells (sensitization). If the individual is re-exposed to the allergen, cross-linkage of the membrane-bound IgE occurs. This results in the immediate release of mediators (e.g., histamine, kininogen), which induce vasodilation, smooth-muscle contraction, mucus secretion, edema, and/or skin blisters. Most allergens are small proteins that can easily diffuse through the skin or mucosa. They are frequently proteases and are active at very low doses. IL-4 favors differentiation of TH2 cells. The exact mechanism that leads B cells to produce IgE is not known.

Type II: Antibody-mediated cytotoxic reaction

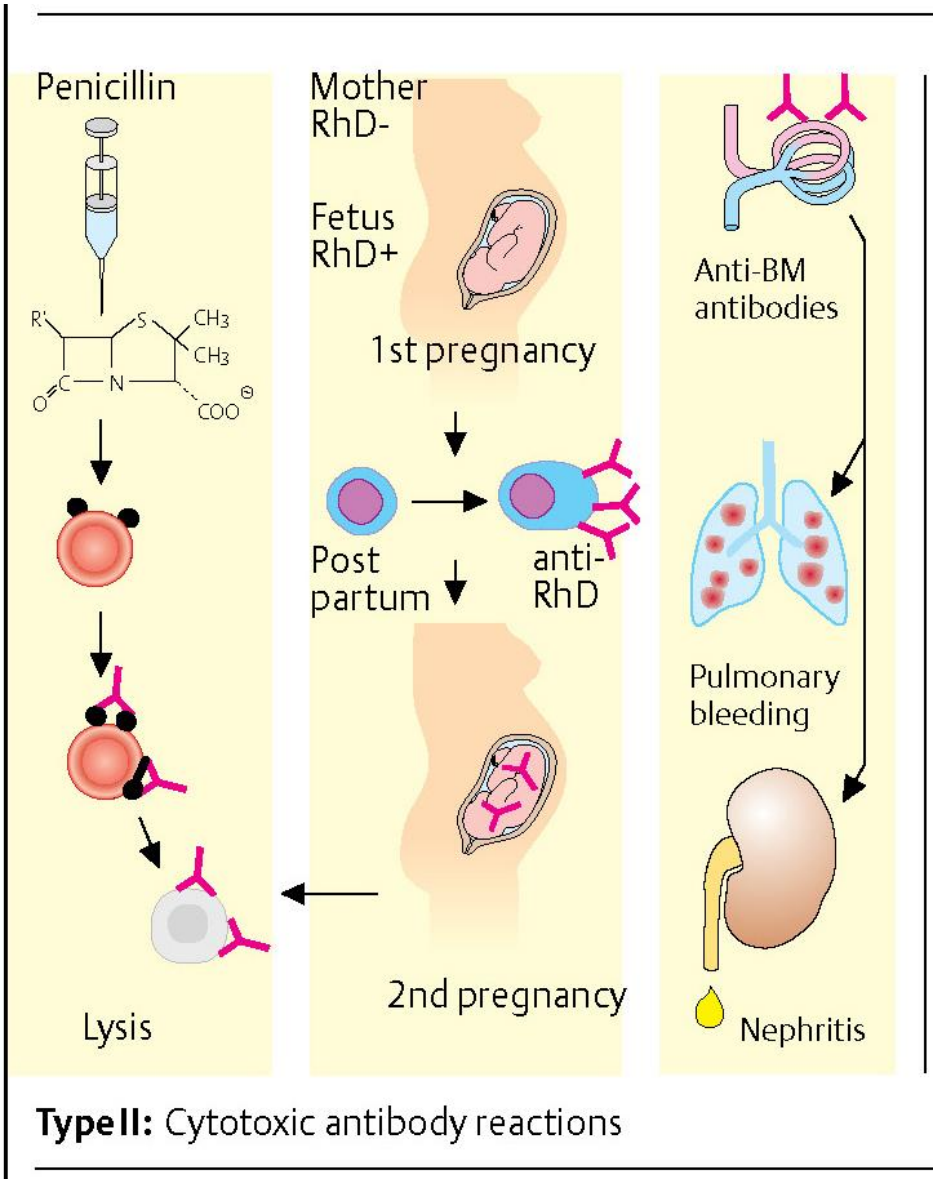

Fig. 15.

The immunization of individuals to erythrocyte antigens during pregnancy is a typical example of a type II reaction. Children who inherit the RhD erythrocyte antigen from their father can induce immunization against the RhD+ antigen in their RhD-mother. Sensitization usually occurs at birth when fetal blood cells come into contact with the maternal immune system. In any subsequent pregnancies, maternal anti-RhD antibodies of the IgG type can pass into the placenta and cause severe hemolysis of fetal RhD+ erythrocytes.

Other examples: Drugs (e.g., penicillin) can passively bind to erythrocytes. Antibodies directed against penicillin then lead to lysis of the erythrocytes. The formation of

antibodies directed against the basement membrane (BM) of the glomerulus can develop during the course of kidney inflammation. Lung damage accompanied by pulmonary hemorrhage and renal inflammation (glomerulonephritis) may occur due to cross-reaction of these antibodies with the basement membrane of the lung (Good-pasture's syndrome).

Type III: Immune complex-mediated reaction

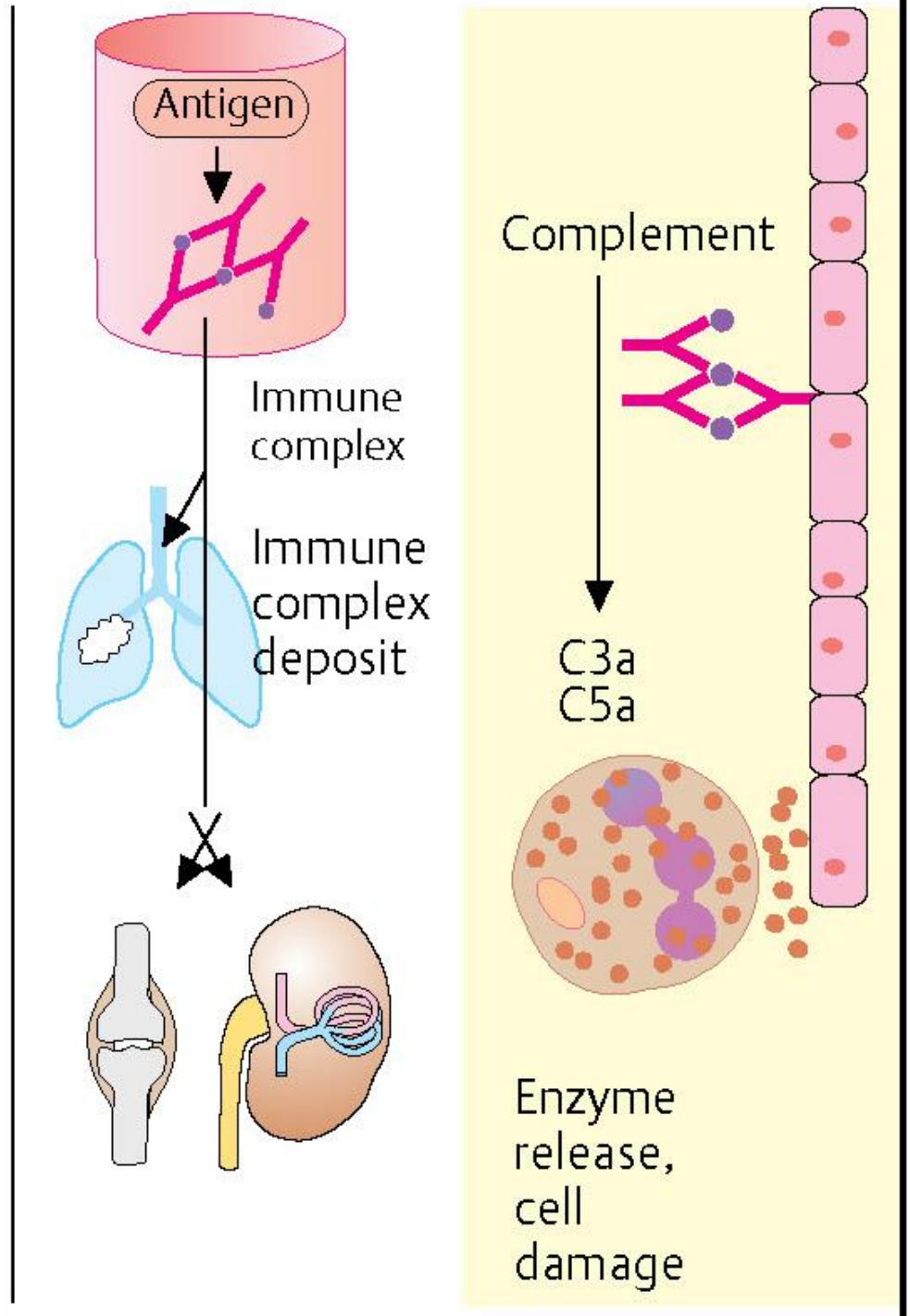
Antigen
Immune complex
Immune complex deposit
Complement
C3a
C5a
Enzyme release, cell damage
Type III: Immune complex reaction

Fig. 16.

Antibody-antigen complexes (immune complexes) can form during an immune response. Immune complexes can settle in vessel walls, the basement membrane of the lungs and/or kidneys, and in the joints (synovia). They can induce inflammatory processes in these structures by binding complement factors C3a and C5a (anaphylatoxins). A particular type III reaction is the Arthus reaction: when an antigen has penetrated the skin of an individual who has preformed IgG antibodies, the immune complexes can bind to Fc receptors of most cells inducing degranulation inflammatory cells are recruited and complement is activated, leading to the release of C5a and local inflammation, platelet accumulation, and eventually to blood vessel occlusion with necrosis.

Type IV: Delayed-type hypersensitivity reaction

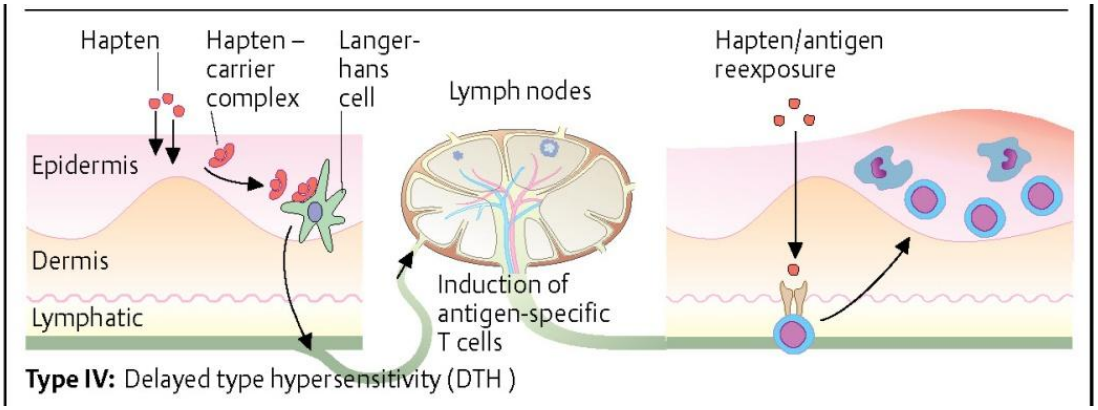

Fig. 17.

Haptens are molecules of very small molecular weight (often < 1 kDa). They are too small to function as antigens, but they can penetrate the epidermis and bind to certain proteins in the skin (carrier proteins). Hapten-carrier complexes are bound by antigen-presenting cells of the skin (Langerhans cells), which then migrate to regional lymph nodes. T-cell stimulation then occurs at the lymph node. The so-called sensitization phase lasts ca. 10-14 days. If the individual is reexposed to the hapten, antigen-specific T cells migrate to the skin, where they accumulate and proliferate. They also cause edema formation and local inflammation with the help of cytokines. Compounds containing nickel or chrome and chemicals such as those found in rubber are typical triggers of type IV hypersensitivity reactions.

5.1.5 Introduction to transplantation

Transplantation is the process of moving cells, tissues, or organs, from one site to another, either within the same person or between a donor and a recipient. If an organ system fails, or becomes damaged as a consequence of disease or injury, it can be replaced with a healthy organ or tissue from a donor. Organ transplantation is a major operation and is only offered when all other treatment options have failed. Consequently, it is often a life-saving intervention. In 2015/16, 4,601 patient lives were saved or improved in the UK by an organ transplant.i Kidney transplants are the most common organ transplanted on the NHS in the UK (3,265 in 2015/16), followed by the liver (925), and pancreas (230).i In addition, a total of 383 combined heart and lung transplants were performed, while in 2015/16. However, whole organs are not the only type of transplant. The cornea, for example, is the most transplanted single tissue, with 5,734 procedures carried out in 2015/16.i Hematopoietic stem cell transplantation (HSCT), often called blood and marrow transplantation (BMT), is another common tissue transplantation procedure. Used to treat a broad spectrum of diseases, though most commonly for blood or bone marrow cancers such as leukaemia and lymphoma, around 3,600 HSCT transplants were undertaken in 2012.

5.1.6 Transplant Immunology

- Transplantation is the process of moving cells, tissues or organs from one site to another for the purpose of replacing or repairing damaged or diseased organs and tissues. It saves thousands of lives each year. However, the immune system poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another.

- Rejection is caused by the immune system identifying the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue. Long term survival of the transplant can be maintained by manipulating the immune system to reduce the risk of rejection.
- Donor and recipient are carefully matched prior to transplantation to minimise the risk of rejection. They are matched based on their blood group, tissue typing, and how the recipient’s blood serum reacts to donor cells.

- Immunosuppressive drugs are used to prevent and to treat transplant rejection by dampening the overall immune response. However, immunosuppressive drugs are non-specific and leave patients more susceptible to disease as well as being associated with numerous unwanted side effects.
- Further research on the immunological mechanisms of rejection will help improve cross matching, diagnosis and treatment, as well as facilitating the discovery of novel strategies for preventing.

The immune system plays a critical role in transplantation. The complex mechanisms of immunity, which under normal circumstances work to identify foreign microbes and direct the immune system to destroy them, pose a significant barrier to successful transplantation. Rejection of a transplant occurs in instances where the immune system identifies the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue.

The intensity of the immune response against the organ or tissue, also commonly referred to as the graft, will depend on the type of graft being transplanted and the genetic disparity between the donor and recipient. To reduce the possibility of rejection, the donor and recipient are carefully matched for immune compatibility prior to transplantation. However, the small pool of eligible donors can make it difficult to find a donor-recipient match and there will always be a degree of rejection against the graft. A critical undersupply of donated organs means that waiting lists for transplants are extremely long. Patients needing a kidney transplantation, for example, wait on average 944 days (more than two and a half years) for a life-saving transplant.

There were 6,943 patients registered for organ transplant in the UK as of March 2015.i Unfortunately, 479 of these patients died during 2015/16 whilst waiting for a transplant due the small pool of transplantable organs.i These figures underline the value of every organ and highlight the importance of a successful transplantation and maintaining long-term transplant survival. Manipulation of the immune system can support longterm survival of the graft ensuring that every transplant is as successful as possible. There are several types of transplantation involving tissues and organs:

Autograft

Transplantation of cells, tissues or organs between sites within the same individual e.g. skin graft.

Allograft

Transplantation of organs or tissues from a donor to a non-genetically identical individual of the same species. Allografts are the most common type of transplant.

Xenograft

Transplantation of an organ or tissue between two different species. 'Pig valves', for example, are commonly used to repair or replace a defective heart valve in humans. In 2015/16, 6,069 xenograft valve replacements were carried out in England by the NHS. Xenotransplantation of whole organs is not currently viable, although it is an area of huge scientific interest as a potential solution for the existing critical undersupply of adequate organs.

ABO incompatible

ABO refers to blood group, which can vary between individuals. For most transplant types, matching of blood group between donor and recipient is a key strategy in reducing rejection risk. However, blood group compatibility is not always required for transplantations. For example, in the case of very young children with immature immune systems, ABO incompatible transplants can be carried out with less risk of transplant rejection.

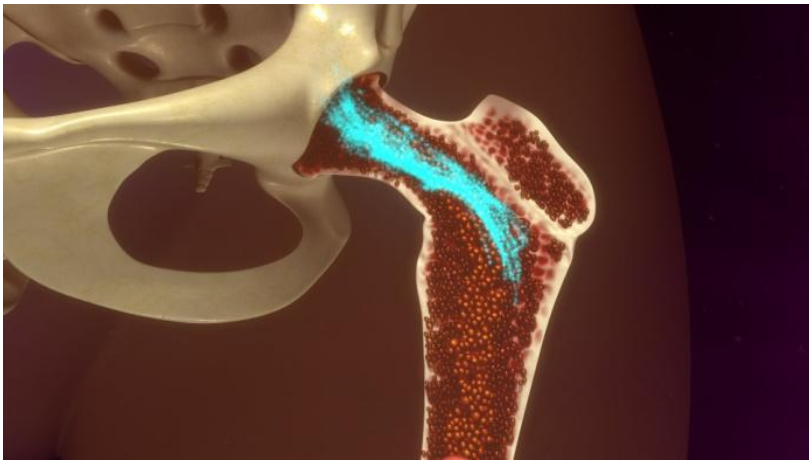

Fig. 18.

Stem cell transplant

Stem cells are cells that have the capacity to develop into a range of different types of cells in the body. Blood stem cells (haematopoietic stem cells) can develop into all the

different cells found in the blood and are donated to replace damaged or destroyed blood cells. Haematopoietic stem cell transplants are used to treat certain types of cancer e.g. leukaemia, and blood diseases where the bone marrow has become damaged preventing the production of healthy blood cells. These stem cells can be harvested either directly from bone marrow (see image on left) or from the cord blood (blood from the placenta and umbilical cord) from consenting mothers following childbirth.

5.1.7 The immunology of transplant rejection

Distinguishing between self and non-self

When the immune system encounters a foreign organism, it mounts an attack against it to protect the body from infection. To prevent an attack on our own cells and tissues (autoimmunity), the immune system must be able to differentiate between our own healthy tissues and foreign invaders.

Foreign invaders are presented to the immune system in the form of small molecules called antigens. Identification of these non-self antigens will trigger an immune response and will stimulate the production of antigen specific antibodies that mark infected cells for destruction by the immune system and help amplify the immune response. The Human Leukocyte Antigen (HLA) complex is a group of genes that encode the proteins responsible for identifying foreign agents to the immune system. These proteins are found on the surface of all cells and act as 'self-markers' telling the immune system not to trigger a response.

Each person will have their own specific set of HLA proteins, based upon their unique genetic make-up, that the immune system will have learned not to react to. Any cell not displaying these specific HLA proteins will be identified as 'non-self' by the immune system and will be treated as a foreign invader.

Mechanism of rejection

Graft rejection occurs when the recipient's immune system attacks the donated graft and begins destroying the transplanted tissue or organ. The immune response is usually triggered by the presence of the donor's own unique set of HLA proteins, which the recipient's immune system will identify as foreign.

The degree of similarity between the HLA genes of the donor and recipient is known as histocompatibility; the more genetically compatible the donor and the recipient, the more tolerant the recipient's immune system should be of the graft. However, unless the donor and recipient are genetically identical (e.g. as in identical twins) there will always be some degree of rejection. As well as nonself HLA proteins, other surface proteins on the donor graft can also be identified as a foreign antigen and illicit an immune response.

In some cases, a patient may experience something known as 'graft versus host reaction' where mature immune cells already present in the donor graft begin attacking the healthy cells of the recipient. Graft versus host reaction, where the donor graft is described as being "immune-competent" (i.e. capable of producing an immune response) is a particular risk with stem cell transplants (bone marrow transplant) and can also occur following blood transfusions.

5.1.8 Clinical stages of rejection

Hyperacute rejection

This occurs within minutes or hours after a transplantation and is caused by the presence of preexisting antibodies of the recipient, that match the foreign antigens of the donor, triggering an immune response against the transplant. These antibodies could have been generated as a result of prior blood transfusions, prior transplantations or multiple pregnancies. The antibodies react with cells in the blood vessels of the graft, causing blood clots to form, which will prevent blood supply from reaching the graft resulting in immediate rejection of the transplant.

Acute rejection

This occurs within the first 6 months after transplantation. Some degree of acute rejection will occur in all transplantations, except between identical twins. Recipients are most at risk in the first 3 months, but rejection can still occur at a later stage. Acute rejection is caused by the formation of antibodies following the detection of non-self antigens in the donated graft. If diagnosed early enough, acute rejection can be treated by suppressing the immune system and permanent damage to the graft can be avoided in some cases.

Chronic rejection

Repeated episodes of acute rejection can ultimately lead to chronic rejection of the graft and failure of the transplant. Chronic rejection commonly manifests as scarring of the tissue or organ which can occur months to years after acute rejection has subsided. At present, there is no cure for chronic rejection other than removal of the graft.

Finding an eligible donor-recipient match

Rejection can be minimised by carefully matching the donor and recipient for compatibility prior to transplantation. The better matched the donor and recipient are the more successful the transplantation is likely to be. Compatibility between donor and recipient is assessed using a combination of tests, including:

ABO blood group compatibility

The donor and recipient are tested for compatible blood groups. This is the first test to be carried out as the transplant will be rapidly rejected if the blood groups do no match. In some transplants, for example young children and also bone marrow transplants, ABO compatibility is not a necessity.

Fig. 19.

Tissue typing

A blood sample is taken from the recipient to identify the HLA antigens present on the surface of the their cells to help find a histone compatible donor. The more alike the HLA types of the donor and recipient are the more likely a transplant will be successful. Family members, in particular siblings, are often the best HLA matches due to their genetic similarity.

Cross matching

Blood samples are taken from both the recipient and donor, and the cells of the donor are mixed with the blood serum of the recipient. If the recipient's antibodies attack the donor cells, they are considered a positive match and transplantation will not be suitable due to increased risk of hyper-acute rejection.

Panel reactive antibody test

The blood serum of patients awaiting transplantation are tested for reactive antibodies against a random panel of cells. Previous exposure to foreign tissue, by blood transfusion, pregnancy or prior transplantations, are likely to increase the number of HLA antibodies in the blood. The more HLA antibodies present, the higher he panel reactive antibody (PRA) level denoted to the patient, and the greater the chance of graft rejection. If PRA levels are high, it may be more difficult to find a match and a higher dosage of immunosuppressive drugs may be required.

Serology screening

For patients undergoing stem cell transplantation they and their donor will undergo pre-transplant serology screening. This is undertaken to detect the immune status of both the donor and a potential recipient against a number of clinically significant infectious organisms, including viruses like HIV, Cytomegalovirus (CMV), and Epstein-Barr Virus (EBV), thus determining potential for re-infection or reactivation of the infection upon immunosuppression. Individuals are often matched according to the CMV and EBV status.

Immunosupressive drugs

To reduce the risk of transplant rejection, patients are treated with immunosuppressive drugs that will dampen their immune response. Immunosuppressive drugs are given in two phases; an initial induction phase involving a high dose, and a later maintenance phase which involves using the drug in the long term at a lower dose.

The combination of drugs, and dosage given, will vary depending on the type of transplant and the chosen treatment regime. If a patient experiences an episode of acute rejection the drug combination is subject to change and the dosage is also likely to increase. Side effects can also cause alternative drugs to be used. Steroids, in the past, have been the most commonly used immunosuppressant drug. However, their use is being reduced due to the adverse side effects associated with them.

All current immunosuppressive drugs come with limitations. One of the major limitations of these drugs is immunodeficiency. As these immunosuppressive drugs are non-specific, they will reduce overall immune system function leaving patients susceptible to opportunistic infection. Additionally, many of these drugs are associated with adverse side effects, such as high blood pressure, impaired renal function, diabetes mellitus, and increased risk of cancer – to name just a few. Patients are required to take a large number of immunosuppressants each day for the rest of their lives, which can have a major impact on their health and lifestyle. A fine balance needs to be reached between suppressing immune function sufficiently to avoid rejection, preventing drug toxicity, and maintaining enough immune function to fight off disease.

Future transplant therapies

As well as new immunosuppressive drugs, with increased specificity and fewer side effects, other new therapies could also one day greatly reduce, or entirely remove, the possibility of rejection.

Stem cells could have a major impact on transplantation in the future beyond their current use in treating blood disorders. Pluripotent stem cells have the capacity to mature into any cell in the body, and this ability can be harnessed to grow tissues and organs. Moreover, the discovery that other cell types can be induced to have stem cell capacities means that the cells used to make the tissue could come directly from the recipient themselves, thus circumventing the risk of rejection.

Another future approach is the manufacture of organ scaffolds using 3D printing and then growing stem cells around these scaffolds to artificially replicate the tissue being replaced. Bio-manufacturing of tissues and organs would not only reduce the risk of transplant rejection, if the patient's own stem cells were used, but would also reduce the strain on the limited organ supply.

However, the UK national strategy for organ transplantation ('Taking organ transplantation to 2020: a UK strategy') states that advances in stem cell treatment are unlikely to have a significant impact on organ donation over the next decade. Therefore, improving currently available therapies and the discovery of novel immunosuppressive regimes remains at the forefront of transplant medicine research.

Improving compatibility testing between donor and recipient could also reduce the risk of transplant rejection and increase the longevity of the transplant. The better matched the donor and the recipient are, the more tolerant the recipient's immune system will be to the transplanted organ or tissue. Additionally, a greater understanding of the disparity between the donor and recipient will better inform treatment strategies after transplantation and help avoid repeated episodes of acute rejection.

Immunological research has led to huge advancements in transplant medicine. However, immune rejection still remains the most formidable barrier to successful transplantation. Continued research is needed to find ways to alleviate the risk of rejection, improve diagnosis and maintain long term survival of the transplant; all of which would have a significant impact on the strained organ supply.

5.1.9 Summary

The human immune system has two levels of immunity: specific and non-specific immunity. Through non-specific immunity, also called innate immunity, the human body protects itself against foreign material that is perceived to be harmful. Microbes as small as viruses and bacteria can be attacked, as can larger organisms such as worms. Collectively, these organisms are called pathogens when they cause disease in the host. All animals have innate immune defenses against common pathogens. These first lines of defense include outer barriers like the skin and mucous membranes. When pathogens breach the outer barriers, for example through a cut in the skin or when inhaled into the lungs, they can cause serious harm.

While healthy phagocytes are critical to good health, they are unable to address certain infectious threats. Specific immunity is a complement to the function of phagocytes and other elements of the innate immune system. In contrast to innate immunity, specific immunity allows for a targeted response against a specific pathogen. Only vertebrates have specific immune responses. Two types of white blood cells called lymphocytes are vital to the specific immune response. Lymphocytes are produced in the bone marrow, and mature into one of several subtypes. The two most common are T cells and B cells.

An antigen is a foreign material that triggers a response from T and B cells. The human body has B and T cells specific to millions of different antigens. We usually think of antigens as part of microbes, but antigens can be present in other settings. For example, if a person received a blood transfusion that did not match his blood type, it could trigger reactions from T and B cells. Infection occurs when a pathogen invades body cells and reproduces. Infection will usually lead to an immune response. If the response is quick and effective, the infection will be eliminated or contained so quickly that the disease will not occur.

Terminal questions

Q.1.

Answer:----------

Q.2.

Answer:----------

Q.3.

Answer:----------

Q.4.

Answer:----------

Q.5.

Answer:----------

Q.6.

Answer:----------

Q.7.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit: 6 Transplantation Immunology

Structure

6.7 Tolerance in physiology and medicine

6.8 Oral tolerance and hypersensitivity

6.9 The tumor microenvironment

6.10 Evolution

6.1.1 Immunology of tumors

6.1.2 Immunoediting

6.1.3 Tumor evasion mechanisms

6.1.6 Relationship to chemotherapy

6.1.7 Cancer

6.1.9.3 "Drivers" of Cancer

6.1.9.4 When Cancer Spreads?

6.1.9.5 Tissue Changes that Are Not Cancer

6.2.0 Types of Cancer

6.2.0.1 Carcinoma

6.2.0.3 Leukemia

6.2.0.4 Lymphoma

6.2.0.5 Hodgkin lymphoma

6.2.0.6 Non-Hodgkin lymphoma

6.2.0.7 Multiple Myeloma

6.2.0.8 Melanoma

6.2.0.9 Brain and Spinal Cord Tumors

6.2.1 Other Types of Tumors

6.2.1.1 Germ Cell Tumors

6.2.1.3 Carcinoid Tumors

6.2.14. Acquired immunodeficiency syndrome (AIDS)

6.2.1.5 Acute infection

6.2.1.6 Main symptoms of acute HIV infection

6.2.1.7 Clinical latency

6.2.1.8 Main symptoms of AIDS

6.2.1.9 Transmission

6.2.2.0 Classifications

6.2.2.1 Sexual contact

6.2.2.2 Vaccination

6.2.2.3 Treatment

6.2.2.4 Antiviral therapy

6.2.2.5 Stribild

6.2.2.6 Opportunistic infections

6.2.2.7 Diet

6.2.2.8 Alternative medicine

6.2.2.9 Epidemiology

6.2.3.0 Trends in new cases and deaths per year from HIV/AIDS

6.2.3.1 Summary

6.2.3.2 Terminal questions

6.1. Introduction

Transplantation is the process of moving cells, tissues, or organs, from one site to another, either within the same person or between a donor and a recipient. If an organ system fails, or becomes damaged as a consequence of disease or injury, it can be replaced with a healthy organ or tissue from a donor. Organ transplantation is a major operation and is only offered when all other treatment options have failed. Consequently, it is often a life-saving intervention. In 2015/16, 4,601 patient lives were saved or improved in the UK by an organ transplant.i Kidney transplants are the most common organ transplanted on the NHS in the UK (3,265 in 2015/16), followed by the liver (925), and pancreas (230).i In addition, a total of 383 combined heart and lung transplants were performed, while in 2015/16. However, whole organs are not the only type of transplant. The cornea, for example, is the most transplanted single tissue, with 5,734 procedures carried out in 2015/16.i Hematopoietic stem cell transplantation (HSCT), often called blood and marrow transplantation (BMT), is another common

tissue transplantation procedure. Used to treat a broad spectrum of diseases, though most commonly for blood or bone marrow cancers such as leukaemia and lymphoma, around 3,600 HSCT transplants were undertaken in 2012.

6.2 Transplant Immunology

- Transplantation is the process of moving cells, tissues or organs from one site to another for the purpose of replacing or repairing damaged or diseased organs and tissues. It saves thousands of lives each year. However, the immune system poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another.

- Rejection is caused by the immune system identifying the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue. Long term survival of the transplant can be maintained by manipulating the immune system to reduce the risk of rejection.
- Donor and recipient are carefully matched prior to transplantation to minimise the risk of rejection. They are matched based on their blood group, tissue typing, and how the recipient's blood serum reacts to donor cells.
- Immunosuppressive drugs are used to prevent and to treat transplant rejection by dampening the overall immune response. However, immunosuppressive drugs are non-specific and leave patients more susceptible to disease as well as being associated with numerous unwanted side effects.
- Further research on the immunological mechanisms of rejection will help improve cross matching, diagnosis and treatment, as well as facilitating the discovery of novel strategies for preventing.

The immune system plays a critical role in transplantation. The complex mechanisms of immunity, which under normal circumstances work to identify foreign microbes and direct the immune system to destroy them, pose a significant barrier to successful transplantation. Rejection of a transplant occurs in instances where the immune system identifies the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue.

The intensity of the immune response against the organ or tissue, also commonly referred to as the graft, will depend on the type of graft being transplanted and the genetic disparity between the donor and recipient. To reduce the possibility of rejection, the donor and recipient are carefully matched for immune compatibility prior to transplantation. However, the small pool of eligible donors can make it difficult to find a donor-recipient match and there will always be a degree of rejection against the graft. A critical undersupply of donated organs means that waiting lists for transplants are extremely long. Patients needing a kidney transplantation, for example, wait on average 944 days (more than two and a half years) for a life-saving transplant.

There were 6,943 patients registered for organ transplant in the UK as of March 2015.i Unfortunately, 479 of these patients died during 2015/16 whilst waiting for a transplant due the small pool of transplantable organs.i These figures underline the value of every organ and highlight the importance of a successful transplantation and maintaining long-term transplant survival. Manipulation of the immune system can support longterm survival of the graft ensuring that every transplant is as successful as possible. There are several types of transplantation involving tissues and organs:

Autograft

Transplantation of cells, tissues or organs between sites within the same individual e.g. skin graft.

Allograft

Transplantation of organs or tissues from a donor to a non-genetically identical individual of the same species. Allografts are the most common type of transplant.

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Transplantation of an organ or tissue between two different species. ‘Pig valves’, for example, are commonly used to repair or replace a defective heart valve in humans. In 2015/16, 6,069 xenograft valve replacements were carried out in England by the NHS. Xenotransplantation of whole organs is not currently viable, although it is an area of huge scientific interest as a potential solution for the existing critical undersupply of adequate organs.

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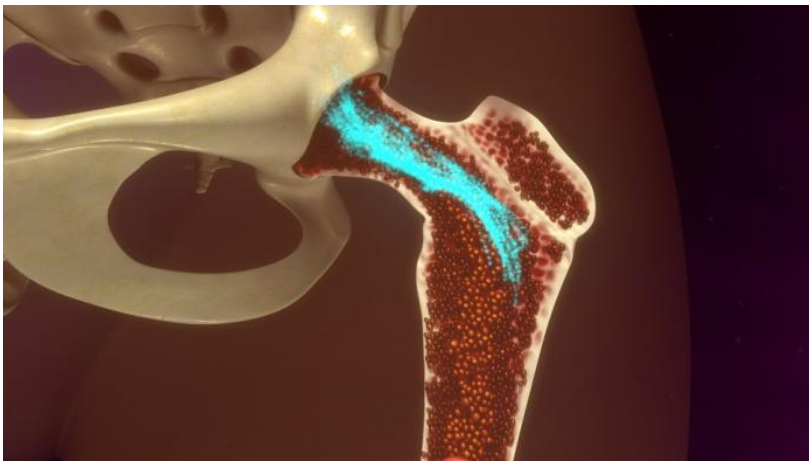

Fig. 1

Stem cell transplant

Stem cells are cells that have the capacity to develop into a range of different types of cells in the body. Blood stem cells (haematopoietic stem cells) can develop into all the different cells found in the blood and are donated to replace damaged or destroyed blood cells. Haematopoietic stem cell transplants are used to treat certain types of cancer e.g. leukaemia, and blood diseases where the bone marrow has become damaged preventing the production of healthy blood cells. These stem cells can be harvested either directly from bone marrow (see image on left) or from the cord blood (blood from the placenta and umbilical cord) from consenting mothers following childbirth.

6.3 The immunology of transplant rejection

Distinguishing between self and non-self

When the immune system encounters a foreign organism, it mounts an attack against it to protect the body from infection. To prevent an attack on our own cells and tissues

(autoimmunity), the immune system must be able to differentiate between our own healthy tissues and foreign invaders.

Foreign invaders are presented to the immune system in the form of small molecules called antigens. Identification of these non-self antigens will trigger an immune response and will stimulate the production of antigen specific antibodies that mark infected cells for destruction by the immune system and help amplify the immune response. The Human Leukocyte Antigen (HLA) complex is a group of genes that encode the proteins responsible for identifying foreign agents to the immune system. These proteins are found on the surface of all cells and act as 'self-markers' telling the immune system not to trigger a response.

Each person will have their own specific set of HLA proteins, based upon their unique genetic make-up, that the immune system will have learned not to react to. Any cell not displaying these specific HLA proteins will be identified as 'non-self' by the immune system and will be treated as a foreign invader.

Mechanism of rejection

Graft rejection occurs when the recipient's immune system attacks the donated graft and begins destroying the transplanted tissue or organ. The immune response is usually triggered by the presence of the donor's own unique set of HLA proteins, which the recipient's immune system will identify as foreign.

The degree of similarity between the HLA genes of the donor and recipient is known as histocompatibility; the more genetically compatible the donor and the recipient, the more tolerant the recipient's immune system should be of the graft. However, unless the donor and recipient are genetically identical (e.g. as in identical twins) there will always be some degree of rejection. As well as nonself HLA proteins, other surface proteins on the donor graft can also be identified as a foreign antigen and illicit an immune response.

In some cases, a patient may experience something known as 'graft versus host reaction' where mature immune cells already present in the donor graft begin attacking the healthy cells of the recipient. Graft versus host reaction, where the donor graft is described as being "immune-competent" (i.e. capable of producing an immune

response) is a particular risk with stem cell transplants (bone marrow transplant) and can also occur following blood transfusions.

6.4 Clinical stages of rejection

Hyperacute rejection

This occurs within minutes or hours after a transplantation and is caused by the presence of preexisting antibodies of the recipient, that match the foreign antigens of the donor, triggering an immune response against the transplant. These antibodies could have been generated as a result of prior blood transfusions, prior transplantations or multiple pregnancies. The antibodies react with cells in the blood vessels of the graft, causing blood clots to form, which will prevent blood supply from reaching the graft resulting in immediate rejection of the transplant.

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Chronic rejection

Repeated episodes of acute rejection can ultimately lead to chronic rejection of the graft and failure of the transplant. Chronic rejection commonly manifests as scarring of the tissue or organ which can occur months to years after acute rejection has subsided. At present, there is no cure for chronic rejection other than removal of the graft.

Finding an eligible donor-recipient match

Rejection can be minimised by carefully matching the donor and recipient for compatibility prior to transplantation. The better matched the donor and recipient are the more successful the transplantation is likely to be. Compatibility between donor and recipient is assessed using a combination of tests, including:

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The donor and recipient are tested for compatible blood groups. This is the first test to be carried out as the transplant will be rapidly rejected if the blood groups do no match. In some transplants, for example young children and also bone marrow transplants, ABO compatibility is not a necessity.

Fig. 2

Tissue typing

A blood sample is taken from the recipient to identify the HLA antigens present on the surface of the their cells to help find a histone compatible donor. The more alike the HLA types of the donor and recipient are the more likely a transplant will be successful. Family members, in particular siblings, are often the best HLA matches due to their genetic similarity.

Cross matching

Blood samples are taken from both the recipient and donor, and the cells of the donor are mixed with the blood serum of the recipient. If the recipient's antibodies attack the donor cells, they are considered a positive match and transplantation will not be suitable due to increased risk of hyper-acute rejection.

Panel reactive antibody test

The blood serum of patients awaiting transplantation are tested for reactive antibodies against a random panel of cells. Previous exposure to foreign tissue, by blood transfusion, pregnancy or prior transplantations, are likely to increase the number of HLA antibodies in the blood. The more HLA antibodies present, the higher he panel

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All current immunosuppressive drugs come with limitations. One of the major limitations of these drugs is immunodeficiency. As these immunosuppressive drugs are non-specific, they will reduce overall immune system function leaving patients susceptible to opportunistic infection. Additionally, many of these drugs are associated with adverse side effects, such as high blood pressure, impaired renal function, diabetes mellitus, and increased risk of cancer – to name just a few. Patients are required to take a large number of immunosuppressants each day for the rest of their lives, which can have a major impact on their health and lifestyle. A fine balance needs to be reached

between suppressing immune function sufficiently to avoid rejection, preventing drug toxicity, and maintaining enough immune function to fight off disease.

Future transplant therapies

As well as new immunosuppressive drugs, with increased specificity and fewer side effects, other new therapies could also one day greatly reduce, or entirely remove, the possibility of rejection.

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Another future approach is the manufacture of organ scaffolds using 3D printing and then growing stem cells around these scaffolds to artificially replicate the tissue being replaced. Bio-manufacturing of tissues and organs would not only reduce the risk of transplant rejection, if the patient's own stem cells were used, but would also reduce the strain on the limited organ supply.

However, the UK national strategy for organ transplantation ('Taking organ transplantation to 2020: a UK strategy') states that advances in stem cell treatment are unlikely to have a significant impact on organ donation over the next decade. Therefore, improving currently available therapies and the discovery of novel immunosuppressive regimes remains at the forefront of transplant medicine research.

Improving compatibility testing between donor and recipient could also reduce the risk of transplant rejection and increase the longevity of the transplant. The better matched the donor and the recipient are, the more tolerant the recipient's immune system will be to the transplanted organ or tissue. Additionally, a greater understanding of the disparity between the donor and recipient will better inform treatment strategies after transplantation and help avoid repeated episodes of acute rejection.

Immunological research has led to huge advancements in transplant medicine. However, immune rejection still remains the most formidable barrier to successful transplantation. Continued research is needed to find ways to alleviate the risk of rejection, improve diagnosis and maintain long term survival of the transplant; all of which would have a significant impact on the strained organ supply.

6.4.5 Immune Tolerance or immunological tolerance

Immune tolerance, or immunological tolerance, or immunotolerance, is a state of unresponsiveness of the immune system to substances or tissue that have the capacity to elicit an immune response in a given organism. It is induced by prior exposure to that specific antigen[1][2] and contrasts with conventional immune-mediated elimination of foreign antigens (see Immune response). Tolerance is classified into central tolerance or peripheral tolerance depending on where the state is originally induced, in the thymus and bone marrow (central) or in other tissues and lymph nodes (peripheral). The mechanisms by which these forms of tolerance are established are distinct, but the resulting effect is similar.

Immune tolerance is important for normal physiology. Central tolerance is the main way the immune system learns to discriminate self from non-self. Peripheral tolerance is key to preventing over-reactivity of the immune system to various environmental entities (allergens, gut microbes, etc.). Deficits in central or peripheral tolerance also cause autoimmune disease, resulting in syndromes such as systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes, autoimmune polyendocrine syndrome type 1 (APS-1), and immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), and potentially contribute to asthma, allergy, and inflammatory bowel disease. And immune tolerance in pregnancy is what allows a mother animal to gestate a genetically distinct offspring with an alloimmune response muted enough to prevent miscarriage.

Tolerance, however, also has its negative tradeoffs. It allows for some pathogenic microbes to successfully infect a host and avoid elimination. In addition, inducing peripheral tolerance in the local microenvironment is a common survival strategy for a number of tumors that prevents their elimination by the host immune system.

Tolerance is the prevention of an immune response against a particular antigen. For instance, the immune system is generally tolerant of self-antigens, so it does not usually attack the body's own cells, tissues, and organs. However, when tolerance is lost, disorders like autoimmune disease or food allergy may occur. Tolerance is maintained in a number of ways:

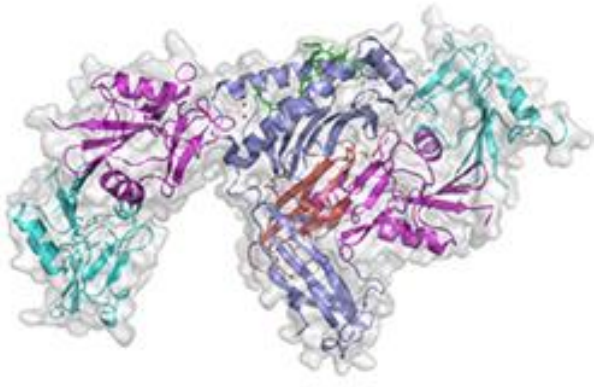


Fig. 3. Inhibitory NK cell receptor (purple and light blue) binds to MHC-I (blue and red), an interaction that prevents immune responses against self.

- When adaptive immune cells mature, there are several checkpoints in place to eliminate autoreactive cells. If a B cell produces antibodies that strongly recognize host cells, or if a T cell strongly recognizes self-antigen, they are deleted.
- Nevertheless, there are autoreactive immune cells present in healthy individuals. Autoreactive immune cells are kept in a non-reactive, or anergic, state. Even though they recognize the body's own cells, they do not have the ability to react and cannot cause host damage.
- Regulatory immune cells circulate throughout the body to maintain tolerance. Besides limiting autoreactive cells, regulatory cells are important for turning an immune response off after the problem is resolved. They can act as drains, depleting areas of essential nutrients that surrounding immune cells need for activation or survival.
- Some locations in the body are called immunologically privileged sites. These areas, like the eye and brain, do not typically elicit strong immune responses.

Part of this is because of physical barriers, like the blood-brain barrier, that limit the degree to which immune cells may enter. These areas also may express higher levels of suppressive cytokines to prevent a robust immune response.

Fetomaternal tolerance is the prevention of a maternal immune response against a developing fetus. Major histocompatibility complex (MHC) proteins help the immune system distinguish between host and foreign cells. MHC also is called human leukocyte antigen (HLA). By expressing paternal MHC or HLA proteins and paternal antigens, a fetus can potentially trigger the mother's immune system. However, there are several barriers that may prevent this from occurring: The placenta reduces the exposure of the fetus to maternal immune cells, the proteins expressed on the outer layer of the placenta may limit immune recognition, and regulatory cells and suppressive signals may play a role.

Transplantation of a donor tissue or organ requires appropriate MHC or HLA matching to limit the risk of rejection. Because MHC or HLA matching is rarely complete, transplant recipients must continuously take immunosuppressive drugs, which can cause complications like higher susceptibility to infection and some cancers. Researchers are developing more targeted ways to induce tolerance to transplanted tissues and organs while leaving protective immune responses intact.

6.5 Central tolerance

Central tolerance refers to the tolerance established by deleting autoreactive lymphocyte clones before they develop into fully immunocompetent cells. It occurs during lymphocyte development in the thymus and bone marrow for T and B lymphocytes, respectively. In these tissues, maturing lymphocytes are exposed to self-antigens presented by medullary thymic epithelial cells and thymic dendritic cells, or bone marrow cells. Self-antigens are present due to endogenous expression, importation of antigen from peripheral sites via circulating blood, and in the case of thymic stromal cells, expression of proteins of other non-thymic tissues by the action of the transcription factor AIRE.

Those lymphocytes that have receptors that bind strongly to self-antigens are removed by induction of apoptosis of the autoreactive cells, or by induction of anergy, a state of non-activity. Weakly autoreactive B cells may also remain in a state of immunological ignorance where they simply do not respond to stimulation of their B cell receptor. Some weakly self-recognizing T cells are alternatively differentiated into natural regulatory T cells (nTreg cells), which act as sentinels in the periphery to calm down potential instances of T cell autoreactivity.

The deletion threshold is much more stringent for T cells than for B cells since T cells alone can cause direct tissue damage. Furthermore, it is more advantageous for the organism to let its B cells recognize a wider variety of antigen so it can produce antibodies against a greater diversity of pathogens. Since the B cells can only be fully activated after confirmation by more self-restricted T cells that recognize the same antigen, autoreactivity is held in check.

This process of negative selection ensures that T and B cells that could initiate a potent immune response to the host's own tissues are eliminated while preserving the ability to recognize foreign antigens. It is the step in lymphocyte education that is key for preventing autoimmunity (entire process detailed here). Lymphocyte development and education is most active in fetal development but continues throughout life as immature lymphocytes are generated, slowing as the thymus degenerates and the bone marrow shrinks in adult life.

6.6 Peripheral tolerance

Peripheral tolerance develops after T and B cells mature and enter the peripheral tissues and lymph nodes. It is established by a number of partly overlapping mechanisms that mostly involve control at the level of T cells, especially CD4+ helper T cells, which orchestrate immune responses and give B cells the confirmatory signals they need in order to produce antibodies. Inappropriate reactivity toward normal self-antigen that was not eliminated in the thymus can occur, since the T cells that leave the thymus are relatively but not completely safe. Some will have receptors (TCRs) that can respond to self-antigens that:

- are present in such high concentration outside the thymus that they can bind to "weak" receptors.
- the T cell did not encounter in the thymus (such as, tissue-specific molecules like those in the islets of Langerhans, brain, or spinal cord not expressed by AIRE in thymic tissues).

Those self-reactive T cells that escape intrathymic negative selection in the thymus can inflict cell injury unless they are deleted or effectively muzzled in the peripheral tissue chiefly by nTreg cells (see central tolerance above). Appropriate reactivity toward certain antigens can also be quieted by induction of tolerance after repeated exposure, or exposure in a certain context. In these cases, there is a differentiation of naïve CD4+ helper T cells into induced Treg cells (iTreg cells) in the peripheral tissue or nearby lymphoid tissue (lymph nodes, mucosal-associated lymphoid tissue, etc.). This differentiation is mediated by IL-2 produced upon T cell activation, and TGF-β from any of a variety of sources, including tolerizing dendritic cells (DCs), other antigen presenting cells, or in certain conditions surrounding tissue.

Treg cells are not the only cells that mediate peripheral tolerance. Other regulatory immune cells include T cell subsets similar to but phenotypically distinct from Treg cells, including TR1 cells that make IL-10 but do not express Foxp3, TGF-β-secreting TH3 cells, as well as other less well-characterized cells that help establish a local tolerogenic environment. B cells also express CD22, a non-specific inhibitor receptor that dampens B cell receptor activation. A subset of B regulatory cells that makes IL-10 and TGF-β also exists. Some DCs can make Indoleamine 2,3-dioxygenase (IDO) that depletes the amino acid tryptophan needed by T cells to proliferate and thus reduce responsiveness. DCs also have the capacity to directly induce anergy in T cells that recognize antigen expressed at high levels and thus presented at steady-state by DCs. In addition, FasL expression by immune privileged tissues can result in activation-induced cell death of T cells.

6.7 Tolerance in physiology and medicine

Allograft tolerance

Immune recognition of non-self-antigens typically complicates transplantation and engrafting of foreign tissue from an organism of the same species (allografts), resulting in graft reaction. However, there are two general cases in which an allograft may be accepted. One is when cells or tissue are grafted to an immune-privileged site that is sequestered from immune surveillance (like in the eye or testes) or has strong molecular signals in place to prevent dangerous inflammation (like in the brain). The second is when a state of tolerance has been induced, either by previous exposure to the antigen of the donor in a manner that causes immune tolerance rather than sensitization in the recipient, or after chronic rejection. Long-term exposure to a foreign antigen from fetal development or birth may result in establishment of central tolerance, as was observed in Medawar's mouse-allograft experiments. In usual transplant cases, however, such early prior exposure is not possible. Nonetheless, a few patients can still develop allograft tolerance upon cessation of all exogenous immunosuppressive therapy, a condition referred to as operational tolerance. CD4+ Foxp3+ Treg cells, as well as CD8+ CD28- regulatory T cells that dampen cytotoxic responses to grafted organs, are thought to play a role. In addition, genes involved in NK cell and γδT cell function associated with tolerance have been implicated for liver transplant patients. The unique gene signatures of these patients implies their physiology may be predisposed toward immune tolerance.

Fetal development

The fetus has a different genetic makeup than the mother, as it also translates its father's genes, and is thus perceived as foreign by the maternal immune system. Women who have borne multiple children by the same father typically have antibodies against the father's red blood cell and major histocompatibility complex (MHC) proteins. However, the fetus usually is not rejected by the mother, making it essentially a physiologically tolerated allograft. It is thought that the placental tissues which interface with maternal tissues not only try to escape immunological recognition by downregulating identifying MHC proteins but also actively induce a marked peripheral tolerance.

Placental trophoblast cells express a unique Human Leukocyte Antigen (HLA-G) that inhibits attack by maternal NK cells. These cells also express IDO, which represses maternal T cell responses by amino acid starvation. Maternal T cells specific for paternal antigens are also suppressed by tolerogenic DCs and activated iTregs or cross-reacting nTregs. Some maternal Treg cells also release soluble fibrinogen-like proteins 2 (sFGL2), which suppresses the function of DCs and macrophages involved in inflammation and antigen presentation to reactive T cells. These mechanisms altogether establish an immune-privileged state in the placenta that protects the fetus. A break in this peripheral tolerance results in miscarriage and fetal loss.

The microbiome

The skin and digestive tract of humans and many other organisms is colonized with an ecosystem of microorganisms that is referred to as the microbiome. Though in mammals a number of defenses exist to keep the microbiota at a safe distance, including a constant sampling and presentation of microbial antigens by local DCs, most organisms do not react against commensal microorganisms and tolerate their presence. Reactions are mounted, however, to pathogenic microbes and microbes that breach physiological barriers. Peripheral mucosal immune tolerance, in particular, mediated by iTreg cells and tolerogenic antigen-presenting cells, is thought to be responsible for this phenomenon. In particular, specialized gut CD103+ DCs that produce both TGF-β and retinoic acid efficiently promotes the differentiation of iTreg cells in the gut lymphoid tissue.[8] Foxp3- TR1 cells that make IL-10 are also enriched in the intestinal lining. Break in this tolerance is thought to underlie the pathogenesis of inflammatory bowel diseases like Crohn's disease and ulcerative colitis.

6.8 Oral tolerance and hypersensitivity

Oral tolerance refers to a specific type of peripheral tolerance induced by antigens given by mouth and exposed to the gut mucosa and its associated lymphoid tissues. The hypo-responsiveness induced by oral exposure is systemic and can reduce hypersensitivity reactions in certain cases. Records from 1829 indicate that American Indians would reduce contact hypersensitivity from poison ivy by consuming leaves of related Rhus species; however, contemporary attempts to use oral tolerance to

ameliorate autoimmune diseases like rheumatoid arthritis and other hypersensitivity reactions have been mixed. The systemic effects of oral tolerance may be explained by the extensive recirculation of immune cells primed in one mucosal tissue in another mucosal tissue, allowing extension of mucosal immunity. The same probably occurs for cells mediating mucosal immune tolerance.

Oral tolerance may depend on the same mechanisms of peripheral tolerance that limit inflammation to bacterial antigens in the microbiome since both involve the gut-associated lymphoid tissue. It may also have evolved to prevent hypersensitivity reactions to food proteins. It is of immense immunological importance, since it is a continuous natural immunologic event driven by exogenous antigen.

Allergy and hypersensitivity reactions in general are traditionally thought of as misguided or excessive reactions by the immune system, possibly due to broken or underdeveloped mechanisms of peripheral tolerance. Usually, Treg cells, TR1, and Th3 cells at mucosal surfaces suppress type 2 CD4 helper cells, mast cells, and eosinophils, which mediate allergic response. Deficits in Treg cells or their localization to mucosa have been implicated in asthma and atopic dermatitis. Attempts have been made to reduce hypersensitivity reactions by oral tolerance and other means of repeated exposure. Repeated administration of the allergen in slowly increasing doses, subcutaneously or sublingually appears to be effective for allergic rhinitis. Repeated administration of antibiotics, which can form haptens to cause allergic reactions, can also reduce antibiotic allergies in children.

6.9 The tumor microenvironment

Immune tolerance is an important means by which growing tumors, which have mutated proteins and altered antigen expression, prevent elimination by the host immune system. It is well recognized that tumors are a complex and dynamic population of cells composed of transformed cells as well as stromal cells, blood vessels, tissue macrophages, and other immune infiltrates. These cells and their interactions all contribute to the changing tumor microenvironment, which the tumor largely manipulates to be immunotolerant so as to avoid elimination. There is an accumulation of metabolic enzymes that suppress T cell proliferation and activation,

including IDO and arginase, and high expression of tolerance-inducing ligands like FasL, PD-1, CTLA-4, and B7.

Pharmacologic monoclonal antibodies targeted against some of these ligands has been effective in treating cancer. Tumor-derived vesicles known as exosomes have also been implicated promoting differentiation of iTreg cells and myeloid derived suppressor cells (MDSCs), which also induce peripheral tolerance. In addition to promoting immune tolerance, other aspects of the microenvironment aid in immune evasion and induction of tumor-promoting inflammation.

6.10 Evolution

Though the exact evolutionary rationale behind the development of immunological tolerance is not completely known, it is thought to allow organisms to adapt to antigenic stimuli that will consistently be present instead of expending considerable resources fighting it off repeatedly. Tolerance in general can be thought of as an alternative defense strategy that focuses on minimizing impact of an invader on host fitness, instead of on destroying and eliminating the invader. Such efforts may have a prohibitive cost on host fitness. In plants, where the concept was originally used, tolerance is defined as a reaction norm of host fitness over a range of parasite burdens, and can be measured from the slope of the line fitting these data. Immune tolerance may constitute one aspect of this defense strategy, though other types of tissue tolerance have been described.

6.1.1 Immunology of tumors

Cancer immunology is an interdisciplinary branch of biology that is concerned with understanding the role of the immune system in the progression and development of cancer; the most well known application is cancer immunotherapy, which utilises the immune system as a treatment for cancer.
Cancer immunosurveillance and immunoediting are based on protection against development of tumors in animal systems and identification of targets for immune recognition of human cancer.

Cancer immunology is an interdisciplinary branch of biology concerned with the role of the immune system in the progression and development of cancer; the most well known application is cancer immunotherapy, where the immune system is used to treat cancer.[1][2] Cancer immunosurveillance is a theory formulated in 1957 by Burnet and Thomas, who proposed that lymphocytes act as sentinels in recognizing and eliminating continuously arising, nascent transformed cells. Cancer immunosurveillance appears to be an important host protection process that decreases cancer rates through inhibition of carcinogenesis and maintaining of regular cellular homeostasis. It has also been suggested that immunosurveillance primarily functions as a component of a more general process of cancer immunoediting.

Tumor antigens

Tumors may express tumor antigens that are recognized by the immune system and may induce an immune response. These tumor antigens are either TSA (Tumor-specific antigen) or TAA (Tumor-associated antigen).

Tumor-specific

Tumor-specific antigens (TSA) are antigens that only occur in tumor cells. TSAs can be products of oncoviruses like E6 and E7 proteins of Human papillomavirus, occurring in cervical carcinoma, or EBNA-1 protein of EBV, occurring in Burkitt's lymphoma cells. Another example of TSAs are abnormal products of mutated oncogenes (e.g. Ras protein) and anti-oncogenes (e.g. p53).

Tumor-associated antigens

Tumor-associated antigens (TAA) are present in healthy cells, but for some reason they also occur in tumor cells. However, they differ in quantity, place or time period of expression. Oncofetal antigens are tumor-associated antigens expressed by embryonic cells and by tumors. Examples of oncofetal antigens are AFP (α-fetoprotein), produced by hepatocellular carcinoma, or CEA (carcinoembryonic antigen), occurring in ovarian and colon cancer. More tumor-associated antigens are HER2/neu, EGFR or MAGE-1.

6.1.2 Immunoediting

Cancer immunoediting is a process in which immune system interacts with tumor cells. It consists of three phases: elimination, equilibrium and escape. These phases are often

referred to as "the three Es" of cancer immunoediting. Both, adaptive and innate immune system participate in immunoediting. In the elimination phase, the immune response leads to destruction of tumor cells and therefore to tumor suppression. However, some tumor cells may gain more mutations, change their characteristics and evade the immune system. These cells might enter the equilibrium phase, in which the immune system doesn't recognise all tumor cells, but at the same time the tumor doesn't grow. This condition may lead to the phase of escape, in which the tumor gains dominance over immune system, starts growing and establishes immunosuppressive environment.

As a consequence of immunoediting, tumor cell clones less responsive to the immune system gain dominance in the tumor through time, as the recognized cells are eliminated. This process may be considered akin to Darwinian evolution, where cells containing pro-oncogenic or immunosuppressive mutations survive to pass on their mutations to daughter cells, which may themselves mutate and undergo further selective pressure. This results in the tumor consisting of cells with decreased immunogenicity and can hardly be eliminated. This phenomenon was proven to happen as a result of immunotherapies of cancer patients.

6.1.3 Tumor evasion mechanisms

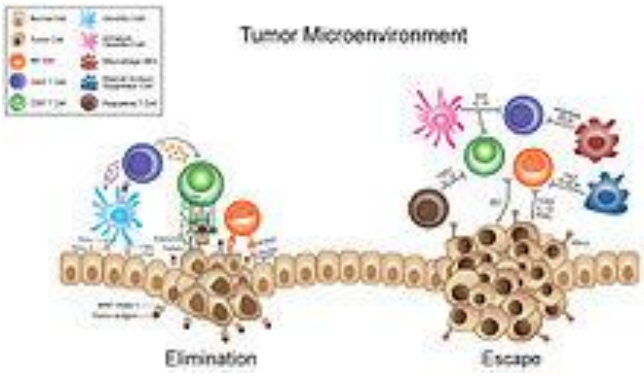


Fig. 4 Multiple factors determine whether tumor cells will be eliminated by the immune system or will escape detection. During the elimination phase immune effector cells such as CTL's and NK cells with the help of dendritic and CD4+ T-cells are able to recognize and eliminate tumor cells.

- CD8+ cytotoxic T cells are a fundamental element of anti-tumor immunity. Their TCR receptors recognise antigens presented by MHC class I and when bound, the Tc cell triggers its cytotoxic activity. MHC I are present on the surface of all nucleated cells. However, some cancer cells lower their MHC I expression and avoid being detected by the cytotoxic T cells. This can be done by mutation of MHC I gene or by lowering the sensitivity to IFN-γ (which influences the surface expression of MHC I). Tumor cells also have defects in antigen presentation pathway, what leads into down-regulation of tumor antigen presentations. Defects are for example in Transporter associated with antigen processing (TAP) or Tapasin. On the other hand, a complete loss of MHC I is a trigger for NK cells. Tumor cells therefore maintain a low expression of MHC I.
- Another way to escape cytotoxic T cells is to stop expressing molecules essential for co-stimulation of cytotoxic T cells, such as CD80 or CD86.
- Tumor cells express molecules to induce apoptosis or to inhibit T lymphocytes:
  - Expression of FasL on its surface, tumor cells may induce apoptosis of T lymphocytes by FasL-Fas interaction.
  - Expression of PD-L1 on the surface of tumor cells leads to suppression of T lymphocytes by PD1-PD-L1 interaction.
- Tumor cells have gained resistance to effector mechanisms of NK and Cytotoxic CD8+ T cell:
  - by loss of gene expression or inhibiton of apoptotic signal pathway molecules: APAF1, Caspase 8, Bcl-2-associated X protein (bax) and Bcl-2 homologous antagonist killer (bak).
  - by induction of expression or overexpression of antiapoptotic molecules: Bcl-2, IAP or XIAP.

Tumor microenvironment

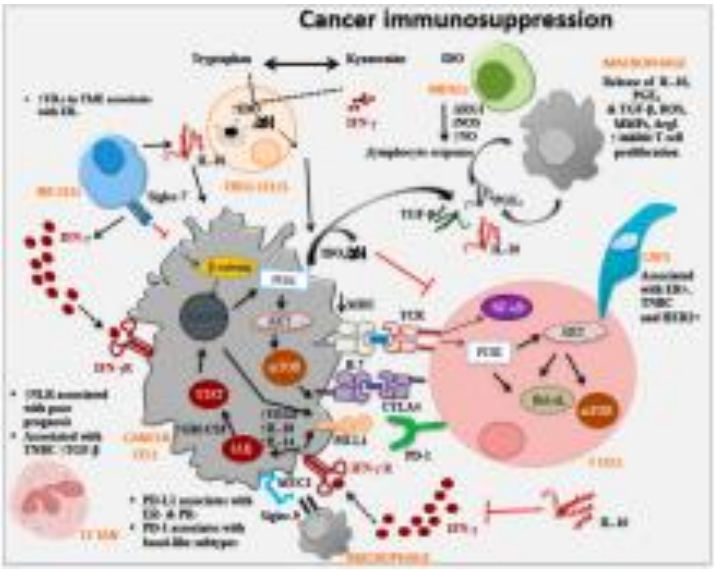


Fig. 5 Immune checkpoints of immunosuppressive actions associated with breast cancer

- Production of TGF-β by tumor cells and other cells (such as Myeloid-derived suppressor cell) leads to conversion of CD4+ T cell into suppressive regulatory T cell (Treg) by a contact dependent or independent stimulation. In a healthy tissue, functioning Tregs are essential to maintain self-tolerance. In a tumor, however, Tregs form an immunosupresive microenviroment.
- Tumor cells produce special cytokines (such as Colony-stimulating factor) to produce Myeloid-derived suppressor cell. These cells are heterogenous collection of cell types including precursors of Dendritic cell, Monocyte and Neutrophil. MDSC have suppressive effects on T-lymphocytes, Dendritic cells and Macrophages. They produce immunosuppressive TGF-β and IL-10.
- Another producer of suppressive TGF-β and IL-10 are Tumor-associated macrophages, these macrophages have mostly phenotype of alternatively activated M2 macrophages. Their activation is promoted by TH type 2 cytokines (such as IL-4 and IL-13). Their main effects are immunosuppression, promotion of tumor growth and angiogenesis.
- Tumor cells have non-classical MHC class I on their surface, for example HLA-G. HLA-G is inducer of Treg, MDSC, polarise macrophages into alternatively activated M2 and has other immunosuppresive effects on immune cells.

Immunomodulation methods

Immune system is the key player in fighting cancer. As described above in mechanisms of tumor evasion, the tumor cells are modulating the immune response in their profit. It is possible to improve the immune response in order to boost the immunity against tumor cells.

- monoclonal anti-CTLA4 and anti-PD-1 antibodies are called immune checkpoint inhibitors:
  - CTLA-4 is a receptor upregulated on the membrane of activated T lymphocytes, CTLA-4 CD80/86 interaction leads to switch off of T lymphocytes. By blocking this interaction with monoclonal anti CTLA-4 antibody we can increase the immune response. An example of approved drug is ipilimumab.
  - PD-1 is also an upregulated receptor on the surface of T lymphocytes after activation. Interaction PD-1 with PD-L1 leads to switching off or apoptosis. PD-L1 are molecules which can be produced by tumor cells. The monoclonal anti-PD-1 antibody is blocking this interaction thus leading to improvement of immune response in CD8+ T lymphocytes. An example of approved cancer drug is nivolumab.
  - **Chimeric Antigen Receptor T cell**
    - This CAR receptors are genetically engineered receptors with extracellular tumor specific binding sites and intracelullar signalling domain that enables the T lymphocyte activation.
  - **Cancer vaccine**
    - Vaccine can be composed of killed tumor cells, recombinant tumor antigens, or dendritic cells incubated with tumor antigens (dendritic cell-based cancer vaccine).

6.1.6 Relationship to chemotherapy

Obeid et al. investigated how inducing immunogenic cancer cell death ought to become a priority of cancer chemotherapy. He reasoned, the immune system would be able to play a factor via a ‘bystander effect’ in eradicating chemotherapy-resistant cancer

cells. However, extensive research is still needed on how the immune response is triggered against dying tumour cells. Professionals in the field have hypothesized that 'apoptotic cell death is poorly immunogenic whereas necrotic cell death is truly immunogenic'. This is perhaps because cancer cells being eradicated via a necrotic cell death pathway induce an immune response by triggering dendritic cells to mature, due to inflammatory response stimulation. On the other hand, apoptosis is connected to slight alterations within the plasma membrane causing the dying cells to be attractive to phagocytic cells. However, numerous animal studies have shown the superiority of vaccination with apoptotic cells, compared to necrotic cells, in eliciting anti-tumor immune responses.

Thus Obeid *et al.* propose that the way in which cancer cells die during chemotherapy is vital. Anthracyclins produce a beneficial immunogenic environment. The researchers report that when killing cancer cells with this agent uptake and presentation by antigen presenting dendritic cells is encouraged, thus allowing a T-cell response which can shrink tumours. Therefore, activating tumour-killing T-cells is crucial for immunotherapy success. However, advanced cancer patients with immunosuppression have left researchers in a dilemma as to how to activate their T-cells. The way the host dendritic cells react and uptake tumour antigens to present to $CD4^+$ and $CD8^+$ T-cells is the key to success of the treatment.

6.1.7 Cancer

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors. Many cancers form solid tumors, which are masses of tissue. Cancers of the blood, such as leukemias, generally do not form solid tumors.

Cancerous tumors are malignant, which means they can spread into, or invade, nearby tissues. In addition, as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor. Unlike malignant tumors, benign tumors do not spread into, or invade, nearby tissues. Benign tumors can sometimes be quite large, however. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening.

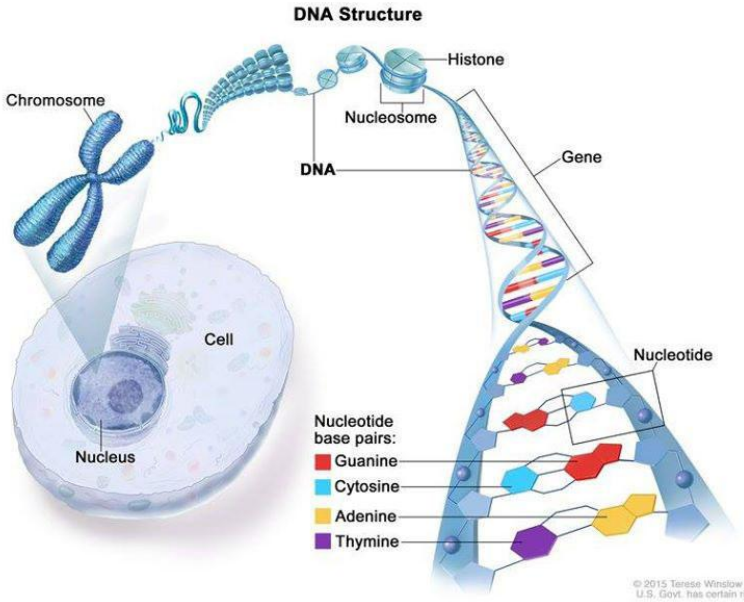
Differences between Cancer Cells and Normal Cells

Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. One important difference is that cancer cells are less specialized than normal cells. That is, whereas normal cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, unlike normal cells, cancer cells continue to divide without stopping. In addition, cancer cells are able to ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or apoptosis, which the body uses to get rid of unneeded cells.

Cancer cells may be able to influence the normal cells, molecules, and blood vessels that surround and feed a tumor—an area known as the microenvironment. For instance, cancer cells can induce nearby normal cells to form blood vessels that supply tumors with oxygen and nutrients, which they need to grow. These blood vessels also remove waste products from tumors. Cancer cells are also often able to evade the immune system, a network of organs, tissues, and specialized cells that protects the body from infections and other conditions. Although the immune system normally removes damaged or abnormal cells from the body, some cancer cells are able to "hide" from the immune system. Tumors can also use the immune system to stay alive and grow. For example, with the help of certain immune system cells that normally prevent a runaway immune response, cancer cells can actually keep the immune system from killing cancer cells.

How Cancer Arises?

Fig. 1

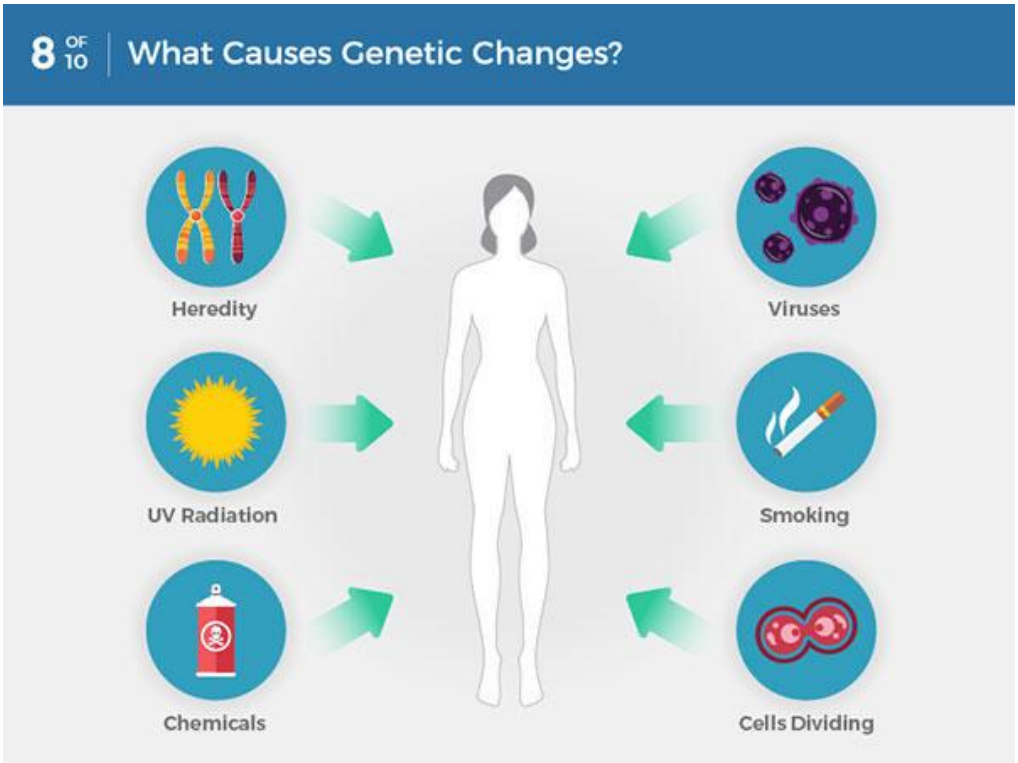


ENLARGE

Fig. 6 Cancer is caused by certain changes to genes, the basic physical units of inheritance. Genes are arranged in long strands of tightly packed DNA called chromosomes.

Cancer is a genetic disease—that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide. Genetic changes that cause cancer can be inherited from our parents. They can also arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to DNA caused by certain environmental exposures. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun. (Our Cancer Causes and Prevention section has more information.)

Each person's cancer has a unique combination of genetic changes. As the cancer continues to grow, additional changes will occur. Even within the same tumor, different cells may have different genetic changes. In general, cancer cells have more genetic changes, such as mutations in DNA, than normal cells. Some of these changes may have nothing to do with the cancer; they may be the result of the cancer, rather than its cause.


8 OF 10
What Causes Genetic Changes?
Heredity
Viruses
UV Radiation
Smoking
Chemicals
Cells Dividing

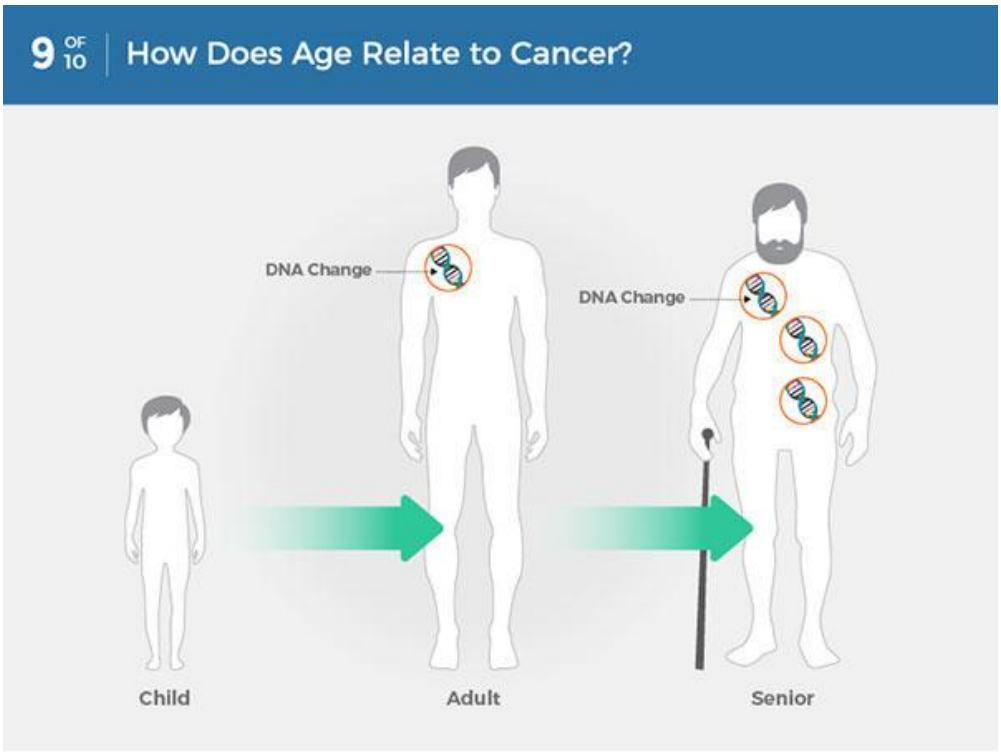


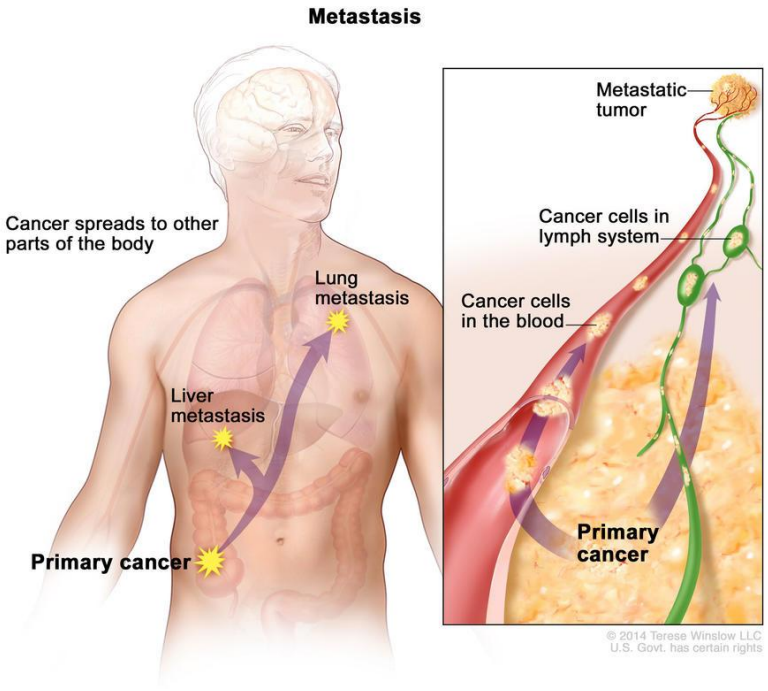
Fig. 7

6.1.9.3 Drivers" of Cancer

The genetic changes that contribute to cancer tend to affect three main types of genes, proto-oncogenes, tumor suppressor genes, and DNA repair genes. These changes are sometimes called “drivers” of cancer. Proto-oncogenes are involved in normal cell growth and division. However, when these genes are altered in certain ways or are more active than normal, they may become cancer-causing genes (or oncogenes), allowing cells to grow and survive when they should not. Tumor suppressor genes are also involved in controlling cell growth and division. Cells with certain alterations in tumor suppressor genes may divide in an uncontrolled manner.

DNA repair genes are involved in fixing damaged DNA. Cells with mutations in these genes tend to develop additional mutations in other genes. Together, these mutations may cause the cells to become cancerous. As scientists have learned more about the molecular changes that lead to cancer, they have found that certain mutations commonly occur in many types of cancer. Because of this, cancers are sometimes characterized by the types of genetic alterations that are believed to be driving them, not just by where they develop in the body and how the cancer cells look under the microscope.

6.1.9.4 When Cancer Spreads?



ENLARGE

Fig. 8

In metastasis, cancer cells break away from where they first formed (primary cancer), travel through the blood or lymph system, and form new tumors (metastatic tumors) in other parts of the body. The metastatic tumor is the same type of cancer as the primary tumor. A cancer that has spread from the place where it first started to another place in the body is called metastatic cancer.

The process by which cancer cells spread to other parts of the body is called metastasis. Metastatic cancer has the same name and the same type of cancer cells as the original, or primary, cancer. For example, breast cancer that spreads to and forms a metastatic tumor in the lung is metastatic breast cancer, not lung cancer. Under a microscope, metastatic cancer cells generally look the same as cells of the original cancer. Moreover, metastatic cancer cells and cells of the original cancer usually have some molecular features in common, such as the presence of specific chromosome changes.

Treatment may help prolong the lives of some people with metastatic cancer. In general, though, the primary goal of treatments for metastatic cancer is to control the

growth of the cancer or to relieve symptoms caused by it. Metastatic tumors can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease.

6.1.9.5 Tissue Changes that Are Not Cancer

Not every change in the body's tissues is cancer. Some tissue changes may develop into cancer if they are not treated, however. Here are some examples of tissue changes that are not cancer but, in some cases, are monitored: Hyperplasia occurs when cells within a tissue divide faster than normal and extra cells build up, or proliferate. However, the cells and the way the tissue is organized look normal under a microscope. Hyperplasia can be caused by several factors or conditions, including chronic irritation.

Dysplasia is a more serious condition than hyperplasia. In dysplasia, there is also a buildup of extra cells. But the cells look abnormal and there are changes in how the tissue is organized. In general, the more abnormal the cells and tissue look, the greater the chance that cancer will form. Some types of dysplasia may need to be monitored or treated. An example of dysplasia is an abnormal mole (called a dysplastic nevus) that forms on the skin. A dysplastic nevus can turn into melanoma, although most do not. An even more serious condition is carcinoma in situ. Although it is sometimes called cancer, carcinoma in situ is not cancer because the abnormal cells do not spread beyond the original tissue. That is, they do not invade nearby tissue the way that cancer cells do. But, because some carcinomas in situ may become cancer, they are usually treated.

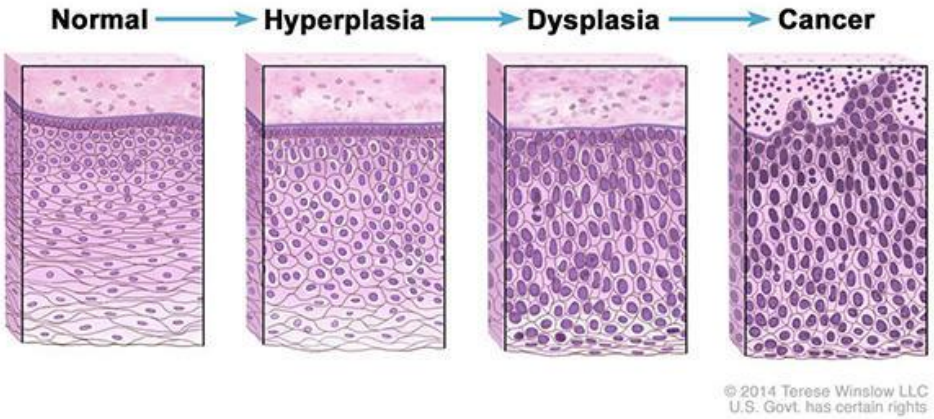


Fig. 9

Normal cells may become cancer cells. Before cancer cells form in tissues of the body, the cells go through abnormal changes called hyperplasia and dysplasia. In hyperplasia, there is an increase in the number of cells in an organ or tissue that appear normal under a microscope. In dysplasia, the cells look abnormal under a microscope but are not cancer. Hyperplasia and dysplasia may or may not become cancer.

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6.2.0 Types of Cancer

There are more than 100 types of cancer. Types of cancer are usually named for the organs or tissues where the cancers form. For example, lung cancer starts in cells of the lung, and brain cancer starts in cells of the brain. Cancers also may be described by the type of cell that formed them, such as an epithelial cell or a squamous cell. Here are some categories of cancers that begin in specific types of cells:

6.2.0.1 Carcinoma

Carcinomas are the most common type of cancer. They are formed by epithelial cells, which are the cells that cover the inside and outside surfaces of the body. There are many types of epithelial cells, which often have a column-like shape when viewed under a microscope. Carcinomas that begin in different epithelial cell types have specific names: Adenocarcinoma is a cancer that forms in epithelial cells that produce fluids or mucus. Tissues with this type of epithelial cell are sometimes called glandular tissues. Most cancers of the breast, colon, and prostate are adenocarcinomas. Basal cell carcinoma is a cancer that begins in the lower or basal (base) layer of the epidermis, which is a person's outer layer of skin.

Squamous cell carcinoma is a cancer that forms in squamous cells, which are epithelial cells that lie just beneath the outer surface of the skin. Squamous cells also line many other organs, including the stomach, intestines, lungs, bladder, and kidneys. Squamous cells look flat, like fish scales, when viewed under a microscope. Squamous cell carcinomas are sometimes called epidermoid carcinomas. Transitional cell carcinoma is a cancer that forms in a type of epithelial tissue called transitional epithelium, or

urothelium. This tissue, which is made up of many layers of epithelial cells that can get bigger and smaller, is found in the linings of the bladder, ureters, and part of the kidneys (renal pelvis), and a few other organs. Some cancers of the bladder, ureters, and kidneys are transitional cell carcinomas.

Sarcoma

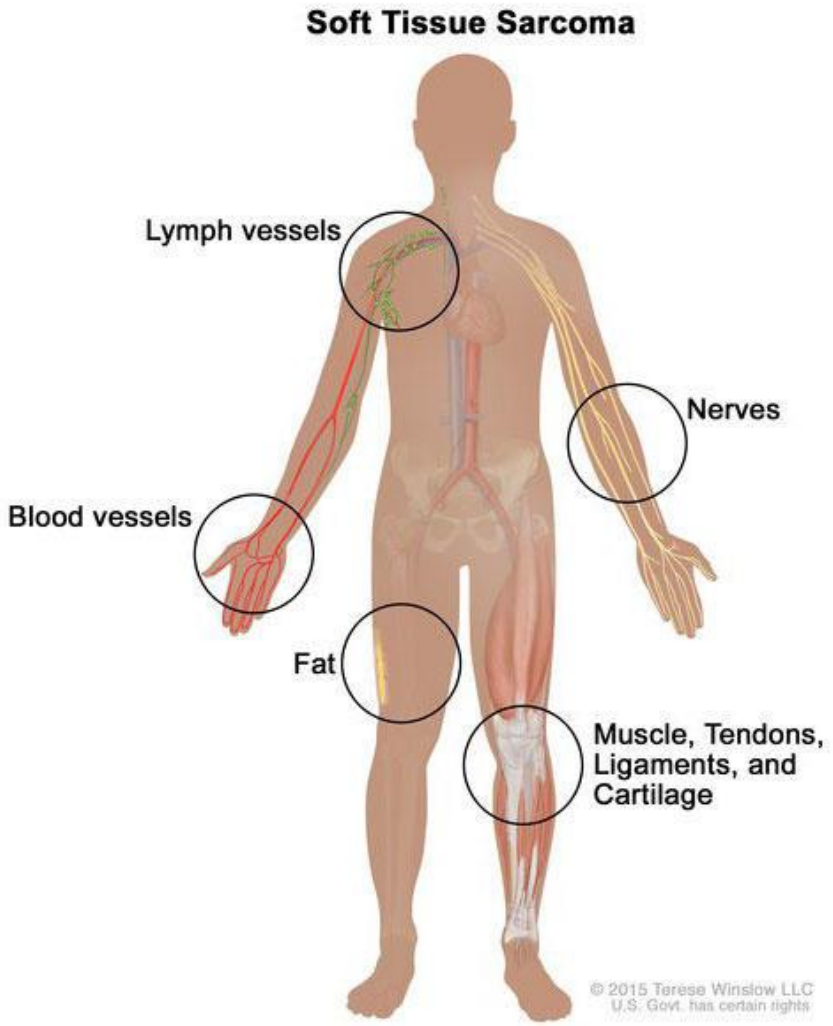

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Fig. 10

Soft tissue sarcoma forms in soft tissues of the body, including muscle, tendons, fat, blood vessels, lymph vessels, nerves, and tissue around joints. Sarcomas are cancers that form in bone and soft tissues, including muscle, fat, blood vessels, lymph vessels, and fibrous tissue (such as tendons and ligaments). Osteosarcoma is the most common

cancer of bone. The most common types of soft tissue sarcoma are leiomyosarcoma, Kaposi sarcoma, malignant fibrous histiocytoma, liposarcoma, and dermatofibrosarcoma protuberans.

6.2.0.3 Leukemia

Cancers that begin in the blood-forming tissue of the bone marrow are called leukemias. These cancers do not form solid tumors. Instead, large numbers of abnormal white blood cells (leukemia cells and leukemic blast cells) build up in the blood and bone marrow, crowding out normal blood cells. The low level of normal blood cells can make it harder for the body to get oxygen to its tissues, control bleeding, or fight infections. There are four common types of leukemia, which are grouped based on how quickly the disease gets worse (acute or chronic) and on the type of blood cell the cancer starts in (lymphoblastic or myeloid).

6.2.0.4 Lymphoma

Lymphoma is cancer that begins in lymphocytes (T cells or B cells). These are disease-fighting white blood cells that are part of the immune system. In lymphoma, abnormal lymphocytes build up in lymph nodes and lymph vessels, as well as in other organs of the body. There are two main types of lymphoma:

6.2.0.5 Hodgkin lymphoma

People with this disease have abnormal lymphocytes that are called Reed-Sternberg cells. These cells usually form from B cells.

6.2.0.6 Non-Hodgkin lymphoma

This is a large group of cancers that start in lymphocytes. The cancers can grow quickly or slowly and can form from B cells or T cells.

6.2.0.7 Multiple Myeloma

Multiple myeloma is cancer that begins in plasma cells, another type of immune cell. The abnormal plasma cells, called myeloma cells, build up in the bone marrow and form tumors in bones all through the body. Multiple myeloma is also called plasma cell myeloma and Kahler disease.

6.2.0.8 Melanoma

Melanoma is cancer that begins in cells that become melanocytes, which are specialized cells that make melanin (the pigment that gives skin its color). Most melanomas form on the skin, but melanomas can also form in other pigmented tissues, such as the eye.

6.2.0.9 Brain and Spinal Cord Tumors

There are different types of brain and spinal cord tumors. These tumors are named based on the type of cell in which they formed and where the tumor first formed in the central nervous system. For example, an astrocytic tumor begins in star-shaped brain cells called astrocytes, which help keep nerve cells healthy. Brain tumors can be benign (not cancer) or malignant (cancer).

6.2.1 Other Types of Tumors

6.2.1.1 Germ Cell Tumors

Germ cell tumors are a type of tumor that begins in the cells that give rise to sperm or eggs. These tumors can occur almost anywhere in the body and can be either benign or malignant. Our page of cancers by body location/system includes a list of germ cell tumors with links to more information.

Neuroendocrine Tumors

Neuroendocrine tumors form from cells that release hormones into the blood in response to a signal from the nervous system. These tumors, which may make higher-than-normal amounts of hormones, can cause many different symptoms. Neuroendocrine tumors may be benign or malignant.

6.2.1.3 Carcinoid Tumors

Carcinoid tumors are a type of neuroendocrine tumor. They are slow-growing tumors that are usually found in the gastrointestinal system (most often in the rectum and small intestine). Carcinoid tumors may spread to the liver or other sites in the body, and they may secrete substances such as serotonin or prostaglandins, causing carcinoid syndrome.

6.2.1.4 Acquired immunodeficiency syndrome (AIDS)

Human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS) is a spectrum of conditions caused by infection with the human immunodeficiency virus (HIV). Following initial infection a person may not notice any symptoms, or may experience a brief period of influenza-like illness. Typically, this is followed by a prolonged period with no symptoms. If the infection progresses, it interferes more with the immune system, increasing the risk of developing common infections such as tuberculosis, as well as other opportunistic infections, and tumors which are otherwise rare in people who have normal immune function. These late symptoms of infection are referred to as acquired immunodeficiency syndrome (AIDS). This stage is often also associated with unintended weight loss.

HIV is spread primarily by unprotected sex (including anal and oral sex), contaminated blood transfusions, hypodermic needles, and from mother to child during pregnancy, delivery, or breastfeeding. Some bodily fluids, such as saliva, sweat and tears, do not transmit the virus. HIV is a member of the group of viruses known as retroviruses.

Methods of prevention include safe sex, needle exchange programs, treating those who are infected, and pre- & post-exposure prophylaxis. Disease in a baby can often be prevented by giving both the mother and child antiretroviral medication. There is no cure or vaccine; however, antiretroviral treatment can slow the course of the disease and may lead to a near-normal life expectancy. Treatment is recommended as soon as the diagnosis is made. Without treatment, the average survival time after infection is 11 years.

In 2018, about 37.9 million people were living with HIV and it resulted in 770,000 deaths. An estimated 20.6 million of these live in eastern and southern Africa. Between the time that AIDS was identified (in the early 1980s) and 2018, the disease caused an estimated 32 million deaths worldwide. HIV/AIDS is considered a pandemic-a disease outbreak which is present over a large area and is actively spreading. HIV made the jump from other primates to humans in west-central Africa in the early-to-mid 20th century.[18] AIDS was first recognized by the United States Centers for Disease Control

and Prevention (CDC) in 1981 and its cause—HIV infection—was identified in the early part of the decade.

HIV/AIDS has had a large impact on society, both as an illness and as a source of discrimination. The disease also has large economic impacts. There are many misconceptions about HIV/AIDS, such as the belief that it can be transmitted by casual non-sexual contact. The disease has become subject to many controversies involving religion, including the Catholic Church's position not to support condom use as prevention. It has attracted international medical and political attention as well as large-scale funding since it was identified in the 1980s. There are three main stages of HIV infection: acute infection, clinical latency, and AIDS.

6.2.1.5 Acute infection

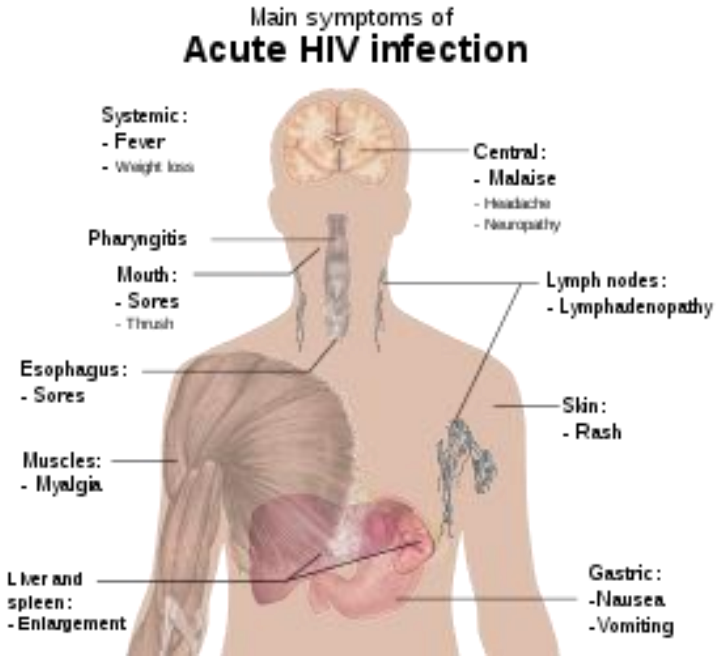

Fig. 11

6.2.1.6 Main symptoms of acute HIV infection

The initial period following the contraction of HIV is called acute HIV, primary HIV or acute retroviral syndrome. Many individuals develop an influenza-like illness or a mononucleosis-like illness 2–4 weeks after exposure while others have no significant

symptoms. Symptoms occur in 40–90% of cases and most commonly include fever, large tender lymph nodes, throat inflammation, a rash, headache, tiredness, and/or sores of the mouth and genitals. The rash, which occurs in 20–50% of cases, presents itself on the trunk and is maculopapular, classically. Some people also develop opportunistic infections at this stage. Gastrointestinal symptoms, such as vomiting or diarrhea may occur. Neurological symptoms of peripheral neuropathy or Guillain–Barré syndrome also occurs. The duration of the symptoms varies, but is usually one or two weeks.

Owing to their nonspecific character, these symptoms are not often recognized as signs of HIV infection. Even cases that do get seen by a family doctor or a hospital are often misdiagnosed as one of the many common infectious diseases with overlapping symptoms. Thus, it is recommended that HIV be considered in people presenting with an unexplained fever who may have risk factors for the infection.

6.2.1.7 Clinical latency

The initial symptoms are followed by a stage called clinical latency, asymptomatic HIV, or chronic HIV. Without treatment, this second stage of the natural history of HIV infection can last from about three years to over 20 years (on average, about eight years). While typically there are few or no symptoms at first, near the end of this stage many people experience fever, weight loss, gastrointestinal problems and muscle pains. Between 50% and 70% of people also develop persistent generalized lymphadenopathy, characterized by unexplained, non-painful enlargement of more than one group of lymph nodes (other than in the groin) for over three to six months.

Although most HIV-1 infected individuals have a detectable viral load and in the absence of treatment will eventually progress to AIDS, a small proportion (about 5%) retain high levels of $CD4^+$ T cells (T helper cells) without antiretroviral therapy for more than five years. These individuals are classified as "HIV controllers" or long-term nonprogressors (LTNP). Another group consists of those who maintain a low or undetectable viral load without anti-retroviral treatment, known as "elite controllers" or "elite suppressors". They represent approximately 1 in 300 infected persons.

Acquired immunodeficiency syndrome

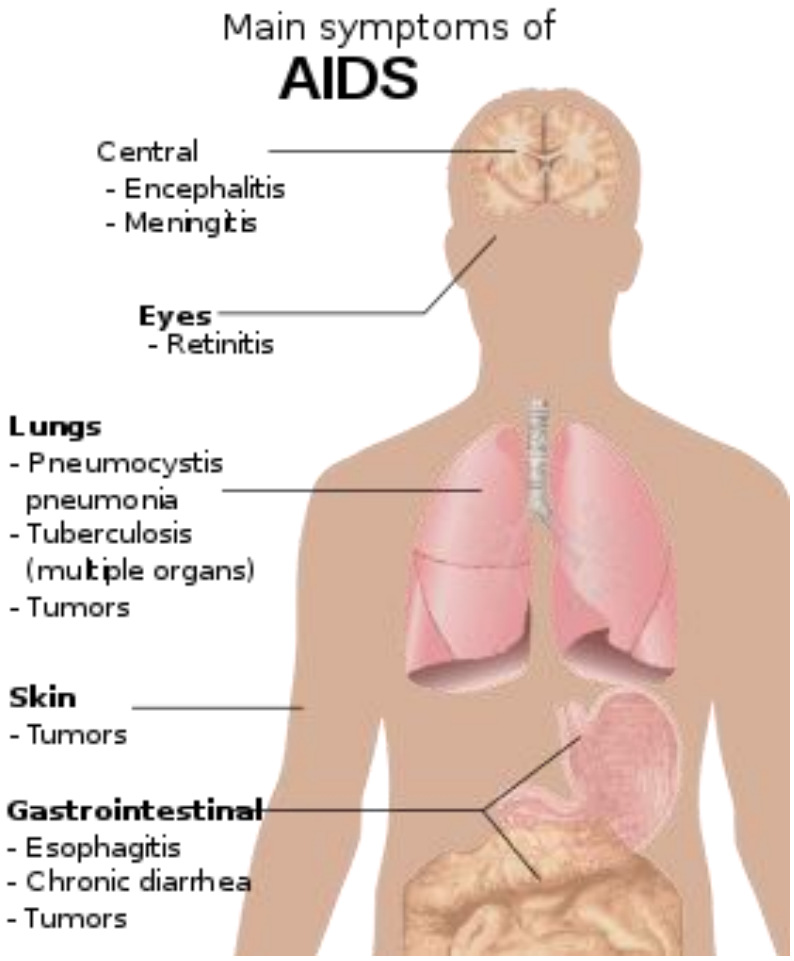

Fig. 12

6.2.1.8 Main symptoms of AIDS

Acquired immunodeficiency syndrome (AIDS) is defined as an HIV infection with either a $CD4^+$ T cell count below 200 cells per µL or the occurrence of specific diseases associated with HIV infection. In the absence of specific treatment, around half of people infected with HIV develop AIDS within ten years. The most common initial conditions that alert to the presence of AIDS are pneumocystis pneumonia (40%), cachexia in the form of HIV wasting syndrome (20%), and esophageal candidiasis. Other common signs include recurrent respiratory tract infections. Opportunistic infections may be caused by bacteria, viruses, fungi,

and parasites that are normally controlled by the immune system. Which infections occur depends partly on what organisms are common in the person's environment. These infections may affect nearly every organ system.

Average per act risk of getting HIV
by exposure route to an infected source

Exposure route	Chance of infection
Blood transfusion	90%
Childbirth (to child)	25%
Needle-sharing injection drug use	0.67%
Percutaneous needle stick	0.30%
Receptive anal intercourse*	0.04–3.0%
Insertive anal intercourse*	0.03%
Receptive penile-vaginal intercourse*	0.05–0.30%
Insertive penile-vaginal intercourse*	0.01–0.38%

Receptive oral intercourse[*§]	0–0.04%
Insertive oral intercourse[*§]	0–0.005%

[*] Assuming no condom use

[§] source refers to oral intercourse performed on a man

People with AIDS have an increased risk of developing various viral-induced cancers, including Kaposi's sarcoma, Burkitt's lymphoma, primary central nervous system lymphoma, and cervical cancer. Kaposi's sarcoma is the most common cancer, occurring in 10% to 20% of people with HIV. The second-most common cancer is lymphoma, which is the cause of death of nearly 16% of people with AIDS and is the initial sign of AIDS in 3% to 4%. Both these cancers are associated with human herpesvirus 8 (HHV-8). Cervical cancer occurs more frequently in those with AIDS because of its association with human papillomavirus (HPV). Conjunctival cancer (of the layer that lines the inner part of eyelids and the white part of the eye) is also more common in those with HIV.

Additionally, people with AIDS frequently have systemic symptoms such as prolonged fevers, sweats (particularly at night), swollen lymph nodes, chills, weakness, and unintended weight loss. Diarrhea is another common symptom, present in about 90% of people with AIDS. They can also be affected by diverse psychiatric and neurological symptoms independent of opportunistic infections and cancers.

6.2.1.9 Transmission

HIV is spread by three main routes: sexual contact, significant exposure to infected body fluids or tissues, and from mother to child during pregnancy, delivery, or breastfeeding (known as vertical transmission). There is no risk of acquiring HIV if exposed to feces, nasal secretions, saliva, sputum, sweat, tears, urine, or vomit unless these are contaminated with blood. It is also possible to be co-infected by more than one strain of HIV-a condition known as HIV superinfection.

6.2.1.9.1 Sexual

The most frequent mode of transmission of HIV is through sexual contact with an infected person. However, an HIV-positive person who has an undetectable viral load as a result of long-term treatment has effectively no risk of transmitting HIV sexually. The existence of functionally noncontagious HIV-positive people on antiretroviral therapy was controversially publicized in the 2008 Swiss Statement, and has since become accepted as medically sound.

Globally, the most common mode of HIV transmission is via sexual contacts between people of the opposite sex; however, the pattern of transmission varies among countries. As of 2017, most HIV transmission in the United States occurred among men who had sex with men (82% of new HIV diagnoses among males aged 13 and older and 70% of total new diagnoses). In the US, gay and bisexual men aged 13 to 24 accounted for an estimated 92% of new HIV diagnoses among all men in their age group and 27% of new diagnoses among all gay and bisexual men.

With regard to unprotected heterosexual contacts, estimates of the risk of HIV transmission per sexual act appear to be four to ten times higher in low-income countries than in high-income countries. In low-income countries, the risk of female-to-male transmission is estimated as 0.38% per act, and of male-to-female transmission as 0.30% per act; the equivalent estimates for high-income countries are 0.04% per act for female-to-male transmission, and 0.08% per act for male-to-female transmission.

The risk of transmission from anal intercourse is especially high, estimated as 1.4–1.7% per act in both heterosexual and homosexual contacts. While the risk of transmission from oral sex is relatively low, it is still present. The risk from receiving oral sex has been described as "nearly nil"; however, a few cases have been reported. The per-act risk is estimated at 0–0.04% for receptive oral intercourse. In settings involving prostitution in low-income countries, risk of female-to-male transmission has been estimated as 2.4% per act, and of male-to-female transmission as 0.05% per act.

Risk of transmission increases in the presence of many sexually transmitted infections and genital ulcers. Genital ulcers appear to increase the risk approximately fivefold. Other sexually transmitted infections, such

as gonorrhea, chlamydia, trichomoniasis, and bacterial vaginosis, are associated with somewhat smaller increases in risk of transmission.

The viral load of an infected person is an important risk factor in both sexual and mother-to-child transmission. During the first 2.5 months of an HIV infection a person's infectiousness is twelve times higher due to the high viral load associated with acute HIV. If the person is in the late stages of infection, rates of transmission are approximately eightfold greater.

Commercial sex workers (including those in pornography) have an increased likelihood of contracting HIV. Rough sex can be a factor associated with an increased risk of transmission. Sexual assault is also believed to carry an increased risk of HIV transmission as condoms are rarely worn, physical trauma to the vagina or rectum is likely, and there may be a greater risk of concurrent sexually transmitted infections.

6.2.1.9.2 Body fluids

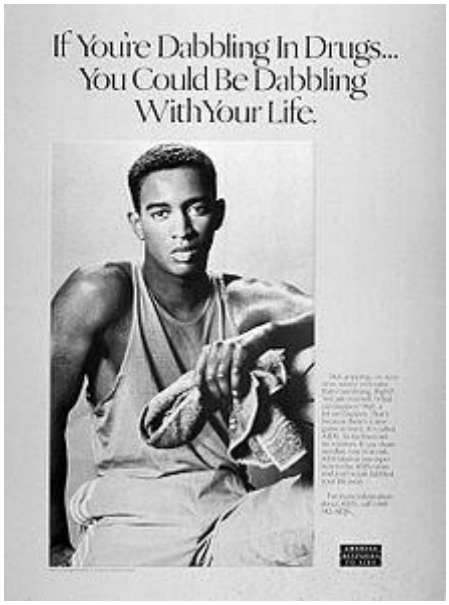

Fig. 13

CDC poster from 1989 highlighting the threat of AIDS associated with drug use

The second-most frequent mode of HIV transmission is via blood and blood products. Blood-borne transmission can be through needle-sharing during intravenous drug use, needle-stick injury, transfusion of contaminated blood or blood product, or medical injections with unsterilized equipment. The risk from sharing a needle

during drug injection is between 0.63% and 2.4% per act, with an average of 0.8%. The risk of acquiring HIV from a needle stick from an HIV-infected person is estimated as 0.3% (about 1 in 333) per act and the risk following mucous membrane exposure to infected blood as 0.09% (about 1 in 1000) per act. This risk may, however, be up to 5% if the introduced blood was from a person with a high viral load and the cut was deep. In the United States intravenous drug users made up 12% of all new cases of HIV in 2009, and in some areas more than 80% of people who inject drugs are HIV-positive.

HIV is transmitted in about 90% of blood transfusions using infected blood.[41] In developed countries the risk of acquiring HIV from a blood transfusion is extremely low (less than one in half a million) where improved donor selection and HIV screening is performed; for example, in the UK the risk is reported at one in five million and in the United States it was one in 1.5 million in 2008. In low-income countries, only half of transfusions may be appropriately screened (as of 2008),[74] and it is estimated that up to 15% of HIV infections in these areas come from transfusion of infected blood and blood products, representing between 5% and 10% of global infections. It is possible to acquire HIV from organ and tissue transplantation, although this is rare because of screening.

Unsafe medical injections play a role in HIV spread in sub-Saharan Africa. In 2007, between 12% and 17% of infections in this region were attributed to medical syringe use. The World Health Organization estimates the risk of transmission as a result of a medical injection in Africa at 1.2%. Risks are also associated with invasive procedures, assisted delivery, and dental care in this area of the world. People giving or receiving tattoos, piercings, and scarification are theoretically at risk of infection but no confirmed cases have been documented. It is not possible for mosquitoes or other insects to transmit HIV.

6.2.1.9.3 Mother-to-child

HIV can be transmitted from mother to child during pregnancy, during delivery, or through breast milk, resulting in the baby also contracting HIV. As of 2008, vertical transmission accounted for about 90% of cases of HIV in children. In the absence of

treatment, the risk of transmission before or during birth is around 20%, and in those who also breastfeed 35%. Treatment decreases this risk to less than 5%.

Antiretrovirals when taken by either the mother or the baby decrease the risk of transmission in those who do breastfeed. If blood contaminates food during pre-chewing it may pose a risk of transmission. If a woman is untreated, two years of breastfeeding results in an HIV/AIDS risk in her baby of about 17%. Due to the increased risk of death without breastfeeding in many areas in the developing world, the World Health Organization recommends either exclusive breastfeeding or the provision of safe formula. All women known to be HIV-positive should be taking lifelong antiretroviral therapy.

6.2.1.9.4 Virology

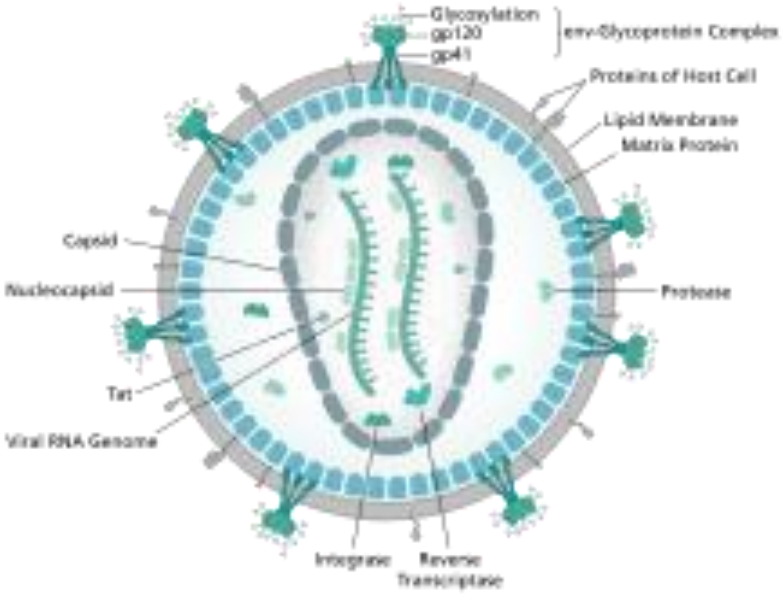

Fig. 14 Diagram of a HIV virion structure

Fig. 15 Scanning electron micrograph of HIV-1, colored green, budding from a cultured lymphocyte.

HIV is the cause of the spectrum of disease known as HIV/AIDS. HIV is a retrovirus that primarily infects components of the human immune system such as $CD4^+$ T cells, macrophages and dendritic cells. It directly and indirectly destroys $CD4^+$ T cells.

HIV is a member of the genus *Lentivirus*, part of the family *Retroviridae*. Lentiviruses share many morphological and biological characteristics. Many species of mammals are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period. Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle.

The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors. Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle anew.

HIV is now known to spread between $CD4^+$ T cells by two parallel routes: cell-free spread and cell-to-cell spread, i.e. it employs hybrid spreading mechanisms. In the cell-free spread, virus particles bud from an infected T cell, enter the blood/extracellular

fluid and then infect another T cell following a chance encounter. HIV can also disseminate by direct transmission from one cell to another by a process of cell-to-cell spread. The hybrid spreading mechanisms of HIV contribute to the virus's ongoing replication against antiretroviral therapies.

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was originally discovered (and initially referred to also as LAV or HTLV-III). It is more virulent, more infective, and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 as compared with HIV-1 implies that fewer people exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa.

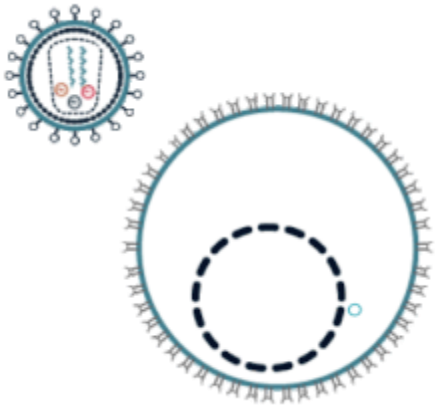

Fig. 16

HIV replication cycle

After the virus enters the body there is a period of rapid viral replication, leading to an abundance of virus in the peripheral blood. During primary infection, the level of HIV may reach several million virus particles per milliliter of blood. This response is accompanied by a marked drop in the number of circulating $CD4^+$ T cells. The acute viremia is almost invariably associated with activation of $CD8^+$ T cells, which kill HIV-infected cells, and subsequently with antibody production, or seroconversion. The $CD8^+$ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the $CD4^+$ T cell counts recover. A good $CD8^+$ T cell response has

been linked to slower disease progression and a better prognosis, though it does not eliminate the virus.[99]

Ultimately, HIV causes AIDS by depleting $CD4^+$ T cells. This weakens the immune system and allows opportunistic infections. T cells are essential to the immune response and without them, the body cannot fight infections or kill cancerous cells. The mechanism of $CD4^+$ T cell depletion differs in the acute and chronic phases.[100] During the acute phase, HIV-induced cell lysis and killing of infected cells by $CD8^+$ T cells accounts for $CD4^+$ T cell depletion, although apoptosis may also be a factor. During the chronic phase, the consequences of generalized immune activation coupled with the gradual loss of the ability of the immune system to generate new T cells appear to account for the slow decline in $CD4^+$ T cell numbers.

Although the symptoms of immune deficiency characteristic of AIDS do not appear for years after a person is infected, the bulk of $CD4^+$ T cell loss occurs during the first weeks of infection, especially in the intestinal mucosa, which harbors the majority of the lymphocytes found in the body. The reason for the preferential loss of mucosal $CD4^+$ T cells is that the majority of mucosal $CD4^+$ T cells express the CCR5 protein which HIV uses as a co-receptor to gain access to the cells, whereas only a small fraction of $CD4^+$ T cells in the bloodstream do so. A specific genetic change that alters the CCR5 protein when present in both chromosomes very effectively prevents HIV-1 infection.

HIV seeks out and destroys CCR5 expressing $CD4^+$ T cells during acute infection. A vigorous immune response eventually controls the infection and initiates the clinically latent phase. $CD4^+$ T cells in mucosal tissues remain particularly affected. Continuous HIV replication causes a state of generalized immune activation persisting throughout the chronic phase. Immune activation, which is reflected by the increased activation state of immune cells and release of pro-inflammatory cytokines, results from the activity of several HIV gene products and the immune response to ongoing HIV replication. It is also linked to the breakdown of the immune surveillance system of the gastrointestinal mucosal barrier caused by the depletion of mucosal $CD4^+$ T cells during the acute phase of disease.

6.2.1.9.5 Diagnosis

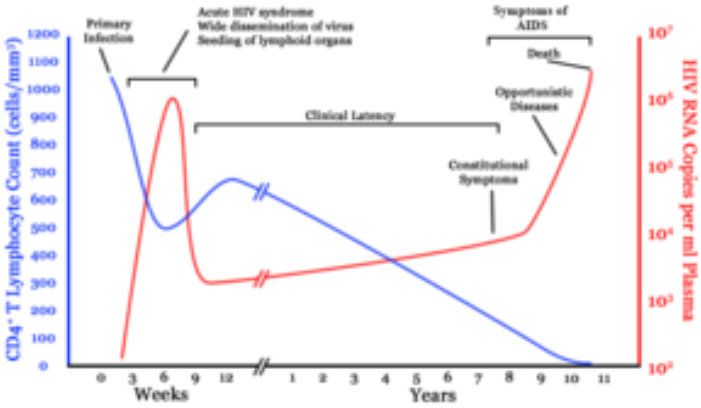

Fig. 17 A generalized graph of the relationship between HIV copies (viral load) and $CD4^+$ T cell counts over the average course of untreated HIV infection.

$CD4^+$ T Lymphocyte count (cells/mm^3)

HIV RNA copies per mL of plasma

Days after exposure needed for the test to be accurate

Blood test	**Days**
Antibody test (rapid test, ELISA 3rd gen)	23–90
Antibody and p24 antigen test (ELISA 4th gen)	18–45
PCR	10–33

HIV/AIDS is diagnosed via laboratory testing and then staged based on the presence of certain signs or symptoms. HIV screening is recommended by the United States Preventive Services Task Force for all people 15 years to 65 years of age, including all pregnant women. Additionally, testing is recommended for those at high risk, which includes anyone diagnosed with a sexually transmitted illness. In many areas of the world, a third of HIV carriers only discover they are infected at an advanced stage of the disease when AIDS or severe immunodeficiency has become apparent.

6.2.1.9.6 HIV testing

HIV Rapid Test being administered

Fig. 18

Fig. 19 Oraquick

Most people infected with HIV develop specific antibodies (i.e. seroconvert) within three to twelve weeks after the initial infection. Diagnosis of primary HIV before seroconversion is done by measuring HIV-RNA or p24 antigen. Positive results

obtained by antibody or PCR testing are confirmed either by a different antibody or by PCR.

Antibody tests in children younger than 18 months are typically inaccurate, due to the continued presence of maternal antibodies. Thus HIV infection can only be diagnosed by PCR testing for HIV RNA or DNA, or via testing for the p24 antigen. Much of the world lacks access to reliable PCR testing, and people in many places simply wait until either symptoms develop or the child is old enough for accurate antibody testing. In sub-Saharan Africa between 2007 and 2009, between 30% and 70% of the population were aware of their HIV status. In 2009, between 3.6% and 42% of men and women in sub-Saharan countries were tested; this represented a significant increase compared to previous years.

6.2.2.0 Classifications

Two main clinical staging systems are used to classify HIV and HIV-related disease for surveillance purposes: the WHO disease staging system for HIV infection and disease, and the CDC classification system for HIV infection. The CDC's classification system is more frequently adopted in developed countries. Since the WHO's staging system does not require laboratory tests, it is suited to the resource-restricted conditions encountered in developing countries, where it can also be used to help guide clinical management. Despite their differences, the two systems allow comparison for statistical purposes.

The World Health Organization first proposed a definition for AIDS in 1986. Since then, the WHO classification has been updated and expanded several times, with the most recent version being published in 2007. The WHO system uses the following categories:

- Primary HIV infection: May be either asymptomatic or associated with acute retroviral syndrome.
- Stage I: HIV infection is asymptomatic with a $CD4^+$ T cell count (also known as CD4 count) greater than 500 per microlitre (µl or cubic mm) of blood. May include generalized lymph node enlargement.

- Stage II: Mild symptoms, which may include minor mucocutaneous manifestations and recurrent upper respiratory tract infections. A CD4 count of less than 500/µl.
- Stage III: Advanced symptoms, which may include unexplained chronic diarrhea for longer than a month, severe bacterial infections including tuberculosis of the lung, and a CD4 count of less than 350/µl.
- Stage IV or AIDS: severe symptoms, which include toxoplasmosis of the brain, candidiasis of the esophagus, trachea, bronchi, or lungs, and Kaposi's sarcoma. A CD4 count of less than 200/µl.

The United States Center for Disease Control and Prevention also created a classification system for HIV, and updated it in 2008 and 2014. This system classifies HIV infections based on CD4 count and clinical symptoms, and describes the infection in five groups. In those greater than six years of age it is:

- Stage 0: the time between a negative or indeterminate HIV test followed less than 180 days by a positive test.
- Stage 1: CD4 count ≥ 500 cells/µl and no AIDS-defining conditions.
- Stage 2: CD4 count 200 to 500 cells/µl and no AIDS-defining conditions.
- Stage 3: CD4 count ≤ 200 cells/µl or AIDS-defining conditions.
- Unknown: if insufficient information is available to make any of the above classifications.

For surveillance purposes, the AIDS diagnosis still stands even if, after treatment, the $CD4^+$ T cell count rises to above 200 per µL of blood or other AIDS-defining illnesses are cured.

6.2.2.1 Sexual contact

People wearing AIDS awareness signs. on the left: "Facing AIDS a condom and a pill at a time"; on the right: "I am Facing AIDS because people are infected." Consistent condom use reduces the risk of HIV transmission by approximately 80% over the long term. When condoms are used consistently by a couple in which one person is infected, the rate of HIV infection is less than 1% per year. There is some evidence to suggest that female condoms may provide an equivalent level of protection. Application of a vaginal gel containing tenofovir (a reverse transcriptase

inhibitor) immediately before sex seems to reduce infection rates by approximately 40% among African women. By contrast, use of the spermicide nonoxynol-9 may increase the risk of transmission due to its tendency to cause vaginal and rectal irritation.

Circumcision in Sub-Saharan Africa "reduces the acquisition of HIV by heterosexual men by between 38% and 66% over 24 months". Owing to these studies, both the World Health Organization and UNAIDS recommended male circumcision in 2007 as a method of preventing female-to-male HIV transmission in areas with high rates of HIV. However, whether it protects against male-to-female transmission is disputed, and whether it is of benefit in developed countries and among men who have sex with men is undetermined. The International Antiviral Society, however, does recommend it for all sexually active heterosexual males and that it be discussed as an option with men who have sex with men. Some experts fear that a lower perception of vulnerability among circumcised men may cause more sexual risk-taking behavior, thus negating its preventive effects.

Programs encouraging sexual abstinence do not appear to affect subsequent HIV risk. Evidence of any benefit from peer education is equally poor. Comprehensive sexual education provided at school may decrease high-risk behavior. A substantial minority of young people continues to engage in high-risk practices despite knowing about HIV/AIDS, underestimating their own risk of becoming infected with HIV. Voluntary counseling and testing people for HIV does not affect risky behavior in those who test negative but does increase condom use in those who test positive. Enhanced family planning services appear to increase the likelihood of women with HIV using contraception, compared to basic services. It is not known whether treating other sexually transmitted infections is effective in preventing HIV.

6.2.2.1.1 Pre-exposure

Antiretroviral treatment among people with HIV whose CD4 count $\leq$ 550 cells/μL is a very effective way to prevent HIV infection of their partner (a strategy known as treatment as prevention, or TASP). TASP is associated with a 10- to 20-fold reduction in transmission risk. Pre-exposure prophylaxis (PrEP) with a daily dose of the

medications tenofovir, with or without emtricitabine, is effective in people at high risk including men who have sex with men, couples where one is HIV-positive, and young heterosexuals in Africa. It may also be effective in intravenous drug users, with a study finding a decrease in risk of 0.7 to 0.4 per 100 person years. The USPSTF, in 2019, recommended PrEP in those who are at high risk.

Universal precautions within the health care environment are believed to be effective in decreasing the risk of HIV. Intravenous drug use is an important risk factor, and harm reduction strategies such as needle-exchange programs and opioid substitution therapy appear effective in decreasing this risk.

6.2.2.1.2 Post-exposure

A course of antiretrovirals administered within 48 to 72 hours after exposure to HIV-positive blood or genital secretions is referred to as post-exposure prophylaxis (PEP). The use of the single agent zidovudine reduces the risk of a HIV infection five-fold following a needle-stick injury. As of 2013, the prevention regimen recommended in the United States consists of three medications-tenofovir, emtricitabine and raltegravir, is this may reduce the risk further.

PEP treatment is recommended after a sexual assault when the perpetrator is known to be HIV-positive, but is controversial when their HIV status is unknown. The duration of treatment is usually four weeks and is frequently associated with adverse effects-where zidovudine is used, about 70% of cases result in adverse effects such as nausea (24%), fatigue (22%), emotional distress (13%) and headaches (9%).

6.2.2.1.3 Mother to child

Programs to prevent the vertical transmission of HIV (from mothers to children) can reduce rates of transmission by 92–99%. This primarily involves the use of a combination of antiviral medications during pregnancy and after birth in the infant, and potentially includes bottle feeding rather than breastfeeding. If replacement feeding is acceptable, feasible, affordable, sustainable and safe, mothers should avoid breastfeeding their infants; however, exclusive breastfeeding is recommended during the first months of life if this is not the case. If exclusive breast feeding is carried out, the provision of extended antiretroviral prophylaxis to the infant decreases the risk of

transmission. In 2015, Cuba became the first country in the world to eradicate mother-to-child transmission of HIV.

6.2.2.2 Vaccination

Currently there is no licensed vaccine for HIV or AIDS. The most effective vaccine trial to date, RV 144, was published in 2009; it found a partial reduction in the risk of transmission of roughly 30%, stimulating some hope in the research community of developing a truly effective vaccine. Further trials of the RV 144 vaccine are ongoing.

6.2.2.3 Treatment

There is currently no cure, nor an effective HIV vaccine. Treatment consists of highly active antiretroviral therapy (HAART) which slows progression of the disease. As of 2010 more than 6.6 million people were receiving this in low- and middle-income countries. Treatment also includes preventive and active treatment of opportunistic infections. As of March 2020, two persons have been successfully cleared of HIV. Rapid initiation of anti-retroviral therapy within one week of diagnosis appear to improve treatment outcomes in low and medium-income settings.

6.2.2.4 Antiviral therapy

Fig. 20

6.2.2.5 Stribild

a common once-daily ART regime consisting of elvitegravir, emtricitabine, tenofovir and the booster cobicistat Current HAART options are combinations (or "cocktails") consisting of at least three medications belonging to at least two types, or "classes", of antiretroviral agents. Initially, treatment is typically a non-nucleoside reverse transcriptase inhibitor (NNRTI) plus two nucleoside analog reverse transcriptase inhibitors (NRTIs). Typical NRTIs include: zidovudine (AZT) or tenofovir (TDF) and lamivudine (3TC) or emtricitabine (FTC). As of 2019, dolutegravir/lamivudine/tenofovir is listed by the World Health Organization as the first-line treatment for adults, with tenofovir/lamivudine/efavirenz as an alternative. Combinations of agents that include protease inhibitors (PI) are used if the above regimen loses effectiveness.

The World Health Organization and the United States recommend antiretrovirals in people of all ages (including pregnant women) as soon as the diagnosis is made, regardless of CD4 count. Once treatment is begun, it is recommended that it is continued without breaks or "holidays". Many people are diagnosed only after treatment ideally should have begun. The desired outcome of treatment is a long-term plasma HIV-RNA count below 50 copies/mL. Levels to determine if treatment is effective are initially recommended after four weeks and once levels fall below 50 copies/mL checks every three to six months are typically adequate. Inadequate control is deemed to be greater than 400 copies/mL. Based on these criteria treatment is effective in more than 95% of people during the first year.

Benefits of treatment include a decreased risk of progression to AIDS and a decreased risk of death. In the developing world, treatment also improves physical and mental health. With treatment, there is a 70% reduced risk of acquiring tuberculosis. Additional benefits include a decreased risk of transmission of the disease to sexual partners and a decrease in mother-to-child transmission. The effectiveness of treatment depends to a large part on compliance. Reasons for non-adherence to treatment include poor access to medical care, inadequate social supports, mental illness and drug abuse. The complexity of treatment regimens (due to pill numbers and dosing frequency) and adverse effects may reduce adherence. Even though cost is an important issue with some medications, 47% of those who needed them were taking

them in low- and middle-income countries as of 2010, and the rate of adherence is similar in low-income and high-income countries.

Specific adverse events are related to the antiretroviral agent taken. Some relatively common adverse events include: lipodystrophy syndrome, dyslipidemia, and diabetes mellitus, especially with protease inhibitors. Other common symptoms include diarrhea, and an increased risk of cardiovascular disease. Newer recommended treatments are associated with fewer adverse effects. Certain medications may be associated with birth defects and therefore may be unsuitable for women hoping to have children.

Treatment recommendations for children are somewhat different from those for adults. The World Health Organization recommends treating all children less than five years of age; children above five are treated like adults. The United States guidelines recommend treating all children less than 12 months of age and all those with HIV RNA counts greater than 100,000 copies/mL between one year and five years of age.

The European Medicines Agency (EMA) has recommended the granting of marketing authorizations for two new antiretroviral (ARV) medicines, rilpivirine (Rekambys) and cabotegravir (Vocabria), to be used together for the treatment of people with human immunodeficiency virus type 1 (HIV-1) infection. The two medicines are the first ARVs that come in a long-acting injectable formulation. This means that instead of daily pills, people receive intramuscular injections monthly or every two months.

The combination of Rekambys and Vocabria injection is intended for maintenance treatment of adults who have undetectable HIV levels in the blood (viral load less than 50 copies/ml) with their current ARV treatment, and when the virus has not developed resistance to certain class of anti-HIV medicines called non-nucleoside reverse transcriptase inhibitors (NNRTIs) and integrase strand transfer inhibitors (INIs).

6.2.2.6 Opportunistic infections

Measures to prevent opportunistic infections are effective in many people with HIV/AIDS. In addition to improving current disease, treatment with antiretrovirals reduces the risk of developing additional opportunistic infections. Adults and adolescents who are living with HIV (even on anti-retroviral therapy) with no evidence

of active tuberculosis in settings with high tuberculosis burden should receive isoniazid preventive therapy (IPT); the tuberculin skin test can be used to help decide if IPT is needed. Vaccination against hepatitis A and B is advised for all people at risk of HIV before they become infected; however, it may also be given after infection. Trimethoprim/sulfamethoxazole prophylaxis between four and six weeks of age, and ceasing breastfeeding of infants born to HIV-positive mothers, is recommended in resource-limited settings.

It is also recommended to prevent PCP when a person's CD4 count is below 200 cells/uL and in those who have or have previously had PCP. People with substantial immunosuppression are also advised to receive prophylactic therapy for toxoplasmosis and MAC. Appropriate preventive measures reduced the rate of these infections by 50% between 1992 and 1997. Influenza vaccination and pneumococcal polysaccharide vaccine are often recommended in people with HIV/AIDS with some evidence of benefit.

6.2.2.7 Diet

The World Health Organization (WHO) has issued recommendations regarding nutrient requirements in HIV/AIDS. A generally healthy diet is promoted. Dietary intake of micronutrients at RDA levels by HIV-infected adults is recommended by the WHO; higher intake of vitamin A, zinc, and iron can produce adverse effects in HIV-positive adults, and is not recommended unless there is documented deficiency. Dietary supplementation for people who are infected with HIV and who have inadequate nutrition or dietary deficiencies may strengthen their immune systems or help them recover from infections; however, evidence indicating an overall benefit in morbidity or reduction in mortality is not consistent.

Evidence for supplementation with selenium is mixed with some tentative evidence of benefit. For pregnant and lactating women with HIV, multivitamin supplement improves outcomes for both mothers and children. If the pregnant or lactating mother has been advised to take anti-retroviral medication to prevent mother-to-child HIV transmission, multivitamin supplements should not replace these treatments. There is some evidence that vitamin A supplementation in children with an HIV infection reduces mortality and improves growth.

6.2.2.8 Alternative medicine

In the US, approximately 60% of people with HIV use various forms of complementary or alternative medicine, whose effectiveness has not been established.There is not enough evidence to support the use of herbal medicines. There is insufficient evidence to recommend or support the use of medical cannabis to try to increase appetite or weight gain. The primary causes of death from HIV/AIDS are opportunistic infections and cancer, both of which are frequently the result of the progressive failure of the immune system. Risk of cancer appears to increase once the CD4 count is below 500/μL. The rate of clinical disease progression varies widely between individuals and has been shown to be affected by a number of factors such as a person's susceptibility and immune function; their access to health care, the presence of co-infections; and the particular strain (or strains) of the virus involved.

Tuberculosis co-infection is one of the leading causes of sickness and death in those with HIV/AIDS being present in a third of all HIV-infected people and causing 25% of HIV-related deaths. HIV is also one of the most important risk factors for tuberculosis. Hepatitis C is another very common co-infection where each disease increases the progression of the other. The two most common cancers associated with HIV/AIDS are Kaposi's sarcoma and AIDS-related non-Hodgkin's lymphoma. Other cancers that are more frequent include anal cancer, Burkitt's lymphoma, primary central nervous system lymphoma, and cervical cancer. Even with anti-retroviral treatment, over the long term HIV-infected people may experience neurocognitivedisorders, osteoporosis, neuropathy, cancers, nephropathy, an d cardiovascular disease. Some conditions, such as lipodystrophy, may be caused both by HIV and its treatment.

6.2.2.9 Epidemiology

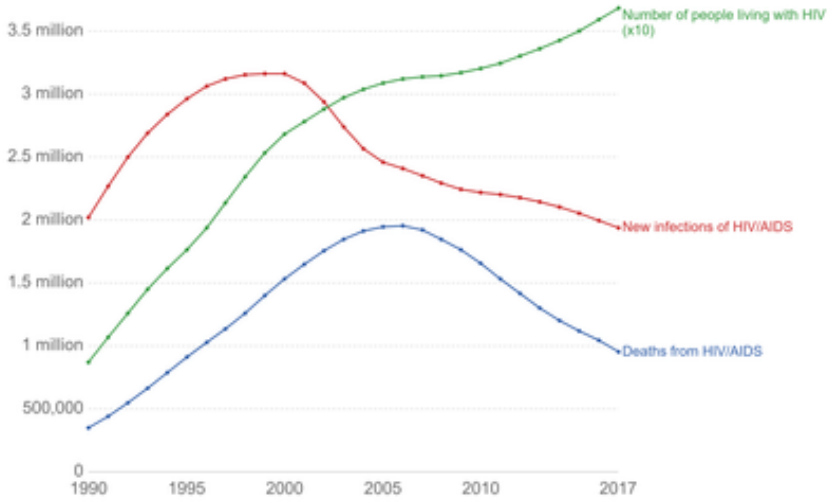

Fig. 21

6.2.2.9 Trends in new cases and deaths per year from HIV/AIDS

HIV/AIDS is a global pandemic. As of 2016 approximately 36.7 million people worldwide have HIV, the number of new infections that year being about 1.8 million. This is down from 3.1 million new infections in 2001. Slightly over half the infected population are women and 2.1 million are children. It is resulted in about 1 million deaths in 2016, down from a peak of 1.9 million in 2005.

Sub-Saharan Africa is the region most affected. In 2010, an estimated 68% (22.9 million) of all HIV cases and 66% of all deaths (1.2 million) occurred in this region. This means that about 5% of the adult population is infected and it is believed to be the cause of 10% of all deaths in children. Here, in contrast to other regions, women comprise nearly 60% of cases. South Africa has the largest population of people with HIV of any country in the world at 5.9 million. Life expectancy has fallen in the worst-affected countries due to HIV/AIDS; for example, in 2006 it was estimated that it had dropped from 65 to 35 years in Botswana. Mother-to-child transmission in Botswana and South Africa, as of 2013, has decreased to less than 5%, with improvement in many other African nations due to improved access to antiretroviral therapy.

South & South East Asia is the second most affected; in 2010 this region contained an estimated 4 million cases or 12% of all people living with HIV resulting in approximately 250,000 deaths. Approximately 2.4 million of these cases are in India.

During 2008 in the United States approximately 1.2 million people were living with HIV, resulting in about 17,500 deaths. The US Centers for Disease Control and Prevention estimated that in that year, 20% of infected Americans were unaware of their infection. As of 2016 about 675,000 people have died of HIV/AIDS in the US since the beginning of the HIV epidemic. In the United Kingdom as of 2015, there were approximately 101,200 cases which resulted in 594 deaths. In Canada as of 2008, there were about 65,000 cases causing 53 deaths. Between the first recognition of AIDS (in 1981) and 2009, it has led to nearly 30 million deaths. Rates of HIV are lowest in North Africa and the Middle East (0.1% or less), East Asia (0.1%), and Western and Central Europe (0.2%). The worst-affected European countries, in 2009 and 2012 estimates, are Russia, Ukraine, Latvia, Moldova, Portugal and Belarus, in decreasing order of prevalence.

6.2.3.1 Summary

6.2.3.2 Terminal questions

Q.8.

Answer:----------

Q.9.

Answer:----------

Q.10.

Answer:----------

Q.11.

Answer:----------

Q.12.

Answer:----------

Q.13.

Answer:----------

Q.14.

Answer:----------

1.14. Further readings

6. Biochemistry- Lehninger A.L.

7. Biochemistry –J.H.Weil.

8. Biochemistry fourth edition-David Hames and Nigel Hooper.

9. Textbook of Biochemistry for Undergraduates - Rafi, M.D.

10. Biochemistry and molecular biology- Wilson Walker.