COURSE INTRODUCTION

The objective of this course is to provide basic introduction and concept to immunology in concerned to immune system, immunity, immunoglobulins and antigen-antibody etc. The aim is to provide concept of types of immunity, types of immunoglobulins and different types of immunological disorders. The course is organized into following two blocks as under:

Block I

The block 1st is organized into following five units as under:

Unit 1 It covers the immune system

Unit 2 It covers immunity and its types

Unit 3 To know the concept of antigen and antibody

Unit 4 It relates various types of immunoglobulins

Unit 5 To know about diversity in immune system

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Introduction

This is the first block on Immunology. It consists of five units. The first unit is as under:

Unit: 1

Our immune system is essential for our survival. Without an immune system, our bodies would be open to attack from bacteria, viruses, parasites, and more. It is our immune system that keeps us healthy as we drift through a sea of pathogens. This vast network of cells and tissues is constantly on the lookout for invaders, and once an enemy is spotted, a complex attack is mounted. The immune system is spread throughout the body and involves many types of cells, organs, proteins, and tissues. Crucially, it can distinguish our tissue from foreign tissue — self from non-self. Dead and faulty cells are also recognized and cleared away by the immune system. If the immune system encounters a pathogen, for instance, a bacterium, virus, or parasite, it mounts a so-called immune response. Later, we will explain how this works, but first, we will introduce some of the main characters in the immune system.

Unit-1

Structure

1.3.3 Immunity

1.3.4 Types of immunity:

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

1.3.4.1.1 Physical Barriers

2. Physiological Barriers

1.3.4.1.2 Cellular Barriers

1.3.4.3 Acquired Immunity (= Adaptive or Specific Immunity):

1.3.4.3.1 Characteristics of Acquired Immunity:

(i) Specificity:

(ii) Diversity:

(iii) Discrimination between Self and Non-self:

(iv) Memory:

1.3.4.3.2 Components of Acquired Immunity:

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

1.3.5 Formation of Plasma B cells and Memory B cells:

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

(b) Memory B Cells:

1.3.6 Role of AMIS:

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

1.3.7 Types of T-cells and their Functions:

1. Helper T cells (T_H):

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

3. Memory T Cells (Primed Cells):

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

Natural Killer (NK) Cells:

1.3.8 Types of Acquired Immunity:

1.3.8.1 Active Immunity:

1.3.8.2 Passive Immunity:

1.3.9 Summary

1.4.0 Terminal questions

Further readings

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 &** the adaptive immune system relies upon the innate immune system to alert it to potential targets, and shape its response to them.

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.

Objectives

This is the first unit on Immunology. Under first unit (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?

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.

1.3 History of Immunology

1.3.1 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.

1.3.2 Immune system

The immune system is a network of biological processes that protects an organism from diseases. It detects and responds to a wide variety of pathogens, from viruses to parasitic worms, as well as cancer cells and objects such as wood splinters, distinguishing them from the organism's own healthy tissue. Many species have two major subsystems of the immune system. The innate immune system provides a preconfigured response to broad groups of situations and stimuli. The adaptive immune system provides a

tailored response to each stimulus by learning to recognize molecules it has previously encountered. Both use molecules and cells to perform their functions.

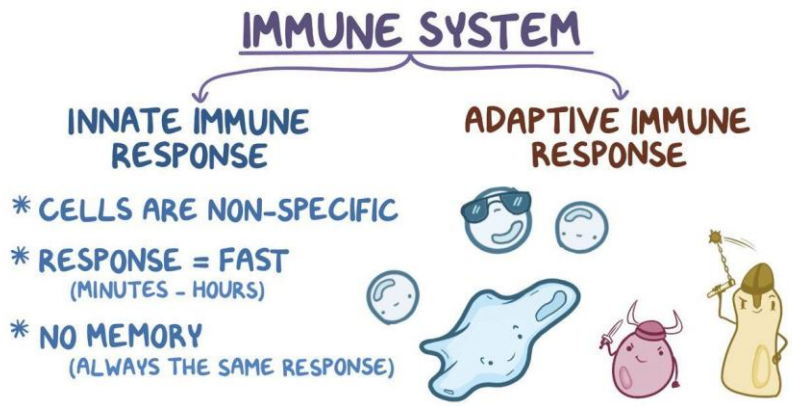

Fig. 1 The immune system

Nearly all organisms have some kind of immune system. Bacteria have a rudimentary immune system in the form of enzymes that protect against virus infections. Other basic immune mechanisms evolved in ancient plants and animals and remain in their modern descendants. These mechanisms include phagocytosis, antimicrobial peptides called defensins, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defense mechanisms, including the ability to adapt to recognize pathogens more efficiently. Adaptive (or acquired) immunity creates an immunological memory leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.

Dysfunction of the immune system can cause autoimmune diseases, inflammatory diseases and cancer. Immunodeficiency occurs when the immune system is less active than normal, resulting in recurring and life-threatening infections. In humans, immunodeficiency can be the result of a genetic disease such as severe combined immunodeficiency, acquired conditions such as HIV/AIDS, or the use of immunosuppressive medication. Autoimmunity results from a hyperactive immune system attacking normal tissues as if they were foreign organisms. Common autoimmune diseases include Hashimoto's thyroiditis, rheumatoid arthritis, diabetes mellitus type 1, and systemic lupus erythematosus. Immunology covers the study of all aspects of the immune system.

1.3.3 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.

1.3.4 Types of immunity:

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

1.3.4.1 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 is an important component of the host defense against infection. It is the only host defense system in nonvertebrate animals and synergizes adaptive immunity in vertebrates. Virtually all cells can contribute to innate immunity by producing certain innate cytokines, particularly the type 1 IFNs, and by responding to these cytokines to induce new and elevated intracellular molecular mechanisms for fighting off infections. Macrophages, DCs, and NK cells, however, are the main immune cellular constitutes responsible for innate responses. Macrophages and DCs carry PRRs that respond to PAMPs, motifs common to large classes of infectious agents but often absent in eukaryotic organisms. For viruses, ss- and dsRNA—distinct from normal cellular RNAs—are the major PAMPs. Once PRRs have been ligated, they set off intracellular biochemical cascades that lead to cellular activation. Activated cells initiate phagocytosis and secretion of many cytokines, such as type I IFN, that in turn induce inflammation and other antiviral responses. Other cytosolic receptors function

to detect viruses and induce IFN production by a wider range of infected cell types. NK cells carry unique sensors in the form of activating and inhibitory receptors. The balance of the engagement of these receptors acts to protect normal cells from the detrimental effects of NK cells while activating them to kill virus-infected target cells.

Vertebrate Immunity		
Innate Immune System		Adaptive Immune System
Physical Barriers	Internal Defenses	
• Skin, hair, cilia • Mucus membranes • Mucus and chemical secretions • Digestive enzymes in mouth • Stomach acid	• Inflammatory response • Complement proteins • Phagocytic cells • Natural killer (NK) cells	• Antibodies and the humoral immune response • Cell-mediated immune response • Memory response

Table 1 Innate Immunity – Concepts of Biology

Innate immunity is initiated within hours and provides a rapid array of defenses, whereas the antigen-specific adaptive immune responses are induced during the first weeks after infection. Furthermore, selected innate responses—such as the activation of DCs, initiation of antigen processing, migration to draining lymph nodes, up-regulation of co-stimulatory molecules, and the composition of the early cytokine profiles to shape downstream adaptive responses—are the essential first steps in induction of acquired immunity. Although there is a detailed understanding of their general functions during acute primary viral infections, much remains to be learned about how the innate and adaptive arms of immunity interact under conditions of sustained viral burdens and/or during subsequent viral infections, and about how genetic variations in the human population uniquely shape their responses and functions. Innate immunity consists of four types of barriers— physical, physiological, cellular and cytokine barriers.

1.3.4.2.1 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.

1.3.4.1.2 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.

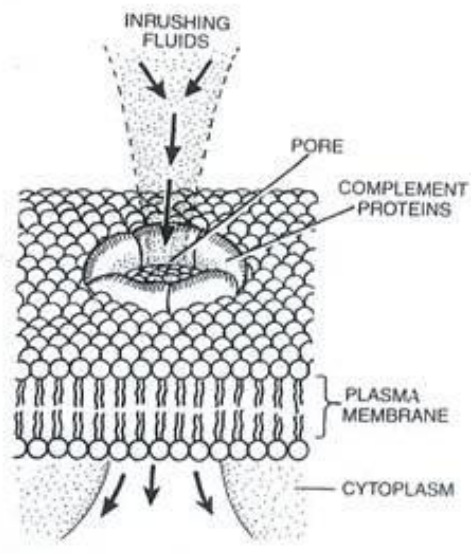

Fig.2 Complement proteins creating a hole in the plasma membrane

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.

1.3.4.3 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.

1.3.4.3.1 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.

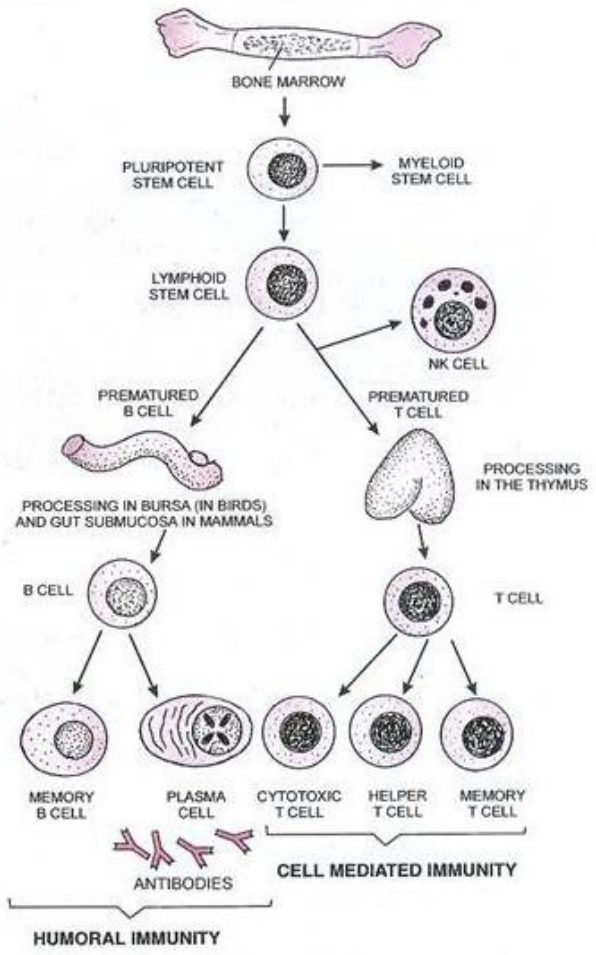

Fig. 3 Development of B and T lymphocytes

1.3.4.3.2 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.

1.3.5 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.

1.3.6 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.

1.3.7 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.

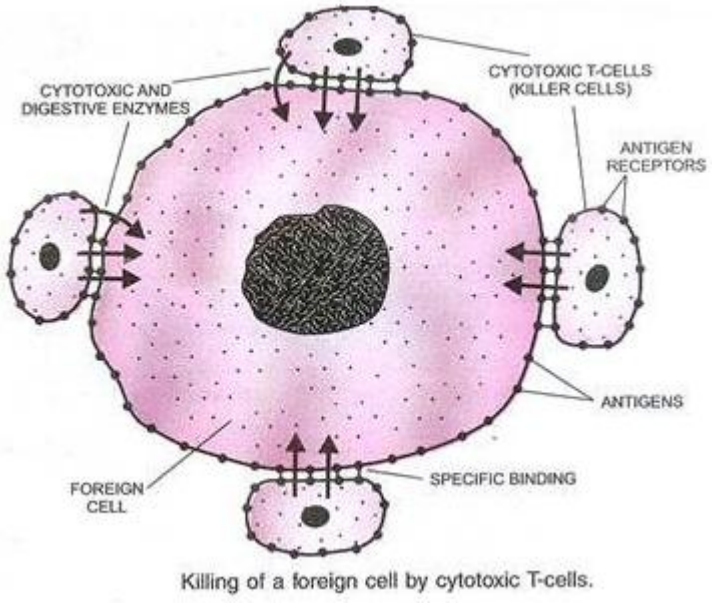

Fig. 4 Killing of a foreign body by cytotoxic T cells

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.

1.3.8 Types of Acquired Immunity:

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

1.3.8.1 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.

1.3.8.2 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.

1.3.9 Summary

Under this unit we summarize the immune system and immunity. Our immune system is essential for our survival. Without an immune system, our bodies would be open to attack from bacteria, viruses, parasites, and more. It is our immune system that keeps us healthy as we drift through a sea of pathogens. This vast network of cells and tissues is constantly on the lookout for invaders, and once an enemy is spotted, a complex attack is mounted. The immune system is spread throughout the body and involves many types of cells, organs, proteins, and tissues. Crucially, it can distinguish our tissue from foreign tissue — self from non-self. Dead and faulty cells are also recognized and cleared away by the immune system. If the immune system encounters a pathogen, for instance, a bacterium, virus, or parasite, it mounts a so-called immune response. Later, we will explain how this works, but first, we will introduce some of the main characters in the immune system.

The immune system has innate and adaptive components. Innate immunity is present in all metazoans, while adaptive immunity only occurs in vertebrates. The innate component of the immunity system involves the recognition of certain foreign (non-self) molecules to

generate one of two types of innate immune responses: inflammatory responses and phagocytosis. The adaptive component, on the other hand, involves more advanced lymphatic cells that can distinguish between specific "non-self" substances in the presence of "self". The reaction to foreign substances is etymologically described as inflammation while the non-reaction to self substances is described as immunity. The two components of the immune system create a dynamic biological environment where "health" can be seen as a physical state where the self is immunologically spared, and what is foreign is inflammatorily and immunologically eliminated. "Disease" can arise when what is foreign cannot be eliminated or what is self is not spared.

1.4.0 Terminal questions

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

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

Q.2. What are the differences between innate and acquired immunity?

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 immune system.

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.

Unit-2

Structure

2.6 Harnessing the Innate Immune System

2.7 Component 1. Physical and Chemical or Anatomical Barriers:

2.7.1 Skin:

2.7.2 Gastro-intestinal tract:

2.7.3 Urinogenital tract:

2.7.4 Mammary gland:

2.7.5 Respiratory tract:

2.7.6 Component 2. Phagocytic Barriers:

2.7.6.1 Neutrophils:

2.7.6.2 Macrophages:

2.7.6.3 Endocytosis, phagocytosis and killing of microbes:

2.8 Immunology of Barrier Surfaces

2.9 Cells and soluble mediators of innate immunity

2.10 Cytokines

2.11 Discovery

2.12 Difference from hormones

2.13 Nomenclature

2.14 Classification

2.14.1 Structural

2.14.2 Functional

2.14.3 Receptors

2.15 Cellular effects

2.16 Roles in health and disease

2.17 Adverse effects

2.18 Medical use as drugs

2.19 Summary

2.20 Terminal questions

Further readings

2.1 Introduction

The innate immune system is the phylogenically oldest component of the human immune system. Although it is ancient, the innate immune system is highly complex and consists of

barriers to infection (epithelia of skin, gastrointestinal, respiratory, genitourinary tracts), antimicrobial peptides and proteins, humoral components (i.e. complement and opsonins) and cellular components (i.e. neutrophils, monocytes/macrophages, dendritic cells, and innate lymphoid cells). Innate immunity serves as the front line of host defense and plays an essential role in preventing infection while tolerating normal host flora. Defects in innate immunity are associated with invasive, life-threatening infection. Inappropriate activation of the innate immune system can lead to autoinflammatory states. The innate immune system directs the subsequent development of adaptive immune responses. Its proper function is thus critical for health.

Many of these pathogen-specific molecules are recognized by Toll-like receptor proteins, which are found in plants and in invertebrate and vertebrate animals. In vertebrates, microbial surface molecules also activate complement, a group of blood proteins that act together to disrupt the membrane of the microorganism, to target microorganisms for phagocytosis by macrophages and neutrophils, and to produce an inflammatory response. The phagocytic cells use a combination of degradative enzymes, antimicrobial peptides, and reactive oxygen species to kill the invading microorganisms. In addition, they release signaling molecules that trigger an inflammatory response and begin to marshal the forces of the adaptive immune system. Cells infected with viruses produce interferons, which induce a series of cell responses to inhibit viral replication and activate the killing activities of natural killer cells and cytotoxic T lymphocytes.

Objectives

This is the second unit on Immunology. Under second unit we have following objectives. These are as under:

- To know about innate immunity and adaptive immunity
- To know about components of immune system.
- To know about cytokines and its types.
- To discuss macrophages, neutrophiles, receptors and use of drugs

2.2 Immune Response: The Good, The Bad, The Controlled

The immune system protects the body from disease. Over the past decade, however, researchers have come to understand that the innate immune system — the part of the immune

system that serves as the body's first line of defense — is implicated in an enormous number of disease processes that affect many millions of people around the world.

Sometimes the immune system runs too hot, erroneously attacking the body. Sometimes, it runs too cold — its defenses insufficient to defend against invaders. We now know that the tipping point between running too hot or too cold often depends on the innate immune response, but we don't know how to prevent it from going off course. The CIIID's goal is to determine how to fine-tune the innate immune system (so that it knows exactly when to turn on and how to protect the body), and then how to turn it off before it causes any collateral damage.

2.3 The Two Halves of the Immune System

Manipulating immune function — enhancing it or suppressing it — will rest on our ability to control the two aspects of the immune system: innate immunity, mentioned above, and adaptive immunity.

2.4 The innate immune system: the first line of defense

The innate immune system is the first part of the body to detect invaders such as viruses, bacteria, parasites and toxins, or to sense wounds or trauma. Upon detection of these agents or events, the innate immune system activates cells to attack and destroy the outsider, or to initiate repair, while also informing and modulating the *adaptive* immune response that follows this first line of defense.

2.5 The adaptive immune system: the second, specific response

Adaptive immune cells are the second and specific line of defense, and they are called to action by the innate immune system. After recognizing the invader, the cells can multiply and combat it, leading to recovery from disease and protection against its return.

2.6 Harnessing the Innate Immune System

The idea of using the immune system to help the body fight disease has been in existence for some time; vaccines, which allow the body to remember disease-causing pathogens, provide an excellent example. There are many diseases, though, in which the desired outcome is not to *enhance* the immune system, but to *suppress* its unwanted effects. Lupus, rheumatoid arthritis and multiple sclerosis, for instance, are autoimmune diseases in which the immune system erroneously attacks the body. Chronic inflammation, connected with wound trauma,

diabetes, cardiovascular disease and neurodegenerative disease, is another example of immune-system overreaction. The idea of using innate immunity to either enhance or suppress overall immunity is a new avenue by which we can have profound impact on improving human health.

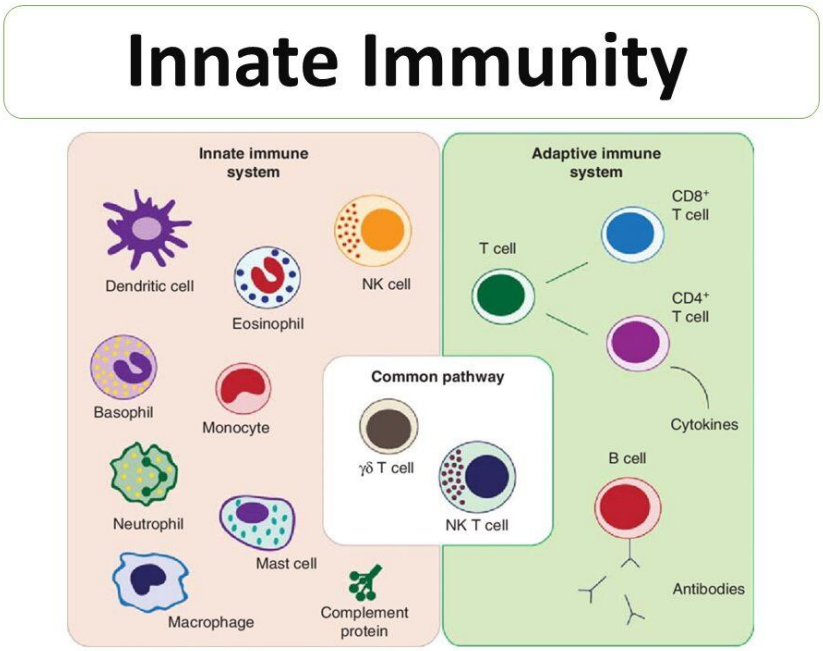

Fig. 1 Innate immunity: description and functions

In striving to understand the innate immune system, the CIIID's ultimate goal is to regulate overall immune response to distinct disease processes, thus using the power of immunity to improve human health. In doing so, we will improve the body's ability to fight pathogens and to control the immune response when it goes off track — and we will change medicine forever.

Innate immunity is an important component of the host defense against infection. It is the only host defense system in nonvertebrate animals and synergizes adaptive immunity in vertebrates. Virtually all cells can contribute to innate immunity by producing certain innate cytokines, particularly the type 1 IFNs, and by responding to these cytokines to induce new and elevated intracellular molecular mechanisms for fighting off infections. Macrophages, DCs, and NK cells, however, are the main immune cellular constitutes responsible for innate responses. Macrophages and DCs carry PRRs that respond to PAMPs, motifs common to

large classes of infectious agents but often absent in eukaryotic organisms. For viruses, ss- and dsRNA—distinct from normal cellular RNAs—are the major PAMPs. Once PRRs have been ligated, they set off intracellular biochemical cascades that lead to cellular activation. Activated cells initiate phagocytosis and secretion of many cytokines, such as type I IFN, that in turn induce inflammation and other antiviral responses.

Other cytosolic receptors function to detect viruses and induce IFN production by a wider range of infected cell types. NK cells carry unique sensors in the form of activating and inhibitory receptors. The balance of the engagement of these receptors acts to protect normal cells from the detrimental effects of NK cells while activating them to kill virus-infected target cells.

Innate immunity is initiated within hours and provides a rapid array of defenses, whereas the antigen-specific adaptive immune responses are induced during the first weeks after infection. Furthermore, selected innate responses—such as the activation of DCs, initiation of antigen processing, migration to draining lymph nodes, up-regulation of co-stimulatory molecules, and the composition of the early cytokine profiles to shape downstream adaptive responses—are the essential first steps in induction of acquired immunity. Although there is a detailed understanding of their general functions during acute primary viral infections, much remains to be learned about how the innate and adaptive arms of immunity interact under conditions of sustained viral burdens and/or during subsequent viral infections, and about how genetic variations in the human population uniquely shape their responses and functions.

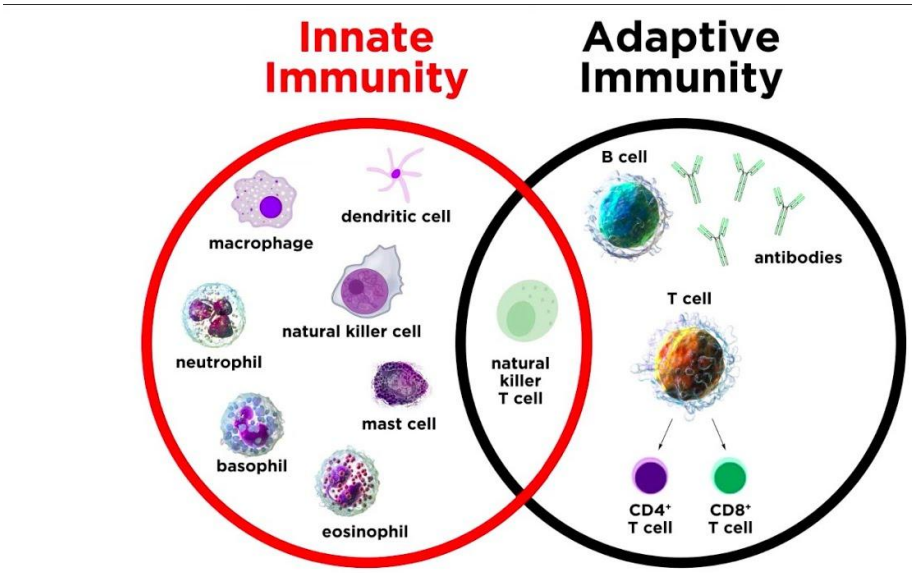

Fig. 2 Introduction to Innate Immunity

The following points highlight the four main components of innate immunity. The components are:

1. Physical and Chemical or Anatomical Barriers
2. Phagocytic Barriers
3. Blood Proteins
4. Cytokines.

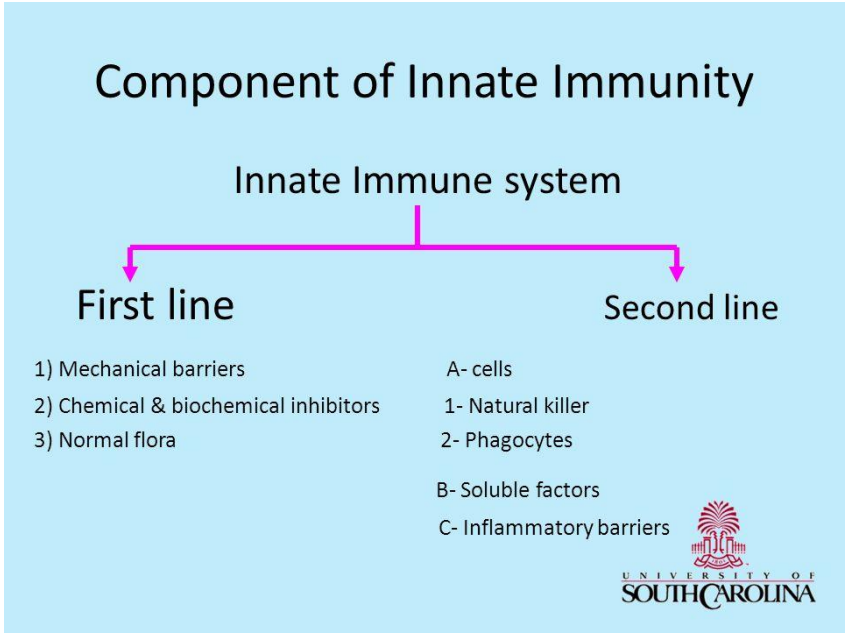

Fig. 3 Components of immunity

2.7 Component 1. Physical and Chemical or Anatomical Barriers:

Physical and Chemical or Anatomical barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection. The skin and the surface of mucous membranes are included in this category because they provide an effective barrier to the entry of most microorganisms.

2.7.1 Skin:

The skin consists of two distinct layers: a relatively thin outer layer—the epidermis—and a thicker inner layer—the dermis. The epidermis contains several layers of tightly packed epithelial cells. The outer epidermal layer consists of dead cells and is filled with a water-proofing protein called keratin. The skin acts as a major barrier to various invading microorganisms. Breaks in the skin results from scratches, wounds or abrasions. These breaks are the routes of infection. The skin may also be penetrated by biting insects e.g., mosquitoes, mites, ticks, fleas and sandflies. On the surface of the skin, dense population of resident bacterial growth occurs. Growth of these bacteria is accelerated by the improper entrance due to the presence of sebum (from sebaceous glands of dermis), desiccation, etc.

2.7.2 Gastro-intestinal tract:

The gastro-intestinal tract contains residential bacteria, which not only help in digestion of certain polysaccharides (as cellulose in herbivores) but also play an important role in the control of potential pathogens. The natural development of the immune system depends on continuous antigenic stimulus provided by those sufficiently low pH which is maintained within the stomach by gastric juices and has bactericidal and viricidal actions. In the intestine, low pH and anaerobic condition are maintained.

2.7.3 Urinogenital tract:

The flow of the urine and low pH provides the lumen of the urinogenital tract sufficient protection. In women, the vaginal wall is lined by squamous epithelium which is composed of rich amount of glycogen. In response to oestrogen, glycogen is deposited upon the epithelial surface just prior to ovulation. This is degraded anaerobically by the lactobacilli to produce lactic acid which act as a deterrent of pathogenic infection.

2.7.4 Mammary gland:

Milk contains bacterial inhibitors called lactenius. The lactenius include complement, lysozymes, an iron-binding protein called lactoferrin and an enzyme called lacto-peroxidase. Lactoferrin competes with bacteria for iron and thus inhibits their growth. In the presence of exogenous hydrogen-peroxide, lacto-peroxidase reacts with thiocyanate ions of milk and convert them into sulphurdicyanide, which is bacteriostatic.

2.7.5 Respiratory tract:

The walls of the respiratory tract are mucous covered when the suspended particles (~5 μm) present in the air enter the respiratory tract, they get stuck to these mucous covered walls. The mucous layer of the upper respiratory tract is provided with antiseptic properties by virtue of the presence of lysosomes and IgA in it. The epithelial lining of GI, respiratory and genitourinary tracts produce various micro-peptides, which act as endogenous, natural antibiotics or disinfectants. Antimicrobial peptides are also produced in glandular secretions and by the phagocytes and other cells. These are defensins, cathelicidins, protegrins, granulysin, histamin, secretory leuco-protease inhibitor (SLPI).

2.7.6 Component 2. Phagocytic Barriers:

The function of ingesting and destroying microbes is mediated by phagocytes, like neutrophils, macrophages and natural killer cells (NK cells).

2.7.6.1 Neutrophils:

Neutrophils or polymorphonuclear leukocytes are the most abundant population of circulating white blood cells. Neutrophils are spherical cells of about 12-15 μm diameter and with numerous ciliary projections. The cytoplasm is granular. The granules are of two types. Specific granules are filled with degradative enzymes, such as lysozyme, collagenase and elastase.

2.7.6.2 Macrophages:

Macrophages and their precursors, called the monocytes play a central role in both innate and acquired immunity. Macrophages may assume different morphologic forms, some develop abundant cytoplasm and are called epitheloid cells. Macrophages are found in sub-epithelial connective tissue, in the interstice of parenchymal organs, in the lining of the vascular sinusoids in the liver and spleen and in the lymphatic sinuses of lymph nodes.

2.7.6.3 Endocytosis, phagocytosis and killing of microbes:

Innate immunity is that defense mechanism which ingest extracellular macromolecules via endocytosis and particulate material via phagocytosis. In endocytosis, macromolecules within the extracellular tissue fluid are taken by cell via the invagination and pinching off a small regions of the plasma membrane. Endocytosis occurs through either pinocytosis or receptor-mediated endocytosis, both help to internalize extracellular macromolecules either by nonspecific membrane invagination or by binding to specific membrane receptors.

After that endocytic vesicles fuse with each other and ultimately fuse with endosomes. These help to dissociate ligands of macromolecules from their receptors so that they can fuse with primary lysosomes to form secondary lysosomes. Primary lysosomes originate from Golgi, primary lysosome includes degrading enzyme with proteases, nucleases, lipases and hydrolytic enzyme, which digest macromolecules within secondary lysosomes and break into certain small products.

Phagocytosis is a cytoskeleton dependent process of engulfment of large particulate material which may also include the whole pathogenic molecules. During phagocytosis the plasma membrane expands around the particulate material to form large vesicle called phagosome. Before forming phagosome, macrophages and neutrophils are attracted by and more towards a variety of substances generated in an immune response. Phagosomes are several times larger than endocytic vesicles.

2.8 Immunology of Barrier Surfaces

Barrier surfaces are the first to come into contact with pathogens and have overlapping and unique immunological mechanisms to prevent infection. The lung, gut and skin form major physcial and immunological barriers to infection. These organs are the main portal of entry for a variety of air and food borne pathogens, allergens and other environmental pollutants. They have the unique ability to maintain homeostasis in the face of constant external provocation. Once this property is jeopardized, different types of diseases ensue. Although the underlying mechanisms of some of these diseases are not known, it is now becoming clear that immune imbalances contribute to many of these disorders. Many hematopoeitic cell types orchestrate immunologic responses to pathogens in these organs. Using animal models of disease (asthma, pulmonary fibrosis, IBD, systemic sclerosis), inducible transgenic techniques

and genomic and proteomic approaches, we are involved in studying both basic mechanisms of dendritic cell maturation, ILCs activation, T cell differentiation and the relevance of these interactions in disease and in tolerance.

Microorganisms can infect a host through various portals of entry. The host attempts to counter microbial infection and dissemination using many physical, chemical and immunological strategical barriers. Among them, epithelial cell layer comprises the outer barrier defence of the body being in constant contact with microorganisms and full- time communication with adjacent immune cells. In this matter, host immune system needs to develop the ability to distinguish members of the microbiota from pathogens in order to provide protection against harmful invaders without inciting unnecessary immune responses. In this article we will discuss the mechanisms by which innate and adaptive immune system can sense the external environment and through the crosstalk with epithelial cells can coordinate effective immune response in skin and mucosal surfaces. The skin and the mucosal surfaces comprise the major portals of entrance for microbes' colonisation and infection. From birth, the skin and mucosal surfaces are naturally populated by microorganisms (bacteria, fungi, viruses) called microbiota, which helps to educate the host immune system to distinguish infectious agents from harmless particles.

The immune system encompasses nonspecific immune responses nominated innate immunity and highly- specialised deference mechanisms called adaptive immunity. Lamina propria consists the connective tissue immediately adjacent to epithelial cell layer rich of cells including fibroblasts, innate and adaptive immune cells. Along with the epithelium and the basement membrane, lamina propria constitutes the mucosa which is present in the several tubes of the body such as respiratory, genitourinary and gastrointestinal tract. The epithelial cell layer acts as a physical barrier and releases chemical substances that play a key role in innate immune responses. Mucosal surfaces harbour a great number of T cells which due to the presence of T- cell receptor (TCR) can recognise specific microorganisms and coordinate a specific response against the invader. Cytokines and chemokines are chemical substances that signal between cells to communicate and activate cells and perform the immune responses.

2.9 Cells and soluble mediators of innate immunity

Soluble factors in blood plasma have a substantial impact on both the innate and adaptive immune responses. The complement system, antibodies, and anti-microbial proteins and peptides can directly interact with potential pathogens, protecting against systemic infection. Levels of these innate effector proteins are generally lower in neonatal circulation at term delivery than in adults, and lower still at preterm delivery. The extracellular environment also has a critical influence on immune cell maturation, activation, and effector functions, and many of the factors in plasma, including hormones, vitamins, and purines, have been shown to influence these processes for leukocytes of both the innate and adaptive immune systems. The ontogeny of plasma factors can be viewed in the context of a lower effectiveness of immune responses to infection and immunization in early life, which may be influenced by the striking neonatal deficiency of complement system proteins or enhanced neonatal production of the anti-inflammatory cytokine IL-10, among other ontogenic differences. Accordingly, we survey here a number of soluble mediators in plasma for which age-dependent differences in abundance may influence the ontogeny of immune function, particularly direct innate interaction and skewing of adaptive lymphocyte activity in response to infectious microorganisms and adjuvanted vaccines.

Plasma, the fluid component of blood, is a complex mixture of water, proteins, electrolytes, lipids, sugars, hormones, and gas molecules. Plasma components also infiltrate the extravascular space and tissues and have a considerable influence on many physiological processes, including being an efficient transport medium for systemic signaling. The study of plasma is complicated by the complexity of its composition – several hundred distinct proteins and hundreds of small molecules have been analyzed in plasma by mass spectrometry. While many of these molecules have uncharacterized functions, there is a growing evidence that many of the factors in plasma that are well-characterized help to shape the response to infection, inflammation, and immunity.

Many plasma molecules vary in concentration as a function of age, and we seek here to describe both the immunoregulatory capacity of some of the best-studied molecules and the age-dependent regulation of their abundance in circulation in the context of well-described deficits in neonatal immune system function. Particular consideration is given to molecules, including cytokines, hormones, lipids, vitamins, and purines that influence the differentiation,

activation, and effector functions of subsets of T cells. Additionally, several classes of proteins, including immunoglobulins (Igs), the complement system, and anti-microbial proteins and peptides (APPs), aid in the innate response to invading microorganisms and display age-dependent maturation. The critical role that plasma components play in immune function also highlights the importance of including autologous or pooled species- and age-specific plasma in the extracellular milieu in *in vitro* assay systems, instead of xenologous media (e.g., fetal calf serum), which is more commonly utilized.

2.10 Cytokines

Cytokines are a broad and loose category of small proteins (~5–25 kDa) important in cell signaling. Cytokines are peptides and cannot cross the lipid bilayer of cells to enter the cytoplasm. Cytokines have been shown to be involved in autocrine, paracrine and endocrine signaling as immunomodulating agents. Their definite distinction from hormones is still part of ongoing research. Cytokines include chemokines, interferons, interleukins, lymphokines, and tumour necrosis factors, but generally not hormones or growth factors (despite some overlap in the terminology). Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells; a given cytokine may be produced by more than one type of cell.

They act through cell surface receptors and are especially important in the immune system; cytokines modulate the balance between humoral and cell-based immune responses, and they regulate the maturation, growth, and responsiveness of particular cell populations. Some cytokines enhance or inhibit the action of other cytokines in complex ways. They are different from hormones, which are also important cell signaling molecules. Hormones circulate in higher concentrations, and tend to be made by specific kinds of cells. Cytokines are important in health and disease, specifically in host immune responses to infection, inflammation, trauma, sepsis, cancer, and reproduction.

2.11 Discovery

Interferon-alpha, an interferon type I, was identified in 1957 as a protein that interfered with viral replication. The activity of interferon-gamma (the sole member of the interferon type II class) was described in 1965; this was the first identified lymphocyte-derived

mediator. Macrophage migration inhibitory factor (MIF) was identified simultaneously in 1966 by John David and Barry Bloom. In 1969, Dudley Dumonde proposed the term "lymphokine" to describe proteins secreted from lymphocytes and later, proteins derived from macrophages and monocytes in culture were called "monokines". In 1974, pathologist Stanley Cohen, M.D. published an article describing the production of MIF in virus-infected allantoic membrane and kidney cells, showing its production is not limited to immune cells. This led to his proposal of the term cytokine. Ogawa described the early acting growth factors, intermediate acting growth factors and late acting growth factors.

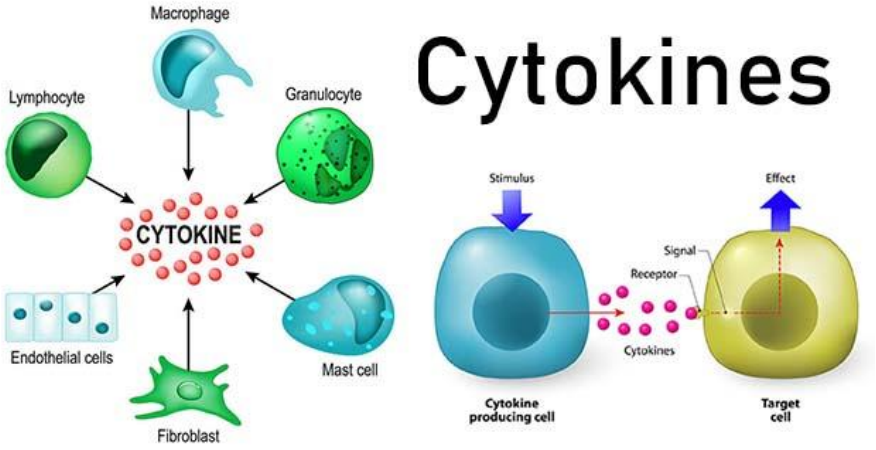

Fig. 4 Cytokines

2.12 Difference from hormones

Classic hormones circulate in aqueous solution in nanomolar (10^{-9} M) concentrations that usually vary by less than one order of magnitude. In contrast, some cytokines (such as IL-6) circulate in picomolar (10^{-12} M) concentrations that can increase up to 1,000 times during trauma or infection. The widespread distribution of cellular sources for cytokines may be a feature that differentiates them from hormones. Virtually all nucleated cells, but especially endo/epithelial cells and resident macrophages (many near the interface with the external environment) are potent producers of IL-1, IL-6, and TNF-α. In contrast, classic hormones, such as insulin, are secreted from discrete glands such as the pancreas.[12] The current terminology refers to cytokines as immunomodulating agents.

A contributing factor to the difficulty of distinguishing cytokines from hormones is that some immunomodulating effects of cytokines are systemic (*i.e.*, affecting the whole

organism) rather than local. For instance, to accurately utilize hormone terminology, cytokines may be autocrine or paracrine in nature, and chemotaxis, chemokinesis and endocrine as a pyrogen. Essentially, cytokines are not limited to their immunomodulatory status as molecules.

2.13 Nomenclature

Cytokines have been classed as lymphokines, interleukins, and chemokines, based on their presumed function, cell of secretion, or target of action. Because cytokines are characterised by considerable redundancy and pleiotropism, such distinctions, allowing for exceptions, are obsolete.

- The term *interleukin* was initially used by researchers for those cytokines whose presumed targets are principally white blood cells (leukocytes). It is now used largely for designation of newer cytokine molecules and bears little relation to their presumed function. The vast majority of these are produced by T-helper cells.
- *Lymphokines*: produced by lymphocytes
- *Monokines*: produced exclusively by monocytes
- *Interferons*: involved in antiviral responses
- *Colony stimulating factors*: support the growth of cells in semisolid media
- *Chemokines*: mediate chemoattraction (chemotaxis) between cells.

2.14 Classification

2.14.1 Structural

Structural homogeneity has been able to partially distinguish between cytokines that do not demonstrate a considerable degree of redundancy so that they can be classified into four types:

- The four-α-helix bundle family (InterPro: *IPR009079*): member cytokines have three-dimensional structures with a bundle of four α-helices. This family, in turn, is divided into three sub-families:
  1. The IL-2 subfamily. This is the largest family. It contains several non-immunological cytokines including erythropoietin (EPO) and thrombopoietin (TPO). They can be grouped into *long-chain* and *short-chain* cytokines by topology. Some members share the common gamma chain as part of their receptor.

2. The interferon (IFN) subfamily.
3. The IL-10 subfamily.

- The IL-1 family, which primarily includes IL-1 and IL-18.
- The cysteine knot cytokines (IPR029034) include members of the transforming growth factor beta superfamily, including TGF-β1, TGF-β2 and TGF-β3.
- The IL-17 family, which has yet to be completely characterized, though member cytokines have a specific effect in promoting proliferation of T-cells that cause cytotoxic effects.

2.14.2 Functional

A classification that proves more useful in clinical and experimental practice outside of structural biology divides immunological cytokines into those that enhance cellular immune responses, type 1 (TNFα, IFN-γ, etc.), and those that enhance antibody responses, type 2 (TGF-β, IL-4, IL-10, IL-13, etc.). A key focus of interest has been that cytokines in one of these two sub-sets tend to inhibit the effects of those in the other. Dysregulation of this tendency is under intensive study for its possible role in the pathogenesis of autoimmune disorders. Several inflammatory cytokines are induced by oxidative stress. The fact that cytokines themselves trigger the release of other cytokines and also lead to increased oxidative stress makes them important in chronic inflammation, as well as other immunoresponses, such as fever and acute phase proteins of the liver (IL-1,6,12, IFN-a). Cytokines also play a role in anti-inflammatory pathways and are a possible therapeutic treatment for pathological pain from inflammation or peripheral nerve injury. There are both pro-inflammatory and anti-inflammatory cytokines that regulate this pathway.

2.14.3 Receptors

In recent years, the cytokine receptors have come to demand the attention of more investigators than cytokines themselves, partly because of their remarkable characteristics and partly because a deficiency of cytokine receptors has now been directly linked to certain debilitating immunodeficiency states. In this regard, and also because the redundancy and pleomorphism of cytokines are, in fact, a consequence of their homologous receptors, many authorities think that a classification of cytokine receptors would be more clinically and experimentally useful. A classification of cytokine receptors based on their three-dimensional

structure has, therefore, been attempted. Such a classification, though seemingly cumbersome, provides several unique perspectives for attractive pharmacotherapeutic targets.

- Immunoglobulin (Ig) superfamily, which are ubiquitously present throughout several cells and tissues of the vertebrate body, and share structural homology with immunoglobulins (antibodies), cell adhesion molecules, and even some cytokines. Examples: IL-1 receptor types.
- Hemopoietic Growth Factor (type 1) family, whose members have certain conserved motifs in their extracellular amino-acid domain. The IL-2 receptor belongs to this chain, whose γ-chain (common to several other cytokines) deficiency is directly responsible for the x-linked form of Severe Combined Immunodeficiency (X-SCID).
- Interferon (type 2) family, whose members are receptors for IFN β and γ.
- Tumor necrosis factors (TNF) (type 3) family, whose members share a cysteine-rich common extracellular binding domain, and includes several other non-cytokine ligands like CD40, CD27 and CD30, besides the ligands on which the family is named.
- Seven transmembrane helix family, the ubiquitous receptor type of the animal kingdom. All G protein-coupled receptors (for hormones and neurotransmitters) belong to this family. Chemokine receptors, two of which act as binding proteins for HIV (CD4 and CCR5), also belong to this family.
- Interleukin-17 receptor (IL-17R) family, which shows little homology with any other cytokine receptor family. Structural motifs conserved between members of this family include: an extracellular fibronectin III-like domain, a transmembrane domain and a cytoplasmic SERIF domain. The known members of this family are as follows: IL-17RA, IL-17RB, IL-17RC, IL17RD and IL-17RE.

2.15 Cellular effects

Each cytokine has a matching cell-surface receptor. Subsequent cascades of intracellular signaling then alter cell functions. This may include the upregulation and/or downregulation of several genes and their transcription factors, resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition. The effect of a particular cytokine on a given cell depends

on the cytokine, its extracellular abundance, the presence and abundance of the complementary receptor on the cell surface, and downstream signals activated by receptor binding; these last two factors can vary by cell type. Cytokines are characterized by considerable redundancy, in that many cytokines appear to share similar functions. It seems to be a paradox that cytokines binding to antibodies have a stronger immune effect than the cytokine alone. This may lead to lower therapeutic doses.

It has been shown that inflammatory cytokines cause an IL-10-dependent inhibition of T-cell expansion and function by up-regulating PD-1 levels on monocytes, which leads to IL-10 production by monocytes after binding of PD-1 by PD-L. Adverse reactions to cytokines are characterized by local inflammation and/or ulceration at the injection sites. Occasionally such reactions are seen with more widespread papular eruptions.

2.16 Roles in health and disease

Cytokines are often involved in several developmental processes during embryonic development. Cytokines are crucial for fighting off infections and in other immune responses. However, they can become dysregulated and pathological in inflammation, trauma, sepsis, and hemorrhagic stroke. Dysregulated cytokine secretion in the aged population can lead to inflammaging, and render these individuals more vulnerable to age-related diseases like neurodegenerative diseases and diabetes.

2.17 Adverse effects

Adverse effects of cytokines have been linked to many disease states and conditions ranging from schizophrenia, major depression and Alzheimer's disease to cancer. T regulatory cells (Tregs) and related-cytokines are effectively engaged in the process of tumor immune escape and functionally inhibit immune response against the tumor. Forkhead box protein 3 (Foxp3) as a transcription factor is an essential molecular marker of Treg cells. Foxp3 polymorphism (rs3761548) might be involved in cancer progression like gastric cancer through influencing Tregs function and the secretion of immunomodulatory cytokines such as IL-10, IL-35, and TGF-β. Normal tissue integrity is preserved by feedback interactions between diverse cell types mediated by adhesion molecules and secreted cytokines; disruption of normal feedback mechanisms in cancer threatens tissue integrity.

Over-secretion of cytokines can trigger a dangerous cytokine storm syndrome. Cytokine storms may have been the cause of severe adverse events during a clinical trial of TGN1412. Cytokine storms are also suspected to be the main cause of death in the 1918 "Spanish Flu" pandemic. Deaths were weighted more heavily towards people with healthy immune systems, because of their ability to produce stronger immune responses, with dramatic increases in cytokine levels. Another example of cytokine storm is seen in acute pancreatitis. Cytokines are integral and implicated in all angles of the cascade, resulting in the systemic inflammatory response syndrome and multi-organ failure associated with this intra-abdominal catastrophe. In the COVID-19 pandemic, some deaths from COVID-19 have been attributable to cytokine release storms. Current data suggest cytokine storms may be the source of extensive lung tissue damage and dysfunctional coagulation in COVID-19 infections.

2.18 Medical use as drugs

Some cytokines have been developed into protein therapeutics using recombinant DNA technology. Recombinant cytokines being used as drugs as of 2014 include:

- Bone morphogenetic protein (BMP), used to treat bone-related conditions
- Erythropoietin (EPO), used to treat anemia
- Granulocyte colony-stimulating factor (G-CSF), used to treat neutropenia in cancer patients
- Granulocyte macrophage colony-stimulating factor (GM-CSF), used to treat neutropenia and fungal infections in cancer patients
- Interferon alfa, used to treat hepatitis C and multiple sclerosis
- Interferon beta, used to treat multiple sclerosis
- Interleukin 2 (IL-2), used to treat cancer.
- Interleukin 11 (IL-11), used to treat thrombocytopenia in cancer patients.
- Interferon gamma is used to treat chronic granulomatous disease and osteopetrosis

2.19 Summary

Under this unit we have summarized immunity, immunological barriers and cytokines.The innate immune responses are the first line of defense against invading pathogens. They are also required to initiate specific adaptive immune responses. Innate immune responses rely on the body's ability to recognize conserved features of pathogens that are not present in the

uninfected host. These include many types of molecules on microbial surfaces and the double-stranded RNA of some viruses.

Innate immunity encompasses anatomical and physiological barriers, cellular internalization mechanisms, and inflammatory responses that are rapidly induced by the presence of antigen. Innate immune mechanisms inhibit pathogen entry, prevent the establishment of infection, and clear both host and microbial debris. Some innate mechanisms are completely antigen nonspecific, while others involve broadly specific pattern recognition molecules (PRMs) that play a role in clearing a limited range of pathogens. Some PRMs are pattern recognition receptors (PRRs) expressed on effector cell surfaces, whereas others are soluble molecules that mark pathogens for clearance. Innate immunity either succeeds in eliminating the pathogen, or helps to hold infection in check until the slower, lymphocyte-mediated adaptive immune responses can develop. In addition, cells of the innate immune response release cytokines that are critical in lymphocyte activation and differentiation, influencing both the extent and the type of adaptive immune response to a given pathogen.

2.20 Terminal questions

Q. 1 What do you mean by immune response? Describe it.

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

Q. 2 Describe endocytosis, phagocytosis and macrophages.

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

Q. 3 Describe the mechanism of MHC I.

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

Q. 4 What are the medical use of drugs?

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

Q. 5 What are the differences between physical and chemical components?

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

Q. 6 Write a short note on neutrophiles.

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-3

Structure

3.1 Introduction

In immunology, an **antigen** (Ag) is a molecule or molecular structure or any foreign particulate matter or a pollen grain that can bind to a specific antibody or T-cell receptor. The presence of antigens in the body may trigger an immune response. The term *antigen* originally referred to a substance that is an antibody generator. Antigens can be proteins, peptides (amino acid chains), polysaccharides (chains of monosaccharides/simple

sugars), lipids, or nucleic acids. Antigens are recognized by antigen receptors, including antibodies and T-cell receptors. Diverse antigen receptors are made by cells of the immune system so that each cell has a specificity for a single antigen. Upon exposure to an antigen, only the lymphocytes that recognize that antigen are activated and expanded, a process known as clonal selection. In most cases, an antibody can only react to and bind one specific antigen; in some instances, however, antibodies may cross-react and bind more than one antigen.

Objectives

This is the third unit on Immunology. Under third unit we have following objectives. These are as under:

- To know about antigen presenting cells, B cells and dendritic cells.
- To know about antigens and its types.
- To know about haptens, human leukocyte antigen and tumour antigen.
- What are the immunoglobulins and their types?

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

The antigen may originate from within the body ("self-protein") or from the external environment ("non-self"). The immune system identifies and attacks "non-self" external antigens and usually does not react to self-protein due to negative selection of T cells in the thymus and B cells in the bone marrow. Vaccines are examples of antigens in an immunogenic form, which are intentionally administered to a recipient to induce the memory function of the adaptive immune system towards antigens of the pathogen invading that recipient. The vaccine for seasonal influenza is a common example.

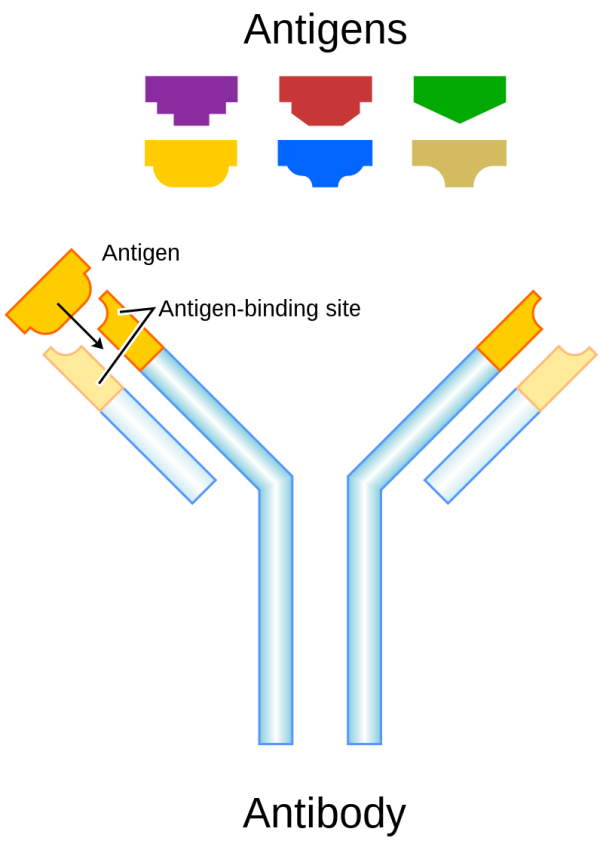

Fig. 1 Antigens

3.2 Antigen-presenting cells

An antigen-presenting cell (APC) or accessory cell is a cell that displays antigen bound by major histocompatibility complex (MHC) proteins on its surface; this process is known as antigen presentation. T cells may recognize these complexes using their T cell receptors (TCRs). APCs process antigens and present them to T-cells.

Almost all cell types can present antigens in some way. They are found in a variety of tissue types. Professional antigen-presenting cells, including macrophages, B cells and dendritic cells, present foreign antigens to helper T cells, while virus-infected cells (or cancer cells) can present antigens originating inside the cell to cytotoxic T cells. In addition to the MHC family of proteins, antigen presentation relies on other specialized signaling molecules on the surfaces of both APCs and T cells.

Antigen-presenting cells are vital for effective adaptive immune response, as the functioning of both cytotoxic and helper T cells is dependent on APCs. Antigen presentation allows for specificity of adaptive immunity and can contribute to immune responses against both

intracellular and extracellular pathogens. It is also involved in defense against tumors. Some cancer therapies involve the creation of artificial APCs to prime the adaptive immune system to target malignant cells.

Antigen-presenting cells present antigens in the form of peptides on histocompatibility molecules. The T cells selectively recognize the antigens; depending on the antigen and the type of the histocompatibility molecule, different types of T cells will be activated. For T-cell receptor (TCR) recognition, the peptide must be processed into small fragments inside the cell and presented by a major histocompatibility complex (MHC). The antigen cannot elicit the immune response without the help of an immunologic adjuvant. Similarly, the adjuvant component of vaccines plays an essential role in the activation of the innate immune system.

An immunogen is an antigen substance (or adduct) that is able to trigger a humoral (innate) or cell-mediated immune response. It first initiates an innate immune response, which then causes the activation of the adaptive immune response. An antigen binds the highly variable immunoreceptor products (B-cell receptor or T-cell receptor) once these have been generated. Immunogens are those antigens, termed immunogenic, capable of inducing an immune response.

At the molecular level, an antigen can be characterized by its ability to bind to an antibody's paratopes. Different antibodies have the potential to discriminate among specific epitopes present on the antigen surface. A hapten is a small molecule that can only induce an immune response when attached to a larger carrier molecule, such as a protein. Antigens can be proteins, polysaccharides, lipids, nucleic acids or other biomolecules. This includes parts (coats, capsules, cell walls, flagella, fimbriae, and toxins) of bacteria, viruses, and other microorganisms. Non-microbial non-self antigens can include pollen, egg white, and proteins from transplanted tissues and organs or on the surface of transfused blood cells.

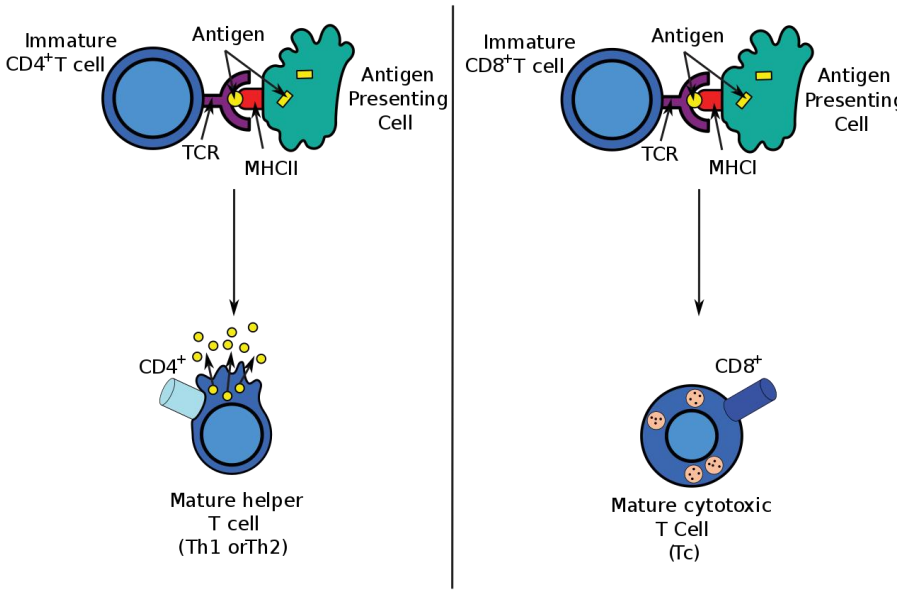

Fig. 2 Antigen presentation stimulates immature T cells to become either mature "cytotoxic" CD8+ cells or mature "helper" CD4+ cells.

3.3 Types and functions

Antigen-presenting cells fall into two categories: professional and non-professional. Those that express MHC class II molecules along with co-stimulatory molecules and pattern recognition receptors are often called professional antigen-presenting cells. The non-professional APCs express MHC class I molecules.

T cells must be activated before they can divide and perform their function. This is achieved by interacting with a professional APC which presents an antigen recognized by their T cell receptor. The APC involved in activating T cells is usually a dendritic cell. T cells cannot recognize (and therefore cannot respond to) "free" or soluble antigens. They can only recognize and respond to antigen that has been processed and presented by cells via carrier molecules like MHC molecules. Helper T cells can recognize exogenous antigen presented on MHC class II; cytotoxic T cells can recognize endogenous antigen presented on MHC class I. Most cells in the body can present antigen to CD8+ cytotoxic T cells via MHC class I; however, the term "antigen-presenting cell" is often used specifically to describe professional APCs. Such cells express MHC class I and MHC class II molecules and can stimulate CD4+ helper T cells as well as cytotoxic T cells.

APCs can also present foreign and self lipids to T cells and NK cells by using the CD1 family of proteins, which are structurally similar to the MHC class I family.

3.4 Professional APCs

Professional APCs specialize in presenting antigens to T cells. They are very efficient at internalizing antigens, either by phagocytosis (e.g. macrophages), or by receptor-mediated endocytosis (B cells), processing the antigen into peptide fragments and then displaying those peptides (bound to a class II MHC molecule) on their membrane. The T cell recognizes and interacts with the antigen-class II MHC molecule complex on the membrane of the antigen-presenting cell. An additional co-stimulatory signal is then produced by the antigen-presenting cell, leading to activation of the T cell. The expression of co-stimulatory molecules and MHC class II are defining features of professional APCs. All professional APCs also express MHC class I molecules as well. The main types of professional antigen-presenting cells are dendritic cells, macrophages and B cells.

3.5 Dendritic cells (DCs)

Dendritic cells have the broadest range of antigen presentation and are necessary for activation of naive T cells. DCs present antigen to both helper and cytotoxic T cells. They can also perform cross-presentation, a process by which they present exogenous antigen on MHC class I molecules to cytotoxic T cells. Cross-presentation allows for the activation of these T cells. Dendritic cells also play a role in peripheral tolerance, which contributes to prevention of auto-immune disease.

Prior to encountering foreign antigen, dendritic cells express very low levels of MHC class II and co-stimulatory molecules on their cell surface. These immature dendritic cells are ineffective at presenting antigen to T helper cells. Once a dendritic cell's pattern-recognition receptors recognize a pathogen-associated molecular pattern, antigen is phagocytosed and the dendritic cell becomes activated, upregulating the expression of MHC class II molecules. It also upregulates several co-stimulatory molecules required for T cell activation, including CD40 and B7. The latter can interact with CD28 on the surface of a CD4+ T cell. The dendritic cell is then a fully mature professional APC. It moves from the tissue to lymph nodes, where it encounters and activates T cells.

3.6 Macrophages

Macrophages can be stimulated by T cell secretion of interferon. After this activation, macrophages are able to express MHC class II and co-stimulatory molecules, including the B7 complex and can present phagocytosed peptide fragments to helper T cells. Activation can assist pathogen-infected macrophages in clearing the infection. Deriving from a monocyte, type of white blood cell, they will circulate the blood and enter affected sites and differentiate from monocytes to macrophages. At the affected site, the macrophage surrounds the site of infection or tissue damage with its membrane in a mechanism called phagocytosis.

3.7 B cells

B cells can internalize antigen that binds to their B cell receptor and present it to helper T cells.[1] Unlike T cells, B cells can recognize soluble antigen for which their B cell receptor is specific. They can then process the antigen and present peptides using MHC class II molecules. When a T helper cell with a TCR specific for that peptide binds, the B cell marker CD40 binds to CD40L on the T cell surface. When activated by a T cell, a B cell can undergo antibody isotype switching, affinity maturation, as well as formation of memory cells.

3.8 In cancer therapy

APCs naturally have a role in fighting tumors, via stimulation of B and cytotoxic T cells to respectively produce antibodies against tumor-related antigen and kill malignant cells. Dendritic cells, presenting tumor-specific antigen to T cells, are key to this process. Cancer therapies have included treating the patient with increased numbers of dendritic cells or cancer-specific T cells. However, newer therapies have turned to genetically engineered artificial antigen-presenting cells designed to prime the immune system to attack malignant cells. Some artificial APCs are derived from human cells; others are acellular, containing MHC proteins, co-stimulatory molecules and the necessary peptides. The APC activator IMP321 is being tested in clinical trials to accelerate the immune reaction to eliminate metastatic breast cancer or melanoma.

3.9 Sources

Antigens can be classified according to their source.

3.9.1 Exogenous antigens

Exogenous antigens are antigens that have entered the body from the outside, for example, by inhalation, ingestion or injection. The immune system's response to exogenous antigens is often subclinical. By endocytosis or phagocytosis, exogenous antigens are taken into the antigen-presenting cells (APCs) and processed into fragments. APCs then present the fragments to T helper cells ($CD4^+$) by the use of class II histocompatibility molecules on their surface. Some T cells are specific for the peptide:MHC complex. They become activated and start to secrete cytokines, substances that activate cytotoxic T lymphocytes (CTL), antibody-secreting B cells, macrophages and other particles. Some antigens start out as exogenous and later become endogenous (for example, intracellular viruses). Intracellular antigens can be returned to circulation upon the destruction of the infected cell.

3.9.2 Endogenous antigens

Endogenous antigens are generated within normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection. The fragments are then presented on the cell surface in the complex with MHC class I molecules. If activated cytotoxic $CD8^+$ T cells recognize them, the T cells secrete various toxins that cause the lysis or apoptosis of the infected cell. In order to keep the cytotoxic cells from killing cells just for presenting self-proteins, the cytotoxic cells (self-reactive T cells) are deleted as a result of tolerance (negative selection).Endogenousantigensinclude xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens. Sometimes antigens are part of the host itself in an autoimmune disease.

3.9.3 Autoantigens

An autoantigen is usually a self-protein or protein complex (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease. Under normal conditions, these self-proteins should not be the target of the immune system, but in autoimmune diseases, their associated T cells are not deleted and instead attack.

3.9.4 Neoantigens

Neoantigens are those that are entirely absent from the normal human genome. As compared with nonmutated self-proteins, neoantigens are of relevance to tumor control, as the quality of the T cell pool that is available for these antigens is not affected by central T cell tolerance.

Technology to systematically analyze T cell reactivity against neoantigens became available only recently. Neoantigens can be directly detected and quantified through a method called MANA-SRM developed by a molecular diagnostics company, Complete Omics Inc., through collaborating with a team in Johns Hopkins University School of Medicine.

3.9.5 Viral antigens

For virus-associated tumors, such as cervical cancer and a subset of head and neck cancers, epitopes derived from viral open reading frames contribute to the pool of neoantigens.

3.9.6 Tumor antigens

Tumor antigens are those antigens that are presented by MHC class I or MHC class II molecules on the surface of tumor cells. Antigens found only on such cells are called tumor-specific antigens (TSAs) and generally result from a tumor-specific mutation. More common are antigens that are presented by tumor cells and normal cells, called tumor-associated antigens (TAAs). Cytotoxic T lymphocytes that recognize these antigens may be able to destroy tumor cells. Tumor antigens can appear on the surface of the tumor in the form of, for example, a mutated receptor, in which case they are recognized by B cells. For human tumors without a viral etiology, novel peptides (neo-epitopes) are created by tumor-specific DNA alterations.

3.9.7 Process

A large fraction of human tumor mutations is effectively patient-specific. Therefore, neoantigens may also be based on individual tumor genomes. Deep-sequencing technologies can identify mutations within the protein-coding part of the genome (the exome) and predict potential neoantigens. In mice models, for all novel protein sequences, potential MHC-binding peptides were predicted. The resulting set of potential neoantigens was used to assess T cell reactivity. Exome–based analyses were exploited in a clinical setting, to assess reactivity in patients treated by either tumor-infiltrating lymphocyte (TIL) cell therapy or checkpoint blockade. Neoantigen identification was successful for multiple experimental model systems and human malignancies.

The false-negative rate of cancer exome sequencing is low—i.e.: the majority of neoantigens occur within exonic sequence with sufficient coverage. However, the vast majority of

mutations within expressed genes do not produce neoantigens that are recognized by autologous T cells. As of 2015 mass spectrometry resolution is insufficient to exclude many false positives from the pool of peptides that may be presented by MHC molecules. Instead, algorithms are used to identify the most likely candidates. These algorithms consider factors such as the likelihood of proteasomal processing, transport into the endoplasmic reticulum, affinity for the relevant MHC class I alleles and gene expression or protein translation levels.

The majority of human neoantigens identified in unbiased screens display a high predicted MHC binding affinity. Minor histocompatibility antigens, a conceptually similar antigen class are also correctly identified by MHC binding algorithms. Another potential filter examines whether the mutation is expected to improve MHC binding. The nature of the central TCR-exposed residues of MHC-bound peptides is associated with peptide immunogenicity.

3.9.8 Nativity

A native antigen is an antigen that is not yet processed by an APC to smaller parts. T cells cannot bind native antigens, but require that they be processed by APCs, whereas B cells can be activated by native ones.

3.9.9 Antigenic specificity

Antigenic specificity is the ability of the host cells to recognize an antigen specifically as a unique molecular entity and distinguish it from another with exquisite precision. Antigen specificity is due primarily to the side-chain conformations of the antigen. It is measurable and need not be linear or of a rate-limited step or equation. Both T cells and B cells are cellular components of adaptive immunity.

Antigen, substance that is capable of stimulating an immune response, specifically activating lymphocytes, which are the body’s infection-fighting white blood cells. In general, two main divisions of antigens are recognized: foreign antigens (or heteroantigens) and autoantigens (or self-antigens). Foreign antigens originate from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals. Autoantigens, on the other hand, originate within the body.

Normally, the body is able to distinguish self from nonself, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. An antigen that induces an immune response—i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly—is called an immunogen.

On the surface of antigens are regions, called antigenic determinants, that fit and bind to receptor molecules of complementary structure on the surface of the lymphocytes. The binding of the lymphocytes' receptors to the antigens' surface molecules stimulates the lymphocytes to multiply and to initiate an immune response—including the production of antibody, the activation of cytotoxic cells, or both—against the antigen. The amount of antibody formed in response to stimulation depends on the kind and amount of antigen involved, the route of entry to the body, and individual characteristics of the host.

3.10 Human leukocyte antigen (HLA)

Human leukocyte antigen (HLA)**,** any of the numerous antigens (substances capable of stimulating an immune response) involved in the major histocompatibility complex (MHC) in humans. The HLA genes encode the cell-surface proteins that are part of the MHC. HLA antigens are programmed by a highly variable gene complex consisting of more than 200 genes, all of which occur on chromosome 6. HLA genes are divided into three distinct groups: class I, class II, and class III. The possibility of numerous variations in these genes serves a key role in providing the immune system with the ability to defend against a wide range of antigens.

3.11 Criterion of antigen city

Antigenicity reflects the pattern of antibody responses which the host develops against a virus. For each virus, the host develops an array of distinct antibodies derived from an array of germline segments and usually refined by somatic mutation to include some with high affinity for the antigen. The particular structural regions of proteins recognized by these antibodies are designated as antigenic sites. Upon re-exposure to the viral antigen, the host immune memory reacts promptly and efficiently to produce high-affinity antibodies, which contributes to antibody-mediated immunity and a reduced risk of severe infections in the host. Under the principle of natural evolution, the virus evolves to adapt to survive pressure from the environment and host through the generation of genetic diversity. Since many viruses have a very high mutation rate this generally occurs quickly and the

accumulation of genetic mutations potentially results in the modification of viral structural proteins. Antibody recognition occurs over a reasonably extensive area of the protein surface (the antibody footprint) but within this region changes in individual key residues might completely prevent recognition, for instance a larger side chain may sterically block antibody engagement, this would be an escape mutation. By improving the survival fitness of the virus, such structural changes located at or near the antigenic site will lead to the emergence of these antigenic variants in the host and environment.

Antigenicity or antigenic reactivity refers to the capacity of viruses to bind to specific antibody molecules. The antigenicity of nonenveloped viruses resides in the antigenic sites or B-cell epitopes of capsid proteins that are recognized by the binding sites of antibodies. Protein epitopes are classified as either continuous or discontinuous depending on whether the amino acids included in the epitope are contiguous in the polypeptide chain or not. Most epitopes are discontinuous and since they consist of surface residues brought together by the folding of the peptide chain, their antigenic reactivity depends on the native conformation of the protein.

The quaternary structure of viral capsids gives rise to epitopes known as neotopes. Neotopes arise either through conformational changes in the capsid proteins induced by intersubunit interactions or by the juxtaposition of residues from neighboring subunits forming a novel epitope. Immunogenicity is the ability of a protein to give rise to an immune response in a competent host and it can be defined only in the biological context of the host. Knowledge of the viral antigenic sites recognized by antibodies does not necessarily indicate which immunogenic structure initiated the production of antibodies in the immunized host. Failure to differentiate between antigenicity and immunogenicity is responsible for the lack of success in developing synthetic peptide vaccines against viral diseases.

3.12 Haptens

Haptens are small molecules that elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself (in general, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body). Once the body has generated antibodies to a hapten-

carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody, a process called *hapten inhibition*.

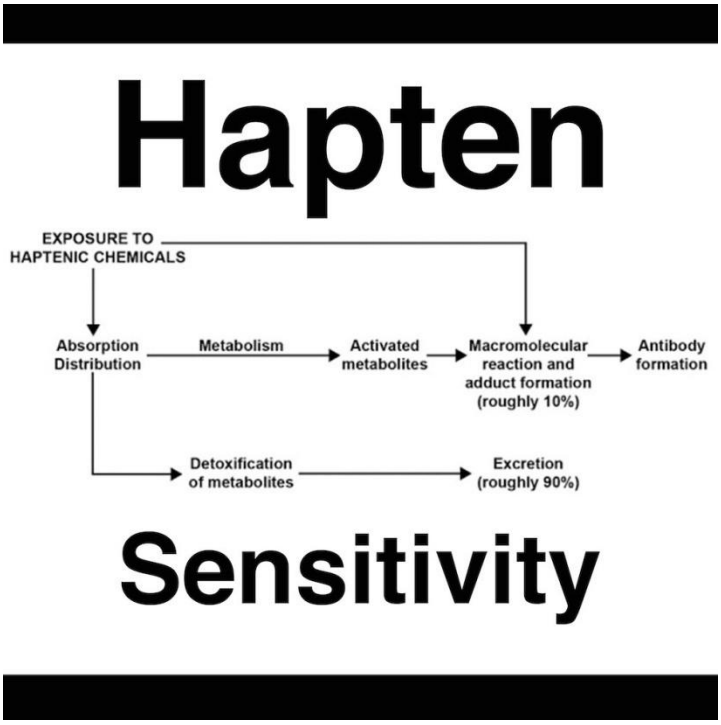

Fig. 3 Haptens

The mechanisms of absence of immune response may vary and involve complex immunological mechanisms, but can include absent or insufficient co-stimulatory signals from antigen-presenting cells. Haptens have been used to study allergic contact dermatitis (ACD) and the mechanisms of inflammatory bowel disease (IBD) to induce autoimmune-like responses. The concept of haptens emerged from the work of Karl Landsteiner, who also pioneered the use of synthetic haptens to study immunochemical phenomena.

3.13 Examples of haptens

The first researched haptens were aniline and its carboxyl derivatives (o-, m-, and p-aminobenzoic acid). A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive quinone-type molecule, which then reacts with skin proteins to form hapten adducts. Usually, the first

exposure causes only sensitization, in which there is a proliferation of effector T-cells. After a subsequent, second exposure, the proliferated T-cells can become activated, generating an immune reaction that produces typical blisters of a poison ivy exposure.

Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure-lowering drug that occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the mechanism by which the anaesthetic gas halothane can cause a life-threatening hepatitis, as well as the mechanism by which penicillin-class drugs cause autoimmune hemolytic anemia. Other haptens that are commonly used in molecular biology applications include fluorescein, biotin, digoxigenin, and dinitrophenol.

Nickel allergy is caused by nickel metal ions penetrating the skin and binding to skin proteins. Antibodies have successfully been raised against endogenous & unreactive small molecules such as some neurotransmitters (e.g. serotonin (5HT), glutamate, dopamine, GABA, tryptamine, glycine, noradrenaline), amino acids (e.g. tryptophan, 5-hydroxytryptophan, 5-metoxytryptophan), by using glutaraldehyde to crosslink these molecules to carrier proteins suitable for immune recognition. Notably, detection of such small molecules in tissues requires the tissue to be glutaraldehyde-fixed, as the glutaraldehyde covalent-linkage on the molecule of interest often forms a portion of the antibody recognized epitope.

3.14 Antibody

An **antibody** (**Ab**), also known as an **immunoglobulin** (**Ig**), is a large, Y-shaped protein used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses. The antibody recognizes a unique molecule of the pathogen, called an antigen. Each tip of the "Y" of an antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can *tag* a microbe or an infected cell for attack by other parts of the immune system, or can neutralize it directly (for example, by blocking a part of a virus that is essential for its invasion).

To allow the immune system to recognize millions of different antigens, the antigen-binding sites at both tips of the antibody come in an equally wide variety. In contrast, the remainder of

the antibody is relatively constant. It only occurs in a few variants, which define the antibody's *class* or *isotype*: IgA, IgD, IgE, IgG, or IgM. The constant region at the trunk of the antibody includes sites involved in interactions with other components of the immune system. The class hence determines the function triggered by an antibody after binding to an antigen, in addition to some structural features. Antibodies from different classes also differ in where they are released in the body and at what stage of an immune response.

Together with B and T cells, antibodies comprise the most important part of the adaptive immune system. They occur in two forms: one that is attached to a B cell, and the other, a soluble form, that is unattached and found in extracellular fluids such as blood plasma. Initially, all antibodies are of the first form, attached to the surface of a B cell – these are then referred to as B-cell receptors (BCR). After an antigen binds to a BCR, the B cell activates to proliferate and differentiate into either plasma cells, which secrete soluble antibodies with the same paratope, or memory B cells, which survive in the body to enable long-lasting immunity to the antigen. Soluble antibodies are released into the blood and tissue fluids, as well as many secretions. Because these fluids were traditionally known as humors, antibody-mediated immunity is sometimes known as, or considered a part of, humoral immunity. The soluble Y-shaped units can occur individually as monomers, or in complexes of two to five units. Antibodies are glycoproteins belonging to the immunoglobulin superfamily. The terms antibody and immunoglobulin are often used interchangeably, though the term 'antibody' is sometimes reserved for the secreted, soluble form, i.e. excluding B-cell receptors.

3.15 Structure

Antibodies are heavy (~150 kDa) proteins of about 10 nm in size, arranged in three globular regions that roughly form a Y shape. In humans and most mammals, an antibody unit consists of four polypeptide chains; two identical *heavy chains* and two identical *light chains* connected by disulfide bonds. Each chain is a series of domains: somewhat similar sequences of about 110 amino acids each. These domains are usually represented in simplified schematics as rectangles. Light chains consist of one variable domain V_L and one constant domain C_L, while heavy chains contain one variable domain V_H and three to four constant domains C_H1, C_H2.

Structurally an antibody is also partitioned into two antigen-binding fragments (Fab), containing one V_L, V_H, C_L, and C_H1 domain each, as well as the crystallisable fragment (Fc),

forming the trunk of the Y shape. In between them is a hinge region of the heavy chains, whose flexibility allows antibodies to bind to pairs of epitopes at various distances, to form complexes (dimers, trimers, etc.), and to bind effector molecules more easily.

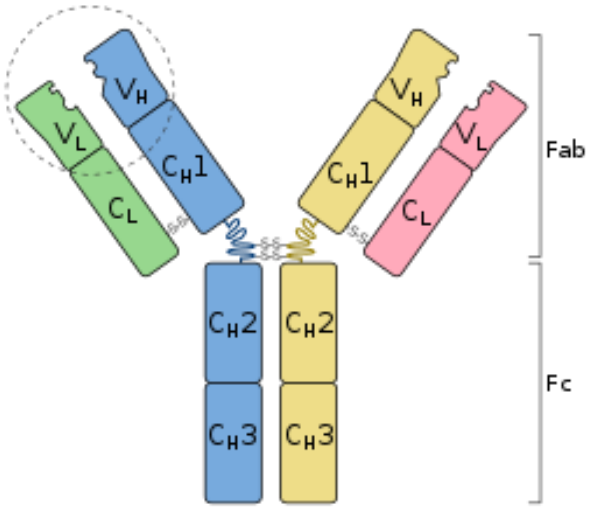

Fig. 4 Schematic structure of an antibody: two heavy chains (blue, yellow) and the two light chains (green, pink). The antigen binding site is circled

In an electrophoresis test of blood proteins, antibodies mostly migrate to the last, gamma globulin fraction. Conversely, most gamma-globulins are antibodies, which is why the two terms were historically used as synonyms, as were the symbols Ig and γ. This variant terminology fell out of use due to the correspondence being inexact and due to confusion with γ heavy chains which characterize the IgG class of antibodies.

3.16 Antigen-binding site

The variable domains can also be referred to as the F_V region. It is the subregion of Fab that binds to an antigen. More specifically, each variable domain contains three *hypervariable regions* – the amino acids seen there vary the most from antibody to antibody. When the protein folds, these regions give rise to three loops of β-strands, localized near one another on the surface of the antibody. These loops are referred to as the complementarity-determining regions (CDRs), since their shape complements that of an antigen. Three CDRs from each of the heavy and light chains together form an antibody-binding site whose shape can be anything from a pocket to which a smaller antigen binds, to a larger surface, to a protrusion

that sticks out into a groove in an antigen. Typically however only a few residues contribute to most of the binding energy.

The existence of two identical antibody-binding sites allows antibody molecules to bind strongly to multivalent antigen (repeating sites such as polysaccharides in bacterial cell walls, or other sites at some distance apart), as well as to form antibody complexes and larger antigen-antibody complexes. The resulting cross-linking plays a role in activating other parts of the immune system.

The structures of CDRs have been clustered and classified by Chothia et al. and more recently by North et al. and Nikoloudis et al. However, describing an antibody's binding site using only one single static structure limits the understanding and characterization of the antibody's function and properties. To improve antibody structure prediction and to take the strongly correlated CDR loop and interface movements into account, antibody paratopes should be described as interconverting states in solution with varying probabilities. In the framework of the immune network theory, CDRs are also called idiotypes. According to immune network theory, the adaptive immune system is regulated by interactions between idiotypes.

3.17 Fc region

The Fc region (the trunk of the Y shape) is composed of constant domains from the heavy chains. Its role is in modulating immune cell activity: it is where effector molecules bind to, triggering various effects after the antibody Fab region binds to an antigen. Effector cells (such as macrophages or natural killer cells) bind via their Fc receptors (FcR) to the Fc region of an antibody, while the complement system is activated by binding the C1q protein complex. IgG or IgM can bind to C1q, but IgA cannot, therefore IgA does not activate the classical complement pathway.

Another role of the Fc region is to selectively distribute different antibody classes across the body. In particular, the neonatal Fc receptor (FcRn) binds to the Fc region of IgG antibodies to transport it across the placenta, from the mother to the fetus. Antibodies are glycoproteins, that is, they have carbohydrates (glycans) added to conserved amino acid residues. These conserved glycosylation sites occur in the Fc region and influence interactions with effector molecules.

3.18 Protein structure

The N-terminus of each chain is situated at the tip. Each immunoglobulin domain has a similar structure, characteristic of all the members of the immunoglobulin superfamily: it is composed of between 7 (for constant domains) and 9 (for variable domains) β-strands, forming two beta sheets in a Greek key motif. The sheets create a "sandwich" shape, the immunoglobulin fold, held together by a disulfide bond.

3.19 Antibody complexes

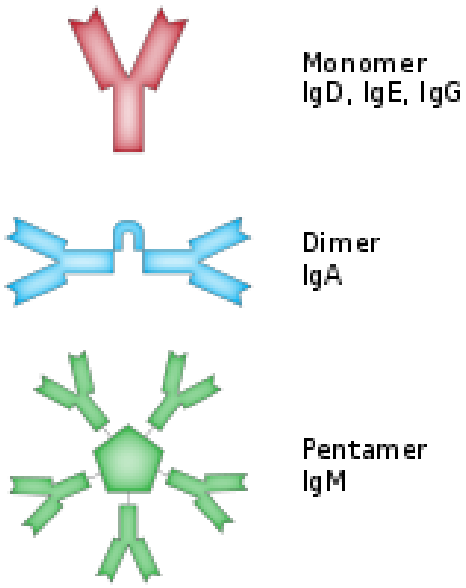

Fig. 5 Some antibodies form complexes that bind to multiple antigen molecules

Secreted antibodies can occur as a single Y-shaped unit, a monomer. However, some antibody classes also form dimers with two Ig units (as with IgA), tetramers with four Ig units (like teleost fish IgM), or pentamers with five Ig units (like shark IgW or mammalian IgM, which occasionally forms hexamers as well, with six units).

Antibodies also form complexes by binding to antigen: this is called an antigen-antibody complex or *immune complex*. Small antigens can cross-link two antibodies, also leading to the formation of antibody dimers, trimers, tetramers, etc. Multivalent antigens (e.g., cells with multiple epitopes) can form larger complexes with antibodies. An extreme example is the clumping, or agglutination, of red blood cells with antibodies in the Coombs test to determine blood groups: the large clumps become insoluble, leading to visually apparent precipitation.

3.20 B cell receptors

The membrane-bound form of an antibody may be called a *surface immunoglobulin* (sIg) or a *membrane immunoglobulin* (mIg). It is part of the *B cell receptor* (BCR), which allows a B cell to detect when a specific antigen is present in the body and triggers B cell activation. The BCR is composed of surface-bound IgD or IgM antibodies and associated Ig-α and Ig-β heterodimers, which are capable of signal transduction. A typical human B cell will have 50,000 to 100,000 antibodies bound to its surface. Upon antigen binding, they cluster in large patches, which can exceed 1 micrometer in diameter, on lipid rafts that isolate the BCRs from most other cell signaling receptors. These patches may improve the efficiency of the cellular immune response. In humans, the cell surface is bare around the B cell receptors for several hundred nanometers, which further isolates the BCRs from competing influences

3.21 Classes

Antibodies can come in different varieties known as *isotypes* or *classes*. In placental mammals there are five antibody classes known as IgA, IgD, IgE, IgG, and IgM, which are further subdivided into subclasses such as IgA1, IgA2. The prefix "Ig" stands for *immunoglobulin*, while the suffix denotes the type of heavy chain the antibody contains: the heavy chain types α (alpha), γ (gamma), δ (delta), ε (epsilon), μ (mu) give rise to IgA, IgG, IgD, IgE, IgM, respectively. The distinctive features of each class are determined by the part of the heavy chain within the hinge and Fc region.

The classes differ in their biological properties, functional locations and ability to deal with different antigens, as depicted in the table. For example, IgE antibodies are responsible for an allergic response consisting of histamine release from mast cells, often a sole contributor to asthma (though other pathways exist as do exist symptoms very similar to yet not technically asthma). The antibody's variable region binds to allergic antigen, for example house dust mite particles, while its Fc region (in the ε heavy chains) binds to Fc receptor ε on a mast cell, triggering its degranulation: the release of molecules stored in its granules.

3.22 Antigenic determinants of immunoglobulins

A. Isotypes

These determinants are present on all molecules of each class and subclass of im-munoglobulin heavy chains and on each type of light chains; they are defined serologically by antisera directed against the constant regions of H and L chains. The antisera are pro-duced in animals, which, upon injection of purified human immunoglobulins, recognize the structural differences between constant regions of H and L chains. Isotypic determinants are common to all members of a given species, hence they cannot be used as genetic mark-ers. Their practical importance results from the fact that they allow the identification of classes and subclasses of immunoglobulins through the heavy-chain isotypes and types of light chains (k, λ). All classes and subclasses of normal immunoglobulins share the two light-chain isotypes.

B. Idiotypes

The antigen-combining site in the V region of the immunoglobulin molecule, in addition to determining specificity for antigen binding, can also act as an antigen and induce produc-tion of antibodies against it. Such antigenic determinants, usually associated with hyper-variable regions, are known as idiotypes.

C. Allotypes

These are hereditary antigenic determinants of Ig polypeptide chains that may differ be-tween individuals of the same species. The loci controlling allotypic determinants are codominant (i.e., both are expressed phenotypically in a heterozygote) autosomal genes that follow Mendelian laws of heredity. All allotypic markers that have so far been identi-fied on human immunoglobulin molecules, with one exception (see later), are present in the C regions of H chains of IgG, IgA, IgE, and on k-type L chains. Since different individu-als of the same species may have different allotypes, these determinants can be used as ge-netic markers.

The most common technique used for allotype determination is hemagglutination-in-hibition. For this purpose, ORh+ red cells are coated with IgG immunoglobulins of known allotypes. The coated cells will agglutinate when exposed to specific antibody. The agglu-tination, however, will be inhibited if the antiserum recognizing the allotype of the im-munoglobulin coating the red cell is preincubated with soluble IgG carrying the same allo-type. Thus, in a first step, the anti-allotypic antiserum and an unknown serum to be typed are mixed. In a second step, red cells coated with the relevant allotype are added to dilu-tions of the mixture.

If agglutination is inhibited, it can be concluded that the allotype was present in the unknown serum.

3.23 Summary

Under this unit we have summarized antigen-antibody interaction, antigenicity, haptens and immunoglobulins. Antigen-antibody interaction, or antigen-antibody reaction, is a specific chemical interaction between antibodies produced by B cells of the white blood cells and antigens during immune reaction. The antigens and antibodies combine by a process called agglutination. It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. The immune complex is then transported to cellular systems where it can be destroyed or deactivated. Antigen is a substances usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody or specially sensitized T cells or both. Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Molecules that stimulate immune responses are called

Haptens are small molecules that elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself (in general, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body). Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody, a process called *hapten inhibition*. The five primary classes of immunoglobulins are IgG, IgM, IgA, IgD and IgE. These are distinguished by the type of heavy chain found in the molecule. IgG molecules have heavy chains known as gamma-chains; IgMs have mu-chains; IgAs have alpha-chains; IgEs have epsilon-chains; and IgDs have delta-chains.

3.24 Terminal questions

Q. 1 What do you mean by immunogenicity? Describe it.

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

Q.2 Describe haptens with examples.

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

Q.3 Describe immunoglobulin with its types.

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

Q. 4 What are antigen presenting cells?

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

Q. 5 What do you mean by antigens? Describe different types of antigens.

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

Q.8. Write a short note on dendritic cells, macrophages and B cells.

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

Further readings

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

Unit-4

Structure

4.1 Introduction

Immunology is the branch of biomedical science that deals with the response of an organism to antigenic challenge and its recognition of what is self and what is not. It deals with the defence mechanisms including all physical, chemical and biological properties of the organism that help it to combat its susceptibility to foreign organisms, material, etc. The immune system is divided into those which are static, or innate to the organism, and those which are responsive, or adaptive to a potential pathogen or foreign substance. The innate system of immunity is on evolutionary terms, the older system that forms the first line of defence. It is non-specific and the resistance is static (it does not improve with repeated exposure and there is no memory on subsequent exposures). This includes physical defences such as skin & epithelial surfaces, cilia, commensual flora, acidic gastric contents, fever etc. Others are biochemical defences such as soluble - lysosyme, acute phase reactants and complement, fibronectin, interferons. Cellular components include natural killer cells, RES phagocytes.

The adaptive system is the second line of defence and is activated once the innate system has been overwhelmed. It is specific to the infective agent and can store the information about the invader as memory to show an enhanced response to subsequent challenge. Immunology deals with physiological functioning of the immune system in states of both health and disease as well as malfunctions of the immune system in immunological disorders like allergies, hypersensitivities, immune deficiency, transplant rejection and autoimmune disorders. Immunology deals with physical, chemical and physiological characteristics of the components of the immune system in vitro, in situ, and in vivo. Immunology has a vast array of uses in several disciplines of science and medical science.

Objectives

This is the fourth unit on Immunology of block I. Under this unit we have following objectives. These are as under:

- To know about immunoglobulins, functions and their types
- To discuss the generation of antibody diversity.
- To know B-cell activation, plasma and memory cells.
- To discuss clonal selection theory by supported theories

4.2 Immunoglobulins

Immunoglobulins (Ig) are major proteins of colostrum and milk that provide essential immunological protection against life threatening bacterial and viral infections to offspring. They act as first line of defense. Bovine secretion contains IgG as predominant Ig, along with IgA and IgM. Their concentrations in colostrum make them principal protein fraction which tends to decrease with progression of lactation to traces in mature milk. In case of human milk, IgA is the predominant Ig. All Igs have common basic Y-shaped structure of IgG molecule. These Igs are important considering their therapeutic roles in offspring, lactating animals as well as human beings. Thus, several Ig rich preparations have emerged in global nutraceutical market.

Immunoglobulins are produced by B-lymphocytes, with certain classes of immunoglobulins serving as receptors on B cells. For example, naive B cells have monomeric IgM and IgD on their surface, whereas more mature B cells have Ig G. The B cell is stimulated when antigen binds to the Fab regions of these surface receptors. The B cell matures to a plasma cell and the plasma cell produces antibodies of the same specificity (Fab region) as the receptor. Although a given B cell produces antibodies specific for a particular antigen, the B cell can undergo class switching (related to the Fc region), such that the effector function of the resultant immune response evolves. There was initially a great deal of confusion as to the structure of the gene (or genes) for Ab. That is, for there to be sufficient diversity of Ab specificity (i.e., antibodies specific for each possible Ag that may be encountered), the amount of DNA needed would exceed that which exists within cells. Therefore, it was speculated, and later proven, that there is not a separate gene for each specific Ab. Instead, it was discovered that genes for the Fab and the Fc regions are closely linked and that the Fab region is made up of closely linked genes that have a unique way of recombining to impart the diversity to Fab

products with a minimal amount of genetic material. Specifically, the Fab region is divided into V, D, J segments that are linked to the C segments

4.3 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.

4.3.1 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.

4.3.2 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.

4.3.3 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.

4.3.4 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.

4.3.5 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.

4.3.6 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.

4.3.7 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.

4.3.8 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.

4.3.9 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.

4.4 The Generation of Antibody Diversity

Even in the absence of antigen stimulation, a human can probably make more than 10^{12} different antibody molecules—its *preimmune antibody repertoire*. Moreover, the antigen-binding sites of many antibodies can cross-react with a variety of related but different antigenic determinants, making the antibody defense force even more formidable. The preimmune repertoire is apparently large enough to ensure that there will be an antigen-binding site to fit almost any potential antigenic determinant, albeit with low affinity. After repeated stimulation by antigen, B cells can make antibodies that bind their antigen with much higher affinity—a process called *affinity maturation.* Thus, antigen stimulation greatly increases the antibody arsenal.

Antibodies are proteins, and proteins are encoded by genes. Antibody diversity therefore poses a special genetic problem: how can an animal make more antibodies than there are genes in its genome? (The human genome, for example, contains fewer than 50,000 genes.) This problem is not quite as formidable as it might first appear. Recall that the variable regions of both the light and heavy chains of antibodies usually form the antigen-binding site. Thus, an animal with 1000 genes encoding light chains and 1000 genes encoding heavy chains could, in principle, combine their products in 1000 × 1000 different ways to make 10^6 different antigen-binding sites (although, in reality, not every light chain can combine with every heavy chain to make an antigen-binding site). Nonetheless, the mammalian immune system has evolved unique genetic mechanisms that enable it to generate an almost unlimited number of different light and heavy chains in a remarkably economical way, by joining separate gene segments together before they are transcribed. Birds and fish

use very different strategies for diversifying antibodies, and even sheep and rabbits use somewhat different strategies from mice and humans. We shall confine our discussion to the mechanisms used by mice and humans.

We begin this unit by discussing the mechanisms that B cells use to produce antibodies with an enormous diversity of antigen-binding sites. We then consider how a B cell can alter the tail region of the antibody it makes, while keeping the antigen-binding site unchanged. This ability allows the B cell to switch from making membrane-bound antibody to making secreted antibody, or from making one class of antibody to making another, all without changing the antigen-specificity of the antibody.

4.5 Antibody Genes Are Assembled From Separate Gene Segments During B Cell Development

The first direct evidence that DNA is rearranged during B cell development came in the 1970s from experiments in which molecular biologists compared DNA from early mouse embryos, which do not make antibodies, with the DNA of a mouse B cell tumor, which makes a single species of antibody molecule. The specific variable (V)-region and constant (C)-region coding sequences that the tumor cells used were present on the same DNA restriction fragment in the tumor cells but on two different restriction fragments in the embryos. This showed that the DNA sequences encoding an antibody molecule are rearranged at some stage in B cell development.

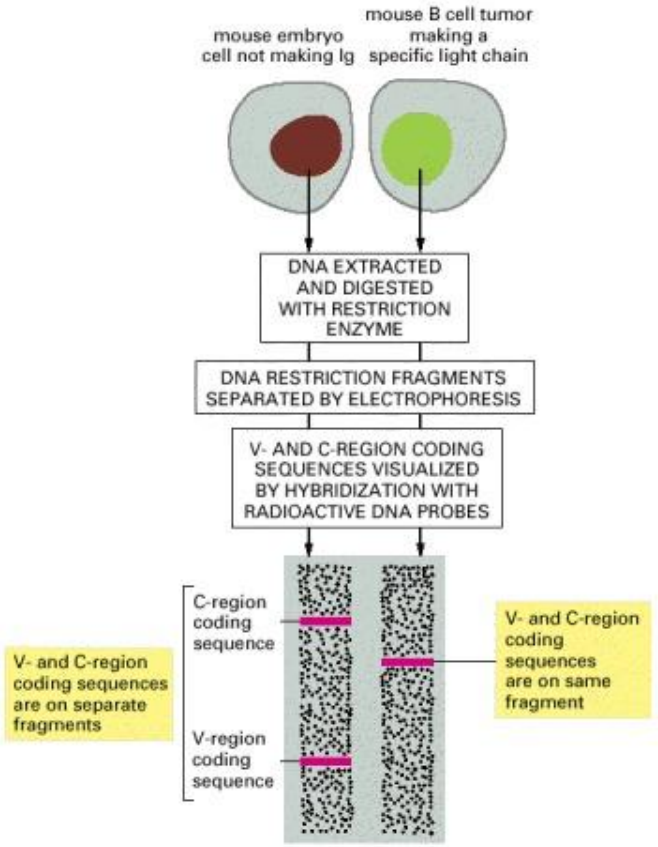

Fig.1 Drawing of an experiment that directly demonstrates that DNA is rearranged during B cell development

We now know that each type of antibody chain—κ light chains, λ light chains, and heavy chains—has a separate pool of gene segments and exons from which a single polypeptide chain is eventually synthesized. Each pool is on a different chromosome and contains a large number of gene segments encoding the V region of an antibody chain and, a smaller number of exons encoding the C region. During the development of a B cell, a complete coding sequence for each of the two antibody chains to be synthesized is assembled by site-specific genetic recombination. In addition to bringing together the separate gene segments and the C-region exons of the antibody gene, these rearrangements also activate transcription from the gene promoter through changes in the relative positions of the enhancers and silencers acting on the promoter. Thus, a complete antibody chain can be synthesized only after the DNA has been rearranged. As we shall see, the process of joining gene segments contributes to the diversity of antigen-binding sites in several ways.

4.6 Each Variable Region Is Encoded by More Than One Gene Segment

When genomic DNA sequences encoding V and C regions were first analyzed, it was found that a single region of DNA encodes the C region of an antibody chain, but two or more regions of DNA have to be assembled to encode each V region. Each light-chain V region is encoded by a DNA sequence assembled from two gene segments—a long ***V* gene segment** and a short *joining*, or ***J* gene segment** (not to be confused with the protein *J chain*, which is encoded elsewhere in the genome).

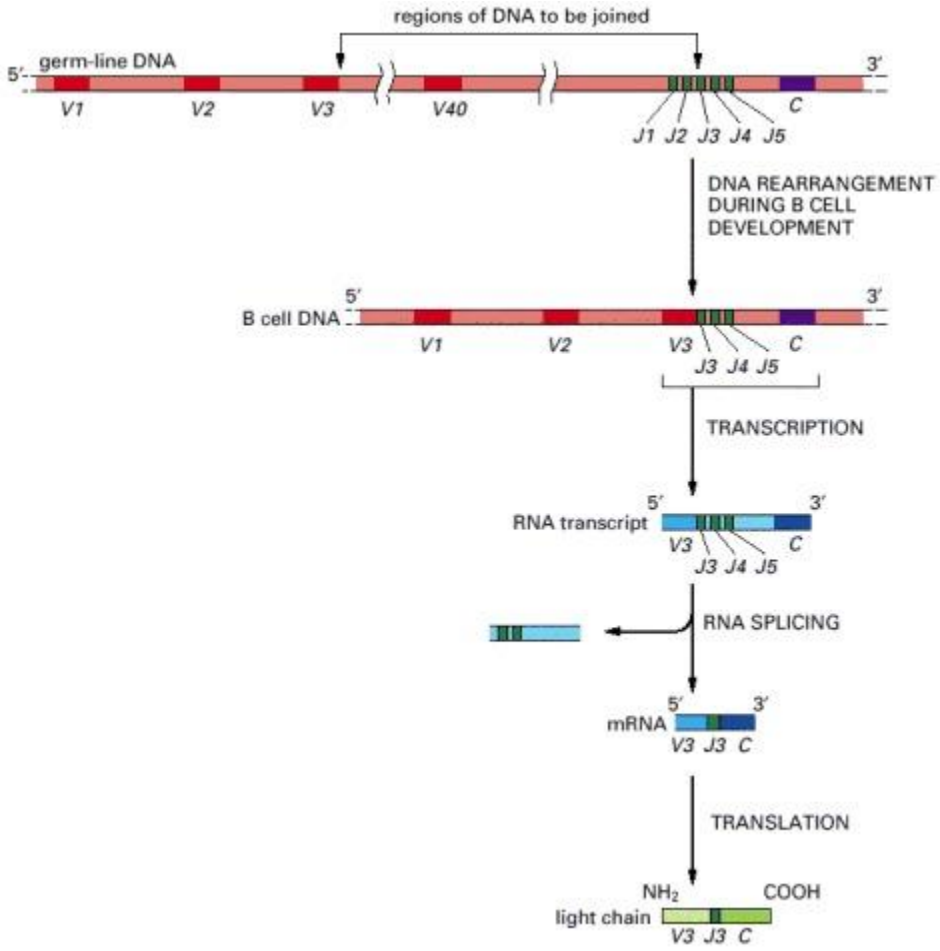

Fig. 2 The *V-J* joining process involved in making a human κ light chain

Each heavy-chain V region is encoded by a DNA sequence assembled from three gene segments—a *V* segment, a *J* segment, and a *diversity segment*, or ***D* gene segment**. The figure shows the number and organization of the gene segments used in making human heavy chains.

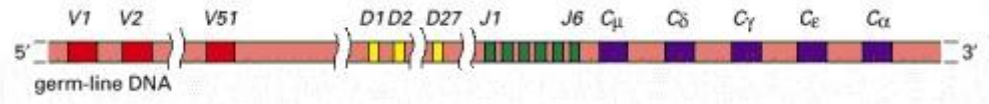

Fig. 3 The human heavy-chain gene-segment pool

The large number of inherited *V, J,* and *D* gene segments available for encoding antibody chains makes a substantial contribution on its own to antibody diversity, but the combinatorial joining of these segments (called *combinatorial diversification)* greatly increases this contribution. Any of the 40 *V* segments in the human κ light-chain gene-segment pool, for example, can be joined to any of the 5 *J* segments, so that at least 200 (40 × 5) different κ-chain V regions can be encoded by this pool. Similarly, any of the 51 *V* segments in the human heavy-chain pool can be joined to any of the 6 *J* segments and any of the 27 *D* segments to encode at least 8262 (51 × 6 × 27) different heavy-chain V regions.

The combinatorial diversification resulting from the assembly of different combinations of inherited *V, J,* and *D* gene segments just discussed is an important mechanism for diversifying the antigen-binding sites of antibodies. By this mechanism alone, a human can produce 287 different V_L regions (200 κ and 116 λ) and 8262 different V_H regions. In principle, these could then be combined to make about 2.6×10^6 (316 × 8262) different antigen-binding sites. In addition, as we discuss further, the joining mechanism itself greatly increases this number of possibilities (probably more than 10^8-fold), making it much greater than the total number of B cells (about 10^{12}) in a human.

4.7 Imprecise Joining of Gene Segments Greatly Increases the Diversity of V Regions

During B cell development, the *V* and *J* gene segments (for the light chain) and the *V, D,* and *J* gene segments (for the heavy chain) are joined together to form a functional V_L- or V_H-region coding sequence by a process of site-specific recombination called **V(D)J joining**. Conserved DNA sequences flank each gene segment and serve as recognition sites for the joining process, ensuring that only appropriate gene segments recombine. Thus, for example, a *V* segment will always join to a *J* or *D* segment but not to another *V* segment. Joining is mediated by an enzyme complex called the **V(D)J recombinase**. This complex contains two proteins that are specific to developing lymphocytes, as well as enzymes that help repair damaged DNA in all our cells.

The lymphocyte-specific proteins of the V(D)J recombinase are encoded by two closely linked genes called *rag-1* and *rag-2* (*rag* = *r*ecombination *a*ctivating *g*enes). The **RAG proteins** introduce double-strand breaks at the flanking DNA sequences, and this is followed by a rejoining process that is mediated by both the RAG proteins and the enzymes involved in general DNA double-strand repair. Thus, if both *rag* genes are artificially expressed in a fibroblast, the fibroblast is now able to rearrange experimentally introduced antibody gene segments just as a developing B cell normally does. Moreover, individuals who are deficient in either *rag* gene or in one of the general repair enzymes are highly susceptible to infection because they are unable to carry out *V(D)J* joining and consequently do not have functional B or T cells. (T cells use the same recombinase to assemble the gene segments that encode their antigen-specific receptors.)

In most cases of site-specific recombination, DNA joining is precise. But during the joining of antibody (and T cell receptor) gene segments, a variable number of nucleotides are often lost from the ends of the recombining gene segments, and one or more randomly chosen nucleotides may also be inserted. This random loss and gain of nucleotides at joining sites is called junctional diversification, and it enormously increases the diversity of V-region coding sequences created by recombination, specifically in the third hypervariable region. This increased diversification comes at a price, however. In many cases, it will result in a shift in the reading frame that produces a nonfunctional gene. Because roughly two in every three rearrangements are "nonproductive" in this way, many developing B cells never make a functional antibody molecule and consequently die in the bone marrow. B cells making functional antibody molecules that bind strongly to self antigens in the bone marrow are stimulated to re-express the RAG proteins and undergo a second round of *V(D)J* rearrangements, thereby changing the specificity of the cell-surface antibody they make—a process referred to as **receptor editing**. Self-reactive B cells that fail to change their specificity in this way are eliminated through the process of clonal deletion.

4.8 Antigen-Driven Somatic Hypermutation Fine-Tunes Antibody Responses

As mentioned earlier, with the passage of time after immunization, there is usually a progressive increase in the affinity of the antibodies produced against the immunizing antigen. This phenomenon, known as affinity maturation, is due to the accumulation of point mutations specifically in both heavy-chain and light-chain V-region coding sequences. The mutations

occur long after the coding regions have been assembled, when B cells are stimulated by antigen and helper T cells to generate memory cells in a lymphoid follicle in a peripheral lymphoid organ. They occur at the rate of about one per V-region coding sequence per cell generation. Because this is about a million times greater than the spontaneous mutation rate in other genes, the process is called somatic hypermutation. The molecular mechanism is still uncertain, but it is believed to involve some form of error-prone DNA repair process targeted to the rearranged V-region coding sequence by specific regions of DNA brought together by V(D)J joining. Surprisingly, an enzyme involved in RNA editing is required, but its function in the hypermutation process is unknown.

Only a small minority of the altered antigen receptors generated by hypermutation have an increased affinity for the antigen. The few B cells expressing these higher-affinity receptors, however, are preferentially stimulated by the antigen to survive and proliferate, whereas most other B cells die by apoptosis. Thus, as a result of repeated cycles of somatic hypermutation, followed by antigen-driven proliferation of selected clones of memory B cells, antibodies of increasingly higher affinity become abundant during an immune response, providing progressively better protection against the pathogen.

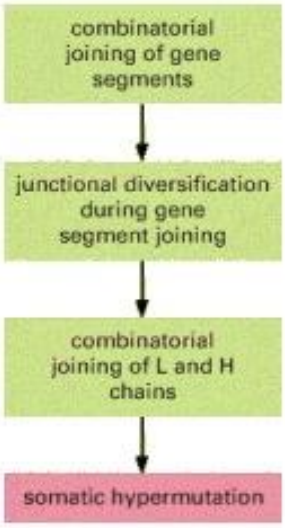

Fig. 4 The four main mechanisms of antibody diversification

The Control of V(D) J Joining Ensures That B Cells Are Monospecific

As the clonal selection theory predicts, B cells are *monospecific*. That is, all the antibodies that any one B cell produces have identical antigen-binding sites. This property enables antibodies to cross-link antigens into large aggregates, thereby promoting antigen elimination. It also means that an activated B cell secretes antibodies with the same specificity as that of the membrane-bound antibody on the B cell that was originally stimulated.

The requirement of monospecificity means that each B cell can make only one type of V_L region and one type of V_H region. Since B cells, like most other somatic cells, are diploid, each cell has six gene-segment pools encoding antibody chains: two heavy-chain pools (one from each parent) and four light-chain pools (one κ and one λ from each parent). If DNA rearrangements occurred independently in each heavy-chain pool and each light-chain pool, a single cell could make up to eight different antibodies, each with a different antigen-binding site.

In fact, however, each B cell uses only two of the six gene-segment pools: one of the two heavy-chain pools and one of the four light-chain pools. Thus, each B cell must choose not only between its κ and λ light-chain pools, but also between its maternal and paternal light-chain and heavy-chain pools. This second choice is called allelic exclusion, and it also occurs in the expression of genes that encode T cell receptors. For most other proteins that are encoded by autosomal genes, both maternal and paternal genes in a cell are expressed about equally.

Allelic exclusion and κ versus λ light-chain choice during B cell development depend on negative feedback regulation of the *V(D)J* joining process. A functional rearrangement in one gene-segment pool suppresses rearrangements in all remaining pools that encode the same type of polypeptide chain. In B cell clones isolated from transgenic mice expressing a rearranged μ-chain gene, for example, the rearrangement of endogenous heavy-chain genes is usually suppressed. Comparable results have been obtained for light chains. The suppression does not occur if the product of the rearranged gene fails to assemble into a receptor that inserts into the plasma membrane. It has therefore been proposed that either the receptor assembly process itself or extracellular signals that act on the receptor are involved in the suppression of further gene rearrangements.

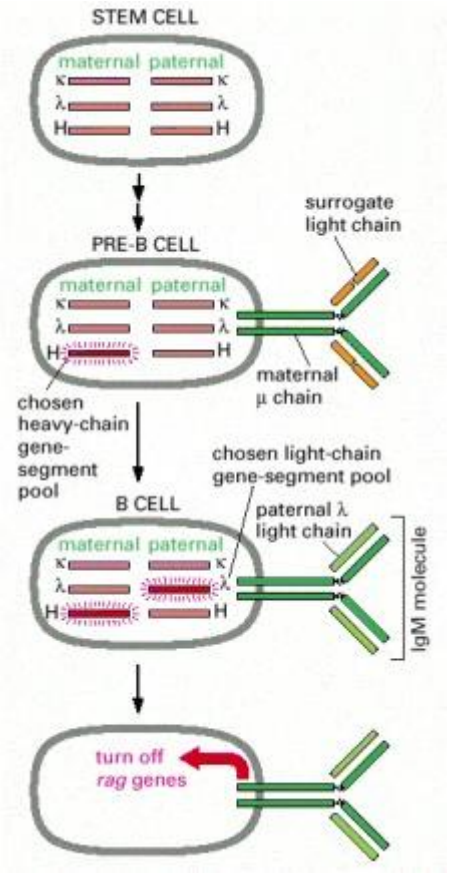

Fig. 5 Antibody gene-pool selection in B cell development

Although no biological differences between the constant regions of κ and λ light chains have been discovered, there is an advantage in having two separate pools of gene segments encoding light chain variable regions. Having two separate pools increases the chance that a pre-B cell that has successfully assembled a V_H-region coding sequence will go on to assemble successfully a V_L-region coding sequence to become a B cell. This chance is further increased because, before a developing pre-B cell produces ordinary light chains, it makes surrogate light chains, which assemble with μ heavy chains. The resulting receptors are displayed on the cell surface and allow the cell to proliferate, producing large numbers of progeny cells, some of which are likely to succeed in producing bona fide light chains.

4.4.0 When Activated by Antigen, a B Cell Switches From Making a Membrane-Bound Antibody to Making a Secreted Form of the Same Antibody

We now turn from the genetic mechanisms that determine the antigen-binding site of an antibody to those that determine its biological properties—that is, those that determine what

form of heavy-chain constant region is synthesized. The choice of the particular gene segments that encode the antigen-binding site is a commitment for the life of a B cell and its progeny, but the type of C_H region that is made changes during B cell development. The changes are of two types: changes from a membrane-bound form to a secreted form of the same C_H region and changes in the class of the C_H region made.

All classes of antibody can be made in a membrane-bound form, as well as in a soluble, secreted form. The membrane-bound form serves as an antigen receptor on the B cell surface, while the soluble form is made only after the cell is activated by antigen to become an antibody-secreting effector cell. The sole difference between the two forms resides in the C-terminus of the heavy chain. The heavy chains of membrane-bound antibody molecules have a hydrophobic C-terminus, which anchors them in the lipid bilayer of the B cell's plasma membrane. The heavy chains of secreted antibody molecules, by contrast, have instead a hydrophilic C-terminus, which allows them to escape from the cell. The switch in the character of the antibody molecules made occurs because the activation of B cells by antigen (and helper T cells) induces a change in the way in which the H-chain RNA transcripts are made and processed in the nucleus.

4.4.1 B Cells Can Switch the Class of Antibody They Make

During B cell development, many B cells switch from making one class of antibody to making another—a process called class switching. All B cells begin their antibody-synthesizing lives by making IgM molecules and inserting them into the plasma membrane as receptors for antigen. After the B cells leave the bone marrow, but before they interact with antigen, they switch and make both IgM and IgD molecules as membrane-bound antigen receptors, both with the same antigen-binding sites. On stimulation by antigen and helper T cells, some of these cells are activated to secrete IgM antibodies, which dominate the primary antibody response. Later in the immune response, the combination of antigen and the cytokines that helper T cells secrete induce many B cells to switch to making IgG, IgE, or IgA antibodies. These cells generate both memory cells that express the corresponding classes of antibody molecules on their surface and effector cells that secrete the antibodies. The IgG, IgE, and IgA molecules are collectively referred to as *secondary* classes of antibodies, both because they are produced only after antigen stimulation and because they dominate

secondary antibody responses. As we saw earlier, each different class of antibody is specialized to attack microbes in different ways and in different sites.

The constant region of an antibody heavy chain determines the class of the antibody. Thus, the ability of B cells to switch the class of antibody they make without changing the antigen-binding site implies that the same assembled V_H-region coding sequence (which specifies the antigen-binding part of the heavy chain) can sequentially associate with different C_H-coding sequences. This has important functional implications. It means that, in an individual animal, a particular antigen-binding site that has been selected by environmental antigens can be distributed among the various classes of antibodies, thereby acquiring the different biological properties of each class.

When a B cell switches from making IgM and IgD to one of the secondary classes of antibody, an irreversible change at the DNA level occurs—a process called *class switch recombination.* It entails deletion of all the C_H-coding sequences between the assembled VDJ-coding sequence and the particular C_H-coding sequence that the cell is destined to express. Switch recombination differs from *V(D)J* joining in several ways: (1) it involves noncoding sequences only and therefore leaves the coding sequence unaffected; (2) it uses different flanking recombination sequences and different enzymes; (3) it happens after antigen stimulation; and (4) it is dependent on helper T cells.

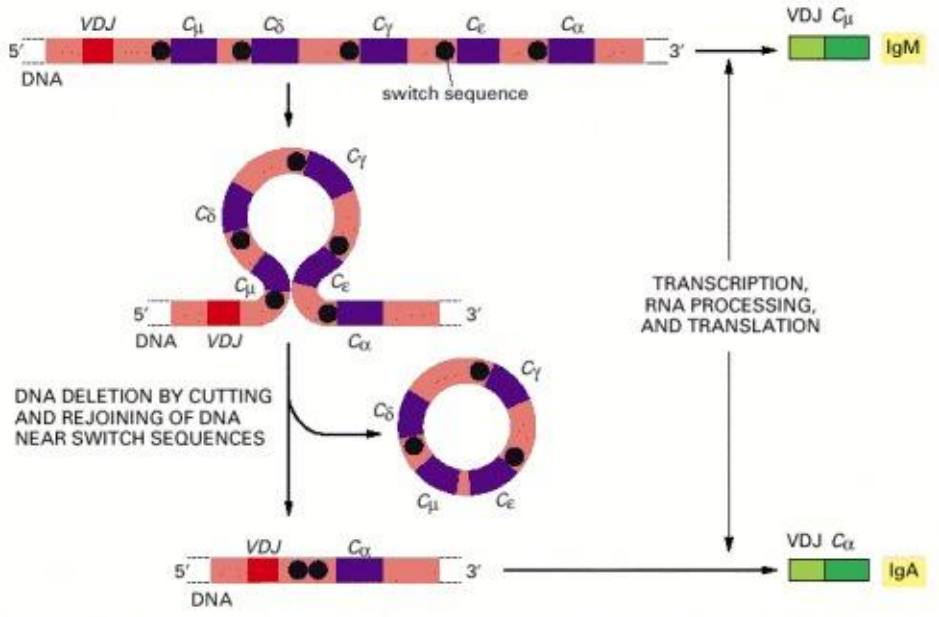

Fig. 6 An example of the DNA rearrangement that occurs in class switch recombination

4.4.2 B cell activation and the germinal centre response

4.4.3 B cell activation

B cells are activated when their B cell receptor (BCR) binds to either soluble or membrane bound antigen. This activates the BCR to form microclusters and trigger downstream signalling cascades. The microcluster eventually undergoes a contraction phase and forms an immunological synapse, this allows for a stable interaction between B and T cells to provide bidirectional activation signals. Once activated B cells may undergo class switch recombination. In their inactivated state B cells express IgM/IgD but once activated they may express IgA, IgE, IgG or retain IgM expression. They do this by excision of the unwanted isotypes (Figure 1). Cytokines produced by T cells and other cells are important in determining what isotype the B cells express.

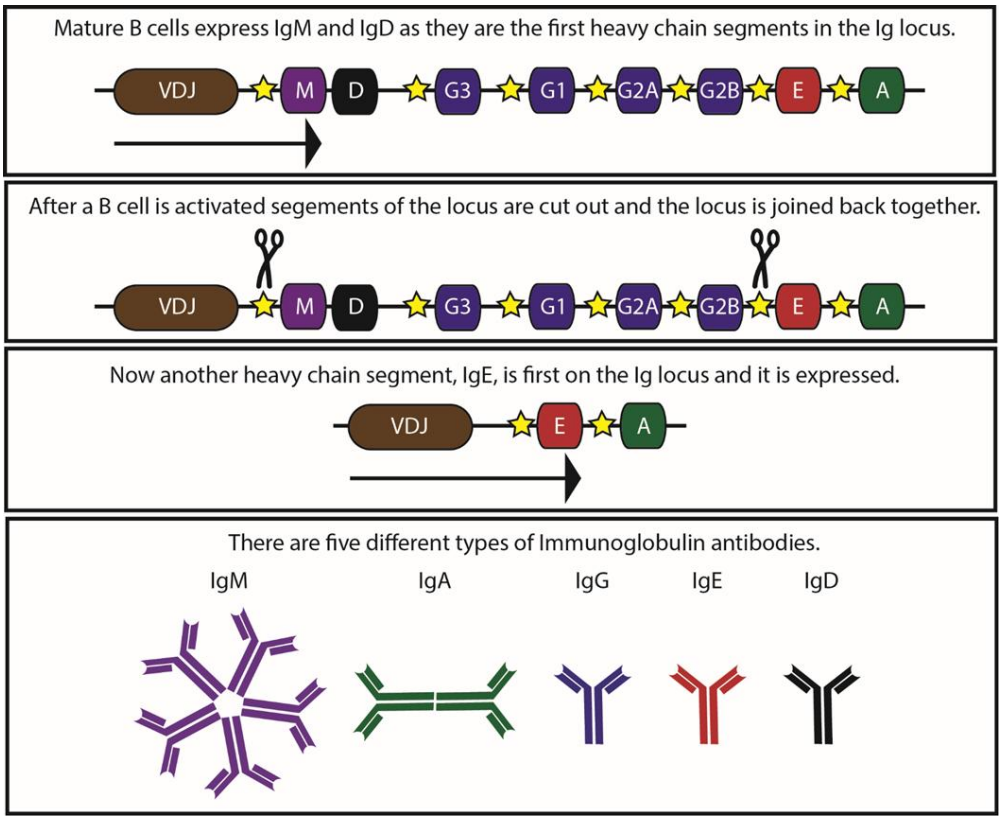

Fig. 7 Class switch recombination. After VDJ recombination class switch recombination may occur. In this process unwanted Immunoglobulin (Ig) genes are excised so that the desired gene can be expressed. In this depiction excision occurs and IgE is expressed. There are five isotypes which can be found in difference circumstances. For example, IgE is common in allergic responses such as asthma.

4.4.4 The germinal centre

B cells have two main types of immune responses. In a T-Independent immune response B cells can respond directly to the antigen. In a T-dependent immune response the B cells need assistance from T cells in order to respond. In this situation activated B cells move to the border of the T cell zone to interact with T cells CD40 ligand is found on these T helper cells and interacts with CD40 on the B cells to form a stable attraction. Cytokines secreted by T cells encourage proliferation and isotype switching and maintain germinal centre size and longevity. Without these signals the germinal centre response will quickly collapse.

B cells that have encountered antigen and begun proliferating may exit the follicle and differentiate into short-lived plasma cells called plasmablasts. They secrete antibody as an early attempt to neutralize the foreign antigen. They do not survive more than three days but the antibody produced can provide important assistance to stop fast-dividing pathogens such as viruses.

The germinal centre has a light zone and a dark zone. The germinal centre response begins in the dark zone where the B cells rapidly proliferate and undergo somatic hypermutation. During somatic hypermutation, random mutations are generated in the variable domains of the BCR by the enzyme activation-induced cytidine deaminase (AID). B cells then enter the light zone and compete with each other for antigen. If the mutation resulted in a BCR with an improved affinity to the antigen the B cell clone can out-compete other clones and survive. The light zone is also thought to be where B cells undergo class switch recombination, although a germinal centre is not crucial for this process. The B cells may migrate between both zones to undergo several rounds of somatic hypermutation and class switch recombination. The ultimate goal of the germinal centre is to produce B cells with a BCR which has high affinity for the initial antigen.

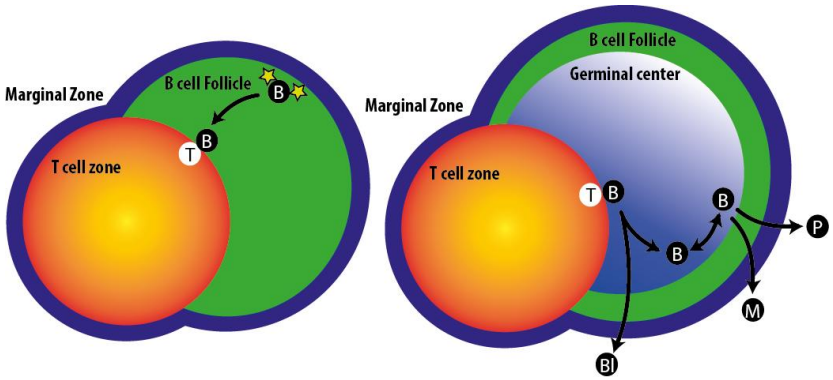

Fig. 8 The migration of B cells in an immune response. When B cells (B) first encounter antigen (★) they migrate to the T-B border to receive survival signals from T cells (T). If they receive survival signals they will begin to proliferate and either become plasmablasts (Bl) or form a germinal centre (Blue). B cells can migrate between the light zone and dark zone of the germinal centre to undergo somatic hypermutation and class switch recombination. Eventually they may leave the GC as high-affinity memory cells (M) or plasma cells (P).

4.4.5 Plasma and memory cells

B cells leave the germinal centre response as high-affinity plasma cells and memory B cells. Plasma cells secrete antigen-binding antibodies for weeks after activation. They migrate to the bone marrow soon after formation where they can reside indefinitely, ready to encounter the antigen again and respond. Memory B cells circulate throughout the body on the lookout for antigen with a high-affinity for their BCR and then quickly respond to the antigen, stopping infection. This is how vaccination works. As your body has been previously exposed to the antigen the immune cells can quickly respond to remove the antigen if it is encountered again, stopping you getting sick.

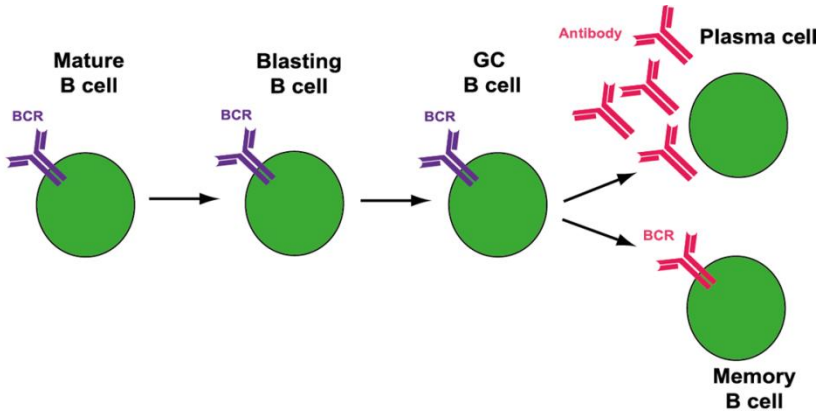

Figure 9 B cell differentiation after activation. When a mature B cell encounters antigen that binds to its B cell receptor it becomes activated. It then proliferates and becomes a blasting B cell. These B cells form germinal centres. The germinal centre B cells undergo somatic hypermutation and class switch recombination. Plasma cells and memory B cells with a high-affinity for the original antigen stimuli are produced. These cells are long lived and plasma cells may secrete antibody for weeks after the initial infection.

4.4.6 Clonal selection theory

Clonal selection theory is a scientific theory in immunology that explains the functions of cells of the immune system (lymphocytes) in response to specific antigens invading the body. The concept was introduced by Australian doctor 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.

The theory states that in a pre-existing group of lymphocytes (specifically B cells), a specific antigen activates (i.e. selects) only its counter-specific cell, which then induces that particular cell to multiply, producing identical clones for antibody production. This activation occurs in secondary lymphoid organs such as the spleen and the lymph nodes. 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.

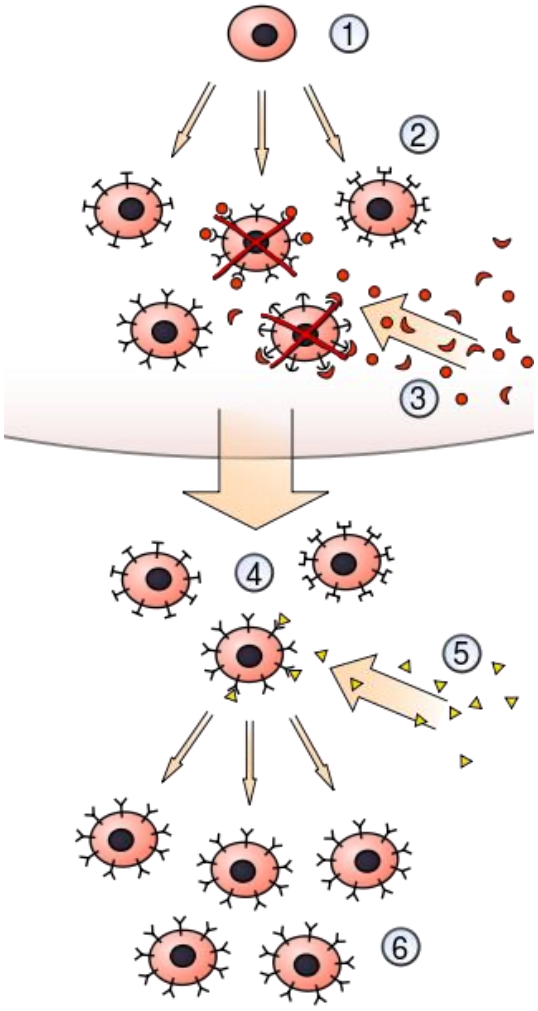

Fig. 10 Clonal selection theory of lymphocytes: 1) A hematopoietic stem cell undergoes differentiation and genetic rearrangement to produce 2) immature lymphocytes with many different antigen receptors. Those that bind to 3) antigens from the body's own tissues are destroyed, while the rest mature into 4) inactive lymphocytes. Most of these never encounter a matching 5) foreign antigen, but those that do are activated and produce 6) many clones of themselves.

4.4.7 Burnet's clonal selection theory

Later in 1957, Australian immunologist Frank Macfarlane Burnet published a paper titled "A modification of Jerne's theory of antibody production using the concept of clonal selection" in the rather obscure *Australian Journal of Science*. In it Burnet expanded the ideas of Talmage and named the resulting theory the "clonal selection theory". He further formalised the theory in his 1959 book *The Clonal Selection Theory of Acquired Immunity*. He

explained immunological memory as the cloning of two types of lymphocyte. One clone acts immediately to combat infection whilst the other is longer lasting, remaining in the immune system for a long time and causing immunity to that antigen. According to Burnet's hypothesis, among antibodies are molecules that can probably correspond with varying degrees of precision to all, or virtually all, the antigenic determinants that occur in biological material other than those characteristic of the body itself. Each type of pattern is a specific product of a clone of lymphocytes and it is the essence of the hypothesis that each cell automatically has available on its surface representative reactive sites equivalent to those of the globulin they produce.

When an antigen enters the blood or tissue fluids it is assumed that it will attach to the surface of any lymphocyte carrying reactive sites that correspond to one of its antigenic determinants. Then the cell is activated and undergoes proliferation to produce a variety of descendants. In this way, preferential proliferation is initiated of all those clones whose reactive sites correspond to the antigenic determinants on the antigens present in the body. The descendants are capable of active liberation of soluble antibody and lymphocytes, the same functions as the parental forms. In 1958, Gustav Nossal and Joshua Lederberg showed that one B cell always produces only one antibody, which was the first direct evidence supporting the clonal selection theory.

4.4.8 Theories supported by clonal selection

Burnet and Peter Medawar worked together on understanding immunological tolerance, a phenomenon also explained by clonal selection. This is the organism's ability to tolerate the introduction of cells prior to the development of an immune response as long as it occurs early in the organism's development. There are a vast number of lymphocytes occurring in the immune system, ranging from cells that tolerate self tissue to cells that do not. However, only cells tolerant of self tissue survive the embryonic stage. If non-self tissue is introduced, lymphocytes that develop are the ones that include the non-self tissues as self tissue.

In 1959, Burnet proposed that under certain circumstances, tissues could be successfully transplanted into foreign recipients. This work has led to a much greater understanding of the immune system and also great advances in tissue transplantation. Burnet and Medawar shared the Nobel Prize in Physiology or Medicine in 1960. In 1974, Niels Kaj Jerne proposed that the

immune system functions as a network that is regulated via interactions between the variable parts of lymphocytes and their secreted molecules. Immune network theory is firmly based on the concept of clonal selection. Jerne won the Nobel Prize in Physiology or Medicine in 1984, largely for his contributions to immune network theory.

4.4.9 Summary

Under this unit we have summarized immunoglobulins and its types, generation of antibody diversity and theory of clonal selection etc. The innate immune responses are the first line of defense against invading pathogens. They are also required to initiate specific adaptive immune responses. Innate immune responses rely on the body's ability to recognize conserved features of pathogens that are not present in the uninfected host. These include many types of molecules on microbial surfaces and the double-stranded RNA of some viruses.

Immunoglobulins, also known as antibodies, are glycoprotein molecules produced by plasma cells (white blood cells). They act as a critical part of the immune response by specifically recognizing and binding to particular antigens, such as bacteria or viruses, and aiding in their destruction. The antibody immune response is highly complex and exceedingly specific. The various immunoglobulin classes and subclasses (isotypes) differ in their biological features, structure, target specificity and distribution. Hence, the assessment of the immunoglobulin isotype can provide useful insight into complex humoral immune response. Assessment and knowledge of immunoglobulin structure and classes is also important for selection and preparation of antibodies as tools for immunoassays and other detection applications.

The various antibodies produced by plasma cells are classified by isotype, each of which differs in function and antigen responses primarily due to structure variability. Five major antibody classes have been identified in placental mammals: IgA, IgD, IgE, IgG and IgM. This classification is based on differences in amino acid sequence in the constant region (Fc) of the antibody heavy chains. IgG and IgA are further grouped into subclasses (e.g., in human IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) based on additional small differences in the amino acid heavy chain sequences. B cell activation is triggered by the binding of specific antigen to the BCR. The end result of this process will depend on the characteristics of the antigen, the B

cell subpopulation activated, and the co-stimulatory signals provided by the antigen itself, T cells, and the microenvironment.

4.50 Terminal questions

Q.9. What do you mean by immunoglobulins? Describe it.

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

Q.10. Describe biological functions of immunoglobulins .

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

Q.11. Describe the generation of antibody diversity.

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

Q.12. What are the plasma and memory cells?

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

Q.13. Describe clonal selection theory.

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

Q.14. Write a short note on B- cell activation.

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

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.

Unit-5

Structure

5.1 Introduction

Diversity is one of the key characteristics of the vertebrate immune system. Lymphocyte repertoires of at least 3×107 different clonotypes protect humans against infections, while avoiding unwanted immune responses against self-peptides and innocuous antigens. It is this lymphocyte diversity that forms the main difference between the immune systems of invertebrate and vertebrate species. Invertebrates are protected from pathogenic invasions by broad-spectrum pathogen-associated recognition molecules, recognizing conserved pathogenic structures. On top of these innate responses, which have been preserved in vertebrate species, vertebrates evolved an adaptive immune system, which has the capacity to

respond to a virtually infinite variety of antigens. Adaptive immunity evolved when gene rearrangements were employed to generate highly diverse lymphocyte repertoires.

The mammalian immune system has evolved sophisticated germiline and somatic strategies for the generation of an immense repertoire of antigen-specific lymphocytes. The key evolutionary selective forces have been the need to protect the individual against 'unexpected' infections and to avoid autoimmune disease. The germiline is composed of a large tandem array of V segments located upstream of joining elements (D,J) which are themselves proximal 5′ to the constant region coding exons at Ig and TcR genetic loci. During lymphocyte development V genes rearrange to produce complete V[D]J variable regions which are transcribed, translated and the protein chains assembled into functional antigen-specific Ig and TcR receptors. Such receptors are clonally distributed such that any mature B cell or most T cells express only one antigen-specific receptor on their surface membrane.

Objectives

This is the fifth unit on Immunology. Under fifth unit we have following objectives. These are as under:

- To know about diversities in immunology.
- To know about concept of clonal selection theory.
- What are the important aspects generation of antibody diversity
- To discuss antigen receptor structure and signaling pathways

5.2 Diversity in immune system

Combinatorial DNA recombination of the germline encoded elements (V-to-[D]-to-J) together with combinatorial associateion and assembly of complete polypeptide chains can by itself generate a potentially very large recognition repertoire ($>10^9$), although many of these 'random' combinations may not be functional receptors. Additional somatic diversification processes include V to [D]J junctional diversity, nucleotide deletions, N region additions and 'secondary rearrangements' that can lead to receptor replacement and therefore a complete change of clonal specificity. Finally, there is the tightly regulated antigen-driven process of somatic hypermutation of rearranged IgV genes.

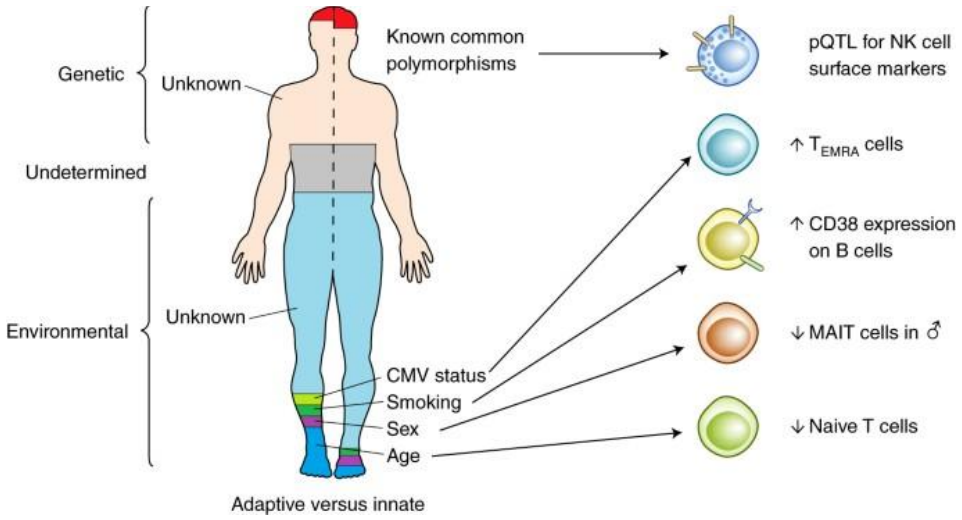

Fig. 1The origins of diversity in human immunity | Nature Immunology

It is confined to a subset of mature B cells during differentiation to memory cells in specialized post-antigenic lymphoid structures called germinal centers. Memory B cells arising from germinal centers express and secrete mutated high affinity antibodies. In this way the specificity of the antibodies is fine tuned during an immune response. It is not known whether a similar process occurs in T cells but if it does it would have to occur during T cell development in the thymus to ensure clonal deletion of autoreactive cells. Positive Darwinian selection drives the development and evolution of both the germline and somatic variable gene repertoires. Indeed, there is emerging evidence from the structure and pattern of germline V gene sequences that acquired somatic mutations in V[D]J genes may be inherited in the germline DNA.

5.3 Clonal selection theory

Clonal selection theory is a scientific theory in immunology that explains the functions of cells of the immune system (lymphocytes) in response to specific antigens invading the body. The concept was introduced by Australian doctor 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.

The theory states that in a pre-existing group of lymphocytes (specifically B cells), a specific antigen activates (i.e. selects) only its counter-specific cell, which then induces that particular cell to multiply, producing identical clones for antibody production. This activation occurs in secondary lymphoid organs such as the spleen and the lymph nodes. 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.

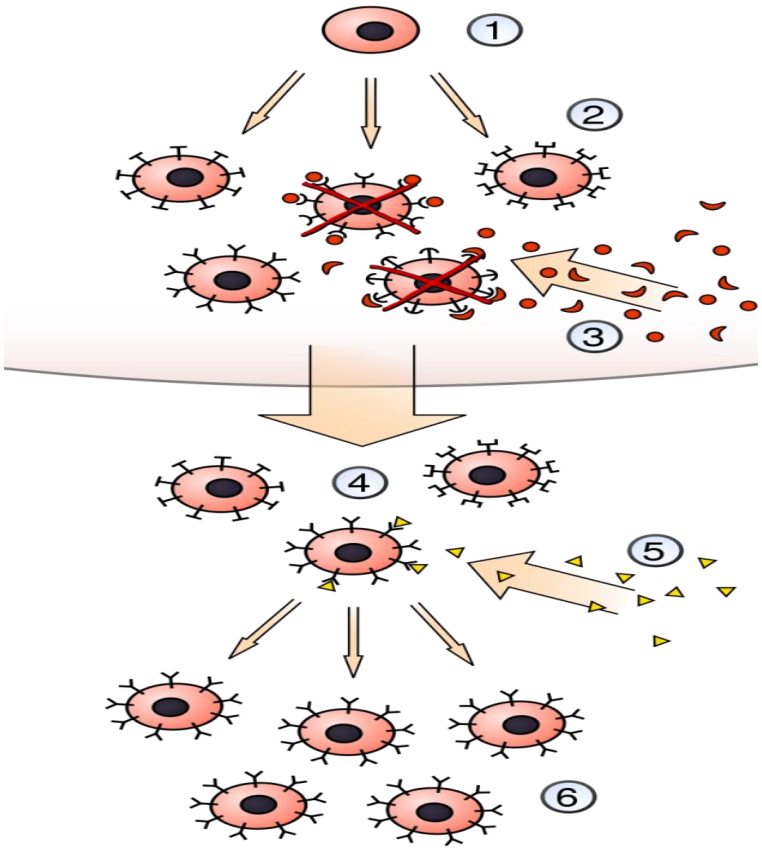

Fig. 2 Clonal selection theory of lymphocytes:

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3) antigens from the body's own tissues are destroyed, while the rest mature into
4) inactive lymphocytes. Most of these never encounter a matching
5) foreign antigen, but those that do are activated and produce 6) many clones of themselves.

5.4 Postulates

The clonal selection theory can be summarised with the following four points:

- ✓ Each lymphocyte bears a single type of receptor with a unique specificity (generated by V(D)J recombination).
- ✓ Receptor occupation is required for cell activation.
- ✓ The differentiated effector cells derived from an activated lymphocyte bear receptors of identical specificity as the parent cell.
- ✓ Those lymphocytes bearing receptors for self molecules (i.e., endogenous antigens produced within the body) are destroyed at an early stage.

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5.7 Antigen receptor structure and signaling pathways

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.

The antigen receptors on B cells (the **B-cell receptor** or **BCR**) and T cells (the T-cell receptor or **TCR**) are multiprotein complexes made up of clonally variable antigen-binding chains—the heavy and light immunoglobulin chains in the B-cell receptor, and the TCRα and TCRβ chains in the T-cell receptor—that are associated with invariant accessory proteins. The invariant chains are required both for transport of the receptors to the cell surface and, most importantly, for initiating signaling when the receptors bind to an extracellular ligand.

Antigen binding to the receptor generates signals that lead ultimately to the activation of nuclear transcription factors that turn on new gene expression and turn off genes typically expressed only in resting cells. In this part of the unit we also see how clustering of the antigen receptors with co-receptors helps to generate these signals.

The antigen receptors of B and T lymphocytes have several features that are important for their functions in adaptive immunity. Although these receptors have many similarities in terms of structure and mechanisms of signaling, there are fundamental differences related to the types of antigenic structures that B cells and T cells recognize.

- ✓ Membrane-bound antibodies, which serve as the antigen receptors of B lymphocytes, can recognize many types of chemical structures, while T cell antigen receptors recognize only peptides bound to major histocompatibility complex (MHC) molecules. B lymphocyte antigen receptors and the antibodies that B cells secrete can recognize the shapes, or conformations, of macromolecules, including proteins, lipids, carbohydrates, and nucleic acids, as well as simpler, smaller chemical moieties. This broad specificity of B cells for structurally different types of molecules in their native form enables the humoral immune system to recognize, respond to, and eliminate diverse microbes and toxins. In striking contrast, T cells see only peptides displayed on antigen-presenting cells (APCs) bound to MHC molecules. This specificity ensures that T cells never interact with free or soluble antigens and that they only interact with microbial or tumor antigens present inside other cells in the body.

- ✓ Antigen receptor molecules consist of regions (domains) involved in antigen recognition—therefore varying between clones of lymphocytes—and other regions required for structural integrity and effector functions—thus relatively conserved among all clones. The antigen-recognizing domains of the receptors are called variable (V) regions, and the conserved portions are the constant (C) regions. Even within each V region, most of the sequence variation is concentrated within short stretches, which are called hypervariable regions, or complementarity-determining regions (CDRs), because they form the parts of the receptor that bind antigens (i.e., they are complementary to the shapes of antigens). By concentrating sequence variation in small regions of the receptor, it is possible to maximize the variability of the antigen-

binding part while retaining the basic structure of the receptors. As discussed later, special mechanisms exist in developing lymphocytes to create genes that encode different variable regions of antigen receptor proteins in individual clones.

- ✓ Antigen receptor chains are associated with invariant membrane proteins whose function is to deliver intracellular signals following antigen recognition. These signals, which are transmitted to the cytosol and the nucleus, may cause a lymphocyte to divide, to differentiate, to perform effector functions, or in certain circumstances to die. Thus, the two functions of lymphocyte receptors for antigen—specific antigen recognition and signal transduction—are mediated by different polypeptides. This again allows variability to be segregated in one set of molecules—the antigen receptors themselves—while leaving the conserved function of signal transduction to the other invariant proteins.

  The set of plasma membrane antigen receptor and signaling molecules in B lymphocytes is called the B cell receptor (BCR) complex, and in T lymphocytes it is called the T cell receptor (TCR) complex. When antigens bind to the extracellular portions of the antigen receptors of lymphocytes, intracellular portions of the associated signaling proteins are phosphorylated on conserved tyrosine residues by enzymes called protein tyrosine kinases. Phosphorylation triggers complex signaling cascades that culminate in the transcriptionalactivation of many genes and the production of numerous proteins that mediate the responses of the lymphocytes.

- ✓ Antibodies exist in two forms—as membrane-bound antigen receptors on B cells and as secreted proteins—but TCRs exist only as membrane receptors on T cells. Secreted antibodies are present in the blood and mucosal secretions, where they provide protection against microbes (i.e., they are the effector molecules of humoral immunity). Antibodies are also called immunoglobulins (Igs), referring to immunity-conferring proteins with the physical characteristics of globulins. Secreted antibodies recognize microbial antigens and toxins by their variable domains, the same as the membrane-bound antigen receptors of B lymphocytes. The constant regions of some secreted antibodies have the ability to bind to other molecules that participate in the

elimination of antigens: these molecules include receptors on phagocytes and proteins of the complement system.

Thus, antibodies serve different functions at different stages of humoral immune responses: membrane-bound antibodies on B cells recognize antigens to initiate B cell activation, and secreted antibodies neutralize and eliminate microbes and their toxins in the effector phase of humoral immunity. In cell-mediated immunity, the effector function of microbe elimination is performed by T lymphocytes themselves and by other leukocytes responding to the T cells. The antigen receptors of T cells are involved only in antigen recognition and T cell activation, and these proteins are not secreted and do not mediate effector functions.

5.8 The variable chains of lymphocyte antigen receptors are associated with invariant accessory chains that carry out the signaling function of the receptor

The antigen-binding portion of the B-cell receptor complex is a cell-surface immunoglobulin that has the same antigen specificity as the secreted antibodies that the B cell will eventually produce. Indeed, it is identical to a secreted monomeric immunoglobulin, except that it is attached to the membrane through the carboxy termini of the paired heavy chains. The mRNA for the cell-surface heavy chain is spliced in such a way that the carboxy terminus of the protein is made up of a transmembrane domain and a very short cytoplasmic tail. The heavy and light chains do not by themselves make up a complete cell-surface receptor, however. When cells were transfected with heavy- and light-chain cDNA derived from a cell expressing surface immunoglobulin, the immunoglobulin that was synthesized remained inside the transfected cell rather than appearing on the surface. This implied that other molecules were required for the immunoglobulin receptor to be expressed on the cell surface. Two proteins associated with heavy chains on the B-cell surface were subsequently identified and called **Igα** and **Igβ**. Transfection of Igα and Igβ cDNA along with that for the immunoglobulin chains results in the appearance of a B-cell receptor on the cell surface.

One Igα chain and one Igβ chain associates with each surface immunoglobulin molecule. Thus the complete B-cell receptor is thought to be a complex of six chains—two identical light chains, two identical heavy chains, one Igα, and one Igβ as in given figure. The Igα and

Igβ genes are closely linked in the genome and encode proteins composed of a single amino-terminal immunoglobulin-like domain connected via a transmembrane domain to a cytoplasmic tail. Igα and Igβ provide the only substantial cytoplasmic domains present in the receptor complex and are crucial for signaling. The transmembrane form of the immunoglobulin heavy chain has a very short cytoplasmic tail and it was hard to understand how this could signal into the cell that the surface immunoglobulin was ligated. The discovery of Igα and Igβ solved this intellectual problem.

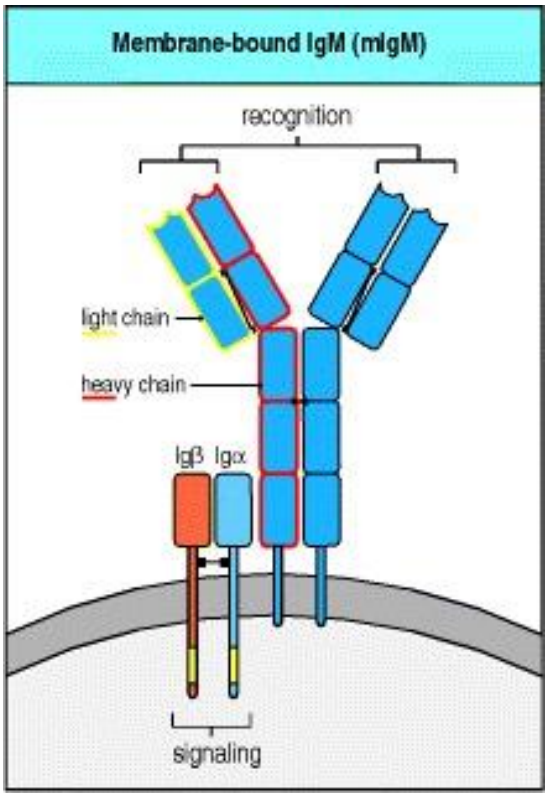

Fig. 3 The B-cell receptor complex is made up of cell-surface immuno-globulin with one each of the invariant proteins Igα and Igβ

Signaling from the B-cell receptor complex depends on the presence in Igα and Igβ of amino acid sequences called immunoreceptor tyrosine-based activation motifs (**ITAMs**). These motifs were originally identified in the cytoplasmic tails of Igα and Igβ, but are now known to be present in the accessory chains involved in signaling from the T-cell receptor, and in the Fc receptors on mast cells, macrophages, monocytes, and natural killer (NK) cells that bind antibody constant regions. ITAMs are composed of two tyrosine residues separated by

around 9–12 amino acids; the canonical ITAM sequence is ...**Y**XX[L/V]$X_{6–9}$**Y**XX[L/V]..., where Y is tyrosine, L is leucine, V is valine, and X represents any amino acid. Igα and Igβ each have a single ITAM in their cytosolic tails, giving the B-cell receptor a total of two ITAMs. When antigen binds, the tyrosines in these ITAMs become phosphorylated by receptor-associated Src-family tyrosine kinases Blk, Fyn, or Lyn. The ITAMs, by virtue of their two precisely spaced tyrosines, are then able to bind with high affinity to the tandem SH2 domains of members of a second family of protein tyrosine kinases. As we will see in Section 6-9, these kinases—Syk in B cells and ZAP-70 in T cells—are important in transmitting the signal onward.

The complete α:β T-cell antigen receptor complex contains several different accessory chains in addition to the highly variable TCRα and TCRβ chains which form heterodimers containing a single antigen-binding site (see Chapter 4). The invariant accessory chains are CD3γ, CD3δ, and CD3ε, which make up the CD3 complex, and the ζ chain, which is present as a largely intracytoplasmic homodimer. Although the exact stoichiometry of the T-cell receptor complex is not definitively established, it seems likely that two α:β heterodimers are associated at the cell surface with one CD3γ, one CD3δ, two CD3ε, and one ζ homodimer. The three CD3 proteins are encoded in adjacent genes that are regulated as a unit and are required for surface expression of the α:β heterodimers and for signaling via the receptor. Optimal expression and maximum signaling, however, also require the ζ chain, which is encoded elsewhere in the genome.

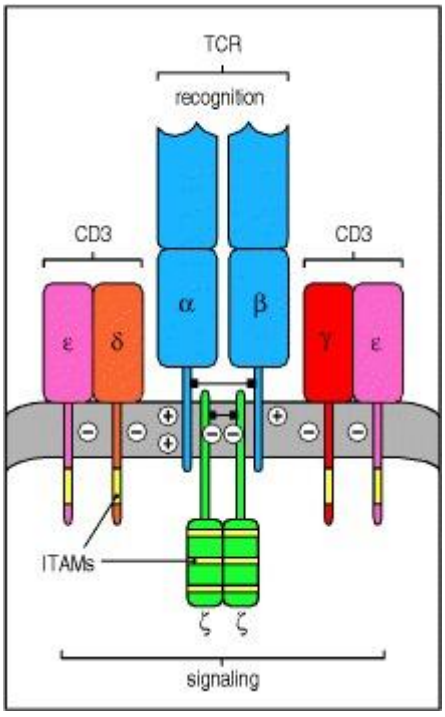

Fig. 4 The T-cell receptor complex is made up of antigen-recognition proteins and invariant signaling proteins

The CD3 proteins resemble Igα and Igβ in having an extracellular immunoglobulin-like domain and a single ITAM in their cytoplasmic tails. The ζ chain is distinct in having only a short extracellular domain, but it has three ITAMs in its cytoplasmic domain. The CD3 chains have negatively charged acidic residues in their transmembrane domains, which are able to interact with the positive charges of the α and β chains, as shown in Fig. 6.8. In total, the T-cell receptor complex is equipped with 10 ITAMs, which might give it greater flexibility in signaling compared with the B-cell receptor, as discussed later in this chapter.

Thus the antigen receptors of B and T lymphocytes are made from distinct sets of proteins but are similarly constructed. Both are molecular complexes made up of two types of functional component: variable chains that recognize the individual antigens and invariant chains that have a role both in the surface expression of the receptors and in transmitting signals to the cell's interior, enabling antigen recognition to be translated into action.

5.9 The Generation of Antibody Diversity

One of the major roles that **B cells** play in an immune response is the production of antibodies, that specifically recognise and bind to proteins on the invading bacteria or virus particles. The binding of specific antibody to its target can prevent viruses from entering cells

or aid **phagocytes** in identifying and destroying the bacteria or viruses. Given that each B cell can only produce antibody with one specificity, and that there are an enormous variety of organisms that can infect us, the immune system needs to generate vast numbers of B cells that each produce a different antibody.

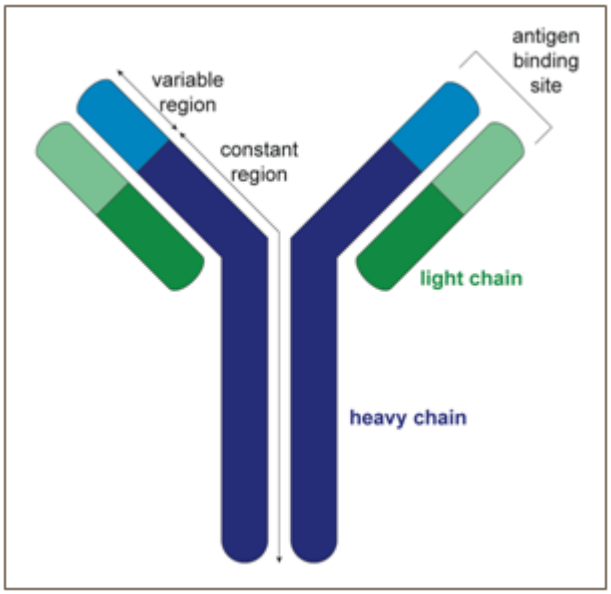

Fig. 5 Schematic diagram of an antibody molecule composed of two heavy chains and two light chains. Both the heavy chain and the light chain comprise a variable and a constant region. The variable regions are responsible for binding of a specific protein called an antigen.

Even in the absence of antigen stimulation, a human can probably make more than 10^{12} different antibody molecules—its preimmune antibody repertoire. Moreover, the antigen-binding sites of many antibodies can cross-react with a variety of related but different antigenic determinants, making the antibody defense force even more formidable. The preimmune repertoire is apparently large enough to ensure that there will be an antigen-binding site to fit almost any potential antigenic determinant, albeit with low affinity. After repeated stimulation by antigen, B cells can make antibodies that bind their antigen with much higher affinity—a process called affinity maturation. Thus, antigen stimulation greatly increases the antibody arsenal.

Antibodies are proteins, and proteins are encoded by genes. Antibody diversity therefore poses a special genetic problem: how can an animal make more antibodies than there are genes in its genome? (The human genome, for example, contains fewer than 50,000 genes.) This problem is not quite as formidable as it might first appear. Recall that the variable regions of both the light and heavy chains of antibodies usually form the antigen-binding site.

Thus, an animal with 1000 genes encoding light chains and 1000 genes encoding heavy chains could, in principle, combine their products in 1000 × 1000 different ways to make 10^6 different antigen-binding sites (although, in reality, not every light chain can combine with every heavy chain to make an antigen-binding site).

Nonetheless, the mammalian immune system has evolved unique genetic mechanisms that enable it to generate an almost unlimited number of different light and heavy chains in a remarkably economical way, by joining separate *gene segments* together before they are transcribed. Birds and fish use very different strategies for diversifying antibodies, and even sheep and rabbits use somewhat different strategies from mice and humans. We shall confine our discussion to the mechanisms used by mice and humans.

We begin this unit by discussing the mechanisms that B cells use to produce antibodies with an enormous diversity of antigen-binding sites. We then consider how a B cell can alter the tail region of the antibody it makes, while keeping the antigen-binding site unchanged. This ability allows the B cell to switch from making membrane-bound antibody to making secreted antibody, or from making one class of antibody to making another, all without changing the antigen-specificity of the antibody.

5.9.1 Important points about antibody diversity

- ✓ The V(D)J recombination that occurs during B-cell development, along with somatic mutation after antigenic stimulation, leads to the generation of antigen-binding diversity.
- ✓ Each individual B cell and all of its progeny express only one heavy chain and one light chain V region sequence; thus all have the same antigenic specificity.
- ✓ Plasma cells are activated B lymphoctyes that secrete antibody molecules.
- ✓ Isotype switching allows functional diversity due to unique properties associated with each antibody isotype.
- ✓ The sources of antibody diversity include the presence of multiple V gene segments, combinatorial diversity resulting from random recombination of V, D, and J segments, diversity due to insertion of nucleotides which result in amino acid

changes in the V-D and D-J junctions, and the coexpression of different heavy and light chain pairs.

- ✓ Somatic hypermutation allows affinity maturation after the plasma cell has encountered antigen and undergone associated stimulation.

Chickens generate antibody diversity in a manner that is quite unlike that seen in mammals. Chickens have only one functional *V* gene and one *J* gene for both light chains and heavy chains, although they do have 16 different *D* genes. Chicken immunoglobulin diversity is, therefore, generated by inserting gene sequences from nonfunctional pseudogenes in a process called *gene conversion*. Although they have only one functional *V* gene, chickens have a large number of *V* pseudogenes that serve as sequence donors. During recombination of the *V* and *J* genes, single bases are also added to each gene (N-region addition), and joining occurs at random. Chicken immunoglobulins are further diversified by somatic hypermutation and imprecise V–J joining. A second major difference involves the timing of this process. In mammals, rearrangement of immunoglobulin genes occurs throughout life.

In chickens, however, immunoglobulin genes are rearranged as a single wave between 10 and 15 days of embryogenesis, when there is clonal expansion of B cells in the bursa of Fabricius. During that 5-day period, birds generate all the antibody specificities they will need for the rest of their lives. After the bursa degenerates at puberty, the chicken must largely make do with the B cell diversity generated in early life. However, once a mature chicken B cell is stimulated by exposure to an antigen, it can generate additional V-region diversity by further gene conversion. The chicken can generate about 10^6 different immunoglobulin molecules. This is approximately one order of magnitude less than in the mouse.

5.10 Summary

Under this unit we have summarized diversity in immune system, clonal selection theory and generation in antibody diversity. Immune systems have evolved to protect organisms against large and unpredictable pathogenic environments. However, immunity always comes at a cost (metabolic and maintenance costs, autoimmune disorders, etc., and this cost must be balanced by the benefits that protection confers. Faced with the problem of evolving a suitable defense, different organisms, from archaea to humans, have developed different strategies to identify and target pathogens, which have given rise to a diversity of mechanisms of immunity.

A large effort has been made to elucidate these mechanisms down to their molecular details in a variety of species Beyond many differences, these studies have revealed many commonalities, which hint at a possible general understanding of the trade-offs that shape their design. For instance, independently of the well-known adaptive immune systems of jawed vertebrates, jawless vertebrates (e.g., lampreys) have developed an alternative adaptive system that uses a distinct molecular family of receptors, but both systems function largely in the same way, relying on the generation of a large number of diverse receptors expressed by two types of lymphocytes (B- or T-like cells). Essential to 'clonal selection' is the availability of a mechanism to focus the antigen to the appropriate B cells and, in so doing, enable the stimulation of those B cells. In 1967 Mitchison and his coworkers indirectly demonstrated that the fine specificity of antibody produced by B cells could be influenced by selectively stimulating these cells with a cross-reactive hapten. This indirect demonstration of a correlation between the B cell's surface receptor and secreted antibody product was later extended by Klinman, who demonstrated that clonal precursors whose potential antibody product was of high affinity could be selectively stimulated with low antigen concentrations.

5.11 Terminal questions

Q.15. What do you mean by diversity in immune system? Explain it.

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

Q.16. Describe clonal selection theory.

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

Q.17. Describe the generation of antibody diversity.

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

Q.18. What is the concept of antigen specific receptor? Explain it.

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

Q.19. Write a short note on generation of antibody diversity.

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

Further readings

11. Biochemistry- Lehninger A.L.

12. Biochemistry –J.H.Weil.

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

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

15. Biochemistry and molecular biology- Wilson Walker.

Unit 6

The objective of this course is to provide basic introduction and concept of antigen & antibody interactions, principle of ELISA and to understand different types of immunological disorders etc. The aim is to provide concept of immunoglobulin and different types of immunoglobulin with their functions. This block also covers the structure, function and distribution of MHC. The course is organized into following units as under:

Block II

Unit 6 To know antigen antibody interactions

Unit 7 To understand Enzyme linked sorbent assay (ELISA)

Unit 8 To understand different types of immunological disorders

Unit 9 It covers the structure, function and distribution of MHC

Unit 10 It covers different types of immunoglobulins (IgG, IgM, IgD, IgA and IgE)

Unit-6

Structure

6.1 Introduction

Antigen**,** substance that is capable of stimulating an immune response, specifically activating lymphocytes, which are the body's infection-fighting white blood cells. In general, two main divisions of antigens are recognized: foreign antigens (or heteroantigens) and autoantigens (or self-antigens). Foreign antigens originate from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals. Autoantigens, on the other hand, originate within the body. Normally, the body is able to distinguish self from nonself, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. An antigen that induces an immune response—i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly—is called an immunogen.

On the surface of antigens are regions, called antigenic determinants, that fit and bind to receptor molecules of complementary structure on the surface of the lymphocytes. The binding of the lymphocytes' receptors to the antigens' surface molecules stimulates the lymphocytes to multiply and to initiate an immune response—including the production of antibody, the activation of cytotoxic cells, or both—against the antigen. The amount of antibody formed in response to stimulation depends on the kind and amount of antigen involved, the route of entry to the body, and individual characteristics of the host.

Objectives

This is the sixth unit on Immunology. Under sixth unit we have following objectives. These are as under:

- To know about antigens, its types and uses in medical science.
- To know about antibody, antibody complexes and its interaction with antigen.
- To distinguish between antigen and antibody
- To know about agglutination, hemagglutination and opsonization.
- What do you mean by leukoagglutination and apoptic cells?

6.2 What is an antigen?

In general, antigens are composed of proteins, peptides, and polysaccharides. Any portion of bacteria or viruses, such as surface protein, coat, capsule, toxins, and cell wall, can serve as antigens. Moreover, a combination of lipid or nucleic acid with proteins or polysaccharides can form more complex antigens, such as lipopolysaccharides. Lipopolysaccharides are major ingredients of endotoxins produced by gram-negative bacteria. An antigen contains distinct sites on its surface, which is called an epitope or antigenic determinant. Antibodies generated against an antigen recognize and interact with specific epitopes via antigen-binding sites (paratopes) to trigger immune responses.

Antigens are specifically defined as molecules that interact specifically with immunoglobulin receptor of B-cell (or T-cell when complexed with MHC) or antigen is any substance that may be specifically bound by an antibody molecule or T cell receptor. Antigen is a substance which when introduced into living animal evokes specific immune response either by producing specific antibody or by sensitized T-cell. Antigen may be soluble substance, toxin or substance present in bacteria, virus, RBC and other types of cell.

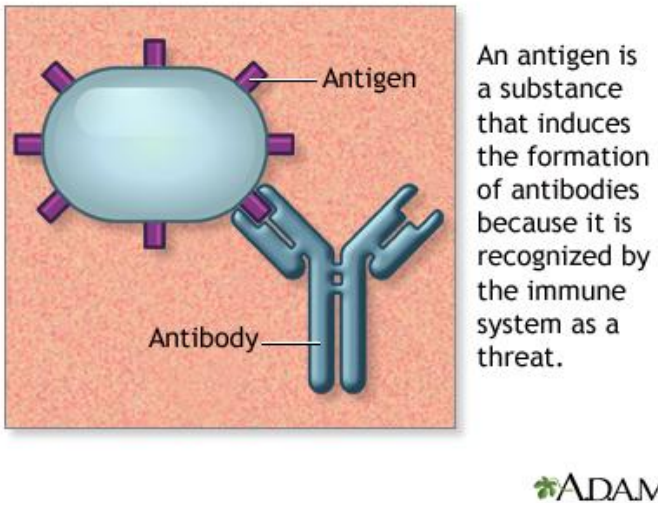

Fig. 1 Antigen

6.3 What are the types of antigens?

Antigens are mainly categorized based on their origins. For example, antigens that enter the body from outside via ingestion, inhalation, or injection are termed as exogenous antigens. These include pathogens, chemicals, toxins, allergens, pollens, etc. Autoantigens or self-antigens are normal cellular proteins or a complex of proteins that are mistakenly attacked by the immune system, leading to autoimmune diseases. A normal self-protein becomes a self-antigen because of impaired immunological tolerance, which can be caused by genetic or environmental factors.

Tumor antigens are produced due to tumor-specific mutations that occur during the neoplastic transformation of normal cells into cancerous cells. These antigens are expressed on the cancer cell surface to be recognized by the immune system. However, despite expressing cell surface antigens, the majority of cancer cells gain the ability to escape immune system-mediated elimination.

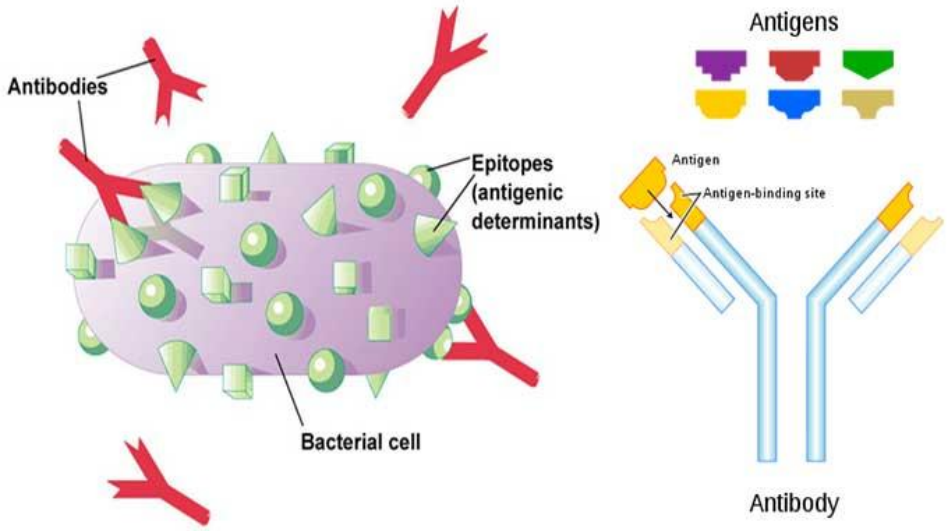

Fig. 2 Difference between antigens and antibodies

6.4 Antigens in medical science

Pathogen-specific antigens can be used as diagnostic markers to detect the current infection status of an individual. Rapid antigen tests are immunoassays used to detect the presence of pathogen-specific proteins in biological samples. Also, pathogen-specific antigens are used in vaccine production. During vaccine production, pathogen-specific antigens are processed so that they can induce desired immune responses without causing disease. In tumor vaccines, tumor-specific antigens are used to trigger immune cells that specifically target and destroy cancer cells.

6.5 Foreign antigens

It originates from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals.

6.6 Auto antigens

It originates within the body. Normally, the body is able to distinguish self from non-self, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. Ribonucleoprotein antigens in lupus-related diseases and mitochondrial antigens in primary biliary cirrhosis (PBC) etc. are examples of autoantigens. Although all antigens are recognized by specific lymphocytes or by

antibodies, only some antigens are capable of activating lymphocytes. Antigens that stimulate immune responses are called **immunogens.** An antigen that induces an immune response i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly is called an immunogen. A substance that induces specific immune response can be called as immunogen.

Antibody binds to only a portion of the antigen, which is called a **determinant or an epitope**. **Epitope** is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants**. The small area of chemical grouping on antigen molecule which determines specific immune response and reacts specifically with antibody is known as epitope.

Antigens typically contain multiple determinants, some of which may be repeated and each of which can be bound by an antibody. The presence of multiple identical determinants in an antigen is referred to as **polyvalency or multivalency**. The spatial arrangement of different epitopes on a single protein molecule may influence the binding of antibodies in several ways. When determinants are well separated, two or more antibody molecules can be bound to the same protein antigen without influencing each other; such determinants are said to be **non-overlapping**. When two determinants are close to one another, the binding of antibody to the first determinant may cause steric interference with the binding of antibody to the second; such determinants are said to be **overlapping**.

6.7 Antibodies

An **antibody** (**Ab**), also known as an **immunoglobulin** (**Ig**), is a large, Y-shaped protein used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses. The antibody recognizes a unique molecule of the pathogen, called an antigen. Each tip of the "Y" of an antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can *tag* a microbe or an infected cell for attack by other parts of the immune system, or can neutralize it directly (for example, by blocking a part of a virus that is essential for its invasion).

To allow the immune system to recognize millions of different antigens, the antigen-binding sites at both tips of the antibody come in an equally wide variety. In contrast, the remainder of the antibody is relatively constant. It only occurs in a few variants, which define the antibody's *class* or *isotype*: IgA, IgD, IgE, IgG, or IgM. The constant region at the trunk of the antibody includes sites involved in interactions with other components of the immune system. The class hence determines the function triggered by an antibody after binding to an antigen, in addition to some structural features. Antibodies from different classes also differ in where they are released in the body and at what stage of an immune response.

Together with B and T cells, antibodies comprise the most important part of the adaptive immune system. They occur in two forms: one that is attached to a B cell, and the other, a soluble form, that is unattached and found in extracellular fluids such as blood plasma. Initially, all antibodies are of the first form, attached to the surface of a B cell – these are then referred to as B-cell receptors (BCR). After an antigen binds to a BCR, the B cell activates to proliferate and differentiate into either plasma cells, which secrete soluble antibodies with the same paratope, or memory B cells, which survive in the body to enable long-lasting immunity to the antigen. Soluble antibodies are released into the blood and tissue fluids, as well as many secretions. Because these fluids were traditionally known as humors, antibody-mediated immunity is sometimes known as, or considered a part of, humoral immunity. The soluble Y-shaped units can occur individually as monomers, or in complexes of two to five units.

Antibodies are glycoproteins belonging to the immunoglobulin superfamily. The terms antibody and immunoglobulin are often used interchangeably, though the term 'antibody' is sometimes reserved for the secreted, soluble form, i.e. excluding B-cell receptors.

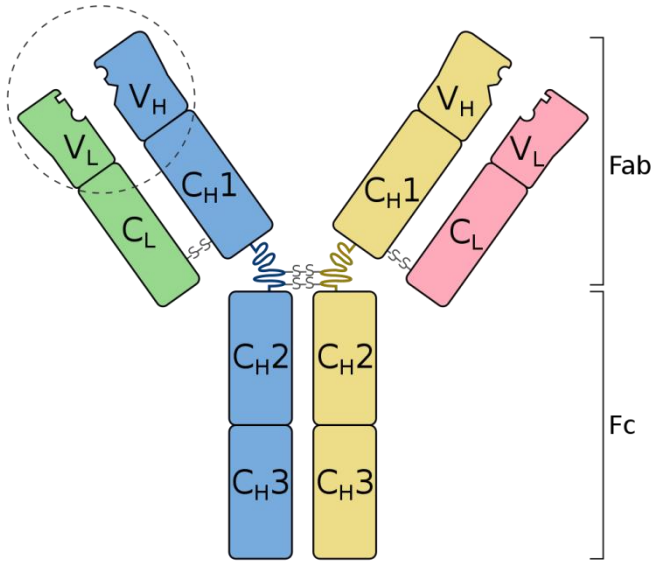

Fig. 3 Schematic structure of an antibody: two heavy chains (blue, yellow) and the two light chains (green, pink). The antigen binding site is circled.

6.8 Antigen-binding site

The variable domains can also be referred to as the F_V region. It is the subregion of Fab that binds to an antigen. More specifically, each variable domain contains three *hypervariable regions* – the amino acids seen there vary the most from antibody to antibody. When the protein folds, these regions give rise to three loops of β-strands, localized near one another on the surface of the antibody. These loops are referred to as the complementarity-determining regions (CDRs), since their shape complements that of an antigen. Three CDRs from each of the heavy and light chains together form an antibody-binding site whose shape can be anything from a pocket to which a smaller antigen binds, to a larger surface, to a protrusion that sticks out into a groove in an antigen. Typically however only a few residues contribute to most of the binding energy.

The existence of two identical antibody-binding sites allows antibody molecules to bind strongly to multivalent antigen (repeating sites such as polysaccharides in bacterial cell walls, or other sites at some distance apart), as well as to form antibody complexes and larger antigen-antibody complexes. The resulting cross-linking plays a role in activating other parts of the immune system.

The structures of CDRs have been clustered and classified by Chothia et al. and more recently by North et al. and Nikoloudis et al. However, describing an antibody's binding site using only one single static structure limits the understanding and characterization of the antibody's function and properties. To improve antibody structure prediction and to take the strongly correlated CDR loop and interface movements into account, antibody paratopes should be described as interconverting states in solution with varying probabilities. In the framework of the immune network theory, CDRs are also called idiotypes. According to immune network theory, the adaptive immune system is regulated by interactions between idiotypes.

6.9 Antibody complexes

Antibodies are also called immunoglobulins or Ig. They are Y-shaped proteins made by your immune system’s B lymphocytes or B cells. B cells attack and eliminate viruses and other toxins outside the cell. They do this by making specific antibodies for a single type of antigen. These tailored antibodies lock on to their specific antigens and tag them for attack. Antibodies also block these antigens, keeping them away from your healthy cells. Ultimately, antibodies kill these antigens, stopping infection. The main types of antibodies (immunoglobulins) include:

- **IgG.** These are the most abundant types of antibodies in your plasma. They detoxify harmful substances and provide long-term protection.
- **IgM.** These are the first antibodies made by B cells in response to antigens.
- **IgA.** These antibodies collect antigens and remove them from your body in your mucus or other body fluids.
- **IgE.** These antibodies trigger allergies and protect against parasites. Small amounts are in your skin, lungs, and mucosal membranes.
- **IgD.** These antibodies bind to B cells and signal them to release IgM antibodies.

Each antibody guards against its target antigen, and many types of antibodies are found throughout your body. They play a vital role in your body’s defense against illness and disease.

Secreted antibodies can occur as a single Y-shaped unit, a monomer. However, some antibody classes also form dimers with two Ig units (as with IgA), tetramers with four Ig units (like teleost fish IgM), or pentamers with five Ig units (like shark IgW or mammalian IgM, which occasionally forms hexamers as well, with six units).

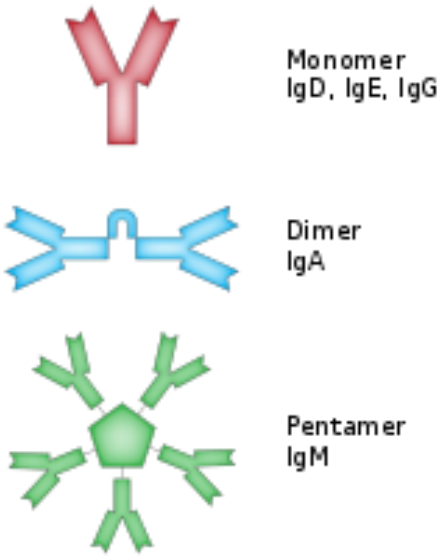

Fig. 4 Some antibodies form complexes that bind to multiple antigen molecules.

Antibodies also form complexes by binding to antigen: this is called an antigen-antibody complex or *immune complex*. Small antigens can cross-link two antibodies, also leading to the formation of antibody dimers, trimers, tetramers, etc. Multivalent antigens (e.g., cells with multiple epitopes) can form larger complexes with antibodies. An extreme example is the clumping, or agglutination, of red blood cells with antibodies in the Coombs test to determine blood groups: the large clumps become insoluble, leading to visually apparent precipitation.

6.10 B cell receptors

The membrane-bound form of an antibody may be called a *surface immunoglobulin* (sIg) or a *membrane immunoglobulin* (mIg). It is part of the *B cell receptor* (BCR), which allows a B cell to detect when a specific antigen is present in the body and triggers B cell activation. The BCR is composed of surface-bound IgD or IgM antibodies and associated Ig-α and Ig-β heterodimers, which are capable of signal transduction. A typical human B cell will have 50,000 to 100,000 antibodies bound to its surface. Upon antigen binding, they cluster in large

patches, which can exceed 1 micrometer in diameter, on lipid rafts that isolate the BCRs from most other cell signaling receptors. These patches may improve the efficiency of the cellular immune response. In humans, the cell surface is bare around the B cell receptors for several hundred nanometers, which further isolates the BCRs from competing influences.

6.11 Classes

Antibodies can come in different varieties known as *isotypes* or *classes*. In placental mammals there are five antibody classes known as IgA, IgD, IgE, IgG, and IgM, which are further subdivided into subclasses such as IgA1, IgA2. The prefix "Ig" stands for *immunoglobulin*, while the suffix denotes the type of heavy chain the antibody contains: the heavy chain types α (alpha), γ (gamma), δ (delta), ε (epsilon), μ (mu) give rise to IgA, IgG, IgD, IgE, IgM, respectively. The distinctive features of each class are determined by the part of the heavy chain within the hinge and Fc region.

The classes differ in their biological properties, functional locations and ability to deal with different antigens, as depicted in the table. For example, IgE antibodies are responsible for an allergic response consisting of histamine release from mast cells, often a sole contributor to asthma (though other pathways exist as do exist symptoms very similar to yet not technically asthma). The antibody's variable region binds to allergic antigen, for example house dust mite particles, while its Fc region (in the ε heavy chains) binds to Fc receptor ε on a mast cell, triggering its degranulation: the release of molecules stored in its granules.

Class	Subclass	Description
IgA	2	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.
IgD	1	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens.[28] It has been shown to activate basophils and mast cells to produce antimicrobial factors.
IgE	1	Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Humans and other animals evolved IgE to protect against parasitic worms, though in the present, IgE is primarily related to allergies and asthma.
IgG	4	In its four forms, provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity

		to the fetus.
IgM	1	Expressed on the surface of B cells (monomer) and in a secreted form (pentamer) with very high avidity. Eliminates pathogens in the early stages of B cell-mediated (humoral) immunity before there is sufficient IgG.

Table 1

6.12 Antibody–antigen interactions

The antibody's paratope interacts with the antigen's epitope. An antigen usually contains different epitopes along its surface arranged discontinuously, and dominant epitopes on a given antigen are called determinants. Antibody and antigen interact by spatial complementarity (lock and key). The molecular forces involved in the Fab-epitope interaction are weak and non-specific – for example electrostatic forces, hydrogen bonds, hydrophobic interactions, and van der Waals forces. This means binding between antibody and antigen is reversible, and the antibody's affinity towards an antigen is relative rather than absolute. Relatively weak binding also means it is possible for an antibody to cross-react with different antigens of different relative affinities.

6.13 Functions

The main categories of antibody action include the following:

- Neutralisation, in which neutralizing antibodies block parts of the surface of a bacterial cell or virion to render its attack ineffective
- Agglutination, in which antibodies "glue together" foreign cells into clumps that are attractive targets for phagocytosis
- Precipitation, in which antibodies "glue together" serum-soluble antigens, forcing them to precipitate out of solution in clumps that are attractive targets for phagocytosis
- Complement activation (fixation), in which antibodies that are latched onto a foreign cell encourage complement to attack it with a membrane attack complex, which leads to the following:
    - Lysis of the foreign cell
    - Encouragement of inflammation by chemotactically attracting inflammatory cells

More indirectly, an antibody can signal immune cells to present antibody fragments to T cells, or down regulate other immune cells to avoid autoimmunity. Activated B

cells differentiate into either antibody-producing cells called plasma cells that secrete soluble antibody or memory cells that survive in the body for years afterward in order to allow the immune system to remember an antigen and respond faster upon future exposures.

At the prenatal and neonatal stages of life, the presence of antibodies is provided by passive immunization from the mother. Early endogenous antibody production varies for different kinds of antibodies, and usually appear within the first years of life. Since antibodies exist freely in the bloodstream, they are said to be part of the humoral immune system. Circulating antibodies are produced by clonal B cells that specifically respond to only one antigen (an example is a virus capsid protein fragment). Antibodies contribute to immunity in three ways: They prevent pathogens from entering or damaging cells by binding to them; they stimulate removal of pathogens by macrophages and other cells by coating the pathogen; and they trigger destruction of pathogens by stimulating other immune responses such as the complement pathway. Antibodies will also trigger vasoactive amine degranulation to contribute to immunity against certain types of antigens (helminths, allergens).

6.14 Differences Between Antigen and Antibody

Antibodies, also called immunoglobulins, Y-shaped molecules are proteins manufactured by the body that help fight against foreign substances called **antigens**. **Antigens** are any substance that stimulates the immune system to produce antibodies. **Antigens** can be bacteria, viruses, or fungi that cause infection and disease. Following are some of the differences between Antigen and Antibody:

S.N.	Antigen	Antibody
1	Generally proteins but can be lipids, carbohydrates or nucleic acids.	Antibodies are proteins.
2	Triggers the formation of antibodies.	Variable sites has the antigen binding domain.
3	There are three basic kinds of antigens. (Exogenous, Endogenous and Autoantigens)	There are five basic kinds of antibodies. (Immunoglobulins M, G, E, D and A)

4	The region of the antigen that interacts with the antibodies is called epitopes.	The variable region of the antibody that specially binds to an epitope is called paratope.
5	Cause disease or allergic reactions.	Protects the body by immobilization or lysis of antigenic material.

Table 2

6.15 Antigen-Antibody interaction

The interactions between antigens and antibodies are known as antigen-antibody reactions. The reactions are highly specific, and an antigen reacts only with antibodies produced by itself or with closely related antigens. Antibodies recognize molecular shapes (epitopes) on antigens. Generally, the better the fit of the epitope (in terms of geometry and chemical character) to the antibody combining site, the more favorable the interactions that will be formed between the antibody and antigen and the higher the affinity of the antibody for antigen. The affinity of the antibody for the antigen is one of the most important factors in determining antibody efficacy in vivo.

The antigen- antibody interaction is bimolecular irreversible association between antigen and antibody. The association between antigen and antibody includes various non-covalent interactions between epitope (antigenic determinant) and variable region (VH/VL) domain of antibody. Chemical Bonds Responsible for the Antigen-Antibody Reaction The interaction between the Ab-binding site and the epitope involves exclusively noncovalent bonds, in a similar manner to that in which proteins bind to their cellular receptors, or enzymes bind to their substrates. The binding is reversible and can beprevented or dissociated by high ionic strength or extreme pH.

The following intermolecular forces are involved in Ag–Ab binding:

- Electrostatic bonds: This result from the attraction between oppositely charged ionic groups of two protein side chains; for example, an ionized amino group (NH4 +) on a lysine in the Ab, and an ionized carboxyl group (COO_) on an aspartate residue in the Ag.

- Hydrogen bonding: When the Ag and Ab are in very close proximity, relatively weak hydrogen bonds can be formed between hydrophilic groups (e.g., OH and C=O, NH and C=O, and NH and OH groups).
- Hydrophobic interactions: Hydrophobic groups, such as the side chains of valine, leucine, and phenylalanine, tend to associate due to Van der Waals bonding and coalesce in an aqueous environment, excluding water molecules from their surroundings. As a consequence, the distance between them decreases, enhancing the energies of attraction involved. This type of interaction is estimated to contribute up to 50% of the total strength of the Ag–Ab bond.
- Van der Waals bonds: These forces depend upon interactions between the "electron clouds" that surround the Ag and Ab molecules. The interaction has been compared to that which might exist between alternating dipoles in two molecules, alternating in such a way that, at any given moment, oppositely oriented dipoles will be present in closely apposed areas of the Ag and Ab molecules.

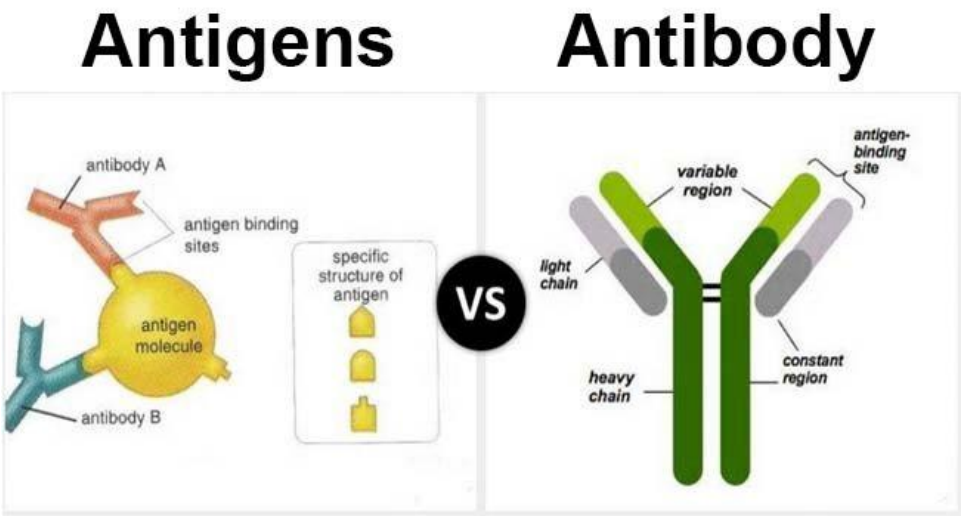

Fig. 5 What is the difference between Antigens and Antibodies?

6.16 Agglutination

Agglutination is the clumping of particles. The word *agglutination* comes from the Latin *agglutinare* (glueing to). Agglutination is the process that occurs if an antigen is

mixed with its corresponding antibody called isoagglutinin. This term is commonly used in blood grouping. This occurs in biology in two main examples:

1. The clumping of cells such as bacteria or red blood cells in the presence of an antibody or complement. The antibody or other molecule binds multiple particles and joins them, creating a large complex. This increases the efficacy of microbial elimination by phagocytosis as large clumps of bacteria can be eliminated in one pass, versus the elimination of single microbial antigens.
2. When people are given blood transfusions of the wrong blood group, the antibodies react with the incorrectly transfused blood group and as a result, the erythrocytes clump up and stick together causing them to agglutinate. The coalescing of small particles that are suspended in a solution; these larger masses are then (usually) precipitated.

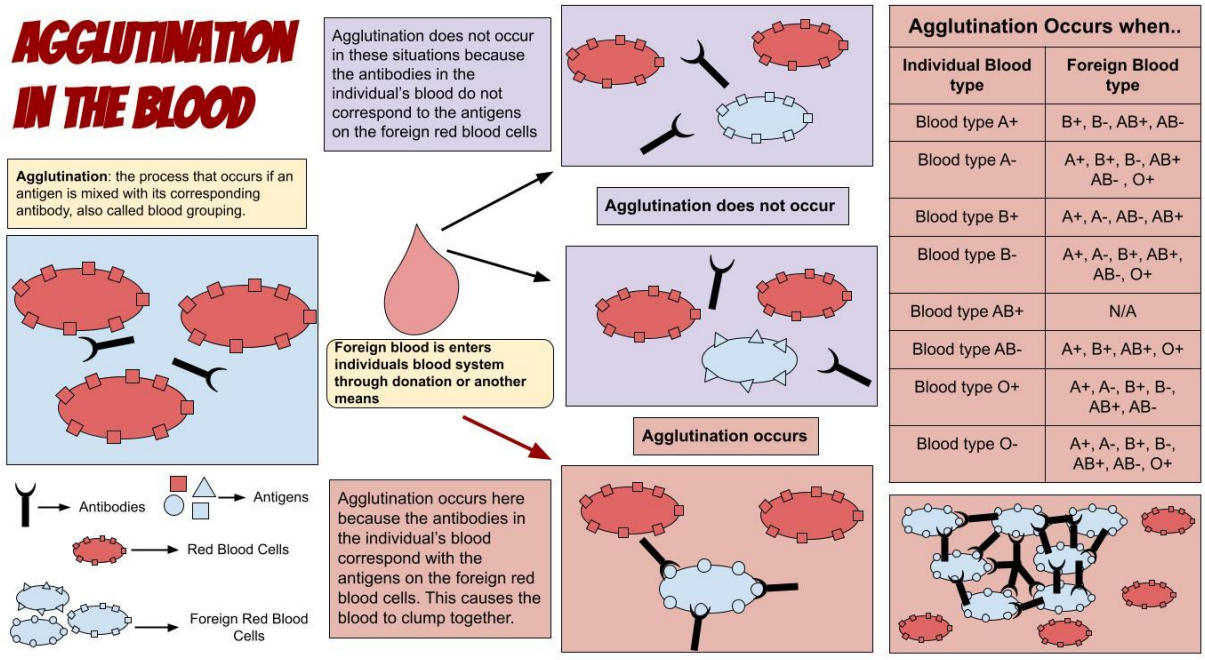

Fig. 6 This image explains agglutination in the blood

6.17 In hematology

In hematology, red cell agglutination or autoagglutination is a phenomenon in which red blood cells clump together, forming aggregates. It is caused by the surface of the red cells being coated with antibodies. This often occurs in cold agglutinin disease, a type

of autoimmune hemolytic anemia in which people produce antibodies (termed cold agglutinins) that bind to their red blood cells at cold temperatures and destroy them. People may develop cold agglutinins from lymphoproliferative disorders, from infection with *Mycoplasma pneumoniae* or Epstein-Barr virus, or idiopathically (without any apparent cause). Red cell agglutination can also occur in paroxysmal nocturnal hemoglobinuria and warm autoimmune hemolytic anemia. In cases of red cell agglutination, the direct antiglobulin test can be used to demonstrate the presence of antibodies bound to the red cells.

6.18 Hemagglutination

Hemagglutination is the process by which red blood cells agglutinate, meaning clump or clog. The agglutin involved in hemagglutination is called hemagglutinin. In cross-matching, donor red blood cells and the recipient's serum or plasma are incubated together. If agglutination occurs, this indicates that the donor and recipient blood types are incompatible. When a person produces antibodies against their own red blood cells, as in cold agglutinin disease and other autoimmune conditions, the cells may agglutinate spontaneously. This is called autoagglutination and it can interfere with laboratory tests such as blood typing and the complete blood count.

6.18.1 Leukoagglutination

Leukoagglutination occurs when the particles involved are white blood cells. An example is the PH-L form of phytohaemagglutinin.

6.18.2 In microbiology

Agglutination is commonly used as a method of identifying specific bacterial antigens and the identity of such bacteria, and therefore is an important technique in diagnosis.

6.18.3 Precipitation

Precipitation reactions are based on the interaction of antibodies and antigens. They are based on two soluble reactants that come together to make one insoluble product, the precipitate. These reactions depend on the formation of lattices (cross-links) when antigen and antibody exist in optimal proportions. Excess of either component reduces lattice formation and subsequent precipitation. Precipitation reactions differ from agglutination reactions in the size and solubility of the antigen and sensitivity. Antigens are soluble molecules and larger in size

in precipitation reactions. There are several precipitation methods applied in clinical laboratory for the diagnosis of disease. These can be performed in semisolid media such as agar or agarose, or non-gel support media such as cellulose acetate.

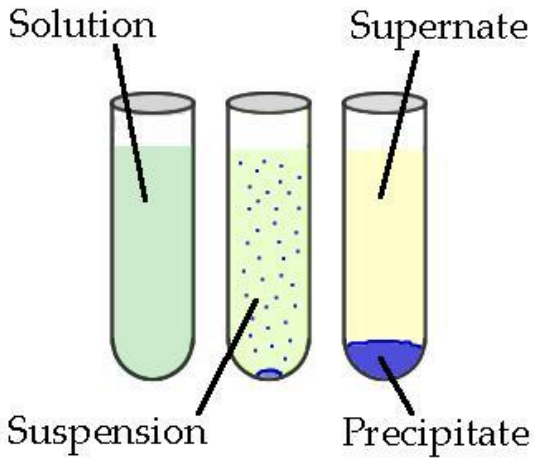

Fig. 7 Precipitation reaction: Difference in the visual appearance of an aggregate and a precipitate.

Precipitation methods include double immunodiffusion (qualitative gel technique that determines the relationship between antigen and antibody), radial immunodiffusion (semi-quantitation of proteins by gel diffusion using antibody incorporated in agar), and electroimmuno diffusion (variation of the double immunodiffusion method reaction that uses an electric current to enhance the mobility of the reactants toward each other). Precipitation reactions are less sensitive than agglutination reactions but remain gold standard serological techniques. The most commonly used serologic precipitation reactions are the Ouchterlony test (based on double immunodiffusion and named after the Swedish physician who invented it), and the Mancini method (based on single radial immunodiffusion). In the double immunodiffusion technique, three basic reaction patterns result from the relationship of antigens and antibodies. These patterns are identity, non-identity, and partial identity. The Mancini method results in precipitin ring formation on a thin agarose layer. The diameter of the ring correlates with the concentration of proteins in the precipitin.

6.18.4 Opsonization

Opsonization is the mechanism by which targeting of particles for destruction through phagocytosis becomes enhanced. Opsonins are molecules that mark foreign particles for phagocytosis. Phagocytosis is the cellular process for removes pathogens and dead or dying

cells. Opsonization is the second step of phagocytosis, with chemotaxis first causing the recruitment of the phagocyte towards the site of infection or cell death. Opsonization, or the attachment of opsonins, then makes the pathogen more visible to the phagocyte, and the opsonized pathogen is then ingested by the phagocyte before intracellular destruction through digestion.

6.18.5 Mechanism of Opsonization and Types of Opsonins

Opsonization occurs through the binding of an opsonin to an epitope of the pathogen or dead cells. Immune cells and pathogens all have negatively charged cell membranes. This causes the phagocyte and pathogen to be repelled away from each other. The opsonin molecule overcomes the repellant force of the negative charges through the interaction between the opsonin and the cell surface receptors on the immune cells. Specific antibodies can act as opsonins as well as compliment proteins of the innate immune response, and circulating proteins secreted from pattern recognition receptors can also be opsonins.

6.19 Antibodies

Antibodies are proteins made by immune cells which target specific molecules. These molecules can range from proteins present on the surface of bacteria, molecules secreted by bacteria or molecules present on host cells. These proteins are Y-shaped, and the “branches” are where the recognition occurs. Antibodies facilitate phagocytosis as the “stalk” of the antibody molecule is recognized by the phagocyte.

The Compliment System and Opsonization

The compliment system is a part of the innate immune response that bridges the innate (non-specific) and adaptive (specific) immune responses. The system is comprised of distinct plasma proteins that facilitate opsonization to reduce inflammation and remove pathogens. The compliment molecules C3b, C4b and C1q can all serve as opsonins.

Opsonization of Pathogens by Complement Proteins

The compliment system can lead to the removal of pathogens through three pathways:

1. The classical pathway.
2. The alternative pathway.
3. The mannose-binding lectin pathway.

The classical pathway is activated by the binding of C1q to antigen-antibody complexes, thus forming a link between innate and adaptive immunity. C1q can also bind directly to the pathogen surface in the absence of an antibody. The C1q protein is a part of the C1 complex which acts on C4 and C3 molecules in a series of cleavage reactions to produce the classical C3 convertase, made up of components including C4b and C3b.

The alternative pathway involves the spontaneous activation of a compliment component binding to the surface of the pathogen. It requires factors such as B and D interacting with each other and C3b to form the alternative C3 convertase, C3bBb.
The mannose-binding lectin pathway begins with the binding of mannose-binding lectin (MBL) to mannose-containing carbohydrates on the pathogen. This leads to the production of MBL-associated serine proteases which in turn activate C4 and C3 to form the classical C3 convertase. All three pathways converge at this point with C3 convertase cleaving C3 to produce C3b that binds to the pathogen cell membrane and opsonizes the pathogen.

6.20 Opsonization of Apoptotic Cells

The controlled death of cells through apoptosis and the clearance of these dead cells are important processes for preventing the accumulation of dead cells during inflammation. Poor removal of dead cells can lead to an active immune response that aggravates chronic inflammation. Opsonization removes apoptotic cells via soluble innate pattern-recognition proteins (sPRPs) that are able to recognize a diverse range of surface ligands on apoptotic cells. These circulating proteins include pentraxins, collectins and ficolins.
Phosphatidylcholine (PC) is a pentraxin molecule that is hydrolyzed in the later stages of apoptosis to form lysophosphatidylcholine (lysoPC), exposed on the surface of the dying, or apoptotic, cell. The soluble lysoPC acts as a chemo-attractant so the phagocyte can easily recognize the late apoptotic cell. Ligands on the dead cell act as a signal for the phagocyte to begin ingestion, with vital cells not presenting the same signals.

6.21 Ouchterlony double immunodiffusion

Ouchterlony double immunodiffusion (also known as passive double immunodiffusion) is an immunological technique used in the detection, identification and quantification of antibodies and antigens, such as immunoglobulins and extractable nuclear antigens. The technique is named after Örjan Ouchterlony, the Swedish physician who invented the test in 1948.

Fig. 8 Picture of an Ouchterlony double immunodiffusion plate, after immunodiffusion has taken place. In this, titre value of an antigen is quantified. The central well has an antibody, and the surrounding wells have decreasing concentration of the corresponding antigen.

6.21.1 Procedure

A gel plate is cut to form a series of holes ("wells") in an agar or agarose gel. A sample extract of interest (for example human cells harvested from tonsil tissue) is placed in one well, and sera or purified antibodies are placed in another well and the plate left for 48 hours to develop. During this time the antigens in the sample extract and the antibodies each diffuse out of their respective wells. Where the two diffusion fronts meet, if any of the antibodies recognize any of the antigens, they will bind to the antigens and form an immune complex. The immune complex precipitates in the gel to give a thin white line (precipitin line), which is a visual signature of antigen recognition.

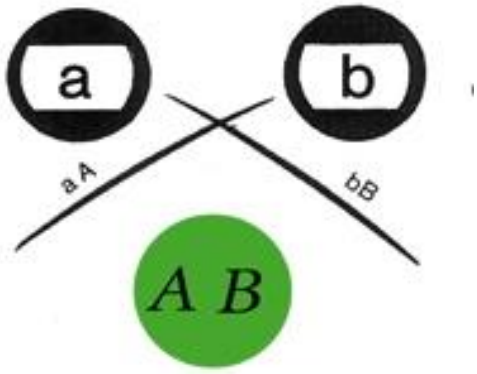

Fig. 9 Ouchterlony patterns showing no identity between upper spots

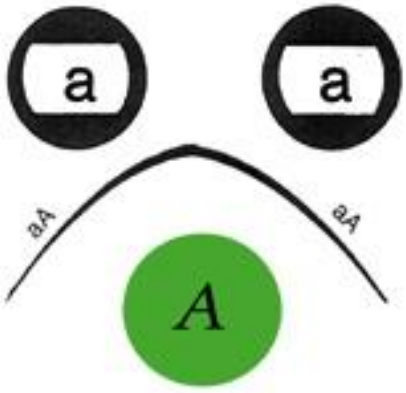

Fig. 10 Ouchterlony patterns showing full identity between upper spots

The method can be conducted in parallel with multiple wells filled with different antigen mixtures and multiple wells with different antibodies or mixtures of antibodies, and antigen-antibody reactivity can be seen by observing between which wells the precipitate is observed. When more than one well is used there are many possible outcomes based on the reactivity of the antigen and antibody selected. The zone of equivalence lines may give a full identity (i.e. a continuous line), partial identity (i.e. a continuous line with a spur at one end), or a non-identity (i.e. the two lines cross completely).

6.21.2 Theory

Precipitation occurs with most antigens because the antigen is multivalent (i.e. has several antigenic determinants per molecule to which antibodies can bind). Antibodies have at least two antigen binding sites (and in the case of Immunoglobulin M there is a multimeric complex with up to 10 antigen binding sites), thus large aggregates or gel-like lattices of antigen and antibody are formed. Experimentally, an increasing amount of antigen is added to a constant amount of antibody in solution. Initially at low antigen concentration, all of the antibody is contained in the precipitate. This is called the antibody-excess zone (i.e. prozone phenomenon). As more antigen is added, the amount of protein precipitated increases until the antigen/antibody molecules are at an optimal ratio. This is known as the zone of equivalence or equivalence point. When the amount of antigen in solution exceeds the

amount of antibody, the amount of precipitation will decrease. This is known as the antigen excess zone.

6.22 Summary

Under this unit we have summarized antigen, antibody, interaction between both and agglutination and other facts. During the first half of the 20th century, a series of scientific discoveries resolved that antibody-mediated immunity is the cornerstone of the specific immune response. Since their first use as immunolabeling research tools in the early 1970s, antibody technologies have vastly improved, and antibodies have become critical tools for most areas of life science research. The basic principle of any immunochemical technique is that a specific antibody will combine with its specific antigen to generate an exclusive antibody-antigen complex. In the following pages we will discuss the nature of this bond, and the use of this robust and specific binding as a molecular tag for research.

An antibody is defined as "an immunoglobulin capable of specific combination with the antigen that caused its production in a susceptible animal." Antibodies are produced in response to the invasion of foreign molecules in the body. An antibody, abbreviated as Ab, is commonly referred to as an immunoglobulin or Ig. Human immunoglobulins are a group of structurally and functionally similar glycoproteins (82-96% protein and 4-18% carbohydrate) that confer humoral immunity.

Agglutination is defined as the formation of clumps of cells or inert particles by specific antibodies to surface antigenic components (direct agglutination) or to antigenic components adsorbed or chemically coupled to red cells or inert particles (passive hemagglutination and passive agglutination, respectively). Erythrocytes are also agglutinated by nonantibody substances such as plant proteins, viruses, salts of heavy metals, inorganic colloidal acids and bases, and basic proteins (protamines, histones). Agglutination inhibition or hemagglutination inhibition refers to the inhibition of these reactions by soluble antigen which reacts with the combining sites of the antibodies and thereby prevents their binding to and agglutination of the particles.

6.23Terminal questions

Q. 1 What do you mean by antigen? Explain it.

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

Q. 2 What do you mean by antibody? Explain it.

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

Q. 3 Describe the interaction between antigen and antibody.

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

Q. 4 What are the differences between antigen and antibody?

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

Q. 5 What is mechanism of Opsonization? Explain it.

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

Q.20. Write a short note on agglutination.

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

Q.21. Explain hemagglutination and leukoagglutination .

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

Further readings

16. Biochemistry- Lehninger A.L.

17. Biochemistry –J.H.Weil.

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

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

20. Biochemistry and molecular biology- Wilson Walker.

Unit-7

Structure

7.1 Introduction

Our immune system is essential for our survival. Without an immune system, our bodies would be open to attack from bacteria, viruses, parasites, and more. It is our immune system that keeps us healthy as we drift through a sea of pathogens. This vast network of cells and tissues is constantly on the lookout for invaders, and once an enemy is spotted, a complex attack is mounted.

The immune system is spread throughout the body and involves many types of cells, organs, proteins, and tissues. Crucially, it can distinguish our tissue from foreign tissue — self from non-self. Dead and faulty cells are also recognized and cleared away by the immune system. If the immune system encounters a pathogen, for instance, a bacterium, virus, or parasite, it

mounts a so-called immune response. Later, we will explain how this works, but first, we will introduce some of the main characters in the immune system.

This is the seventh unit on Immunology. Under seventh unit we have following objectives. These are as under:

- To know about immune response, B lymphocyte and T lymphocyte.
- To know about role of helper T cells and killer T cells.
- To know about immunity and immunizations.
- To discuss ELISA, autoimmunity and hypersensitivity.
- To know about applications of ELISA and generation of TCR diversity.

7.2 White blood cells

White blood cells are also called leukocytes. They circulate in the body in blood vessels and the lymphatic vessels that parallel the veins and arteries. White blood cells are on constant patrol and looking for pathogens. When they find a target, they begin to multiply and send signals out to other cell types to do the same. Our white blood cells are stored in different places in the body, which are referred to as lymphoid organs. These include the following:

- **Thymus** — a gland between the lungs and just below the neck.
- **Spleen** — an organ that filters the blood. It sits in the upper left of the abdomen.
- **Bone marrow** — found in the center of the bones, it also produces red blood cells.
- **Lymph nodes** —small glands positioned throughout the body, linked by lymphatic vessels.

There are two main types of leukocyte:

1. Phagocytes

These cells surround and absorb pathogens and break them down, effectively eating them. There are several types, including:

- **Neutrophils** — these are the most common type of phagocyte and tend to attack bacteria.
- **Monocytes** — these are the largest type and have several roles.
- **Macrophages** — these patrol for pathogens and also remove dead and dying cells.

- **Mast cells** — they have many jobs, including helping to heal wounds and defend against pathogens.

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). These two cell types have different roles:

- **B lymphocytes** — they produce antibodies and help alert the T lymphocytes.
- **T lymphocytes** — they destroy compromised cells in the body and help alert other leukocytes.

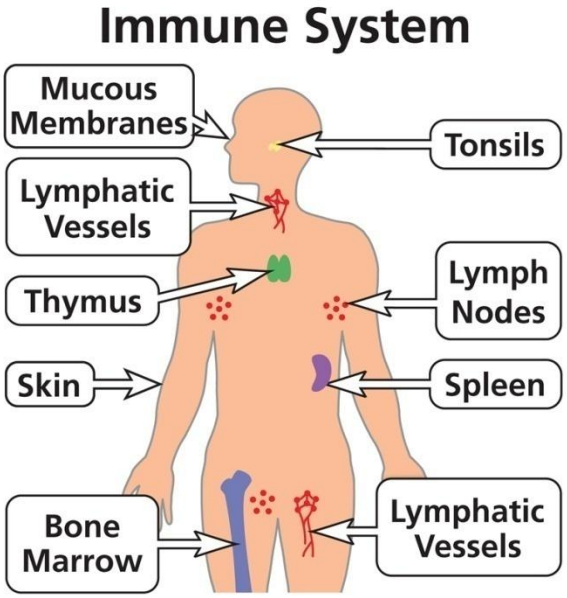

Fig. 1 Immune system

7.3 How an immune response works

The immune system needs to be able to tell self from non-self. It does this by detecting proteins that are found on the surface of all cells. It learns to ignore its own or self proteins at an early stage.

An antigen is any substance that can spark an immune response.

In many cases, an antigen is a bacterium, fungus, virus, toxin, or foreign body. But it can also be one of our own cells that is faulty or dead. Initially, a range of cell types works together to recognize the antigen as an invader.

7.4 The role of B lymphocytes

Once B lymphocytes spot the antigen, they begin to secrete antibodies (antigen is short for "antibody generators"). Antibodies are special proteins that lock on to specific antigens. Each B cell makes one specific antibody. For instance, one might make an antibody against the bacteria that cause pneumonia, and another might recognize the common cold virus. Antibodies are part of a large family of chemicals called immunoglobulins, which play many roles in the immune response:

- **Immunoglobulin G (IgG)** — marks microbes so other cells can recognize and deal with them.
- **IgM** — is expert at killing bacteria.
- **IgA** — congregates in fluids, such as tears and saliva, where it protects gateways into the body.
- **IgE** — protects against parasites and is also to blame for allergies.
- **IgD** — stays bound to B lymphocytes, helping them to start the immune response.

Antibodies lock onto the antigen, but they do not kill it, only mark it for death. The killing is the job of other cells, such as phagocytes.

7.5 The role of T lymphocytes

There are distinct types of T lymphocytes:

7.5.1 Helper T cells (Th cells)

They coordinate the immune response. Some communicate with other cells, and some stimulate B cells to produce more antibodies. Others attract more T cells or cell-eating phagocytes.

7.5.2 Killer T cells (cytotoxic T lymphocytes)

As the name suggests, these T cells attack other cells. They are particularly useful for fighting viruses. They work by recognizing small parts of the virus on the outside of infected cells and destroy the infected cells.

7.6 Immunity

Everyone's immune system is different but, as a general rule, it becomes stronger during adulthood as, by this time, we have been exposed to more pathogens and developed more immunity. That is why teens and adults tend to get sick less often than children.Once an antibody has been produced, a copy remains in the body so that if the same antigen appears again, it can be dealt with more quickly. That is why with some diseases, such as chickenpox, you only get it once as the body has a chickenpox antibody stored, ready and waiting to destroy it next time it arrives. This is called immunity. There are three types of immunity in humans called innate, adaptive, and passive:

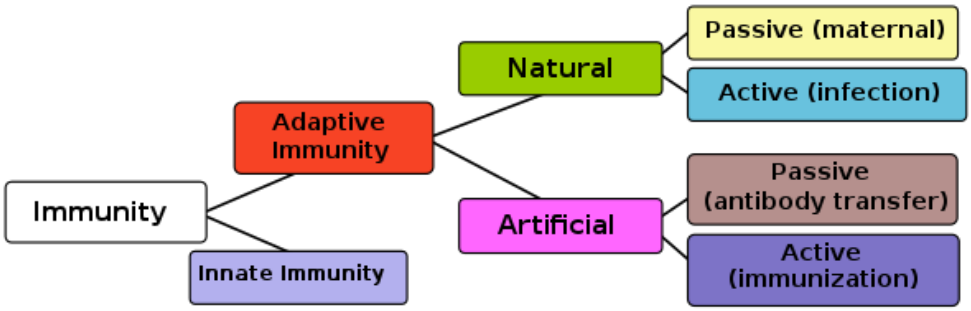

Fig. 2 Immunity

7.6.1 Innate immunity

We are all born with some level of immunity to invaders. Human immune systems, similarly to those of many animals, will attack foreign invaders from day one. This innate immunity includes the external barriers of our body — the first line of defense against pathogens — such as the skin and mucous membranes of the throat and gut. This response is more general and non-specific. If the pathogen manages to dodge the innate immune system, adaptive or acquired immunity kicks in.

7.6.2 Adaptive (acquired) immunity

This protect from pathogens develops as we go through life. As we are exposed to diseases or get vaccinated, we build up a library of antibodies to different pathogens. This is sometimes referred to as immunological memory because our immune system remembers previous enemies.

7.6.3 Passive immunity

This type of immunity is "borrowed" from another source, but it does not last indefinitely. For instance, a baby receives antibodies from the mother through the placenta before birth and in breast milk following birth. This passive immunity protects the baby from some infections during the early years of their life.

7.7 Immunizations

Immunization introduces antigens or weakened pathogens to a person in such a way that the individual does not become sick but still produces antibodies. Because the body saves copies of the antibodies, it is protected if the threat should reappear later in life.

7.8 Immune system disorders

Because the immune system is so complex, there are many potential ways in which it can go wrong. Types of immune disorder fall into three categories:

7.9 Immunodeficiencies

These arise when one or more parts of the immune system do not function. Immunodeficiencies can be caused in a number of ways, including age, obesity, and alcoholism. In developing countries, malnutrition is a common cause. AIDS is an example of an acquired immunodeficiency. In some cases, immunodeficiencies can be inherited, for instance, in chronic granulomatous disease where phagocytes do not function properly.

7.10 Autoimmunity

In autoimmune conditions, the immune system mistakenly targets healthy cells, rather than foreign pathogens or faulty cells. In this scenario, they cannot distinguish self from non-self. Autoimmune diseases include celiac disease, type 1 diabetes, rheumatoid arthritis, and Graves' disease.

7.11 Hypersensitivity

With hypersensitivity, the immune system overreacts in a way that damages healthy tissue. An example is anaphylactic shock where the body responds to an allergen so strongly that it can be life-threatening.

7.12 In a nutshell

The immune system is incredibly complicated and utterly vital for our survival. Several different systems and cell types work in perfect synchrony (most of the time) throughout the body to fight off pathogens and clear up dead cells.

7.13 Enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is a commonly used analytical biochemistry assay, first described by Eva Engvall and Peter Perlmann in 1971. The assay uses a solid-phase type of enzyme immunoassay (EIA) to detect the presence of a ligand (commonly a protein) in a liquid sample using antibodies directed against the protein to be measured. ELISA has been used as a diagnostic tool in medicine, plant pathology, and biotechnology, as well as a quality control check in various industries.

In the most simple form of an ELISA, antigens from the sample to be tested are attached to a surface. Then, a matching antibody is applied over the surface so it can bind the antigen. This antibody is linked to an enzyme and then any unbound antibodies are removed. In the final step, a substance containing the enzyme's substrate is added. If there was binding, the subsequent reaction produces a detectable signal, most commonly a color change.

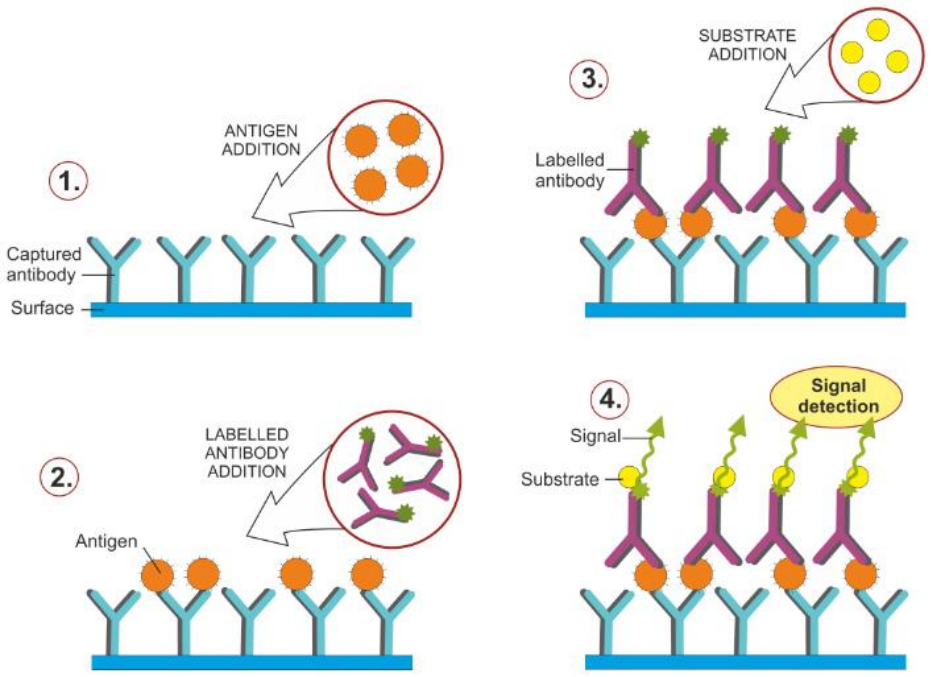

Fig. 3 enzyme-linked immunosorbent assay (ELISA)

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are non-specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

Of note, ELISA can perform other forms of ligand binding assays instead of strictly "immuno" assays, though the name carried the original "immuno" because of the common use and history of development of this method. The technique essentially requires any ligating reagent that can be immobilized on the solid phase along with a detection reagent that will bind specifically and use an enzyme to generate a signal that can be properly quantified. In between the washes, only the ligand and its specific binding counterparts remain specifically

bound or "immunosorbed" by antigen-antibody interactions to the solid phase, while the nonspecific or unbound components are washed away. Unlike other spectrophotometric wet lab assay formats where the same reaction well (e.g., a cuvette) can be reused after washing, the ELISA plates have the reaction products immunosorbed on the solid phase, which is part of the plate, and so are not easily reusable.

7.14 Principle

As an analytical biochemistry assay and a "wet lab" technique, ELISA involves detection of an analyte (i.e., the specific substance whose presence is being quantitatively or qualitatively analyzed) in a liquid sample by a method that continues to use liquid reagents during the analysis (i.e., controlled sequence of biochemical reactions that will generate a signal which can be easily quantified and interpreted as a measure of the amount of analyte in the sample) that stays liquid and remains inside a reaction chamber or well needed to keep the reactants contained. This is in contrast to "dry lab" techniques that use dry strips. Even if the sample is liquid (e.g., a measured small drop), the final detection step in "dry" analysis involves reading of a dried strip by methods such as reflectometry and does not need a reaction containment chamber to prevent spillover or mixing between samples.

As a heterogenous assay, ELISA separates some component of the analytical reaction mixture by adsorbing certain components onto a solid phase which is physically immobilized. In ELISA, a liquid sample is added onto a stationary solid phase with special binding properties and is followed by multiple liquid reagents that are sequentially added, incubated, and washed, followed by some optical change (e.g., color development by the product of an enzymatic reaction) in the final liquid in the well from which the quantity of the analyte is measured.

The quantitative "reading" is usually based on detection of intensity of transmitted light by spectrophotometry, which involves quantitation of transmission of some specific wavelength of light through the liquid (as well as the transparent bottom of the well in the multiple-well plate format). The sensitivity of detection depends on amplification of the signal during the analytic reactions. Since enzyme reactions are very well known amplification processes, the signal is generated by enzymes which are linked to the detection reagents in fixed proportions to allow accurate quantification, and thus the name "enzyme-linked.

The analyte is also called the ligand because it will specifically bind or ligate to a detection reagent, thus ELISA falls under the bigger category of ligand binding assays. The ligand-specific binding reagent is "immobilized," i.e., usually coated and dried onto the transparent bottom and sometimes also side wall of a well (the stationary "solid phase"/"solid substrate" here as opposed to solid microparticle/beads that can be washed away), which is usually constructed as a multiple-well plate known as the "ELISA plate." Conventionally, like other forms of immunoassays, the specificity of antigen-antibody type reaction is used because it is easy to raise an antibody specifically against an antigen in bulk as a reagent. Alternatively, if the analyte itself is an antibody, its target antigen can be used as the binding reagent.

7.15 Applications

The ELISA was the first screening test widely used for HIV because of its high sensitivity. In an ELISA, a person's serum is diluted 400 times and applied to a plate to which HIV antigens are attached. If antibodies to HIV are present in the serum, they may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. A specially prepared "secondary antibody"—an antibody that binds to other antibodies—is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus, the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the "cut-off" point between a positive and a negative result.

A cut-off point may be determined by comparing it with a known standard. If an ELISA test is used for drug screening at workplace, a cut-off concentration, 50 ng/ml, for example, is established, and a sample containing the standard concentration of analyte will be prepared. Unknowns that generate a stronger signal than the known sample are "positive." Those that generate weaker signal are "negative".

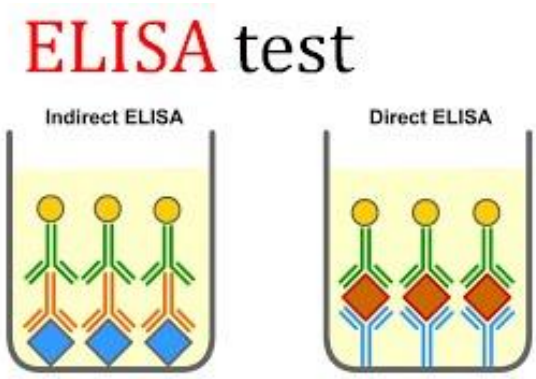

Fig. 4 ELISA Test

There are ELISA tests to detect various kind of diseases, such as dengue, malaria, Chagas disease, Johne's disease, and others. ELISA tests also are extensively employed for *in vitro* diagnostics in medical laboratories. The other uses of ELISA include:

- Detection of SARS-CoV-2 antibodies in blood samples

7.16 Types of ELSA

There are four major types of ELISA:

- Direct ELISA (antigen-coated plate; screening antibody)
- Indirect ELISA (antigen-coated plate; screening antigen/antibody)
- Sandwich ELISA (antibody-coated plate; screening antigen)
- Competitive ELISA (screening antibody)

7.16.1 Direct ELISA

Both direct and indirect ELISAs begin with the coating of antigen to the ELISA plates. The first binding step involves adding antigen to the plates, which is incubated for one hour at 37 degrees C or can be incubated at 4 degrees C overnight. Once the incubation step is completed, the next step is to wash the plates of any potential unbound antibody and block any unbound sites on the ELISA plate using agents like BSA, ovalbumin, aprotinin, or other animal proteins. This second step is important because it prevents the binding of any non-specific antibodies to the plate and minimizes false-positive results. After adding the buffer, the plate is rewashed, and a selected *enzyme-conjugated primary detection antibody* is added. The plate is further incubated for one hour.

In a direct ELISA, the primary detection antibody binds directly to the protein of interest. Next, the plate is rewashed to remove any unbound antibody and followed by the addition of a substrate/chromophore, such as alkaline phosphatase (AP) or Horseradish Peroxidase (HRP) to the plate, which results in a color change. The color change of the sample occurs by either the hydrolysis of phosphate groups from the substrate by AP or by the oxidation of substrates by HRP. The advantages of using direct ELISA include eliminating secondary antibody cross-reactivity, and due to fewer steps, it is rapid compared to indirect ELISA. Its disadvantages include its low sensitivity compared to the other types of ELISA and its high cost of reaction.[

7.16.2 Indirect ELISA

The steps of the indirect ELISA are identical to the direct ELISA, except for an additional wash step and the types of antibody added after the buffer is removed. Indirect ELISA requires two antibodies, a primary detection antibody that sticks to the protein of interest and a secondary enzyme-linked antibody complementary to the primary antibody. The primary antibody is added first, followed by a wash step, and then the enzyme-conjugated secondary antibody is added and incubated. After this, the steps are the same as the direct ELISA, which includes a wash step, the addition of substrate, and detection of a color change.

The indirect ELISA has a higher sensitivity when compared to the direct ELISA. It is also less expensive and more flexible due to the many possible primary antibodies that can be used. The only major disadvantage with this type of ELISA is the risk of cross-reactivity between the secondary detection antibodies.

7.16.3 Sandwich ELISA

Unlike direct and indirect ELISA, the sandwich ELISA begins with a capture antibody coated onto the wells of the plate. It is termed a “sandwich” because the antigens are sandwiched between two layers of antibodies (capture and detection antibodies). After adding the capture antibody to the plates, the plates are then covered and incubated overnight at 4°C. Once the coating step is complete, the plates are washed with PBS, then buffered/blocked with BSA. The buffer washes are carried out for at least 1-2 hours at room temperature. Finally, the plate is washed with PBS once again before the addition of the antigen.

The antigen of interest is then added to the plates to bind to the capture antibody and incubated for 90 min at 37 degrees C. The plate is rewashed, and the primary detection antibody is then added to the plate and incubated for another 1 to 2 hours at room temperature, followed by a buffer wash. Then the secondary enzyme-conjugated antibody is added and incubated for another 1 to 2 hours. The plate is rewashed, and the substrate is added to produce a color change. The sandwich ELISA has the highest sensitivity among all the ELISA types. The major disadvantages of this type of ELISA are the time and expense and the necessary use of "matched pair" (divalent/multivalent antigen) and secondary antibodies.

7.14.4 Competitive ELISA

The competitive ELISA tests for the presence of an antibody specific for antigens in the test serum. This type of ELISA utilizes two specific antibodies, an enzyme-conjugated antibody and another antibody present in the test serum (if the serum is positive). Combining the two antibodies into the wells will allow for a competition for binding to antigen. The presence of a color change means that the test is negative because the enzyme-conjugated antibody bound the antigens (not the antibodies of the test serum). The absence of color indicates a positive test and the presence of antibodies in the test serum. The competitive ELISA has a low specificity and cannot be used in dilute samples. However, the benefits are that there is less sample purification needed, it can measure a large range of antigens in a given sample, can be used for small antigens, and has low variability.

7.15.6 T-cell receptor (TCR)

The **T-cell receptor** (**TCR**) is a protein complex found on the surface of T cells, or T lymphocytes, that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules. The binding between TCR and antigen peptides is of relatively low affinity and is degenerate: that is, many TCRs recognize the same antigen peptide and many antigen peptides are recognized by the same TCR. The TCR is composed of two different protein chains (that is, it is a heterodimer). In humans, in 95% of T cells the TCR consists of an alpha (α) chain and a beta (β) chain (encoded by *TRA* and *TRB*, respectively), whereas in 5% of T cells the TCR consists of gamma and delta (γ/δ) chains (encoded by *TRG* and *TRD*, respectively). This ratio changes during ontogeny and in diseased states (such as leukemia). It also differs between

species. Orthologues of the 4 loci have been mapped in various species. Each locus can produce a variety of polypeptides with constant and variable regions.

When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T lymphocyte is activated through signal transduction, that is, a series of biochemical events mediated by associated enzymes, co-receptors, specialized adaptor molecules, and activated or released transcription factors. Based on the initial receptor triggering mechanism, the TCR belongs to the family of non-catalytic tyrosine-phosphorylated receptors (NTRs).

7.17 History

In 1982, Nobel laureate James P. Allison first discovered the T-cell receptor.[6] Then, Tak Wah Mak and Mark M. Davis[8] identified the cDNA clones encoding the human and mouse TCR respectively in 1984. These findings allowed the entity and structure of the elusive TCR, known before as the "Holy Grail of Immunology", to be revealed. This allowed scientists from around the world to carry out studies on the TCR, leading to important studies in the fields of CAR-T, cancer immunotherapy and checkpoint inhibition.

7.18 Structural characteristics

The TCR is a disulfide-linked membrane-anchored heterodimeric protein normally consisting of the highly variable alpha (α) and beta (β) chains expressed as part of a complex with the invariant CD3 chain molecules. T cells expressing this receptor are referred to as α:β (or αβ) T cells, though a minority of T cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, referred as γδ T cells. Each chain is composed of two extracellular domains: Variable (V) region and a Constant (C) region, both of Immunoglobulin superfamily (IgSF) domain forming antiparallel β-sheets. The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the Variable region binds to the peptide/MHC complex. The variable domain of both the TCR α-chain and β-chain each have three hypervariable or complementarity-determining regions (CDRs). There is also an additional area of hypervariability on the β-chain (HV4) that does not normally contact antigen and, therefore, is not considered a CDR.

The residues in these variable domains are located in two regions of the TCR, at the interface of the α- and β-chains and in the β-chain framework region that is thought to be in proximity to the CD3 signal-transduction complex. CDR3 is the main CDR responsible for

recognizing processed antigen, although CDR1 of the alpha chain has also been shown to interact with the N-terminal part of the antigenic peptide, whereas CDR1 of the β-chain interacts with the C-terminal part of the peptide. CDR2 is thought to recognize the MHC. CDR4 of the β-chain is not thought to participate in antigen recognition, but has been shown to interact with superantigens. The constant domain of the TCR consists of short connecting sequences in which a cysteine residue forms disulfide bonds, which form a link between the two chains.

The TCR is a member of the immunoglobulin superfamily, a large group of proteins involved in binding, recognition, and adhesion; the family is named after antibodies (also called immunoglobulins). The TCR is similar to a half-antibody consisting of a single heavy and single light chain, except the heavy chain is without its crystallisable fraction (Fc). The two subunits of TCR are twisted together. Whereas the antibody uses its Fc region to bind to Fc Receptors on leukocytes, TCR is already docked onto the cell membrane. However, it is not able to mediate signal transduction itself due to its short cytoplasmic tail, so TCR still requires CD3 and zeta to carry out the signal transduction in its place, just as antibodies require binding to FcRs to initiate signal transduction. In this way the MHC-TCR-CD3 interaction for T cells is functionally similar to the antigen (Ag)-immunoglobulin(Ig)-FcR interaction for myeloid leukocytes, and Ag-Ig-CD79 interaction for B cells.

7.19 Generation of the TCR diversity

The generation of TCR diversity is similar to that for antibodies and B-cell antigen receptors. It arises mainly from genetic recombination of the DNA-encoded segments in individual somatic T cells by somatic V(D)J recombination using RAG1 and RAG2 recombinases. Unlike immunoglobulins, however, TCR genes do not undergo somatic hypermutation, and T cells do not express activation-induced cytidine deaminase(AID). The recombination process that creates diversity in BCR (antibodies) and TCR is unique to lymphocytes (T and B cells) during the early stages of their development in primary lymphoid organs (thymus for T cells, bone marrow for B cells). Each recombined TCR possess unique antigen specificity, determined by the structure of the antigen-binding site formed by the α and β chains in case of αβ T cells or γ and δ chains on case of γδ T cells.

- The TCR *alpha chain* is generated by VJ recombination, whereas the *beta chain* is generated by VDJ recombination (both involving a random joining of gene segments to generate the complete TCR chain).
- Likewise, generation of the TCR *gamma chain* involves VJ recombination, whereas generation of the TCR *delta chain* occurs by VDJ recombination.

The intersection of these specific regions (V and J for the alpha or gamma chain; V, D, and J for the beta or delta chain) corresponds to the CDR3 region that is important for peptide/MHC recognition (see above). It is the unique combination of the segments at this region, along with palindromic and random nucleotide additions (respectively termed "P-" and "N-"), which accounts for the even greater diversity of T-cell receptor specificity for processed antigenic peptides. Later during development, individual CDR loops of TCR can be re-edited in the periphery outside thymus by reactivation of recombinases using a process termed TCR revision (editing) and change its antigenic specificity.

7.20 Concept of autoimmunity

Autoimmunity is the system of immune responses of an organism against its own healthy cells, tissues and other body normal constituents. Any disease resulting from this type of immune response is termed an "autoimmune disease". Prominent examples include celiac disease, post-infectious IBS, diabetes mellitus type 1, Henloch Scholein Pupura (HSP) sarcoidosis, systemic lupus erythematosus (SLE), Sjögren syndrome, eosinophilic granulomatosis with polyangiitis, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, Addison's disease, rheumatoid arthritis (RA), ankylosing spondylitis, polymyositis (PM), dermatomyositis (DM) and multiple sclerosis (MS). Autoimmune diseases are very often treated with steroids.

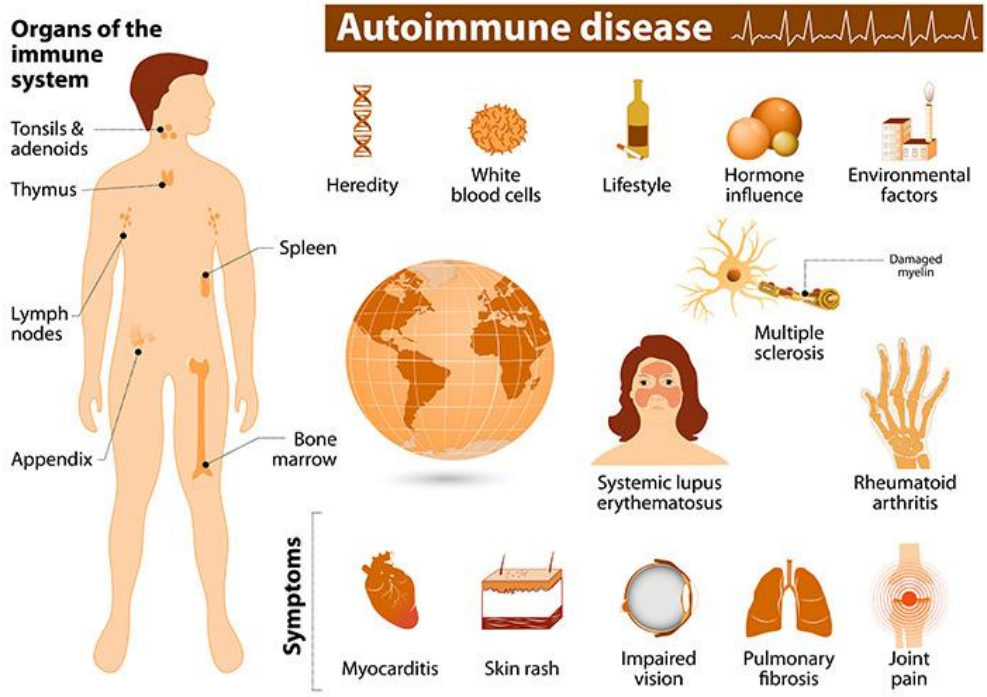

Fig. 5 Autoimmune diseases

Autoimmunity means presence of antibodies or T cells that react with self-protein and is present in all individuals, even in normal health state. It causes autoimmune diseases if self-reactivity can lead to tissue damage. An autoimmune disease is a condition in which your immune system mistakenly attacks your body. The immune system normally guards against germs like bacteria and viruses. When it senses these foreign invaders, it sends out an army of fighter cells to attack them. Normally, the immune system can tell the difference between foreign cells and your own cells. In an autoimmune disease, the immune system mistakes part of your body, like your joints or skin, as foreign. It releases proteins called autoantibodies that attack healthy cells. Some autoimmune diseases target only one organ. Type 1 diabetes damages the pancreas. Other diseases, like systemic lupus erythematosus (SLE), affect the whole body.

Immune system disorders cause abnormally low activity or over activity of the immune system. In cases of immune system overactivity, the body attacks and damages its own tissues (autoimmune diseases). Immune deficiency diseases decrease the body's ability to fight invaders, causing vulnerability to infections. In response to an unknown trigger, the immune system may begin producing antibodies that instead of fighting infections, attack the body's own tissues.

Treatment for autoimmune diseases generally focuses on reducing immune system activity. Examples of autoimmune diseases include:

7.21 Rheumatoid arthritis

The immune system produces antibodies that attach to the linings of joints. Immune system cells then attack the joints, causing inflammation, swelling, and pain. If untreated, rheumatoid arthritis causes gradually causes permanent joint damage. Treatments for rheumatoid arthritis can include various oral or injectable medications that reduce immune system overactivity. See charts that list rheumatoid arthritis drugs and their side effects.

7.22 Systemic lupus erythematosus (lupus)

People with lupus develop autoimmune antibodies that can attach to tissues throughout the body. The joints, lungs, blood cells, nerves, and kidneys are commonly affected in lupus. Treatment often requires daily oral prednisone, a steroid that reduces immune system function. Read an overview on lupus symptoms and treatments.

7.23 Inflammatory bowel disease (IBD)

The immune system attacks the lining of the intestines, causing episodes of diarrhea, rectal bleeding, urgent bowel movements, abdominal pain, fever, and weight loss. Ulcerative colitis and Crohn's disease are the two major forms of IBD. Oral and injected immune-suppressing medicines can treat IBD. Learn about the differences between ulcerative colitis and Crohn's disease.

7.24 Multiple sclerosis (MS)

The immune system attacks nerve cells, causing symptoms that can include pain, blindness, weakness, poor coordination, and muscle spasms. Various medicines that suppress the immune system can be used to treat multiple sclerosis. Read more on multiple sclerosis drugs and their side effects.

7.25 Type 1 diabetes mellitus.

Immune system antibodies attack and destroy insulin-producing cells in the pancreas. At diagnosis, people with type 1 diabetes require insulin injections to survive. Learn about the symptoms to look for in type 1 diabetes.

7.26 Guillain-Barre syndrome

The immune system attacks the nerves controlling muscles in the legs and sometimes the arms and upper body. Weakness results, which can sometimes be severe. Filtering the blood with a procedure called plasmapheresis is the main treatment for Guillain-Barre syndrome.

7.27 Chronic inflammatory demyelinating polyneuropathy

Similar to Guillain-Barre, the immune system also attacks the nerves in CIDP, but symptoms last much longer. About 30% of patients can become confined to a wheelchair if not diagnosed and treated early. Treatment for CIDP and GBS are essentially the same. Find out what the treatment options are for CIDP.

7.28 Psoriasis

In psoriasis, immune system blood cells called T-cells collect in the skin. The immune system activity stimulates skin cells to reproduce rapidly, producing silvery, scaly plaques on the skin. See a photo of what psoriasis looks like.

7.29 Graves' disease

The immune system produces antibodies that stimulate the thyroid gland to release excess amounts of thyroid hormone into the blood (hyperthyroidism). Symptoms of Graves' disease can include bulging eyes as well as weight loss, nervousness, irritability, rapid heart rate, weakness, and brittle hair. Destruction or removal of the thyroid gland, using medicines or surgery, is usually required to treat Graves' disease. Learn more about treatments for Graves' disease.

7.30 Hashimoto's thyroiditis

Antibodies produced by the immune system attack the thyroid gland, slowly destroying the cells that produce thyroid hormone. Low levels of thyroid hormone develop (hypothyroidism), usually over months to years. Symptoms include fatigue, constipation, weight gain, depression, dry skin, and sensitivity to cold. Taking a daily oral synthetic thyroid hormone pill restores normal body functions. Find out more on treatments for an underactive thyroid.

7.31 Myasthenia gravis

Antibodies bind to nerves and make them unable to stimulate muscles properly. Weakness that gets worse with activity is the main symptom of myasthenia gravis. Mestinon (pyridostigmine) is the main medicine used to treat myasthenia gravis. Read an overview on the symptoms of myasthenia gravis.

7.32 Vasculitis

The immune system attacks and damages blood vessels in this group of autoimmune diseases. Vasculitis can affect any organ, so symptoms vary widely and can occur almost anywhere in the body. Treatment includes reducing immune system activity, usually with prednisone or another corticosteroid. Learn more about vasculitis symptoms and treatments.

7.33 Summary

Under this unit we have summarize immunity, ELISA and autoimmune diseases etc. In medicine, the immune system's way of protecting the body against an infectious disease. The three types of immunity are innate, adaptive, and passive. Innate immunity includes barriers, such as skin and mucous membranes, that keep harmful substances from entering the body. It is the first response of the body's immune system to a foreign substance. Adaptive immunity occurs in response to being infected with or vaccinated against a microorganism. The body makes an immune response, which can prevent future infection with the microorganism. Adaptive immunity can last a person's entire life. Passive immunity occurs when a person receives antibodies to a disease rather than making them through his or her own immune system. Passive immunity provides immediate protection but only lasts a few weeks or months.

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. In an ELISA, the antigen (target macromolecule) is immobilized on a solid surface (microplate) and then complexed with an antibody that is linked to a reporter enzyme. Detection is accomplished by measuring the activity of the reporter enzyme via incubation with the appropriate substrate to produce a measurable product. The most crucial element of an ELISA is a highly specific antibody-antigen interaction.

n autoimmune disease is a condition in which your immune system mistakenly attacks your body. The immune system normally guards against germs like bacteria and viruses. When it senses these foreign invaders, it sends out an army of fighter cells to attack them. Normally, the immune system can tell the difference between foreign cells and your own cells. In an autoimmune disease, the immune system mistakes part of your body, like your joints or skin, as foreign. It releases proteins called autoantibodies that attack healthy cells. Some

autoimmune diseases target only one organ. Type 1 diabetes damages the pancreas. Other diseases, like systemic lupus erythematosus (SLE), affect the whole body.

7.34 Terminal questions

Q.22. What do you mean by innate immunity?

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

Q.23. Describe the role of B-lymphocyte and T-lymphocyte.

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

Q.24. Describe the immunodeficiency diseases.

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

Q.25. What are the applications of ELISA?

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

Q.26. What do you mean by autoimmunity? Explain it.

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

Q.27. Write a short note on ELISA.

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

Q.28. Explain rheumatoid arthritis and multiple sclerosis.

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

Further readings

21. Biochemistry- Lehninger A.L.

22. Biochemistry –J.H.Weil.

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

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

25. Biochemistry and molecular biology- Wilson Walker.

Unit-8

Structure

8.1 Introduction

Immune system disorder**,** any of various failures in the body's defense mechanisms against infectious organisms. Disorders of immunity include immune deficiency diseases, such as AIDS, that arise because of a diminution of some aspect of the immune response. Other types of immune disorders, such as allergies and autoimmune disorders, are caused when the body develops an inappropriate response to a substance—either to a normally harmless foreign substance found in the environment, in the case of allergies, or to a component of the body, in the case of autoimmune diseases. Lymphocytes (white blood cells of the immune system) can become cancerous and give rise to tumours called leukemias, lymphomas, and myelomas. This unit discusses various immune

deficiencies, allergies, autoimmune disorders, and lymphocyte cancers. For additional information on leukemias, lymphomas, and myelomas.

Objectives

This is the eighth unit on Immunology. Under eighth unit we have following objectives. These are as under:

- To know about immune disorder and overactive immune system.
- To know about the concept of sexually transmitted diseases and HIV.
- What are the immune tolerance and fetomaternal tolerance
- To discuss AIDS and its prevention
- To know oral tolerance and hypersensitivity

Your immune system is your body's defense against infections and other harmful invaders. Without it, you would constantly get sick from bacteria or viruses. Your immune system is made up of special cells, tissues, and organs that work together to protect you.

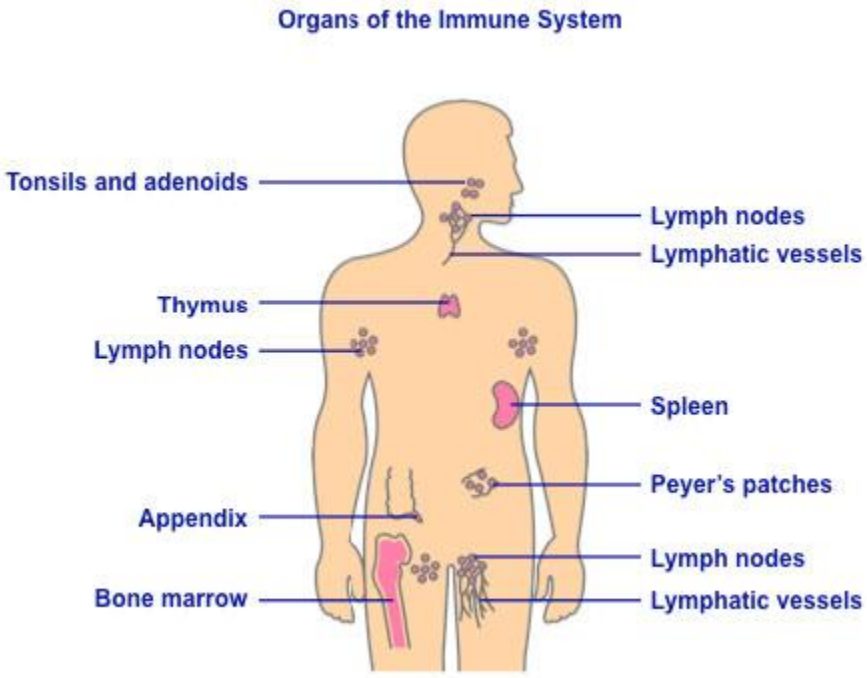

Fig.1

The lymph, or lymphatic, system is a major part of the immune system. It's a network of lymph nodes and vessels. Lymphatic vessels are thin tubes that branch, like blood

vessels, throughout the body. They carry a clear fluid called lymph. Lymph contains tissue fluid, waste products, and immune system cells. Lymph nodes are small, bean-shaped clumps of immune system cells that are connected by lymphatic vessels. They contain white blood cells that trap viruses, bacteria, and other invaders, including cancer cells. White blood cells are the cells of the immune system. They are made in one of your lymph organs, the bone marrow. Other lymph organs include the spleen and thymus.

8.2 What can go wrong with your immune system?

When your immune system doesn't work the way it should, it is called an immune system disorder. You may:

- Be born with a weak immune system. This is called primary immune deficiency.
- Get a disease that weakens your immune system. This is called acquired immune deficiency.
- Have an immune system that is too active. This may happen with an allergic reaction.
- Have an immune system that turns against you. This is called autoimmune disease.

8.3 An immune response

An immune response pertains to any of the body’s response to a foreign substance, such as an antigen. The response intends to protect the body from disease-causing viruses, fungi, bacteria, and parasites. A transplanted organ may also incite an immune response when it is identified as non-self. In vertebrates, including humans, an immune response may be in the form of antibody production, induction of cell-mediated immunity, complement activation, or development of immunological tolerance. Antigens may be exogenous, endogenous, or autoantigens. Exogenous antigens are those that are able to gain entry inside the human body. Examples include allergens, proteins from transplanted tissues and organs, substances on the surface of foreign cells, toxins, and other foreign particles. Endogenous antigens are those that are produced from within the cell and presented on the cell surface, for instance, when the cell harbors bacteria or viruses. Endogenous antigens that are mistaken by the immune system as nonself are referred to as autoantigens.

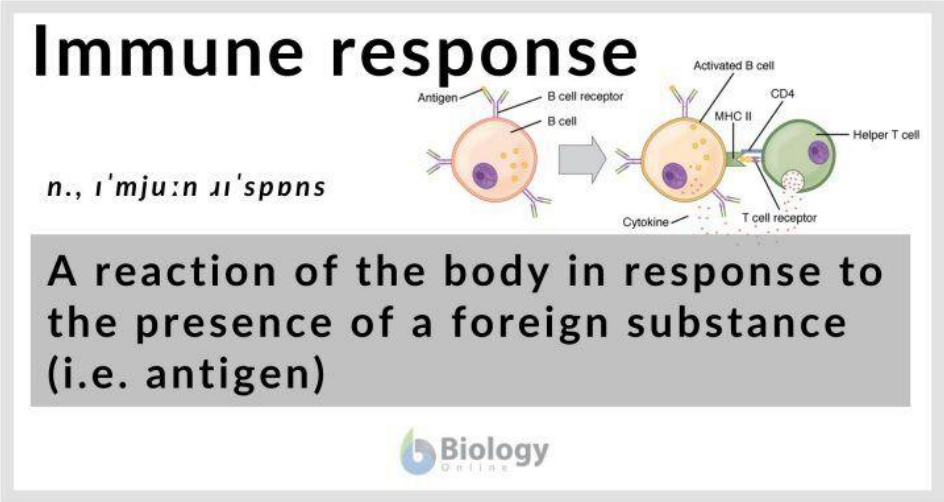

Fig. 2 Immune response

8.4 Immune system disorders

Here are some common examples:

- Severe combined immunodeficiency (SCID). This is an example of an immune deficiency that is present at birth. Children are in constant danger of infections from bacteria, viruses, and fungi. This disorder is sometimes called “bubble boy disease.” In the 1970s, a boy had to live in a sterile environment inside a plastic bubble. Children with SCID are missing important white blood cells.
- Temporary acquired immune deficiencies. Your immune system can be weakened by certain medicines, for example. This can happen to people on chemotherapy or other drugs used to treat cancer. It can also happen to people following organ transplants who take medicine to prevent organ rejection. Also, infections like the flu virus, mono (mononucleosis), and measles can weaken the immune system for a brief time. Your immune system can also be weakened by smoking, alcohol, and poor nutrition.
- AIDS. HIV, which causes AIDS, is an acquired viral infection that destroys important white blood cells and weakens the immune system. People with HIV/AIDS become seriously ill with infections that most people can fight off. These infections are called “opportunistic infections” because they take advantage of weak immune systems.

8.5 An overactive immune system

If you are born with certain genes, your immune system may react to substances in the environment that are normally harmless. These substances are called allergens. Having an allergic reaction is the most common example of an overactive immune system. Dust, mold, pollen, and foods are examples of allergens. Some conditions caused by an overactive immune system are:

- **Asthma.** The response in your lungs can cause coughing, wheezing, and trouble breathing. Asthma can be triggered by common allergens like dust or pollen or by an irritant like tobacco smoke.
- **Eczema.** An allergen causes an itchy rash known as atopic dermatitis.
- **Allergic rhinitis.** Sneezing, a runny nose, sniffling, and swelling of your nasal passages from indoor allergens like dust and pets or outdoor allergens like pollens or molds.

8.6 Autoimmune disease

In autoimmune diseases, the body attacks normal, healthy tissues. The cause is unknown. It is probably a combination of a person's genes and something in the environment that triggers those genes. Three common autoimmune diseases are:

- **Type 1 diabetes.** The immune system attacks the cells in the pancreas that make insulin. Insulin removes sugar from the blood to use as energy.
- **Rheumatoid arthritis.** This type of arthritis causes swelling and deformities of the joints. An auto-antibody called rheumatoid factor is in the blood of some people with rheumatoid arthritis.
- **Lupus.** This disease that attacks body tissues, including the lungs, kidneys, and skin. Many types of auto-antibodies are found in the blood of people with lupus.

No one knows exactly what causes autoimmune diseases, but many factors seem to be involved. If you have an immune system disorder, learn as much as you can about it. And work closely with your healthcare providers to manage it.

8.7 Acquired immunodeficiency

Acquired immunodeficiency syndrome (AIDS) is a chronic, potentially life-threatening condition caused by the human immunodeficiency virus (HIV). By damaging your immune system, HIV interferes with your body's ability to fight infection and disease. HIV is a sexually transmitted infection (STI). It can also be spread by contact with infected blood or from mother to child during pregnancy, childbirth or breast-feeding. Without medication, it may take years before HIV weakens your immune system to the point that you have AIDS. There's no cure for HIV/AIDS, but medications can dramatically slow the progression of the disease. These drugs have reduced AIDS deaths in many developed nations.

8.7.1 Symptoms

The symptoms of HIV and AIDS vary, depending on the phase of infection.

Primary infection (Acute HIV)

Some people infected by HIV develop a flu-like illness within two to four weeks after the virus enters the body. This illness, known as primary (acute) HIV infection, may last for a few weeks. Possible signs and symptoms include:

- Fever
- Headache
- Muscle aches and joint pain
- Rash
- Sore throat and painful mouth sores
- Swollen lymph glands, mainly on the neck
- Diarrhea
- Weight loss
- Cough
- Night sweats

These symptoms can be so mild that you might not even notice them. However, the amount of virus in your bloodstream (viral load) is quite high at this time. As a result, the infection spreads more easily during primary infection than during the next stage.

8.8 Clinical latent infection (Chronic HIV)

In this stage of infection, HIV is still present in the body and in white blood cells. However, many people may not have any symptoms or infections during this time. This stage can last

for many years if you're not receiving antiretroviral therapy (ART). Some people develop more severe disease much sooner.

8.9 Symptomatic HIV infection

As the virus continues to multiply and destroy your immune cells — the cells in your body that help fight off germs — you may develop mild infections or chronic signs and symptoms such as:

- Fever
- Fatigue
- Swollen lymph nodes — often one of the first signs of HIV infection
- Diarrhea
- Weight loss
- Oral yeast infection (thrush)
- Shingles (herpes zoster)
- Pneumonia

8.10 Progression to AIDS

When AIDS occurs, your immune system has been severely damaged. You'll be more likely to develop opportunistic infections or opportunistic cancers — diseases that wouldn't usually cause illness in a person with a healthy immune system. The signs and symptoms of some of these infections may include:

- Sweats
- Chills
- Recurring fever
- Chronic diarrhea
- Swollen lymph glands
- Persistent white spots or unusual lesions on your tongue or in your mouth
- Persistent, unexplained fatigue

- Weakness
- Weight loss
- Skin rashes or bumps

Causes

HIV is caused by a virus. It can spread through sexual contact or blood, or from mother to child during pregnancy, childbirth or breast-feeding.

How does HIV become AIDS?

HIV destroys CD4 T cells — white blood cells that play a large role in helping your body fight disease. The fewer CD4 T cells you have, the weaker your immune system becomes. You can have an HIV infection, with few or no symptoms, for years before it turns into AIDS. AIDS is diagnosed when the CD4 T cell count falls below 200 or you have an AIDS-defining complication, such as a serious infection or cancer.

8.11 How HIV spreads

To become infected with HIV, infected blood, semen or vaginal secretions must enter your body. This can happen in several ways:

- **By having sex.** You may become infected if you have vaginal, anal or oral sex with an infected partner whose blood, semen or vaginal secretions enter your body. The virus can enter your body through mouth sores or small tears that sometimes develop in the rectum or vagina during sexual activity.
- **By sharing needles.** Sharing contaminated IV drug paraphernalia (needles and syringes) puts you at high risk of HIV and other infectious diseases, such as hepatitis.
- **From blood transfusions.** In some cases, the virus may be transmitted through blood transfusions. American hospitals and blood banks now screen the blood supply for HIV antibodies, so this risk is very small.
- **During pregnancy or delivery or through breast-feeding.** Infected mothers can pass the virus on to their babies. Mothers who are HIV-positive and get treatment for the infection during pregnancy can significantly lower the risk to their babies.

8.12 How HIV doesn't spread

You can't become infected with HIV through ordinary contact. That means you can't catch HIV or AIDS by hugging, kissing, dancing or shaking hands with someone who has the infection. HIV isn't spread through the air, water or insect bites.

8.12.1 Risk factors

Anyone of any age, race, sex or sexual orientation can be infected with HIV/AIDS. However, you're at greatest risk of HIV/AIDS if you:

- **Have unprotected sex.** Use a new latex or polyurethane condom every time you have sex. Anal sex is more risky than is vaginal sex. Your risk of HIV increases if you have multiple sexual partners.
- **Have an STI.** Many STIs produce open sores on your genitals. These sores act as doorways for HIV to enter your body.
- **Use IV drugs.** People who use IV drugs often share needles and syringes. This exposes them to droplets of other people's blood.

8.12.2 Complications

HIV infection weakens your immune system, making you much more likely to develop many infections and certain types of cancers.

8.12.3 Infections common to HIV/AIDS

- **Pneumocystis pneumonia (PCP).** This fungal infection can cause severe illness. Although it's declined significantly with current treatments for HIV/AIDS, in the U.S. PCP is still the most common cause of pneumonia in people infected with HIV.
- **Candidiasis (thrush).** Candidiasis is a common HIV-related infection. It causes inflammation and a thick, white coating on your mouth, tongue, esophagus or vagina.
- **Tuberculosis (TB).** In resource-limited nations, TB is the most common opportunistic infection associated with HIV. It's a leading cause of death among people with AIDS.
- **Cytomegalovirus.** This common herpes virus is transmitted in body fluids such as saliva, blood, urine, semen and breast milk. A healthy immune system inactivates the virus, and

it remains dormant in your body. If your immune system weakens, the virus resurfaces — causing damage to your eyes, digestive tract, lungs or other organs.

- **Cryptococcal meningitis.** Meningitis is an inflammation of the membranes and fluid surrounding your brain and spinal cord (meninges). Cryptococcal meningitis is a common central nervous system infection associated with HIV, caused by a fungus found in soil.
- **Toxoplasmosis.** This potentially deadly infection is caused by Toxoplasma gondii, a parasite spread primarily by cats. Infected cats pass the parasites in their stools, which may then spread to other animals and humans. Toxoplasmosis can cause heart disease, and seizures occur when it spreads to the brain.

8.12.4 Cancers common to HIV/AIDS

- **Lymphoma.** This cancer starts in the white blood cells. The most common early sign is painless swelling of the lymph nodes in your neck, armpit or groin.
- **Kaposi's sarcoma.** A tumor of the blood vessel walls, Kaposi's sarcoma usually appears as pink, red or purple lesions on the skin and mouth. In people with darker skin, the lesions may look dark brown or black. Kaposi's sarcoma can also affect the internal organs, including the digestive tract and lungs.

8.12.5 Other complications

- **Wasting syndrome.** Untreated HIV/AIDS can cause significant weight loss, often accompanied by diarrhea, chronic weakness and fever.
- **Neurological complications.** HIV can cause neurological symptoms such as confusion, forgetfulness, depression, anxiety and difficulty walking. HIV-associated neurocognitive disorders (HAND) can range from mild symptoms of behavioral changes and reduced mental functioning to severe dementia causing weakness and inability to function.
- **Kidney disease.** HIV-associated nephropathy (HIVAN) is an inflammation of the tiny filters in your kidneys that remove excess fluid and wastes from your blood and pass them to your urine. It most often affects black or Hispanic people.
- **Liver disease.** Liver disease is also a major complication, especially in people who also have hepatitis B or hepatitis C.

8.12.6 Prevention

There's no vaccine to prevent HIV infection and no cure for AIDS. But you can protect yourself and others from infection. To help prevent the spread of HIV:

- **Use treatment as prevention (TasP).** If you're living with HIV, taking HIV medication can keep your partner from becoming infected with the virus. If you make sure your viral load stays undetectable — a blood test doesn't show any virus — you won't transmit the virus to anyone else. Using TasP means taking your medication exactly as prescribed and getting regular checkups.
- **Use post-exposure prophylaxis (PEP) if you've been exposed to HIV.** If you think you've been exposed through sex, needles or in the workplace, contact your doctor or go to the emergency department. Taking PEP as soon as possible within the first 72 hours can greatly reduce your risk of becoming infected with HIV. You will need to take medication for 28 days.
- **Use a new condom every time you have sex.** Use a new condom every time you have anal or vaginal sex. Women can use a female condom. If using a lubricant, make sure it's water-based. Oil-based lubricants can weaken condoms and cause them to break. During oral sex use a nonlubricated, cut-open condom or a dental dam — a piece of medical-grade latex.
- **Consider preexposure prophylaxis (PrEP).** The combination drugs emtricitabine plus tenofovir (Truvada) and emtricitabine plus tenofovir alafenamide (Descovy) can reduce the risk of sexually transmitted HIV infection in people at very high risk. PrEP can reduce your risk of getting HIV from sex by more than 90% and from injection drug use by more than 70%, according to the Centers for Disease Control and Prevention. Descovy hasn't been studied in people who have receptive vaginal sex.

  Your doctor will prescribe these drugs for HIV prevention only if you don't already have HIV infection. You will need an HIV test before you start taking PrEP and then every three months as long as you're taking it. Your doctor will also test your kidney function before prescribing Truvada and continue to test it every six months. You need to take the drugs every day. They don't prevent other STIs, so you'll still need to practice

safe sex. If you have hepatitis B, you should be evaluated by an infectious disease or liver specialist before beginning therapy.

- **Tell your sexual partners if you have HIV.** It's important to tell all your current and past sexual partners that you're HIV-positive. They'll need to be tested.
- **Use a clean needle.** If you use a needle to inject drugs, make sure it's sterile and don't share it. Take advantage of needle-exchange programs in your community. Consider seeking help for your drug use.
- **If you're pregnant, get medical care right away.** If you're HIV-positive, you may pass the infection to your baby. But if you receive treatment during pregnancy, you can significantly cut your baby's risk.
- **Consider male circumcision.** There's evidence that male circumcision can help reduce the risk of getting HIV infection.

8.13 Immune 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 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:

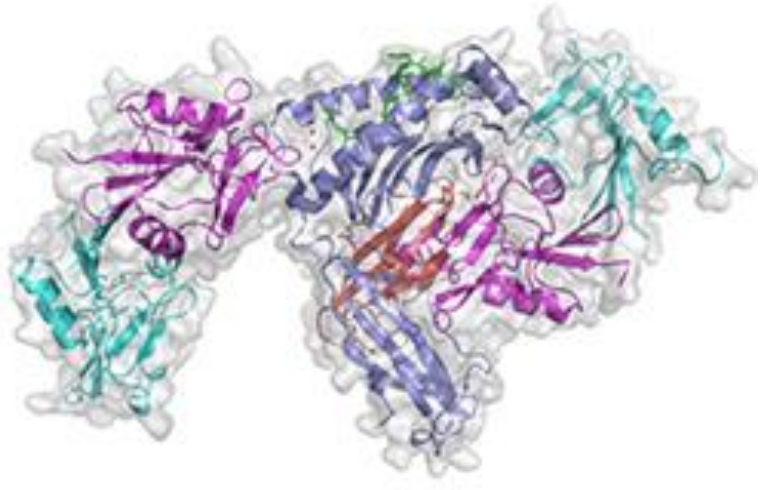

Fig. 3 Inhibitory NK cell receptor (purple and light blue) binds to MHC-I (blue and red), an interaction that prevents immune responses against self.

- 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.

8.14 Fetomaternal tolerance

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.

8.15 Transplantation

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.

8.16 Tolerance in physiology and medicine

8.16.1 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.

8.16.2 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.[25] (for more information, see Immune tolerance in pregnancy).

8.16.3 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. 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.

Hypersensitivity (also called hypersensitivity reaction or intolerance) refers to undesirable reactions produced by the normal immune system, including allergies and autoimmunity. They are usually referred to as an over-reaction of the immune system and these reactions may be damaging and uncomfortable. This is an immunologic term and is not to be confused with the psychiatric term of being hypersensitive which implies to an individual who may be overly sensitive to physical (ie sound, touch, light, etc.) and/or emotional stimuli. Although there is a relation between the two - studies have shown that those individuals that have ADHD (a psychiatric disorder) are more likely to have hypersensitivity reactions such as allergies, asthma, eczema than those who do not have ADHD. Hypersensitivity reactions can be classified into four types.

Type I: IgE mediated immediate reaction

Type II: Antibody-mediated reaction (IgG or IgM antibodies)

Type III: Immune complex-mediated reaction

Type IV: Cytotoxic, cell-mediated, delayed hypersensitivity reaction

The first three types are considered immediate hypersensitivity reactions because they occur within 24 hours. The fourth type is considered a delayed hypersensitivity reaction because it usually occurs more than 12 hours after exposure to the allergen, with a maximal reaction time between 48 and 72 hours.

8.16.4 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.

8.17 Treatment/Management

8.17.1 Immediate hypersensitivity reactions

The treatment of immediate hypersensitivity reactions includes the management of anaphylaxis with intramuscular adrenaline (epinephrine), oxygen, intravenous (IV) antihistamine, support blood pressure with IV fluids, avoid latex gloves and equipment in patients who are allergic, and surgical procedures such as tracheotomy if there is severe laryngeal edema.

1. Allergic bronchial asthma can be treated with any of the following: inhaled short- and long-acting bronchodilators (anticholinergics) along with inhaled corticosteroids, leukotriene antagonists, use of disodium cromoglycate, and environmental control. Experimentally, a low dose of methotrexate or cyclosporin and omalizumab (a monoclonal anti-IgE antibody) has been used.
2. Treatment of autoimmune disorders (e.g., SLE) include one or a combination of NSAIDs and hydroxychloroquine, azathioprine, methotrexate, mycophenolate, cyclophosphamide, low dose IL-2, intravenous immunoglobulins, and belimumab.
3. Omalizumab is a monoclonal antibody that interacts with the binding site of the high-affinity IgE receptor on mast cells. It is an engineered, humanized recombinant immunoglobulin. Moderate to severe allergic bronchial asthma can improve with omalizumab.

8.17.2 Delayed hypersensitivity reactions

Treatment of type 4 HR involves the treatment of the eliciting cause.

1. The most common drugs to treat tuberculosis include isoniazid, rifampin, ethambutol, and pyrazinamide. For drug-resistant TB, a combination of antibiotics such as amikacin, kanamycin, or capreomycin should be used.

2. The most common drugs to treat leprosy include rifampicin and clofazimine in combination with dapsone for multibacillary leprosy. A single dose of antimicrobial combination to cure single lesion paucibacillary leprosy comprises ofloxacin, rifampicin, and minocycline.
3. Praziquantel can be useful for treating infections caused by all *Schistosoma* species.
4. Hydroxychloroquine and chloroquine can use in the therapy of sarcoidosis involving the skin, lungs, and the nervous system.
5. The use of anti-TNF monoclonal antibodies such as adalimumab and certolizumab have been approved for Crohn disease.

8.18 Summary

Under this unit we have summarized immune disorder, autoimmune disease, HIV and AIDS etc. to understand the proper concept of the unit. Your immune system is your body's defense against infections and other harmful invaders. Without it, you would constantly get sick from bacteria or viruses. Your immune system is made up of special cells, tissues, and organs that work together to protect you. White blood cells are the cells of the immune system. They are made in one of your lymph organs, the bone marrow. Other lymph organs include the spleen and thymus. An autoimmune disease is a condition in which your immune system mistakenly attacks your body.

The immune system normally guards against germs like bacteria and viruses. When it senses these foreign invaders, it sends out an army of fighter cells to attack them. Normally, the immune system can tell the difference between foreign cells and your own cells. In an autoimmune disease, the immune system mistakes part of your body, like your joints or skin, as foreign. It releases proteins called autoantibodies that attack healthy cells. Some autoimmune diseases target only one organ. Type 1 diabetes damages the pancreas. Other diseases, like systemic lupus erythematosus (SLE), affect the whole body.

HIV (human immunodeficiency virus) is a virus that attacks the body's immune system. If HIV is not treated, it can lead to AIDS (acquired immunodeficiency syndrome). Learning the basics about HIV can keep you healthy and prevent HIV transmission. You can also download materials to share or watch videos on basic information about HIV. The virus can be transmitted through contact with infected blood, semen or vaginal fluids. Within a few weeks

of HIV infection, flu-like symptoms such as fever, sore throat and fatigue can occur. Then the disease is usually asymptomatic until it progresses to AIDS. AIDS symptoms include weight loss, fever or night sweats, fatigue and recurrent infections.

No cure exists for AIDS, but strict adherence to antiretroviral regimens (ARVs) can dramatically slow the disease's progress as well as prevent secondary infections and complications. HIV is a sexually transmitted infection (STI). It can also be spread by contact with infected blood or from mother to child during pregnancy, childbirth or breast-feeding. Without medication, it may take years before HIV weakens your immune system to the point that you have AIDS.

There's no cure for HIV/AIDS, but medications can dramatically slow the progression of the disease. These drugs have reduced AIDS deaths in many developed nations.

8.19 Terminal questions

Q. 1 What do you mean by immune response? Explain it.

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

Q. 2 Describe immune system disorder.

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

Q. 3 Describe acquired immunodeficiency syndrome (AIDS).

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

Q. 4 What do you mean by overactive immune system?

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

Q. 5 What do you mean by AIDS? How it can be control.

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

Q. 6 Write a short note on HIV.

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

Q. 7 Explain immune tolerance and fetomaternal tolerance.

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-9

Structure

9.1 Introduction

In the late 19th century Hippocratic tradition medicine system, the immune system was imagined into two branches: humoral immunity, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or serum) and **cellular immunity**, for which the protective function of immunization was associated with cells. CD4 cells or helper T cells provide protection against different pathogens. Naive T cells, which are immature T cells that have yet to encounter an antigen, are converted into activated effector T cells after encountering antigen-presenting cells (APCs). These APCs, such as macrophages, dendritic cells, and B cells in some circumstances, load antigenic peptides

onto the major histocompatibility complex (MHC) of the cell, in turn presenting the peptide to receptors on T cells. The most important of these APCs are highly specialized dendritic cells; conceivably operating solely to ingest and present antigens.

Objectives

This is the ninth unit of block II on Immunology. Under this unit we have following objectives. These are as under:

- To know about cell mediated immunity and T cell development.
- To know about the major histocompatibility complex (MHC).
- To discuss glycoproteins in health & disease

9.2 Cell-mediated immunity (CMI)

The cell-mediated or cellular immunity is that where the T-lymphocytes destroy other cells having antigens on their surface without any mediation by antibodies. 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.

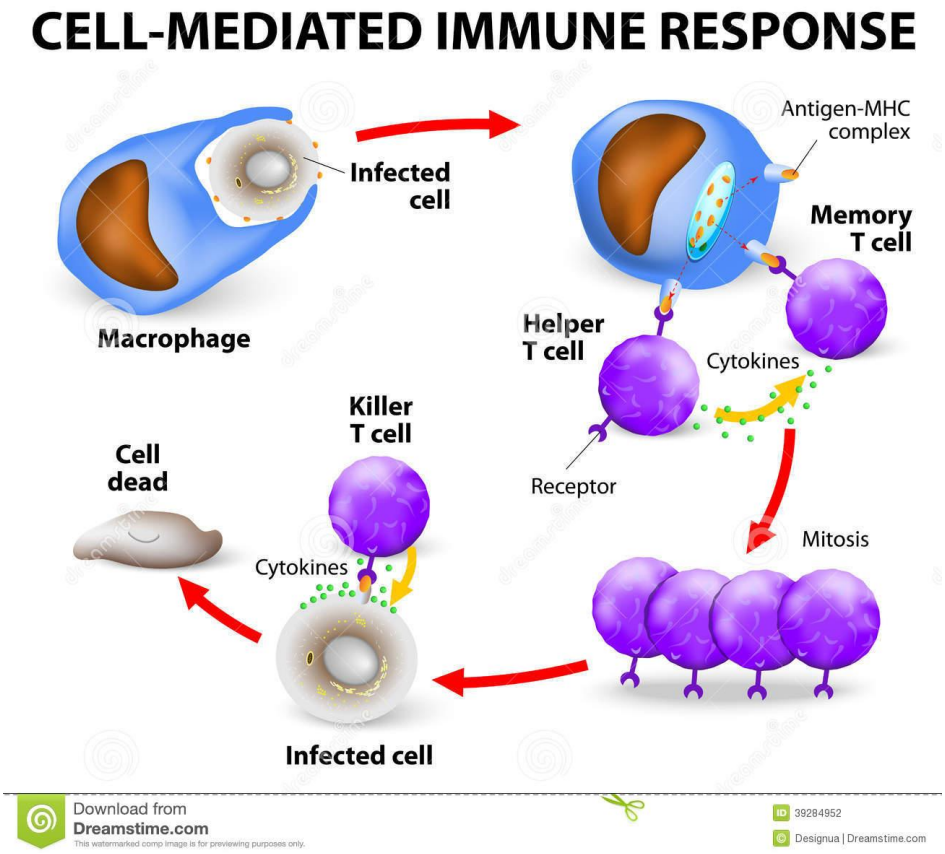

Fig. 1 Cell mediated immune response

The cytotoxic T-lymphocytes, in addition with other T-lymphocytes, release biologically active soluble factors collectively called lymphokines which act as a biochemical mediators of cellular immunity. Unlike B-lymphocytes which are normally stimulated by free antigens in the circulatory system of the body, 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

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.). In such infections the antibodies (hence the antibody-mediated or humoral immunity) prove to be ineffective because the antibodies are

unable to penetrate and attack intracellular pathogens multiplying within the host cells. In addition, the cellular immunity is considered to play an important role in monitoring and regulating the proliferation of abnormal type of cells, (e.g., would be tumor cells), and thus, inhibiting the tumor development.

Activated effector T cells can be placed into three functioning classes, detecting peptide antigens originating from various types of pathogen: The first class being 1) Cytotoxic T cells, which kill infected target cells by apoptosis without using cytokines, 2) T_h1 cells, which primarily function to activate macrophages, and 3) T_h2 cells, which primarily function to stimulate B cells into producing antibodies. In another ideology, the innate immune system and the adaptive immune system each comprise both humoral and cell-mediated components. Cell-mediated immunity (CMI) is an immune response that does not involve antibodies but rather involves the activation of macrophages and NK-cells, the production of antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Cellular immunity protects the body by:

1. Activating antigen-specific cytotoxic T-lymphocytes (CTLs) that are able to destroy body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;
2. Activating macrophages and NK cells, enabling them to destroy intracellular pathogens; and
3. Stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in destroying virus-infected cells, intracellular bacteria, and cancers. It also plays a major role in delayed transplant rejection. Cellular immunity protects the body through:

- T-cell mediated immunity or T-cell immunity: activating antigen-specific cytotoxic T cells that are able to induce apoptosis in body cells displaying epitopes of foreign antigen

on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;

- Macrophage and natural killer cell action: enabling the destruction of pathogens via recognition and secretion of cytotoxic granules (for natural killer cells) and phagocytosis (for macrophages); and
- Stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily at microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in removing virus-infected cells, but also participates in defending against fungi, protozoans, cancers, and intracellular bacteria. It also plays a major role in transplant rejection. Type 1 immunity is directed primarily at viruses, bacteria, and protozoa and is responsible for activating macrophages, turning them into potent effector cells. This is achieved by the secretion of interferon gamma and TNF.

9.3 T cell development

A **T cell** is a type of lymphocyte. T cells are one of the important white blood cells of the immune system and play a central role in the adaptive immune response. T cells can be distinguished from other lymphocytes by the presence of a T-cell receptor (TCR) on their cell surface. T cells are born from hematopoietic stem cells, found in the bone marrow. Developing T cells then migrate to the thymus gland to develop (or mature). T cells derive their name from the thymus. After migration to the thymus, the precursor cells mature into several distinct types of T cells. T cell differentiation also continues after they have left the thymus. Groups of specific, differentiated T cell subtypes have a variety of important functions in controlling and shaping the immune response.

One of these functions is immune-mediated cell death, and it is carried out by two major subtypes: CD8+ "killer" and CD4+ "helper" T cells. (These are named for the presence of the cell surface proteins CD8 or CD4.) CD8+ T cells, also known as "killer T cells", are cytotoxic – this means that they are able to directly kill virus-infected cells, as well as cancer cells. CD8+ T cells are also able to use small signaling proteins, known as cytokines, to recruit other types of cells when mounting an immune response. A different population of T cells, the CD4+ T cells, function as "helper cells". Unlike CD8+ killer T cells, the CD4+

helper T (T_H) cells function by further activating memory B cells and cytotoxic T cells, which leads to a larger immune response. The specific adaptive immune response regulated by the T_H cell depends on its subtype, which is distinguished by the types of cytokines they secrete.

Regulatory T cells are yet another distinct population of T cells that provide the critical mechanism of tolerance, whereby immune cells are able to distinguish invading cells from "self". This prevents immune cells from inappropriately reacting against one's own cells, known as an "autoimmune" response. For this reason, these regulatory T cells have also been called "suppressor" T cells. These same regulatory T cells can also be co-opted by cancer cells to prevent the recognition of, and an immune response against, tumor cells.

9.4 Development

Origin, early development and migration to the thymus

All T cells originate from c-kit$^+$Sca1$^+$ haematopoietic stem cells (HSC) which reside in the bone marrow. In some cases, the origin might be the fetal liver during embryonic development. The HSC then differentiate into multipotent progenitors (MPP) which retain the potential to become both myeloid and lymphoid cells. The process of differentiation then proceeds to a common lymphoid progenitor (CLP), which can only differentiate into T, B or NK cells. These CLP cells then migrate via the blood to the thymus, where they engraft:. Henceforth they are known as thymocytes, the immature stage of a T cell.

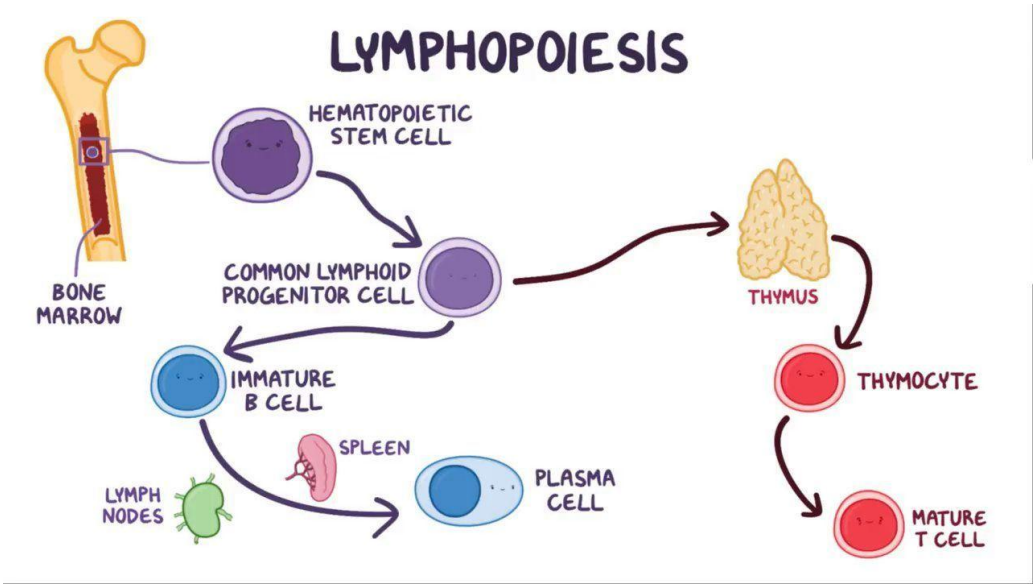

Fig. T-cell development

The earliest cells which arrived in the thymus are termed *double-negative*, as they express neither the CD4 nor CD8 co-receptor. The newly arrived CLP cells are $CD4^{-}CD8^{-}CD44^{+}CD25^{-}ckit^{+}$ cells, and are termed early thymic progenitor (ETP) cells. These cells will then undergo a round of division and downregulate c-kit and are termed *double-negative one* (DN1) cells. To become T cells, the thymocytes must undergo multiple DN stages as well as positive selection and negative selection.

Double negative thymocytes can be identified by the surface expression of CD2, CD5 and CD7. Still during the double negative stages, CD34 expression stops and CD1 is expressed. Expression of both CD4 and CD8 makes them *double positive*, and matures into either CD4+ or CD8+ cells.

9.5 TCR development

A critical step in T cell maturation is making a functional T cell receptor (TCR). Each mature T cell will ultimately contain a unique TCR that reacts to a random pattern, allowing the immune system to recognize many different types of pathogens. This process is essential in developing immunity to threats that the immune system has not encountered before, since due to random variation there will always be at least one TCR to match any new pathogen.

A thymocyte can only become an active T cell when it survives the process of developing a functional TCR. The TCR consists of two major components, the alpha and beta chains. These both contain random elements designed to produce a wide variety of different TCRs, but due to this huge variety they must be tested to make sure they work at all. First, the thymocytes attempt to create a functional beta chain, testing it against a 'mock' alpha chain. Then they attempt to create a functional alpha chain. Once a working TCR has been produced, the cells then must test if their TCR will identify threats correctly, and to do this it is required to recognize the body’s major histocompatibility complex (MHC) in a process known as positive selection. The thymocyte must also ensure that it does not react adversely to "self" antigens, called negative selection. If both positive and negative selection are successful, the TCR becomes fully operational and the thymocyte becomes a T cell.

9.6 TCR β-chain selection

At the DN2 stage ($CD44^{+}CD25^{+}$), cells upregulate the recombination genes RAG1 and RAG2 and re-arrange the TCRβ locus, combining V-D-J recombination and constant region genes in

an attempt to create a functional TCRβ chain. As the developing thymocyte progresses through to the DN3 stage ($CD44^{-}CD25^{+}$), the thymocyte expresses an invariant α-chain called pre-Tα alongside the TCRβ gene. If the rearranged β-chain successfully pairs with the invariant α-chain, signals are produced which cease rearrangement of the β-chain (and silence the alternate allele). Although these signals require the pre-TCR at the cell surface, they are independent of ligand binding to the pre-TCR. If the chains successfully pair a pre-TCR forms, and the cell downregulates CD25 and is termed a DN4 cell ($CD25^{-}CD44^{-}$). These cells then undergo a round of proliferation, and begin to re-arrange the TCRα locus during the *double-positive* stage.

9.7 Positive selection

The process of positive selection takes a number of days. Double-positive thymocytes ($CD4^{+}/CD8^{+}$) migrate deep into the thymic cortex, where they are presented with self-antigens. These self-antigens are expressed by thymic cortical epithelial cells on MHC molecules, which reside on the surface of cortical epithelial cells. Only thymocytes that interact well with MHC-I or MHC-II will receive a vital "survival signal", while those that cannot interact strongly enough will receive no signal and die from neglect. This process ensures that the surviving thymocytes will have an 'MHC affinity' that means they can serve useful functions in the body, responding to MHC molecules to assist immune responses. The vast majority of developing thymocytes will not pass positive selection, and die during this process.

A thymocyte's fate is determined during positive selection. Double-positive cells ($CD4^{+}/CD8^{+}$) that interact well with MHC *class II* molecules will eventually become $CD4^{+}$ "helper" cells, whereas thymocytes that interact well with MHC *class I* molecules mature into $CD8^{+}$ "killer" cells. A thymocyte becomes a $CD4^{+}$ cell by down-regulating expression of its CD8 cell surface receptors. If the cell does not lose its signal, it will continue downregulating CD8 and become a $CD4^{+}$, both $CD8^{+}$ and $CD4^{+}$ cells are now *single positive* cells. This process does not filter for thymocytes that may cause autoimmunity. The potentially autoimmune cells are removed by the following process of negative selection, which occurs in the thymic medulla.

9.8 Negative selection

Negative selection removes thymocytes that are capable of strongly binding with "self" MHC molecules. Thymocytes that survive positive selection migrate towards the boundary of the cortex and medulla in the thymus. While in the medulla, they are again presented with a self-antigen presented on the MHC complex of medullary thymic epithelial cells (mTECs). mTECs must be Autoimmune regulator positive ($AIRE^+$) to properly express self-antigens from all tissues of the body on their MHC *class I* peptides.

Some mTECs are phagocytosed by thymic dendritic cells; this makes them $AIRE^-$ antigen presenting cells (APCs), allowing for presentation of self-antigens on MHC *class II* molecules (positively selected $CD4^+$ cells must interact with these MHC class II molecules, thus APCs, which possess MHC class II, must be present for $CD4^+$ T-cell negative selection). Thymocytes that interact too strongly with the self-antigen receive an apoptotic signal that leads to cell death. However, some of these cells are selected to become Treg cells. The remaining cells exit the thymus as mature naive T cells, also known as recent thymic emigrants. This process is an important component of central tolerance and serves to prevent the formation of self-reactive T cells that are capable of inducing autoimmune diseases in the host.

β-selection is the first checkpoint, where thymocytes that are able to form a functional pre-TCR (with an invariant alpha chain and a functional beta chain) are allowed to continue development in the thymus. Next, positive selection checks that thymocytes have successfully rearranged their TCRα locus and are capable of recognizing MHC molecules with appropriate affinity. Negative selection in the medulla then eliminates thymcytes that bind too strongly to self-antigens expressed on MHC molecules. These selection processes allow for tolerance of self by the immune system. Typical naive T cells that leave the thymus (via the corticomedullary junction) are self-restricted, self-tolerant, and single positive.

9.9 Thymic output

About 98% of thymocytes die during the development processes in the thymus by failing either positive selection or negative selection, whereas the other 2% survive and leave the thymus to become mature immunocompetent T cell[]. The thymus contributes fewer cells as a person ages. As the thymus shrinks by about 3% a year throughout middle age, a

corresponding fall in the thymic production of naive T cells occurs, leaving peripheral T cell expansion and regeneration to play a greater role in protecting older people.

9.9 Types of T cells

T cells are grouped into a series of subsets based on their function. CD4 and CD8 T cells are selected in the thymus, but undergo further differentiation in the periphery to specialized cells which have different functions. T cell subsets were initially defined by function, but also have associated gene or protein expression patterns.

9.10 Major histocompatibility complex (MHC)

The major histocompatibility complex (MHC) is a large locus on vertebrate DNA containing a set of closely linked polymorphic genes that code for cell surface proteins essential for the adaptive immune system. These cell surface proteins are called **MHC molecules**. This locus got its name because it was discovered via the study of transplanted tissue compatibility. Later studies revealed that tissue rejection due to incompatibility is an experimental artifact masking the real function of MHC molecules: binding an antigen derived from self-proteins, or from pathogens, and bringing the antigen presentation to the cell surface for recognition by the appropriate T-cells. MHC molecules mediate the interactions of leukocytes, also called white blood cells (WBCs), with other leukocytes or with body cells. The MHC determines donor compatibility for organ transplant, as well as one's susceptibility to autoimmune diseases.

In a cell, protein molecules of the host's own phenotype or of other biologic entities are continually synthesized and degraded. Each MHC molecule on the cell surface displays a small peptide (a molecular fraction of a protein) called an epitope. The presented self-antigens prevent an organism's immune system from targeting its own cells. The presentation of pathogen-derived proteins results in the elimination of the infected cell by the immune system.

Diversity of an individual's self-antigen presentation, mediated by MHC self-antigens, is attained in at least three ways: (1) an organism's MHC repertoire is polygenic (via multiple, interacting genes); (2) MHC expression is codominant (from both sets of inherited alleles); (3) MHC gene variants are highly polymorphic (diversely varying from organism to organism within a species). Sexual selection has been observed in male mice making mate choices of

females with different MHCs and thus demonstrating sexual selection. Also, at least for MHC I presentation, there has been evidence of antigenic peptide splicing, which can combine peptides from different proteins, vastly increasing antigen diversity.

9.10.1 MHC class I

MHC class I molecules are expressed in all nucleated cells and also in platelets—in essence all cells but red blood cells. It presents epitopes to killer T cells, also called cytotoxic T lymphocytes (CTLs). A CTL expresses CD8 receptors, in addition to T-cell receptors (TCR)s. When a CTL's CD8 receptor docks to a MHC class I molecule, if the CTL's TCR fits the epitope within the MHC class I molecule, the CTL triggers the cell to undergo programmed cell death by apoptosis. Thus, MHC class I helps mediate cellular immunity, a primary means to address intracellular pathogens, such as viruses and some bacteria, including bacterial L forms, bacterial genus *Mycoplasma*, and bacterial genus *Rickettsia*. In humans, MHC class I comprises HLA-A, HLA-B, and HLA-C molecules.

The first crystal structure of Class I MHC molecule, human HLA-A2, was published in 1989. The structure revealed that MHC-I molecules are heterodimers, they have polymorphic heavy α-subunit whose gene occurs inside the MHC locus and small invariant β_2 microglobulin subunit whose gene is located usually outside of it. Polymorphic heavy chain of MHC-I molecule contains N-terminal extra-cellular region composed by three domains, α1, α2, and α3, transmembrane helix to hold MHC-I molecule on the cell surface and short cytoplasmic tail. Two domains, α1 and α2 form deep peptide-binding groove between two long α-helices and the floor of the groove formed by eight β-strands. Immunoglobulin-like domain α3 involved in the interaction with CD8 co-receptor. β_2 microglobulin provides stability of the complex and participates in the recognition of peptide-MHC class I complex by CD8 co-receptor. The peptide is non-covalently bound to MHC-I, it is held by the several pockets on the floor of the peptide-binding groove. Amino acid side-chains that are most polymorphic in human alleles fill up the central and widest portion of the binding groove, while conserved side-chains are clustered at the narrower ends of the groove.

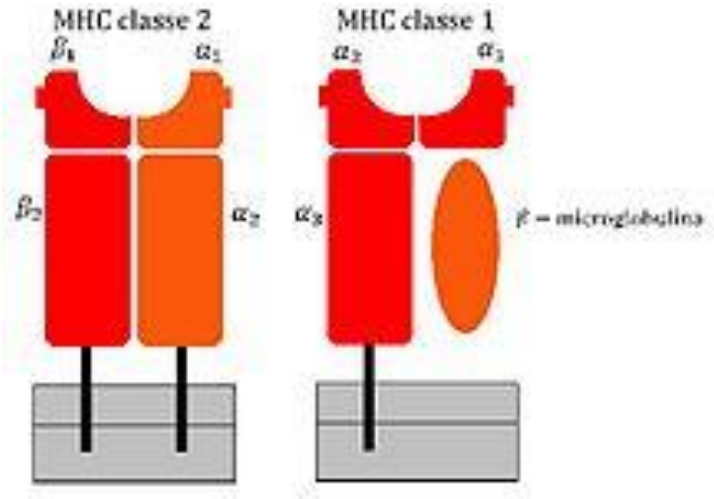

Fig. 3 Schematic view of MHC class I and MHC class II molecules

Classical MHC molecules present epitopes to the TCRs of CD8+ T lymphocytes. **Nonclassical molecules** (MHC class IB) exhibit limited polymorphism, expression patterns, and presented antigens; this group is subdivided into a group encoded within MHC loci (e.g., HLA-E, -F, -G), as well as those not (e.g., stress ligands such as ULBPs, Rae1, and H60); the antigen/ligand for many of these molecules remain unknown, but they can interact with each of CD8+ T cells, NKT cells, and NK cells. The evolutionary oldest nonclassical MHC class I lineage in human was deduced to be the lineage that includes the CD1 and PROCR (alias EPCR) molecules, and this lineage may have been established before the origin of tetrapod species. However, the only nonclassical MHC class I lineage for which evidence exists that it was established before the evolutionary separation of Actinopterygii (ray-finned fish) and Sarcopterygii (lobe-finned fish plus tetrapods) is lineage Z of which members are found, together in each species with classical MHC class I, in lungfish and throughout ray-finned fishes; why the Z lineage was well conserved in ray-finned fish but lost in tetrapods is not understood.

9.10.2 MHC class II

MHC class II can be conditionally expressed by all cell types, but normally occurs only on "professional" antigen-presenting cells (APCs): macrophages, B cells, and especially dendritic cells (DCs). An APC takes up an antigenic protein, performs antigen processing, and returns a molecular fraction of it—a fraction termed the epitope—and displays it on the APC's surface

coupled within an MHC class II molecule (antigen presentation). On the cell's surface, the epitope can be recognized by immunologic structures like T-cell receptors (TCRs). The molecular region which binds to the epitope is the paratope.

On surfaces of helper T cells are CD4 receptors, as well as TCRs. When a naive helper T cell's CD4 molecule docks to an APC's MHC class II molecule, its TCR can meet and bind the epitope coupled within the MHC class II. This event primes the naive T cell. According to the local milieu, that is, the balance of cytokines secreted by APCs in the microenvironment, the naive helper T cell (Th_0) polarizes into either a memory Th cell or an effector Th cell of phenotype either type 1 (Th_1), type 2 (Th_2), type 17 (Th_{17}), or regulatory/suppressor (T_{reg}), as so far identified, the Th cell's terminal differentiation.

MHC class II thus mediates immunization to—or, if APCs polarize Th_0 cells principally to T_{reg} cells, immune tolerance of—an antigen. The polarization during primary exposure to an antigen is key in determining a number of chronic diseases, such as inflammatory bowel diseases and asthma, by skewing the immune response that memory Th cells coordinate when their memory recall is triggered upon secondary exposure to similar antigens. B cells express MHC class II to present antigens to Th_0, but when their B cell receptors bind matching epitopes, interactions which are not mediated by MHC, these activated B cells secrete soluble immunoglobulins: antibody molecules mediating humoral immunity.

Class II MHC molecules are also heterodimers, genes for both α and β subunits are polymorphic and located within MHC class II subregion. Peptide-binding groove of MHC-II molecules is forms by N-terminal domains of both subunits of the heterodimer, α1 and β1, unlike MHC-I molecules, where two domains of the same chain are involved. In addition, both subunits of MHC-II contain transmembrane helix and immunoglobulin domains α2 or β2 that can be recognized by CD4 co-receptors. In this way MHC molecules chaperone which type of lymphocytes may bind to the given antigen with high affinity, since different lymphocytes express different T-Cell Receptor (TCR) co-receptors.

MHC class II molecules in humans have five to six isotypes. **Classical molecules** present peptides to CD4+ lymphocytes. **Nonclassical molecules**, accessories, with intracellular functions, are not exposed on cell membranes, but in internal membranes, assisting with the loading of antigenic peptides onto classic MHC class II molecules. The important nonclassical

MHC class II molecule DM is only found from the evolutionary level of lungfish, although also in more primitive fishes both classical and nonclassical MHC class II are found.

9.10.3 Differences between MHC class I and MHC class II molecules

Sr. No	Feature[	Class I MHC	Class II MHC
1	Constituting polypeptide chains	α chain (45KDa in humans) β_2 chain (12 KDa in humans)	α chain (30-34 KDa in humans) β chain (26-29 KDa in humans)
2	Antigen binding domain	α1and α2 domains	α_1 and β_1 domains
3	Binds protein antigens of	8-10 amino acids residues	13-18 amino acids residues
4	Peptide bending cleft	Floor formed by β sheets and sides by α helices, blocked at both the ends	Floor formed by β sheets and sides by α helices, opened at both the ends
5	Antigenic peptide motifs involved in binding	Anchor residues located at amino and carbon terminal ends	Anchor residues located almost uniformly along the peptide
6	Presents antigenic peptide to	CD8+ T cells	CD4+ T cells

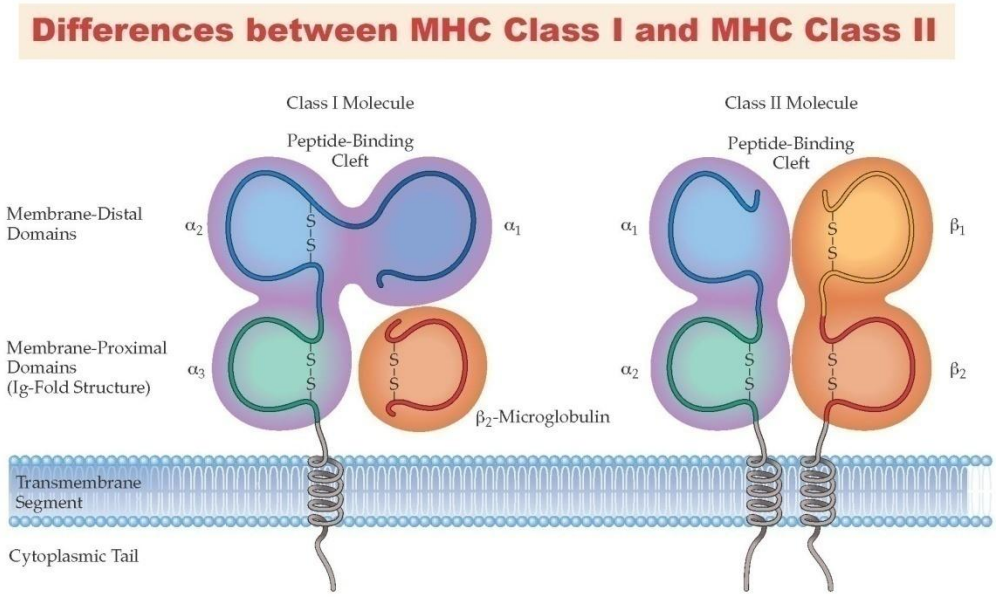

Fig. Differences between MHC Class I vs. MHC Class II Protein

9.10.4 MHC class III

Class III molecules have physiologic roles unlike classes I and II, but are encoded between them in the short arm of human chromosome 6. Class III molecules include several secreted proteins with immune functions: components of the complement system (such as C2, C4, and B factor), cytokines (such as TNF-α, LTA, and LTB), and heat shock proteins.

9.11 Functions

MHC is the tissue-antigen that allows the immune system (more specifically T cells) to bind to, recognize, and tolerate itself (autorecognition). MHC is also the chaperone for intracellular peptides that are complexed with MHCs and presented to T cell receptors (TCRs) as potential foreign antigens. MHC interacts with TCR and its co-receptors to optimize binding conditions for the TCR-antigen interaction, in terms of antigen binding affinity and specificity, and signal transduction effectiveness.

Essentially, the MHC-peptide complex is a complex of auto-antigen/allo-antigen. Upon binding, T cells should in principle tolerate the auto-antigen, but activate when exposed to the allo-antigen. Disease states occur when this principle is disrupted. Antigen presentation: MHC molecules bind to both T cell receptor and CD4/CD8 co-receptors on T lymphocytes, and the antigen epitope held in the peptide-binding groove of the MHC molecule interacts with the variable Ig-Like domain of the TCR to trigger T-cell activation

Autoimmune reaction: Having some MHC molecules increases the risk of autoimmune diseases more than having others. HLA-B27 is an example. It is unclear how exactly having the HLA-B27 tissue type increases the risk of ankylosing spondylitis and other associated inflammatory diseases, but mechanisms involving aberrant antigen presentation or T cell activation have been hypothesized. Tissue allorecognition: MHC molecules in complex with peptide epitopes are essentially ligands for TCRs. T cells become activated by binding to the peptide-binding grooves of any MHC molecule that they were not trained to recognize during positive selection in the thymus.

9.12 What are glycoproteins?

Glycoproteins are proteins containing glycans attached to amino acid side chains. Glycans are oligosaccharide chains; which are saccharide polymers, that can attach to either lipids (glycolipids) or amino acids (glycoproteins). Typically, these bonds are formed through a process called glycosylation. Glycosylation occurs on a majority of proteins post-

translationally with most RER synthesized proteins undergoing glycosylation. There are different forms of glycosylation that attach specific glycans to proteins and lipids. For example, N-glycosylation (attachment of glycans to nitrogen on the amine side chain of asparagine) and O-glycosylation (attachment of glycans to oxygen on serine and/or threonine). Specific sugars (glycans) that can be attached to proteins or lipids in humans include β-D-Glucose *(Glc)* and β-D-Galactose *(Gal).*

9.13 Glycoproteins in health & disease

Glycoproteins are incredibly diverse and serve many functions in the body. Some provide structure e.g. collagens, others are involved in immunity e.g. immunoglobulins (such as IgG). Mucins are secreted into mucus of the respiratory and digestive tracts where the specific mucins can retain water thus allowing mucus to serve as an effective lubricant. Specific glycoproteins (and glycolipids) present on the surface of red blood cells determine blood group type. A-oligosaccharide for A group, B-oligosaccharide for B group, both A & B oligosaccharides for AB group, and the absence of both A & B for O group (H-oligosaccharide precursor only). The presence of Rh factor (an antigen) determines Rh^+ groups, whereas the absence of the Rh antigen leads to Rh^- groups after ABO determination.

Certain hormones are glycoproteins including follicle-stimulating hormone (FSH) – a gonadotropin hormone that has several functions in development, growth, puberty, and reproduction. Others include erythropoietin – a cytokine secreted by the kidneys that stimulate red blood cell production in bone marrow in high levels in response to hypoxia (low levels normally). Many viruses have surface glycoproteins called spike domains; S which enable viruses to bind to their target receptors and enter cells.

Normally these surface glycoproteins can also serve as natural neutralizing targets for antibodies produced by the body in fighting off an infection and conferring some degree of future immunity. Some viruses including HIV, however, have heavily glycosylated S-domains with an abundance of glycans that interfere with antibody binding and recognition thus making viruses such as HIV more evasive and difficult to fully treat.

9.14 Cell-mediated immunity

Cell-mediated immunity is an immune response that does not involve antibodies. Rather, cell-mediated immunity is the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. In the late 19th century Hippocratic tradition medicine system, the immune system was imagined into two branches: humoral immunity, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or serum) and **cellular immunity**, for which the protective function of immunization was associated with cells. CD4 cells or helper T cells provide protection against different pathogens. Naive T cells, which are immature T cells that have yet to encounter an antigen, are converted into activated effector T cells after encountering antigen-presenting cells (APCs). These APCs, such as macrophages, dendritic cells, and B cells in some circumstances, load antigenic peptides onto the major histocompatibility complex (MHC) of the cell, in turn presenting the peptide to receptors on T cells.

The most important of these APCs are highly specialized dendritic cells; conceivably operating solely to ingest and present antigens. Activated effector T cells can be placed into three functioning classes, detecting peptide antigens originating from various types of pathogen: The first class being 1) Cytotoxic T cells, which kill infected target cells by apoptosis without using cytokines, 2) T_h1 cells, which primarily function to activate macrophages, and 3) T_h2 cells, which primarily function to stimulate B cells into producing antibodies. In another ideology, the innate immune system and the adaptive immune system each comprise both humoral and cell-mediated components.

Cellular immunity protects the body through:

- T-cell mediated immunity or T-cell immunity: activating antigen-specific cytotoxic T cells that are able to induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;
- Macrophage and natural killer cell action: enabling the destruction of pathogens via recognition and secretion of cytotoxic granules (for natural killer cells) and phagocytosis (for macrophages); and
- Stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily at microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in removing virus-infected cells, but also participates in defending against fungi, protozoans, cancers, and intracellular bacteria. It also plays a major role in transplant rejection. Type 1 immunity is directed primarily at viruses, bacteria, and protozoa and is responsible for activating macrophages, turning them into potent effector cells. This is achieved by the secretion of interferon gamma and TNF.

9.15 Summary

Under this unit we have summarized cell mediated immunity, major histocompatibility complex and glycoproteins. cell-mediated immunity, unlike humoral immunity, does not rely on antibodies to perform adaptive immunological activities. Mature T cells, macrophages, and the production of cytokines in response to an antigen are the main drivers of cell-mediated immunity. To recognize intracellular target antigens, T cells that participate in cell-mediated immunity rely on antigen-presenting cells that have membrane-bound MHC class I proteins. The maturation and differentiation of naive T cells into helper or killer T cells are dependent on the binding specificity of MHC proteins to external antigens. Cell-mediated immunity is activated when cells in the body are infected by a virus, bacterium, or fungus (intracellular invaders). T lymphocytes can detect malignant cells with the help of MHC class I proteins. Helper T cells, killer T cells, and macrophages are the three main kinds of lymphocytes involved in cell-mediated immunity. Major histocompatibility complex (MHC)**,** group of genes that code for proteins found on the surfaces of cells that help the immune system recognize foreign substances. MHC proteins are found in all higher vertebrates. In human beings the complex is also called the human leukocyte antigen (HLA) system.

Glycoproteins are molecules that comprise protein and carbohydrate chains that are involved in many physiological functions including immunity. Many viruses have glycoproteins that help them enter bodily cells, but can also serve to be important therapeutic or preventative targets. Glycoproteins are incredibly diverse and serve many functions in the body. Some provide structure e.g. collagens, others are involved in immunity e.g. immunoglobulins (such as IgG). Mucins are secreted into mucus of the respiratory and digestive tracts where the specific mucins can retain water thus allowing mucus to serve as an effective lubricant. Specific glycoproteins (and glycolipids) present on the surface of red blood cells determine

blood group type. A-oligosaccharide for A group, B-oligosaccharide for B group, both A & B oligosaccharides for AB group, and the absence of both A & B for O group (H-oligosaccharide precursor only).

9.16 Terminal questions

Q.29. What do you mean by cell mediated immunity? Explain it.

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

Q.30. Describe major histocompatibility complex (MHC) with their types.

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

Q.31. Describe the mechanism of MHC class I.

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

Q. 4 What are the differences between MHC class I and MHC class II molecules?

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

Q.32. What are glycoproteins? Explain it

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

Q.33. Write a short note on cell-mediated immunity.

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

Q. 6 Explain T cell development.

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

Further readings

- Biochemistry- Lehninger A.L.
- Biochemistry –J.H.Weil.
- Biochemistry fourth edition-David Hames and Nigel Hooper.
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Unit-10

Structure

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10.1 Introduction

Immunoglobulins (Ig) or antibodies are glycoproteins that are produced by plasma cells. B cells are instructed by specific immunogens, for, example, bacterial proteins, to differentiate into plasma cells. Plasma cells are protein-making cells that participate in humoral immune responses against bacteria, viruses, fungi, parasites, cellular antigens, chemicals, and synthetic substances. Immunoglobulins constitute about 20% of the protein in plasma.

This is the tenth unit on immunology of block II. Under this unit we have following objectives. These are as under:

- To know about different immunoglobulin and their structure.
- To know about receptors, genetics and clinical significance of immunoglobulins.
- To know about pros and cons of active immunity and passive immunity
- To discuss AIDS, SARS and Hepatitis

The immunogen or antigen reacts with a B-cell receptor (BCR) on the cell surface of B lymphocytes, and a signal is produced that directs the activation of transcription factors to stimulate the synthesis of antibodies, which are highly specific for the immunogen that stimulated the B cell. Furthermore, one clone of a B cell makes an immunoglobulin (specificity). The immune system remembers the antigens that caused a previous reaction (memory) due to the development of memory B cells. These are intermediate, differentiated B cells with the capability to quickly become plasma cells. Circulating antibodies recognize antigen in tissue fluids and serum. The following are 5 types of immunoglobulins in humans:

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

10.2 Functions

10.3 Basic immunoglobulin Structure and Function

Antibodies or immunoglobulins have two light chains and two heavy chains in a light-heavy-heavy-light structure arrangement. The heavy chains differ among classes. They have one Fc

region that mediates biological functions (e.g., the binding capacity to cellular receptors) and a Fab region that contains the antigen-binding sites. The chains are folded into regions called domains. There are 4 or 5 domains in the heavy chain, depending on their class, and two domains in the light chain. The hypervariable regions (HRR) contain the antigen-binding sites. There are three HRR in the V domains of each light and heavy chain. These fold into regions that produce 2 antigen-binding sites at the tip of each monomer. All antibodies exhibit one or more functions (bifunctional) including activation of the complement system, opsonization of microbes to be easily phagocytosed, prevention of attachment of the microbes to mucosal surfaces, and neutralization of toxins and viruses.

10.3.1 Immunoglobulin M

IgM has a molecular weight of 970 Kd and an average serum concentration of 1.5 mg/ml. It is mainly produced in the primary immune response to infectious agents or antigens. It is a pentamer and activates the classical pathway of the complement system. IgM is regarded as a potent agglutinin (e.g., anti-A and anti-B isoagglutinin present in type B and type A blood respectively) and a monomer of IgM is used as a B cell receptor (BCR).

10.3.2 Immunoglobulin G

IgG is a monomer with an approximate molecular weight of 146 Kd and a serum concentration of 9.0 mg/mL. IgG is said to be divalent i-e it has two identical antigen-binding sites that comprise 2 L chains and 2 H chains joined by disulfide bonds. IgG is synthesized mostly in the secondary immune response to pathogens. IgG can activate the classical pathway of the complement system, and it also is highly protective. The four subclasses of IgG include IgG1, IgG2, IgG3, and IgG4. IgG1 is around 65% of the total IgG. IgG2 forms an important host defense against bacteria that are encapsulated. IgG is the only immunoglobulin that crosses the placenta as its Fc portion binds to the receptors present on the surface of the placenta, protecting the neonate from infectious diseases. IgG is thus the most abundant antibody present in newborns.

10.3.3 Immunoglobulin A

IgA appears in 2 different molecular structures: monomeric (serum) and dimeric structure (secretory). The serum IgA has a molecular weight of 160 Kd and a serum concentration of 3 mg/mL. Secretory IgA (sIgA) has a molecular weight of 385 Kd and a mean serum concentration of 0.05 mg/mL. Being the major antibody in secretions IgA is found in saliva,

tears, colostrum, and intestinal, genital tract, and respiratory secretions. It appears in mucosa membranes as a dimer (with J chain when secreted) and protects the epithelial surfaces of the respiratory, digestive, and the genitourinary system. IgA possesses a secretory component that prevents its enzymatic digestion. It activates the alternative pathway of activation of the complement system.

10.3.4 Immunoglobulin E

IgE is a monomer. It has a molecular weight of 188 Kd and a serum concentration of 0.00005 mg/mL. It protects against parasites and also binds to high-affinity receptors on mast cells and basophils causing allergic reactions. IgE is regarded as the most important host defense against different parasitic infections which include Strongyloides stercoralis, Trichinella spiralis, Ascaris lumbricoides, and hookworms Necator americanus and Ancylostoma duodenale.

10.3.5 Immunoglobulin D

IgD is a monomer with a molecular weight of 184 Kd. IgD is present in a meager amount in the serum (0.03 mg/mL) and has an unknown function against pathogens. It is regarded as a BCR. IgD may play an essential role in antigen-triggered lymphocyte differentiation.

10.3.6 Receptors for Immunoglobulins

For immunoglobulins to fulfill various biological functions, they should interact with receptors that are mainly expressed on mononuclear cells, mast cells, neutrophils, natural killer cells, and eosinophils. Again, binding to these receptors is essential for immunoglobulin functions. It promotes several activities including phagocytosis of bacteria (opsonization); mast cell degranulation (as seen in type I hypersensitivity or allergic response); killing of tumors; and activation of antigen-presenting cells including macrophages and dendritic cells, which present antigens to T lymphocytes for the generation of cellular and humoral immune responses.

The following are immunoglobulin receptors:

1. Fc gamma RI (CD64) binds to monomeric IgG is expressed on phagocytes and is involved in the phagocytosis of immune complexes.

2. Fc gamma RII (CD32) attaches to B-cells, monocyte/macrophages (phagocytes), and granulocytes. On B cells regulates cell activation in the presence of a high titer of antibodies.

3. Fc gamma RIII (CD16) has 2 types. Fc gamma RIIIa is expressed on macrophages, NK cells, and some T cells. Fc gamma RIIIb is expressed on granulocytes and has a low affinity for IgG.
4. Fc epsilon RI is a high-affinity receptor for IgE that is shown on mast cells and basophils. It involves an allergic response.
5. Fc epsilon RII is expressed on leukocytes and lymphocytes and has homology with mannose-binding lectin.

10.4 Genetics of Immunoglobulins

The immune system can respond to many antigens by generating a vast diversity in immunoglobulins produced by plasma cells. V and J gene segments encode immunoglobulin light chains. The above genes, in addition to D gene segments, encode the heavy chains. The mechanisms that contribute to this great diversity of immunoglobulin specificities include somatic mutation (immunoglobulin heavy and light chain genes undergo structural modifications after antigen stimulation) and the presence of multiple V-region genes in the germline (antibody diversity also arises when numerous V genes are recombining with J and D segments). Gene conversion, recombinational inaccuracies, nucleotide addition, and assorted heavy and light chains also contribute to the diversity of immunoglobulin molecules.

10.5 Clinical Significance

Immunoglobulins or antibodies play an essential role in the protection against bacteria, viruses, and fungi. When there is a deficiency of these glycoproteins, recurrent infectious diseases occur as seen in the following antibody deficiency disorders.

- X-linked agammaglobulinemia
- Transient hypogammaglobulinemia of infancy
- IgA deficiency
- IgG subclass deficiency
- Immunodeficiency with increased IgM
- Common variable immunodeficiency

The most common immunodeficiency is Selective IgA deficiency, characterized by recurrent infections that affect the respiratory, digestive, and genitourinary systems. Recurrent pneumonia, Giardia lamblia infestation, and urinary sepsis are prevalent. The majority of

patients can, however, be asymptomatic. They are at higher risks for autoimmune diseases, atopy, and anaphylaxis to IgA-containing products.

Another common problem is the transient hypogammaglobulinemia of infancy. During the first 3 to 5 months the child is healthy, but he becomes sick because of a physiological deficit of immunoglobulins. This disease is characterized by recurrent bacterial infections including pneumonia, meningitis, otitis, arthritis, osteomyelitis, among others. This problem diminishes once the child starts producing immunoglobulins.

X-linked agammaglobulinemia is also called Bruton agammaglobulinemia. It occurs due to a defect in Bruton Tyrosine Kinase (BTK) gene that prevents B-cell maturation. This condition is X-linked recessive and seen mostly in males. They present with recurrent bacterial and enteroviral infections after 6 months, once the maternal IgG is low. No B cells are seen in peripheral blood and immunoglobulins of all classes are absent. Patients also have absent or scanty lymph nodes and tonsils. Live vaccines are contraindicated.

In common variable immunodeficiency (CVID), individuals acquire the immunodeficiency in the second or third decade of life or later. Both males and females can develop this problem. CVID may follow a viral infection, such as infectious mononucleosis. Giardia lamblia infestation and recurrent pyogenic infections characterize CVID. It may be due to a defect in B-cell differentiation. The patients have a risk of autoimmune disease, bronchiectasis, lymphoma, and sinopulmonary infections.

10.6 Laboratory Assessment of Immunoglobulins

The quantification of immunoglobulins and the study of their functions are vital for the immunodiagnosis of immunodeficiencies, autoimmunity, hypersensitivity reactions, and inflammatory disorders. The following examinations are routinely performed for the study of the behavior of antibodies:

10.7 Quantitative serum immunoglobulins (classes and subclasses)

- IgG
- IgM
- IgA
- IgE

This assay is used to test for the presence of immunodeficiency disorders such as those in X-linked agammaglobulinemia. There are insufficient amounts of all classes of immunoglobulins, or they are absent. The presence of low IgA may be associated with recurrent diarrhea and lung and sinus infections. Low IgG is associated with pyogenic infections, and a high IgE may be found in parasitic infections.

10.8 IgG antibodies (post-immunization)

- Tetanus toxoid
- Diphtheria toxoid
- Pneumococcal polysaccharide
- Polio

This assay evaluates the quality of the immune response after vaccination. In healthy individuals, there is at least a 1:16 titer of antibody.

10.9 IgG antibodies (post-exposure)

- Measles
- Varicella-Zoster

This test is to evaluate the production of antibodies against antigens after the infectious disease has occurred.

10.10 Detection of isohemagglutinins (IgM)

- Anti-type A blood
- Anti-type B blood

Isohemagglutinins are IgM antibodies produced by the immune system in response to bacterial antigens present in the digestive system. It has been shown that their titers may be below 1:4 in antibody deficiency disorders.

10.11 Other assays

- Test for heterophile antibody to measure the presence of antibodies against Epstein-Barr virus
- Serum protein electrophoresis evaluates the level of antibodies qualitatively. For example, in multiple myeloma, it shows a monoclonal peak in the gamma region of the electrophoresis that is consistent with a monoclonal antibody.

10.12 Clinical use of immunoglobulins

Immunoglobulins or antibodies can be used as a form of immunotherapy. Like drugs, they are prepared from a pool of blood donated at blood collection centers and processed through fractionation to separate the protein fraction from the cellular component. The purified immunoglobulin can be used in the treatment of many immunological problems, including antibody deficiencies, severe combined immunodeficiency disorders (SCID), multiple sclerosis, myasthenia gravis, Kawasaki disease, systemic lupus erythematosus (SLE), organ transplantations, and many others.

10.13 Immunity

Immunity is defined as the body's ability to protect itself from an infectious disease. When you are immune to a disease, your immune system can fight off infection from it. Immunity is either innate or adaptive. Innate immunity, also known as natural or genetic immunity, is immunity that an organism is born with. This type of immunity is encoded in one's genes. Genetic immunity protects an organism throughout their entire life. Innate immunity consists of:

- **External defenses:** Known as the first line of defense, external defenses work to protect an organism from pathogen exposure. External defenses include things like the skin, tears, and stomach acid.
- **Internal defenses:** Known as the second line of defense, internal defenses address a pathogen once it has entered the body. Internal defenses include things like inflammation and fevers.

Adaptive immunity, also known as acquired immunity, is the third line of defense. Adaptive immunity protects an organism from a specific pathogen. Adaptive immunity is further broken down into two subgroups: active immunity and passive immunity. In this article, we will explore active and passive immunity.

10.14 What is active immunity?

Active immunity is defined as immunity to a pathogen that occurs following exposure to said pathogen. When the body is exposed to a novel disease agent, B cells, a type of white blood cell, create antibodies that assist in destroying or neutralizing the disease agent. Antibodies are y-shaped proteins that are capable of binding to sites on toxins or pathogens called antigens. Antibodies are disease-specific, meaning that each antibody protects the body from only one

disease agent. For instance, antibodies produced when the body detects the virus that causes mumps will not provide any defense against cold or flu viruses.

When B cells encounter a pathogen, they create memory cells in addition to antibodies. Memory cells are a type of B cell produced following the primary infection that can recognize the pathogen. Memory cells can survive for decades, waiting within the body until the pathogen invades again. When the body is exposed to the pathogen for a second time, the immune response is more robust, quickly addressing the disease agent. Immunity does not happen immediately upon disease exposure. It can take days or weeks after the first exposure for active immunity to develop. But once it does so, the protection can last an entire lifetime. Active immunity can occur in one of two ways: naturally or via an immunization.

10.14.1 Natural immunity

Natural immunity is created when a person becomes infected by a disease. Take, for instance, someone who becomes infected with chickenpox. After the initial infection, the body builds immunity against the disease. This natural active immunity is why people who catch chicken pox are immune for many decades against the disease.

10.14.2 Vaccine-induced immunity

Also known as artificial active immunity, a person can build a resistance to a disease following an immunization. An immunization is defined as the process by which someone becomes protected against a specific disease via the administration of a vaccine. Vaccines use a weakened or dead form of a disease to stimulate an immune response. Vaccines are typically administered using an injection. However, there are vaccinations administered via the mouth or as a nasal spray. When a person's immune system detects the weakened or dead pathogen, it begins to take steps to destroy it. This includes forming new antibodies and memory cells specific to that pathogen. In the future, if the body is exposed to said pathogen, antibodies will be created to protect the body. Vaccination and immunity are essential for keeping large populations of people safe from infectious diseases. For instance, the flu vaccine prevents millions of people from becoming infected with the flu every year.

10.14.3 What is passive immunity?

Passive immunity is protection from a disease provided by antibodies created outside of the body. Passive immunity:

- Does not require previous exposure to a disease agent
- Takes effect immediately
- Does not last long (up to a few months)

What is the difference between artificial passive immunity and natural passive immunity?

Passive immunity is either maternal or artificial. Maternal passive immunity, or natural passive immunity, is immunity passed along from mother to child. Before the child is born, antibodies are passed through the placenta to protect the child from illness. After birth, an infant continues to receive passive immunity to disease from antibodies found in breast milk. Artificial passive immunity comes from injected antibodies created within a different person or an animal. These antibody-containing preparations are termed antiserum. The rabies vaccine and snake antivenom are two examples of antiserums that yield passive immunity.

10.15 Differences Between Active and Passive Immunity

When strange bacteria and pathogens find their way into your body, they're usually destroyed by your immune system. Before getting to your immune system, foreign material has to go through a few lines of defense that your body has in place. Active and passive immunity are the two most common ways that your immunity is strengthened. Your immunity grows stronger when there are antibodies to illnesses and diseases present. Antibodies' purpose is to damage or kill foreign organisms that enter your body. Active and passive immunity both serve this purpose but are different in how antibodies are created.

10.15.1 Active Immunity

Active immunity is more common in our bodies than passive immunity. Our individual immune systems build up active immunity instinctively as we're exposed to new bacteria and strange pathogens.

Active immunity happens in response to breathing new air, eating new food, and touching new things. People with average immune systems don't get sick every time something new enters their body because active immunity is constantly working to neutralize foreign agents. Examples of active immunity are numberless because your body is exposed to and reacts to new pathogens every day.

10.15.2 Passive Immunity

Any contributions not made by the body are considered passive immunity. These are less common, but they are incredibly important because they let our bodies take a proactive defense against dangerous illnesses and diseases.

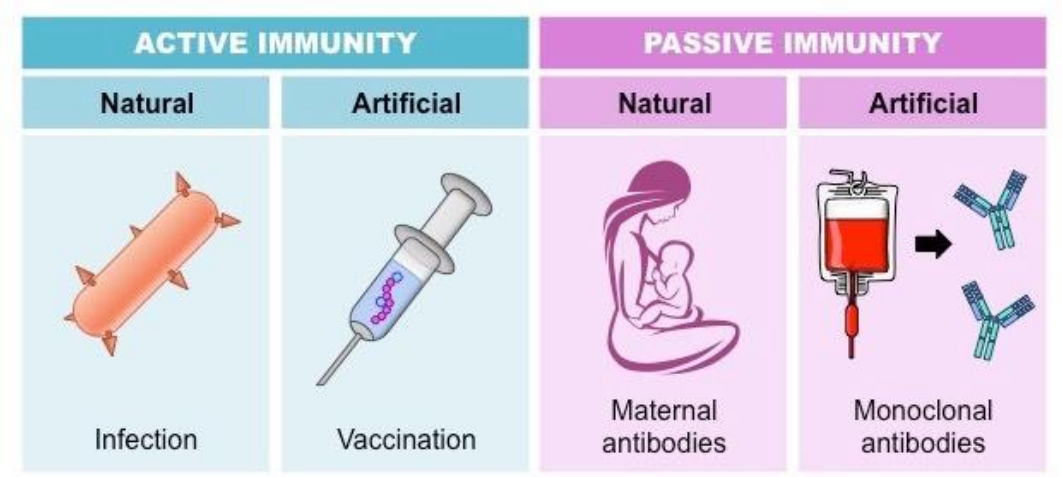

Fig. 1 Types of Immunity

10.15.3 Active vs passive immunity

	Active Immunity	**Passive Immunity**
Antibodies	Produced inside of the body	Introduced from outside of the body
Results from	• Direct infection • Vaccination	• Breast milk • Injection • Mother to baby through the placenta
Takes effect	Over time (typically several weeks)	Immediately
Length of efficacy	Long-term to lifelong	Short-term
Produced by memory cells?	Yes	No

Table 1

10.15.4 Pros and Cons of Active Immunity

A benefit of active immunity is that it lasts longer than passive immunity. Active immunity creates a certain kind of cell that has a long memory, and when they recognize a dangerous pathogen, their memory is triggered. The cells multiply and alert other parts of the immune system that something familiar is back, and they work together to fight something they know exactly how to defeat. Although you typically take in foreign substances that aren't life-threatening, it's possible that you may come across something dangerous. Because active immunity is random, there's more room for illness and disease. Similarly, active immunity doesn't protect you against mutations of diseases that your body already has antibodies to. When diseases mutate, they change structure in ways that your immune system isn't prepared to fight.

10.15.5 Pros and Cons of Passive Immunity

Passive immunity is valuable to your health because you can be immediately prepared to fight specific, dangerous illnesses and diseases. It protects your body from things it might not be able to overcome on its own. Additionally, passive immunity gives your immune system a boost immediately. The greatest downside to passive immunity is that these antibodies don't stay in the body for very long. Because your body isn't continually reacting to specific pathogens, the antibodies that fight them will die off without restocking.

10.15.6 Examples of Passive Immunity

One of the most common instances of passive immunity happens between mothers and their children. Babies benefit from passive immunity via their mothers before they're born and for a period of time afterwards. Their mother's placenta and breastmilk offer something called maternal antibodies to help keep them healthy.

10.15.7 Placenta

Pregnant women give their babies nutrition and defense against illness through placentas and blood circulation. With blood, maternal antibodies and other immunity defenses travel to the unborn child. Although the baby is mostly safe from bacteria and illness before birth, immediately after leaving its mother's body the baby is susceptible to them.

10.15.8 Breastmilk

Breastmilk offers maternal antibodies, too. Specifically, the colostrum produced by mothers immediately after birth helps pass along immunity. Colostrum has extremely high levels of antibodies that help protect the intestines and other important systems. Immunity from the mother's system prepares the child for whatever they come into contact with before they can build up their own immune system.

Vaccines are another common form of passive immunity. When you receive a vaccine, you are given a tiny dose of pathogens that your body is likely to defeat. After killing the foreign substances, your body builds up a temporary defense. For a period of time that varies by vaccine, your immune system is well-equipped to battle the same pathogens. Your body needs to be continually introduced to new pathogens and other substances in order to stay healthy. Active and passive immunity both contribute to a well-equipped, strong immune system.

10.16 Acquired immunodeficiency syndrome (AIDS)

Acquired immunodeficiency syndrome (AIDS) is a chronic, potentially life-threatening condition caused by the human immunodeficiency virus (HIV). By damaging your immune system, HIV interferes with your body's ability to fight infection and disease. HIV is a sexually transmitted infection (STI). It can also be spread by contact with infected blood or from mother to child during pregnancy, childbirth or breast-feeding. Without medication, it may take years before HIV weakens your immune system to the point that you have AIDS. There's no cure for HIV/AIDS, but medications can dramatically slow the progression of the disease. These drugs have reduced AIDS deaths in many developed nations.

10.17 Symptoms

The symptoms of HIV and AIDS vary, depending on the phase of infection.

Primary infection (Acute HIV)

Some people infected by HIV develop a flu-like illness within two to four weeks after the virus enters the body. This illness, known as primary (acute) HIV infection, may last for a few weeks. Possible signs and symptoms include:

- Fever
- Headache
- Muscle aches and joint pain
- Rash

- Sore throat and painful mouth sores
- Swollen lymph glands, mainly on the neck
- Diarrhea
- Weight loss
- Cough
- Night sweats

These symptoms can be so mild that you might not even notice them. However, the amount of virus in your bloodstream (viral load) is quite high at this time. As a result, the infection spreads more easily during primary infection than during the next stage.

10.18 Clinical latent infection (Chronic HIV)

In this stage of infection, HIV is still present in the body and in white blood cells. However, many people may not have any symptoms or infections during this time.

This stage can last for many years if you're not receiving antiretroviral therapy (ART). Some people develop more severe disease much sooner.

10.19 Symptomatic HIV infection

As the virus continues to multiply and destroy your immune cells — the cells in your body that help fight off germs — you may develop mild infections or chronic signs and symptoms such as:

- Fever
- Fatigue
- Swollen lymph nodes — often one of the first signs of HIV infection
- Diarrhea
- Weight loss
- Oral yeast infection (thrush)
- Shingles (herpes zoster)
- Pneumonia

Progression to AIDS

The better antiviral treatments, most people with HIV in the U.S. today don't develop AIDS. Untreated, HIV typically turns into AIDS in about 8 to 10 years. When AIDS occurs, your immune system has been severely damaged. You'll be more likely to develop opportunistic

infections or opportunistic cancers — diseases that wouldn't usually cause illness in a person with a healthy immune system. The signs and symptoms of some of these infections may include:

- Sweats
- Chills
- Recurring fever
- Chronic diarrhea
- Swollen lymph glands
- Persistent white spots or unusual lesions on your tongue or in your mouth
- Persistent, unexplained fatigue
- Weakness
- Weight loss
- Skin rashes or bumps

When to see a doctor

If you think you may have been infected with HIV or are at risk of contracting the virus, see a doctor as soon as possible.

Causes

HIV is caused by a virus. It can spread through sexual contact or blood, or from mother to child during pregnancy, childbirth or breast-feeding.

How does HIV become AIDS?

HIV destroys CD4 T cells — white blood cells that play a large role in helping your body fight disease. The fewer CD4 T cells you have, the weaker your immune system becomes. You can have an HIV infection, with few or no symptoms, for years before it turns into AIDS. AIDS is diagnosed when the CD4 T cell count falls below 200 or you have an AIDS-defining complication, such as a serious infection or cancer.

How HIV spreads

To become infected with HIV, infected blood, semen or vaginal secretions must enter your body. This can happen in several ways:

- **By having sex.** You may become infected if you have vaginal, anal or oral sex with an infected partner whose blood, semen or vaginal secretions enter your body. The virus can

enter your body through mouth sores or small tears that sometimes develop in the rectum or vagina during sexual activity.

- **By sharing needles.** Sharing contaminated IV drug paraphernalia (needles and syringes) puts you at high risk of HIV and other infectious diseases, such as hepatitis.
- **From blood transfusions.** In some cases, the virus may be transmitted through blood transfusions. American hospitals and blood banks now screen the blood supply for HIV antibodies, so this risk is very small.
- **During pregnancy or delivery or through breast-feeding.** Infected mothers can pass the virus on to their babies. Mothers who are HIV-positive and get treatment for the infection during pregnancy can significantly lower the risk to their babies.

How HIV doesn't spread

You can't become infected with HIV through ordinary contact. That means you can't catch HIV or AIDS by hugging, kissing, dancing or shaking hands with someone who has the infection. HIV isn't spread through the air, water or insect bites.

Risk factors

Anyone of any age, race, sex or sexual orientation can be infected with HIV/AIDS. However, you're at greatest risk of HIV/AIDS if you:

- **Have unprotected sex.** Use a new latex or polyurethane condom every time you have sex. Anal sex is more risky than is vaginal sex. Your risk of HIV increases if you have multiple sexual partners.
- **Have an STI.** Many STIs produce open sores on your genitals. These sores act as doorways for HIV to enter your body.
- **Use IV drugs.** People who use IV drugs often share needles and syringes. This exposes them to droplets of other people's blood.

Complications

HIV infection weakens your immune system, making you much more likely to develop many infections and certain types of cancers.

Infections common to HIV/AIDS

- **Pneumocystis pneumonia (PCP).** This fungal infection can cause severe illness. Although it's declined significantly with current treatments for HIV/AIDS, in the U.S. PCP is still the most common cause of pneumonia in people infected with HIV.
- **Candidiasis (thrush).** Candidiasis is a common HIV-related infection. It causes inflammation and a thick, white coating on your mouth, tongue, esophagus or vagina.
- **Tuberculosis (TB).** In resource-limited nations, TB is the most common opportunistic infection associated with HIV. It's a leading cause of death among people with AIDS.
- **Cytomegalovirus.** This common herpes virus is transmitted in body fluids such as saliva, blood, urine, semen and breast milk. A healthy immune system inactivates the virus, and it remains dormant in your body. If your immune system weakens, the virus resurfaces — causing damage to your eyes, digestive tract, lungs or other organs.
- **Cryptococcal meningitis.** Meningitis is an inflammation of the membranes and fluid surrounding your brain and spinal cord (meninges). Cryptococcal meningitis is a common central nervous system infection associated with HIV, caused by a fungus found in soil.
- **Toxoplasmosis.** This potentially deadly infection is caused by Toxoplasma gondii, a parasite spread primarily by cats. Infected cats pass the parasites in their stools, which may then spread to other animals and humans. Toxoplasmosis can cause heart disease, and seizures occur when it spreads to the brain.

Cancers common to HIV/AIDS

- **Lymphoma.** This cancer starts in the white blood cells. The most common early sign is painless swelling of the lymph nodes in your neck, armpit or groin.
- **Kaposi's sarcoma.** A tumor of the blood vessel walls, Kaposi's sarcoma usually appears as pink, red or purple lesions on the skin and mouth. In people with darker skin, the lesions may look dark brown or black. Kaposi's sarcoma can also affect the internal organs, including the digestive tract and lungs.

Other complications

- **Wasting syndrome.** Untreated HIV/AIDS can cause significant weight loss, often accompanied by diarrhea, chronic weakness and fever.
- **Neurological complications.** HIV can cause neurological symptoms such as confusion, forgetfulness, depression, anxiety and difficulty walking. HIV-associated neurocognitive disorders (HAND) can range from mild symptoms of behavioral changes and reduced mental functioning to severe dementia causing weakness and inability to function.
- **Kidney disease.** HIV-associated nephropathy (HIVAN) is an inflammation of the tiny filters in your kidneys that remove excess fluid and wastes from your blood and pass them to your urine. It most often affects black or Hispanic people.
- **Liver disease.** Liver disease is also a major complication, especially in people who also have hepatitis B or hepatitis C.

Prevention

There's no vaccine to prevent HIV infection and no cure for AIDS. But you can protect yourself and others from infection. To help prevent the spread of HIV:

- **Use treatment as prevention (TasP).** If you're living with HIV, taking HIV medication can keep your partner from becoming infected with the virus. If you make sure your viral load stays undetectable — a blood test doesn't show any virus — you won't transmit the virus to anyone else. Using TasP means taking your medication exactly as prescribed and getting regular checkups.
- **Use post-exposure prophylaxis (PEP) if you've been exposed to HIV.** If you think you've been exposed through sex, needles or in the workplace, contact your doctor or go to the emergency department. Taking PEP as soon as possible within the first 72 hours can greatly reduce your risk of becoming infected with HIV. You will need to take medication for 28 days.
- **Use a new condom every time you have sex.** Use a new condom every time you have anal or vaginal sex. Women can use a female condom. If using a lubricant, make sure it's water-based. Oil-based lubricants can weaken condoms and cause them to break. During

oral sex use a nonlubricated, cut-open condom or a dental dam — a piece of medical-grade latex.

- **Consider preexposure prophylaxis (PrEP).** The combination drugs emtricitabine plus tenofovir (Truvada) and emtricitabine plus tenofovir alafenamide (Descovy) can reduce the risk of sexually transmitted HIV infection in people at very high risk. PrEP can reduce your risk of getting HIV from sex by more than 90% and from injection drug use by more than 70%, according to the Centers for Disease Control and Prevention. Descovy hasn't been studied in people who have receptive vaginal sex.

  Your doctor will prescribe these drugs for HIV prevention only if you don't already have HIV infection. You will need an HIV test before you start taking PrEP and then every three months as long as you're taking it. Your doctor will also test your kidney function before prescribing Truvada and continue to test it every six months.

  You need to take the drugs every day. They don't prevent other STIs, so you'll still need to practice safe sex. If you have hepatitis B, you should be evaluated by an infectious disease or liver specialist before beginning therapy.

- **Tell your sexual partners if you have HIV.** It's important to tell all your current and past sexual partners that you're HIV-positive. They'll need to be tested.
- **Use a clean needle.** If you use a needle to inject drugs, make sure it's sterile and don't share it. Take advantage of needle-exchange programs in your community. Consider seeking help for your drug use.
- **If you're pregnant, get medical care right away.** If you're HIV-positive, you may pass the infection to your baby. But if you receive treatment during pregnancy, you can significantly cut your baby's risk.
- **Consider male circumcision.** There's evidence that male circumcision can help reduce the risk of getting HIV infection.

10.20 Severe acute respiratory syndrome (SARS)

Severe acute respiratory syndrome (SARS) is a contagious and sometimes fatal respiratory illness. SARS first appeared in China in November 2002. Within a few months, SARS spread worldwide, carried by unsuspecting travelers. SARS showed how quickly infection can spread

in a highly mobile and interconnected world. On the other hand, a collaborative international effort allowed health experts to quickly contain the spread of the disease. There has been no known transmission of SARS anywhere in the world since 2004.

Symptoms

SARS usually begins with flu-like signs and symptoms — fever, chills, muscle aches, headache and occasionally diarrhea. After about a week, signs and symptoms include:

- Fever of 100.5 F (38 C) or higher
- Dry cough
- Shortness of breath

When to see a doctor

SARS is a serious illness that can lead to death. If you have signs or symptoms of a respiratory infection, or if you have flu-like signs and symptoms with fever after traveling abroad, see your doctor right away.

Causes

SARS is caused by a strain of coronavirus, the same family of viruses that causes the common cold. Previously, these viruses had never been particularly dangerous to humans. Coronaviruses can, however, cause severe disease in animals, and that's why scientists suspected that the SARS virus might have crossed from animals to humans. It now seems likely that that the virus evolved from one or more animal viruses into a new strain.

How SARS spreads

Most respiratory illnesses, including SARS, spread through droplets that enter the air when someone with the disease coughs, sneezes or talks. Most experts think SARS spreads mainly through close personal contact, such as caring for someone with SARS. The virus may also be spread on contaminated objects — such as doorknobs, telephones and elevator buttons.

Risk factors

In general, people at greatest risk of SARS are those who have had direct, close contact with someone who's infected, such as family members and health care workers.

Complications

Many people with SARS develop pneumonia, and breathing problems can become so severe that a mechanical respirator is needed. SARS is fatal in some cases, often due to respiratory

failure. Other possible complications include heart and liver failure. People older than 60 — especially those with underlying conditions such as diabetes or hepatitis — are at the highest risk of serious complications.

Prevention

Researchers are working on several types of vaccines for SARS, but none has been tested in humans. If SARS infections reappear, follow these safety guidelines if you're caring for someone who may have a SARS infection:

- **Wash your hands.** Clean your hands frequently with soap and hot water or use an alcohol-based hand rub containing at least 60% alcohol.
- **Wear disposable gloves.** If you have contact with the person's body fluids or feces, wear disposable gloves. Throw the gloves away immediately after use and wash your hands thoroughly.
- **Wear a surgical mask.** When you're in the same room as a person with SARS, cover your mouth and nose with a surgical mask. Wearing eyeglasses also may offer some protection.
- **Wash personal items.** Use soap and hot water to wash the utensils, towels, bedding and clothing of someone with SARS.
- **Disinfect surfaces.** Use a household disinfectant to clean any surfaces that may have been contaminated with sweat, saliva, mucus, vomit, stool or urine. Wear disposable gloves while you clean and throw the gloves away when you're done.

Follow all precautions for at least 10 days after the person's signs and symptoms have disappeared. Keep children home from school if they develop a fever or respiratory symptoms within 10 days of being exposed to someone with SARS.

10.21 Hepatitis

Hepatitis is an inflammation of the liver. Alcohol consumption, several health conditions, and some medications can all cause this condition. However, viral infections are the most common cause of hepatitis. In this unit, we detail the different types of hepatitis, their common symptoms, causes, and how to treat and prevent the condition.

What is hepatitis?

Hepatitis refers to an inflammatory condition of the liver. It is commonly the result of a viral infection, but there are other possible causes of hepatitis. These include autoimmune hepatitis and hepatitis that occurs as a secondary result of medications, drugs, toxins, and alcohol. Autoimmune hepatitis is a disease that occurs when your body makes antibodies against your liver tissue. The five main viral classifications of hepatitis are hepatitis A, B, C, D, and E. A different virus is responsible for each type of viral hepatitis. The World Health Organization (WHO) estimates that 354 millionTrusted Source people currently live with chronic hepatitis B and C globally.

Hepatitis A

Hepatitis A is the result of an infection with the hepatitis A virus (HAV). This type of hepatitis is an acute, short-term disease.

Hepatitis B

The hepatitis B virus (HBV) causes hepatitis B. This is often an ongoing, chronic condition. The Centers for Disease Control and Prevention (CDC) estimates that around 826,000Trusted Source people are living with chronic hepatitis B in the United States and around 257 million people worldwide.

Hepatitis C

Hepatitis C comes from the hepatitis C virus (HCV). HCV is among the most common bloodborne viral infections in the United States and typically presents as a long-term condition. According to the CDC, approximately 2.4 million AmericansTrusted Source are currently living with a chronic form of this infection.

Hepatitis D

This is a rare form of hepatitis that only occurs in conjunction with hepatitis B infection. The hepatitis D virus (HDV) causes liver inflammation like other strains, but a person cannot contract HDV without an existing hepatitis B infection. Globally, HDV affects almost 5 percentTrusted Source of people with chronic hepatitis B.

Hepatitis E

Hepatitis E is a waterborne disease that results from exposure to the hepatitis E virus (HEV). Hepatitis E is mainly found in areas with poor sanitation and typically results from ingesting fecal matter that contaminates the water supply. This disease is uncommonTrusted Source in

the United States, according to the CDC. Hepatitis E is usually acute but can be particularly dangerous in pregnant women.

Causes of hepatitis

Type of hepatitis	Common route of transmission
hepatitis A	exposure to HAV in food or water
hepatitis B	contact with HBV in body fluids, such as blood, vaginal secretions, or semen
hepatitis C	contact with HCV in body fluids, such as blood, vaginal secretions, or semen
hepatitis D	contact with blood containing HDV
hepatitis E	exposure to HEV in food or water

Table 2

Causes of noninfectious hepatitis

Although hepatitis is most commonly the result of an infection, other factors can cause the condition.

Alcohol and other toxins

Excess alcohol consumption can cause liver damage and inflammation. This may also be referred to as alcoholic hepatitis. The alcohol directly injures the cells of your liver. Over time, it can cause permanent damage and lead to thickening or scarring of liver tissue (cirrhosis) and liver failure. Other toxic causes of hepatitis include misuse of medications and exposure to toxins.

Autoimmune system response

In some cases, the immune system mistakes the liver as harmful and attacks it. This causes ongoing inflammation that can range from mild to severe, often hindering liver function. It’s three times more common in women than in men.

Common symptoms of hepatitis

If you are living with a chronic form of hepatitis, like hepatitis B and C, you may not show symptoms until the damage affects liver function. By contrast, people with acute hepatitis may present with symptoms shortly after contracting a hepatitis virus. Common symptoms of infectious hepatitis include:

- fatigue
- flu-like symptoms
- dark urine
- pale stool
- abdominal pain
- loss of appetite
- unexplained weight loss
- yellow skin and eyes, which may be signs of jaundice

How hepatitis is diagnosed

It is crucial to understand what is causing hepatitis in order to treat it correctly. Doctors will progress through a series of tests to accurately diagnose your condition.

History and physical exam

To diagnose all forms of hepatitis, your doctor will first take your history to determine any risk factors you may have. During a physical examination, your doctor may press down gently on your abdomen to see if there's pain or tenderness. Your doctor may also check for any swelling of the liver and any yellow discoloration in your eyes or skin.

Liver function tests

Liver function tests use blood samples to determine how efficiently your liver works. Abnormal results of these tests may be the first indication that there is a problem, especially if you don't show any signs on a physical exam of liver disease. High liver enzyme levels may indicate that your liver is stressed, damaged, or not functioning correctly.

Other blood tests

If your liver function tests are abnormal, your doctor will likely order other blood tests to detect the source of the problem. These tests can determine Trusted Source if you have infectious hepatitis by checking for the presence of hepatitis viruses or antibodies your body produces to combat them. Doctors may also use blood tests to check for any signs of autoimmune hepatitis.

Liver biopsy

When diagnosing hepatitis, doctors will also assess your liver for potential damageTrusted Source. A liver biopsy is a procedure that involves taking a sample of tissue from your liver. A medical professional may take this sample through your skin with a needle, meaning there is no need for surgery. They will typically use an ultrasound scan for guidance during this procedure. This test allows your doctor to determine how infection or inflammation has affected your liver.

Ultrasound

An abdominal ultrasound uses ultrasound waves to create an image of the organs within your abdomen. This test allows your doctor to take a close look at your liver and nearby organs. It can reveal:

- fluid in your abdomen
- liver damage or enlargement
- liver tumors
- abnormalities of your gallbladder

Sometimes the pancreas shows up on ultrasound images as well. This can be a useful test in determining the cause of your abnormal liver function.

How hepatitis is treated

Treatment options will vary by the type of hepatitis you have and whether the infection is acute or chronic.

10.21.1 Hepatitis A

Hepatitis A is a short-term illness and may not require treatment. However, if symptoms cause a great deal of discomfort, bed rest may be necessary. In addition, if you experience vomiting or diarrhea, your doctor may recommend a dietary program to maintain your hydration and nutrition.

10.21.2 Hepatitis B

There is no specific treatment program for acute hepatitis B. However, if you have chronic hepatitis B, you will require Trusted Source antiviral medications. This form of treatment can be costly, as you may have to continue it for several months or years. Treatment for chronic hepatitis B also requires regular medical evaluations and monitoring to determine if the virus is responding to treatment.

10.21.3 Hepatitis C

Antiviral medications can treat both acute and chronic forms of hepatitis C. Typically, people who develop chronic hepatitis C will use a combination of antiviral drug therapies. They may also need further testing to determine the best form of treatment. People who develop cirrhosis or liver disease due to chronic hepatitis C may be candidates for a liver transplant.

10.21.4 Hepatitis D

The WHO Trusted Source lists pegylated interferon alpha as a treatment for hepatitis D. However, this medication can have severe side effects. As a result, it's not recommended for people with cirrhosis liver damage, those with psychiatric conditions, and people with autoimmune diseases.

10.21.5 Hepatitis E

Currently, no specific medical therapies are available Trusted Source to treat hepatitis E. Because the infection is often acute, it typically resolves on its own. Doctors will typically advise people with this infection to get adequate rest, drink plenty of fluids, get enough nutrients, and avoid alcohol. However, pregnant women who develop this infection require close monitoring and care.

10.21.6 Autoimmune hepatitis

Corticosteroids, like prednisone or budesonide, are extremely important in the early treatment of autoimmune hepatitis. They're effective in about 80 percent of people with this condition. Azathioprine (Imuran), a drug that suppresses the immune system, may also be a part of treatment programs. People may use this with or without steroids. Other immune-suppressing drugs like mycophenolate (CellCept), tacrolimus (Prograf), and cyclosporine (Neoral) can also replace azathioprine in treatment.

Tips to prevent hepatitis

There are vaccines that can help protect against many hepatitis viruses. Minimizing your risk of exposure to substances containing these viruses can also be an important preventive measure.

Vaccines

A vaccine for hepatitis A is available and can help prevent the contraction of HAV. The hepatitis A vaccine is a series of two doses and most children begin vaccination at age 12 to

23 monthsTrusted Source. This is also available for adults and can also include the hepatitis B vaccine. The CDC Trusted Source recommends hepatitis B vaccinations for all newborns. Doctors typically administer the series of three vaccines over the first 6 months of childhood.

The CDC also recommends the vaccine for all healthcare and medical personnel. Vaccination against hepatitis B can also prevent hepatitis D. There are currently no vaccines for hepatitis C or E.

Reducing exposure

Hepatitis viruses can transmit from person to person through contact with bodily fluids, water, and foods containing infectious agents. Minimizing your risk of contact with these substances can help to prevent contracting hepatitis viruses. Practicing effective hygiene is one way to avoid contracting hepatitis A and E. The viruses that cause these conditions can be present Trusted Source in water. If you're traveling to a country where there is a high prevalence of hepatitis, you should avoid:

- local water
- ice
- raw or undercooked shellfish and oysters
- raw fruit and vegetables

The hepatitis B, C, and D viruses can transmit through contact with bodily fluids containing these infectious agents. You can reduce your risk Trusted Source of coming into contact with fluids containing these viruses by:

- not sharing needles
- not sharing razors
- not using someone else's toothbrush
- not touching spilled blood

Hepatitis B and C can carry through sexual intercourse and sexual contact. Using barrier methods, such as condoms and dental dams, during sexual activity can help decrease the risk of infection.

Complications of hepatitis

Chronic hepatitis B or C can lead to more severe health problems. Because the virus affects the liver, people with chronic hepatitis B or C are at risk of:

- chronic liver disease
- cirrhosis
- liver cancer

When your liver stops functioning normally, liver failure can occur. Complications of liver failure include:

- bleeding disorders
- a buildup of fluid in your abdomen, known as ascites
- increased blood pressure in portal veins that enter your liver, known as portal hypertension
- kidney failure
- hepatic encephalopathy, which can involve fatigue, memory loss, and diminished mental abilities
- hepatocellular carcinoma, which is a form of liver cancer
- death

People with chronic hepatitis B and C should avoid alcohol as it can accelerate liver disease and failure. Certain supplements and medications can also affect liver function. If you have chronic hepatitis B or C, check with your doctor before taking any new medications.

10.22Terminal questions

Q. 1 What do you mean by basic immunoglobulin structure and function?

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

Q. 2 Describe different types of immunoglobulins.

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

Q. 3 Describe the clinical uses of immunoglobulins.

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

Q. 4 What are the pros and cons of active and passive immunity?

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

Q. 5 Describe hepatitis and its types.

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

Q. 6 Write a short note AIDS.

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

Q.34. Explain symptomatic HIV infection.

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

Q. 7 Write a short note SARS.

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

Further readings

26. Biochemistry- Lehninger A.L.

27. Biochemistry –J.H.Weil.

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

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

30. Biochemistry and molecular biology- Wilson Walker.