ALAGAPPA UNIVERSITY

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DIRECTORATE OF DISTANCE EDUCATION

M.Sc.(Zoology)

III SEMESTER

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IMMUNOLOGY

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IMMUNOLOGY

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Structure

BLOCK 1 INTRODUCTION TO IMMUNOLOGY

UNIT I HISTORICAL PERSPECTIVES AND SCOPE OF IMMUNOLOGY

Structure

1.1 Introduction

There was no rational approach to the origin of disease developed until the late eighteenth century in the universe. If one survived a disease, the person thereafter became "immune" to any subsequent exposures. However, this was never recognized as evidence of some type of internal defense system until the later part of the seventeenth century.

Most historical accounts credit Edward Jenner for the development of the first immunization process. Immunity to smallpox was conferred by inserting the dried exudates of smallpox pustules into the nose. This technique for the transfer of smallpox, as a form of limited infection, travelled to the west from China along the traditional trade routes to Constantinople where it spread throughout Europe. Hearing of this practice, the Royal family of England had their children inoculated against the disease in 1721.

In 1798, Edward Jenner, noticed that milkmaids were protected from smallpox if they had been first infected with cowpox. It was not his intention to make medical history, as his interests were mostly scholarly and involved the transfer of infections from one species to another, especially from animals to humans. However, Jenner's work led him to the conclusion, that inoculation with cowpox (a bovine analogue of smallpox) could confer immunity to smallpox. Thus, the concept of vaccination was initiated. Jenner's ideas was not accepted medically and socially as they were in opposition to both the church and popular beliefs., However, the use of vaccinations gradually became widely accepted and most European countries had some form of compulsory program within fifty years of Jenner's discovery.

The idea that a pathogenic organism caused disease was not fully realized until certain technological advances had occurred. Initially, Antoni van Leeuwenhoek 's development of the microscope and the subsequent realization that entities existed that were not visible to the human eye, allowed the concept of germs to be appreciated. That these organisms were the causative agent of disease was not recognized until Louis Pasteur developed his germ theory of disease. His original interests were in fermentation in wine and beer, and he was the first to isolate the organisms that caused the fermentation process. Pasteur's work eventually led him to the development of pasteurization (heating) as a means of halting fermentation.

Finally, in 1878, Pasteur accidentally used an attenuated (weakened) chicken cholera culture and realized, when he repeated the experiment using a fresh culture, that the weakened form protected the chickens from the virulent form of the disease. Pasteur went on to develop an attenuated vaccine against rabies and swine

Pasteur was not the only proponent of the germ theory of disease. His chief competitor was Robert Koch . Koch was the first to isolate the anthrax microbe and, unaware of Pasteur's work, he was able to show that it caused the disease. Then in 1882, Koch was able to demonstrate that the germ theory of disease applied to human ailments as well as animals, when he isolated the microbe that caused tuberculosis. His "Koch's postulates" are still used to identify infective organisms. Much of the basis for modern

medicine, as well as the field of immunology, can be traced back to these two scientists,

Later, Emil von Behring and ShibasaburoKitasato were able to demonstrate passive immunity when they took serum from animals infected with diphtheria and injected into healthy animals. These same animals were found to be resistant to the disease. Eventually these serum factors were recognized in 1930 as antibodies. However, thirty years before antibodies were finally isolated and identified, Paul Ehrlich and others, recognized that a specific antigen elicited the production of a specific antibody. Ehrlich hypothesized that these antibodies were specialized molecular structures with specific receptor sites that fit each pathogen like a lock and key.

The idea that specific cells could be directly involved with defending the body was first suggested in 1884 by Élie Metchnikoff. His field was zoology and he studied phagocytosis in single cell organisms. Metchnikoff postulated that vertebrates could operate in a similar manner to remove pathogens. However, it was not until the 1940s that his theories were accepted and the cell mediated, as opposed to the humoral, immune response was recognized.

The clarification of the immune response and the science of immunology did not progress in a systematic or chronological order. This could not have been accomplished without the concomitant development of molecular biology. Louis Pasteur is traditionally considered as the progenitor of modern immunology because of his studies in the late nineteenth century that popularized the germ theory of disease, and that introduced the hope that all infectious diseases could be prevented by prophylactic vaccination, as well as also treated by therapeutic vaccination, if applied soon enough after infection.

1.2 Objectives

- ✓ An understanding of humoral and cellular immunity and their relative significances to transfusion science theory and practice.
- ✓ An understanding of the characteristics of antigens and antibodies.
- ✓ An understanding of the nature of antigen-antibody reactions.
- ✓ An appreciation of the importance of immunology as a foundation of transfusion medicine theory and practice.

1.3 TYPES OF IMMUNE CELLS

The bone marrow is the source of many immune and blood cells in the healthy adult animal. In the adult animal, all immune cells originate from hematopoietic Stem cells located in the bone marrow. Stem cells in the bone marrow are the progenitors of all immune cells. Cytokines are small signalling proteins that guide the development of immune cells. The types

of immune cells are polymorphonuclear cells, mast cells, monocytes, macrophages, and lymphocytes.

If the bone is split lengthwise, a marked difference in tissue is noticed. Part of the tissue is red, which is the source of red and white blood cells. The other portion is yellow adipose tissue that is inactive. During an infection, the yellow marrow can be reactivated to become red marrow to help in the production of larger numbers of immune cells. In the adult animal, all immune cells originate from hematopoietic stem cells located in the bone marrow. Stems cells constantly divide and differentiate into various types of immune cells under the influence of cytokines. They help to regulate the behaviour of the cells of the body. The bone marrow is ultimately responsible for the synthesis of eight types of cells such as red blood cells, platelets, neutrophils, basophils, eosinophils, mast cells, monocytes/macrophages, T lymphocytes and B lymphocytes. Some of these cell types mature in the bone marrow itself, while others migrate through the circulatory system and undergo final maturation in other tissues.

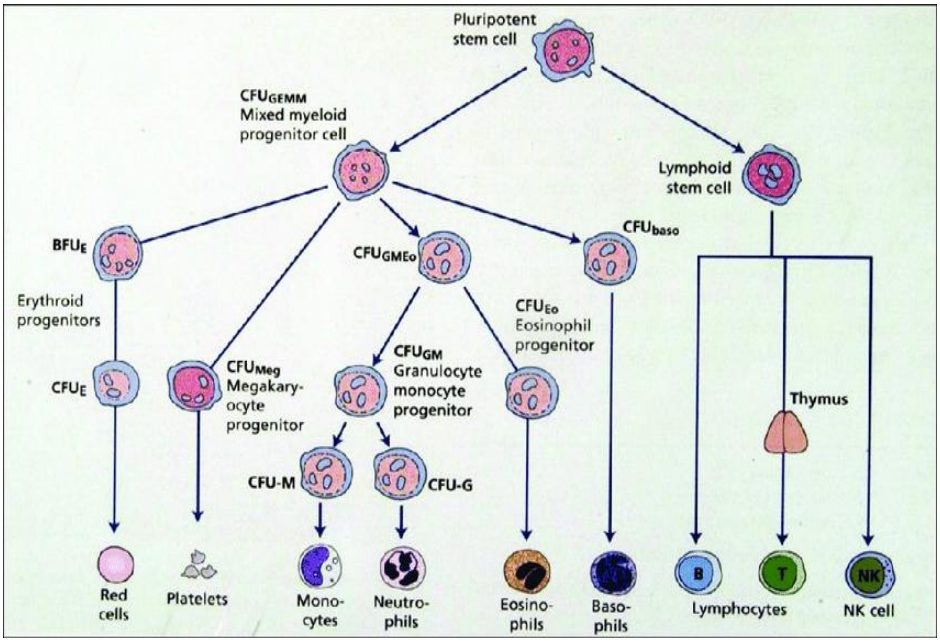

Fig.1.1.

1.4 Immune Cells made in the bone marrow

The major cell type made by the bone marrow is red blood cells. Platelets also form in the bone marrow and assist in formation of blood clots following any kind of injury. Neither of these cell types plays a role in the immune response, but we mention them here because they also originate from bone marrow stem cells and are essential components of the blood.

1.4.1 Neutrophils

Polymorphonuclear granulocytes is the general term given to neutrophils, . The first half of the name describes the appearance of the nucleus that seems to be split into a number of different lobes contains many in foldings, which give it a polynuclear appearance. The cytoplasm is full of granules that contain compounds and enzymes important in fulfilling the function of each cell type. Polymorphonuclear granulocytes make up 50-70% of the white blood cells found in blood. They last only about three days and have to be replaced at a rate of 80 million cells per minute. Neutrophils are the most common type of polymorphonuclear cells, making up 90% of granulocytes in the blood. These cells function as phagocytes in attacking and destroying infectious agents.

1.4.2 Eosinophils

They make up 2-5% of granulocytes in the blood, but this number can rise considerably in people with parasitic diseases as well as asthma, eczema or other diseases associated with allergies. The granules in these cells bind the red dye eosin, giving the cells their name. Eosinophil granules contain a number of different enzymes including, acid phosphatase, glucuronidase, cathepsins, RNAse, and arylsulfatase and peroxidase. They also produce toxic basic proteins. They respond to the chemical signals put out by other immune cells and can then participate in an immune response. The major reactions take three forms.

They can down-regulate an immune response by destroying histamine secreted by mast cells using the enzyme histinase. Eosinophils also liberate arylsulphatase that breaks down the slow reactive substance of anaphylaxis (a dangerous form of allergic response) that is released by mast cells. Eosinophils combat antigenic challenges too big to be attacked by phagocytes. Examples of such challenges are parasitic worms or helminths. In battling these infections, the body first covers the worm with antibody. This then activates eosinophils, which bind to the parasite and release the contents of their granules, thus causing external digestion of the worm.

1.4.3 Basophils

They are small cells that make up less than 1% of all white blood cells. The granules of these cells contain heparin, histamine, decarboxylase, histidine, dehydrogenase and diaphorase. Heparin is an important anti-clotting compound, and histamine finds its use modulating the immune response. Histidine is converted to histamine by decarboxylase. The role of basophils in the immune response is not yet clear, but they seem to play a role in the defense against parasitic worms and in severe allergic reactions. They have a very high affinity for IgE antibodies and they are usually found coated

with IgE in tissues. Basophils may be cellular alarms that notify the rest of the immune system and help to concentrate the point of attack.

1.4.4 Mast cells

They are closely related to basophils but are distinct in their reactions to antigens. They are found throughout the body in lymph nodes, spleen, bone marrow, around blood vessels, nerves, glands and in the skin. Mast cells have granules that, like basophils, contain heparin and histamine. They have a high affinity for IgE as well and their activation by antigen triggers histamine release. Until recently, they were mostly thought to trigger unwanted allergic reactions, but it is now becoming clear they participate in immune responses to gram-negative bacteria. Their wide distribution indicates that they are important in many immune responses.

It is also known as a mastocyte or a labrocyte. Specifically, it is a type of granulocyte derived from the myeloid stem cell that is a part of the immune and neuroimmune systems. Mast cells also are important for defense against parasites. Mast cells are found in tissues and can mediate allergic reactions by releasing inflammatory chemicals like histamine. Mast cells originate from the bone marrow where they develop from the hematopoietic stem cells (HSC) via multipotent progenitors (MPP), common myeloid progenitors (CMPs) and granulocyte/monocyte progenitors (GMPs).Mast cells are now considered to be part of the immune system.

1.4.5 Monocytes and macrophages

They are long-lived specialized phagocytic cells. Monocytes are migrating phagocytic cells found in the bloodstream and when they enter other tissues, they differentiate into macrophages. Macrophages are found in the brain, lungs, liver, spleen, lymph nodes, joints and peritoneum. The key functions of monocytes and macrophages are to remove our own dead cells when they reach the end of their useful life and also to remove pathogens. For example macrophages in the liver, called Kupffer cells, Phagocytize old erythrocytes from the blood and remove them. Another one of their functions is the creation of important immune proteins and peptides. They are responsible for synthesizing transferrin (an iron-binding protein), complement proteins and various cytokines necessary for immune function.

1.4.6 B lymphocytes or B cells

They are antibody-producing cells. They are very important in fighting many different types of infections, especially, bacterial infections.

1.4.7 T lymphocytes or T cells

They are involved in regulating the immune system and destroying host cells that are out of control, either due to a breakdown in cell division regulation (cancer) or infection by a virus or even an intracellular parasite. The thymus is a fist-sized organ located above the heart that is involved in the maturation of T lymphocytes. T cells produced by the bone marrow are immature and journey to the thymus through the bloodstream. The blood vessels that supply the thymus with oxygen and other nutrients also contain a blood-thymus barrier that only allows immature T cells in and mature T cells out. The thymus is also connected to the lymphatic system through lymph vessels.

Check your progress

Note: write your answer in the space given below

1. What are the types of immune cells?

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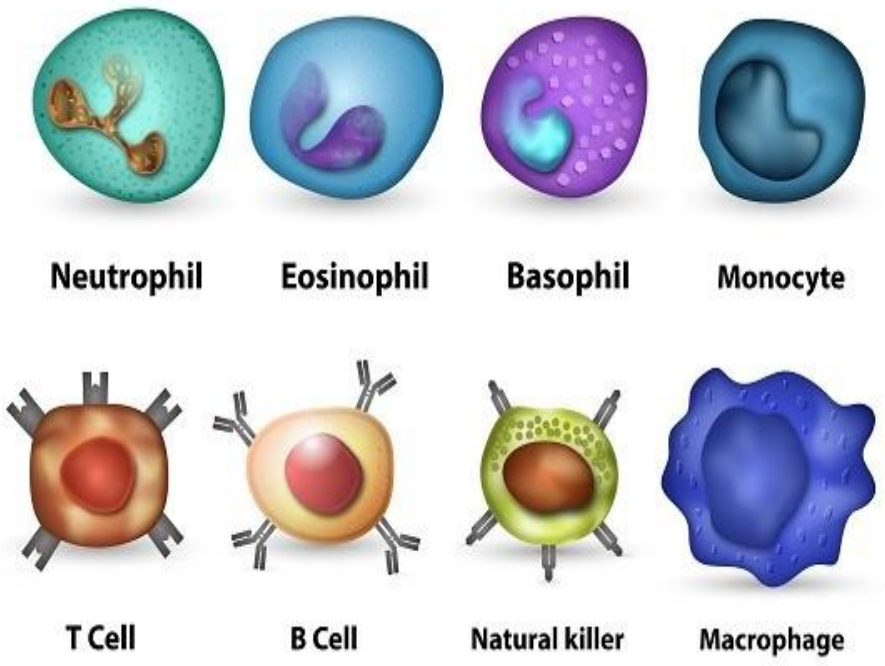


Fig.1.2.

1.5 Dendritic cells

Dendritic cells (DCs) are antigen-presenting cells (also known as accessory cells) of the mammalian immune system. Their main function is to process antigen material and present it on the cell surface to the T cells of the immune system. They act as messengers between the innate and the adaptive immune systems. Dendritic cells are an important antigen-presenting cell (APC), and they also can develop from monocytes. Antigens are molecules from pathogens, host cells, and allergens that may be recognized by adaptive immune cells. APCs like DCs are responsible for processing large molecules into "readable" fragments (antigens) recognized by adaptive B or T cells. However, antigens alone cannot activate T cells. They must be presented with the appropriate major histocompatibility complex (MHC) expressed on the APC. MHC provides a checkpoint and helps immune cells distinguish between host and foreign cells.

Dendritic cells are present in those tissues that are in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called the Langerhans cell) and the inner lining of the nose, lungs, stomach and intestines. They can also be found in an immature state in the blood. Once activated, they migrate to the lymph nodes where they interact with T cells and B cells to initiate and shape the adaptive immune response. At certain development stages they grow branched projections, the dendrites that give the cell its name in Greek meaning tree. Immature dendritic cells are also called veiled cells, as they possess large cytoplasmic 'veils' rather than dendrites.

1.6 Natural killer (NK) cells

They have features of both innate and adaptive immunity. They are important for recognizing and killing virus-infected cells or tumorcells. They contain intracellular compartments called granules, which are filled with proteins that can form holes in the target cell and also cause apoptosis, the process for programmed cell death. It is important to distinguish between apoptosis and other forms of cell death like necrosis. Apoptosis, unlike necrosis, does not release danger signals that can lead to greater immune activation and inflammation. Through apoptosis, immune cells can discreetly remove infected cells and limit bystander damage. Recently, researchers have shown in mouse models that NK cells, like adaptive cells, can be retained as memory cells and respond to subsequent infections by the same pathogen.

Natural killer cells, or NK cells, are a type of cytotoxic lymphocyte critical to the innate immune system. The role NK cells play is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to virus-infected cells, acting at around 3 days after infection, and respond to tumor formation. Typically, immune

cells detect major histocompatibility complex (MHC) presented on infected cell surfaces, triggering cytokine release, causing lysis or apoptosis. NK cells are unique, however, as they have the ability to recognize stressed cells in the absence of antibodies and MHC, allowing for a much faster immune reaction. They were named "natural killers" because of the initial notion that they do not require activation to kill cells that are missing "self" markers of MHC class 1. This role is especially important because harmful cells that are missing MHC I markers cannot be detected and destroyed by other immune cells, such as T lymphocyte cells.

NK cells belonging to the group of innate lymphoid cells are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor-generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they then enter into the circulation.[3] NK cells differ from natural killer T cells (NKTs) phenotypically, by origin and by respective effector functions; often, NKT cell activity promotes NK cell activity by secreting interferon gamma.

In addition to the knowledge that natural killer cells are effectors of innate immunity, recent research has uncovered information on both activating and inhibitory NK cell receptors which play important functional roles, including self tolerance and the sustaining of NK cell activity. NK cells also play a role in the adaptive immune response. Numerous experiments have demonstrated their ability to readily adjust to the immediate environment and formulate antigen-specific immunological memory, fundamental for responding to secondary infections with the same antigen. The role of NK cells in both the innate and adaptive immune responses is becoming increasingly important in research using NK cell activity as a potential cancer therapy.

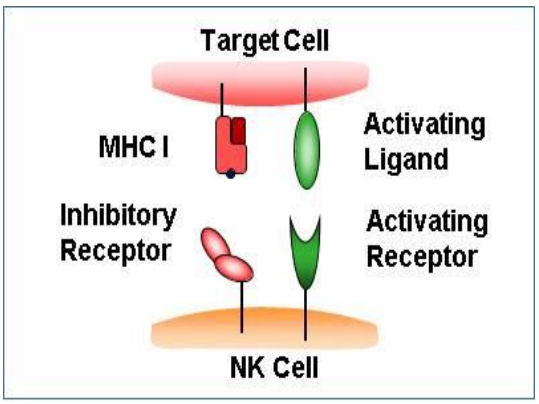


Fig.1.3.

Check your progress

Note: write your answer in the space given below

1. Differentiate T and B Lymphocytes?

.....

NOTES

1.7 LYMPHOID ORGANS:

1.7.1 Types of Lymphoid organs:

Lymphoid organs are those organs where the maturation and proliferation of lymphocytes takes place. There are two types of lymphoid organs: primary lymphoid organs and secondary lymphoid organs.

1.7.2 Primary lymphoid organs:

The primary lymphoid organs are those organs where T lymphocytes and B lymphocytes, mature and acquire their antigen-specific receptors. After maturation, the lymphocytes migrate to secondary lymphoid organs. Primary lymphoid organs include bone marrow and thymus.

i) Bone marrow:

Bone marrow is the main lymphoid organ where all blood cells including lymphocytes are formed. Maturation of B-lymphocytes occurs here. The soft tissue inside the bones where all blood cells, including lymphocytes, are made. Bone marrow produces T cells and other lymphocytes called B cells.

ii) Thymus:

A small organ in your upper chest, behind the breastbone, where lymphocytes called T cells grow and mature during childhood. When you reach adulthood, the mature T cells can divide to make new T cells. Thymus is the site of T lymphocyte maturation. Thymus is situated near the heart. Thymus is quite large in size at the time of birth but keeps reducing with age. T-lymphocytes are responsible for cellular immune response.

1.7.3 Secondary lymphoid organs:

After maturation, B lymphocytes and T lymphocytes migrate via blood vascular and lymphatic system to the secondary lymphoid organs where they undergo proliferation and differentiation. The acquired immune response to antigens usually develops in these organs and become effector cells. In the secondary lymphoid tissues, the lymphocytes do not remain, and move from one lymphoid organ to another through blood and lymph. The secondary lymphoid organs are lymph nodes, spleen, tonsils, Peyer's

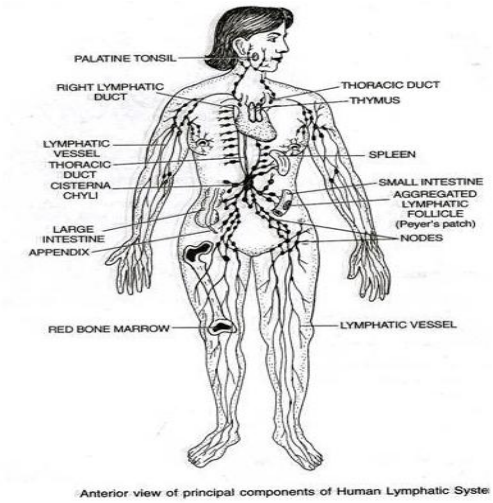
patches of the small intestine and mucosal associated lymphoid tissues (MALT).

Our human body has lymphoid tissue throughout the body. Their job is to trap antigens and present them to lymphocytes to trigger an immune response. Lymphoid tissues along the gastrointestinal tract include the tonsils and adenoids, which are located behind the throat and nose, and the appendix, a small organ attached to the large intestine. Human body has areas of lymphoid tissue along our respiratory system also. Other important parts of the immune system include lymph vessels and nodes. As the lymph moves out of different areas of the body through the lymph vessels, it slows down to be filtered by the regional lymph nodes. For example, lymph from the hand, arm, and under the arm, as well as the chest and upper back areas, drains to the underarm also known as axillary lymph nodes to be filtered.

Lymph vessels pick up wastes such as protein, cellular debris, bacteria, and viruses that leak out of the body's blood vessels. This waste-containing fluid is called lymph, and it travels through the lymph vessels into the lymph nodes. We have lymph vessels throughout our body, in much the same way that we have blood vessels (arteries and veins).The lymphoid tissues throughout your body are constantly monitoring your blood and lymph for the presence of foreign substances that could cause harm and require your immune system to take action.

1.8 Tertiary lymphoid organs

Tertiary lymphoid organs (TLO) are abnormal lymph node–like structures that form in peripheral tissues at sites of chronic inflammation, such as chronic infection, transplanted organs undergoing graft rejection, some cancers, and autoimmune and autoimmune-related diseases. TLOs are regulated differently from the normal process whereby lymphoid tissues are formed during ontogeny, being dependent on cytokines and hematopoietic cells, but still drain interstitial fluid and transport lymphocytes in response to the same chemical messengers and gradients. TLOs typically contain far fewer lymphocytes, and assumes an immune role only when challenged with antigens that result in inflammation. It achieves this by importing the lymphocytes from blood and lymph. The other main function is that of defense in the immune system.



Anterior view of principal components of Human Lymphatic Syste

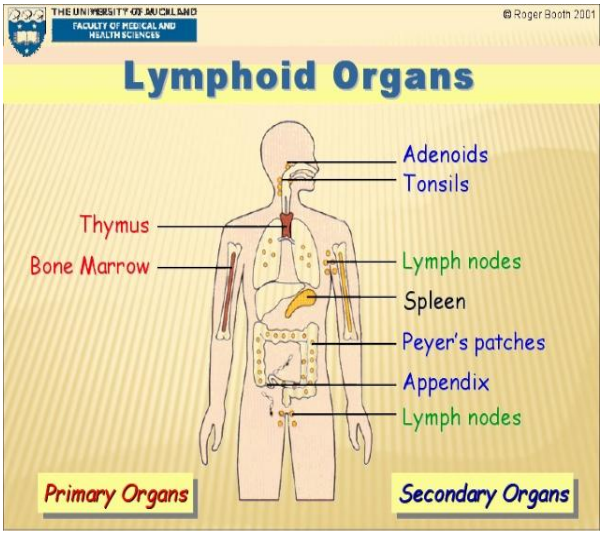


Fig.1.4.

1.9 Lymph

It is very similar to blood plasma. It contains lymphocytes. It also contains waste products and cellular debris together with bacteria and proteins. Associated organs composed of lymphoid tissue are the sites of lymphocyte production. Lymphocytes are concentrated in the lymph nodes. The system also includes all the structures dedicated to the circulation and production of lymphocytes. It is the primary cellular component of lymph, which also includes the bone marrow, and the lymphoid tissue associated with the digestive system.

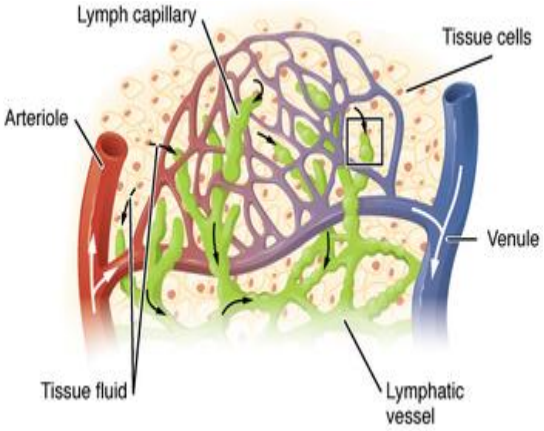


Fig.1.5.

1.10 Lymph nodes:

These are small solid structures found at intervals along the lymphatic system. They are composed of lymphoid tissue and act as filters for the lymph, preventing foreign particles from entering the bloodstream. Lymph nodes also produce lymphocytes and plasma cells. Lymph nodes are small, round organs that filter out bacteria, waste, and other toxins and also contain infection-fighting white blood cells. The nodes play a key role in recognizing and destroying these substances while also signaling the body to launch an immune response. We have clusters of lymph nodes in your groin, under your arms, and in your neck, as well as more nodes located along other lymphatic pathways in the chest, abdomen, and pelvis.

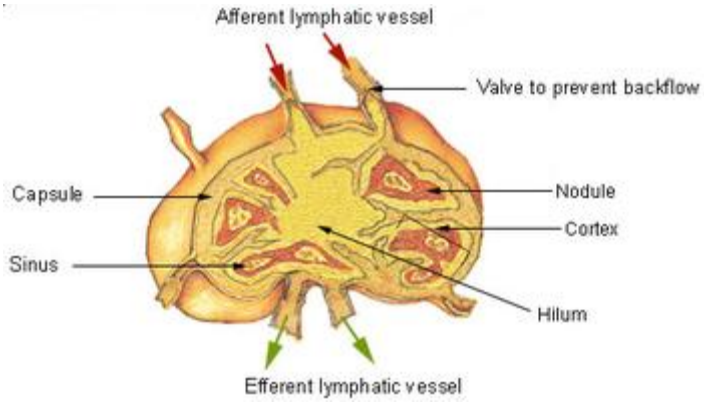


Fig.1.6.

1.11 Spleen:

It is a bean shaped organ which is the largest single mass of lymphoid tissue in the body. In foetus the spleen produces all types of blood cells but in adult it only produces lymphocytes. Macrophages of spleen are phagocytic. A fist-sized organ at the upper left of the abdomen, just behind the stomach. The spleen contains white blood cells that respond to any antigens collected from the blood. The main functions of the spleen are:

- ✓ To produce immune cells to fight antigens

- ✓ To remove particulate matter and aged blood cells, mainly red blood cells.
- ✓ To produce blood cells during fetal life

The spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph node circulation. A study published in 2009 using mice found that the spleen contains, in its reserve, half of the body's monocytes within the red pulp. These monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cells and macrophages while promoting tissue healing. The spleen is a center of activity of the mononuclear phagocyte system and can be considered analogous to a large lymph node, as its absence causes a predisposition to certain infections. Like the thymus, the spleen has only efferent lymphatic vessels. Both the short gastric arteries and the splenic artery supply it with blood. Up to the fifth month of prenatal development the spleen creates red blood cells. After birth the bone marrow is solely responsible for hematopoiesis. The spleen stores red blood cells and lymphocytes. It can store enough blood cells to help in an emergency.

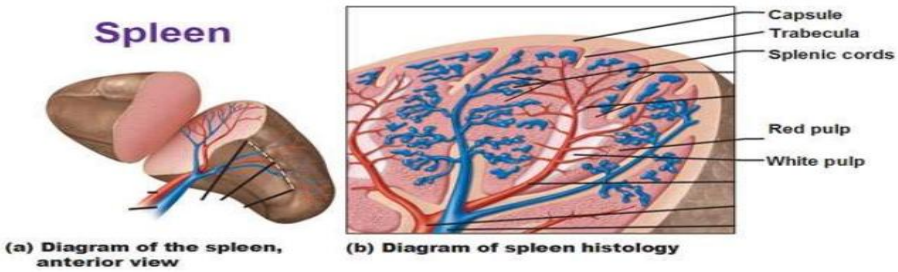


Fig.1.7.

1.12 Tonsils:

Usually there are six tonsils. They act as filters to protect body from bacteria and aid in the formation of white blood cells. Tonsils are collections of lymphoid tissue. The set of lymphatic tissue known as Waldeyer's tonsillar ring includes the adenoid tonsil, two tubal tonsils, two palatine tonsils, and the lingual tonsil. The palatine tonsils and the nasopharyngeal tonsil are lympho-epithelial tissues located near the oropharynx and nasopharynx (parts of the throat).

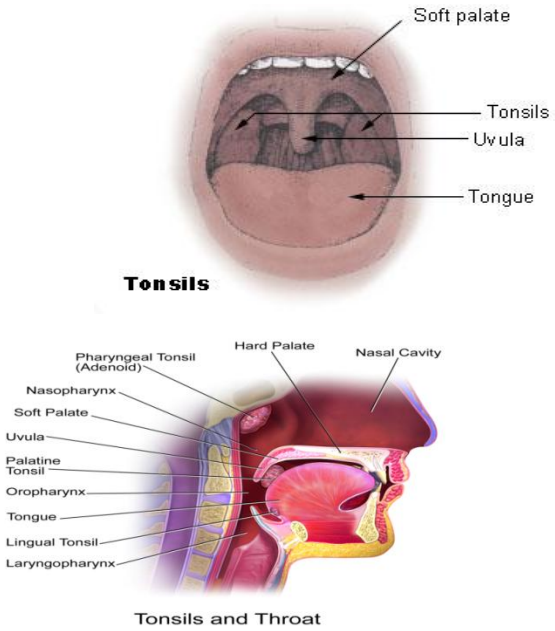


Fig.1.8.

1.13 Peyer's patches:

These are clusters of lymph nodules found in small intestine, especially along the ileum. They produce lymphocytes. Peyer's patches are small masses of lymphatic tissue found throughout the ileum region of the small intestine. Also known as aggregated lymphoid nodules, they form an important part of the immune system by monitoring intestinal bacteria populations and preventing the growth of pathogenic bacteria in the intestines

Fig.1.9.

1.14 Mucosal-Associated Lymphoid Tissues (MALT)

MALT are significant aggregations of lymphoid tissues which are seen in relation to the mucosa of the major tracts like respiratory, alimentary canal and urinogenital tracts. It constitutes about 50 percent of the lymphoid tissue in human body. They do not serve as filters of lymph. Larger aggregations extend into the submucosa. However, they are centres of

lymphocyte production. Apart from B-lymphocytes and T-lymphocytes, phagocytic macrophages and dendritic cells are present.

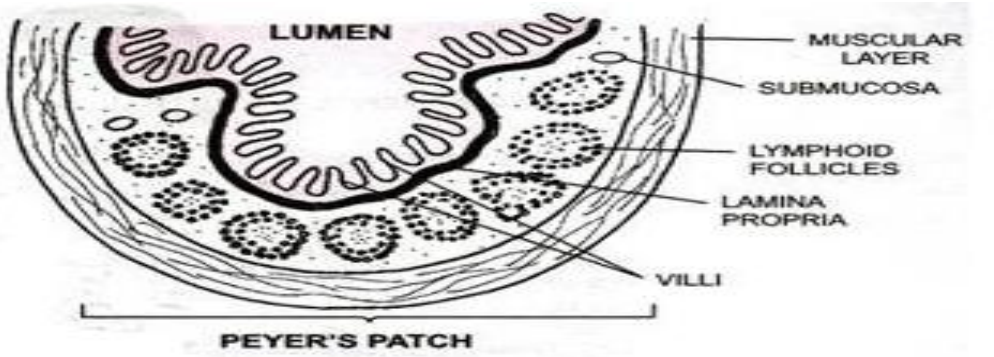


Diagram showing Peyer's patch in the submucosa of small intestine.

1.15 LET US SUME UP

In this unit you have learnt about the meaning ,need, objectives and important concept of primary and secondary lymphoid organ. It may helpful to differentiate primary lymphoid organ and secondary lymphoid organ.

1. 16 UNIT END EXERCISES

1. Explain thymus
2. What are the functions of lymph?

1.17ANSWERS TO CHECK YOUR PROGRESS

1. The bone marrow is the source of many immune and blood cells in the healthy adult animal. In the adult animal, all immune cells originate from hematopoietic Stem cells located in the bone marrow. Stem cells in the bone marrow are the progenitors of all immune cells. Cytokines are small signalling proteins that guide the development of immune cells. The types of immune cells are polymorphonuclear cells, mast cells, monocytes, macrophages, and lymphocytes.
2. B lymphocytes or B cells

They are antibody-producing cells. They are very important in fighting many different types of infections, especially, bacterial infections.

T lymphocytes or T cells

They are involved in regulating the immune system and destroying host cells that are out of control, either due to a breakdown in cell division regulation (cancer) or infection by a virus or even an intracellular parasite.

1.18 SUGGESTED READINGS

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UNIT II LYMPHOID ORGANS STRUCTURE AND FUNCTIONS OF PRIMARY AND SECONDARY LYMPHOID ORGANS

Structure

2.1 INTRODUCTION

There was no rational approach to the origin of disease developed until the late eighteenth century in the universe. If one survived a disease, the person thereafter became "immune" to any subsequent exposures. However, this was never recognized as evidence of some type of internal defense system until the later part of the seventeenth century.

Most historical accounts credit Edward Jenner for the development of the first immunization process. Immunity to smallpox was conferred by inserting the dried exudates of smallpox pustules into the nose. This technique for the transfer of smallpox, as a form of limited infection, travelled to the west from China along the traditional trade routes to Constantinople where it spread throughout Europe. Hearing of this practice, the Royal family of England had their children inoculated against the disease in 1721.

In 1798, Edward Jenner, noticed that milkmaids were protected from smallpox if they had been first infected with cowpox. It was not his intention to make medical history, as his interests were mostly scholarly and involved the transfer of infections from one species to another, especially from animals to humans. However, Jenner's work led him to the conclusion, that inoculation with cowpox (a bovine analogue of smallpox) could confer immunity to smallpox. Thus, the concept of vaccination was initiated. Jenner's ideas was not accepted medically and socially as they were in opposition to both the church and popular beliefs., However, the use of vaccinations gradually became widely accepted and most European countries had some form of compulsory program within fifty years of Jenner's discovery.

The idea that a pathogenic organism caused disease was not fully realized until certain technological advances had occurred. Initially, Antoni van Leeuwenhoek 's development of the microscope and the subsequent realization that entities existed that were not visible to the human eye, allowed the concept of germs to be appreciated. That these organisms were the causative agent of disease was not recognized until Louis Pasteur developed his germ theory of disease. His original interests were in fermentation in wine and beer, and he was the first to isolate the organisms that caused the fermentation process. Pasteur's work eventually led him to the development of pasteurization (heating) as a means of halting fermentation.

Finally, in 1878, Pasteur accidentally used an attenuated (weakened) chicken cholera culture and realized, when he repeated the experiment using a fresh culture, that the weakened form protected the chickens from the virulent form of the disease. Pasteur went on to develop an attenuated vaccine against rabies and swine.

2.2 OBJECTIVES

- A lymphocyte is a type of white blood cell in the vertebrate immune system.

- NK cells are a part of the innate immune system and play a major role in defending the host from both tumors and virally infected cells.
- T cells are involved in cell-mediated immunity whereas B cells are primarily responsible for humoral immunity (relating to antibodies).
- Helper T-cells coordinate immune responses, while cytotoxic T-cells lyse (break down) pathogens associated with T cell's specific antigen.
- Memory B cells are formed at the end of an adaptive immune response and will produce antibodies more quickly when the antigen is detected again, which is effective at preventing recurrent infections from the same pathogen.
- Sometimes the body will present antigens that aren't harmful (allergy) or antigens from otherwise normally functioning body parts (autoimmunity). The latter can cause severe antibody and T-cell induced immune responses and diseases.

2.3 PRIMARY LYMPHOID ORGANS.

It is also called central lymphoid organs. These are responsible for synthesis and maturation of immunocompetant cells. These include the bone marrow and the thymus.

2.3.1 BONE MARROW

While primary lymphoid organs are concerned with production and maturation of lymphoid cells, the secondary or peripheral lymphoid organs are sites where the lymphocytes localise, recognise foreign antigen and play a key role against it. These include the lymph nodes, spleen, tonsils, adenoids, appendix, and clumps of lymphoid tissue in the small intestine known as Peyer's patches. They trap and concentrate foreign substances, and they are the main sites of production of antibodies. Some lymphoid organs are capsulated such as lymph node and spleen while others are non-capsulated, which include mostly mucosa-associated lymphoid tissue (MALT).

PALS contain mainly T lymphocytes, about 75% of which are CD4+ helper T cells. Attached to this are lymphoid follicles, some of which contain germinal centers. Follicles and germinal center predominantly contain B cells. The PALS and follicles are surrounded by rim of

lymphocytes and macrophages, called marginal zone. Marginal zone is composed of macrophages, B cells, and CD4+ helper T cells. The arterioles end in vascular sinusoids in the red pulp, which in turn end in venules that drain into splenic vein. Antigens and lymphocytes enter the spleen through vascular sinusoids. Activation of B cells occurs at the junction between follicle and PALS. Activated B cells then migrate to the germinal centers or into the red pulp.

2.4 MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT):

Approximately 50% of lymphoid tissue in the body is found associated with the mucosal system. MALT is composed of gut-associated lymphoid tissues (GALT) lining the intestinal tract, bronchus-associated lymphoid tissue (BALT) lining the respiratory tract, and lymphoid tissue lining the genitourinary tract. The respiratory, alimentary and genitourinary tracts are guarded by sub epithelial accumulations of lymphoid tissue that are not covered by connective tissue capsule. They may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and lamina propria of intestine or as clearly organised tissue with well-formed lymphoid follicles. The well-formed follicles include the tonsils (lingual, palatine and pharyngeal), Peyer’s patches in the intestine and appendix. The major function of these organs is to provide local immunity. Diffuse accumulations of lymphoid tissue are seen in the lamina propria of the intestinal wall. The intestinal epithelium overlying the Peyer's patches is specialized to allow the transport of antigens into the lymphoid tissue. This function is carried out by cuboidal absorptive epithelial cells termed "M" cells, so called because they have numerous microfolds on their luminal surface. M cells endocytose, transport and present antigens to subepithelial lymphoid cells. Majority of intra-epithelial lymphocytes are T cells, and most often CD8+ lymphocytes. The intestinal lamina propria contains CD4+ lymphocytes, large number of B cells, plasma cells, macrophages, dendritic cells, eosinophils and mast cells. Peyer’s patches contain both B cells and CD4+ T cells.

2.5 LYMPHOCYTES:

Lymphocytes are stem cells derived cells that mature either in the bone marrow or thymus. Together, the thymus and bone marrow produce

approximately 109 mature lymphocytes each day and the adult human body contains approximately 1012 lymphocytes. Lymphocytes comprise 20-40% (1000 - 4000 cells/µl) of all leukocytes. The lymphocytes are distributed to blood, lymph and lymphoid organs. Typically, lymphocyte is small, round, cell with diameter of 5-10µm, spherical nucleus, densely compacted nuclear chromatin and scanty cytoplasm. Though the cytoplasm contains mitochondria and ribosomes, other organelles are not detectable. Such mature but resting lymphocytes are known as naive cells. They are mitotically inactive but when stimulated can undergo cell division.

2.6 NAIVE LYMPHOCYTES

Naive lymphocytes have a short life span and die in few days after leaving bone marrow or thymus unless they are stimulated. Once the lymphocyte is activated (stimulated), they become large (10-12µm), have more cytoplasm and more organelles. Activated lymphocytes may undergo several successive rounds of cell division over a period of several days. Some of the progeny cells revert to the resting stage and become memory cells, but can survive for several years in the absence of any antigenic stimulus. There are three major types of lymphocyte, B lymphocyte, T lymphocyte and NK cells. Different lymphocytes are identified by certain protein markers on their surface called "cluster of differentiation" or "CD" system. One marker that all leukocytes have in common is CD45. The presence of the markers can be detected using Specific monoclonal antibodies.

2.6.1 Distribution:

They account for 5-15% of lymphocytes (250cells/µl) in circulation and 80-90% in bone marrow, 20-30% in lymph node and 50-60% in spleen. Surface markers: The most important surface marker on the surface of mature B cell is the surface immunoglobulin. The surface immunoglobulins are of IgM and IgD type. B cell will have approximately 109 immunoglubulins of single specificity on its surface. Markers/Receptors on B cells are Surface Immunoglobulin (IgM and IgD), CD40, B7, ICAM-1, LFA-1, MHC II, CD32 (Ig Fc receptor), CD35 (Receptor for complement component) and additional markers that distinguish B cells such as CD19, CD20, CD21 and CD22. Demonstration of B cells: EAC (Erythrocyte Amboceptor Complement) Rosettes: When

sheep RBCs coated with antibody and treated with complement and B cells, a rosette is formed due to the presence of complement receptor on B cells. B cells can be demonstrated by immuno fluorescence with fluorescent-labelled monoclonal antibodies against surface markers such as surface immunoglobulin.

Check your progress

Note: write your answer in the space given below

1. What is the function of peripheral lymphoid organ?

……

2.6.2 Functions of B-cells:

Direct antigen recognition and Antigen presentation B cells may differentiate into plasma cells (which secrete large amounts of antibodies) or into memory B cells. Memory cells can survive 20 years or more. Plasma cells: These are the effector cells of the B-cell lineage and are specialised in secreting immunoglobulins. When activated B cells divide, some of its progeny become memory cells and the reminder become immunoglobulin-secreting plasma cells. Plasma cells are oval or egg shaped, have eccentrically placed nuclei, have abundant cytoplasm containing dense rough endoplasmic reticulum (the site of antibody production), per nuclear Golgi body (where immunoglobulins are converted to final form and packaged). Unlike B cells, immunoglobulins are not present on the surface of plasma cells. They have a short life span of few days to few weeks.

2.7 T LYMPHOCYTE:

Ontogeny: The name "T-cell" is an abbreviation of "thymus dependent lymphocyte". T lymphocytes arise in the bone marrow as T-cell precursors, then migrate to and mature in the thymus. After entry into the thymus T-cell precursors are also referred to as "thymocytes". In the thymus there are rearrangements at gene segments coding for the variable part of the TCR (T Cell Receptor) resulting in generation of diversity. T Cell Receptors are then expressed on the surface, which is followed by expression of either CD8 or CD4 surface molecules. Those cells expressing receptors that can interact with self MHC molecules are positively selected while those cells that express receptors that recognize peptides derived

from self protein in association with self MHC are negatively selected. Such cells undergo clonal deletion or anergy.

2.7.1 Distribution

T cell accounts for 70-80% (1500 cells/µl) lymphocytes in peripheral blood, 5-10% in bone marrow, 70-80% in lymph node and 30-40% in spleen. Surface markers: The most important surface receptor is TCR. TCR are polypeptides that belong to the immunoglobulin super family. The other markers/receptors present on the surface are IL-2R, IL-1R, CD2, CD3, CD4/CD8, CD28, ICAM-1 and LFA-1. Nearly all the mature T lymphocytes express both CD2 and CD3 on their surface. CD3, which is always found closely associated with TCR, is necessary for signal transduction following antigen recognition by the TCR. Subsets of T Cells: There are two major types of T cells, Helper (CD4) and Cytotoxic/Suppressor (CD8) T cells. CD4 cells account for 45% (900/µl) of lymphocytes while CD8 cells account for 30% (600/µl). ¾ Helper T cells (TH) secrete cytokines that promote the proliferation and differentiation of cytotoxic T cells, B cells and macrophages and activation of inflammatory leukocytes. TH cells are identified by the presence of the CD4 marker. They recognize antigen when presented along with Class II MHC molecules.

2.8 LARGEGRANULAR LYMPHOCYTES:

Also called Large Granular Lymphocytes (LGLs), these are large lymphocytes containing azurophilic granules in the cytoplasm. NK cells derive form bone marrow but don't require thymus for development. NK cells are so called because they kill variety of target cells (such as tumour cells, virus-infected cells, and transplanted cells) without the participation of MHC molecules. They can kill target cell without a need for activation unlike cytotoxic T lymphocytes. Hence they mediate a form of natural (innate) immunity.

2.8.1 Distribution

They account for 10-15% of blood lymphocytes. They are rare in lymph nodes and don't circulate through lymph. Surface markers: NK cells lack any surface immunoglobulins, TCR or CD4 makers; instead they have CD16 (Immunoglobulin Fc receptor) and CD56. Approximately 50% of human NK cells express only one form of CD8. Other receptors include

IL-2R, CD2, ICAM-1 and LFA-1. Functions: NK cells are activated by recognition of antibody-coated cells, virus infected cell, cell infected with intracellular bacteria and cells lacking MHC I proteins. Activated NK cells produce cytokines such as IFN-γ, TNFα, GM-CSF and CSF-1 all of which are immunomodulators.

Check your progress

Note: write your answer in the space given below

What is Antibodies?

……

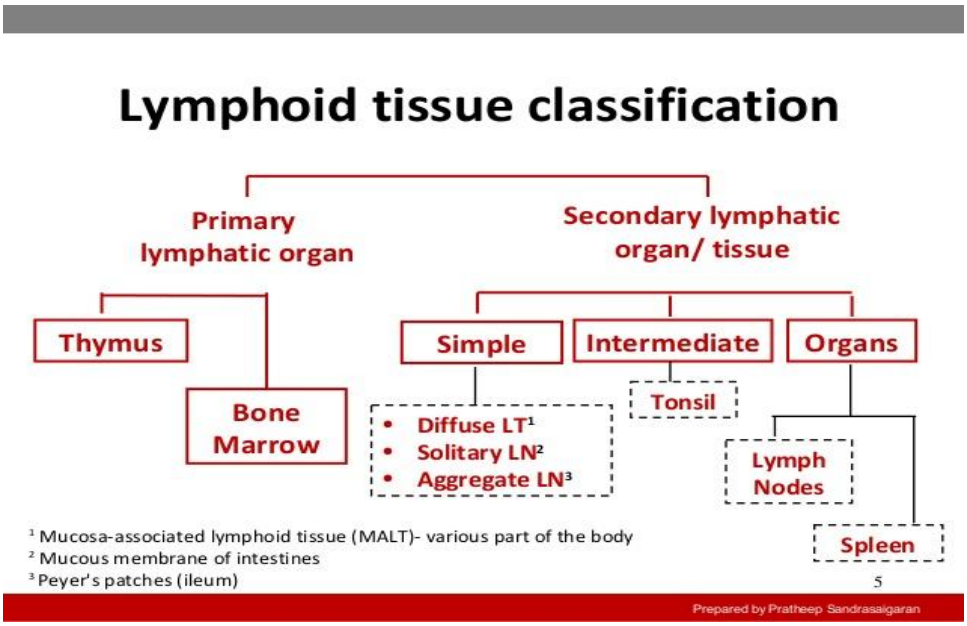

Fig.1.10.

2.9 LET US SUM UP

In this unit you have learnt about the meaning ,need, objectives and important concept of primary and secondary lymphoid organ. It may helpful to differentiate primary lymphoid organ and secondary lymphoid organ.

2.10 UNIT END EXERCISES

1. Explain Secondary Lymphatic organ
2. What is the function of Lymph Nodes?

2.11 ANSWERS TO CHECK YOUR PROGRESS

1. Primary lymphoid organs are concerned with production and maturation of lymphoid cells, the secondary or peripheral lymphoid organs are sites where the lymphocytes localise, recognise foreign antigen and play a key role against it.

2. Antibodies act as the antigen receptor on the surface of B cells and, in response to antigen, are subsequently secreted by plasma cells. Antibodies recognize specific configurations (epitopes, or antigenic determinants) on the surfaces of antigens (eg, proteins, polysaccharides, nucleic acids).

2.12 SUGGESTED READINGS

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UNIT III MOLECULES OF IMMUNE SYSTEM-ANTIBODIES, COMPLEMENTS, CYTOKONES, INTERFERONSE, GENERATION OF SOURCES AND FUNCTIONS. ANTIGEN: CLASSIFICATION AND EPISODE

Structure

3.1 INTRODUCTION

The complement system helps or "complements" the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is

part of the immune system called the"innate immune system" that is not adaptable and does not change over the course of an individual's lifetime. However, it can be recruited and brought into action by the adaptive immune system.

The complement system consists of a number of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors (pro-proteins). When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The end result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex. Over 25 proteins and protein fragments make up the complement system, including serum proteins, serosal proteins, and cell membrane receptors. They account for about 5% of the globulin fraction of blood serum.

3.2 OBJECTIVES

- Three biochemical pathways activate the complement system–the classical complement pathway, the alternative complement pathway, and the lectin pathway.
- The following are the basic functions of the complement: Opsonization (enhancing phagocytosis of antigens); chemotaxis (attracting macrophages and neutrophils); cell lysis (rupturing membranes of foreign cells); and clumping (antigen-bearing agents).
- The complement system consists of a number of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors (pro-proteins).

3.3 MOLECULES OF IMMUNE SYSTEM

3.3.1 Acute phase reactants

Acute phase reactants are plasma proteins whose levels dramatically increase (called positive acute phase reactants) or, in some cases, decrease (called negative acute phase reactants) in response to the elevated circulating levels of IL-1 and IL-6 that occur when infection or tissue damage occurs. Most dramatically increased are:

- C-reactive protein (CRP)

- Mannose-binding lectin
- Alpha-1 acid glycoprotein
- Serum amyloid P component

C-reactive protein and mannose-binding lectin fix complement and act as opsonins. Alpha-1 acid glycoprotein is a transport protein. Serum amyloid P component activates complement. Elevated C-reactive protein levels are a nonspecific indicator of infection or inflammation. Fibrinogen levels also increase and are the main reason the erythrocyte sedimentation rate (ESR) is elevated in acute inflammation. Many acute phase reactants are made in the liver. Collectively, they may help limit tissue injury, enhance host resistance to infection, and promote tissue repair and resolution of inflammation.

3.3.2 Antibodies

Antibodies act as the antigen receptor on the surface of B cells and, in response to antigen, are subsequently secreted by plasma cells. Antibodies recognize specific configurations (epitopes, or antigenic determinants) on the surfaces of antigens (eg, proteins, polysaccharides, nucleic acids). Antibodies and antigens fit tightly together because their shape and other surface properties are complementary. The same antibody moleculecan cross-react with related antigens if their epitopes are similar enough to those of the original antigen.

3.3.3 Antibody structure

Antibodies consist of 4 polypeptide chains (2 identical heavy chains and 2 identical light chains) joined by disulfide bonds to produce a Y configuration. The heavy and light chains are divided into a variable (V) region and a constant (C) region.

3.3.4 B-cell receptor

The B-cell receptor consists of an Ig molecule anchored to the cell's surface. CH (heavy chain constant region), CL (light chain constant region), Fab (antigen-binding fragment) Fc (crystallizable fragment),Ig (immunoglobulin) L-kappa (κ) or lambda (λ) are the 2 types of light chains, VH (heavy chain variable region), VL (light chain variable region).

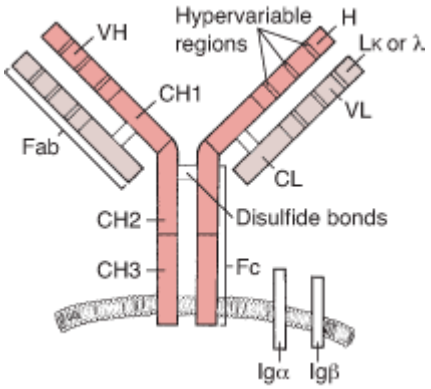

Fig.3.1

V regions are located at the amino-terminal ends of the Y arms. They are called variable because the amino acids they contain are different in different antibodies. Within the V regions, hypervariable regions determine the specificity of the Ig. They also function as antigens idiotypic determinants to which certain natural (anti-idiotype) antibodies can bind. This binding may help regulate B-cell responses.

The C region of the heavy chains contains a relatively constant sequence of amino acids (isotype) that is distinctive for each Ig class. The amino-terminal (variable) end of the antibody binds to antigen to form an antibody-antigen complex. The antigen-binding (Fab) portion of Ig consists of a light chain and part of a heavy chain and contains the V region of the Ig molecule (ie, the combining sites). The crystallizable

fragment (Fc) contains most of the C region of the heavy chains; Fc is responsible for complement activation and binds to Fc receptors on cells.

Check your progress

Note: write your answer in the space given below

What is Antibodies?

……

3.3.5 Antibody classes

Antibodies are divided into 5 classes:

- IgM
- IgG
- IgA
- IgD
- IgE

The classes are defined by their type of heavy chain: mu (μ) for IgM, gamma (γ) for IgG, alpha (α) for IgA, epsilon (ε) for IgE, and delta (δ) for IgD. There are also 2 types of light chains: kappa (κ) and lambda (λ). Each of the 5 Ig classes can bear either kappa or lambda light chains.

i) IgM

IgM is the first antibody formed after exposure to new antigen. It has 5 Y-shaped molecules (10 heavy chains and 10 light chains), linked by a single joining (J) chain. IgM circulates primarily in the intravascular space. it complexes with and agglutinates antigens and can activate complement, thereby facilitating phagocytosis. Isohemagglutinins are predominantly IgM. Monomeric IgM acts as a surface antigen receptor on B cells. Patients with hyper-IgM syndrome have a defect in the genes involved in antibody class switching (eg, genes that encode CD40, CD154.Therefore, IgA, IgG, and IgE levels are low or absent, and levels of circulating IgM are often high.

ii) IgG

IgG is the most prevalent Ig isotype in serum and is also present in intravascular and extra vascular spaces. It coats antigen to activate complement and facilitate phagocytosis by neutrophils and macrophages. IgG is the primary circulating Ig produced after reexposure to antigen

(secondary immune response) and is the predominant isotype contained in commercial gamma-globulin products. IgG protects against bacteria, viruses, and toxins. It is the only Ig isotype that crosses the placenta. Therefore, this class of antibody is important for protecting neonates, but pathogenic IgG antibodies (eg, anti-Rh_0 [D] antibodies, stimulatory anti-thyroid-stimulating hormone receptor autoantibodies), if present in the mother, can potentially cause significant disease in the fetus. There are 4 subclasses of IgG: IgG1, IgG2, IgG3, and IgG4. They are numbered in descending order of serum concentration. IgG subclasses differ functionally mainly in their ability to activate complement; IgG1 and IgG3 are most efficient, IgG2 is less efficient, and IgG4 is inefficient. IgG1 and IgG3 are efficient mediators of antibody-dependent cellular cytotoxicity; IgG4 and IgG2 are less so.

iii) IgA

IgA occurs at mucosal surfaces, in serum, and in secretions such as saliva, tears, respiratory, Gastrointestinal tract secretions, colostrum where it provides an early antibacterial and antiviral defense. J chain links IgA into a dimmer to form secretary IgA. Secretory IgA is synthesized by plasma cells in the sub epithelial regions of the Gastrointestinal and respiratory tracts.

iv) IgD

IgD is co expressed with IgM on the surface of naive B cells. Whether these 2 classes function differently on the surface of the B cell and, if so, how differently are unclear. They may simply be an example of molecular degeneracy. Serum IgD levels are very low, and the function of circulating IgD is unknown.

v) IgE

IgE is present in low levels in serum and in respiratory and gastrointestinal mucous secretions. IgE binds with high affinity to receptors present in high levels on mast cells and basophils and to a lesser extent on several other hematopoietic cells, including dendritic cells. If antigen bridges 2 IgE molecules bound to the mast cell or basophil surface, the cells degranulate, releasing chemical mediators that cause an inflammatory response. IgE levels are elevated in atopic disorders (eg,

allergic or extrinsic asthma, hay fever, atopic dermatitis) and parasitic infections.

Overview of Antibody-Mediated Immunity

3.4 Cytokines

Cytokines are polypeptides secreted by immune and other cells when the cell interacts with a specific antigen, with pathogen-associated molecules such as endotoxin, or with other cytokines. Main categories include

- Chemokines
- Hematopoietic colony-stimulating factors (CSFs)
- Interleukins
- Interferons (IFN-alpha, IFN-beta, IFN-gamma)
- Transforming growth factors (TGFs)
- Tumor necrosis factors (TNF-alpha, lymphotoxin-alpha, lymphotoxin-beta)

Although lymphocyte interaction with a specific antigen triggers cytokine secretion, cytokines themselves are not antigen-specific. They bridge innate and acquired immunity and generally influence the magnitude of inflammatory or immune responses. They act sequentially, synergistically, or antagonistically. They may act in an autocrine or paracrine manner.

Cytokines deliver their signals via cell surface receptors. For example, the IL-2 receptor consists of 3 chains: alpha (α), beta (β), and gamma (γ). The receptor's affinity for IL-2 is high if all 3 chains are expressed, Intermediate if only the beta and gamma chains are expressed, Low if only the alpha chain is expressed. Mutations or deletion of the gamma chain is the basis for X-linked severe combined immunodeficiency.

3.5 Chemokines

Chemokines induce chemotaxis and migration of leukocytes. There are 4 subsets (C, CC, CXC, CX3C), defined by the number and spacing of their amino terminal cysteine residues. Chemokine receptors

(CCR5 on memory T cells, monocytes/macrophages, and dendritic cells; CXCR4 on resting T cells) act as co-receptors for entry of HIV into cells.

3.6 COLONY-STIMULATING FACTORS (CSF)

3.6.1 Granulocyte-colony stimulating factor (G-CSF)

Granulocyte-colony stimulating factor (G-CSF) is produced by endothelial cells and fibroblasts. The main effect of G-CSF is stimulation of neutrophil precursors. Clinical uses of G-CSF include reversal of neutropenia after chemotherapy, radiation therapy, or both

3.6.2 Granulocyte-macrophage colony stimulating factor (GM-CSF)

Granulocyte-macrophage colony stimulating factor (GM-CSF) is produced by endothelial cells, fibroblasts, macrophages, mast cells, and T_H cells. The main effects of GM-CSF are stimulation of growth of monocyte, neutrophil, eosinophil, and basophil precursors, Activation of macrophages, Clinical uses of GM-CSF include reversal of neutropenia after chemotherapy, radiation therapy, or both

3.6.3 Macrophage colony stimulating factor (M-CSF)

Macrophage colony stimulating factor (M-CSF) is produced by endothelial cells, epithelial cells, and fibroblasts. The main effect of M-CSF is Stimulation of monocyte precursor growth Clinical uses of M-CSF include Therapeutic potential for stimulating tissue repair

3.6.4 Stem cell factor (SCF)

Stem cell factor (SCF) is produced by bone marrow stromal cells. The main effect of SCF is stimulation of stem cell division. Clinical uses of SCF include therapeutic potential for stimulating tissue repair.

3.7 INTERFERONS

3.7.1 IFN-alpha

IFN-alpha is produced by leukocytes. The main effects of IFN-alpha are Inhibition of viral replication, Augmentation of class I major histocompatibility complex (MHC) expression Clinical uses of IFN-alpha include treatment of chronic hepatitis C, AIDS-related Kaposi sarcoma, hairy cell leukemia, chronic myeloid leukemia, and metastatic melanoma

3.7.2 IFN-beta

IFN-beta is produced by fibroblasts. The main effects of IFN-beta are Inhibition of viral replication, Augmentation of class I MHC

expression Clinical uses of IFN-beta include reduction of the number of flare-ups in relapsing multiple sclerosis

3.7.3 IFN-gamma

IFN-gamma is produced by natural killer (NK) cells, cytotoxic type 1 (Tc1) cells, and T helper type 1 (Th1) cells. The main effects of IFN-gamma are Inhibition of viral replication, Augmentation of classes I and II MHC expression, Activation of macrophages, Antagonism of several actions of IL-4 Inhibition of Th2 cell proliferation. Clinical uses of IFN-gamma include Control of infection in chronic granulomatous disease. Delay of progression in severe malignant osteopetrosis

3.8 Interleukins

3.8.1 IL-1 (alpha and beta)

IL-1 (alpha and beta) is produced by B cells, dendritic cells, endothelium, macrophages, monocytes, and natural killer (NK) cells. The main effects of IL-1 are Co-stimulation of T-cell activation by enhancing production of cytokines (eg, IL-2 and its receptor),Enhancement of B-cell proliferation and maturation, Enhancement of NK-cell cytotoxicity, Induction of IL-1, IL-6, IL-8, TNF, GM-CSF, and prostaglandin E2 production by macrophages, Proinflammatory activity by inducing chemokines, ICAM-1, and VCAM-1 on endothelium, Induction of sleep, anorexia, release of tissue factor, acute phase reactants, and bone resorption by osteoclasts, Endogenous pyrogenic activity, Clinical relevance of IL-1 includes For **anti**–IL-1 beta monoclonal antibody (mAb), treatment of cryopyrin-associated periodic syndromes, juvenile idiopathic arthritis, and calcium pyrophosphate arthritis.

IL-2 is produced by Th1 cells,The main effects of IL-2 are Induction of activated T- and B-cell proliferation.

IL-4 is produced by mast cells, NK cells, natural killer T (NKT) cells, gamma-delta T cells, Tc2 cells, and Th2 cells. The main effects of IL-4 are Induction of Th2 cells Stimulation of activated B-, T-, and mast cell proliferation, Up regulation of class II MHC molecules on B cells and on macrophages and CD23 on B cells, Down regulation of IL-12 production, thereby inhibiting Th1 cell-differentiation, Augmentation of macrophage phagocytosis.

IL-5 is produced by mast cells and Th2 cells. The main effects of IL-5 are induction of eosinophil and activated B-cell proliferation.

IL-6 is produced by dendritic cells, fibroblasts, macrophages, monocytes, and Th2 cells. The main effects of IL-6 are Induction of differentiation of B cells into plasma cells and differentiation of myeloid stem cell.

IL-7 is produced by bone marrow and thymus stromal cells. The main effects of IL-7 are Induction of differentiation of lymphoid stem cells into T- and B-cell precursors, Activation of mature T cells.

IL-8 (chemokine) is produced by endothelial cells, macrophages, and monocytes. The main effect of IL-8 is mediation of chemotaxis and activation of neutrophils.

IL-9 is produced by T cells. The main effects of IL-9 are induction of thymocyte proliferation. Enhancement of mast cell growth.

IL-10 is produced by B cells, macrophages, monocytes, Tc cells, Th2 cells, and regulatory T cells. The main effects of IL-10 are inhibition of IL-2 secretion by Th1 cells. **IL-12** is produced by B cells, dendritic cells, macrophages, and monocytes. The main effects of IL-12 are critical role in Th1 differentiation.

IL-13 is produced by mast cells and Th2 cells. The main effects of IL-13 are inhibition of activation and cytokine secretion by macrophages, Coactivation of B-cell proliferation.

IL-15 is produced by B cells, dendritic cells, macrophages, monocytes, NK cells, and T cells. The main effects of IL-15 are induction of proliferation of T, NK, and activated B cells, Potential as an immunostimulatory agent in the treatment of cancer.

IL-17 (A and F) is produced by Th17 cells, gamma-delta T cells, NKT cells, and macrophages. The main effects of IL-17 are Proinflammatory action. Stimulation of production of cytokines (eg, TNF, IL-1 beta, IL-6, IL-8, G-CSF).

IL-18 is produced by monocytes, macrophages, and dendritic cells. The main effects of IL-18 are induction of IFN-gamma production by T cells.

IL-21 is produced by NKT cells and Th cells. The main effects of IL-21 are stimulation of B-cell proliferation after CD40 cross-linking, Stimulation of NK cells, Costimulation of T cells, Stimulation of bone marrow precursor cell proliferation.

IL-22 is produced by NK cells, Th17 cells, and gamma-delta cells. The main effects of IL-22 are proinflammatory activity.

IL-23 is produced by dendritic cells and macrophages. The main effect of IL-23 is induction of Th-cell proliferation.

IL-24 is produced by B cells, macrophages, monocytes, and T cells. The main effects of IL-24 are suppression of tumor cell growth induction of apoptosis in tumor cells.

IL-27 is produced by dendritic cells, monocytes, and macrophages. The main effect of IL-27 is induction of Th1 cells.

IL-32 is produced by NK cells and T cells. The main effects of IL-32 are proinflammatory activity, Participation in activation-induced T cell apoptosis.

IL-33 is produced by endothelial cells, stromal cells, and dendritic cells. The main effects of IL-33 are induction of Th2 cytokines.

IL-35 is produced by regulatory T cells, macrophages, and dendritic cells. The main effect of IL-35 is suppression of inflammation, eg, by inducing regulatory T and B cells and inhibiting Th17 cells
Clinical uses of IL-35 include

3.8.2 Transforming growth factors (TGF)

TGF-beta is produced by B cells, macrophages, mast cells, and Th3 cells.

The main effects of TGF-beta are pro inflammatory activity (eg, by chemo attraction of monocytes and macrophages) but also anti-inflammatory activity (eg, by inhibiting lymphocyte proliferation)

3.8.3 Tumor necrosis factors (TNFs)

TNF-alpha (cachectin) is produced by B cells, dendritic cells, macrophages, mast cells, monocytes, NK cells, and T_H cells. The main effects of TNF-alpha include cytotoxicity to tumor cells induction of secretion of several cytokines (eg, IL-1, GM-CSF, IFN-gamma),Induction

of E-selectin on endothelium, activation of macrophages, antiviral activity

TNF-beta (lymphotoxin) is produced by Tc cells, and Th1 cells. The main effects of TNF-beta include cytotoxicity to tumor cells, antiviral activity, enhancement of phagocytosis by neutrophils and macrophages,involvement in lymphoid organ development.

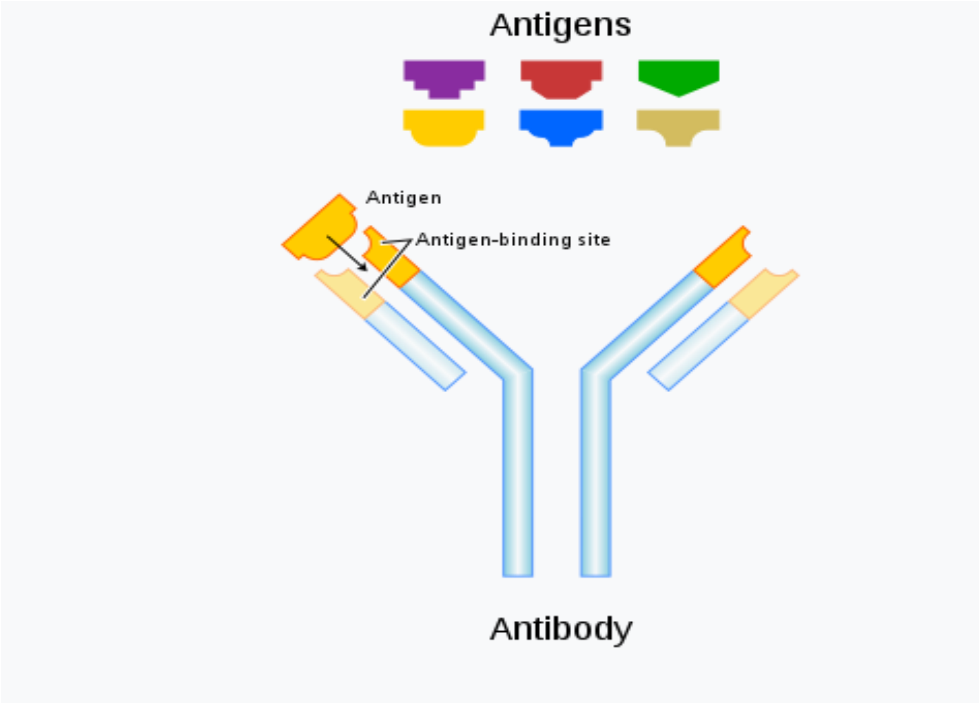

Fig 3.1.

3.9 Antigens

Antigens (**Ag**) are structures specifically bound by antibodies (Ab) or a cell surface version of Ab called B cell antigen receptor (BCR). The term antigen originally described a structural molecule that binds specifically to an antibody only in the form of native antigen. It was expanded later to refer to any molecule or a linear molecular fragment after processing the native antigen that can be recognized by T-cell receptor (TCR). BCR and TCR are both highly variable antigen receptors. Both T cells and B cells are cellular components of adaptive immunity.

Antigens are "targeted" by antibodies. Each antibody is specifically produced by the immune system to match an antigen after cells in the immune system come into *contact* with it. This allows a precise identification or matching of the antigen and the initiation of a tailored

response. The antibody is said to "match" the antigen in the sense that it can bind to it due to an adaptation in a region of the antibody, because of this, many different antibodies are produced, each able to bind a different antigen while sharing the same basic structure. In most cases, an adapted antibody can only react to and bind one specific antigen; in some instances, however, antibodies may cross-react and bind more than one antigen.

Also, an antigen is a molecule that binds to Ag-specific receptors, but cannot necessarily induce an immune response in the body by itself.[3] Antigens are usually proteins, peptides (amino acid chains) and polysaccharides (chains of monosaccharides/simple sugars) but lipids and nucleic acids become antigens only when combined with proteins and polysaccharides.[4] In general, saccharides and lipids (as opposed to peptides) qualify as antigens but not as immunogens since they cannot elicit an immune response on their own. Furthermore, for a peptide to induce an immune response (activation of T-cells by antigen-presenting cells) it must be a large enough size, since peptides too small will also not elicit an immune response.

The antigen may originate from within the body ("self-antigen") or from the external environment ("non-self"). The immune system is supposed to identify and attack "non-self" invaders from the outside world or modified/harmful substances present in the body and usually does not react to self-antigens under normal homeostatic conditions due to negative selection of Tcells in the thymus.

Vaccines are examples of antigens in an immunogenic form, which are intentionally administered to a recipient to induce the memory function of adaptive immune system toward the antigens of the pathogen invading that recipient.

Paul Ehrlich coined the term antibody (in German *Antikörper*) in his side-chain theory at the end of the 19th century.[6] In 1899, Ladislas Deutsch (Laszlo Detre) (1874–1939) named the hypothetical substances halfway between bacterial constituents and antibodies called antigenic or immunogenic substances). He originally believed those substances to be precursors of antibodies, just as zymogen is a precursor of an enzyme. But,

by 1903, he understood that an antigen induces the production of immune bodies called antibodies.

3.10 EPITOPE

The distinct surface features of an antigen, its *antigenic determinant*. Antigenic molecules, normally "large" biological polymers, usually present surface features that can act as points of interaction for specific antibodies. Any such feature constitutes an epitope. Most antigens have the potential to be bound by multiple antibodies, each of which is specific to one of the antigen's epitopes. Using the "lock and key" metaphor, the antigen can be seen as a string of keys (epitopes) each of which matches a different lock (antibody).Different antibody idiotypes, each have distinctly formed complementarity determining regions.

3.10.1 Classification of antigens on the basis of its role

Allergen – A substance capable of causing an allergic reaction. The (detrimental) reaction may result after exposure via ingestion, inhalation, injection, or contact with skin.

Superantigen – A class of antigens that cause non-specific activation of T-cells, resulting in polyclonal T-cell activation and massive cytokine release.

Tolerogen – A substance that invokes a specific immune non-responsiveness due to its molecular form. If its molecular form is changed, a tolerogen can become an immunogen.

Immunoglobulin-binding protein – Proteins such as protein A, protein G, and protein L that are capable of binding to antibodies at positions outside of the antigen-binding site. While antigens are the "target" of antibodies, immunoglobulin-binding proteins "attack" antibodies.

T-dependent antigen – Antigens that require the assistance of T cells to induce the formation of specific antibodies.

T-independent antigen – Antigens that stimulate B cells directly.

Immunodominant antigens – Antigens that dominate (over all others from a pathogen) in their ability to produce an immune response. T cell responses typically are directed against a relatively few immunodominant epitopes, although in some cases (e.g., infection with the malaria pathogen *Plasmodium spp.*) it is dispersed over a relatively large number of parasite antigens.[8]

3.10.2 Antigen presenting cells

Antigen presenting cells present antigens in the form of peptides on histocompatibility molecules. The T cell selectively recognizes 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 variable Fab region. Different antibodies have the potential to discriminate among specific epitopes present on the antigen surface. A hapten is a small molecule that changes the structure of an antigenic epitope. In order to induce an immune response, it needs to be attached to a large carrier molecule such as a protein (a complex of peptides). Antigens are usually carried by proteins and polysaccharides, and less frequently, lipids. This includes parts (coats, capsules, cell walls, flagella, fimbriae, and toxins) of bacteria, viruses, and

other microorganisms. Lipids and nucleic acids are antigenic only when combined with proteins and polysaccharides. 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.

3.10.3 Antigens can be classified according to their source.

i) 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.

ii) 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. Sometimes antigens are part of the host itself in an autoimmune disease.

iii) Autoantigens

An autoantigen is usually a normal 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 antigens should not be the target of the immune system,

but in autoimmune diseases, their associated T cells are not deleted and instead attack.

iv) Neoantigens

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 neo antigens became available only recently.

v) 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.

vi) 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. Antigens that are presented by tumor cells and normal cells, called tumor-associated antigens (TAAs). Cytotoxic Tlymphocytes that recognize these antigens may be able to destroy tumor cells. Tumor antigens can appear on the surface of the tumor 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.

Check your progress

Note: write your answer in the space given below

Define viral Antigen

……

vii) Native Antigen

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.

3.11 AN EPITOPE

It is also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. For example, the epitope is the specific piece of the antigen to which an antibody binds. The part of an antibody that binds to the epitope is called a paratope. Although epitopes are usually non-self proteins, sequences derived from the host that can be recognized (as in the case of autoimmune diseases) are also epitopes.

The epitopes of protein antigens are divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the paratope. Conformational and linear epitopes interact with the paratope based on the 3-D conformation adopted by the epitope, which is determined by the surface features of the involved epitope residues and the shape or tertiary structure of other segments of the antigen. A conformational epitope is formed by the 3-D conformation adopted by the interaction of discontiguous amino acid residues. In contrast, a linear epitope is formed by the 3-D conformation adopted by the interaction of contiguous amino acid residues. A linear epitope is not determined solely by the primary structure of the involved amino acids. Residues that flank such amino acid residues, as well as more distant amino acid residues of the antigen affect the ability of the primary structure residues to adopt the epitope's 3-D conformation. The proportion of epitopes that are conformational is unknown

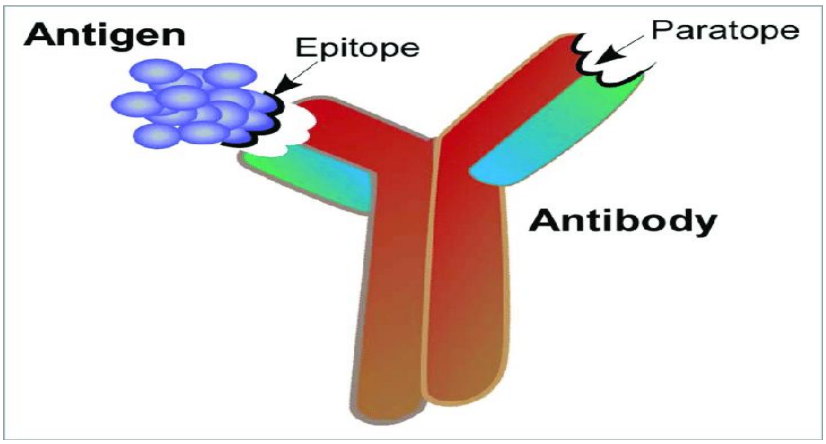

Fig.1.13.

3.12 LET US SUM UP

In this unit you have learnt about the meaning, need, objectives and important concept of primary and secondary lymphoid organ. It may helpful to molecules of immune system**.**

3.13 UNIT END EXERCISES

1. Define the epitope
2. What are the functions of Antigen?

3.14 ANSWERS TO CHECK YOUR PROGRESS

1. Antibodies act as the antigen receptor on the surface of B cells and, in response to antigen, are subsequently secreted by plasma cells. Antibodies recognize specific configurations (epitopes, or antigenic determinants) on the surfaces of antigens (eg, proteins, polysaccharides, nucleic acids).

2. 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.

3.15 SUGGESTED READINGS

1. Matthew Helbert,Immunology For Medical Students. Elsevier, 2016

2. Sunil Kumar Mohant, Dr.Sai Leela Text book of immunology Jaypee Brothers Medical Publishers (P) Ltd.Second edition 2014.

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UNIT IV ELEMENTS OF IMMUNE SYSTEM

Structure

4.1 INTRODUCTION

The immune system is a host defense system comprising many biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, known as pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. In many species, there are two major subsystems of the immune system: the innate immune system and the adaptive immune system. Both subsystems use humoral immunity and cell-mediated immunity to perform their functions. In humans, the blood–brain barrier, blood–cerebrospinal fluid barrier, and similar fluid–brain barriers separate the peripheral immune system from the neuroimmune system, which protects the brain.

Pathogens can rapidly evolve and adapt, and thereby avoid detection and neutralization by the immune system; however, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms evolved in ancient eukaryotes and remain in their modern descendants, such as plants and invertebrates. These mechanisms include phagocytosis, antimicrobial peptides called defensins, and the complement system. Jawed vertebrates, including humans, have even

more sophisticated defense mechanisms,[1] including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (or acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination

4.2 OBJECTIVES

- The lymphatic system contains lymph: a fluid that bathes tissues and organs and contains white blood cells (not red blood cells).
- Once B and T cells mature, the majority of them enter the lymphatic system, where they are stored in lymph nodes until needed.
- Lymph nodes also store dendritic cells and macrophages; as antigens are filtered through the lymphatic system, these cells collect them so as to present them to B and T cells.
- The spleen, which is to blood what lymph nodes are to lymph, filters foreign substances and antibody -complexed pathogens from the blood.

4.3 HEMATOPOIESIS

It is the production of all of the cellular components of blood and blood plasma. It occurs within the hematopoietic system, which includes organs and tissues such as the bone marrow, liver, and spleen. Simply, hematopoiesis is the process through which the body manufactures blood cells. Hematopoiesis is the production of all of the cellular components of blood and blood plasma. It occurs within the hematopoietic system, which includes organs and tissues such as the bone marrow, liver, and spleen.

Hematopoiesis begins during the first weeks of embryonic development. All blood cells and plasma develop from a stem cell that can develop into any other cell. The blood is made up of more than 10 different cell types. Each of these cell types falls into one of three broad categories:

1. Red blood cells (erythrocytes): These transport oxygen and hemoglobin throughout the body.

2. White blood cells (leukocytes): These support the immune system. There are several different types of white blood cells:

- **Lymphocytes**: Including T cells and B cells, which help fight some viruses and tumors.
- **Neutrophils**: These help fight bacterial and fungal infections.
- **Eosinophils**: These play a role in the inflammatory response, and help fight some parasites.
- **Basophils**: These release the histamines necessary for the inflammatory response.
- **Macrophages**: These engulf and digest debris, including bacteria.

3. Platelets (Thrombocytes): These help the blood to clot.

Current research endorses a theory of haematopoiesis called the monophyletic theory. This theory says that one type of stem cell produces all types of blood cells.

4.3.1 Hematopoiesis in the embryo

Hematopoiesis in the embryo provides organs with oxygen. Sometimes called primitive hematopoiesis, hematopoiesis in the embryo produces only red blood cells that can provide developing organs with oxygen. At this stage in development, the yolk sac, which nourishes the embryo until the placenta is fully developed, controls hematopoiesis. As the embryo continues to develop, the hematopoiesis process moves to the liver, the spleen, and bone marrow, and begins producing other types of blood cells. In adults, hematopoiesis of red blood cells and platelets occurs primarily in the bone marrow. In infants and children, it may also continue in the spleen and liver.

The lymph system, particularly the spleen, lymph nodes, and thymus, produces a type of white blood cell called lymphocytes. Tissue in the liver, spleen, lymph nodes and some other organs produce another type of white blood cells, called monocytes.

Check your progress

Note: write your answer in the space given below

1. List out the white blood cells (leukocytes)

.....

4.3.2 The process of Haematopoiesis

The rate of hematopoiesis depends on the body's needs. The body continually manufactures new blood cells to replace old ones White blood cells have the shortest life span, sometimes surviving just a few hours to a few days, while red blood cells can last up to 120 days or so. The process of haematopoiesis begins with an unspecialized stem cell. This stem cell multiplies, and some of these new cells transform into precursor cells. These are cells that are destined to become a particular type of blood cell but are not yet fully developed. However, these immature cells soon divide and mature into blood components, such as red and white blood cells, or platelets. Although researchers understand the basics of hematopoiesis, there is an-ongoing scientific debate about how the stem cells that play a role in hematopoiesis are formed. Although mature lymphocytes all look pretty much alike, they are extraordinarily diverse in their functions. The most abundant lymphocytes are

4.4 B LYMPHOCYTES (B CELLS) and T LYMPHOCYTES (T CELLS).

T cells (thymus cells) and B cells (bone marrow- or bursa-derived cells) are the major cellular components of the adaptive immune response. T cells are involved in cell-mediated immunity, whereas B cells are primarily responsible for humoral immunity. The function of T cells and B cells is to recognize specific "non-self" antigens, during a process known as antigen presentation. Once they have identified an invader, the cells generate specific responses that are tailored maximally to eliminate specific pathogens or pathogen-infected cells. B cells respond to pathogens by producing large quantities of antibodies which then neutralize foreign objects like bacteria and viruses. In response to pathogens some T cells, called T helper cells, produce cytokines that direct the immune response, while other T cells, called cytotoxic T cells, produce toxic granules that contain powerful enzymes which induce the death of pathogen-infected cells. Following activation, B cells and T cells leave a lasting legacy of the

antigens they have encountered, in the form of *memory cells*. Throughout the lifetime of an animal, these memory cells will "remember" each specific pathogen encountered, and are able to mount a strong and rapid response if the same pathogen is detected again; this is known as acquired immunity.

Some of the differences between B Cells and T Cells are as follows:

S.No.	Properties	**B-Cells**	**T-Cells**
1	**Name**	B lymphocytes	T lymphocytes
2	**Origin**	Bone Marrow	Thymus
3	**Position**	Outside Lymph Node	Interior of Lymph Node
4	**Membrane receptor**	BCR (= immunoglobulin) for antigen	TCR for antigen
5	**Connections**	B-cells can connect to antigens right on the surface of the invading virus or bacteria.	T-cells can only connect to virus antigens on the outside of infected cells.
6	**Tissue Distribution**	Germinal centres of lymph nodes, spleen, gut, respiratory tract; also subcapsular and medullary cords of lymph nodes	Parafollicular areas of cortex in nodes, periarteriolar in spleen
7	**Life Span**	Life span is short	Life span is long
8	**Surface Antibodies**	Surface Antibodies present	Absence of surface antibodies
9	**Secretion**	They secrete antibodies	They secrete Lymphokines
10	**Function**	B-cells form humoral or antibody-mediated immune system (AMI).	T-cells form cell-mediated immune system (CMI).

11	**Blood**	20% of lymphocytes	80% of lymphocytes; CD4 > CD8
12	**Formation**	They form plasma cells and memory cells.	They form killer, helper and suppressor cells.
13	**Movement to Infection Site**	Plasma cells do not move to the site of infection.	Lymphoblasts move to the site of infection.
14	**Function**	Plasma cells do not react against transplants and cancer cells.	Killer cells react against transplants and cancer cells.
15	**Function**	Plasma cells have no inhibitory effect on immune system.	Suppressor cells inhibit immune system.
16	**Function**	They defend against viruses and bacteria that enter the blood and lymph.	They defend against pathogens including protists and fungi that enter the cells.

4.5 SPECIFICITY AND DIVERSITY OF THE IMMUNE SYSTEM.

1965, several theories to explain how animals generate specific and diverse immune response

1) Instructive Theory-According to this theory

- A limited number of antibodies are made.
- The antigen causes the antibody to fold into a certain structure allowing the antibody to bind to the antigen with high affinity.

2) Clonal selection According to this theory

- A large number of different antibodies are produced in animals.

- In response to antigen, a small subset of antibodies were stimulated to proliferate.
- Post-translational modification by glycosylation or phosphorylation could increase the number of different molecules produce from a given number of genes. It could alternative the splicing (pre-mRNA splicing not discovered until 1977).

In actuality, a little of both instinctive theory and clonal selection mechanisms contribute to diversity and specificity of the immune response

- Mostly clonal selection is responsible
- In the end, the genome does contain the capacity to code for large numbers of different antibodies.
- But the antigen is instructive and does have an effect on specificity of antibody produced.

3 ways that diversity and specificity are generated

- 1. Somatic Recombination
- 2. Joining
- 3. Somatic Mutation

1. **Somatic Recombination**

- DNA in B cells rearranges
- DNA arrangement in B cells is different from the DNA arrangement in other cells of the body.
- Site-specific recombination
- DNA of both heavy and light chains encode multiple variable region gene segments and so they are adjacent to each other in the chromosome.
- They recombine in different combinations
- DNA rearrangement occurs in both heavy and light chains and produces the variable antigen-binding regions.

Check your progress

Note: write your answer in the space given below
Difference between T and B cells function
.....

4.5.1 Kappa light chain rearrangement

V1 V2 V3 V4Š..V45Š..V75 V76 J1 J2 J3 J4 J5 C

Above is shown the germ-line pre-DNA arrangement. A DNA recombination event brings 1 V and 1 J segment together. This is a B cell specific event. We will analyze what happens when V45 and J4 are recombined together. The region between V45 and J4 is lost when V45 and J4 are put together, yielding: V1 V2 V3 V4 Š..V45 J4 J5 C The above is B cell DNA after rearrangement. After transcription, the following pre-mRNA is yielded: V45 J4 J5 C splicing occurs and introns removed yielding V45 J4 C

Heavy chains have an additional segment

- In light chains, V and J recombine
- In heavy chains, V, D (diversity), and J recombine
- You have 2 recombination events for heavy chains where the first recombination involves D and J recombining into DJ.
- The second recombination brings V and DJ together
- Then transcription and splicing occurs.

How much diversity?

- Heavy chain
  - 300 V, 20 D, 6 J
  - 300 x 20 x 6 = 36,000 different combinations
- Light chain
  - 300V x 6J-C = 1800
  - 80V x 5J = 400
  - 1800 + 400 = 2200
- TOTAL different combinations = 8×10^7

Somatic Recombination

- DNA rearrangement
- yields approximately 10^8 combinations.

2. Joining

- Diversity is introduced during the site specific recombination.
- Site specific recombination is where extra nucleotides are added in randomly at recombination joints
- Diversity is introduced in conserved sequences that flank the gene segments to be recombined

At the Kappa locus:

- 3' to all 76 Variable gene segments the consensus sequence heptamer-12bp-nonamer appears
- 5' to all J segments the consensus sequence nonamer-23bp-heptamer appears

Implications

- 1/3 of time get productive rearrangement
- 2/3 of the time, you ends up with a V and J out of frame which is bad and leads to a nonproductive antibody. But this is the price paid to increase diversity of the antibodies produces

SUMMARY: Joining

- Light chain &endash; assume 2 amino acids randomized &endash; 20^2 = 400 different combinations
- Heavy chain &endash; assume 2 amino acids randomized at each join so 400 x 400
- 6 x 10^7 different additional combinations of light and heavy chains.

Somatic Muations &endash; bizarre!

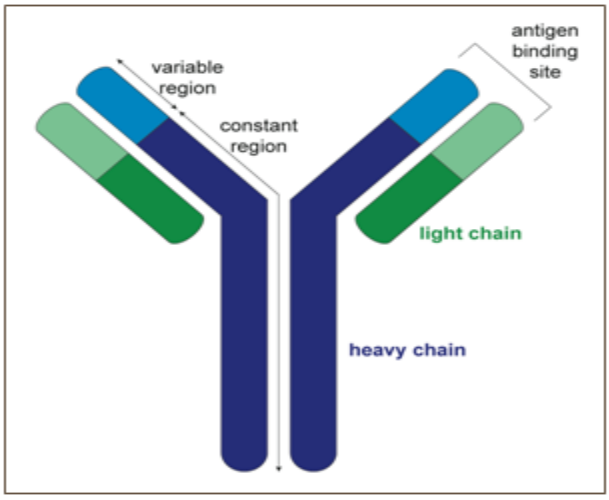
- entails random mutagenesis of VH
- this was first noticed when people started sequencing VH from B cells that underwent rearrangement because this DNA of VH sequence was different from any VH sequence in germline.
- Idea: somatic mutations increase affinity of antibody for antigen. A small % of these mutations lead to increased affinity of antibody to antigen but most lead to a decreased affinity. Apparently, the immune system considers it worthwhile.
- 5 amino acids randomized, 20^5 = 3.2 x 10^6 different combinations

Total diversity

- (somatic recombination) X (joining) X (somatic mutation)
- (8×10^7) X (6×10^7) X (3.2×10^6) = ~ 10^{21} !!!!
- lymphocytes = 2×10^{12}
- Ability to generate diversity exceeds the number of cells in the immune system.

4.5.2 Generation of B-cell / 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.



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.

The specificity of a particular antibody, i.e. what the antibody recognises, is determined by the shape of **its** variable region (**Fig:4.1**); a

particular antibody will bind to a protein that has a region with a complementary structure to the antibody's own variable region. Diversity in the specificity of antibodies is initially generated at the earliest stages of B-cell development. While still at the B-cell progenitor stage **in the** bone marrow**,** B cells randomly rearrange their variable (V)**,** diversity (D)**, and** joining (J) genes to form the blueprint for the variable regions of their antibodies. Diversity comes from the fact that there are multiple copies of the V, D and J genes that can be joined together in different combinations (**Fig.2**). In a majority of mammals, each antibody molecule is composed of both **a** heavy and light chain **(**Fig.1), which each have their own V and J genes to rearrange (only the heavy chain has D genes). Further diversity is added to the variable region genes by an enzyme called terminal deoxynucleotidyl transferase **(**TdT) that adds extra nucleotides between the V, D and J regions, changing the structure of the variable regions that will be produced.

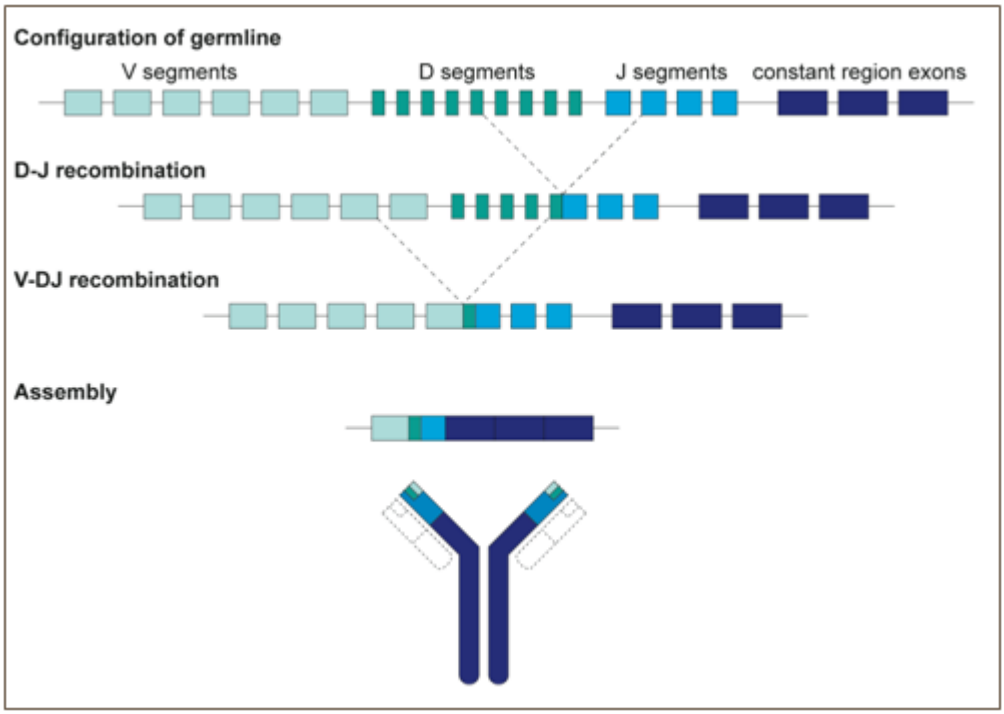


Fig. 4.2 Schematic representation of the rearrangement of variable region genes

During the course of an infection, B cells can further alter the specificity of the antibody they produce. When a mature B cell meets an antigen that its **B**-cell receptor recognises (the B-cell receptor comprises

the antibody the cell produces anchored on the cell surface) then the B cell can undergo a process called somatic hypermutation**.** Here an enzyme called activation-induced cytidine deaminase **(**AID**)** makes random mutations in the antibody variable region genes. If the mutations result in an antibody that more strongly binds to their targets then these B cells will survive and may differentiate into antibody-producing plasma cells with the new specificity.

4.6 LET US SUM UP

In this unit you have learnt about the meaning, need, objectives and important concept of primary and secondary lymphoid organ. It may helpful to molecules of elements of immune system and difference for T & B Lymphocyte.

4.7 UNIT END EXERCISES

1. What are the functions of B lymphocyte?
2. What is haematopoiesis?

4.8ANSWERS TO CHECK YOUR PROGRESS

1. These support the immune system. There are several different types of white blood cells:

- **Lymphocytes**: Including T cells and B cells, which help fight some viruses and tumors.
- **Neutrophils**: These help fight bacterial and fungal infections.
- **Eosinophils**: These play a role in the inflammatory response, and help fight some parasites.
- **Basophils**: These release the histamines necessary for the inflammatory response.
- **Macrophages**: These engulf and digest debris, including bacteria.

2. T cells plasma cells have no inhibitory effect on immune system.

B cells suppressor cells inhibit immune system.

4.9 SUGGESATED READINGS

1. Matthew Helbert,Immunology For Medical Students. Elsevier, 2016

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UNIT-V
ANTIGEN PROCESSING AND PRESENTATION, SUBSECTS OF T CELLS, MEMERY CELL, HELPER AND SUPPRESSOR CELLSMYELOIDE CELLS, MAJOR HISTOCOMPATIABILITYCOMPLEX (MCH)

Structure

5.1 INTRODUCTION

Antigen presentation describes a vital immune process which is essential for T cell immune response triggering. Because T cells recognise only fragmented antigens displayed on cell surfaces, antigen processing must occur before the antigen fragment, now bound to the major histocompatibility complex (MHC), is transported to the surface of the cell, a process known as presentation, where it can be recognized by a T cell receptor. If there has been an infection with viruses or bacteria, the cell will present an endogenous or exogenous peptide fragment derived from the antigen bound to MHC molecules. There are two types of MHC molecules which differ in the of the antigens: MHC class I molecules (MHC-I) bind peptides from the cell cytosol, while peptides generated in the endocytic

vesicles after internalisation are bound to MHC class II (MHC-II). Cellular membranes separate these two cellular environments - intracellular and extracellular.

5.2 OBJECTIVES

- T cells (also called T lymphocytes) are one of the main components of the **adaptive immune system**. They are vital in hosting an immune response against pathogens.
- T cells play a major role in defence against intracellular pathogens such as viruses, protozoa and intracellular bacteria, and in immunity to extracellular pathogens by providing help for the antibody response.
- This article shall discuss the production of T cells, the different types present in the immune system and relevant clinical conditions.

5.3 ANTIGEN PROCESSING AND PRESENTATION

AP is the display of peptide antigens (created via antigen processing) on the cell surface together with either MHC class I or class II molecules, which permit T cells to recognize antigens on the cell surface of an antigen-presenting cell (APC).In order to be capable of engaging the key elements of adaptive immunity (specificity, memory, diversity, self/nonself discrimination), antigens have to be processed and presented to immune cells. Antigen presentation is mediated by MHC class I molecules, and the class II molecules found on the surface of antigen-presenting cells (APCs) and certain other cells.

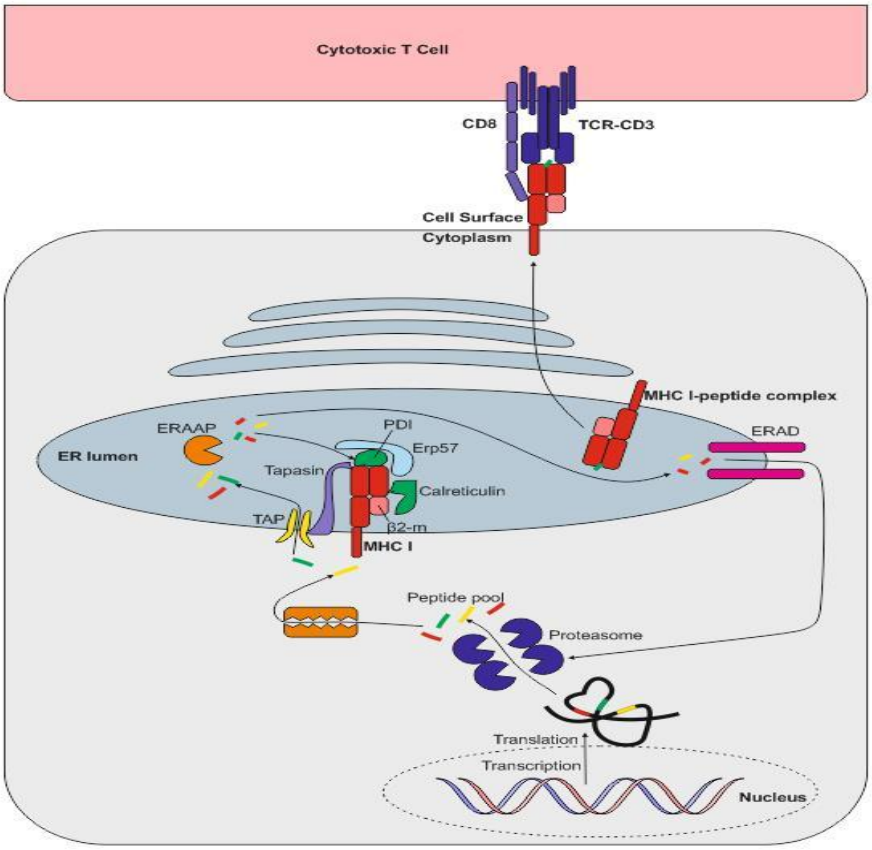

Fig.1.16. The MHC class I antigen-presentation pathway.

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

5.3.1 Antigen presentation

On the surface of a single cell, MHC class I molecules provide a readout of the expression level of up to 10,000 proteins. This array is interpreted by cytotoxic T lymphocytes and Natural Killer cells, allowing them to monitor the events inside the cell and detect infection and tumorigenesis. MHC class I complexes at the cell surface may dissociate as time passes and the heavy chain can be internalised. When MHC class I

molecules are internalised into the endosome, they enter the MHC class-II presentation pathway. Some of the MHC class I molecules can be recycled and present endosomal peptides as a part of a process which is called cross-presentation.

The usual process of antigen presentation through the MHC I molecule is based on an interaction between the T-cell receptor and a peptide bound to the MHC class I molecule. There is also an interaction between the CD8+ molecule on the surface of the T cell and non-peptide binding regions on the MHC class I molecule. Thus, peptide presented in complex with MHC class I can only be recognised by CD8+ T cells. This interaction is a part of so-called 'three-signal activation model', and actually represents the first signal. The next signal is the interaction between CD80/86 on the APC and CD28 on the surface of the T cell, followed by a third signal – the production of cytokines by the APC which fully activates the T cell to provide a specific response.

5.4 MHC class I presentation

MHC class I molecules are expressed by all nucleated cells. MHC class I molecules are assembled in the endoplasmic reticulum **(ER**) and consist of two types of chain–a polymorphic heavy chain and a chain called β2-microglobulin. The heavy chain is stabilised by the chaperone calnexin**,** prior to association with the β2-microglobulin. Without peptides, these molecules are stabilised by chaperone proteins: calreticulin, Erp57, protein disulfide isomerase (PDI) and tapasin. The complex of TAP, tapasin, MHC class I, ERp57 and calreticulin is called the peptide-loading complex **(PLC).** Tapasin interacts with the transport protein TAP (transporter associated with antigen presentation) which translocates peptides from the cytoplasm into the ER. Prior to entering the ER, peptides are derived from the degradation of proteins, which can be of viral- or self origin. Degradation of proteins is mediated by cytosolic- and nuclear proteasomes, and the resulting peptides are translocated into the ER by means of TAP. TAP translocates peptides of 8 –16 amino acids and they may require additional trimming in the ER before binding to MHC class I molecules. This is possibly due to the presence of ER aminopeptidase (ERAAP) associated with antigen processing.

It should be noted that 30–70% of proteins are immediately degraded after synthesis (they are called DRiPs – defective ribosomal products, and they are the result of defective transcription or translation). This process allows viral peptides to be presented very quickly – for example, influenza virus can be recognised by T cells approximately 1.5 hours post-infection. When peptides bind to MHC class I molecules, the chaperones are released and peptide–MHC class I complexes leave the ER for presentation at the cell surface. In some cases, peptides fail to associate with MHC class I and they have to be returned to the cytosol for degradation. Some MHC class I molecules never bind peptides and they are also degraded by the ER-associated protein degradation (ERAD) system.

There are different proteasomes that generate peptides for MHC class-I presentation**:** 26S proteasome, which is expressed by most cells; the immunoproteasome**,** which is expressed by many immune cells; and the thymic-specific proteasome expressed by thymic epithelial cells.

Check your progress
Note: write your answer in the space given below

Abbreviation

(i) MHC (ii) PLC (iii) APC (iv) ERAD

.....

5.4.1 MHC class I polymorphism

Human MHC class I molecules are encoded by a series of genes – HLA-A, HLA-B and HLA-C (HLA stands for 'Human Leukocyte Antigen', which is the human equivalent of MHC molecules found in most vertebrates). These genes are highly polymorphic, which means that each individual has his/her own HLA allele set. The consequences of these polymorphisms are differential susceptibilities to infection and autoimmune diseases that may result from the high diversity of peptides that can bind to MHC class I in different individuals. Also, MHC class I polymorphisms make it virtually impossible to have a perfect tissue match between donor and recipient, and thus are responsible for graft rejection.

5.4.2 MHC CLASS II PRESENTATION

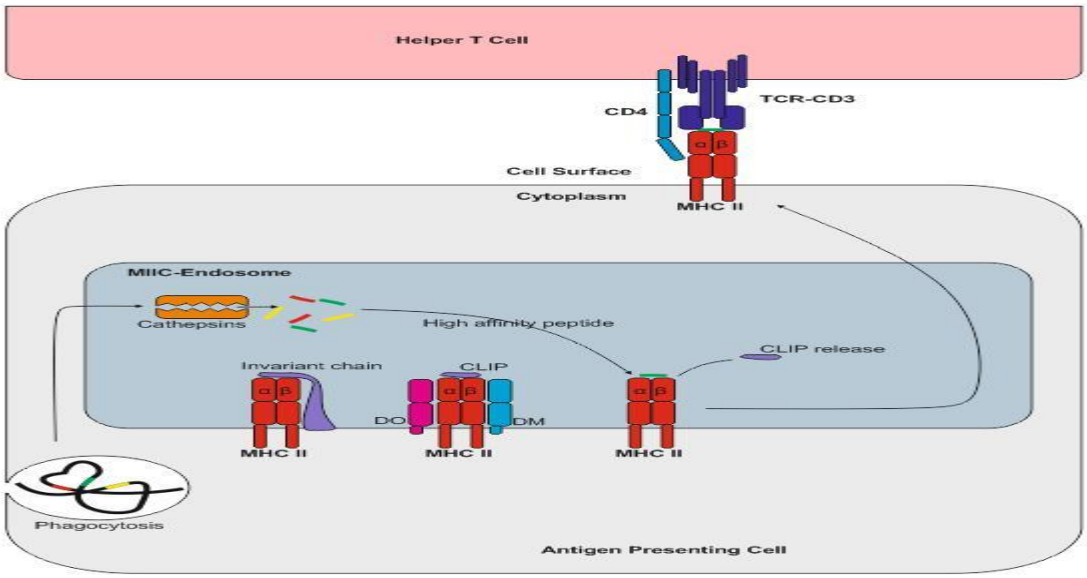

Fig.1.17. The MHC class II antigen-presentation pathway

MHC class II molecules are expressed by APCs, such **as** dendritic cells **(DC),** macrophages **and** B cells (and, under IFNγ stimuli, by mesenchymal stromal cells, fibroblasts and endothelial cells, as well as by epithelial cells and enteric glial cells). MHC class II molecules bind to peptides that are derived from proteins degraded in the endocytic pathway. MHC class II complexes consists of α- and β-chains that are assembled in the ER and are stabilised by invariant chain (Ii). The complex of MHC class II and invariant chain (Ii)is transported through the Golgi into a compartment which is termed the MHC class II compartment (MIIC). Due to acidic pH, proteases cathepsin S and cathepsin L are activated and digest Ii, leaving a residual class II-associated Ii peptide (CLIP) in the peptide-binding groove of the MHC class II. Later, the CLIP is exchanged for an antigenic peptide derived from a protein degraded in the endosomal pathway. This process requires the chaperone HLA-DM, and, in the case of B cells, the HLA-DO molecule. MHC class II molecules loaded with foreign peptide are then transported to the cell membrane to present their cargo to CD4+ T cells. Thereafter, the process of antigen presentation by means of MHC class II molecules basically follows the same pattern as for MHC class I presentation.

As opposed to MHC class I, MHC class II molecules do not dissociate at the plasma membrane. The mechanisms that control MHC class II degradation have not been established yet, but MHC class II molecules can be ubiquitinated and then internalised in an endocytic pathway.

5.4.3 MHC class II polymorphism

Like the MHC class I heavy chain, human MHC class II molecules are encoded by three polymorphic genes: HLA-DR, HLA-DQ and HLA-DP. Different MHC class II alleles can be used as genetic markers for several autoimmune diseases, possibly owing to the peptides that they present.

5.5 SUBSETS OF T CELLS,

5.5.1 T helper type 1 (Th1) cells are required for host defense against intracellular viral and bacterial pathogens. The most common markers used to identify Th1 cells are IFN-gamma production and expression of the T-bet transcription factor.

5.5.2 T helper type 2 (Th2) cells are important for host defense against large extracellular pathogens and are responsible for allergic responses. Among the cytokines secreted by Th2 cells, IL-4 is the most commonly used marker for Th2 cell identification..

5.5.3 T helper type 9 (Th9) cells protect against parasitic helminth infections, but can also cause asthma symptoms and induce experimental autoimmune encephalomyelitis. IL-9 production, together with a lack of IL-4, IL-5, and IL-13 production, is most commonly used as a marker for Th9 cells.

5.5.4 T helper type 17 (Th17) cells are involved in mucosal immunity and autoimmune disorders. These cells are Proinflammatory, as they can inhibit the expansion of regulatory T (Treg) cells. Th17 cells are most commonly identified by IL-17 production in both mice and humans.

5.5.5 T helper type 22 (Th22) cells are recruited to skin where they defend against microbial pathogens, but are also associated with

inflammatory skin disorders. High IL-22 production, along with low IL-17 production, is utilized most commonly as a marker for Th22 cells..

5.5.6 Follicular helper T (Tfh) cells are highly involved in the regulation and development of antigen-specific B cell immunity. The most common surface markers for Tfh cell identification are CXCR5 along with ICOS and/or PD-1..

5.5.7 Regulatory T (Treg) cells comprise 5 - 10 % of total $CD4^+$ cells. These cells are responsible for maintaining immune homeostasis via inhibition of differentiation and activity of pro-inflammatory T helper cells. FoxP3 expression is the most commonly used marker for Treg cells in mice and humans. Human Treg cells are further differentiated by low expression levels of CD127 (CD127lo).

5.5.8 Natural killer T (NKT) cells consist of type I cells, which express an invariant TCR alpha chain and one of a small number of TCR beta chains, and type II cells, which express a wider range of TCR alpha chains. NKT cells in mice are most commonly identified by the alpha-GalCerCD1d-tetramer and TCR-beta surface markers. Human NKT cells, however, are most commonly identified by the Va24 invariant TCR alpha chain.

5.5.9 Gamma delta T cells are so named because they express TCR chains encoded by the gamma and delta gene loci. These cells have roles in both innate and adaptive immune responses. The gamma delta TCR is the most commonly used marker to identify these cells..

5.5.10 $CD8^+$ cytotoxic T lymphocytes (CTLs) are a subset of T cells that express an alpha beta T cell receptor (TCR) and are responsible for the direct killing of infected, damaged, and dysfunctional cells, including tumor cells. CD8 expression and IFN-gamma production are the most commonly used markers for CTL identification. Additionally, Perforin and Granzyme B, which are required for CTL-mediated cell death, are commonly utilized as secondary markers. Activated $CD8^+$ cells express XCL1, a chemokine that attracts XCR1-expressing dendritic cells and is

required for maximal priming and expansion of CTLs. The population of activated CTLs is heterogeneous. Bottom of Form

5.6 MEMORY CELLS

When a disease strikes the body the memory cells instruct the body on how to produce antibodies. Once created, these antibodies are released into the bloodstream. Then the antibodies find the disease and destroy it. If a disease that was not previously encountered is introduced into the body, then the immune system (hopefully) destroys it and the memory cells record how it was done. If your body fights a virus once, the same virus will probably try to attack again. After all the work it took to get rid of that first infection, it would be a shame to have to do it all over again. An amazing feature of your immune system is that it remembers the infections it has fought. This makes it much easier to fight the same virus or bacteria a second, or third, or fourth time.

Toward the end of each battle to stop an infection, some T-cells and B-cells turn into Memory T-cells and Memory B-cells. As you would expect from their names, these cells remember the virus or bacteria they just fought. These cells live in the body for a long time, even after all the viruses from the first infection have been destroyed. They stay in the ready-mode to quickly recognize and attack any returning viruses or bacteria.

Quickly making lots of antibodies can stop an infection in its tracks. The first time your body fights a virus, it can take up to 15 days to make enough antibodies to get rid of it. With the help of Memory B-cells, the second time your body sees that virus, it can do the same in thing 5 days. It also makes 100 times more antibodies than it did the first time. The faster your body makes antibodies, the quicker the virus can be destroyed. With the help of Memory B-cells, you might get rid of it before you even feel sick. This is called gaining immunity.

Check your progress

Note: write your answer in the space given below

Write a note on memory cells

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5.7 SUPPRESSOR CELL

Suppressor cells are part of both the adaptive and innate immune systems and include different immune cell populations with defined characteristics that have been identified at the phenotypic and functional levels.Regulatory immune cells in patients with cancer. Suppressor cells can be broadly defined as regulatory elements necessary for maintaining homeostasis within host tissues and the immune system. More than 35 years ago, Gershon coined the term to describe CD8 T lymphocytes capable of blocking functions of otherT cells. Today, it is clear that various immune cells can acquire and exercise regulatory function. While Treg and MDSC are the two regulatory cell types most commonly associated with immune suppression in cancer, other immune cells, including plasmacytoid, NK and B cells, are also capable of suppression in the tumor microenvironment. Accumulations of activated suppressor cells in tumors and the peripheral blood of cancer patients have been associated with tumor progression and poor prognosis.

5.8 T Regulatory/Suppressor Cells

Historically, cytotoxic suppressor cells were identified with function in regulating cellular (T cell) responses. Recently, it has become clear that this group represents a separate subpopulation of CD4+ lymphocytes generated in the thymus, which reflects consequent activities of T cells to produce cytokines that down regulate developmental pathways of T_H responsiveness. This population is identified by expression of both the CD4 marker and the IL-2 receptor α chain (CD25), often with co-expression of CD45. Specific engagement of the TCR is required for function. Regulation is controlled via the transcriptional regulator *Foxp3*, mutations in which result in incidence of autoimmunity as well as uncontrolled lymph proliferation. Mechanisms of immunosuppression/tolerance by CD4(+)CD25(+) Tregs include the local secretion of cytokines such as tumor growth factor–β and IL-10, and direct cell contact through binding of cell surface molecules such as CTLA-4 (CD152) on Tregs to CD80 and CD86 molecules on effector T cells.

5.9 MYELOID CELLS

Myeloid and lymphoid lineages both are involved in dendritic cell formation. Myeloid cells include monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, and megakaryocytes to platelets. Lymphoid cells include T cells, B cells, and natural killer cells.

5.10 THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

The major histocompatibility complex (**MHC**) is a set of genes that code for cell surface proteins essential for the acquired immune system to recognize foreign molecules in vertebrates, which in turn determines histocompatibility.

MHC molecules is to bind peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T cells. The consequences are almost always deleterious to the pathogen—virus-infected cells are killed, macrophages are activated to kill bacteria living in their intracellular vesicles, and B cells are activated to produce antibodies that eliminate or neutralize extracellular pathogens. Thus, there is strong selective pressure in favor of any pathogen that has mutated in such a way that it escapes presentation by an MHC molecule.

Two separate properties of the MHC make it difficult for pathogens to evade immune responses in this way. First, the MHC is polygenic: it contains several different MHC class I and MHC class II genes, so that every individual possesses a set of MHC molecules with different ranges of peptide-binding specificities. Second, the MHC is highly polymorphic. Without these, there would be no presentation of internal or external antigens to the T cells. The importance of MHC proteins is that they allow T cells to distinguish self from non-self. In every cell in your body, antigens are constantly broken up and presented to passing T cells. Without this presentation, other aspects of the immune response cannot occur.

Class I MHC proteins (found on all nucleated cell surfaces) present antigens to cytotoxic T lymphocytes (**CTLs**). Most CTLs possess both T-cell receptors (**TCR**) and CD8 molecules On their surfaces. These TCRs

are able to recognize peptides when they are expressed in complexes with MHC Class I molecules.

The MHC Class II proteins (found only on B lymphocytes, macrophages, and other cells that present antigens to T cells), which primarily present peptides which have been digested from external sources, are needed for T-cell communication with B-cells and macrophages. Class II MHC proteins presenting antigens are detected by a different group of T cells (called T-helper or TH cells) to Class I MHC proteins (which are detected by CTLs cells).

The MHC proteins, and several closely associated with them in the carrying out of their functions, are coded for by loci that are close together within the Human Genome. Major Histocompatibility Complex proteins and their associated molecules are fundamental in the process of antigen presentation.

5.11 LET US SUM UP

In this unit you have learnt about the meaning, need, objectives and important concept of antigen processing and presentation, Antigen presentation, MHC class I presentation, MHC class I polymorphism, MHC class II presentation, MHC class II polymorphism, **subsets of T cells** Bottom of Form memory cells, suppressor cell T regulatory/suppressor cells myeloid cells the major histocompatibility complex (MHC) .It may helpful to antigen processing and presentation, Antigen presentation, MHC class I presentation, MHC class I polymorphism, MHC class II presentation, MHC class II polymorphism, **subsets of T cells** Bottom of Form memory cells, suppressor cell T regulatory/suppressor cells myeloid cells the major histocompatibility complex (MHC).

5.12 UNIT END EXERCISES

1. What are the primary lymphoid organs?
2. What are the types of immune cells?

5.13 ANSWERS TO CHECK YOUR PROGRESS

1. (i) Major Histocompatibility Complex.

   (ii) Peptide-loading complex

(iii) Antigen-presenting cell

(iv) ER-associated protein degradation

2. Once created, these antibodies are released into the bloodstream. Then the antibodies find the disease and destroy it. If a disease that was not previously encountered is introduced into the body, then the immune system (hopefully) destroys it and the memory cells record how it was done.

5.14 SUGGESTED READINGS.

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BLOCK II: IMUNITY AND IMMUNE RESPONSE

UNIT VI IMMUNITY, TYPES OF IMMUNITY, INNATE, ADAPTIVE IMMUNITY

Structure

6.1 INTRODUCTION

In biology, immunity is the balanced state of multicellular organisms having adequate biological defenses to fight infection, disease, or other unwanted biological invasion, while having adequate tolerance to avoid allergy, and autoimmune diseases.

6.2 OBJECTIVES

1. Describe the path physiology of the following:

a. Immune system

b. Hepatitis

c. Influenza

d. Herpes zoster virus

e. Human Immunodeficiency Virus (HIV)

f. Anaphylaxis

2. Recognize risk factors for altered immune system functioning.
3. Recognize when an individual has altered immune system functioning, either suppressed or exaggerated.
4. Identify appropriate nursing and collaborative interventions to optimize the immune response and minimize the complications of an altered immune system.
5. Describe the path physiology of viral and retroviral infections.
6. Identify core drug knowledge and core patient variables relevant to drugs used to treat suppressed or exaggerated immune responses, viral and retroviral infections.
7. Relate the interaction of core drug knowledge to core patient variables for drugs used to treat suppressed or exaggerated immune responses, viral and retroviral infections.

6.3 DEFINITION

Immunity is the ability of the body to protect against all types of foreign bodies like bacteria, virus, toxic substances, etc. which enters the body. Immunity is also called disease resistance. The lack of immunity is known as susceptibility. The science dealing with the various phenomena of immunity, induced sensitivity and allergy is called immunology.

6.4 TYPES OF IMMUNITY

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

6.4.1 Innate or Natural or Nonspecific Immunity

As its name nonspecific suggests that it lacks specific responses to specific invaders. Innate immunity or nonspecific immunity is well done by providing different barriers to the entry of the foreign agents into our body. Innate immunity consists of four types of barriers namely physical, physiological, cellular and cytokine barriers.

(a) Physical Barriers:

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

(i) Skin

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

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.

(b) 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,
- ✓ Sebum (sweat). It forms a protective acid film over the skin surface that inhibits growth of many microbes.

Check your progress

Note: write your answer in the space given below

What is innate immunity?

……..

(c) Cellular Barriers:

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

(i) Certain Leucocytes:

Neutrophils and monocytes are major phagocytic leucocytes.

- ❖ **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.

❖ **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.

6.4.2 **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 macrophages and (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

6.4.3 Natural Killer Cells (NK Cells):

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: 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. 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. Thus functions of NK cells are to destroy target cells by cytolysis and apoptosis. NK cells constitute 5%-10% of the peripheral blood lymphocytes in humans.

(i) 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.

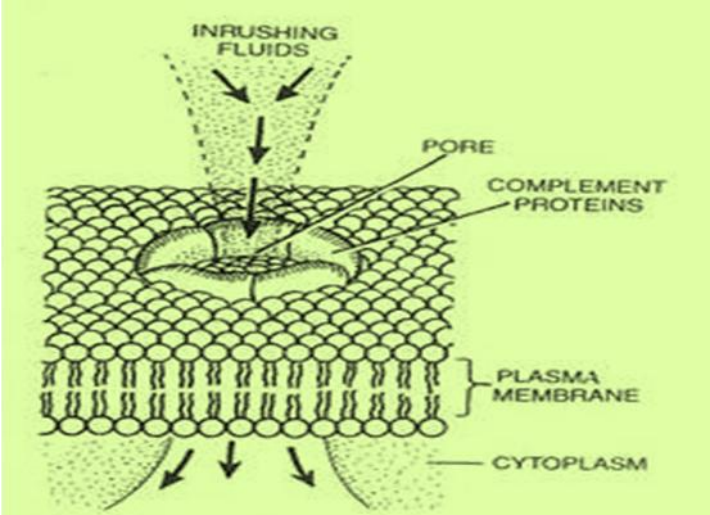


Fig. 8.7. Complement proteins creating a hole in the plasma membrane.

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 (i) cytolysis (ii) inflammation and (iii) phagocytosis. These proteins also prevent excessive damage of the host tissues.

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.

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.

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, tumour necrosis factor and interferon's (IFNs). Interferon's protect against viral infection of cells.

6.5 Types of innate immunity:

1. Species immunity
2. Racial immunity
3. Individual immunity

6.5.1 Species immunity:

- If one species is resistant to certain infection and the other species is susceptible to the same infection then it is called as species immunity.
- Anatomic, physiological and metabolic differences between species determine species immunity. For example, Birds are resistant to anthrax but Human are susceptible. It is simply because higher body temperature of birds kills *Bacillus anthracis*.
- Anatomic differences between species also determine species immunity. For example, Human are more susceptible to skin infection whereas Cattles are more resistant to the same skin infection. It is because of tough and hairy skin (hides) of Cattles.

6.5.2 Racial immunity:

- If one race is susceptible while other race is resistant to same infection, then it is called racial immunity.

- For examples; certain African race are more resistant to malaria and yellow fever where are Asian or Americans are susceptible to same infection. Similarly Orientals (East Asia) are relatively resistant to syphilis.
- Racial immunity is determined by difference in Socio-economic status, habitat, culture feeding habits, environments, genetic, etc.

6.5.3 Individual immunity:

- If one individual of certain race or cast is resistant while other individuals of same race or cast are susceptible to certain infection, then it is called as individual immunity
- Individual immunity is determined by various factors such as health status, nutritional status, previous illness, personal hygiene, genetic differences etc.
- For examples; Individual with genetic deficiency of glucose-6 phosphate dehydrogenase are resistant to Malaria.

6.6 ACQUIRED OR DEVELOPED IMMUNITY OR ADAPTIVE IMMUNITY.

- Immunity which is developed later in life after microbial infection in host is called as Acquired or developed immunity. For example, if an individual is infected with chicken pox virus, he/she become resistant to same virus in later life.
- Acquired immunity is provided by Antibodies and certain T-lymphocytes.
- Components of acquired immunity such as Antibodies and T- cells are specific to particular microorganism. Therefore acquired immunity is also known as Specific immunity.

6.6.1 Types of acquired immunity:

1. Active immunity
2. Passive immunity

(i) Active immunity:

- If host itself produces antibodies, it is called active immunity.
- It is of two types; artificial active immunity and natural active immunity.
- **Artificial active immunity:** Immunity provided by vaccination.
- **Natural active immunity:** immunity provided by natural infection.

(ii) Passive immunity:

- If host does not produce antibodies itself but antibodies produced in other host provides immunity, than it is known as Passive immunity.
- It is of two types; natural passive immunity and Artificial passive immunity
- **Natural passive immunity:** IgG antibody produced in mother cross placenta and protects fetus up to 6 month old age.
- **Artificial passive immunity:** if preformed antibody are injected into host for immunity. Eg. Anti-venom, Rabies vaccine (* it is not a vaccine, it is preformed anti rabies antibody)

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.

6.6.2 Characteristics of Acquired immunity:

- Specificity
- Self/non-self recognition
- Immunological memory
- Diversity

(i) Specificity

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

(ii) Diversity

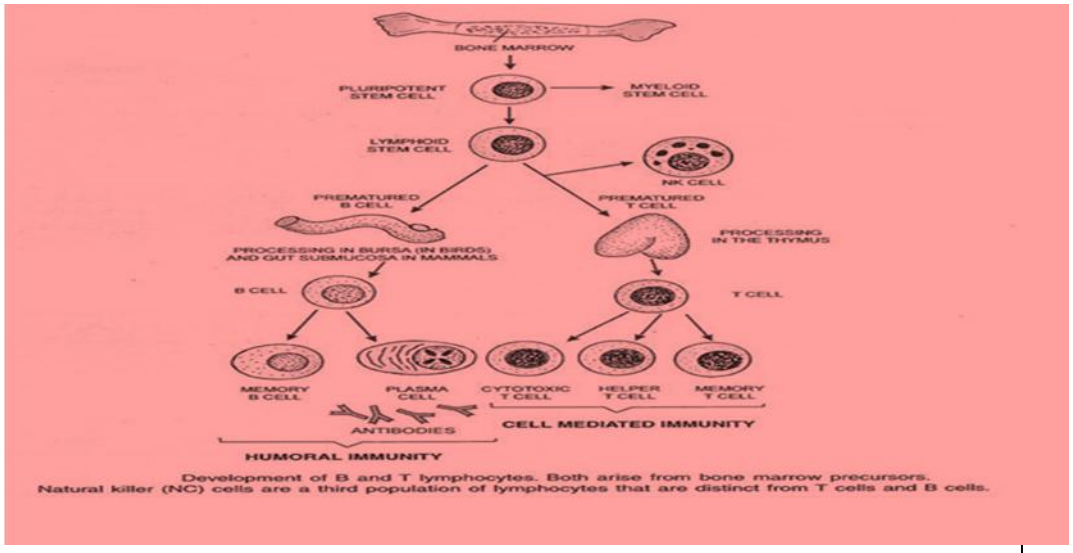
It can recognise a vast variety of foreign molecules (foreign antigens).

(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.



Development of B and T lymphocytes. Both arise from bone marrow precursors. Natural killer (NC) cells are a third population of lymphocytes that are distinct from T cells and B cells.

6.7 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).

6.7.1 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.

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

6.7.3 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.

6.8 Types of T-cells and their Functions

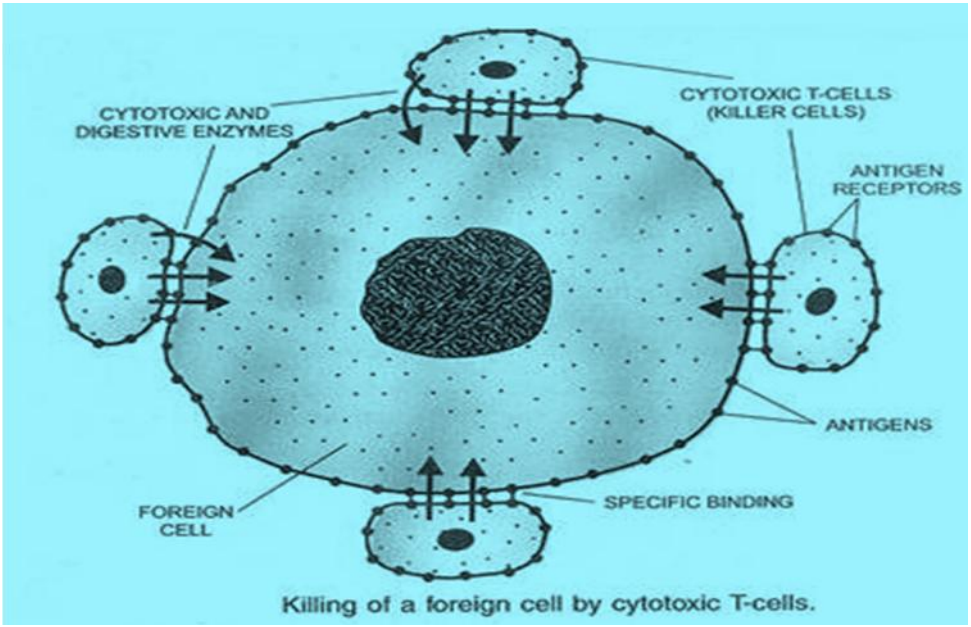
6.8.1 Helper T cells (T_H)

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.

6.8.2 Cytotoxic T cells (Tc) 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 intestinal 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.



Killing of a foreign cell by cytotoxic T-cells.

6.8.3 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.

Check your progress

Note: write your answer in the space given below

What is function of T_H cells?

……

6.8.4 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.

6.8.5 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.

6.9 Determinants of Acquired Specific Immunity.

6.9.1 Form

The natural ability of antigen to induce an immune response may be enhanced by altering or mixing it with another substance known as adjuvant. The antigen can be absorbed on a mineral gel such as aluminium hydroxide or aluminium phosphate e.g. Alum precipitated antigen. Such particulate form of antigen seems to be able to initiate antibody production much more effectively than the same antigen in non-particulate form. The effect is not yet fully understood, but it may be due to the direct effect of the particulate antigen on the lymphoid cell membrane leading to more effective transformation of the cell for antibody formation than that can be brought about by antigen in solution. Another method has been developed to enhance antibody response i.e. preparation of a water-in-oil emulsion. The emulsion forms a depot of antigen in the tissues from which small quantities of antigen are released continuously, sometimes for a year or more. Example,Influenza vaccine.

6.9.2 Route:

If an antigen is given intravenously, most of the antibody is produced by spleen, and some in lung, bone marrow, if given subcutaneously or intradermal the antigen travels via lymphatic's to local lymph nodes where antibody is produced initially.

6.9.3 Dose:

The antibody response is proportionate to the dose of antigen. If the antigen dose is increased, the immune response is small, so if a particular level of antigen is increased then there will be specific paralysis of the antibody forming tissues Immunological tolerance.

6.10 LET US SUM UP

In this unit you have learnt about the meaning, need, objectives and important concept of primary and secondary lymphoid organ. It may helpful to differentiate primary lymphoid organ and secondary lymphoid organ.

6.11 UNIT END EXERCISES

1. Determinants of Acquired Specific Immunity
2. What about the function of T_H helper cells?

6.12 ANSWERS TO CHECK YOUR PROGRESS

1. Immunity is the ability of the body to protect against all types of foreign bodies like bacteria, virus, toxic substances, etc. which enters the body. Immunity is also called disease resistance.

2. 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.

6.13 SUGGESTED READINGS

1. Matthew Helbert ,Immunology For Medical Students. Elsevier, 2016

2.Sunil Kumar Mohant, Dr.Sai Leela Text book of immunology Jaypee Brothers Medical Publishers (P) Ltd.Second edition 2014.

3. “Immunology” by Roitt I and Male Brost off. Mosby-Year Book; 4th edition (January 1996)

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UNIT VII IMMUNE RESPONSE: TYPES OF IMMUNE RESPONSE EFFECTOR MECHANISM OF HUMORAL AND CELL MEDIATED IMMUNE RESPONSES

Structure

7.1 INTRODUCTION

Many of the bacteria that cause infectious disease in humans multiply in the extracellular spaces of the body, and most intracellular pathogens spread by moving from cell to cell through the extracellular fluids. The extracellular spaces are protected by the humoral immune response, in which antibodies produced by B cells cause the destruction of extracellular microorganisms and prevent the spread of intracellular infections.

7.2 OBJECTIVES

- Differentiate between innate and adaptive immunity.
- State the origin of cells of the immune system in terms of myeloid or lymphoid origin. Identify the tissues that contribute to immunity
- Describe the roles of cytokines, chemokines, and colony-stimulating factors in the immune response

7.3 Types of immune response

Once the non-specific barriers to infection have been breached, the specific immune responses to pathogens come into play: acquired immunity .Hallmarks of acquired immunity include-**specificity** (discrimination between self and non-self) and **memory** (rapid response to previously encountered antigen).

7.3.1 Two types of immune response

(i) Humoral immune response - Serum transfer, antibodies

(ii) Cellular immune response - T lymphocytes and activated macrophages

(iii) Antibody soluble proteins produced by B cell, they interact with specific antigens

(iv) Antigen a molecule capable of interacting with components of the immune systems (antibodies or immune cells)

(v) Immunogen

Molecule capable of inducing an immune response (immunogens are antigens but not all antigens are immunogens). Immunogens include proteins, glycoproteins and lipoproteins, many polysaccharides, some nucleic acids and techoic acids. Most antigens or immunogens have multiple antigenic determinants; the portion of the molecule that is actually recognized by the Ab or cell - for a protein ~ 4 - 6 amino acids. All are derived from precursor cells in the bone marrow, immune responses usually involve the interaction of more than one cell, the cells communicate through direct contact or through secreted signals - cytokines (lymphokines are cytokines produced by lymphocytes).

Large Phagocyticcells. Play a central role in the immune response. They not only phagocytize and destroy pathogens and foreign material, but also process part of the ingested antigen and place it back on the surface of the macrophage. Thus they are one of the major antigen-presenting cells (APC). The antigen (either ingested or, in the case of viruses, produced internally) is bound to a molecule of MHC (major histocompatibility complex, marks the cell as "self") class I (internal peptides) or class II (Exocytose external peptide) and presented on the surface of the cell, where it can be seen by other cells or molecules of the immune system.

(vi) B cells

Antibody producing cells.Are responsible for the production of the soluble antibodies which play a role in production against a variety of infections. B cells have antibody on their surface, if the corresponding antigen is bound, cell division - clonal expansion and formation of **plasma cells** and **memory cells**. B cells mature in bone marrow and peripheral lymphoid tissue (lymph nodes, spleen, gut-associated lymphatics)

(vii) T cells

T cells include both effector and regulator cells. They react with antigens but use a T cell receptor (TCR) rather than antibody. TCRs, like the antibodies on B cells, are highly specific, and there are probably as much specificity for TCRs as for Ab. However, the structure of the molecule is different, and TCRs recognize antigen associated with MHC.

Different T cell types can be distinguished by different surface markers: CD4, CD8 and other markers. TH - helper T cells, CD4 - stimulate other cells, esp. B cells, to enhance the immune response, TS - supressor T cells, CD8 - down regulation, TC - cytotoxic T cells, CD8 - recognize and kill infected host cells, TD - delayed type hypersensitivity cells, CD4, recruit and activate non- specific effector cells, esp. macrophages. T cells mature in the thymus (where many self clones are eliminated) before migration to peripheral lymphatics, recirculate through blood and lymph

Check your progress

Note: write your answer in the space given below

What is T cells role?

…….

7.4 Humoral immunity: B cell response

Structure and classes of antibodies antibodies are found on surface of B-cells as membrane bound markers and are secreted Antibody binds to antigen - **affinity** refers to how well they fit **avidity** refers to the total binding strength (i.e. depends on valence as well as affinity)

IgG - major secreted Ab
IgA - secretions on body surfaces
IgM - multivalent
IgD - B cell receptor
IgE - usually bound to mast cells or basophils, allergic response

IgM on the surface first, then IgD on mature B-cells. A single B cell switches class but all the antibody produced has the same antigen binding site. The antigen on the surface and the antibody secreted by the cell have the same specificity. Generation of diversity-millions of different specificities available, binding of antigen selects that clone and allow it to expand. If 1 gene = 1 antibody, enormous numbers of genes would be required. Instead, DNA rearrangement of a limited number of genes is used

to create diversity. Probably even greater diversity of T-cell receptors. Ab made, predominantly IgM made in response to first exposure to Ag, some IgG. On second and subsequent exposure IgM pattern the same but more rapid and quantitatively greater IgG response.

1. Shorter lag
2. Ab produced at a faster rate and is more persistent
3. Ab concentration higher at peak response
4. IgG rather than IgM predominates
5. Ab's produced have a higher affinity for Ag

Stimulation of B cells and production of Ab involves a complex series of events.Ag (e.g. influenza virus) --phagocytosis - processing by macrophage which "presents" Ag to B and T cells. Some B cells mature to plasma cell - some divide to produce memory cells.If Ag binds to B cell with low affinity - more Ag required stimulating division - thus higher affinity Ab's tend to predominate. This example shows response to a single antigen. Pathogens are complex and possess many antigenic determinants, e.g. typical Salmonella.

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

7.4.1 The primary immune response

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

Check your progress

Note: Write your answer in the space given below

Define primary immune response.

……

7.4.2 The secondary immune response

Memory cells may remain in the body for decades. Every new encounter with the same antigen results in a rapid proliferation of memory cells. This is also called “booster response”. The antibody titer after subsequent encounters is far greater than during a primary response and consists mainly of IgG antibodies. This accelerated, more intense response is called the secondary immune response. Antibodies produced during a secondary response have an even higher affinity for the antigen. A person who had been suffering from diseases like measles, small pox or chicken pox becomes immune to subsequent attacks of these diseases. It includes

spleen, lymph nodes, tonsils, Peyer's patches of small intestine and appendix. The increased power and duration of the secondary immune response explain why immunization (method of providing immunity artificially, it is called vaccination) is usually accomplished by injecting antigen in multiple doses.

7.5 LET US SUM UP

In this unit you have learnt about the meaning, need, objectives and important concept of types of immune response effector mechanism of humoral and cell mediated immune responses. It may helpful to types of immune response effector mechanism of humoral and cell mediated immune responses.

7.6 UNIT END EXERCISES

1. What is the response of B cell?
2. Explain Primary immune response

7.4 ANSWERS TO CHECK YOUR PROGRESS

1. T cells include both effector and regulator cells. They react with antigens but use a T cell receptor (TCR) rather than antibody. TCRs, like the antibodies on B cells, are highly specific, and there are probably as much specificity for TCRs as for Ab.

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

7.5 SUGGESTED READINGS

1. Matthew Helbert ,Immunology For Medical Students. Elsevier, 2016
2.Sunil Kumar Mohant, Dr.Sai Leela Text book of immunology Jaypee Brothers Medical Publishers (P) Ltd.Second edition 2014.
3. "Immunology" by Roitt I and Male Brost off. Mosby-Year Book; 4th edition (January 1996)
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UNIT -VIII ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC)

Structure

8.1 INTRODUCTION

A type of immune reaction in which a target cell or microbe is coated with antibodies and killed by certain types of white blood cells. The white blood cells bind to the antibodies and release substances that kill the target cells or microbes. Also called ADCC and antibody-dependent

cellular cytotoxicity. Antibody-dependent cellular cytotoxicity also referred to as antibody-dependent cell-mediated cytotoxicity, is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies. It is one of the mechanisms through which antibodies, as part of the humoral immune response, can act to limit and contain infection.

ADCC is independent of the immune complement system that also lyses targets but does not require any other cell. ADCC requires an effector cell which classically is known to be natural killer (NK) cells that typically interact with IgG antibodies. However, macrophages, neutrophils and eosinophils can also mediate ADCC, such as eosinophils killing certain parasitic worms known as helminths via IgE antibodies. ADCC is part of the adaptive immune response due to its dependence on a prior antibody response. The coating of target cells with antibodies is sometimes referred to as opsonization.

8.2 OBJECTIVES

The environment contains a wide variety of potentially harmful organisms (pathogens), such as bacteria, viruses, fungi, protozoa and multicellular parasites, which will cause disease if they enter the body and are allowed to multiply. The body protects itself through a various defence mechanisms to physically prevent pathogens from entering the body or to kill them if they do.

The immune system is an extremely important defence mechanism that can identify an invading organism and destroy it. Immunisation prevents disease by enabling the body to more rapidly respond to attack and enhancing the immune response to a particular organism.

Each pathogen has unique distinguishing components, known as antigens, which enable the immune system to differentiate between 'self' (the body) and 'non-self' (the foreign material). The first time the immune system sees a new antigen, it needs to prepare to destroy it. During this time, the pathogen can multiply and cause disease. However, if the same

antigen is seen again, the immune system is poised to confine and destroy the organism rapidly. This is known as adaptive immunity.

8.3 ADCC steps

First, the B-cell produces antibodies directed against specific antigens present on a pathogen or a foreign body. These antibodies bind to the target antigen and the constant region of the antibody (Fc) is recognized by the effector cells of the immune system via Fc receptors on their surfaces. Following recognition, the effector cells release chemicals which then lyse and kill the target cell bound to the antibody. This mechanism forms a crucial part of the immune response.

8.3.1Activation of ADCC

(i) Specific binding of antibodies

In most cases, the activation of ADCC involves the binding of IgG antibodies to antigens, although infestation by invasive helminthes and some other parasites, IgE antibodies may be bound to the antigen on the pathogen surface.

(ii) Recruitment of immune cells by the Fc fragment of the antibody

The constant region of the antibody can recruit different kinds of immune cells, including granulocytes such as neutrophils, monocytes, eosinophills, as well as macrophages and natural killer cells.

(iii) Effector cells

Different kinds of cells are recruited to the site of target cell-antibody reaction following the recognition of the antibody's Fc fragment, and these cells act via different methods to kill the target cell

(iv) Macrophages

Macrophages are involved in killing the target cell by releasing lytic and toxic enzymes at the target site.

Check your progress

Note: write your answer in the space given below

What is macrophage function?

……..

8.4 Natural killer cells, or NK cells

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

They are the type of cytotoxic lymphocyte critical to the innate immune system. The role NK cells play is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to virus-infected cells, acting at around 3 days after infection, and respond to tumor formation. Typically, immune cells detect the major histocompatibility complex (MHC) presented on infected cell surfaces, triggering cytokine release, causing lysis or apoptosis. NK cells are unique. As they have the ability to recognize stressed cells in the absence of antibodies and MHC,allowing for a much faster immune reaction. They were named "natural killers" because of the initial notion that they do not require activation to kill cells that are missing "self" markers of MHC class 1. This role is especially important because harmful cells that are missing MHC I markers cannot be detected and destroyed by other immune cells, such as T lymphocyte cells.

NK cells belonging to the group of innate lymphoid cells are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor-generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they enter into the circulation. NK cells differ from natural killer T cells (NKTs) phenotypically, by origin and by respective effector function. NKT cell activity promotes NK cell activity by secreting interferon gamma. In contrast to NKT cells, NK cells do not express T-cell antigen receptors (TCR) or T marker CD3 or surface immunoglobulins (Ig) B cell receptors, but they usually express the surface markers CD16 (FcγRIII) and CD56 in humans.

Self - Instructional Material

8.5 Mechanisms of cytotoxicity

It is now established that Fc receptors need to be cross linked to initiate the signaling cascade which leads to cytotoxicity. Binding of

monomeric IgG is not sufficient. This process of crosslinking is based on the recognition of several signals which in turn depend upon the antibody and the Fc receptor, other signaling molecules, and the effector cells.

Check your progress

Note: write your answer in the space given below

Define mechanism of cytotoxicity?

……..

8.6.1Calcium-dependent processes

In certain processes, after the target is recognized, a calcium-dependent exocytic cascade is activated which leads to the release of intracellular granules from an effector cell which target the pathogen. One such molecule is perforin, which attaches to the cell membrane and forms a pore. The subsequent leakage of intracellular material and ionic imbalance leads to cell lysis. Another is granzyme B, an enzyme which is involved in DNA fragmentation within target cells.

8.6.2 Engagement of Fas

Some target cells express the Fas antigen, which is then engaged by a host cell molecule called Fas ligand. This activates a cascade of signaling which ultimately leads to cell death mediated by NK cells.

8.6.3 Release of reactive oxygen species

Certain effectors cells, such as monocytes, macrophages, and neutrophils can release reactive oxygen species when the Fc receptors are cross-linked. The reactive oxygen species then lead to the death of the target cell.

8.6.4 Perspectives

ADCC is a critical component of host immunity and some of the antibodies specifically directed against tumor cells have also been shown to play a role in treating tumors in mice models in vivo.

ADCC is invoked when antibodies bind with both the antigen present on the target cell and Fc receptors on the effectors cells of the host. This process ultimately leads to the killing of the pathogenic target cell by the host.

8.6.5 Natural killer cells

The typical ADCC involves activation of NK cells by antibodies. A NK cell expresses Fc receptors, mostly CD16. These receptors recognize, and bind to, the Fc portion of an antibody, such as IgG, which has bound to the surface of a pathogen-infected target cell. The most common Fc receptor on the surface of an NK cell is called CD16 or FcγRIII. Once the Fc receptor binds to the Fc region of IgG, the Natural Killer cell releases cytotoxic factors that cause the death of the target cell.

During replication of a virus some of the viral proteins are expressed on the cell surface membrane of the infected cell. Antibodies can then bind to these viral proteins. Next, the NK cells which have Fc Receptors will bind to that antibody, inducing the NK cell to release proteins such as perforin and proteases known as granzymes, which causes the lysis of the infected cell to hinder the spread of the virus.

Furthermore, NK cells are involved in killing tumor cells and other cells that may lack MHC I on their surface, indicating a non-self cell. This is because, generally, all nucleated cells (which excludes RBCs) of the body contain MHCI.

8.7 IMMUNITY TO INFECTIOUS DISEASES

Whether an infectious disease agent is an “old acquaintance” or a new, emerging threat, the immune system’s battle against it is usually the first line of defense it encounters. With vaccines and effective treatments often unavailable, the immune system’s efforts to eradicate infectious

agents or infected cells are frequently the only means to combat them when the body is exposed to viruses, bacteria, fungi, or parasites through an infection or vaccination the immune system creates antibodies and immune cells that inactivate or destroy the specific infectious organism. If we encounter the same organism in the future, the immune system "remembers" that previous exposure and can mount a vigorous defense.

We gain specific immunity in several ways. We gain temporary immunity to some diseases by acquiring antibodies directly from our mothers when we are in the womb. Throughout life, we gain specific immunity as we are exposed to new organisms. Infections create memory cells that can protect us from future infection from the same or related organisms. Vaccines stimulate the same process.

8.7.1 Infection

The immune system recognizes foreign proteins or complex sugars by their unique shapes. One key to specific immunity lies in the body's ability to manufacture large numbers of antibodies that match each of these shapes. Antibodies swarm the infecting organisms, attaching themselves to the foreign organisms and blocking the ability of the organism to attach to human cells. Immune system cells use the attached antibodies as handles to grasp the infectious organisms for destruction or removal.

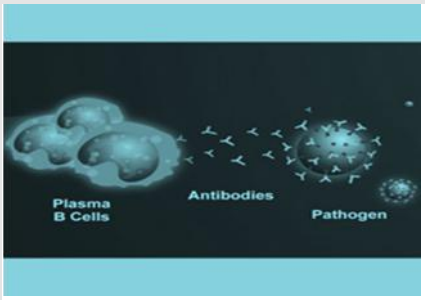
8.7.2 Vaccination

Vaccines present a pathogenic organism's proteins or complex sugars to the immune system. A vaccine stimulates the natural immune response, creating antibodies and a memory of the infection without the danger of an actual infection. The memory helps protect us if we are ever exposed to the pathogen in the future.

8.7.3 Future Exposure

If a person is exposed to the same disease in the future, the body already has a memory of the infection and can quickly fight off the infection.

Immunity to infectious microorganisms can be achieved by active or passive immunization. In each case, immunity can be acquired either by natural processes usually by transfer from mother to fetus or by previous infection by the organism or by artificial means, such as injection of antibodies or vaccines. The agents used for inducing passive immunity include antibodies from humans or animals, whereas active immunization is achieved by inoculation with microbial pathogens that induce immunity but do not cause disease or with antigenic components from the pathogens.



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

8.8 Non-Specific (Innate) Immunity

The human immune system has two levels of immunity: specific and non-specific immunity. Through non-specific immunity, also called innate immunity, the human body protects itself against foreign material that is perceived to be harmful. Microbes as small as viruses and bacteria can be attacked, as can larger organisms such as worms. Collectively, these organisms are called pathogens when they cause disease in the host. All animals have innate immune defenses against common pathogens. These first lines of defense include outer barriers like the skin and mucous membranes. When pathogens breach the outer barriers, for example through a cut in the skin or when inhaled into the lungs, they can cause serious harm. Some white blood cells (phagocytes) fight pathogens that

make it past outer defenses. A phagocyte surrounds a pathogen, takes it in, and neutralizes it.

8.9 Specific Immunity

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

While healthy phagocytes are critical to good health, they are unable to address certain infectious threats. Specific immunity is a complement to the function of phagocytes and other elements of the innate immune system. In contrast to innate immunity, specific immunity allows for a targeted response against a specific pathogen. Only vertebrates have specific immune responses. Depending on the infectious disease, symptoms can vary greatly. Fever is a common response to infection: a higher body temperature can heighten the immune response and provide a hostile environment for pathogens. Inflammation, or swelling caused by an increase in fluid in the infected area, is a sign that white blood cells are on the attack and releasing substances involved in the immune response.

8.10 IMMUNOPROPHYLAXIS

Human immunoglobulins Animal sera Monoclonal antibodies Active immunization (vaccination) Principles of vaccination Vaccine types, their properties and mechanisms of action Prophylaxis of infectious diseases represents the actions taken before or shortly upon exposure of an individual to an infectious agent or its product (e.g. toxin), aimed to prevent infection and disease development. The most important prophylactic method is immunoprophylaxis in which a process of immunization is used for preventing infections. The goal of immunization is to induce immunity (i.e. state of resistance to an infection) in an immunized person for a certain period of time (that may vary from several weeks to several decades). Immunization can be achieved spontaneously without any intentional human activity (called natural immunization) or by a deliberate action of men (so-called artificial immunization), and both of them can be actively induced in an individual by exposure to a pathogen or its components or products (active immunization) or passively adopted through immunoglobulin transfer (passive immunization). Natural immunity is actively induced after each infection, whereas the neonatal protection by maternal antibodies transported across the placenta to the

Self - Instructional Material

fetus (IgG) and via immunoglobulins in milk (breast feeding - predominantly IgA) is an example of natural passive immunization. On the other hand, artificial active immunization is most commonly done by exposure of an individual to nonpathogenic forms or microbes or their components and/or products, a process called vaccination, while artificial passive immunization represents the induction of immunity through administration of human immunoglobulins or animal sera specific for the pathogen or its toxins.

Immunoprophylaxis is potentially of greater value than chemoprophylaxis because the approach would be less complex and would be most likely to prevent late-onset as well as early-onset disease. Passive Immunization of antibody-deficient women or newborns with intravenous gamma globulin is a reasonable consideration. Given that transplacental transfer of antibody does not occur until after the 34th week of gestation, this approach requires timing late in pregnancy. Passive immunization occurs naturally in humans when maternal antibodies of the immunoglobulin G (IgG) class are passed to neonates. Such antibodies provide protection against many communicable bacterial and viral diseases for a period of months, during the period when the immune system has not yet fully developed.

Immunoprophylaxis is also the prevention of disease by the production of active or passive immunity. The incidence of diseases, such as diphtheria, measles, mumps, pertussis (whooping cough), rubella (German measles), poliomyelitis, and tetanus, has declined dramatically as vaccination has become more common.

Immunologic approaches to the prevention and treatment of bacterial infections date back to the antecedent century. Recent interest and controversy has centered about the efficacy of gram-negative bacillary vaccines, antiserum against endotoxin, and pneumococcal vaccines. Immunization of cancer patients with Pseudomonas lipopolysaccharides vaccines has yielded Inconsistent results. Factors limiting the further application of this approach are the poor immune responses in neutropenic patients and the marked pyrogenicity.

Similarly, patients being treated for neoplasms of the hematopoietic system are not likely to show good antibody responses to pneumococcal antigens, even though they are not toxic. Pneumococcal immunization appears to be effective, at least as measured in terms of antibody titers, in those patients with lymphoma who have not undergone splenectomy and are not receiving chemotherapy at the time of immunization. The most reliable approach towards immuno prophylaxis may be the passive one, with antibody being produced in normal donors. The antibodies are short-lived, and this type of prophylaxis still needs to be evaluated in controlled trials. In a recently completed controlled therapeutic trial, the therapeutic application of an antiserum against core end toxin antigens resulted in a significant reduction in deaths and increased recovery from shock complicating gram-negative sepsis.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

Vaccination is a cost-effective weapon for disease prevention. Use of vaccines has contributed solely in the eradication of small-pox, one of mankind's long-standing and most terrible diseases. Since October 1977, not a single naturally acquired smallpox case has been reported anywhere in the world. Other diseases like diphtheria, pertussis, tetanus, measles, mumps, rubella, and poliomyelitis, also known as "vaccine preventable diseases" have been successfully brought down to negligible levels in most developed nations and in some cases in the developing nations as well.

8.11 VACCINES AND IMMUNIZATION SCHEDULE

According to the World Health Organization (WHO), "immunization is the process whereby a person is made immune or resistant to an infectious disease, typically by the administration of a vaccine. Vaccines stimulate the body's own immune system to protect the person against subsequent infection or disease."A person becomes immune to a disease when the body has been exposed to it either through illness or vaccination. The immune system develops antibodies to the disease so that it cannot make you sick again. Immunization describes the actual changes your body goes through after receiving a vaccine.

Self - Instructional Material

8.11.1 Passive Immunization

Artificial passive immunization is mediated by the administration of antibodies of human or animal origin to an individual. These products have immediate action, but induce short-lived immunity that lasts weeks to several months, which is determined by the half-life of immunoglobulins (around three weeks for IgG and only few days for the other isotypes). In general, passive immunization is used for prophylactic purposes, to protect immuno deficient patients (prematurely born children or patients with defects in humoral immunity) against various infections or to prevent disease development after exposure of an individual to a particular pathogen (for example, after accidental injury by the contaminated needle or bite by the animals infected by rabies virus). However, in some cases, passive immunization (owing to its immediate action) can also be used for therapy with the aim to reduce the clinical symptoms of the disease and often represents the life-saving therapeutic method. For example, it is used for toxin-mediated diseases such as diphtheria 3 and botulism, or when the person is exposed to some toxins from animals, such as snake venoms.

Human serum globulin (gammaglobulin or intravenous immunoglobulins, IVIG) contains antibodies that are specific for various pathogens that are commonly encountered by the majority of people and are present in the blood of most adults (the normal repertoire of antibodies in human population). They are extracted from the plasma of thousands of randomly selected blood donors. These products are used for the prophylaxis of infectious diseases in patients with a deficit in antibody production (hypo- or Agammaglobulinemia. Also, they are sometimes used for prevention of specific infectious diseases (e.g. measles, hepatitis A, rubella in the first trimester of pregnancy etc. Specific immunoglobulins or high-titer immunoglobulins (also called hyperimmune globulins) contain high titer of antibodies specific for particular pathogen and they are used in the prophylaxis or therapy for specific infectious disease.

Check your progress

Note: write your answer in the space given below

Differentiate Vaccine and immunization?

……..

8.11.2 Active Immunization (Vaccination)

Vaccination is the most commonly used form of artificial immunization aimed to actively induce protective immune response against a certain pathogen in an individual and, in the event of subsequent exposure to that pathogen, prevent disease development in an immunized person. The term vaccine is derived from the Latin word given that the first recorded successful vaccination of a child against smallpox (Edward Jenner, 1796) was carried out using vaccinia virus that causes cowpox. Moreover, the only human disease that has been eradicated by human intervention was smallpox, and this was achieved by a worldwide program of vaccination. Therefore, vaccination is considered to be one of the greatest successes of immunology and medicine in general.

Finally, the vaccine has to fulfill certain practical requirements, such as stability (so that it can be easily transported and last for longer periods), ease of application (advantage of oral and nasal administration in comparison to injection), low price (vaccines should be available in developing countries), etc. In general, vaccines that contain one or few antigens of a pathogen, such as subunit vaccines (see below), are associated with less adverse effects compared with whole-cell vaccines, but are less immunogenic (i.e. induce weaker immune response). There are two ways for overcoming this disadvantage, either by adding adjuvants that increase the immunogenicity of the vaccines or by revaccination (administration of several additional doses, so called booster doses, over a longer period of time). Adjuvants are believed to stimulate innate immunity by acting on dendritic cells and other antigen-presenting cells,

8.12 Vaccine

The US Centers for Disease Control and Prevention defines a vaccine as "a product that stimulates a person's immune system to produce immunity to a specific disease, protecting the person from that disease. Vaccines are usually administered through needle injections, but can also be administered by mouth or sprayed into the nose."

Types of vaccines, their properties and mechanisms of action.All vaccines can be divided, based on their properties, into several groups or types: live, inactivated, subunit, conjugated and combined vaccines (Features of different vaccine types are presented in Table 2).

8.12.1 Live vaccines

Live vaccines are composed of viable microorganisms with limited capacity to induce disease in humans. These strains of pathogens usually infect other animal species (e.g. cow in case of smallpox vaccine) or their virulence has been reduced through a process called attenuation (so called attenuated strains), so these vaccines are also called attenuated vaccines.

8.12.2 Attenuation

It is usually performed by repeated pathogen passage in cell cultures in the absence of host immune mechanisms under conditions that are different from those present in human body (e.g. on lower temperature or in animal cells which normally cannot be infected by that pathogen). During that process, the accumulation of mutations and the adaptation of a pathogen to such new conditions results in a loss of its capacity to induce disease in humans. Recently, new approach of attenuation has been introduced based on genetic manipulation of a pathogen with the goal to induce mutations in genes coding for important virulence factors of that pathogen. Most vaccines against viral diseases belong to a group of live (attenuated) vaccines, such as vaccines against mumps, measles, rubella, chickenpox and polio (oral Sabin polio vaccine), as well as some vaccines against bacterial infections (e.g. BCG vaccine against tuberculosis, containing attenuated strain of Mycobacterium bovis). Special type of live

vaccines is that obtained by genetic recombination or reassortment of homologous gene segments between the related viruses (e.g. vaccine for rotavirus that is made by genetic reassortment between human and bovine rotavirus).

8.12.3 Inactivated vaccines

Inactivated vaccines (also called killed vaccines) contain whole microorganisms that were killed using various chemicals (e.g. formaldehyde) or high temperatures, but their antigenic properties and immunogenicity were preserved. These vaccines are usually used for preventing disease where pathogen cannot be successfully attenuated. Inactivated vaccines 7 are stable and safe (except for the people allergic to vaccine components, e.g. egg) and they act mainly through induction of antibodies.

8.12.4 Subunit vaccines

Subunit vaccines represent a special form of inactivated vaccines. They are composed of structural components of microorganisms or their products (e.g. toxins) that can induce protective immune response in recipients, which is mediated primarily by antibodies, mainly neutralizing. Since they are composed of individual antigens, these vaccines are also called antigenic vaccines. Those antigens are obtained by isolation and purification of pathogen products, or, more often, by using recombinant DNA techniques (as recombinant proteins produced by yeast cells.

8.14 IMMUNIZATION SCHEDULE

Most parents are overwhelmed at the number of vaccines that are recommended for their babies starting just after birth. It may seem like it's too much to give an infant three or four shots at a time every couple of months during the first year of his life.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

Immunization Schedule (NIS) for Infants, Children and Pregnant Women

Vaccine	When to give	Max age	Dose	Diluent	Route	Site
For Children						
DPT Booster-1	16-24 months	7 years of age	0.5 ml	NO	Intra-muscular	Antero-lateral side of mid-thigh – LEFT
Measles / Rubella 2nd dose ##	16-24 months	5 years of age	0.5 ml	YES Manufacturer supplied diluent (Sterile water)	Sub-cutaneous	Upper Arm - RIGHT
OPV Booster	16-24 months	5 Years	2 drops	NO	Oral	Oral
Japanese Encephalitis – 2 @ (Where applicable)	16-24 months @	till 15 years of age	0.5 ml	YES Manufacturer supplied diluent (Phosphate Buffer Solution)	Sub-cutaneous	Upper Arm - LEFT
Vitamin A $ (2nd to 9th dose)	At 16 months. Then, one dose every 6 months.	up to the age of 5 years	2 ml (2 lakh IU)	-	Oral	Oral
DPT Booster-2	5-6 years	7 Years of age	0.5 ml	NO	Intra-muscular	Upper Arm
TT	10 years & 16 years	16 Years	0.5 ml	NO	Intra-muscular	Upper Arm

* Give TT-2 or Booster doses before 36 weeks of pregnancy. However, give these even if more than 36 weeks have passed. Give TT to a woman in labour, if she has not previously received TT.

** Pentavalent vaccine is introduced in place of DPT and HepB 1, 2 and 3.

‡ Rotavirus vaccine is being in troduced in phases.

MR vaccine introduced in phases replacing measles vaccine in the UIP schedule. **If first dose delayed beyond 12 months ensure minimum 1 month gap between 2 MR doses.**

@ JE Vaccine has been introduced in select endemic districts. **If first dose delayed beyond 12 months ensure minimum 3 months gap between 2 JE doses.**

$ The 2nd to 9th doses of Vitamin A can be administered to children 1-5 years old during biannual rounds, in collaboration with ICDS.

- Human Papilloma Virus (HPV) Vaccine – presently not in schedule.
- Td - Tetanus diphtheria to replace TT - to be added in schedule

Self - Instructional Material

8.15 LET US SUM UP

In this unit you have learnt about the meaning, need, objectives and important concept of types of mechanisms of cytotoxicity, mechanism of cell destruction, Calcium-dependent processes**,** Engagement of Fas Release of reactive oxygen species**,** Perspectives Natural killer cells immunity to infectious diseases. It may helpful to types of immune response of vaccine and Immunization cell mediated immune responses.

8.16 UNIT END EXERCISES

1. What is the vaccination?
2. Explain innate immunity

8.17 ANSWERS TO CHECK YOUR PROGRESS

1. It is now established that Fc receptors need to be cross linked to initiate the signaling cascade which leads to cytotoxicity. Binding of monomeric IgG is not sufficient. This process of cross linking is based on the recognition of several signals which in turn depend upon the antibody and the Fc receptor, other signaling molecules, and the effector cells.

2. Vaccines stimulate the body's own immune system to protect the person against subsequent infection or disease."A person becomes immune to a disease when the body has been exposed to it either through illness or vaccination. The immune system develops antibodies to the disease so that it cannot make you sick again. Immunization describes the actual changes your body goes through after receiving a vaccine.

8.18. SUGGESTED READINGS

1. Matthew Helbert, Immunology For Medical Students. Elsevier, 2016

2. Sunil Kumar Mohant, Dr.Sai Leela Text book of immunology Jaypee Brothers Medical Publishers (P) Ltd.Second edition 2014.

3. "Immunology" by Roitt I and Male Brost off. Mosby-Year Book; 4th edition (January 1996)

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5. The Elements of Immunology” by Fahim Halim Khan. Pearson Education India, 2009.

BLOCK – III: IMMUNE DISORDERS

UNIT IX INFECTIOUS DISEASES; HYPERSENSITIVITY – TYPE I, II, III AND IV.

Structure

9.1 Introduction

The response of the host to the presence of foreign substances can trigger four types of hypersensitivity reactions:

- Immediate
- Cytotoxic
- Immune complex
- Cell-mediated

Type I: **Immediate and anaphylactic reaction**: The main mediator is IgE. Examples include Hay fever, allergic asthma food allergies, anaphylactic shock. They result from antibody antigen reaction.

Type II: **Cytotoxic:** Including transfusion reactions, hemolytic disease of the new born. The main mediators are IgM, IgG and complement.

Type III: **Mediated by immune complexes:** Examples Intrinsic allergisalveolitis, serum sickness. The principal agents are antigen/antibody complexes like IgG.

Type IV: **Delayed Type Hypersensitivity:** Examples are Mantoux Reaction, Contact hypersensitivity. The main role is played by sensitized $CD4^{+}$T lymphocytes.

Frequently, a particular clinical condition disease may involve more than one type of reaction.

9.2 Objectives:

Identify and compare the distinguishing characteristics, mechanisms, and major examples of type I, II, III, and IV hypersensitivities.

- Discuss the prevalence of dentinal hypersensitivity and common contributing factors.
- Explain the hydrodynamic theory, widely accepted as the cause for dentinal sensitivity.
- Discuss common diagnostic tools.
- List common ingredients used in at-home and in-office desensitizing products.
- Discuss the mode of action of common ingredients.
- Discuss the clinical evidence behind common treatment approaches including in-office treatments.

9.3 TYPES OF HYPERSENSITIVITY REACTIONS

Hypersensitivity:

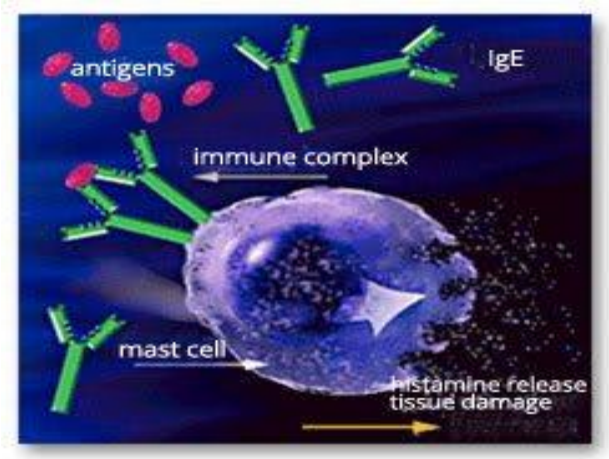
Hypersensitivity reactions are the commonest type among all types which is mainly induced by certain type of antigens i.e. allergens. Actually anaphylaxis means “Opposite of protection” and is mediated by IgE antibodies through interaction with an allergen.

9.3.1 Type I: Immediate Hypersensitivity (Anaphylactic Reaction)

These allergic reactions are systemic or localized, as in allergic dermatitis (e.g., hives, wheal and Erythema reactions). The reaction is the

result of an antigen cross-linking with membrane-bound IgE antibody of a mast cell or basophile. Histamine, serotonin, bradykinin, and lipid mediators (e.g., platelet activating factor, prostaglandins, and leukotrienes) are released during the anaphylactic reaction. These released substances have the potential to cause tissue damage.

Figure 9.1 These reactions are IgE mediated



The IgE antibodies are formed to an antigen (or allergen), with an individual's tendency towards making IgE being determined by many factors including genetic, T cell responsiveness and antigenic burden. The IgE binds to high-affinity IgE receptors on the surfaces of mast cells and basophils, and these cells are now primed to react the next time the cells come into close proximity with the allergen. The cross-linking of IgE on the cell surfaces causes rapid cellular degranulation and liberation of a number of chemical mediators. The mediators released by mast cell degranulation include the preformed molecules histamine, protease enzymes, proteoglycans (heparin) and chemotactic factors. Reaction of antigen with IgE on mast cells also stimulates synthesis and release of platelet activating factor (PAF), leukotrienes (B4, C4 and D4) and prostaglandins (mainly PGD2).(Figure 1)

Mediator **Pharmacological effect**

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

Mediator	Pharmacological effect
Histamine	Vasodilatation, capillary permeability, bronchoconstriction
Heparin	Control of histamine release
Leukotrienes (various)	Bronchoconstriction, airway tissue oedema
Prostaglandins (various)	Potent mediators of inflammatory response
Platelet activating factor	Platelet aggregation
Tryptase	Proteolytic enzyme activates C3
Kininogenase	Kinins → vasodilatation → oedema
Cytokines (IL-5, IL-8, TNFs)	Chemo attractants

The actions of histamine depend on the site of release. In the airways, it induces smooth muscle contraction; in the skin, it causes the hallmark wheal and flare response. Widespread activation of mast cells leads to systemic effects of circulatory shock, hypotension, collapse, chest tightness and, in the most severe cases, respiratory arrest and death: this is anaphylactic shock. Type I hypersensitivity reactions occur rapidly (within approximately 20 min of an insult) and are also called 'immediate hypersensitivity reactions'.

Type I hypersensitivity is an allergic reaction. Exposure to the allergen may be by ingestion, inhalation, injection or direct contact. The difference between a normal immune response and a type I hypersensitivity response is that plasma cells secrete IgE antibodies that bind to mast cells and basophils that then release histamines, a vasodilator, and heparin, a blood thinner. These cause inflammation at the site as blood flow to the affected tissues is increased. The reaction may be either local or systemic. Symptoms vary from mild irritation to sudden death from anaphylactic shock. This is why allergies are manifested as red and watery eyes, runny nose and hives. Asthma is a form of anaphylaxis, as a combination of oedema and airway constriction prevents tissues from getting sufficient oxygen.

Self - Instructional Material

Examples of type I hypersensitivity include

- Allergic asthma
- Allergic conjunctivitis
- Allergic rhinitis ('hay fever')
- Anaphylaxis
- Angio-oedema
- Atopic dermatitis (eczema)
- Eosinophilia
- Urticaria (hives).

Check your progress

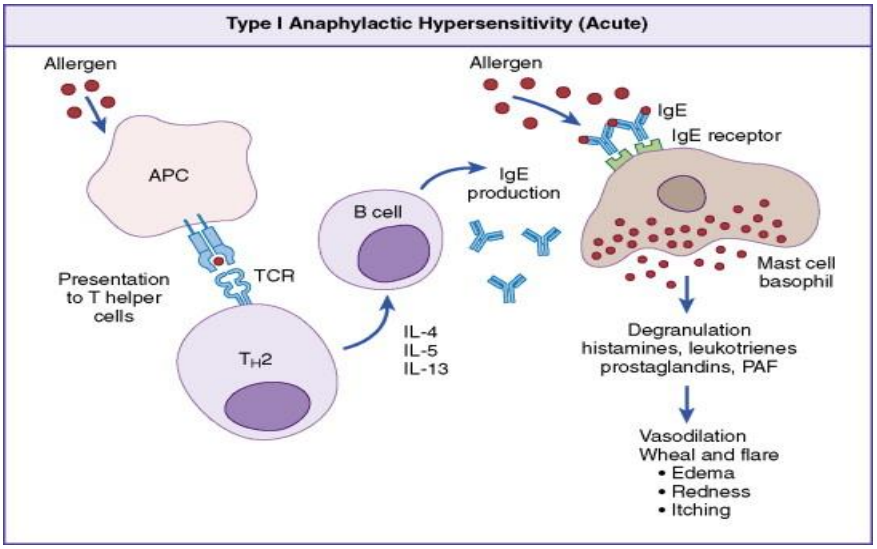
Note: write your answer in the space given below

1. What are the types of hypersensitivity?

……..

9.3.2Immunoglobulin E–Mediated Immediate Hypersensitivity

Type I hypersensitivity (also called immediate hypersensitivity) is due to aberrant production and activity of IgE against normally nonpathogenic antigens (commonly called **allergens**). Common antigenic allergens include animal dander, chemical additives, foods, insect stings, pollens, and even drugs. Antigen presentation to T_H2 cells leads to IgE isotype production. The IgE binds to mast cells or basophils via high-affinity IgE receptors. Subsequent antigen exposure results in cross-linking of cell-bound IgE with activation (degranulation) of mast cells or basophils to release preformed mediators (e.g., vasoactive amines, histamine, leukotrienes, prostaglandin D_2) and to synthesize new mediators (i.e., chemotaxins, cytokines). **Figure 9.2**

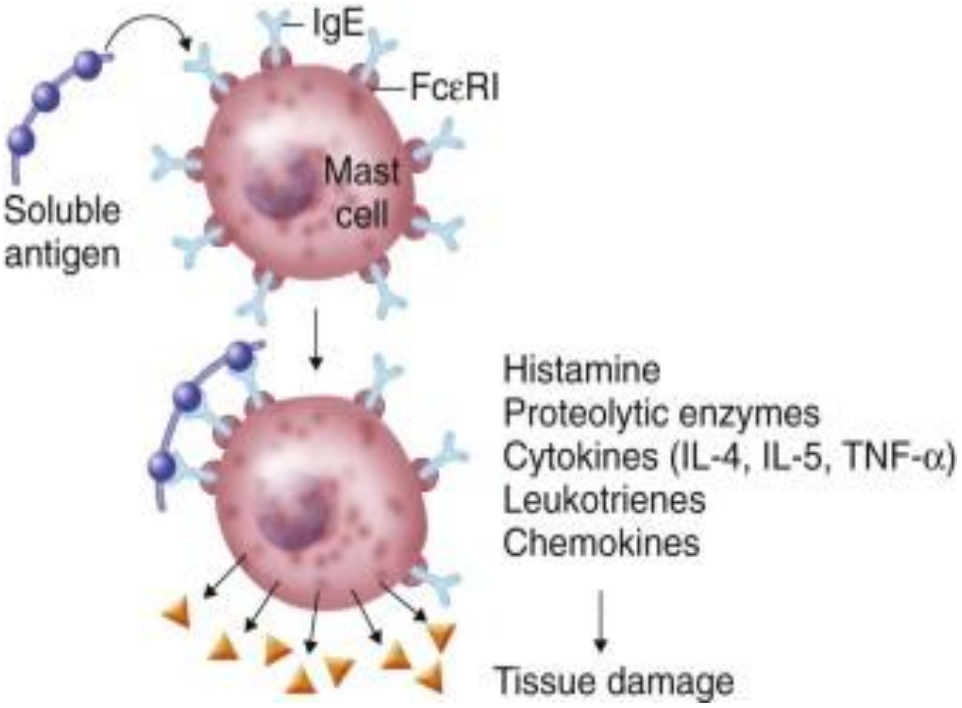


These mediators are responsible for the signs and symptoms of allergic diseases. In severe cases, anaphylactic shock may occur, characterized by a sudden and sharp drop in blood pressure, urticaria, and breathing difficulties caused by exposure to a foreign substance (such as bee venom or drug reactivity).**Figure 9.2** Emergency treatment includes epinephrine injections, used as a heart stimulant, vasoconstrictor, and bronchial relaxant.

9.3.3 Mechanisms of Immune-Mediated Tissue Injury

Type I hypersensitivity reactions are triggered by the interaction of antigen with antigen-specific IgE bound to Receptors on mast cells, which causes mast cell activation. Proteolytic enzymes and toxic mediators, such as histamine, are released immediately from preformed granules, and chemokines, cytokines, and leukotrienes are synthesized after activation. Together, these mediators increase vascular permeability, break down tissue matrix proteins, promote Eosinophils production and activation (IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor [GM-CSF]), and amplification of T_H2 cell responses (IL-4 and IL-13).

Eosinophils and basophils, activated through cell surface Receptors, rapidly release highly toxic granular proteins (major basic protein, Eosinophils peroxidase, and collagenase) and, over a longer period, produce cytokines (IL-3, IL-5, and GM-CSF), chemokines (IL-8), prostaglandins, and leukotrienes that activate epithelial cells, leukocytes, and Eosinophils to augment local inflammation and tissue damage. **Figure 9.3**



9.3.4 Inflammation, Inflammatory Mediators, and Immune-Mediated Disease:

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Type I hypersensitivity is dependent on the presence of preformed IgE antibodies. The release of large amounts of histamine and serotonin results in massive vasodilatation, diminished blood pressure, and marked bronchoconstriction. Widespread activation of mast cells can result in a systemic response to antigen that can cause anaphylactic shock. Other mediators released by activated mast cells recruit Eosinophils and other inflammatory cells (Eosinophils and neutrophils chemotactic factors). Activated mast cells also produce PAF, prostaglandins, and leukotrienes. PGD_2 and leukotrienes are potent bronchospastic agents that can cause transient respiratory insufficiency in anaphylaxis. **Figure 9.4**

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

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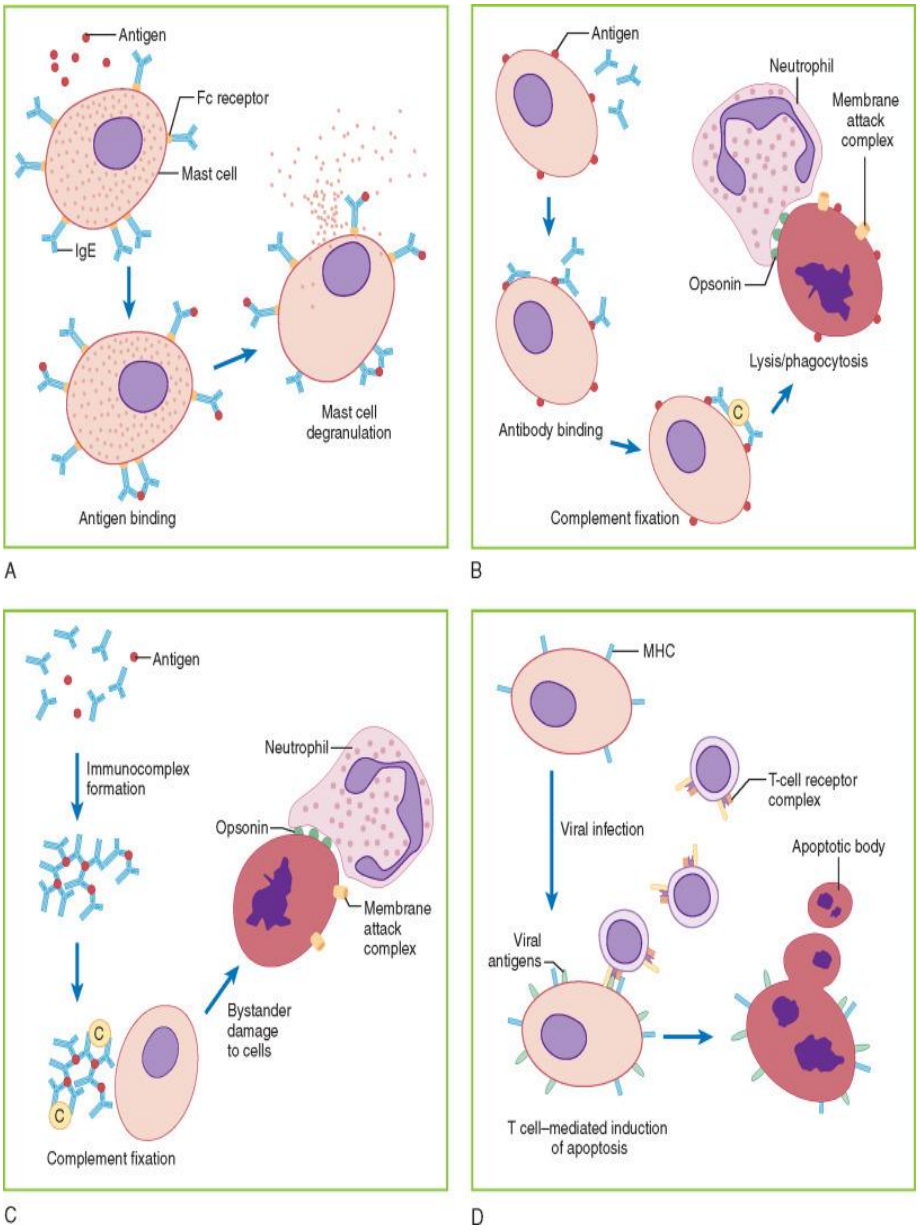
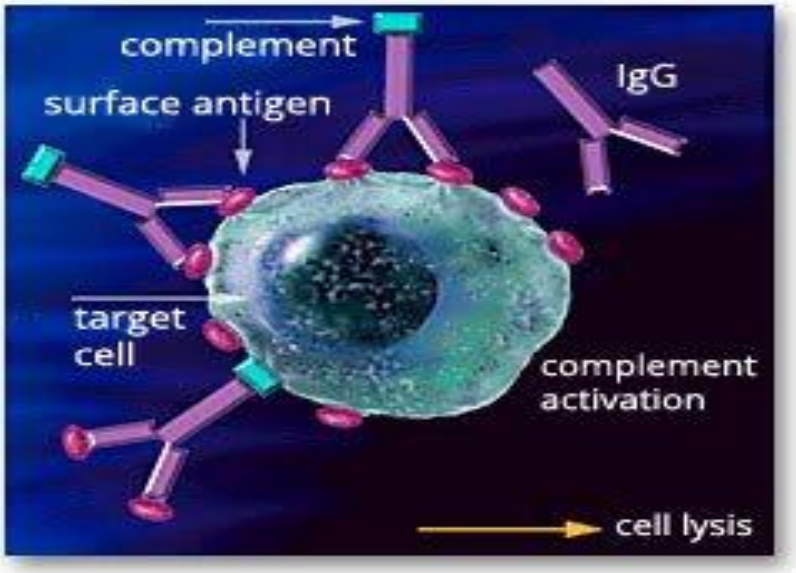


9.4 Type II Hypersensitivity

9.4.1 Cytotoxic Reaction (Antibody-dependent)

In a cytotoxic reaction, the antibody reacts directly with the antigen that is bound to the cell membrane to induce cell lysis through complement activation. These antigens may be intrinsic or "self" as in autoimmune reactions or extrinsic or "non-self." Cytotoxic reactions are mediated by IgG and IgM. Examples of cytotoxic reaction are the Rh incompatibility of a newborn, blood transfusion reactions, and autoimmune diseases like Pemphigus Vulgaris, Bullous Pemphigoid, auto immune hemolytic anemia **(Figure 9.4)**

Self - Instructional Material



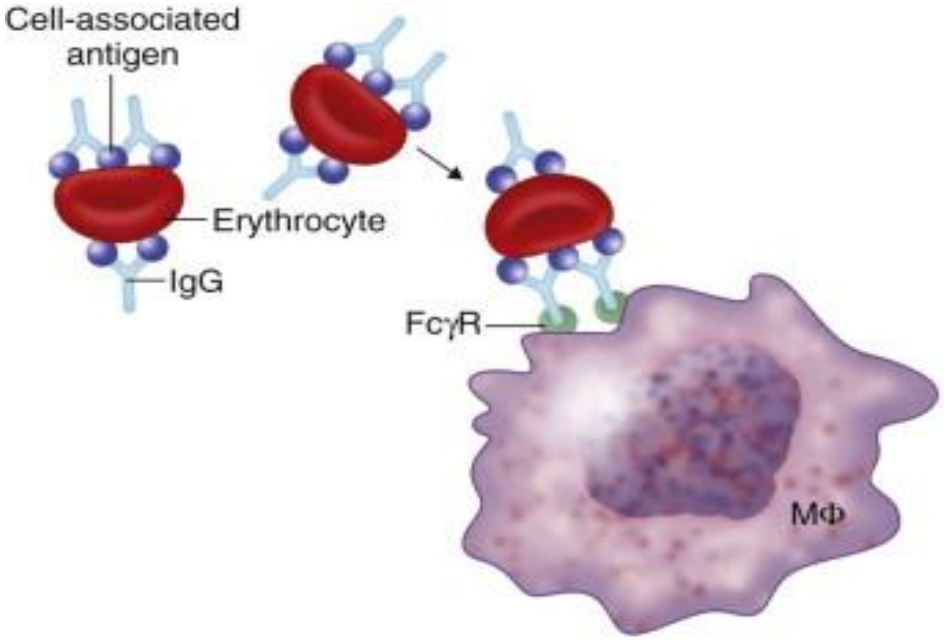
9.4.2 Adaptive Immune Response and Hypersensitivity Type II

Type II hypersensitivity is an antibody-dependent process in which specific antibodies bind to antigens, resulting in tissue damage or destruction. If the antigen is present on cell surfaces, antibody binding can result in cell lysis through the in situ fixation of complement. IgM antibodies (Multimeric) are often more effective in fixing complement than are than IgG antibodies (monomeric). Type II hypersensitivity is typified by a transfusion reaction in which mismatched red blood cells are rapidly destroyed by specific preformed antibodies (anti-ABO or -Rh) and complement. Although fixation of complement can result in direct cell lysis, opsonization and recruitment of inflammatory cells is often a more important cause of cell injury.

9.4.3 Mechanisms of Immune-Mediated Tissue Injury

Type II Hypersensitivity Reactions

Type II hypersensitivity reactions are caused by chemical modification of cell surface or matrix-associated antigens that generates "foreign" epitopes to which the immune system is not tolerant. B cells respond to this antigenic challenge by producing IgG, which binds to these modified cells and renders them susceptible to destruction through complement activation, phagocytosis, and antibody-dependent cytotoxicity. (Figure 9.5)



9.4.4 Molecular Basis of Diseases of Immunity

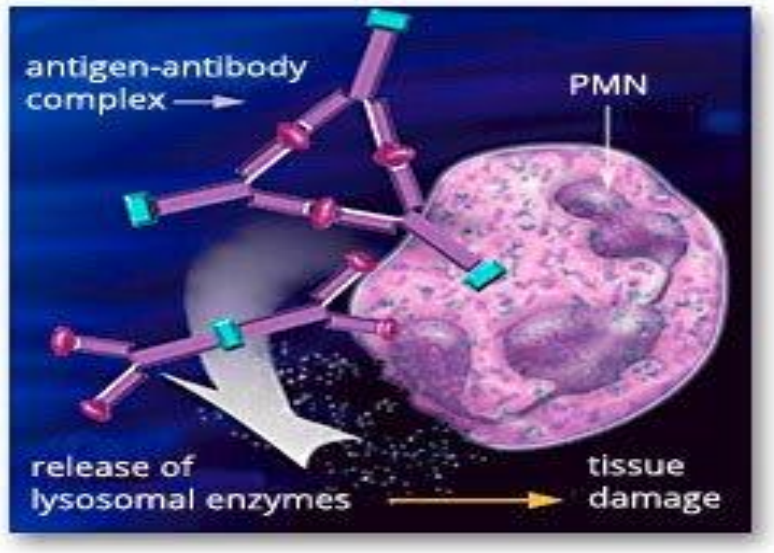
Antibody-Mediated Hypersensitivity

Type II hypersensitivity is mediated by IgM or IgG targeting membrane-associated antigens. A sensitization phase leads to production of antibodies that recognize substances or metabolites that accumulate in cellular membrane structures. In the effector phase, target cells become coated with antibodies, a process termed opsonization, which leads to cellular destruction by three mechanisms: (i) phagocytosis, (ii) complement-dependent cytotoxicity (CDC), and (iii) ADCC. This leads to deposition of C3b, which can mediate phagocytosis. Complement activation also leads to production. The mechanisms involved in type II hypersensitivity also play a role in cellular destruction by autoantibodies.

9.6 Type III Hypersensitivity:

9.6.1 Immune Complex Reaction

IgG and IgM bind antigen, forming antigen-antibody (immune) complexes. These activate complement, which results in PMN chemotaxis and activation. PMNs then release tissue damaging enzymes. Tissue damage present in autoimmune diseases (e.g., systemic lupus erythematosus), and chronic infectious diseases (e.g., leprosy) can be attributed, in part, to immune complex reactions. (Figure 9.6)



Type III hypersensitivity reactions are also termed immune complex reactions. Complexes of antigen and antibody form in the circulation and are then deposited in susceptible tissues; they may also form directly in the tissue. The latter mechanism is termed the Arthus reaction, and is typically seen with repeated insect stings, where a red swollen lesion develops after a sting. The tissue damaging mechanisms are similar to those described for the antigen-antibody complexes that form in type II responses. The response times of types II and III hypersensitivity reactions are slower than that of type I reactions; they typically develop 3–6 h after exposure to antigen. The response can also become chronic, particularly in autoimmune reactions, where antigen persists.

The clinical manifestations of type III hypersensitivity reactions relate to the tissue deposition, for example Vasculitis (skin), serum sickness (systemic), nephritis (kidneys) and extrinsic allergic alveoli is (lungs).

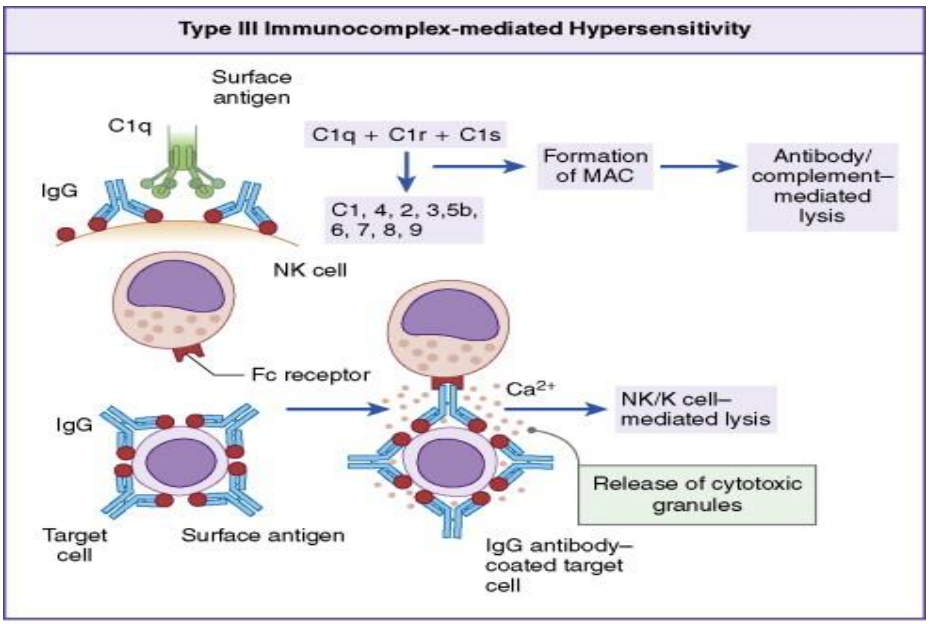
9.6.2 Immunocomplex-Mediated Hypersensitivity:

TypeIII hypersensitivity results from soluble antigen-antibody immunocomplexes that activate complement. The antigens may be self or foreign (i.e., microbial). Such complexes are deposited on membrane surfaces of various organs (e.g., kidney, lung, synovium). The by-products of complement activation (C3a, C5a) are chemotaxins for acute inflammatory cells, resulting in infiltration by polymorph nuclear (*PMN*) cells. Lysosomal enzymes are released that result in tissue injury. Platelet aggregation occurs, resulting in micro thrombus formation

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

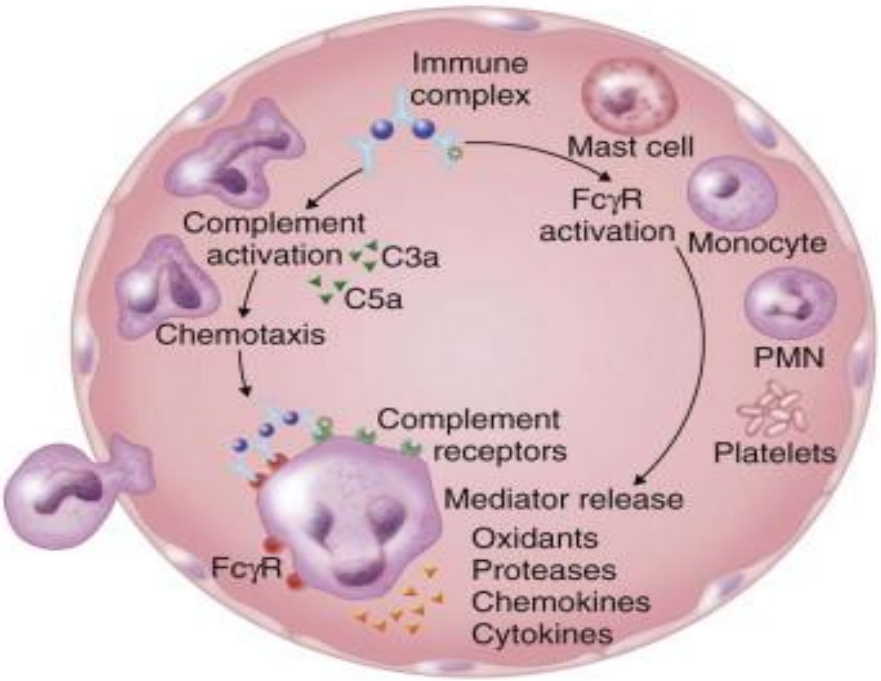
in the vasculature. Type III reactions and accompanying inflammatory injury are seen in diseases such as rheumatoid arthritis, systemic lupus erythematosus, and post infectious arthritis. (Figure 9.7)



Type III immunocomplex-’’mediated hypersensitivity. Deposition of immunocomplexes in vascular beds results in platelet aggregation, complement fixation, and subsequent polymorph nuclear infiltration.

9.6.3 Mechanisms of Immune-Mediated Tissue Injury

Type III hypersensitivity reactions are caused by tissue deposition of small soluble immune complexes that contain antigens and high-affinity IgG antibodies directed at these antigens. (Figure 9.8)



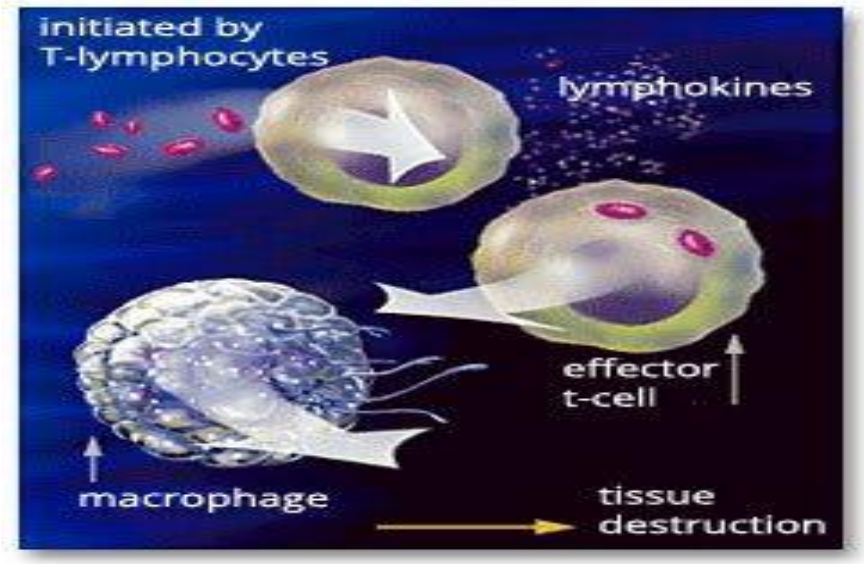
Self - Instructional Material

Serum sickness is a systemic type III hypersensitivity reaction, historically described in patients injected with therapeutic horse antiserum for the treatment of bacterial infections. In general, serum sickness occurs after the injection of large quantities of a soluble antigen. Clinical features include chills, fever, rash, urticaria, arthritis, and glomerulonephritis. Disease manifestations become evident 7 to 10 days after exposure to the antigen, when antibodies are generated against the foreign protein and form immune complexes with these circulating antigens. Immune complexes are deposited in blood vessels, where they activate phagocytes and complement, producing widespread tissue injury and clinical symptoms. The effects are transient, however, and resolve after the antigen is cleared.

9.7 Type IV Hypersensitivity

9.7.1 Cell-Mediated (Delayed Hypersensitivity)

Cell mediated reactions are initiated by T-lymphocytes and mediated by effectors T-cells and macrophages. This response involves the interaction of antigens with the surface of lymphocytes. Sensitized lymphocytes can produce cytokines, which are biologically active substances that affect the functions of other cells. This type of reaction takes 48-72 hours, or longer, after contact with the antigen to fully develop. Many chronic infectious diseases, including tuberculosis and fungal infections, exhibit delayed hypersensitivity. Evidence suggests that hypersensitivity reactions, particularly Type III and IV, may be involved in the pathogenesis of periodontal disease. (Figure 9.9)



Several types of hypersensitive reactions can be identified, reflecting differences in the effector molecules generated in the course of the reaction. Gell and Coomb described four types of hypersensitivity reactions (Types I, II, III and IV). The first three types are antibody-mediated and the fourth type is cell mediated mainly by T-cell.

Check your progress

Note: write your answer in the space given below

2. Define cell-Mediated Hypersensitivity?

……..

9.7.2 Type IV or Delayed-Type Hypersensitivity.

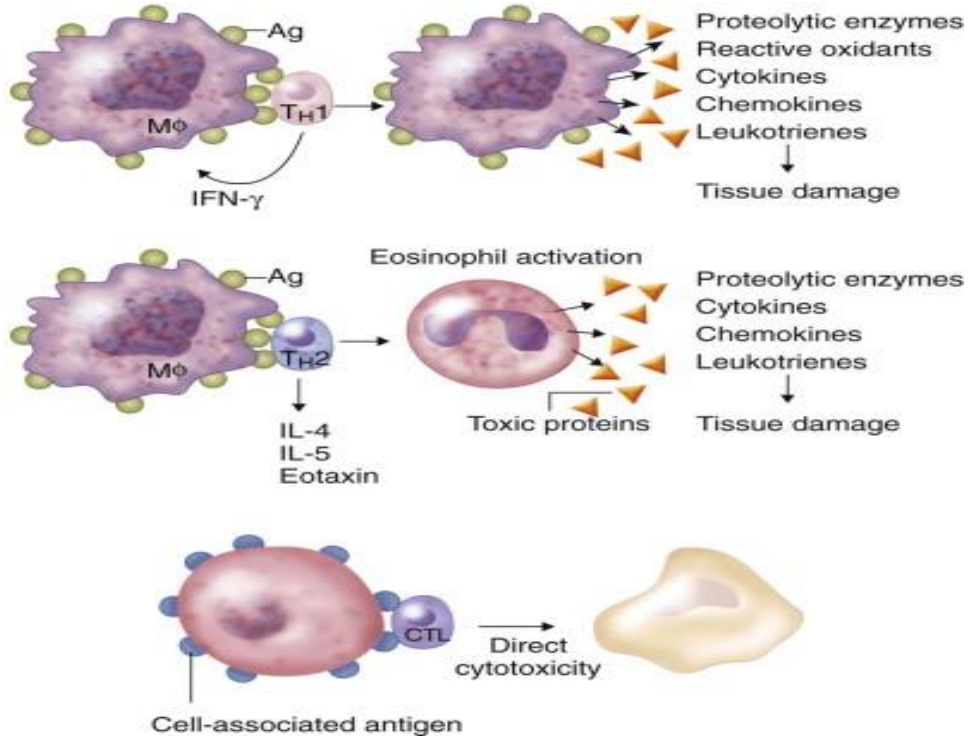
Type IV hypersensitivity reaction is also called delayed Type hypersensitivity reaction because it takes 2 to 3 days for the reaction to develop after exposure to the particular substance. Type IV hypersensitivity reaction can occur in many parts of the body. Generally, they include: Skin: Atopic dermatitis.

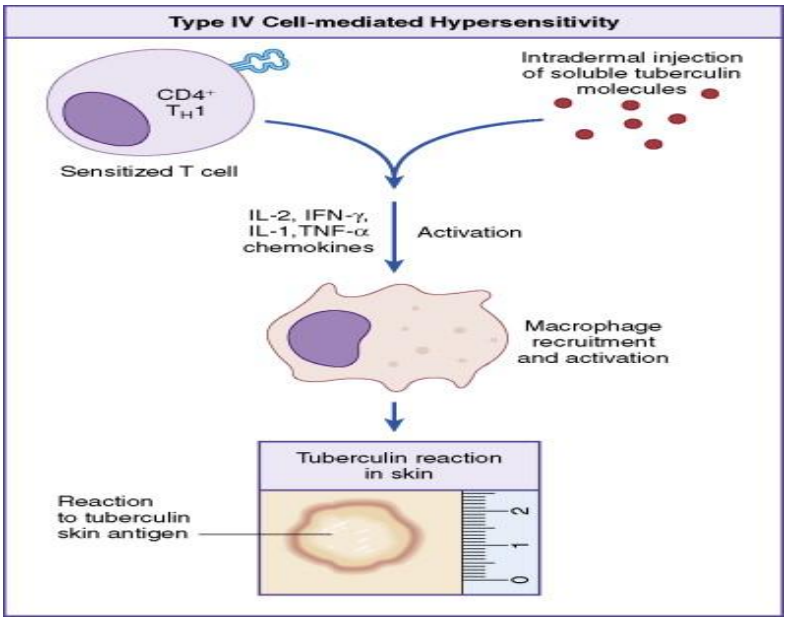
Type IV hypersensitivity typically occurs at least 48 hours after exposure to an antigen. It involves activated T cells, which release cytokines and chemokines, and macrophages and cytotoxic $CD8^+$ T cells. Delayed-type hypersensitivity and granuloma play a major role in tissue damage observed during infections with slow-growing intracellular organisms, such as *M. tuberculosis* (tuberculosis), *M. leprae* (leprosy) and *H. capsulatum*. Many of the clinical manifestations of chlamydial disease, in particular trachoma, seem to result from a delayed-type hypersensitivity triggered by chlamydial heat shock proteins.

9.7.3 Mechanisms of Immune-Mediated Tissue Injury

Type IV hypersensitivity reactions also known as delayed-type hypersensitivity reactions, are mediated by antigen-specific effector T cells. They are distinguished from other hypersensitivity reactions by the lag time from exposure to the antigen until the response is evident (1 to 3 days).Antigen is taken up, processed, and presented by macrophages or dendritic cells. Type 1 helper T (T_H1) effector cells that recognize the specific antigen (these are scarce and take time to arrive) are stimulated to

release chemokines, which recruit macrophages to the site and release cytokines that mediate tissue injury. IFN-γ activates macrophages and enhances their release of inflammatory mediators, whereas TNF-α and TNF-β activate endothelial cells, enhance vascular permeability, and damage local tissue. (Figure 9.10)





The prototypical type IV hypersensitivity reaction is the tuberculin test, but similar reactions can occur after contact with sensitizing antigens (e.g., poison ivy, certain metals) and lead to epidermal reactions

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

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characterized by Erythema, cellular infiltration, and vesicles. $CD8^+$ T cells also may mediate damage by direct toxicity.

Table 11.1: Gell and Coombs classification of hypersensitive reactions

Type	Descriptive name	Initiation time	Mechanism	Typical manifestations
			Delayed Reactions	
Type I	IgE-mediated hypersensitivity	2-30 min	Ag induces cross-linkage of IgE bound to mast cells and basophils with release of vasoactive mediators	Systemic anaphylaxis Localized anaphylaxis Hay fever Asthma Hives Food allergies Eczema
Type II	Antibody mediated cytotoxic hypersensitivity	5-8 h	Ab directed against cell surface antigens mediates cell destruction via complement activation or ADCC	Blood-transfusion reactions Erythroblastosis fetalis Autoimmune haemolytic anaemia
Type III	Immune complex mediated hypersensitivity	2-8 h	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response	Localized Arthus reaction Generalized reactions Serum sickness Glomerulonephritis Rheumatoid arthritis Systemic lupus erythematosus
Type IV	Cell-mediated hypersensitivity	24-72 h	Sensitized T_{DTH} cells release cytokines that activate macrophages or T_C cells, which mediate direct cellular damage	Contact dermatitis Tubercular lesions Graft rejection

9.8 LET US SOME UP

In this Unit, You have learnt about the meaning, definition, need, Objectives, Importance and concept of hypersensitivity. This knowledge would make you understand what Hypersensitivity, types and Mechanism. It can be practice at typical manifestation of the knowledge .Thus, the introduction unit of hypersensitivity would your closer to know the concept of hypersensitivity in your carrier.

9.9 UNIT - END EXERCISES

1. Define type IV Hypersensitivity?
2. What is immune complex Reaction?

9.10 ANSWERS TO CHECK YOUR PROGRESS

1. Type I: Immediate and anaphylactic reaction

   Type II: Cytotoxic

   Type III: Mediated by immune complexes

   Type IV: Delayed Type Hypersensitivity

2. Cell mediated reactions are initiated by T-lymphocytes and mediated by effectors T-cells and macrophages. This response involves the interaction of antigens with the surface of lymphocytes. Sensitized lymphocytes can

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produce cytokines, which are biologically active substances that affect the functions of other cells.

9.11. SUGGESTED READINGS

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Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

UNIT X AUTOIMMUNE DISORDER AND IMMUNODEFICIENCY DISESES. ORAN TRANSPLANTATION ANTIBODY ENGINEERING

Structure

Self - Instructional Material

10.1 Introduction

Cause abnormally low activity or over activity of the immune system. In cases of immune system over activity, the body attacks and damages its own tissues (autoimmune diseases). Immune deficiency diseases decrease the body's ability to fight invaders, causing vulnerability to tissues infection.

10.2 Objectives

To bring novel diagnostic sand therapeutics for autoimmune diseases.

To clinical use through studies of monogenic autoimmune diseases.

10.3 Definition

An autoimmune disease is a condition in which immune system mistakenly attacks its own body unknowingly. 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. Autoimmune diseases result from a dysfunction of the immune system. The immune system protects you from disease and infection. Sometimes, though, the immune system can produce auto antibodies that attack healthy cells, tissues, and organs. This can lead to autoimmune disease.

10.4Autoimmune diseases

Autoimmune diseases can affect any part of the body. More than 80 autoimmune diseases have been identified. Some are relatively well known, such as type 1 diabetes, multiple sclerosis, lupus, and rheumatoid arthritis, while others are rare and difficult to diagnose. The causes of autoimmune diseases remain largely unknown. There is growing consensus that autoimmune diseases likely result from interactions between genetic and environmental factors. The National Institute of Environmental Health Sciences (NIEHS) is supporting research to understand how these factors work together to compromise the body's ability to defend itself, and develop into autoimmune diseases. NIEHS hopes to find clues that will lead to treatments and cures, or ways to prevent the development of these diseases.

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:

10.4.1 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 over activity.

10.4.2 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.

10.4.3 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.

10.4.4 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.

10.4.5 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.

10.4.6 Chronic inflammatory demyelinating polyneuropathy

Similar to Guillian-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.

10.4.7 Psoriasis:

In psoriasis, overactive 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.

10.4.8 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.

10.4.9 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.

Check your progress

Note: write your answer in the space given below

2.What is immune disorders?

……..

10.4.10 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.

10.4.11Vasculitis:

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.

10.5 Autoimmune disorder and immunodeficiency:

One common type of autoimmunity is when the immune system makes antibodies against normal cells and/or tissues of the body which are known as "autoantibodies." However, certain primary immunodeficiency diseases have autoimmune disease as their primary problem.

The immune system is a complex set of organs, cells, proteins and other substances that function to prevent infection. Primary immunodeficiency diseases are characterized by abnormalities in specific components of the immune system that lead to an increased susceptibility to infection. Many times, abnormalities in the immune system that lead to primary immunodeficiency diseases also result in immune dysregulation, which is an immune response that is not properly controlled or restrained. This can lead to autoimmunity, one form of immune dysregulation in which the immune response is directed against normal parts of the body such as cells, tissues or organs (called auto-antigens). Put another way, it is when the immune system attacks the body in which it resides.

10.5.1 Definition of Autoimmunity in Primary Immunodeficiency

A normal immune system makes proteins known as antibodies that recognize and prevent foreign organisms (bacteria, viruses) from causing infection. One common type of autoimmunity is when the immune system makes antibodies against normal cells and/or tissues of the body which are known as "autoantibodies." Sometimes people with primary immunodeficiency diseases cannot make "good" antibodies to protect against infection but only make "bad" autoantibodies, which then cause autoimmune disease. Sometimes these antibodies themselves are harmless but suggest the presence of an autoimmune disease. In other autoimmune diseases, the cellular immune system may also react against a body's auto-antigens.

10.5.2Autoimmune Cytopenias

The development of autoantibodies that bind to and destroy blood cells is the most common autoimmune disease seen in primary immunodeficiency diseases. The blood cells affected are the red blood cells (RBCs), platelets and white blood cells (WBCs).

10.5.3 Red Blood Cells

The RBCs carry oxygen to the body's tissues. Oxygen is necessary for the body's tissues to perform their function. Anemia is the term used to describe a low number of RBCs. Autoantibodies against the RBCs can

cause destruction of these cells and is called autoimmune hemolytic anemia (AIHA).Symptoms associated with AIHA include fatigue, headache, dizziness, fainting and poor exercise tolerance. The person sometimes looks pale. In severe cases the individual can develop a yellow discoloration to the skin and eyes known as jaundice. The spleen may become enlarged as it traps the damaged red blood cells. The body tries to compensate for the decreased capacity to carry oxygen by working the lungs and heart harder.

10.5.4 Platelets

Injuries to the tissues can cause bleeding. Platelets help create blood clots to stop bleeding. A low number of platelets is called thrombocytopenia. When autoantibodies are formed against the platelets and cause thrombocytopenia, it is known as idiopathic thrombocytopenic purpura (ITP). ITP can cause abnormal bleeding.

The urine may have an orange, pink or red color. Stools may appear black and tarry, which can indicate bleeding in the intestinal tract. Rarely, bleeding in the brain can cause altered mental status or death.

10.5.5 White Blood Cells

There are many different types of WBCs. Neutrophils are WBCs that have a major role in responding to infections. A low number of neutrophils are called neutropenia. Autoimmune neutropenia (AIN) occurs when antibodies are produced against neutrophils. The most significant symptom associated with AIN is fever, as this may indicate a serious infection. Other signs of infection such as cough, vomiting, diarrhea and rash may also be present. Serious infections can progress rapidly in people with AIN, and they may require evaluation in the emergency room or admission to the hospital. Antibiotic therapy is urgently needed in these cases. Patients with AIN may also have ulcers or sores develop in the mouth, esophagus or intestine. The gums may also become inflamed and red.

10.6 Diagnosis of Autoimmune Cytopenias

A specialist such as a clinical immunologist, hematologist or oncologist typically evaluates patients for these disorders. Sometimes a bone marrow sample needs to be obtained to determine whether there is a problem with production of blood cells.

10.7 Treatment of Autoimmune Cytopenias

Autoimmune cytopenias may be temporary and require little to no treatment. If treated, the goal of therapy is to remove the autoantibodies and let the body replenish the blood cells. Several treatments have been used including intravenous immunoglobulin (IVIG), steroids, chemotherapy drugs and drugs such as anti-CD20, which is used to specifically deplete B-cells that produce antibodies. The therapy that is best for a particular patient is based on many factors.

Autoimmune cytopenias often respond well to therapy. At times however, symptoms may recur or may require long-term treatment. Patients rarely require blood transfusions except in extreme circumstances. In all cases, patients with cytopenias require close follow-up by their specialist.

10.8 Autoimmune Lung Disease

Abnormal accumulation of white blood cells in the lung tissues, causing inflammation and damage. Sometimes white blood cells accumulate in a specific part of the lung known as the interstitium. This is called interstitial lung disease and interferes with the ability of oxygen to be absorbed into the bloodstream.

10.8.1 Symptoms

Patients may notice a decrease in their endurance with everyday activities. They may find themselves having to cut back on exercise such as biking or running. These changes are often attributed to other causes, which may delay the diagnosis of the lung disease itself. Patients often complain of a cough, which is usually non-productive.

10.8.2 Diagnosis of Pulmonary Complications

Radiology tests can be helpful in identifying lung problems. Chest X-rays are useful for diagnosing infections (pneumonia). However, a chest

X-ray can sometimes be normal, even when there is still significant lung disease present. A chest CT scan can frequently pick up abnormalities not seen on a routine chest X-ray. Breathing tests, called pulmonary function tests (PFTs), can indicate the degree of lung impairment. In some cases, a lung biopsy is needed to make the correct diagnosis and define the correct treatment course. A lung biopsy is a surgical procedure usually done by making a small incision in the chest and inserting a small scope and instruments to obtain a piece of lung tissue.

10.8.3 Treatment

Patients with malignancies are referred to an oncologist (cancer doctor) for continuing care. Patients with infections are treated with antibiotics. Inflammatory changes in the lung are usually treated with immunosuppressant drugs that suppress or alter the immune system.

The most common medicine used is corticosteroids (like prednisone), which can be given by inhalation, orally or intravenously. Steroids can be effective, but sometimes may not provide long-term improvement. Prolonged oral or IV steroid use is associated with significant side effects such as high blood pressure, high blood sugar, osteopenia (weak bones), hyperlipidemia (high cholesterol), and stress on the kidney and eyes. Other immune suppressive medicines such as cyclosporine and Sirolimus are sometimes helpful.

10.9 Autoimmune Skin Disease

Skin conditions due to autoimmunity or immune dysregulation are not unique to people with primary immunodeficiency diseases. Common skin conditions like eczema or psoriasis are seen in people with normal immune systems as well. Sometimes, skin disease is one of the earliest symptoms of a primary immunodeficiency disease and can lead to further clinical or laboratory evaluation to identify immune deficiency.

10.9.1 Hair and Skin Pigmentation Changes

Multiple primary immunodeficiency diseases can have autoimmunity that affects the hair and skin pigment. Some patients develop alopecia, or patches of baldness as a result of autoantibodies against hair producing cells. Alopecia areata refers to round circular areas of hair loss.

Some patients also develop vitiligo, or loss of the pigment in the skin. The affected area of skin will appear white in color.

10.9.2 Diagnosis of Skin Diseases

Biopsies are typically taken from the area where the rash is most evident using a sharp "punch" that cuts and removes a small circular core of skin tissue that can be evaluated microscopically by a pathologist to determine what type of rash it is. This is typically a very minor procedure that can be done in the office with local numbing of the skin.

10.9.3 Treatment

Mild skin conditions can be diagnosed and treated by a primary care provider or an Immunologist but more severe skin conditions often require diagnosis and treatment by a dermatologist. Treatment for most conditions typically begins with local application of moisturizing lotions and steroid ointments directly to the rash. If this is not sufficient to control the symptoms, ointments containing more potent steroids or other immunosuppressant medications can be applied. In rare cases, oral or IV immunosuppressant medications may be needed to treat severe disease.

10.10 Eczema

Eczema, also known as atopic dermatitis, is generally a mild skin disease and is the most common skin disease in primary immunodeficiency diseases. Often referred to as "the itch that rashes," eczema typically begins as patches of dry, itchy skin which worsen and erupt into rash as they are scratched. It is not unusual for patients with primary immunodeficiency diseases who have other autoimmune manifestations to also have eczema.

10.11 Psoriasis

Psoriasis is another type of autoimmune skin disease that is more severe than eczema. Psoriasis plaques are typically red, raised, itchy and painful. They are characterized by the presence of a silvery scale on the surface of the plaques that often bleeds if it is removed.

10.12 Autoimmune Gastrointestinal Disease

Autoimmune gastrointestinal diseases are common among patients with primary immunodeficiency diseases, particularly patients with CVID,

CGD.As a result, the immune system plays a particularly important role in maintaining the barrier function of the intestines and in protecting the body from invasion by the bacteria present in the bowel.

10.13 Mucosal Changes

Autoimmune or inflammatory diseases of the gastrointestinal tract can disrupt the mucous membranes that line the mouth, esophagus, stomach, and intestines. This can cause a variety of symptoms including: geographic tongue, an abnormal appearance of the tongue that can be mistaken for an oral yeast infection (thrush); gingivitis or inflammation of the gums; oral ulcers or canker sores; abdominal pain; diarrhea that may be watery or bloody.

10.13.1 Autoimmune Musculoskeletal Disease

There is no evidence that the incidence of osteoarthritis is higher in patients with primary immunodeficiency diseases but some primary immunodeficiency diseases are associated with a higher incidence of certain autoimmune arthritis syndromes. For example, both Di George syndrome and Selective IgA Deficiency have been associated with an increased risk for developing Juvenile Idiopathic Arthritis (JIA), a type of arthritis that affects children.

10.13.2 Symptoms

Typical signs and symptoms of arthritis include pain and stiffness of the joints, joint swelling, and sometimes warmth or redness over the joints that have arthritis. The stiffness is often worst after not moving the joint, like in the morning after sleep or after resting.

10.13.3 Treatment

Treatment of arthritis typically requires the use of immunosuppressants. Steroids like prednisone are among the most commonly used. These can be given by mouth, injected into the blood through an IV or injected directly into the inflamed joints. They are often very effective for a time but may not provide a long-term effect.

10.14 Liver Inflammation

Autoimmune or inflammatory disease of the liver, which can occur in primary immunodeficiency diseases, can cause temporary or permanent

damage that can disrupt one or more of the liver's important functions. This may lead to accumulation of fluid in the abdomen (ascites), elevated bilirubin in the blood leading to jaundice, blood clotting abnormalities, etc.

10.14.1 Autoimmune Kidney Disease

Destruction of the glomeruli leads to progressive loss of filtering capacity and decreased kidney function. Glomerulonephritis is a common feature of patients with complement deficiencies, particularly those affecting complement components C1, C2, C3, or C4. Autoimmune kidney disease can also be seen less commonly in other primary immunodeficiency diseases including CVID and APECED.

10.14.1 Symptoms

In many cases, the first sign of autoimmune kidney disease is elevated blood pressure. This is often accompanied by the appearance of blood or protein in the urine. In the setting of active glomerulonephritis, blood in the urine may not appear pink, but instead is more likely to cause the urine to have a color closer to that of tea or cola. Blood and protein are easily detected in the urine using readily available test strips that are frequently called urine "dipsticks." If there is substantial protein loss in the urine, it can lead to fluid retention and swelling (edema) of the legs and feet.

10.14.2 Diagnosis of Kidney Complications

. The biopsy is evaluated by a pathologist, who performs a variety of tests on the kidney tissue including a microscopic examination.

10.14.3 Treatment

Patients with autoimmune kidney disease are often referred to a nephrologist (kidney doctor) for evaluation and management of the kidney problems. Blood pressure medications are typically prescribed to manage the elevated blood pressure, and immunosuppressants are used to control the autoimmune process.

Check your progress

Note: write your answer in the space given below

2. Explain the Symptoms of Kidney Disease?

…….

10.15 Diabetes

Diabetes (abnormally elevated blood sugar levels) results from either not being able to produce enough insulin (Type I diabetes) or as a result of the cells of the body becoming resistant to the effects of insulin (Type II diabetes). Type I diabetes (T1D) is the form caused by autoimmune attack on the islet cells in the pancreas that produce insulin. Once islet cells are destroyed, they do not recover.

When the number of Islet cells producing insulin drops below a particular threshold, patients develop diabetes

T1D is typically diagnosed by screening for the presence of glucose (sugar) in the urine and by measuring blood glucose levels. If these do not decrease as expected after eating or if they are high even when a patient is fasting, then diabetes may have developed. Identification of autoantibodies directed toward proteins in the pancreas (anti-islet cell antibodies) can help confirm that the process is autoimmune.

Treatment of T1D typically involves the administration of insulin either via shots or via an insulin pump. Even though T1D is autoimmune mediated, it is not yet clear whether the use of potent immunosuppressive drugs early in the course of disease will change the need for insulin treatment or not, but there are a number of therapeutic trials have been designed to address this question.

Immuno Deficiencies disorders involve malfunction of the immune system, resulting in infection that develop and recur frequently, are more severe, and last longer than usual.

- Immunodeficiency disorders usually result from use of a drug or from a long-lasting serious disorder (such as cancer) but occasionally are inherited.

- People usually have frequent, unusual or unusually severe or prolonged infections and may develop an autoimmune disorder or cancer.
- Doctors suspect immunodeficiency based on symptoms and do blood tests to identify the particular disorder.
- People may be given antimicrobial drugs (such as antibiotics) to prevent and treat infections.
- Immune globulin may be given if there are too few antibodies (immunoglobulins) or they are not functioning normally.
- If the disorder is severe, stem cell transplantation is sometimes done.
- Good control of blood sugar levels can help white blood cells and function better then prevent infections.

10.15.1 Treatment

- General measures and certain vaccines to prevent infections
- Antibiotics and antiviral when needed
- Sometimes immunoglobulin
- Sometimes stem cell transplantation

Treatment of immunodeficiency disorders usually involves preventing infections, treating infections when they occur, and replacing parts of the immune system that are missing when possible. With appropriate treatment, many people with an immunodeficiency disorder have a normal life span. However, some require intensive and frequent treatments throughout life. Others, such as those with severe combined immunodeficiency, die during infancy unless they are given a stem cell transplant.

10.16 There are two types of immunodeficiency disorders:

10.16.1 Primary immunodeficiency

These disorders are usually present at birth and are genetic disorders that are usually hereditary. They typically become evident during infancy or childhood. However, some primary immunodeficiency disorders (such as common variable immunodeficiency) are not recognized until adulthood. There are more than 100 primary immunodeficiency disorders. All are relatively rare. Primary immunodeficiency disorders may be caused by mutations, sometimes in a specific gene. If the mutated gene is on the X (sex) chromosome, the resulting disorder is called an X-linked disorder. X-linked disorders occur more often in boys. About 60% of people with primary immunodeficiency disorders are male. Primary immunodeficiency disorders are classified by which part of the immune system is affected. The affected component of the immune system may be missing, reduced in number or abnormal and malfunctioning.

10.16.2 Secondary immunodeficiency

These disorders generally develop later in life and often result from use of certain drugs or from another disorder, such as diabetes or human immunodeficiency virus (HIV) infection. They are more common than primary immunodeficiency disorders. Some immunodeficiency disorders shorten life span. Others persist throughout life but do not affect life span, and a few resolve with or without treatment. These disorders can result from prolonged (chronic) and/or serious disorders such as diabetes or cancer, Drugs, Rarely, radiation therapy. Immunodeficiency disorders may result from almost any prolonged serious disorder. For example, diabetes can result in an immunodeficiency disorder because white blood cells do not function well when the blood sugar level is high. Human immunodeficiency virus (HIV) infection results in acquired immunodeficiency syndrome (AIDS), the most common severe acquired immunodeficiency disorder.

Many types of cancer can cause an immunodeficiency disorder. For example, any cancer that affects the bone marrow (such

as leukemia and lymphoma) can prevent the bone marrow from producing normal white blood cells (B cells and T cells), which are part of the immune system.

10.16.3 Corticosteroids

A type of immunosuppressant, are used to suppress inflammation due to various disorders, such as rheumatoid arthritis.

10.17 Immunodeficiency in older people

As people age, the immune system becomes less effective in several ways. For example, as people age, they produce fewer T cells. T cells help the body recognize and fight foreign or abnormal cells. Under nutrition, which is common among older people, impairs the immune system. Two nutrients that are particularly important to immunity-calcium and zinc-may be deficient in older people. Calcium deficiency becomes more common among older people, partly because as people age, the intestine becomes less able to absorb calcium. Also, older people may not get enough calcium in their diet.

Zinc deficiency is very common among older people who are institutionalized or homebound. Certain disorders (such as diabetes and chronic kidney disease), which are more common among older people, and certain therapies (such as immunosuppressants), which older people are more likely to use, can also impair the immune system.

10.17.1 Symptoms

Infections of the mouth, eyes, and digestive tract are common. Thrush, a fungal infection of the mouth, may be an early sign of an immunodeficiency disorder. Sores may form in the mouth. People may have chronic gum disease (gingivitis) and frequent ear and skin infections. Bacterial infections (for example, with staphylococci) may cause pus-filled sores to form (Pyoderma). People with certain immunodeficiency disorders may have many large, noticeable warts (caused by viruses).Many people have fevers and chills and lose their appetite and/or weight. Abdominal pain may develop, possibly because the liver or spleen is enlarged.

Infants or young children may have chronic diarrhea and may not grow and develop as expected (called failure to thrive).

Immunodeficiency may be more severe if symptoms develop in early childhood than if they develop later.

10.17.2 Diagnosis

- Blood tests
- Skin tests
- A biopsy
- Sometimes genetic testing and severe recurring infections.

10.17.3 Physical examination

Results of a physical examination may suggest immunodeficiency and sometimes the type of immunodeficiency disorder. For example, doctors suspect certain types of immunodeficiency disorders when the following are found:

- The spleen is enlarged.
- There are problems with the lymph nodes and tonsils.

In some types of immunodeficiency disorders, the lymph nodes are extremely small. In some other types, lymph nodes and tonsils are swollen and tender.

10.17.4 Tests

Laboratory tests are needed to confirm the diagnosis of immunodeficiency and to identify the type of immunodeficiency disorder.

a.) Blood tests

Including a complete blood count (CBC), are done. CBC can detect abnormalities in blood cells that are characteristic of specific immunodeficiency disorders. A blood sample is taken and analyzed to determine the total number of white blood cells and the percentages of each main type of white blood cell. The white blood cells are examined under a microscope for abnormalities. Doctors also determine immunoglobulin levels and the levels of certain specific antibodies

produced after the person is given vaccines. If any results are abnormal, additional tests are usually done.

b.) Skin tests

May be done if the immunodeficiency is thought to be due to a T-cell abnormality. The skin test resembles the tuberculin skin test, which is used to screen for tuberculosis. Small amounts of proteins from common infectious organisms such as yeast are injected under the skin. If a reaction (redness, warmth, and swelling) occurs within 48 hours, the T cells are functioning normally. No reaction could suggest a T-cell abnormality. To confirm a T-cell abnormality, doctors do additional blood tests to determine the number of T cells and to evaluate T-cell function.

c.)Biopsy

May be done to help doctors identify which specific immunodeficiency disorder is causing the symptoms. For the biopsy, doctors take a sample of tissue from the lymph nodes and or bone marrow. The sample is tested to determine whether certain immune cells are present.

d.) Genetic testing

May be done if doctors suspect a problem with the immune system. The gene mutation or mutations that cause many immunodeficiency disorders have been identified. Thus, genetic testing can sometimes help identify the specific immunodeficiency disorder.

e.) Screening

Genetic testing, usually blood tests, may also be done in people whose families are known to carry a gene for a hereditary immunodeficiency disorder. These people may wish to be tested to learn whether they carry the gene for the disorder and what their chances of having an affected child are. Talking with a genetic counselor before testing is helpful.

Several immunodeficiency disorders, such as X-linked Agammaglobulinemia, Wiskott-Aldrich syndrome, severe combined immunodeficiency, and chronic granulomatous disease, can be detected

in a fetus by testing a sample of the fluid around the fetus (amniotic fluid) or the fetus's blood (prenatal testing). Such testing may be recommended for people with a family history of an immunodeficiency disorder when the mutation has been identified in the family.

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Some experts recommend screening all newborns with a blood test that determines whether they have abnormal T cells or too few T cells--called the T-cell receptor excision circle (TREC) test. This test can identify cellular some immune deficiencies, such as severe combined immunodeficiency. Identifying infants with severe combined immunodeficiency early can help prevent their death at a young age. TREC testing of all newborns is now required in many U.S. states.

f.) Prevention

Some of the disorders that can cause secondary immunodeficiency can be prevented and or treated, thus helping prevent immunodeficiency from developing. The following are examples:

10.18 HIV infection

Measures to prevent HIV infection such as following safe sex guidelines and not sharing needles to inject drugs can reduce the spread of this infection. Also, antiretroviral drugs can usually treat HIV infection effectively.

10.19 Cancer

Successful treatment usually restores the function of the immune system unless people need to continue taking immunosuppressants.

10.20 Preventing infections

Strategies for preventing and treating infections depend on the type of immunodeficiency disorder. For example, people who have an immunodeficiency disorder due to a deficiency of antibodies are at risk of bacterial infections. The following can help reduce the risk:

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- Being treated periodically with immune globulin (antibodies obtained from the blood of people with a normal immune system) given intravenously or under the skin
- Practicing good personal hygiene (including conscientious dental care)
- Not eating undercooked food
- Not drinking water that may be contaminated
- Avoiding contact with people who have infections

Vaccines are given if the specific immunodeficiency disorder does not affect antibody production. Vaccines are given to stimulate the body to produce antibodies that recognize and attack specific bacteria or viruses. If the person's immune system cannot make antibodies, giving a vaccine does not result in the production of antibodies and can even result in illness. For example, if a disorder does not affect production of antibodies, people with that disorder are given the influenza vaccine once a year. Doctors may also give this vaccine to the person's immediate family members and to people who have close contact with the person.

Generally, vaccines that contain live but weakened organisms (viruses or bacteria) are not given to people who have a B- or T-cell abnormality because these vaccines may cause an infection in such people. These vaccines include rotavirus vaccines, measles-mumps-rubella vaccine, and chicken pox (varicella) vaccine, one type of varicella-zoster (shingles) vaccine, bacille Calmette-Guérin (BCG) vaccine, influenza vaccine given as a nasal spray, and oral poliovirus vaccine. The oral poliovirus vaccine is no longer used in the United States but is used in some other parts of the world.

10.21 Treating infections

Antibiotics are given as soon as a fever or another sign of an infection develops and often before surgical and dental procedures, which may introduce bacteria into the bloodstream. If a disorder (such as severe

combined immunodeficiency) increases the risk of developing serious infections or particular infections, people may be given antibiotics to prevent these infections.

10.22 Antiviral drugs

Antiviral drugs are given at the first sign of infection if people have an immunodeficiency disorder that increases the risk of viral infections such as immunodeficiency due to a T-cell abnormality. These drugs include oseltamivir or zanamivir for influenza and acyclovir for herpes or chickenpox.

10.23 ORGAN TRANSPLANTATION

10.23.1 Transplantation

The immune system plays a critical role in transplantation. Rejection of a transplant occurs in instances where the immune system identifies the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue. Transplantation is the process of moving cells, tissues or organs from one site to another for the purpose of replacing or repairing damaged or diseased organs and tissues. It saves thousands of lives each year. However, the immune system poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another.

Rejection is caused by the immune system identifying the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue. Long term survival of the transplant can be maintained by manipulating the immune system to reduce the risk of rejection. Donor and recipient are carefully matched prior to transplantation to minimize the risk of rejection. They are matched based on their blood group, tissue typing, and how the recipient's blood serum reacts to donor cells. Immunosuppressive drugs are used to prevent and to treat transplant rejection by dampening the overall immune response. However, immunosuppressive drugs are non-specific and leave patients more susceptible to disease as well as being associated with numerous unwanted side effects. Further research on the immunological mechanisms of rejection will help improve cross matching, diagnosis and treatment, as well as facilitating the discovery of novel strategies for preventing.

Rejection of a transplant occurs in instances where the immune system identifies the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue. The intensity of the immune response against the organ or tissue, also commonly referred to as the graft, will depend on the type of graft being transplanted and the genetic disparity between the donor and recipient. To reduce the possibility of rejection, the donor and recipient are carefully matched for immune compatibility prior to transplantation. However, the small pool of eligible donors can make it difficult to find a donor-recipient match and there will always be a degree of rejection against the graft. A critical undersupply of donated organs means that waiting lists for transplants are extremely long.

Patients needing kidney transplantation, for example, wait on average 944 days (more than two and a half years) for a life-saving transplant. There were 6,943 patients registered for organ transplant in the UK as of March 2015. Unfortunately, 479 of these patients died during 2015/16 whilst waiting for a transplant due the small pool of transplantable organs. These figures underline the value of every organ and highlight the importance of a successful transplantation and maintaining long-term transplant survival. Manipulation of the immune system can support longterm survival of the graft ensuring that every transplant is as successful as possible.

10.23.2 Replacing missing parts of the immune system

Immune globulin can effectively replace missing antibodies (immunoglobulins) in people with an immunodeficiency that affects antibody production by B cells. Immune globulin may be injected into a vein (intravenously) once a month or under the skin (subcutaneously) once a week or once a month. Subcutaneous immune globulin can be given at home, often by the person with the disorder.

10.23.3 Stem cell transplantation

Stem cell transplantation can correct some immunodeficiency disorders, particularly severe combined immunodeficiency. Stem cells may be obtained from bone marrow or blood (including umbilical cord blood). Stem cell transplantation, which is available at some major medical centers, is usually reserved for severe disorders.

10.24.4 There are several types of transplantation involving tissues and organs:

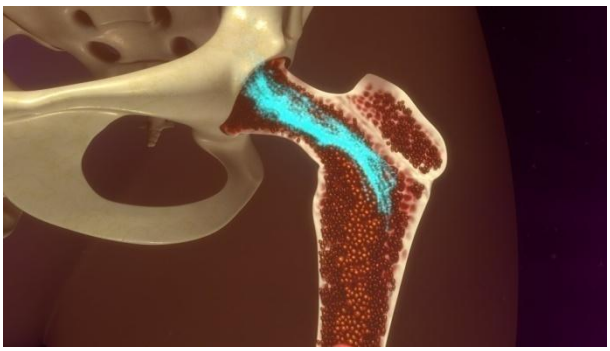
i. Autograft –Transplantation of cells, tissues or organs between sites within the same individual e.g. skin graft.

ii. Allograft – Transplantation of organs or tissues from a donor to a non-genetically identical individual of the same species. Allograft is the most common type of transplant.

iii. Engraft – Transplantation of an organ or tissue between two different species. 'Pig valves', for example, are commonly used to repair or replace a defective heart valve in humans. In 2015/16, 6,069 engraft valve replacements were carried out in England by the NHS.iii Xenotransplantation of whole organs is not currently viable, although it is an area of huge scientific interest as a potential solution for the existing critical undersupply of adequate organs.

10.23.5 ABO incompatible

ABO refers to blood group, which can vary between individuals. For most transplant types, matching of blood group between donor and recipient is a key strategy in reducing rejection risk. However, blood group compatibility is not always required for transplantations.



10.23.6 Stem cell transplant:

Stem cells are cells that have the capacity to develop into a range of different types of cells in the body. Blood stem cells (hematopoietic stem cells) can develop into all the different cells found in the blood and are donated to replace damaged or destroyed blood cells. Hematopoietic stem cell transplants are used to treat certain types of cancer e.g. leukemia, and blood diseases where the bone marrow has become damaged preventing

the production of healthy blood cells. These stem cells can be harvested either directly from bone marrow or from the cord blood (blood from the placenta and umbilical cord) from consenting mothers following childbirth.

10.23.7 The immunology of transplant rejection.

Foreign invaders are presented to the immune system in the form of small molecules called antigens. Identification of these non-self antigens will trigger an immune response and will stimulate the production of antigen specific antibodies that mark infected cells for destruction by the immune system and help amplify the immune response. The Human Leukocyte Antigen (HLA) complex is a group of genes that encode the proteins responsible for identifying foreign agents to the immune system. These proteins are found on the surface of all cells and act as 'self-markers' telling the immune system not to trigger a response.

Each person will have their own specific set of HLA proteins, based upon their unique genetic make-up that the immune system will have learned not to react to. Any cell not displaying these specific HLA proteins will be identified as 'non-self' by the immune system and will be treated as a foreign invader.

10.23.8 Mechanism of rejection

Graft rejection occurs when the recipient's immune system attacks the donated graft and begins destroying the transplanted tissue or organ. The immune response is usually triggered by the presence of the donor's own unique set of HLA proteins, which the recipient's immune system will identify as foreign. The degree of similarity between the HLA genes of the donor and recipient is known as histocompatibility, the more genetically compatible the donor and the recipient, the more tolerant the recipient's immune system should be of the graft. However, unless the donor and recipient are genetically identical (e.g. as in identical twins) there will always be some degree of rejection. As well as non self HLA proteins, other surface proteins on the donor graft can also be identified as a foreign antigen and illicit an immune response.

In some cases, a patient may experience something known as ‘graft versus host reaction’ where mature immune cells already present in the donor graft begin attacking the healthy cells of the recipient. Graft versus host reaction, where the donor graft is described as being “immune-competent” (i.e. capable of producing an immune response) is a particular risk with stem cell transplants (bone marrow transplant) and can also occur following blood transfusions.

10.24.1 Hyper acute rejection

This occurs within minutes or hours after transplantation and is caused by the presence of preexisting antibodies of the recipient, that match the foreign antigens of the donor, triggering an immune response against the transplant. These antibodies could have been generated as a result of prior blood transfusions, prior transplantations or multiple pregnancies. The antibodies react with cells in the blood vessels of the graft, causing blood clots to form, which will prevent blood supply from reaching the graft resulting in immediate rejection of the transplant.

10.24.2 Acute rejection

This occurs within the first 6 months after transplantation. Some degree of acute rejection will occur in all transplantations, except between identical twins. Recipients are most at risk in the first 3 months, but rejection can still occur at a later stage. Acute rejection is caused by the formation of antibodies following the detection of non-self antigens in the donated graft. If diagnosed early enough, acute rejection can be treated by suppressing the immune system and permanent damage to the graft can be avoided in some cases.

10.24.3 Chronic rejection

Repeated episodes of acute rejection can ultimately lead to chronic rejection of the graft and failure of the transplant. Chronic rejection commonly manifests as scarring of the tissue or organ which can occur months to years after acute rejection has subsided. At present, there is no cure for chronic rejection other than removal of the graft.

10.24.5 Finding an eligible donor-recipient match

Rejection can be minimized by carefully matching the donor and recipient for compatibility prior to transplantation. The better matched the donor and recipient are the more successful the transplantation is likely to be. Compatibility between donor and recipient is assessed using a combination of tests, including:

10.24.6 ABO blood group compatibility

The donor and recipient are tested for compatible blood groups. This is the first test to be carried out as the transplant will be rapidly rejected if the blood groups do no match. In some transplants, for example young children and also bone marrow transplants, ABO compatibility is not a necessity.

10.24.7 Tissue typing

A blood sample is taken from the recipient to identify the HLA antigens present on the surface of the cells to help find a histone compatible donor. The more alike the HLA types of the donor and recipient are the more likely a transplant will be successful. Family members, in particular siblings, are often the best HLA matches due to their genetic similarity.

10.24.8 Cross matching

Blood samples are taken from both the recipient and donor, and the cells of the donor are mixed with the blood serum of the recipient. If the recipient's antibodies attack the donor cells, they are considered a positive match and transplantation will not be suitable due to increased risk of hyper-acute rejection.

10.24.9 Panel reactive antibody test

The blood serum of patients awaiting transplantation is tested for reactive antibodies against a random panel of cells. Previous exposure to foreign tissue, by blood transfusion, pregnancy or prior transplantations, is likely to increase the number of HLA antibodies in the blood. The more

HLA antibodies present, the higher he panel reactive antibody (PRA) level denoted to the patient and the greater the chance of graft rejection. If PRA levels are high, it may be more difficult to find a match and a higher dosage of immunosuppressive drugs may be required.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

10.25 Serology screening

For patients undergoing stem cell transplantation they and their donor will undergo pre-transplant serology screening. This is undertaken to detect the immune status of both the donor and a potential recipient against a number of clinically significant infectious organisms, including viruses like HIV, Cytomegalovirus (CMV), and Epstein-Barr Virus (EBV), thus determining potential for re-infection or reactivation of the infection upon immuno suppression. Individuals are often matched according to the CMV and EBV status.

10.26 Immuno suppressive drugs

To reduce the risk of transplant rejection, patients are treated with immunosuppressive drugs that will dampen their immune response. Immunosuppressive drugs are given in two phases; an initial induction phase involving a high dose, and a later maintenance phase which involves using the drug in the long term at a lower dose.

Patients are required to take a large number of immunosuppressants each day for the rest of their lives, which can have a major impact on their health and lifestyle. A fine balance needs to be reached between suppressing immune function sufficiently to avoid rejection, preventing drug toxicity, and maintaining enough immune function to fight off disease.

10.27 Future transplant therapies

As well as new immunosuppressive drugs, with increased specificity and fewer side effects, other new therapies could also one day greatly reduce, or entirely remove, the possibility of rejection. Stem cells could have a major impact on transplantation in the future beyond their current use in treating blood disorders. Pluripotent stem cells have the capacity to mature into any cell in the body, and this ability can be harnessed to grow tissues and organs. Moreover, the discovery that other cell types can be induced to have stem cell capacities means that the cells

Self - Instructional Material

used to make the tissue could come directly from the recipient themselves, thus circumventing the risk of rejection.

Another future approach is the manufacture of organ scaffolds using 3D printing and then growing stem cells around these scaffolds to artificially replicate the tissue being replaced. Bio-manufacturing of tissues and organs would not only reduce the risk of transplant rejection, if the patient's own stem cells were used, but would also reduce the strain on the limited organ supply. Therefore, improving currently available therapies and the discovery of novel immunosuppressive regimes remains at the forefront of transplant medicine research.

Improving compatibility testing between donor and recipient could also reduce the risk of transplant rejection and increase the longevity of the transplant. The better matched the donor and the recipient are, the more tolerant the recipient's immune system will be to the transplanted organ or tissue. Additionally, a greater understanding of the disparity between the donor and recipient will better inform treatment strategies after transplantation and help avoid repeated episodes of acute rejection.

Immunological research has led to huge advancements in transplant medicine. However, immune rejection still remains the most formidable barrier to successful transplantation. Continued research is needed to find ways to alleviate the risk of rejection improve diagnosis and maintain long term survival of the transplant, all of which would have a significant impact on the strained organ supply.

10.28 LET US SOME UP

In this Unit, You have learnt about the meaning, definition, need, Objectives, Importance and concept of autoimmune disorder and immunodeficiency diseases and organ transplantation. This knowledge would make you understand what are the autoimmune disorder types, prevention, treatment and Diagnose of autoimmune disorder and immunodeficiency diseases and organ transplantation. It is may be helpful for differentiate for immune disorders.

10.29 UNIT END EXERCISES

1. Explained transplantation

2. What are the types involve in transplantation

10.30 ANSWERS TO CHECK YOUR PROGRESS

1. Cause abnormally low activity or over activity of the immune system. In cases of immune system over activity, the body attacks and damages its own tissues (autoimmune diseases). Immune deficiency diseases decrease the body's ability to fight invaders, causing vulnerability to tissues infection.

2. In many cases, the first sign of autoimmune kidney disease is elevated blood pressure. This is often accompanied by the appearance of blood or protein in the urine. In the setting of active glomerulonephritis, blood in the urine may not appear pink, but instead is more likely to cause the urine to have a color closer to that of tea or cola. Blood and protein are easily detected in the urine using readily available test strips that are frequently called urine "dipsticks." If there is substantial protein loss in the urine, it can lead to fluid retention and swelling (edema) of the legs and feet.

10.31 SUGGESTED READINGS

1. Matthew Helbert, Immunology for Medical Students. Elsevier, 2016

2. Sunil Kumar Mohant, Dr.Sai Leela Text book of immunology Jaypee Brothers Medical Publishers (P) Ltd.Second edition 2014.

3. "Immunology" by Roitt I and Male Brostoff. Mosby-Year Book; 4th edition (January 1996)

4. "Immunology" by Dulsy Fatima and N Arumugam. Saras Publication,2009.

5. The Elements of Immunology" by Fahim Halim Khan. Pearson Education India, 2009.

UNIT XI CANCER, TYPES AND NATURE, IMMUNOTHERAPY, IMMUNE RESPONSES AGAINST TUMORS AND TRANSPLANTS

Structure

11.1 Introduction:

Cancer, also called malignancy, is an abnormal growth of cells. There are more than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma. Symptoms vary depending on the type. Cancer treatment may include chemotherapy, radiation, and surgery.

Cancer refers to the abnormal growth of cell tissue. Tumours are usually divided into benign and malignant. A benign tumour is localised, develops slowly and does not usually result in the patient's death. Malignant or cancerous tumours develop more rapidly. They are not localised and are often fatal for the patient.

Cancer cells do not always form a compact tumour. Leukaemia, for example, is a cancer in the blood-forming tissue where cancer cells circulate in the body and behave to some extent like healthy cells. Eventually they displace healthy cells, preventing their normal function. Carcinogenesis is a multi-stage process in which damage to a cell's genetic material, changes the cell from normal to malignant. The damage gradually accumulates in the cell's growth regulatory system.

Cancer begins with a genetic defect. Human's genetic factors, meaning genes, are located within the cell structures called chromosomes. Genes control cell functions, such as their distribution. Genes may undergo changes, or mutations, if the cell's regulatory system fails. A single genetic fault will not usually be enough to cause cancer. Cancer develops when mutations take place in genes that play a crucial role in regulating cell growth and differentiation. So-called cancer genes are genes that control a cell's normal functions but which are simply damaged.

11.2 Objectives

The aim of cancer immunotherapy is to reawaken or "reboot" the immune system, enabling it to once again attack the tumour.

Adoptive T cell therapy – As well as targeting the tumour cells, immunotherapies also target the immune cells directly.

11.3 Types of Cancer

- Carcinoma is a cancer that starts in the skin or the tissues that line other organs.
- Sarcoma is a cancer of connective tissues such as bones, muscles, cartilage, and blood vessels.
- Leukemia is a cancer of bone marrow, which creates blood cells.
- Lymphoma and myeloma are cancers of the immune system.

There are two kinds of cancer genes

- Oncogenes, which are cancer generating genes, whose activation causes an uncontrollable distribution of cell tissue;
- Tumour suppressor genes or anti-cancer genes whose cancer inducing effect is due to the cessation of their activity ceases.

Damage to genetic material happens continually in many cells. But the human body contains a defence system developed over a long period, and this is able to repair the damage. If the system breaks down, damaged cells can start to divide uncontrollably, eventually leading to carcinogenesis. A cancerous tumour contains billions of cancer cells. A cancer cell has to divide many thousands of times before a tumour is even the size of a pea. It can take years before a tumour is visible on an X-ray or can be felt by hand. But it can also grow much more rapidly.

11.4 Cancer Growth and Metastasis

In a healthy body, the trillions of cells it's made of grow and divide, as the body needs them to function daily. Healthy cells have a specific life cycle, reproducing and dying off in a way that is determined by the type of cell. New cells take the place of old or damaged cells as they die. Cancer disrupts this process and leads to abnormal growth in cells. It's caused by changes or mutations in DNA.DNA exist in the individual genes of every

cell. It has instructions that tell the cell what functions to perform and how to grow and divide. Mutations occur frequently in DNA, but usually cells correct these mistakes. When a mistake is not corrected, a cell can become cancerous.

Mutations can cause cells that should be replaced to survive instead of die, and new cells to form when they're not needed. These extra cells can divide uncontrollably, causing growths called tumors to form. Tumors can cause a variety of health problems, depending on where they grow in the body. But not all tumors are cancerous. Benign tumors are noncancerous and do not spread to nearby tissues. Sometimes, they can grow large and cause problems when they press against neighboring organs and tissue. Malignant tumors are cancerous and can invade other parts of the body.

Some cancer cells can also migrate through the bloodstream or lymphatic system to distant areas of the body. This process is called metastasis. Cancers that have metastasized are considered more advanced than those that have not. Metastatic cancers tend to be harder to treat and more fatal. Cancers are named for the area in which they begin and the type of cell they are made of, even if they spread to other parts of the body. For example, a cancer that begins in the lungs and spreads to the liver is still called lung cancer.

11.5 Risk Factors and Treatment:

The direct cause of cancer is changes (or mutations) to the DNA in your cells. Genetic mutations can be inherited. They can also occur after birth as a result of environmental forces. Some of these forces include:

- Exposure to cancer-causing chemicals, called carcinogens
- Exposure to radiation
- Unprotected exposure to the sun
- Certain viruses, such as human papilloma virus (HPV)
- Smoking
- Lifestyle choices, such as type of diet and level of physical activity

Cancer risk tends to increase with age. Some existing health conditions that cause inflammation may also increase your risk of cancer. An example is ulcerative colitis, a chronic inflammatory bowel disease. Knowing the factors that contribute to cancer can help you live a lifestyle that decreases your cancer risks. According to experts, these are the seven best ways to prevent cancer: Stop using tobacco and avoid secondhand smoke. Eat a healthy, balanced diet. Limit your intake of processed meats. Consider adopting a "Mediterranean diet" that focuses mainly on plant-based foods, lean proteins, and healthy fats. Avoid alcohol, or drink in moderation. Moderate drinking is defined as one drink a day for women of all ages and men older than 65, and up to two drinks a day for men 65 years of age and younger.

- Keep a healthy weight and stay active by getting at least 30 minutes of physical activity every day.
- Stay protected from the sun.
- Cover up with clothing, sunglasses, and a hat, and apply sunscreen frequently.
- Avoid the sun between 10 a.m. and 4 p.m. This is when the sun's rays are at their strongest.
- Stay in the shade as much as possible when you're outside.
- Avoid tanning beds and sunlight, which can damage your skin just as much as the sun.
- Get vaccinated against viral infections that can lead to cancer, such as hepatitis B and HPV.
- Don't engage in risky behaviors. Practice safe sex and don't share needles when using drugs or prescription medications. Only get tattoos at licensed parlors.
- See your doctor regularly so they can screen you for various types of cancer. This increases your chances of catching any possible cancers as early as possible.

Check your progress

Note: write your answer in the space given below

2. Define Cancer?

……..

11.6 Visit the Cancer Prevention section for more information

Cancer treatment has different objectives, depending on the type of cancer and how advanced it is. These objectives include:

- Finding a cure: This is not possible for all cancers and situations.
- Providing primary treatment: Killing the cancer cells in your body.
- Providing adjuvant treatment: Killing cancer cells that remain after primary treatment to reduce your risk of the cancer coming back.
- Providing palliative treatment: Relieving health symptoms associated with cancer, such as trouble breathing and pain.

The most common types of treatment are:

11.7 Surgery

Surgically removes as much of the cancer as possible.

11.8 Chemotherapy

Uses medications that are toxic to cells to kill rapidly-dividing cancer cell.

11.9 Radiation Therapy

Uses powerful, focused beams of radiation inside (brachytherapy) or outside (external beam radiation) to the patients to kill cancer cells.

11.10 Stem Cell (Bone Marrow) Transplant

Repairs diseased bone marrow with healthy stem cells. Stem cells are undifferentiated cells that can have a variety of functions. These transplants allow doctors to use higher doses of chemotherapy to treat the cancer.

11.11 Immunotherapy (Biological Therapy)

Uses antibodies to help your body's immune system recognize cancer so it can fight it off.

11.12 Hormone Therapy

Removes or blocks hormones that fuel certain cancers to stop cancer cells from growing.

11.13 Targeted Drug Therapy

Uses drugs to interfere with certain molecules that help cancer cells grow and survive.

Clinical Trials

Investigates new ways to treat cancer.

Alternative Medicine**.**

Used to decrease symptoms of cancer and side effects of cancer treatment, such as nausea, fatigue, and pain. Alternative medicine includes:

- acupuncture
- Hypnosis
- Massage
- Yoga
- Meditation
- Relaxation techniques

11.14 The Main types of cancer.

We can group cancer according to the type of cell they start in. There are 5 main types:

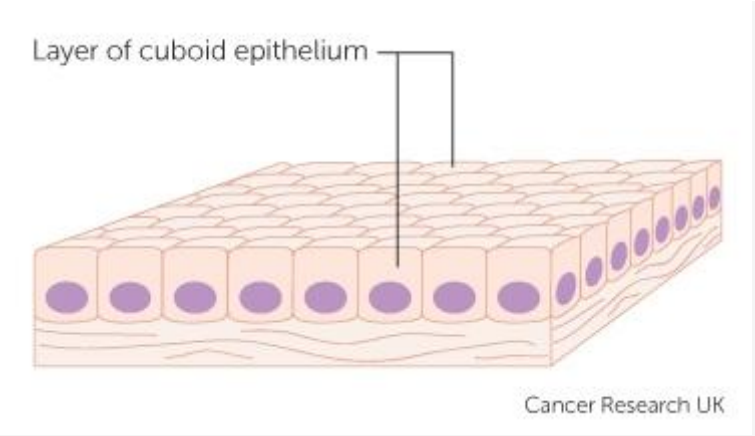
Carcinoma – cancer that begins in the skin or in tissues that line or cover internal organs. There are different subtypes, including adenocarcinoma, basal cell carcinoma, squamous cell carcinoma and transitional cell carcinoma

- **Sarcoma** – cancer that begins in the connective or supportive tissues such as bone, cartilage, fat, muscle or blood vessels
- **Leukaemia** – cancer that starts in blood forming tissue such as the bone marrow and causes abnormal blood cells to be produced and go into the blood
- **Lymphoma and myeloma** – cancers that begin in the cells of the immune system
- **Brain and spinal cord cancers** – these are known as central nervous system cancers

We can also classify cancers according to where they start in the body, such as breast cancer or lung cancer.

11.14.1 Carcinomas

Carcinomas start in epithelial tissues. These cover the outside of the body as the skin. They also cover and line all the organs inside the body, such as the organs of the digestive system. And they line the body cavities, such as the inside of the chest cavity and the abdominal cavity.



Carcinomas are the most common type of cancer.

There are different types of epithelial cells and these can develop into different types of carcinoma. These include those below.

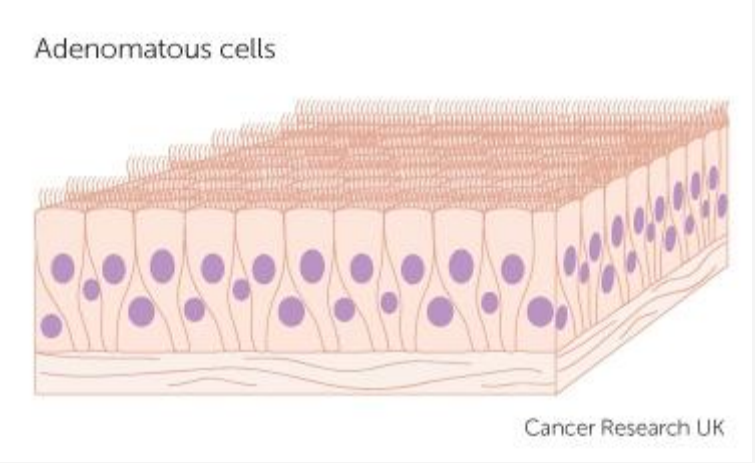
11.14.2 Squamous cell carcinoma

Squamous cell carcinoma starts in squamous cells. These are the flat, surface covering cells found in areas such as the skin or the lining of the throat or food pipe (Oesophagus).

11.14.1 Carcinomas

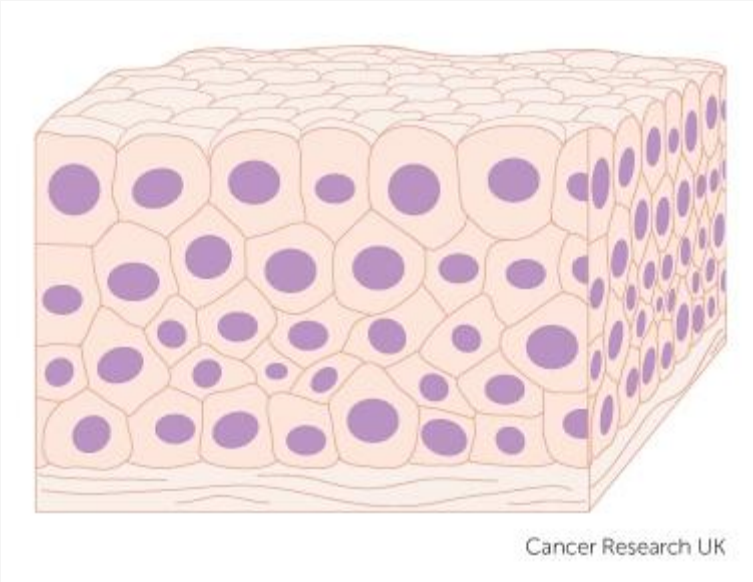
11.14.3 Adenocarcinoma

Adenocarcinoma start in glandular cells called a denomatous cells. Glandular cells produce fluids to keep tissues moist.



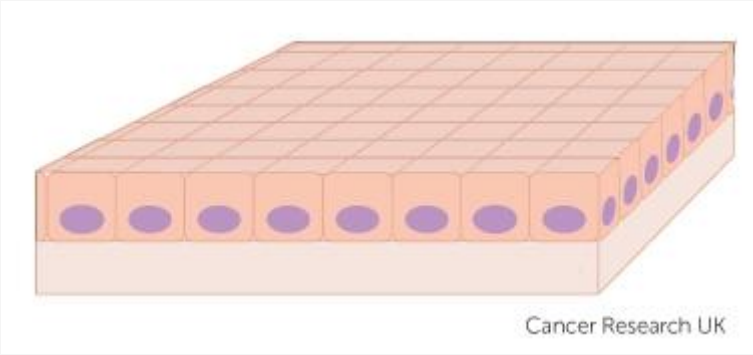
11.14.4 Transitional cell carcinoma

Transitional cells are cells that can stretch as an organ expands. They make up tissues called transitional epithelium. An example is the lining of the bladder. Cancers that start in these cells are called transitional cell carcinoma.



11.14.5 Basal cell carcinoma

Basal cells line the deepest layer of skin cells. Cancers that start in these cells are called basal cell carcinomas.



11.14.6 Sarcomas

Sarcomas start in connective tissues, which are the supporting tissues of the body. Connective tissues include the bones, cartilage, tendons and fibrous tissue that support organs.

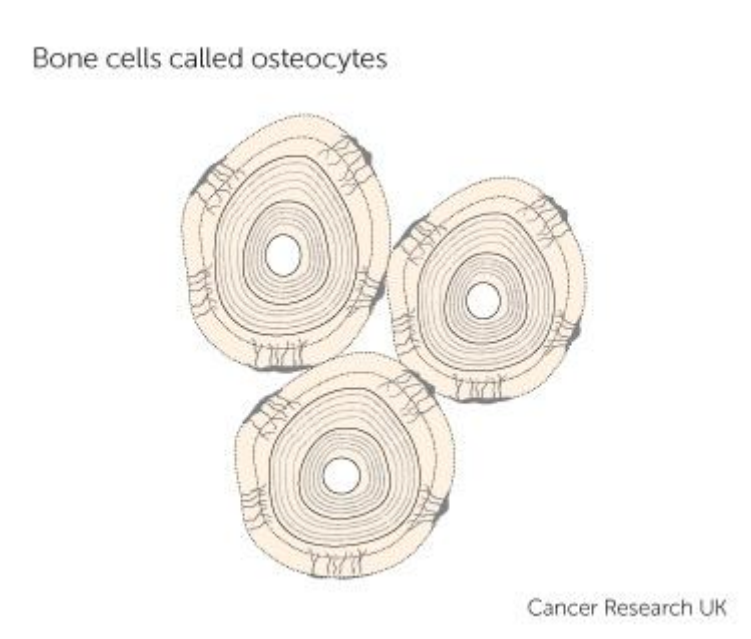
Sarcomas are much less common than carcinomas. They are usually grouped into 2 main types:

- bone sarcomas (Osteosarcoma)
- soft tissue sarcomas

Altogether, these make up less than 1 in every 100 cancers (1%) diagnosed every year.

11.14.7 Bone sarcomas

Sarcomas of bone start from bone cells.



You can read about bone cancers.

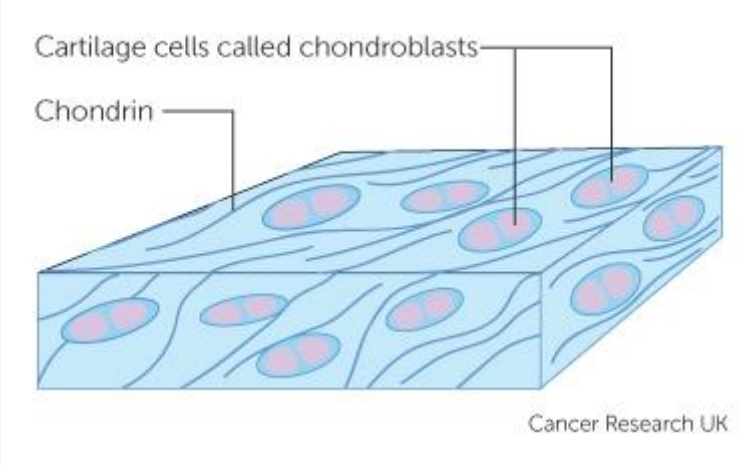
11.14.8 Soft tissue sarcomas

Soft tissue sarcomas are rare but the most common types start in cartilage or muscle.

11.14.9 Cartilage

Cancer of the cartilage is called Chondrosarcoma.

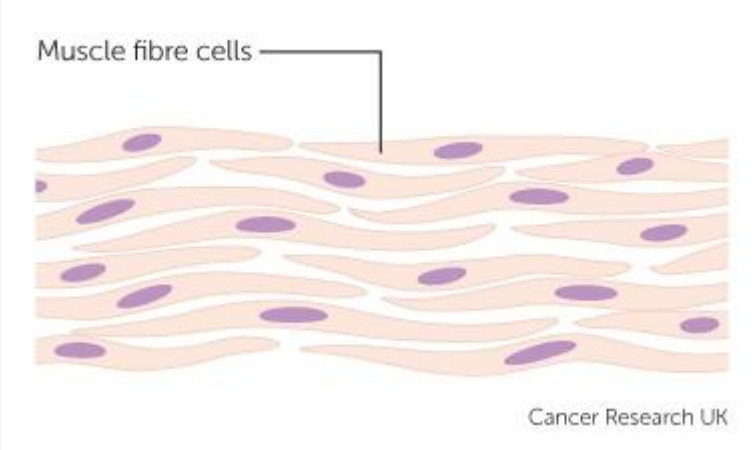
Chondroblasts.



11.14.10 Muscle

Cancer of muscle cells is called rhabdomyosarcoma or leiomyosarcoma.

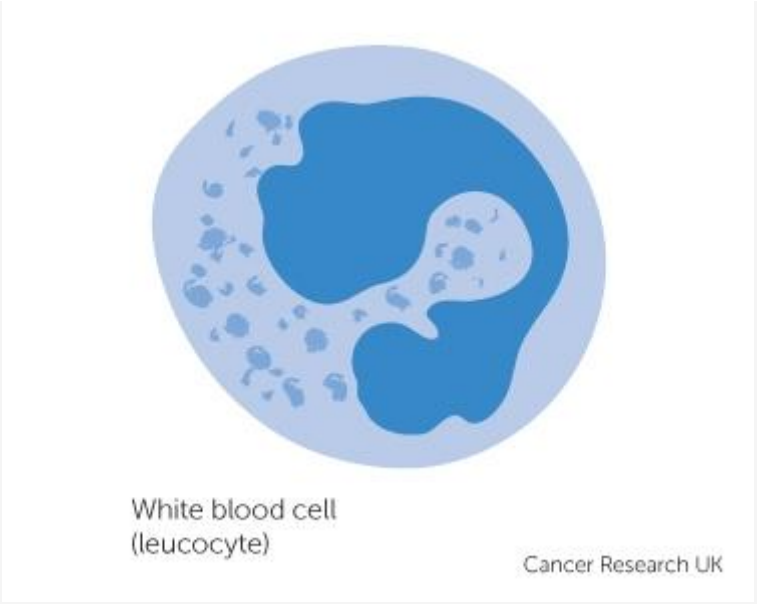
muscle-cells.



Leukaemias – cancers of blood cells

Leukaemia is a condition in which the bone marrow makes too many white blood cells. The blood cells are not fully formed and so they don't work properly. The abnormal cells build up in the blood.

white-blood-cell.



Leukaemias are uncommon and make up only 3 out of 100 of all cancer cases (3%). But they are the most common type of cancer in children.

There are different types of leukemia.

Lymphomas and myeloma

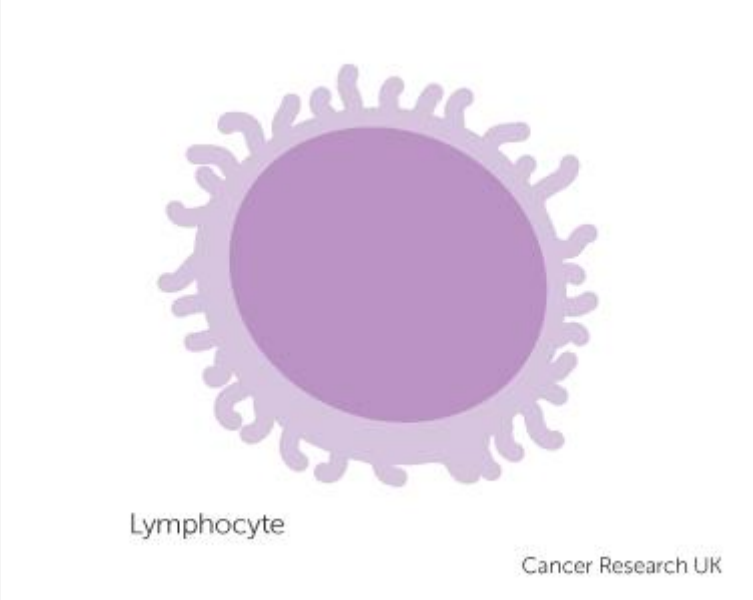
Lymphomas and myeloma are cancers of the lymphatic system. The lymphatic system is a system of tubes and glands in the body that filters body fluid and fights infection.

11.14.11 Lymphomas

Lymphomas start from cells in the lymphatic system. Because the lymphatic system runs all through the body, lymphoma can start just about anywhere.

Some of the lymphatic system white blood cells (lymphocytes) start to divide abnormally and they usually to these cells start to divide before they become fully grown (mature) so they can't fight infection.

lymphocyte.



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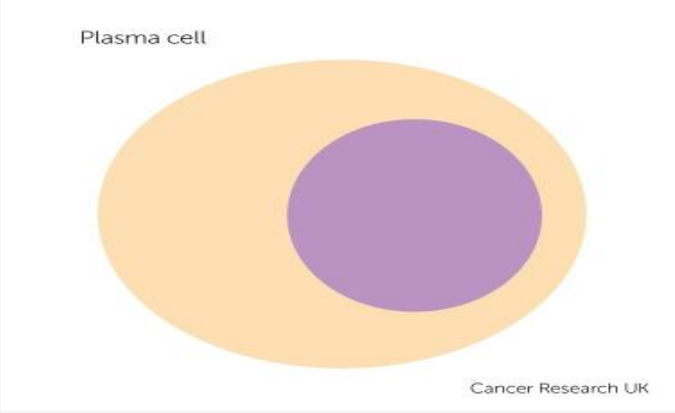
The abnormal lymphocytes start to collect in the lymph nodes or other places such as the bone marrow or spleen. They can then grow into tumours.

11.14.12 Myeloma

Myeloma is also known as multiple myeloma. It is a cancer that starts in plasma cells. Plasma cells are a type of white blood cell made in the bone marrow. They produce antibodies, also called immunoglobulins, to help fight infection

plasma-cell.



Plasma cells can become abnormal, multiply uncontrollably, and only make a type of antibody that doesn't work properly to fight infection.

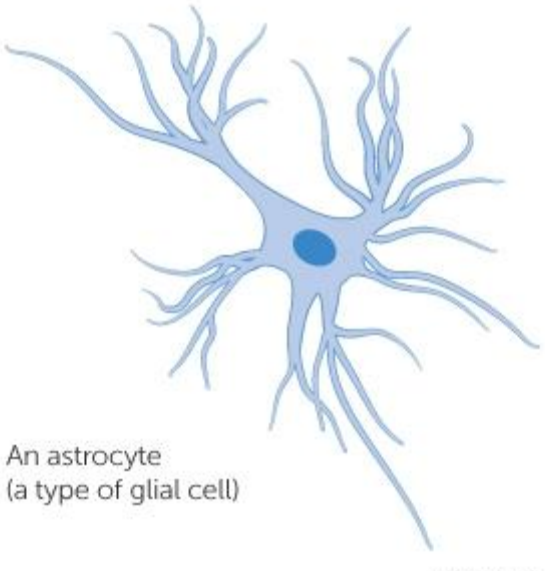
11.15 Brain and spinal cord cancers

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

Cancer can start in the cells of the brain or spinal cord. The brain controls the body by sending electrical messages along nerve fibres. The fibres run out of the brain and join together to make the spinal cord, which also takes messages from the body to the brain. The brain and spinal cord form the central nervous system. The brain is made up of billions of nerve cells called neurones. It also contains special connective tissue cells called glial cells that support the nerve cells. The most common type of brain tumour develops from glial cells and is called glioma. Some tumours that start in the brain or spinal cord are non cancerous (benign) and grow very slowly. Others are cancerous and are more likely to grow and spread.

11.16 GLIAL-CELL.



11.16.1 Cancer treatments

Self - Instructional Material

Cancer treatments are being continually developed. Increasingly more effective and better-targeted treatments are available. As treatment has developed, the outcomes have improved. Treatment outcomes in Finland are outstanding by international comparisons.

11.16.2 The purpose of cancer treatments is that

- The disease is brought under control
- Cancer recurrence is prevented, and
- The symptoms caused by the tumour are alleviated.

The main forms of cancer treatment are cancer surgery (surgical treatment), radiotherapy, chemotherapy and hormone therapy. Nowadays various immunological therapies and so-called smart drug delivery (or targeted drug delivery) are also used. There are a variety of different cancer drugs available. They are usually used in combination. Cancer therapy is personalised, which is why treatments can vary. The choice of treatment is influenced by the location of the tumour, distribution; cell type and the patient's overall condition as well as possible other illnesses.

11.17 Patients' rights

Some cancers progress so slowly that the situation can be monitored for a while before the type of treatment is selected and started. Surgery is often sufficient for the treatment of small malignant tumours. Sometimes it's possible to use only chemotherapy or drug therapy instead. The treatment of large tumours involves various combinations of surgery, radiotherapy and drug therapy. Surgery can be supplemented with other cancer therapies to treat patients.

Everyone with cancer wants to receive treatment as soon as possible. Waiting for treatment can heighten anxiety. However, it is not usually possible to start treatment immediately once diagnosis is confirmed as further tests have to be carried out. Defining the cancer type precisely is important in selecting the right treatment method. The staging classification of the cancer has also to be ascertained as treatment of a cancer that has spread or is in situ can differ greatly.

Many cancers develop slowly over several years. The delay of a few weeks in starting cancer treatment is usually insignificant in terms of the treatment's final outcome. With some cases of acute leukemia or malignant brain tumours, though, even a fairly short delay in treatment can

be important. Cancer therapy is a mental and physical strain on patients. Its side effects commonly include nausea, hair loss, reduced blood count, fatigue and swelling of the lower limbs. The efficacy of cancer therapy (response to treatment) and the adverse effects caused by the treatments are closely monitored.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

11.18 Cancer treatment in practice

Patients receive cancer therapy in hospital. As tests progress, the patient is transferred from care at a health centre to Specialized care. Cancer treatment is always personalised. Treatment is usually carried out under the supervision of an oncologist at an oncology clinic or ward. Treatment plans involve specialists from different medical fields. Rare and particularly difficult cases of cancer are treated at certain university hospitals. Children with cancer are usually treated at the children's clinics of university hospitals. Post-treatment monitoring is carried out on an individual basis. At first, patients are usually under specialist observation and then later at a health centre. Incases of incurable cancer, at some point patients are transferred for specialized treatment at health centers. Individual plans for symptomatic treatment are drawn up for patients. In addition to receiving health centre care, patients may receive palliative, or supportive, care at health care units at the oncology clinics. Palliative care is provided at individual hospices, health centre palliative care units or hospitals.

Check your progress

Note: write your answer in the space given below

2. Explain the purpose of cancer treatments?

……..

11.19Different types of cancer treatment

Self - Instructional Material

11.19.1 Combination therapy

Cancer treatment often involves combination therapy. Combination therapy refers to the combined use of many treatment forms, such as

surgery, radiotherapy and drugs. The purpose of combination therapy is to increase the patient's scope for recovery.

11.19.2 Adjuvant therapy

Adjuvant therapy is used to supplement surgery. Radiotherapy or chemotherapy provided following surgery are forms of adjuvant therapy. Adjuvant therapy ensures that cancer cells are destroyed, thereby improving the patient's prognosis.

11.19.3 Supportive therapy

Supportive cancer therapy alleviates symptoms caused by cancer or its treatment. It can improve the patient's wellbeing during and after the period of treatment. For instance, the anti-nausea medication used during chemotherapy is a form of supportive therapy. Cancer pain treatment is another form of supportive therapy.

11.19.4 Palliative care

Palliative care alleviates the patient's physical and psychological symptoms to improve the quality of life. Palliative care is used in cancer treatment or to treat the symptoms arising from cancer treatment. Palliative care can be provided for months or even years. The most common symptoms treated in palliative care are pain, constipation, nausea, confusion and fatigue. Palliative care is provided in tandem with curative treatment immediately following cancer diagnosis. If cancers cannot be cured, treatment focuses on the patients' symptoms so that their quality of life can be as good as possible. For patients with metastatic cancer, palliative care is of central importance.

11.20 Treatment fatigue

Up to 50 – 90 per cent of cancer patients suffer from treatment fatigue during their illness and periods of treatment. This involves extreme tiredness and fatigue that does not pass simply with sleeping and resting.

11.21 Treatment studies and drug trials

Research is used to develop cancer treatments. Clinical trials or treatment studies are research studies done with patients. Laboratory testing and animal testing precede clinical trials. The advantages and disadvantages of a new form of treatment are studied for a long time before patient trials are started.

Most cancer cells have mutations in both ongoenesis and tumor suppressor genes which lead to their behavior.

11.22. Growth:

Normal cells grow as a part of growth and development such as during childhood, or to repair injured tissue. Cancer cells continue to grow (reproduce) even when further cells are not needed. Cancer cells also fail to listen to signals that tell them to stop growing or commit cell suicide (apoptosis) when the cells become old or damaged.

11.22.1 Ability to invade nearby tissues:

Normal cells respond to signals from other cells which tell them they have reached a boundary. Cancer cells do not respond to these signals and extend into nearby tissues often with finger-like projections. This is one reason why it is difficult at times to surgically remove a cancerous tumor. The word cancer, in fact, is derived from the Greek word *Carcinos* for crab, referring to these claw-like extensions into neighboring tissues.

11.22.2 Ability to spread (metastasize) to other regions of the body:

Normal cells make substances called adhesion molecules that cause them to stick to nearby cells. Cancer cells, lacking the stickiness caused by these adhesion molecules, can break free and float to other regions of the body. They may travel to nearby tissue, or through the bloodstream and lymphatic system to areas of the body far from the original cancer

cell—for example, a lung cancer cell may travel (metastasize) to the lymph nodes, brain, liver, or the bones.

11.22.3 Immortality:

Normal cells, like humans, have a lifespan. When they reach a certain age, they die. Cancer cells, in contrast, have developed a way to "defy" death. On the end of our chromosomes is a structure known as a telomere. Every time a cell divides, its telomeres become shorter. When the telomeres become short enough, the cells die. Cancer cells have figured out a way to restore their telomeres so that they don't continue to shorten as the cell divides, thus, in a way, making them immortal. The ability to invade and metastasize is very important in differentiating a cancer cell from a normal healthy cell, but there are many other important distinctions as well.

11.23 IMMUNO THERAPY

Immunotherapy, also called biologic therapy, is a type of cancer treatment that boosts the body's natural defenses to fight cancer. It uses substances made by the body or in a laboratory to improve or restore immune system function. Stopping or slowing the growth of cancer cells.

The immune system protects us by recognising and attacking things which might make us ill, such as viruses, bacteria, or unhealthy cells.

One of the reasons cancer is so hard to treat is that cancer cells are very similar to our normal, healthy cells. This makes it difficult for our immune systems to recognise them as an enemy, and difficult to design drugs which can tell the difference between cancer and healthy cells.

Immunotherapy is one of the most exciting scientific advances in recent years. The idea is to retrain the immune system to identify and attack cancerous cells.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

NOTES

11.24 Types of Immunotherapy

Several types of immunotherapy are used to treat cancer. These treatments can either help the immune system attack the cancer directly or stimulate the immune system in a more general way.

Types of immunotherapy that help the immune system act directly against the cancer include:

11.24.1 Checkpoint inhibitors

Which are drugs that help the immune system respond more strongly to a tumor? These drugs work by releasing "brakes" that keep T cells (a type of white blood cell and part of the immune system) from killing cancer cells. These drugs do not target the tumor directly. Instead, they interfere with the ability of cancer cells to avoid immune system attack.

11.24.2 Adoptive cell transfer

Which is a treatment that attempts to boost the natural ability of your T cells to fight cancer. In this treatment, T cells are taken from your tumor. Then those that are most active against your cancer are grown in large batches in the lab. The process of growing your T cells in the lab can take 2 to 8 weeks. During this time, you may have treatments such as chemotherapy and radiation therapy to reduce your immune cells. After these treatments, the T cells that were grown in the lab will be given back to you via a needle in your vein.

For more information about a specific type of adoptive cell transfer called CAR T-cell therapy, which uses T cells that are changed in the laboratory, see CAR T-Cell Therapy: Engineering Patients' Immune Cells to Treat Their Cancers.

Self - Instructional Material

11.24.3 Monoclonal antibodies:

Also known as therapeutic antibodies, are immune system proteins produced in the lab. These antibodies are designed to attach to specific targets found on cancer cells. Some monoclonal antibodies mark cancer cells so that they will be better seen and destroyed by the immune system, and these are a type of immunotherapy. Other monoclonal antibodies that are used in cancer treatment do not cause a response from the immune system. Such monoclonal antibodies are considered to be targeted therapy, rather than immunotherapy.
Learn more about targeted therapy.

11.24.4 Treatment vaccines:

Which work against cancer by boosting your immune system's response to cancer cells. Treatment vaccines are different from the ones that help prevent disease.

Types of immunotherapy that enhance the body's immune response to fight the cancer include:

11.24.5 Cytokines

Which are proteins made by your body's cells. They play important roles in the body's normal immune responses and also in the immune system's ability to respond to cancer. The two main types of cytokines used to treat cancer are called interferons and interleukins.

11.24.6 BCG

Which stands for Bacillus Calmette-Guérin, is an immunotherapy that is used to treat bladder cancer. It is a weakened form of the bacteria that causes tuberculosis. When inserted directly into the bladder with a catheter, BCG causes an immune response against cancer cells. It is also being studied in other types of cancer.

11.25 LET US SOME UP

In this Unit, You have learnt about the meaning, definition, need, Objectives, Importance and concept of cancer, types and nature, immunotherapy, immune responses against tumors and transplants**.** This knowledge would make you understand what are the autoimmune disorder types, prevention, treatment and Diagnose of cancer, types and nature, immunotherapy, immune responses against tumors and transplants. It is may be helpful for differentiate for immune disorders.

11.24 UNIT END EXERCISES

1. Explain the types of cancer

2. What is cytokines?

11.25 ANSWERS TO CHECK YOUR PROGRESS

1. Cancer refers to the abnormal growth of cell tissue. Tumours are usually divided into benign and malignant. A benign tumour is localised, develops slowly and does not usually result in the patient's death. Malignant or cancerous tumours develop more rapidly. They are not localised and are often fatal for the patient.

- The disease is brought under control
- Cancer recurrence is prevented, and
- The symptoms caused by the tumour are alleviated.

The main forms of cancer treatment are cancer surgery (surgical treatment), radiotherapy, chemotherapy and hormone therapy. Nowadays various immunological therapies and so-called smart drug delivery (or targeted drug delivery) are also used. There are a variety of different cancer drugs available. They are usually used in combination. Cancer therapy is personalised, which is why treatments can vary. The choice of treatment is influenced by the location of the tumour, distribution; cell type and the patient's overall condition as well as possible other illnesses.

11.26 SUGGESTED READINGS

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BLOCK IV IMMUNOLOGICAL TECHNIQUES

UNIT XII

IMMUNOCYTOCHEMISTRY, ANTIBODY GENERATION AND RADIOIMMUNOASSAY

Structure

12.1 INTRODUCTION

Immunoassay is a biochemical method that identifies and quantifies unknown analytes (protein, lipid, nucleic acid etc.) in solution (serum, urine etc.) using antibody-antigen reactions. There are many different formats and variations of an immunoassay, but the key point is still specific antibody-antigen recognition**.** An antigen is a molecule that is recognized by the immune system, particularly by antibodies. Proteins, polysaccharides, pesticides, antibiotics, toxins, and hormones all can be antigens. But not all

antigens can stimulate the immune system to generate antibodies. An antigen that can elicit an immune response, and particularly antibody synthesis is called an immunogen. Proteins with molecular weights higher than 5-10 kDa are immunogens, while small peptides, pesticides, antibiotics, or hormones are not. These low-molecular-weight substances are called haptens and must be chemically coupled to larger carrier molecules, such as bovine serum albumin or keyhole limpet hemocyanin, in order to elicit specific antibody formation.

12.2 Objectives

Immunocytochemistry is the method to detect tissue antigens by utilizing specific antibodies and visualize antigen-antibody reaction by microscopically visible chromogen**.**

12.3 Immunocytochemistry

Immunocytochemistry (ICC) is a common laboratory technique that is used to anatomically visualize the localization of a specific protein or antigen in cells by use of a specific primary antibody that binds to it. The primary antibody allows visualization of the protein under a fluorescence microscope when it is bound by a secondary antibody that has a conjugated fluorophore. ICC allows researchers to evaluate whether or not cells in a particular sample express the antigen in question. In cases where an immunopositive signal is found, ICC also allows researchers to determine which sub-cellular compartments are expressing the antigen.

Immunocytochemistry is a procedure that is done in a laboratory that visualizes proteins and peptides. Samples are obtained from sections of tissues, mouth swabs, blood, or any other type of sample from human, plants, and animals. Bimolecular antibodies are applied to samples which react with the protein (antigen) that is being tested, causing a change in color to identify the protein. The antibody is associated with a reporter such as an enzyme, fluorescent dye, or fluorophore. The reporter changes the color of the protein which is seen by using a microscope.

12.3.1 Uses of Immunocytochemistry

The body is made up of many different types of proteins, peptides, and antigens that help carry out functions of the immune system. Antigens are single molecules that combine to form peptides. Peptides are called proteins when they contain more than fifty amino acids. The identification of proteins in cells is important in recognizing the body's immune response to various antibodies. Antibodies are used to fight infections in the body. Immunocytochemistry helps researchers determine which antibodies will bind to specific antigens and build immunity.

12.3.2 Technique for Immunocytochemistry

There are four basic steps to complete Immunocytochemistry testing. The first step is cell seeding where the samples are placed on glass slides or transparent plates. Samples require different incubation times before they can be used. Some samples are used right away while others must set for several hours or even twenty-four hours.

The next step consists of fixation of the cells and then immunostaining the cells. A fixative solution is applied to the cells so that they will not move on the slide. Some slides also need to be permeabilized with another solution in order for the stain to react depending on the type of fixative used. The antibody or immunostain (reporter) is then applied to the slide and later rinsed off to ensure excess antibodies are removed.

There are also two different Immunocytochemistry assays which include indirect and direct Immunocytochemistry technique. For indirect Immunocytochemistry assay, the protocol mainly include preparation and culture of cells, cells fixation, serum blocking, primary antibody incubation, marked second antibody incubation, staining, result judgment and imaging. For direct Immunocytochemistry assay, there are only marked primary antibody been incubated without second antibody and other steps are same with indirect Immunocytochemistry.

Check your progress

Note: write your answer in the space given below

1. What are the uses of Immunocytochemistry?

……

12.4 Generation of Antibodies

12.4.1Polyclonal and Monoclonal Antibodies

Antibodies are normally produced by B cells, which are part of the immune system, in response to the introduction of foreign substances, such as infectious agents, into the animal's body. The antibodies bind to the antigens that cause their generation for destruction, thus helping to fight infection. This inherent ability of the animal's body to generate antibodies that bind to specific molecules.

Target-specific antibodies can be used to isolate and identify molecules of interest. Antibodies have become one of the most important tools in life science research, allowing the detection, quantization, and determination of changes in proteins and other molecules with respect to time. Many of the antibodies used in immunochemical techniques are raised by repeated immunization of a suitable animal, e.g., rabbit, goat, donkey, or sheep, with an appropriate antigen. Serum is harvested at the peak of antibody production. Specific IgG concentrations of approximately 1 to 10 mg/mL serum can be obtained by this method. Weakly antigenic molecules may require the addition of an adjuvant, which allows for the slow release of the antigen, making it more readily trapped by macrophages. Smaller molecules, such as drugs, must be coupled to more antigenic structures (i.e. carrier proteins) to stimulate an immune response.

One characteristic of large antigen molecules is that they induce the activation of many antibody-producing B cell clones in the immunized animal. This polyclonal mixture of resulting antibodies may then recognize a variety of epitopes on the antigen, which can be a useful feature in some experimental

procedures. Because these polyclonal mixtures of antibodies react with multiple epitopes on the surface of the antigen, they will be more tolerant of minor changes in the antigen, e.g., polymorphism, heterogeneity of glycosylation, or slight denaturation, will increase monoclonal (homogenous) antibodies.

Depending upon the antigen that is used to create the antibody, one may use polyclonal antibodies to identify proteins of high homology to the immunogen protein or to screen for the target protein in tissue samples from species other than that of the immunogen. Along the same lines, it is especially important when working with polyclonal antibodies to learn as much as possible about the immunogen that has been used for production of the polyclonal antibody and the potential for undesired cross-reactivity within the sample being analyzed. Peptide immunogens are often used to generate polyclonal antibodies that target unique epitopes, especially for protein families of high homology.

12.4.2 Production of monoclonal vs. polyclonal antibodies.

A homogeneous population of antibodies (i.e. monoclonal antibodies) can be raised by fusion of B lymphocytes with immortal cell cultures to produce hybridomas. Hybridomas will produce many copies of the exact same antibody. This impressive phenomenon has been instrumental in the development of antibodies for diagnostic applications because monoclonal antibodies react with one epitope on the antigen. However, they are more vulnerable to the loss of epitope through chemical treatment of the antigen than are polyclonal antibodies. This can be analysed by pooling two or more monoclonal antibodies to the same antigen.

12.4.3 Some Useful Properties of Polyclonal Antibodies

- Polyclonal antibodies often recognize multiple epitopes, making them more tolerant of small changes in the nature of the antigen. Polyclonal antibodies are often the preferred choice for detection of denatured proteins.

- Polyclonal antibodies may be generated in a variety of species, including rabbit, goat, sheep, donkey, chicken, and others, giving the users many options in experimental design.
- Polyclonal antibodies are sometimes used when the nature of the antigen in an untested species is not known.
- Polyclonal antibodies target multiple epitopes and so they generally provide detection in very convenient way

12.4.4 Advantages of Polyclonal Antibodies

- Animal death can terminate the source of antibody. Relatively easy to generate and more cost-effective.
- Multiple epitopes on the same protein can generate many antibodies. Hence, they provide more robust signals.
- Polyclonal antibodies can generate better signals with proteins expressed in low levels.
- They are compatible with a broader range of applications.
- Polyclonal antibodies provide more flexibility in antigen recognition. For example, they may bind the antigen in spite of polymorphism, heterogeneity of glycosylation etc. Hence, they can identify proteins of high homology or from different species. Better suited for the detection of denatured proteins.

12.4.5 Disadvantage of Polyclonal Antibodies.

- Different bleeds may give different results. Immunization of a new animal with the same antigen may lead to different epitopes and different clones may be generated.
- Shared epitopes on different proteins can lead to labeling of proteins other than the antigen protein.
- Greater batch-to-batch variability is possible.

- May produce nonspecific antibodies that can add to background signal

12.4.6 Monoclonal Antibodies

(i) Some Useful Properties of Monoclonal Antibodies

- Because of their specificity, monoclonal antibodies are excellent as the primary antibody in an assay, or for detecting antigens in tissue, and will often result in significantly less background signal than polyclonal antibodies.
- When compared to that of polyclonal antibodies, homogeneity of monoclonal antibodies is very high.
- If experimental conditions are kept constant, results from monoclonal antibodies will be highly reproducible between experiments.
- Specificity of monoclonal antibodies makes them extremely efficient for binding of antigen within a mixture of related molecules, such as in the case of affinity purification.

(ii) **Advantage**

- Different clones of antibodies can be generated to different epitopes on a single antigen.
- Hybridoma cells can serve as an infinite source of the same antibody.
- The high specificity of monoclonal antibodies minimizes background and eliminates cross-reactivity.
- Their homogeneity is very high and they provide consistent, reproducible results.
  They bind only to one antigen in a mixture of related proteins.

(iii)Disadvantage

- Production of monoclonal antibodies is more labor-intensive. More work is required, especially in the cloning and selection process.

- They may be limited in their applications. A vast majority of monoclonal antibodies are produced in mice because of a robust myeloma cell line.
- High specificity of monoclonal antibodies limits their use in multiple species.

- Monoclonal antibodies are more susceptible to the loss of epitope through chemical treatment of the antigen.

(iv) Clone Numbers

Each clone number represents a specific cell line that was used to produce the antibody. Since antibodies are produced by more than one host, each cloned cell line receives a unique clone number. Each hybridoma cell clone produces only one single pure antibody type.

- An animal injected with an antigen will generate multiple antibodies to many epitopes. Since antibodies are produced by B cells, a single clone of B cells can produce antibodies to only a single epitope.
- Monoclonal antibodies are derived from a single clone of cells and can be generated in larger quantities.
- Polyclonal antibodies contain multiple clones of antibodies produced to different epitopes on the antigen. For example, if there are four epitopes on the antigen then four different clones of antibodies will be produced.
- Different antibody clones may have different properties and may even be of different isotypes. They may also work in different applications. Hence, it is best to select an antibody clone that will work optimally in your choice of application.
- It is important to recognize that a clone number is not synonymous with the lot number, which often indicates the date of manufacture.

(v) Antibody Formats

As the name implies, the antibody format refers to the presentation or purification state of the antibody. Various formats are described below:

(vi) Importance of purification of Polyclonal antibodies

Polyclonal antibodies are often available in relatively unpurified formats, and are referred to as "antiserum" or simply as "serum". Antiserum refers to the blood from an immunized host from which the clotting proteins and RBCs have been removed. The antiserum, as its name suggests, still possesses antibodies/immunoglobulins of all classes as well as other serum proteins. In addition to antibodies that recognize the target antigen, the antiserum also contains antibodies to various other antigens that can sometimes react nonspecifically in immunological assays. For this reason, raw antiserum is often subjected to purification steps, to eliminate serum proteins and to enrich the fraction of immunoglobulin that specifically reacts with the target antigen.

12.4.6 Antiserum is commonly purified by one of two methods.

Protein A/G purification or antigen affinity chromatography. Protein A/G purification takes advantage of the high affinity of Staphylococcus aurous protein A or Streptococcus protein G for the immunoglobulin Fc domain. While protein A/G purification eliminates the bulk of the serum proteins from the raw antiserum, it does not eliminate the nonspecific immunoglobulin fraction. As a result, the protein A/G purified antiserum may still possess undesirable cross reactivity. Antigen affinity purification takes advantage of the affinity of the specific immunoglobulin fraction for the immunizing antigen against which it was generated. This method may be used to remove unwanted antibodies from a preparation. The preparation of antibodies is passed through a column matrix containing antigens against which the unwanted antibodies are directed. The unwanted antibodies remain bound to

the column, and the effluent contains the desired, affinity-purified antibodies. The resulting affinity purified immunoglobulin will contain primarily the immunoglobulin of desired specificity.Specificity testing can then be performed to confirm that the antibody only recognizes the post-translationally modified form of the Protein.

12.4.7 Biological Effects of Antibodies

Antibodies are widely used for protection from infectious agents. Most vaccines (microbial antigens) induce the production of antibodies that block infection or interfere with microbial invasion of the bloodstream. To achieve this, antibodies must be functional in the sense that they are capable of neutralization or Opsonophagocytosis.

The membrane attack complex (MAC) cytolysis MAC is formed on the surface of pathogenic bacteria cell as a result of the activation of the complement system (both alternative and the classical pathways). The MAC forms transmembrane channels in bacterial walls, disrupting their phospholipid bilayer and leading to cell lysis and death.

12.5 Neutralization of viruses

Antibodies can interfere with virion binding to receptors and block their uptake into cells. Many enveloped viruses are lysed when antiviral antibodies and the complement system disrupt membranes. Certain antibodies can also aggregate virus particles. Non-neutralizing antibodies are also produced following any viral infection. Although these antibodies bind specifically to virus particles, they do not neutralize them. On the contrary, they may enhance infectivity because the virus-antibody complex enters the cell by Endocytosis. This can lead to viral replication. The type of antibody produced can influence the outcome of viral infection. For example, poliovirus can elicit IgM and IgG responses in the blood, but mucosal IgA is vital for blocking infection. The IgA neutralizes poliovirus in the intestine, the site of primary infection. Hence, the live attenuated Sabin poliovirus vaccine is more effective because it elicits a strong mucosal IgA response.

12.6 Immobilization

An antibody can be directed against cilia or flagella of motile bacteria or protozoa that results in cessation of their motility and blocks their ability to move around and spread infection.

12.7 Cytolysis

Certain antibodies can cause disruption of the microbial membrane that result in death of bacterial cells. This requires the participation of the complement system.

12.8 Opsonization

In this process, the pathogenic organism is targeted for digestion by phagocytes. The antibody binds to a receptor on the cell membrane of the bacterium, attracting phagocytes to the site. The F(ab) portion of the antibody binds to the antigen, while the Fc portion of the antibody binds to an Fc receptor on the phagocyte, facilitating phagocytosis. This process is further enhanced by the complement system.

12.9 Neutralization of exotoxins

Antitoxin antibodies can be generated against microbial toxins. The F (ab) region of the antibody made against epitope of the binding site of an exotoxin can block the exotoxin from binding to the exotoxin receptor on the host cell membrane. This blocks the entry of the toxin into the cell.

12.10 Preventing bacterial adhesion to host cells.

The body's innate defenses can physically remove bacteria by constant shedding of surface epithelial cells from the skin and mucous membranes. However, bacteria may resist this by producing pili, cell wall adhesin proteins, and biofilm-producing capsules. The F (ab) region of the antibody can bind to the adhesive tip of the pili, the cell wall adhesins, or the capsular molecules, and blocks bacterial adhesion to host cells.

(i) Agglutination of microorganisms.

The F (ab) sites of IgM and IgA antibodies can link microorganisms together and cause them to agglutinate. The agglutinated microorganisms can be phagocytosized more effectively.

Check your progress

Note: write your answer in the space given below

1. What is Mobilization?

……

12.11 RADIOIMMUNOASSAY

Radioimmunoassay technique for determining antibody levels by introducing an antigen labeled with a radioisotope and measuring the subsequent radioactivity of the antibody component. Radioimmunoassay allows for the measurement of wide range of materials of clinical and biological importance. This technique has a significant impact on medical diagnosis due to the ease with which the tests can be carried out, while assuring precision, specificity and sensitivity. The radioimmunoassay technique achieves sensitivity through the use of radionuclides and specificity that is uniquely associated with immunochemical reactions.

12.11.1 Radioimmunoassay

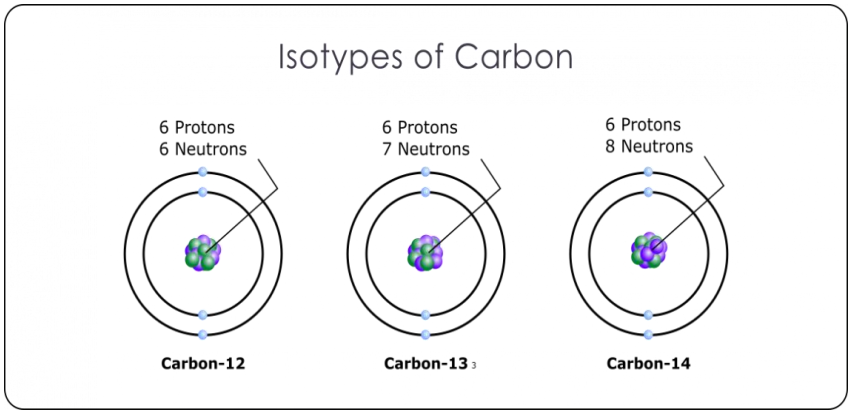
(i) Principle - Uses and Limitations

A radioimmunoassay (RIA) is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes. A RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations (for example, hormone levels in blood) by use of antibodies. Although the RIA technique is extremely sensitive and extremely specific, requiring specialized equipment, it remains among the least expensive methods to perform such measurements. It requires special precautions and licensing, since radioactive substances are used. In contrast, an immuno radiometric assay (IRMA) is an immunoassay that uses radio labeled molecules but in an immediate rather than stepwise way. A radioallergosorbent test (RAST) is an example of radioimmunoassay. It is used to detect the causative allergen for an allergy.

(ii) Radioimmunoassay (RIA) methods

Classically, to perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine, such as 125-I, attached to tyrosine. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free (unbound) antigen remaining in the supernatant is measured using a gamma counter.

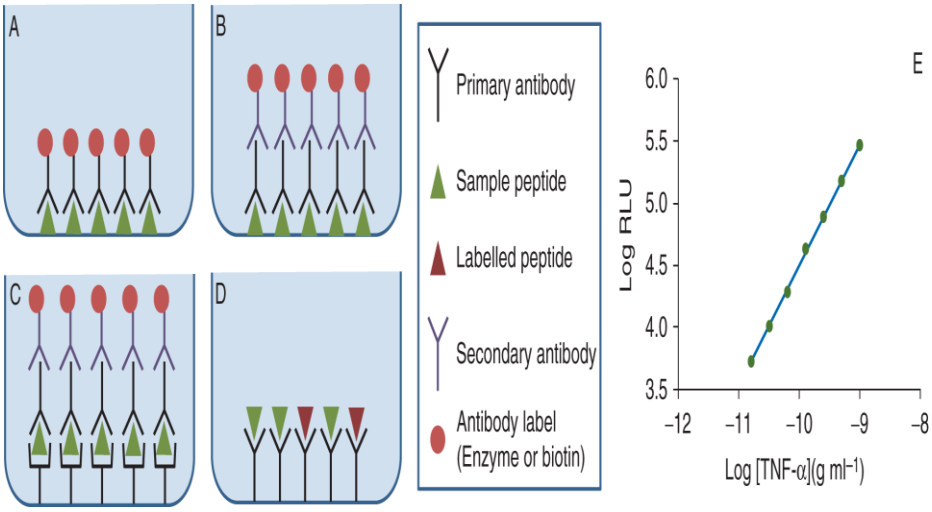
The target antigen is labeled radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added). A sample, for example a blood-serum, is then added in order to initiate a competitive reaction of the labeled antigens from the preparation, and the unlabeled antigens from the serum-sample, with the specific antibodies. The competition for the antibodies will release a certain amount of labeled antigen. This amount is proportional to the ratio of labeled to unlabeled antigen. A binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived.



That means that as the concentration of unlabeled antigen is increased, more of it binds to the antibody, displacing the labeled variant. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigens remaining in the supernatant is measured. A binding curve can be generated using a known standard, which allows the amount of antigens in the patient's serum to be derived. Radioimmunoassay is an old assay technique but it is still a widely used assay and continues to offer distinct advantages in terms of simplicity and sensitivity.

Immunoassays use the high specificity of antibodies, along with their enormous diversity, to target specific molecules of interest and analyse their concentration in a sample. The first immunoassay developed was described by Yalow and Berson in 1959. They used radiolabelled insulin to assess the concentration of insulin in human plasma, and thus developed the first radioimmunoassay (RIA). In 1971, Engvail and Perlman described a technique whereby antigens were immobilized on a microplate well, incubated with antiserum, and then the concentration of antibody in the antiserum was quantified using an enzyme-linked anti-immunoglobulin antibody. This method is the enzyme-linked immunosorbent assay (ELISA). Enzyme immunoassays (EIAs) are very similar to ELISAs, and as such, the terms are often used interchangeably. The EIA was developed by Van Weemen and Schuurs (independently of Engvail and Perlman) for the quantification of antigen rather than antibody.

An RIA requires the following: a sample containing the antigen of interest, a complementary antibody, and a radiolabelled version of the antigen. The sample antigen and antibody are incubated together, allowing the sample antigen to bind with the antibody. The radiolabelled antigen is then added. The radiolabelled antigen competes with the sample antigen and displaces it from the antibody. The more sample antigen present, the less the radio labelled antigen is able to bind to the antibody. A second antibody that binds the primary antibody can then be added, along with serum from the species of the primary antibody, to cause the solution to flocculate and allow for separation of the primary antibody from solution. Since solution containing antigen–antibody complex is more dense than that containing free-antigen, centrifuging this mixture allows separation, resulting in a pellet containing the bound sample antigen/radio labelled antigen. By measuring the radioactivity of the pellet, it is possible to determine the amount of radio labelled antigen that has bound to antibody, and therefore the concentration of antigen in the sample can be measured.



Schematic showing the differences between direct (A), indirect (B), sandwich (C), and competitive (D) EIA methods. (E) Actual standard curve for a sandwich TNF-α assay. Note the way the standard curve is presented varies with the RIA in Figure 1, but analyte samples in biological specimens

should lie on the straight part of the curve. RLU, relative light units signal from the enzyme reaction.

12.12 LET US SUM UP

In this Unit, You have learnt about the meaning, definition, need, Objectives, Importance and concept of Immunocytochemistry, antibody generation and radioimmunoassay**.** This knowledge would make you understand immunocytochemistry, antibody generation and radioimmunoassay It can be practice at typical manifestation of the knowledge.

12.13 UNIT - END EXERCISES

1. What are the functions of radioimmunoassay?

2. What is Opsonization?

12.14 ANSWERS TO CHECK YOUR PROGRESS

1. The body is made up of many different types of proteins, peptides, and antigens that help carry out functions of the immune system. Antigens are single molecules that combine to form peptides. Peptides are called proteins when they contain more than fifty amino acids. The identification of proteins in cells is important in recognizing the body's immune response to various antibodies. Antibodies are used to fight infections in the body. Immunocytochemistry helps researchers determine which antibodies will bind to specific antigens and build immunity.

2. An antibody can be directed against cilia or flagella of motile bacteria or protozoa that results in cessation of their motility and blocks their ability to move around and spread infection.

12.15 SUGGESTED READINGS

1. Matthew Helbert, Immunology for Medical Students. Elsevier, 2016

2. Sunil Kumar Mohant, Dr.Sai Leela Text book of immunology Jaypee Brothers Medical Publishers (P) Ltd.Second edition 2014.

3. “Immunology” by Roitt I and Male Brostoff. Mosby-Year Book; 4th edition (January 1996)

4. “Immunology” by Dulsy Fatima and N Arumugam. Saras Publication,2009.

5. The Elements of Immunology” by Fahim Halim Khan. Pearson Education India, 2009.

UNIT XIII DETECTION OF MOLECULES USING IMMUNOBLOT TECHNIQUES, ELISA AND VACCINE DEVELOPMENT.DETECTION OF MOLECULES USING IMMUNOBLOT TECHNIQUES

Structure

NOTES

13.1 INTRODUCTION

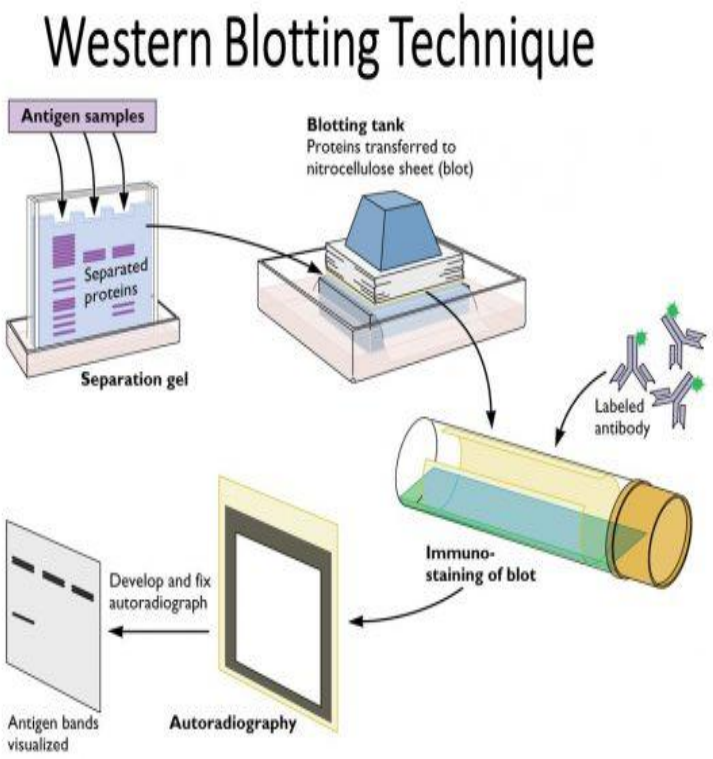
Western blot is often used in research to separate and identify proteins. In this technique a mixture of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein**.**

13.2 OBJECTIVES

- An enzyme-linked immunosorbent assay (ELISA) is used to detect the presence of an antigen in a sample.

- The antigen is immobilized to the well of a plate by adsorption, or captured with a bound, antigen-specific antibody.
- A detection antibody is then added forming a complex with the antigen, if present**.**

13.3 BLOTTING TECHNIQUES



The blotting technique is a tool used in the identification of biomolecules such as DNA, mRNA, and protein during different stages of gene expression. Protein synthesis involves the expression of a DNA segment which gets converted to mRNA to produce the respective protein. Subtypes of blotting such as northern, western & southern depend upon the target molecule that is being sought. When a DNA sequence is a foundation or code for a protein molecule, the particular DNA molecule of interest can be blotted using Southern Blotting technique. During gene expression, when the DNA is expressed as mRNA for a protein production, this process can be identified by Northern blotting. Finally, the coded mRNA produces the concerned protein, this protein identification can be done by Western Blotting.

Western blotting (or immunoblotting) is a widely used method to detect proteins as well as post-translational modifications on proteins, using antibody based probes to obtain specific information about target proteins from complex samples. It is a routine method in a molecular biology, biochemistry and cell biology field with the multitude of applications. It can provide semi-quantitative or quanlitative data about the target protein in simple or complex biological samples.

Since western blotting is a multistep protocol, variations and errors can occur at any step reducing the reliability and reproducibility of this technique. Recent reports suggest that a few key steps, such as the sample preparation method, the amount and source of primary antibody used, as well as the normalization method utilized, are critical for reproducible western blot results. This method relies on the fact that most epitopes (sites recognized by antibodies, generally comprising several amino acids) in spite of denaturation of proteins can still be recognized.

Due to high affinities of antibody toward their epitopes, it is a very sensitive method and even pictogram quantities of a target protein can be detected. The two primary advantages of western blotting are sensitivity and specificity. Western blotting has advantages over other protein detection techniques. Silver staining, another technique of protein detection detects 10 ng of protein and all proteins in a given sample. Whereas, western blotting can detect as little as 0.1ng of protein, and it selectively detects only the protein of interest. Thus a complex mixture containing only traces of the desired protein may be analyzed accurately with this technique. Western blotting was first described by Harry Towbin in 1979. It was in 1981 when W. Neal Burnette developed an improved version of the method and gave the name “western blotting” simply because of the location of laboratory.

Below is a general procedure for blotting, and every step is critical for obtaining high-quality, reliable and analyzable data.

1. Homogenize the sample.

2. Separation of the molecule of interest by an electrophoresis membrane.
3. Transferring the molecules to a nitro cellulosic membrane/ nylon membrane.
4. Hybridization or identification of the molecule

Western blotting is the technique used for separation or identification of protein molecules. This technique can be used for both the active 3D protein and denatured long peptide chains. The 3-D protein in its active structure has sulfur-hydroxyl bonds in the structure. This methodology classifies protein based on the molecular weight and charge. These techniques have application in the identification of a wide variety of infectious diseases like HIV, Hepatitis B, Herpes type 2, and immunodeficiency disease. The identification of these diseases is done by using the antibody of the particular disease as the probe and these probes are produced in vitro condition. Western blotting is also used for research purpose. This technique can be used in the study of the properties and activity of a protein molecule of interest.

13.3.1 PROCEDURE

(i) Sample Preparation

1. Wash cells in the tissue culture flask or dish by adding cold Phosphate Buffered Saline (PBS) and rocking gently. Flask or dish should be kept on ice throughput the process. Discard PBS.
2. Add PBS and use a cell scraper to dislodge the cells. Pipette the mixture into micro centrifuge tubes.
3. Centrifuge at 1500 RPM for 5 minutes. Discard the supernatant.
4. Add 180 ul of ice cold lysis buffer solution to 20 ul of fresh protease inhibitor. This prevents the protease enzyme. Incubate for 30 minutes.
5. Incubate for 30 min on ice, and centrifuge this solution for 10 minutes at 12000 rpm at 4^0c and the sample solution is ready.

6. Transfer supernatant (or protein mix) to a fresh tube and store on ice or frozen at -20°C or -80°C.

7. Measure the concentration of protein using a spectrophotometer and determine the volume of protein extract to ensure 50 µg in each well.

8. Add 5 µl sample buffer to the sample, and make the volume in each lane equalized using double distilled H_2O (dd H_2O). Mix well.

9. Heat the samples with a dry plate for 5 minutes at 100°C.

(ii) Gel Preparation

The gel has two parts stacking gel and separation gel. 10% stacking gel and 6% separating gel are generally used. Add the stacking gel solution into the assembly carefully and then add H_2O to the top. Wait for 15–30 minutes until the gel solidifies. Overlay the stacking gel with the separating gel, after removing the water. Insert the comb, ensuring that there are no air bubbles. Wait until the gel is solidified.

(iii) Electrophoresis

Pour the running buffer into the electrophorator. Place gel inside the electrophorator and connect to a power supply. (Tip: When connecting to the power source always connect red to red, and black to black). Make sure buffer covers the gel completely, and remove the comb carefully. Load marker (6 µl) followed by samples (15 µl) in to each well. Run the gel at 40 volts until the sample reaches the stacking gel and changed into 80 volts from the separation gel. Run the gel for approximately an hour, or until the dye front runs off the bottom of the gel

(iv) Fixing and blotting

Fixing is done 5% of bovine serum albumin solution. Cut 6 filter sheets to fit the measurement of the gel and one polyvinylidene fluoride (PDVF) membrane with the same dimensions. Wet the sponge and filter paper in transfer buffer, and wet the PDVF membrane in methanol. Separate glass

plates and retrieve the gel. Create a transfer sandwich as follows – Sponge, 3 Filter Papers, Gel PVDF, 3 Filter Papers (Ensure there are no air bubbles between the gel and PVDF membrane, and squeeze out extra liquid). Relocate the sandwich to the transfer apparatus, which should be placed on ice to maintain 4°C. Add transfer buffer to the apparatus, and ensure that the sandwich is covered with the buffer. Place electrodes on top of the sandwich, ensuring that the PVDF membrane is between the gel and a positive electrode. Blotting is carried in two ways, capillary blotting or through electro blotting. Usually, Electro blotting is carried out at 40 volts. For capillary blotting, the gel is stacked in the following order: Electrophoresis gel followed by blotting membrane followed by wet tissue and lid glass plate.

(v) Detection

Detection and identification can be carried out by a number of methods like radiography, chemiluminescence, colorimetric and x-ray methods.

13.3.2 Principle

The principle of the western blotting is based around a few broad steps: (a) the extraction of cellular proteins from a complex mixture of intracellular and extracellular proteins (from tissue, cells, etc.); (b) quantification of protein concentration and electrophoretic separation of proteins within a gel matrix; (c) transfer to a membrane with a high affinity for proteins; (d) “blocking” the membrane to reduce non-specific binding; (e) antigen detection by antibodies specific for the protein(s) of interest; (f) incubation with a secondary antibody linked to a label (e.g., chemiluminescent or fluorescent); (g) development and detection of the signal, which is theoretically proportional to the degree of antigen/antibody binding; and (h) quantification of the resulting bands.

13.3.3 Normalization of target protein abundance

To account for possible errors in sample preparation and loading, normalization of samples to remove inter sample/gel variation is paramount. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or β-actin are “housekeeping” proteins (HKP). They are probed for acting as internal loading

controls assuming their expression remains stable under the experimental conditions used. The HKP chosen should be one which is constant between control and experimental samples. It should also remain unaffected by the treatment or intervention undertaken. Errors such as loading more sample within one well will increase the target signal, likely skewing data interpretation. As such, target measurements may be normalized to HKP values, removing loading bias. However, the accuracy and effectiveness of these HKPs are dependent on multiple factors such as oversaturation of the protein, high background, and lack of linearity and can easily suffer from technical errors within the Western blot process. Expression of HKPs such as β-actin has also been shown to be extremely variable between tissue types (i.e., muscle, heart, fat). Alarmingly, however, expression of β-actin has been revealed to not be homogeneous within a single tissue sample, being shown to differ between proximal and distal regions of a single mouse sciatic nerve. Such differences in expression may become more problematic within skeletal muscle. As it is a large complex tissue, with different regions potentially responding differently to stimulation.

In such cases western blot results only provide an average of expression changes within a single sample. The use of a second HKP may help; however, some of the same issues may exist and it will need to be demonstrated that the choice of HKP does not affect the interpretation. Due to the potential changes in HKP expression in response to the experiment and the limited linear range of some, the use of HKPs for normalization may mask or confound potentially relevant changes in protein expression.

A viable alternative to blotting for HKPs is to assess the total amount of protein. Assessing total protein offers distinct advantages over HKPs as it is unbiased with respect to changes in the expression. If stained membranes are utilized it allows evaluation of the blotting process and transfer quality. Membranes may be stained to visualize total protein by several methods (i.e., Ponceau S, colloidal silver, India ink). However, Coomassie staining is a common, simple approach that has been demonstrated to be an unbiased method of total protein assessment. If one of these approaches is chosen, the

quantification of a single random band that is consistent across each lane may be used for normalization. Coomassie staining is reliable quantification of total protein also it removes potential problems associated with individual HKP expression.

The final aspect of the analysis is the requirement to calculate the changes in protein expression, resulting from a treatment/intervention. Depending on the experimental design, multiple blots may be required to analyze all samples, and thus, subtle changes within the process may influence the final data as background or band density may be variable across multiple blots. In this instance, a single quality control sample (typically pooled from multiple controls) is loaded on each gel, providing a control sample across all gels, allowing gel-to-gel comparisons to be made.

Viding band values (i.e., band density) by the quality control sample (as a correction factor) normalizes differences in loading, separation, transfer, and detection that may have occurred. Another approach typically used for human studies is to use an initial basal sample for each individual to assess changes in subsequent samples, permitting the calculation of a fold change from the initial basal levels. Whichever approach is chosen, it must be applied consistently throughout the analysis and may depend on the total number of samples and comparisons needed (i.e., control vs treatment or control vs treatment 1 vs treatment 2).

Determining changes to a protein's total expression in response to a treatment or intervention indicates its capacity to signal since a greater abundance or protein will allow a greater potential for signaling to occur. Measurements of a protein's phosphorylation status (i.e., signaling activation) may change through modification of the individual proteins phosphorylation level or by alterations within the total amount of protein available. Consequently, the normalization of a phospho-protein to its total expression allows the ratio of phosphorylated proteins to be assessed (i.e., the relative proportion of phosphorylated vsnon). For example, within skeletal muscle, the total expression of various anabolic signaling intermediates (e.g., AKT, P70

S6K1) remained unchanged, while phosphorylation of these proteins increased.

As such, the ratio of phosphorylated proteins to total expression increased, demonstrating the increased proportion of phosphor-proteins. Importantly, however, if the treatment undertaken increases both total and phosphorylation levels, this ratio may remain unchanged, masking any potential mechanisms, and thus it may be extremely important for both measures, i.e., total and phosphorylation status, to be made simultaneously.

13.3.4 Conclusions

Western blotting has emerged as an essential tool within physiological research; nevertheless, with poor understanding and implementation, any subsequent analysis can produce misleading and confusing interpretation (Ab specificity and validation). Before a sample is loaded into a gel, careful consideration must be given to often overlook aspects such as the appropriate buffer for homogenization and extraction of the intended target protein for denaturation. Gel composition should effectively separate proteins by size, with changes to concentration giving resolution to the intended target by varying migration speed. However, fluorescent antibodies have a greater dynamic range and may be multiplexed for additional targets if desired.

13.4 Capillary and microchip electrophoresis

Few major limitations of western blotting are time-consuming nature, requirement of a relatively large amount of sample (usually 10-20 µg/assay) and detection of only one protein at a time. Moreover, it requires detection of housekeeping proteins or total proteins on the membrane to normalize the target bands detected. To address these issues, a hybrid between capillary electrophoresis for SDS-PAGE and conventional blotting for the western part referred to as capillary and microchip electrophoresis (MCE) based western blotting was developed. In brief, multiple injections of the same protein samples are loaded in separate tracks on a microchip and are captured on a PVDF membrane for immunoassay.

13.4.1 Automated micro fluidic protein immunoblotting and single cell-resolution western blotting

Another automated micro fluidic protein immunoblotting technique was developed to save time, avoid multiple assay steps and limit equipment and reagents requirements. This automated protein immunoblotting is a programmable controlled technique (i.e. voltage control and pressure) that combines PAGE with blotting in one device. The technique allows the integrated assay steps (PAGE, transfer and in-gel blotting) to be viewed using an epifluorescence microscope equipped with a charge-coupled device camera. In this method photo patterning (photochemical etching) of polyacrylamide gels is done on micro fluid glass devices that act as a platform to integrate the multiple assay steps. The method is rapid and was employed to detect free prostate specific antigen in the human seminal fluid sample in less than 5 min. This method is economical and lowers the consumption of reagents as the glass chips that are used are reusable after simple chemical treatments. The technique is still in process of development to further improve sensitivity and enable protein quantitation.

13.4.2 Single cell-resolution western blotting

It was developed to detect individual cell-to cell variations in protein expression between cells. The micro device used for this assay consists of a thin layered polyacrylamide gel with micro wells. The micro wells in the gel layer are loaded with single cell protein samples and are lysed chemically in each well to obtain single cell lysate to be resolved on PAGE. Subsequently, the proteins are immobilized on the same gel using ultraviolet (UV) light and are probed with antibodies for Micro-loader.

(i) Immunoblotting.

This method involves a funnel-like structure sample micro-loader device to load samples. The device is attached to the top of polyacrylamide gel and filled with 4% stacking gel solution through the outlet of the tips via capillary action. The protein in a sample that travels through the transfer

pipette is concentrated by electrophoresis. The incorporation of micro-loader device in gels improved both protein separation and resolution. This technique was able to detect number of proteins and Phosphoproteins in each sample by loading only 1.5 µg of protein per lane. This technique has the advantage of being relatively simple to do and would be very useful to measure protein expression and phosphorylation in samples that are limited.

13.4.3 Thin-film direct coating with suction-western blotting (TDCS)

This is a highly sensitive and rapid detection method for quantitative analysis of multiple antigen-antibody interactions. The operational time for TCDS is much shorter (about 5 min) than conventional western blotting with increased signal-to noise ratio. Western blot is often used in research to separate and identify proteins. In this technique a mixture of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein. The membrane is then incubated with labels antibodies specific to the protein of interest.

The unbound antibody is washed off leaving only the bound antibody to the protein of interest. The bound antibodies are then detected by developing the film. As the antibodies only bind to the protein of interest, only one band should be visible. The thickness of the band corresponds to the amount of protein present; thus doing a standard can indicate the amount of protein present. The paper will first describe the protocol for western blot, accompanied by pictures to help the reader and theory to rationalize the protocol. This will be followed by the theoretical explanation of the procedure, and in the later section, troubleshooting tips for common problem.

13.5 Enzyme-linked immunosorbent assay (ELISA)

It is a method of target antigen (or antibody) capture in samples using a specific antibody or antigen and of target molecule detection/quantitation using an enzyme reaction with its substrate.

13.5.1 The principle

In ELISA, various antigen-antibody combinations are used, always including an enzyme-labeled antigen or antibody, and enzyme activity is measured calorimetrically.

The enzyme activity is measured using a substrate that changes color when modified by the enzyme. Light absorption of the product formed after substrate addition is measured and converted to numeric values. Depending on the antigen-antibody combination, the assay is called a direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA etc.

NOTES

Check your progress

Note: write your answer in the space given below

1. **Explain the Principle for ELISA**

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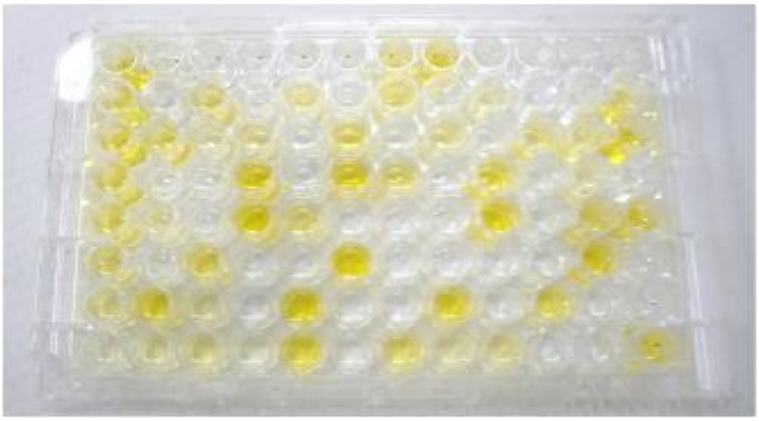
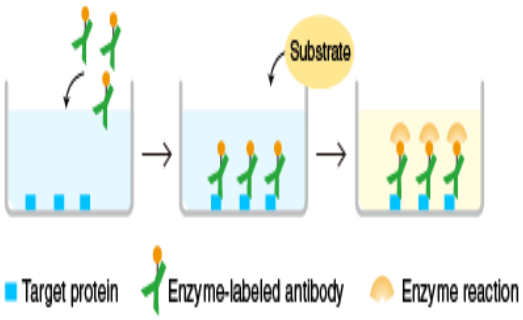
ELISA: An example of an assay using a 96-well plate.

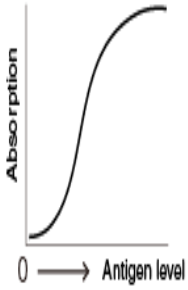


The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.

13.5.2 Direct ELISA

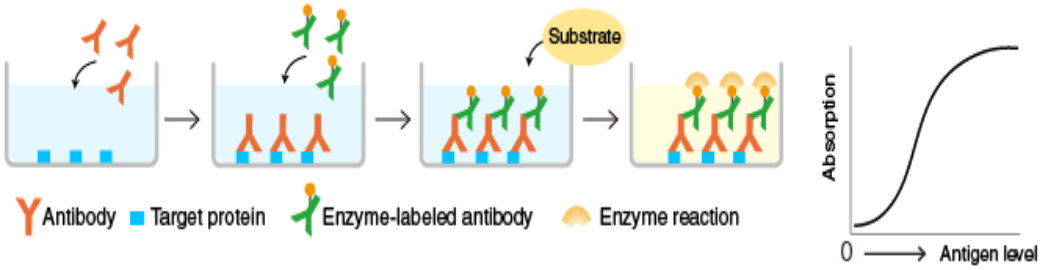
A target protein (or a target antibody) is immobilized on the surface of micro plate wells and incubated with an enzyme-labeled antibody to the target protein (or a specific antigen to the target antibody). After washing, the activity of the micro plate well-bound enzyme is measured.





13.5.3 Indirect ELISA

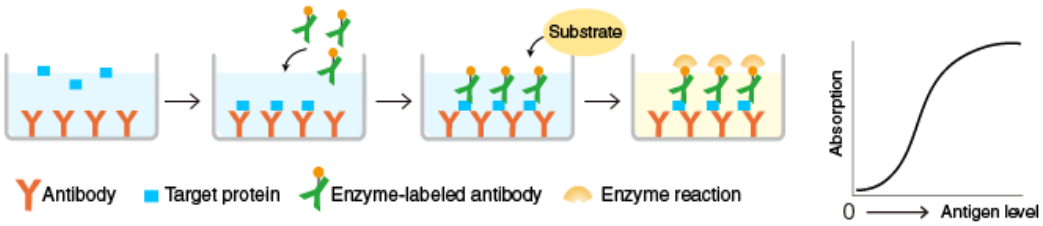
A target protein is immobilized on the surface of micro plate wells and incubated with an antibody to the target protein (the primary antibody), followed by a secondary antibody against the primary antibody. After washing, the activity of the micro plate well-bound enzyme is measured. Although indirect ELISA requires more steps than direct ELISA, labeled secondary antibodies are commercially available, eliminating the need to label the primary antibody.



13.5.4 Sandwich ELISA

NOTES

An antibody to a target protein is immobilized on the surface of micro plate wells and incubated first with the target protein and then with another target protein-specific antibody, which is labeled with an enzyme. After washing, the activity of the micro plate well-bound enzyme is measured. The immobilized antibody (orange) and the enzyme-labeled antibody (green) must recognize different epitopes of the target protein.

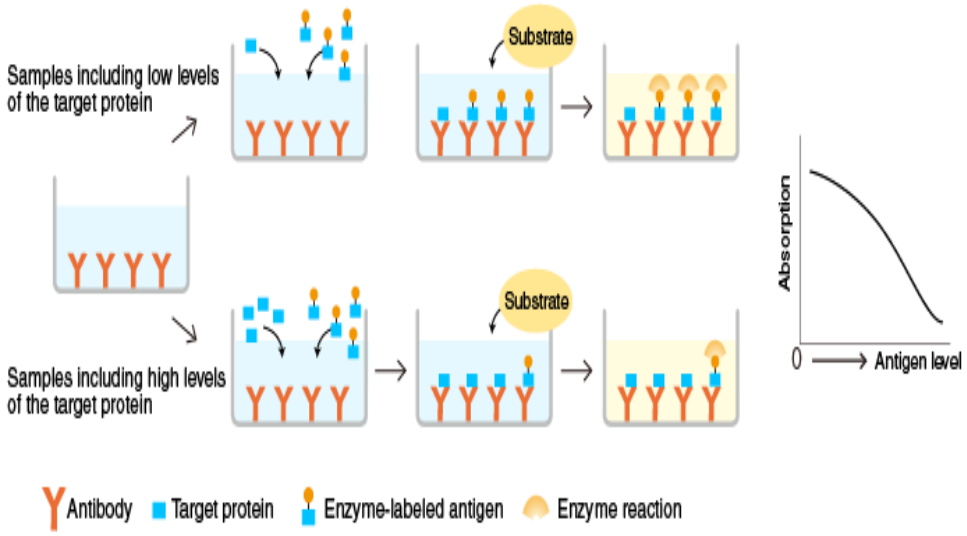


Compared to direct ELISA, the sandwich ELISA (combining antibodies to two different epitopes on the target protein) has a higher specificity. Sandwich ELISA is useful for applications that require a high accuracy.

13.5.5 Competitive ELISA

An antibody specific for a target protein is immobilized on the surface of micro plate wells and incubated with samples containing the target protein and a known amount of enzyme-labeled target protein. After the reaction, the activity of the micro plate well-bound enzyme is measured. When the antigen level in the sample is high, the level of antibody-bound enzyme-labeled antigen is lower and the color is lighter. Conversely, when it is low, the level

of antibody-bound enzyme-labeled antigen is higher and the color, darker. The graph above and to the right illustrates the correlation between absorption and antigen levels in samples.



When a target antigen is a small molecule, such as histamine, pesticide, and dioxin, two antibodies cannot simultaneously bind to the antigen in sandwich ELISA. Competitive ELISA is useful for the measurement of low molecular weight targets.

Check your progress

Note: write your answer in the space given below

2. **Explain the Direct Method for ELISA**

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13.6 Immobilization of antibody/antigen on micro plate wells.

Immobilization is commonly mediated by hydrophobic interaction or covalent bonds. Micro plates that are pre-activated for different immobilization methods are commercially available. The choice of method of immobilization depends on the type of protein to be immobilized. In general, covalent bonding is preferred

for smaller molecules, such as peptides, and hydrophobic interaction (physical adsorption) is preferred for larger molecules, such as proteins. An appropriate method should be determined for each assay system.

Many types of ELISA micro plates are commercially available, including the hydrophobic type, hydrophilic type as well as types surface-activated with amino or carboxyl groups. Surface-activated micro plates are useful for covalent bonding. It is important to select the correct type of ELISA micro plate for the intended use.

NOTES

A more rapid method than Western blot analysis to detect a specific protein in a cell, tissue, organ, or body fluid is enzyme-linked immunosorbent assay, or ELISA. This method, which does not require fractionation of the sample by gel electrophoresis, is based on the property of proteins to readily bind to a plastic surface. To detect viral proteins in serum or clinical samples, a capture antibody, directed against the protein, is linked to a solid support such as a plastic 96 well micro titer plate, or a bead.

The clinical specimen is added, and if viral antigens are present, they will be captured by the bound antibody. The bound viral antigen is then detected by using a second antibody linked to an enzyme. A Chromogenic molecule – one that is converted by the enzyme to an easily detectible product – is then added. The enzyme amplifies the signal because a single catalytic enzyme molecule can generate many product molecules.

To detect antibodies to viruses, viral protein is linked to the plastic support, and then the clinical specimen is added. If antibodies against the virus are present in the specimen, they will bind to the immobilized antigen. The bound antibodies are then detected by using a second antibody that binds to the first antibody.

ELISA is used in both experimental and diagnostic virology. It is a highly sensitive assay that can detect proteins at the Pico molar to nanomolar range (10^{-12} to 10^{-9} moles per liter). It is the mainstay for the diagnosis of

infections by many different viruses, including HIV-1, HTLV-1, adenovirus, and cytomegalovirus.

13.7 LET US SUM UP

In this Unit, You have learnt about the meaning, definition, need, Objectives, Importance and concept of immunoblot techniques, elisa and vaccine development. detection of molecules using immunoblot techniques. This knowledge would make you understand detection of molecules using immunoblot techniques, elisa and vaccine development. detection of molecules using immunoblot techniques. It can be practice at typical manifestation of the knowledge.

13.8 UNIT - END EXERCISES

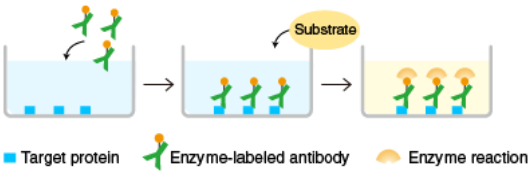
1. What are the functions of immunoassay?
2. What is Immuno Plot?

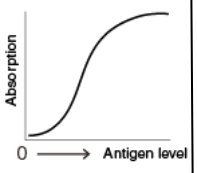
13.9 ANSWERS TO CHECK YOUR PROGRESS

1. In ELISA, various antigen-antibody combinations are used, always including an enzyme-labeled antigen or antibody, and enzyme activity is measured calorimetrically.

The enzyme activity is measured using a substrate that changes color when modified by the enzyme. Light absorption of the product formed after substrate addition is measured and converted to numeric values. Depending on the antigen-antibody combination, the assay is called a direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA etc

2. A target protein (or a target antibody) is immobilized on the surface of micro plate wells and incubated with an enzyme-labeled antibody to the target protein (or a specific antigen to the target antibody). After washing, the activity of the micro plate well-bound enzyme is measured.





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UNIT XIV IMMUNOPRECIPITATION AND IMMUNOFLUORESCENCE MICROSCOPY, ACQUIRED IMMUNO DEFICIENCY SYNDROME (AIDS) DETECTION AND HYBRIDOMA TECHNOLOGY, FACS, IMMUNOFLUORESCENT ASSAY.

Structure

14.1 INTRODUCTION

The first cases of acquired immunodeficiency syndrome (AIDS) were reported in the United States in the spring of 1981. By 1983 the human immunodeficiency virus (HIV), the virus that causes AIDS, had been isolated. Early in the U.S. HIV/AIDS pandemic, the role of substance abuse in the spread of AIDS was clearly established. Injection drug use (IDU) was identified as a direct route of HIV infection and transmission among injection drug users. The largest group of early AIDS cases comprised gay and bisexual men (referred to as men who have sex with men(or MSMs). Early cases of HIV infection that were sexually

transmitted often were related to the use of alcohol and other substances, and the majority of these cases occurred in urban, educated, white MSMs.

Currently, injection drug users represent the largest HIV-infected substance-abusing population in the United States. HIV/AIDS prevalence rates among injection drug users vary by geographic region, with the highest rates in surveyed substance abuse treatment centers in the Northeast, the South, and Puerto Rico. From July 1998 through June 1999, 23 percent of all AIDS cases reported were among men and women who reported IDU (Centers for Disease Control and Prevention [CDC], 1999b).

IDU practices are quick and efficient vehicles for HIV transmission. The virus is transmitted primarily through the exchange of blood using needles, syringes, or other IDU equipment (e.g., cookers, rinse water, cotton) that were previously used by an HIV-infected person. Lack of knowledge about safer needle use techniques and the lack of alternatives to needle sharing (e.g., available supplies of clean, new needles) contribute to the rise of HIV/AIDS.

Another route of HIV transmission among injection drug users is through sexual contacts within relatively closed sexual networks, which are characterized by multiple sex partners, unprotected sexual intercourse, and exchange of sex for money (Friedman et al., 1995). The inclusion of alcohol and other noninjection substances to this lethal mixture only increases the HIV/AIDS caseload (Edlin et al., 1994; Grella et al., 1995). A major risk factor for HIV/AIDS among injection drug users is crack use; one study found that crack abusers reported more sexual partners in the last 12 months, more sexually transmitted diseases (STDs) in their lifetimes, and greater frequency of paying for sex, exchanging sex for drugs, and having sex with injection drug users (Word and Bowser, 1997).

14.2 OBJECTIVES

The Human Immunodeficiency Virus (HIV) targets the immune system and weakens people's defense systems against infections and some types of cancer. As the virus destroys and impairs the function of immune cells, infected individuals gradually become immunodeficient. Immune function is typically measured by CD4 cell count.

Immunodeficiency results in increased susceptibility to a wide range of infections, cancers and other diseases that people with healthy immune systems can fight off.

The most advanced stage of HIV infection is Acquired Immunodeficiency Syndrome (AIDS), which can take from 2 to 15 years to develop depending on the individual. AIDS is defined by the development of certain cancers, infections, or other severe clinical manifestations.

14.3 IMMUNOPRECIPITATION (IP)

Immunoprecipitation is the technique of precipitating a protein antigen out of solution using an antibody that specifically binds to that particular protein. This process can be used to isolate and concentrate a particular protein from a sample containing many thousands of different proteins'

14.4 IMMUNOFLUORESCENCE MICROSCOPY

The specificity of antibodies to their antigen is the base for Immunofluorescence. Immunofluorescence can be used on tissue sections, cultured cell lines, or individual cells, and may be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. Immunofluorescence microscopy is a powerful technique that is widely used by researchers to assess both the localization and endogenous expression levels of their favorite proteins.

(i) Immunofluorescence

- Immunofluorescence: Immunofluorescence is a powerful technique that utilizes fluorescent-labeled antibodies to detect specific target antigens.
- Fluroresceinis dye which emits greenish fluorescence under uv light. It can be tagged to immunoglobulin molecules.
- This technique is sometimes used to make viral plaques more readily visible to the human eye.
- Immunofluorescent labeled tissue section are studied using a fluorescence microscope.

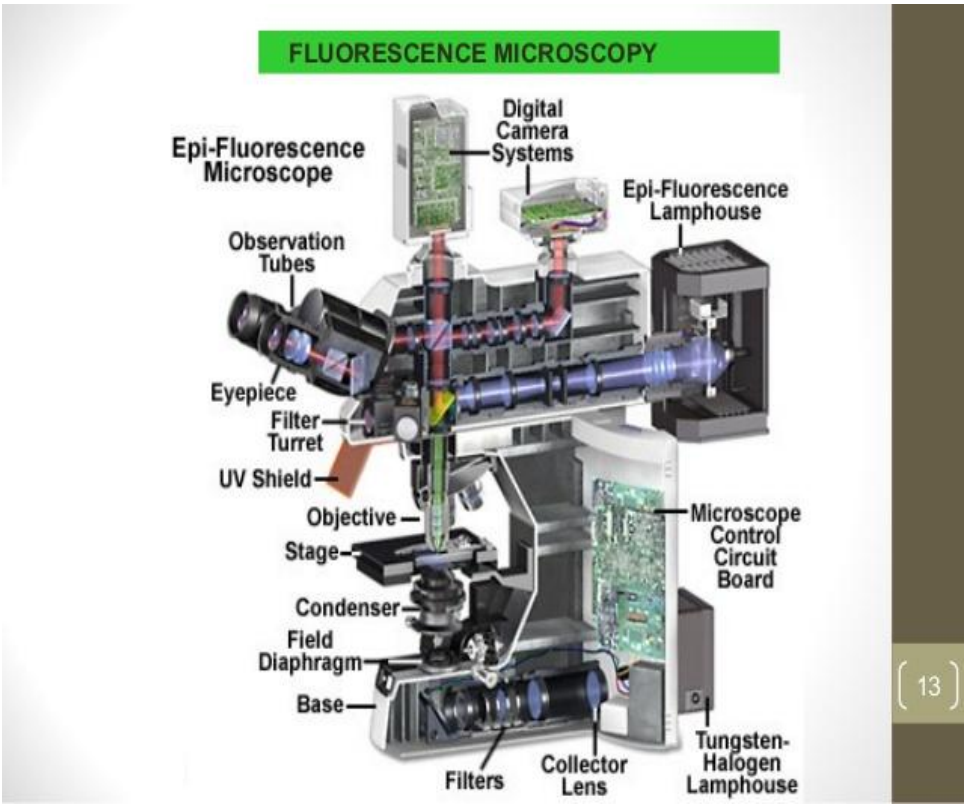
NOTES

(ii) **Fluorescence microscope**

- Same as a conventional light microscope with added features to enhance its capabilities.
- CM-visible light (400-700nanometers)
- FM-higher intensity light soure which excites a fluorescent species in a sample.
- This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source.

(iii) Fluorescence microscope Applications

- Imaging structural components of small specimens,(cells)
- Viability studied on cell populations (alive or dead)
- Imaging the genetic material within a cell (DNAandRNA)
- Viewing specific cells within a larger population with techniques such as fish



14.5 ACQUIRED IMMUNO DEFICIENCY SYNTROME:

Acquired immunodeficiency syndrome (AIDS) is a chronic, potentially life-threatening condition caused by the human immunodeficiency virus (HIV). By damaging the immune system, HIV interferes with the body's ability to fight the organisms that cause 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. There's no cure for HIV/AIDS, but there are medications that can dramatically slow the progression of the disease. These drugs have reduced AIDS deaths in many developed nations.

14.5.1 Symptoms

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

14.5.2 Primary infection (Acute HIV)

Most people infected by HIV develop a flu-like illness within a month or two after the virus enters the body. This illness, known as primary or 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. 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.

14.5.3 Clinical latent infection (Chronic HIV)

In some people, persistent swelling of lymph nodes occurs during this stage. Otherwise, there are no specific signs and symptoms. HIV remains in the body and in infected white blood cells. This stage of HIV infection generally lasts around 10 years if you're not receiving

antiretroviral therapy. But sometimes, even with this treatment, it lasts for decades. Some people develop more severe disease much sooner.

14.5.4 Symptomatic HIV infection

As the virus continues to multiply and destroy the immune cells. It develop mild infections or chronic signs and symptoms such as Fever**,** Fatigue**,** Swollen lymph nodes**,** Diarrhea**,** Weight loss**.**

Check your progress

Note: write your answer in the space given below

2. **Explain the** Clinical latent infection

.....

14.5.5 Progression to AIDS

Untreated, HIV typically turns into AIDS in about 10 years.When AIDS occurs the immune system has been severely damaged.It develop opportunistic infections or opportunistic cancers diseases.The signs and symptoms of some of these infections may include Soaking night sweats**,**Recurring fever**,**Chronic diarrhea**,**Persistent white spots or unusual lesions on your tongue or in your mouth**,**Persistent, unexplained fatigue**,**Weight loss**,**Skin rashes or bumpsHIV destroys CD4 T cells,white blood cells that play a large role in helping your body fight disease.

14.5.6 Complications

HIV infection weakens the immune system to develop numerous infections and certain types of cancers. Infections common to HIV/AIDS are TB. It is the most common opportunistic infection associated with HIV. It's a leading cause of death among people with AIDS. Candidiasis is a common HIV-related infection. It causes inflammation and a thick, white coating on the mucous membranes of your mouth, tongue, esophagus or vagina, Cryptococcus 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. Cryptosporidiosis. This infection is caused by an intestinal parasite that's commonly found in animals. parasite grows in intestines and bile ducts, leading to severe, chronic diarrhea in people with AIDS.

14.5.7 Cancers common to HIV/AIDS

Kaposi's sarcoma. A tumor of the blood vessel walls, this cancer is rare in people not infected with HIV, but common in HIV-positive people. It 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. Lymphoma (type of cancer) starts in the white blood cells. The most common early sign is painless swelling of the lymph nodes in the neck, armpit or groin of HIV infected person

14.5.8 Prevention

Era’s no vaccine to prevent HIV infection and no cure for AIDS. But you can protect yourself and others from infection. To prevent the spread of HIV the following measures are advised. 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 lubricant, make sure it's water-based. Oil-based lubricants can weaken condoms and cause them to break.

Consider the drug Truvada. The drug emtricitabine-tenofovir (Truvada) can reduce the risk of sexually transmitted HIV infection in people at very high risk. Use a clean needle. If you use a needle to inject drugs, make sure it's sterile and don't share it. If you're pregnant, get medical care right away. receive treatment during pregnancy, you can cut your baby's risk significantly. There is evidenced that male circumcision can help reduce a man's risk of getting HIV infection.

NOTES

14.6 HYBRIDOMA TECHNOLOGY

Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell, produces antibodies that bind to the injected antigen. These antibody producing B-cells are then harvested from the mouse and, in turn, fused with immortal B cell cancer cells, a myeloma to produce a hybrid cell line called a hybridoma, which has both the antibody-producing ability of the B-cell and the exaggerated longevity and reproductivity of the myeloma.

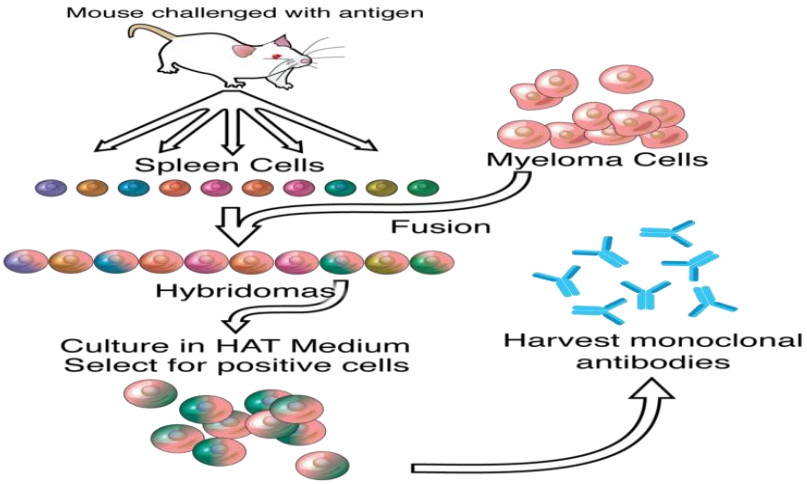
The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal). The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis. In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.

The production of monoclonal antibodies was invented by Milsteinand Georges J. and Kohler in 1975. They shared the Nobel Prize of 1984 for Medicine and Physiology who made other contributions to immunology.

14.7 MONOCLONAL ANTIBODIES

Antibodies are glycoprotein synthesized in blood against specific antigens and give immunity. Such antibodies are heterogenous and are polyclonal antibodies. Therefore they do not have characteristics of specificity. If a specific lymphocyte after isolation and culture invitro becomes capable of producing a single type of antibody which bears specificity against specific antigen, it is known as monoclonal antibody. These monoclonal antibodies are derived from a single clone of cell which recognize only one kind of antigen. Monoclonal antibodies are produced

against a variety of proteins, glycoproteins, glycolipids, nucleic acids and chemically defined groups linked to protein carriers.



Check your progress

Note: write your answer in the space given below

2. **What is Monoclonal Antibody?**

……

14.7.1 Hybridoma technology for production of monoclonal antibodies

Steps in production of monoclonal antibodies:

Step I: Immunization of rabbit or rat and extraction of B-lymphocytes

- In order to isolate B-lymphocyte producing certain antibodies, rabbit or lab rat is immunized through repeated injection of specific antigen (sheep RBCs)
- A sample of B-cells is extracted from spleen of rabbit or rat
- Step II: fusion of myeloma cell with B-lymphocytes:
- The extracted B-lymphocytes is added to a culture of myeloma cell from bone marrow.
- The intended result is the formation of hybridoma cells formed by fusion of B-cell and myeloma cell.
- The fusion is done by using Polyethylene glycol (PEG) or by electrophoration or by using phages.

NOTES

Step III: selection of hybridoma cell

- The next step is selection of hybridoma cells.
- The B-lymphocytes contains HPRT1 gene which codes for enzyme Hypoxanthine-guanine phosphoribosyltransferase (HGPRT). The enzyme HGPRT involved in synthesis of nucleotides from Hypoxanthine present in culture medium. Therefore B- cells can grow in medium containing Hypoxanthine amonopterin thymine (HAT media).
- But myeloma cell lack HPRT1 gene so, it does not produce HGPTR enzyme and it does not grow in HAT medium.
- The myeloma cell fused with another myeloma cell or those do not fused at all die in HAT medium since they do not utilize Hypoxanthine.
- Similarly, B- cell that fuse with another B- cell or those do not fuse at all die eventually because they do not have capacity to divide indefinitely,
- So, only hybridoma cell ie. Fused cell between myeloma and B-cell can survive and divide in HAT medium.
- Screening is done to select hybridoma cells which are the desired cell for monoclonal antibodies production.

Step IV: culture of Hybridoma cell:

- The selected hybridoma cells are cultured in suitable culture medium, often supplemented with insulin, transfer on, ethanol, amine and other additional hormones.
- Some commonly used culture media for hybridoma cell for production of monoclonal antibodies are DMEM (Dulbecco's modified eagle medium),IMDM (Is cove's Modified Dulbecco's Medium),Ham's F12,RPMI 1640 medium (Roswell Park Memorial Institute 1640 medium)

Step V: Inoculation of hybridoma cell into suitable host

- These hybridoma cells are then injected into lab animal so that they starts to produce monoclonal antibodies.

- These hybridoma cells may be frozen and store for future use.

Step VI: extraction and purification of Monoclonal antibodies:

- Monoclonal antibodies from host animal is extracted and purified by one of the following methods;
- Ion exchange chromatography
- Antigen affinity chromatography
- Radial immunoassay
- Immune precipitation

14.7.2 Application of Monoclonal antibodies

- Disease diagnosis:
- ELISA to test HIV, hepatitis, Herpes etc
- RIA- to test viral infection
- Mabs to Human chorionic gonadotropin

14.7.3 Disease treatment

- OKT3- it is an antibody to T3 antigen of T cell which can be used to prevent acute renal allograph rejection in human.
- Different types of Mabs are used in radial immune-detection and radial immune therapy of cancer.

14.7.4 Passive immunization or disease prevention

- Monoclonal antibodies based drugs can be used to treat septic shock
- Used as vaccine

14.7.5 Detection and purification of biomolecules

- Mabs are very useful in determining the presence and absence of specific proteins through western blotting technique.
- Besides that, it can be used to classify strains of a single pathogen. Eg. Neisseria gonorrhea can be typed using Monoclonal antibodies.

NOTES

14.8 FACS

Using FACS a researcher can physically sort a heterogeneous mixture of cells into different populations. By using highly specific antibodies tagged with fluorescent dyes, a researcher can perform FACS analysis and simultaneously gather data on, and sort a sample by a nearly limitless number of different parameters.

Fluorescence-Activated CellSorting. Fluorescence-activated cell sorting (**FACS**) measures the antigen levels on the cell surface quantitatively. Cells are dyed with a fluorescent antibody, then placed in a stream of liquid which passes the focus of a laser, and each cell emits light.

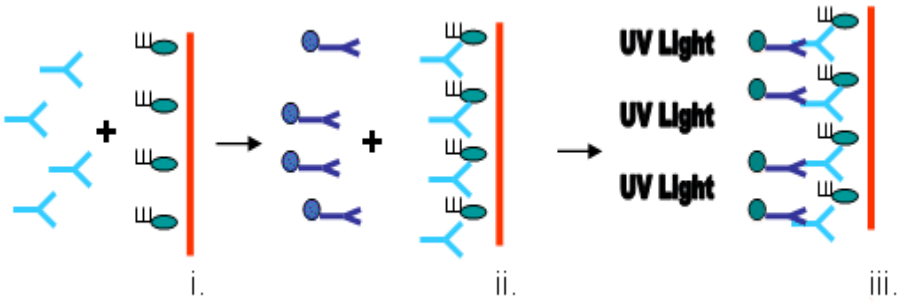
An antibody specific for a particular cell surface protein is associated to a fluorescent molecule and then added to a mixture of cells. For fluorescence when the specific cells pass through a laser beam they are monitored. Droplets containing single cells are given a positive or negative charge, based on whether the cell has limited the fluorescently-tagged antibody or not. Droplets containing a single cell are then detected by an electric field into collection tubes according to their charge.

14.9 IMMUNOFLUORESCENT ASSAY

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

Bio best Laboratories produces Immunofluorescence assay (IFA) test plates and slides by immobilizing cells infected with virus or parasitic tachyzoites by chemical fixation. Samples are added to the test plates or slides at several dilutions and incubated at core body temperature. if antibodies to the antigen are present in the sample they will bind to the antigen during this incubation . The tests are washed to remove any antibodies which have not bound then a suitable anti-species antibody conjugated to a fluorescent marker is added. This secondary antibody will bind to any antibodies which were present in the sample and had bound to the antigen. The tests are incubated then washed again before being read by microscopy using ultra violet illumination. The fluorescent marker glows

with a bright apple green fluorescence under the UV light indicating the presence of specific antibodies bound to the antigen . The pattern of fluorescence observed often confirms the specificity of the reaction. The sample antibody titre is the last dilution of the sample to show this specific fluorescence.



. Detection of Antibodies by immunofluorescence

1. Test serum is incubated with antigen immobilised on a 96-well plate or microscope slide
2. Secondary antibodies labelled with a fluorescence are added
3. After washing, any bound secondary antibodies can be detected by shining UV light on the slide. The label used may be a fluorochrome (IFA test) or be an enzyme which indu

14.9.1 Types of immunofluorescence

There are two classes of immunofluorescence techniques, primary (or direct) and secondary (or indirect).

(i) Primary (direct)

Primary, or direct, Immunofluorescence uses a single antibody that is chemically linked to a fluorophore. The antibody recognizes the target molecule and binds to it, and the fluorophore it carries can be detected via microscopy. This technique has several advantages over the secondary (or indirect) protocol below because of the direct conjugation of the antibody to the fluorophore. This reduces the number of steps in the staining procedure making the process faster and can reduce background signal by avoiding some issues with antibody cross-reactivity or non-specificity. However, since the number of fluorescent molecules that can be bound to

the primary antibody is limited, direct Immunofluorescence is less sensitive than indirect Immunofluorescence

(ii) Secondary (indirect)

Secondary, or indirect, Immunofluorescence uses two antibodies; the unlabeled first (primary) antibody specifically binds the target molecule, and the secondary antibody, which carries the fluorophore, recognises the primary antibody and binds to it. Multiple secondary antibodies can bind a single primary antibody. This provides signal amplification by increasing the number of fluorophore molecules per antigen. This protocol is more complex and time consuming than the primary (or direct) protocol above, but it allows more flexibility because a variety of different secondary antibodies and detection techniques can be used for a given primary antibody.

14.10 LET US SUM UP

In this Unit, You have learnt about the meaning, definition, need, Objectives, Importance and concept of immunofluorescence microscopy, acquired immuno deficiency syndrome (aids) detection and hybridoma technology, facs, immunofluorescent assay. This knowledge would make you understand. It can be practice at typical manifestation of the knowledge.

14.11 UNIT END EXERCISE

1. Discuses HIV Prevention
2. Explain types of Immunofluorescence

14.12 ANSWERS TO CHECK YOUR PROGRESS

1. In some people, persistent swelling of lymph nodes occurs during this stage. Otherwise, there are no specific signs and symptoms. HIV remains in the body and in infected white blood cells. This stage of HIV infection generally lasts around 10 years if you're not receiving antiretroviral therapy. But sometimes, even with this treatment, it lasts for decades. Some people develop more severe disease much sooner.

2. Antibodies are glycoprotein synthesized in blood against specific antigens and give immunity. Such antibodies are heterogenous and are polyclonal antibodies. Therefore they do not have characteristics of specificity. If a specific lymphocyte after isolation and culture invitro becomes capable of producing a single type of antibody which bears specificity against specific antigen, it is known as monoclonal antibody.

14.13 SUGGESTED READINGS

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