UNIT 3 THE IMMUNE SYSTEM

Structure

3.1 INTRODUCTION

Blood, a body fluid, has been rightly termed as an elixir of life. In the last unit we learnt that blood has several important roles to play. What makes it so unique? You would realize that the different blood cells – their functions and significance in maintaining immunity i.e., body's natural defence mechanism against foreign invaders, is what makes blood so unique.

We are living on this globe with many organisms around us. Generally, human beings are scared of some insects or animals. Many are ignorant about the pathogenic organisms around us. Some of them are internal parasites, some are external. There are a number of bacteria, viruses etc. which are pathogenic to human system. We need to have resistance developed against these organisms. Our body is equipped with multiple defence mechanisms, generally known as *immune mechanisms*. In this unit, we will focus on the immune system. What are the components of the immune system? What are the defence mechanisms which protect our body? Why is it essential to have defence mechanisms in our body? These are a few issues discussed in this unit. Finally, we will discuss about the antigen-antibody relationship and the various methods of their in-vitro determination.

Objectives

After studying this unit, you will be able to:

- explain body's immune system,
- discuss the non-specific and specific defence mechanisms in the body,

- differentiate between the innate and specific acquired immunity,
- describe the development, regulation and functions of white blood cells in maintaining immunity in the body, and
- enumerate the methods of in-vitro detection of antigen-antibody interaction.

3.2 THE IMMUNE SYSTEM

The animals and the human beings are forced to encounter many dangerous microbes in their day-to-day life, through water, air and food. Inside our body there is an amazing protective mechanism called the *immune system*. It works silently inside our body for the entire life but we probably know almost nothing about it. What role does it play in our body? The immune system is *designed to protect us from the millions of the microbes, toxins, parasites*. The immune system protects us in three different ways:

1) It creates a barrier that prevents bacteria and viruses from entering our body.
2) If a bacterium or virus does get into the body, the immune system tries to detect and eliminate it before it can make itself at home and reproduce, and
3) If the virus or bacteria are able to reproduce and start causing problems, our immune system is in charge of eliminating it.

You certainly know about your heart, lungs and kidneys. But, do you know what are the organs working inside the body which protect us from germs, infections and other invading substances? Have you ever heard of the *thymus*? Yes, it is there in your chest, right next to your heart. Like thymus, there are other components of the immune system which include:

- Spleen
- Lymph system
- Bone marrow
- White blood cells
- Antibodies
- Complement system
- Hormones

Figure 3.1 illustrates some of these organs. The *thymus* is responsible for producing T cells (we will get to know about them in the next section on WBCs), and is especially important in newborn babies - without a thymus, a baby's immune system collapses and the baby will die. Thymus is important, especially to T-cell maturation. The *spleen* filters the blood looking for foreign cells (the spleen is also looking for old red blood cells in need of replacement). A person missing their spleen gets sick much more often than someone with a spleen. *Bone marrow* produces new blood cells, both red and white. In the case of red blood cells, the cells are fully formed in the marrow and then enter the bloodstream. In the case of some white blood cells, the cells mature elsewhere. The marrow produces all blood cells from *stem cells*. They are called "stem cells" because they can branch off and become many different types of cells – they are precursors to different cell types. Stem cells change into actual, specific types of white blood cells. We shall get to know about the other components as we go along reading the unit.

It is amazing to know that the body develops different types of defence mechanisms. *The study of body's defence mechanisms against invading pathogens is called immunology*. As per the different types of functioning, the defence mechanisms can be of two types. They are:

1) non-specific defence mechanisms, and
2) specific defence mechanisms.

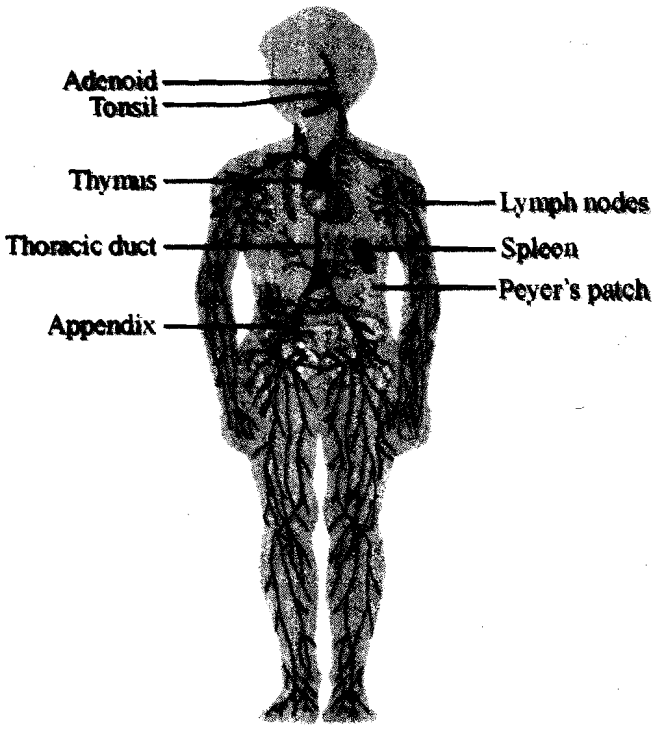

Figure 3.1: Organs of the immune system

One of the most obvious part of the immune system is what you can see i.e. the skin. This is indeed the first line of the defence mechanism the body has in place. The body has yet another second line of defence, about which we will read in a little while. The non-specific defence mechanisms are, therefore, sub divided into two:

a) first line of defence, and
b) second line of defence

Let us learn what these first and the second line of defence mechanisms are.

The first line of defence mechanism (external) includes:

a) skin's horny layer
b) mucous membranes
c) secretions of skin and mucous membranes, and
d) skin and gut bacteria

The second line defence (internal) includes:

a) phagocyte
b) inflammatory reactions
c) fever
d) interferons
e) complement system, and
f) natural killer cells

The mechanisms we have discussed above are all
that our body has yet another third line of
specific mechanism and includes: a)

We shall get to know abo

3.3 NON-SPECIFIC DEFENCE MECHANISM

The non-specific defence mechanism is implemented for different types of infections. It resists infection in two ways, firstly, by blocking the entry of pathogens into the body i.e. through external mechanism and secondly by destroying the microbes, if they enter the body through means other than the antibodies. Let us get to know about these external and internal defence mechanisms.

3.3.1 External Defence Mechanism

The external defence mechanism comprises of physical and chemical barriers to the entry of pathogens. What are the physical barriers? Let's find out first.

1) *Physical barriers*

The physical barriers are primarily of two types – skin and mucous.

A) *Skin:* It provides nice protective covering to the body. The outer layer- horny layer – contains dead keratinized cells. These cells have hard insoluble fibrous proteins called *keratin*, instead of soft protoplasm. This layer is water proof and germ proof. It can prevent entry of virus and bacteria.

B) *Mucous membranes:* The digestive, urinary, genital and respiratory tracts open out at one or both ends. They do not have a direct communication with other parts of the body. The parasites, microbes present are not in the physiological interior of the body. The mucous membranes in these tracts are treated as a part of external defence. These membranes can resist entry of parasites, microbes into tissues. Mucous traps the microorganisms and immobilizes them. How? Mucous, as you may know, is *a clear, sticky substance and has glycoprotein and water.* The microbes get trapped in this sticky fluid. Let us see what role the mucous has in the body systems and organs.

— *The gastrointestinal tract:* The microbes which enter through the mouth are caught in the mucous and eliminated with sputum. A coating of the mucous over the intestinal lining also traps the microbes for removal in the faeces.

— *Respiratory tract:* The microorganisms and dust particles often enter the respiratory tract with air during breathing. Many of these are caught in the hair mesh present in the nostrils. Those which are filtered are trapped in the mucous that covers the tract. The cilia sweeps the mucous loaded with pathogens and dust particles into the pharynx. Further it is thrown out, swallowed or eliminated through the faeces.

— *Eyes:* The secretions from the tear glands, flickering movements of the eyelids flush out the microorganisms setting on the eyeballs from air.

— *Internal tracts:* Various tracts in the body are flushed with fluids such as saliva, digestive juices, bile and urine. All of these can sweep and trap the microbes away.

We have learnt about the physical barriers above. Next, let us get to know about the chemical barriers.

2) *Chemical barriers*

The skin and mucous membranes secrete certain chemicals which dispose the pathogen. These include:

a) *Skin secretions:* The oil and sweat secreted by sebaceous and soporiferous glands contains fatty and lactic acids, which makes the surface of the skin acidic (pH 3-5). These prevent the organisms from infecting the skin. Some friendly bacteria, which reside on the skin checks growth of microbes to a certain extent. *Lysosomal* enzymes of the skin have a bactericidal effect. Thus the skin becomes a self-disinfecting organ.

b) *Saliva:* Saliva contains microorganisms which are not the normal inhabitants. Dead microbes are passively flushed by the saliva and are swallowed.

c) *Gut secretions:* From the saliva and respiratory tract, bacteria reach the gut, here they are killed by the hydrochloric acid and proteolytic enzymes of the gastric juices. They are capable of killing the microbes. If they further escape and reach the large intestine, they are attacked by the gut microbes, which secrete antibiotics that kill many pathogenic bacteria. *Note: Hepatitis A virus survives gastric juice.*

d) *Bile:* It is an alkaline secretion of the liver, which checks the growth of foreign bacteria on the partially digested food.

e) *Tears:* Saline fluid secreted by lachrymal glands, has *lysosomal* activities, which prevents eye infection.

f) *Nasal secretions:* These can destroy harmful organisms due to *lysosomal* activities.

g) *Cerumen (wax of ear):* It traps and kills the bacteria. It contains an effective anti-bacterial component.

h) *Vaginal bacteria:* Certain bacteria normally live in the vagina. They produce lactic acid, which kills the bacteria. Hence, these bacteria form a female's best natural defence against infections.

Skin and mucous membrane may sometimes fail to keep out the invaders. Some parasites make way through the skin e.g. hookworm. Others enter through wounds and openings of sweat glands and hair follicles. Since microorganisms may injure and pass through the thin, moist, relatively vulnerable mucous membranes of digestive, respiratory and urino-genital tracts and get into tissues or blood, it is now that the second line of defence acts for controlling further invasion. We shall learn about these internal defences next.

3.3.2 Internal Defence Mechanism

Body's internal defence mechanism is carried out by white blood corpuscles (WBCs), macrophages, inflammatory reactions, fever, interferons, complement system and natural killer cells, as you may recall reading earlier. All of them together check the damage caused by pathogens. The host usually recovers from such invasions. Let us learn about these internal defence mechanisms, in greater details starting with the WBCs.

1) *The White Blood Corpuscles (Leukocytes)*

The white blood cells are probably the most important part of our immune system. You may recall reading about the white blood cells in the last unit. We learnt that, whenever a germ or infection enters our body, the WBCs snap to attention and destroy the culprit. During an infection, the number of leukocytes increases. The total leukocyte count is about 4000-11000 per cubic mm of blood. Of these, 50 to 70 % are neutrophils, 20-40% are lymphocytes, 2-8% are monocytes, 1-4% eosinophils and 0-1% basophils.

WBCs creep out of the capillaries by amoeboid movement into the intercellular spaces if there is an infection. This process is called *diapedesis* (refer to Figure 3.2).

White blood cells are actually a whole collection of different cells that work together to destroy bacteria and viruses. You may recall studying earlier in Unit 2 that the white blood cells are primarily of two types:

1) *Granulocytes*: Their cytoplasm has granules. The nucleus is lobed and these include:
   - Neutrophils: *Generally three lobes, but may have 2-5 lobes*
   - Eosinophils: *Generally bilobed*
   - Basophils: *Generally bilobed*
2) *Agranulocytes:* Their cytoplasm has no granules. These include:
   - Monocytes: *Nucleus – kidney shaped.*
   - Lymphocytes: *Large nucleus.*

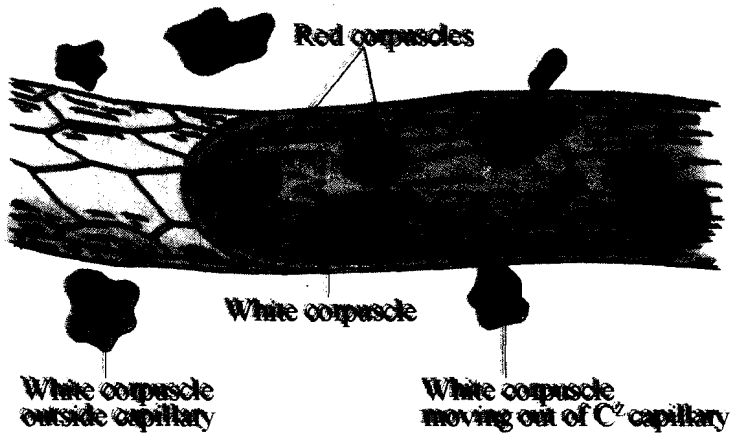

Figure 3.2: Leukocytes moving out through capillary wall into tissue spaces (diapedesis)

You would come across different types, names and classifications of white blood cells – Leukocytes, B-cells, Plasma cells, T-cells, Helper T-cells, Killer T-cells, Suppressor T-cells, Natural killer cells, Phagocytes, Macrophages – while studying about the immune system.

Let us briefly study about some of these different WBCs and their role as internal defence in the body. Later in this unit, in section 3.7, we shall also focus on how granulocytes are formed and what are the factors which influence granulopoiesis.

Neutrophils: They form about 50-70% cells. They have fine dust like particles in the cytoplasm which stain purplish. Tissues, cells damaged by the invading microbes release certain chemicals called *chemokines*, which attract neutrophils from blood. The neutrophils engulf and digest the microorganisms infecting the body tissues. They are called *phagocytes* and the process is known as *phagocytosis.* You may recall reading about this in Unit 2. To refresh your memory, in the phagocytosis process, the cell membrane of phagocyte invaginates and encloses a bacterium in a vacuole known as *phagosome*, the vacuole then fuses with lysosome to form *phagolysosome.* The bacteria are digested by the enzymes present in the lysosome. The doctrine of phagocytosis was advanced by a Russian physiologist, *Elie Metchnikov* in 1882. He got Nobel Prize for medicine and physiology in 1908. A neutrophil may engulf as many as twenty bacteria before it dies. If new entry of an organism is there, then it is trapped by another phagocytic cell known as the *macrophage* (big cells which can eat), about which we will learn in a little while from now.

Basophils: They form only 0.05% of the leukocytes. They release *histamine* that plays a major role in inflammatory reactions. Basophils also secrete *heparin* that prevents clotting of the blood in intact blood vessels. This can prevent clot setting in heart and thus prevent an attack. They help in expression of IgE (immunoglobulin E) mediated allergies.

Eosinophils: They form only 1.5% of leukocytes. They defend against large parasites e.g. blood fluke. They position themselves on the surface of parasite and discharge destructive enzymes. They release histamine and inhibit mast cell degranulation, thus reduce allergic reactions.

Monocytes: They are motile and occasionally show phagocytic activity. They form only 5% of leukocytes, capable of providing more phagocytic defence. They circulate in the blood only for few hours and migrate into tissues change into macrophages. Note monocytes evolve into macrophages.

What are macrophages? Let us get to know about them.

Macrophages: They are large, long living effective phagocytes, which enlarge out of monocytes. It is an irregular cell about 25-50 mm in size. It has a large ovoid nucleus indented on one side. It has more mitochondria and lysosomes than a neutrophil. It engulfs more than 100 bacteria before it dies. Macrophages are of two types:

1) Fixed types, and
2) Wandering types.

The fixed types are located permanently in certain organs, which include:

- Lungs - alveolar macrophages.
- Liver – kupffer cells.
- Renal glomeruli – mesangial cells.
- Brain – microglial cells
- Connective tissues – histocytes.

They are also found in the spleen, lymph nodes and in the endothelium. They catch hold of the microbes and dead cells, that are carried along in the blood and lymph and are trapped in the spleen, lymph nodes etc. The wandering and fixed macrophages together form reticular endothelial system.

What are the functions of the macrophages?

The macrophages:

1) phagocytose microorganisms and inert particles
2) secrete IL-1 (Interlukin 1), TNF (tumor necrosis factor), G-CSF (granulocyte colony-stimulating factor) and M-CSF (macrophage colony-stimulating factor).
3) process and present the antigen to immuno competent cells.
4) destroy old RBC, initiates catabolism of Hb.

Next, let us get to know about lymphocytes, one of the other agranulocytes. We have already studied about the other agranulocyte, i.e. the monocytes above.

Lymphocytes: They are agranulocytes. The nucleus occupies almost the entire cell, as you may recall seeing in Figure 2.25 in Unit 2. They vary in size from 6-18 mm diameter; sometimes divide into small and large lymphocytes. Lymphocytes make up 30% to 40% of all leukocytes. Lymphocytes come in two classes: *B cells (those that mature in bone marrow)* and *T-cells (those that mature in the thymus).* Let us get to know them.

B-cells, when stimulated, mature into plasma cells. These are the cells that produce antibodies. A specific B cell is tuned to a specific germ, and when the germ is present in the body, the B-cell clones itself and produces millions of antibodies designed to eliminate the germ. *T-cells*, on the other hand, actually bump up against cells and kill them. T-cells known as *Killer T-cells* can detect cells in our body that are harboring viruses, and when it detects such a cell, it kills it. Two other types of T-cells, known as *Helper* and *Suppressor T-cells*, help sensitize killer T-cells and control the immune response.

What are the functions of lymphocytes? The lymphocytes handle most of the bacterial and viral infection that we get. In fact, lymphocytes are directly involved with specific acquired immunity. T-Lymphocytes for *cell mediated* and B-Lymphocytes for *humoral immunity*. We shall learn about these immunities later in sections 3.5 and 3.6.

With this, we come to an end of our discussion on white blood cells, one of the internal defence mechanisms of the body. Next, we shall study about the inflammatory responses, which you already know is yet another internal defence mechanism.

2) *Inflammatory responses*

Injury, cut, burn or bite (of an animal) brings foreign matter (bacteria, virus, and fungal spores) to enter the tissue. Certain substances released by damaged cells initiate formation of blood clot. Clotting checks the flow of blood.

From the immune system's standpoint, inflammation is a good thing. It brings in more blood and it dilates capillary walls so that more immune system cells can get to the site of infection. This is how it works. The invading microbes release their own toxic products. The mast cells and basophils release histamine, as you have already learnt earlier. Chemicals from microbes and histamine together cause dilation of capillaries and small blood vessels surrounding the injury and increase the permeability of the capillary wall. As a result, more blood flows to that area, making it red and warm, and fluid leaks out into tissue spaces, causing its swelling. This reaction of the body is called *inflammatory response* and is a part of internal defence. The events in the inflammatory reaction are illustrated in Figure 3.3.

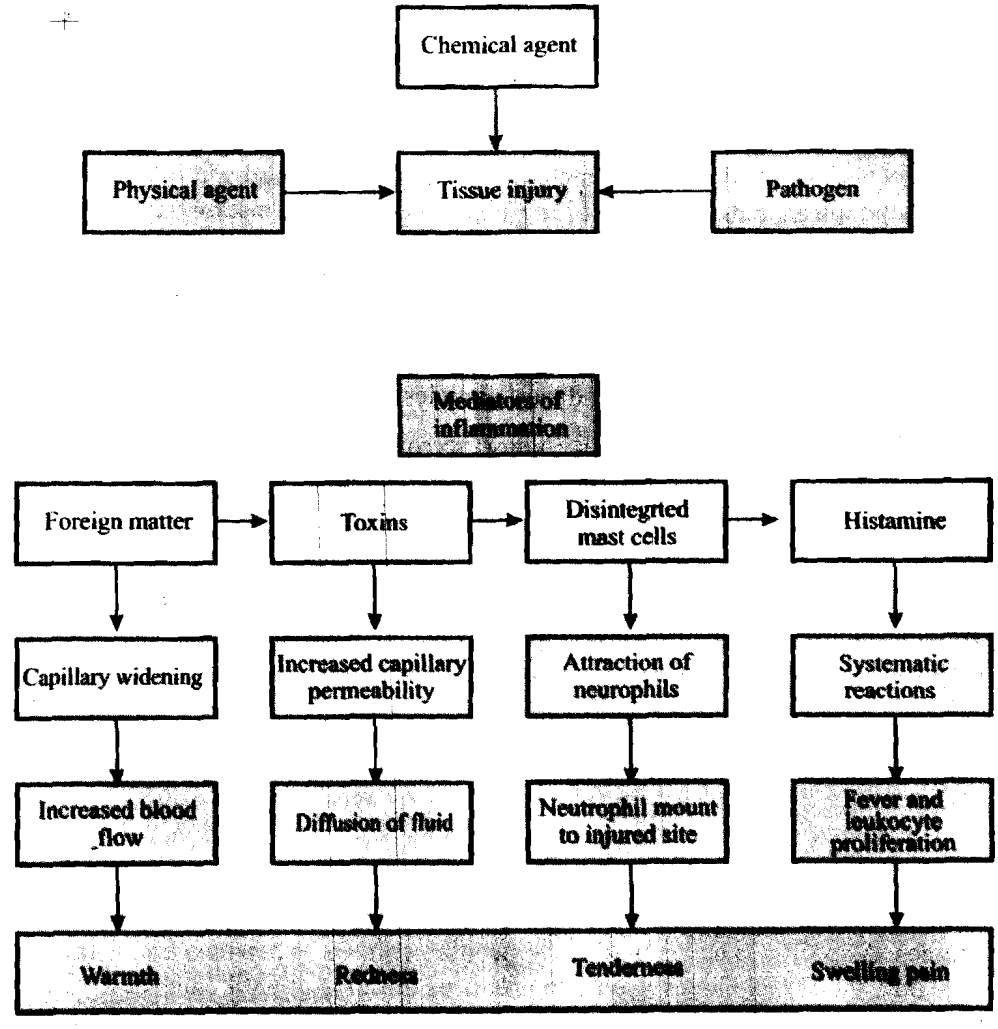

Figure 3.3: Events in the inflammatory reaction

The plasma that accumulates at the injured site dilutes the toxins secreted by bacteria and lessens their effect. Its fibrin forms a network which occludes the lymphatic channels, thus limits the spread of microorganisms. So now you can understand why the inflammatory response is so important.

The neutrophils are attracted to the site of injury or infection by chemicals, by injured cells elaborated by bacteria and histamine. Here they adhere to the lining of capillaries and push between its cells into the tissue spaces. Once the neutrophils reach the site, they move and eat up the microorganisms. By this time, the macrophages of the connective tissue begin their protective action. Monocytes also reach the site of the infection and develop into large macrophages for additional phagocytosis. They would further clean up the tissue and remains of neutrophils. Certain substances in the plasma strongly stimulate phagocytosis, they are called *opsonins,* which in a way make the bacteria 'tastier' to the phagocytes.

The other internal defence mechanism operational in our bodies is pus formation. Let us read about its role in the body, next.

3) *Pus formation*

Once a neutrophil finds a foreign particle or a bacterium, it will engulf it releasing enzymes, hydrogen peroxide and other chemicals from its granules to kill the bacteria. In a site of serious infection (where lots of bacteria have reproduced in the area), pus will form. Pus is simply *dead neutrophils and other cellular debris.* Many neutrophils and macrophages are killed by toxins released by highly virulent invaders. The dead phagocytes, microbes are trapped by still bigger phagocytes and they form a protective barrier. Dead phagocytes, enzymes, fluid, protein, damaged tissue cells etc. leak from capillaries and leave the body in the form of pus. This is a sure sign of infection.

Like pus, fever too is a defence mechanism. Let us find out how.

4) *Fever*

We have seen that due to injury, cut or bite, the inflammatory response develop and this is localized or *systemic*. The localized response is confined to the site of injury only. The *systemic* response affects the body in case of a severe infection or a serious injury. In this case, the WBC count increases. Body temperature rises causing fever. This may be brought about by toxins produced by pathogens and by a protein called *endogenous pyrogen*. When enough pyrogen reaches the brain, the body's thermostat is reset to higher temperature allowing the entire body temperature to rise.

Other components like interferons, complement system are discussed next.

5) *Complement system*

The complement system is *a series of proteins*. There are only a handful of proteins in the complement system and they are floating freely in our blood. The complement proteins are activated by and work with (complement) the antibodies, hence the name. They cause lysing (bursting) of cells and signal to phagocytes that a cell needs to be removed.

6) *Interferon*

Interferon interferes with viruses (hence the name) and is produced by most cells in the body. Interferons, like antibodies and complements, are proteins, and their job is to let cells signal to one another. When a cell detects interferon from other cells, it produces proteins that help prevent viral replication in the cell.

The discussion so far focused on the non-specific defence mechanisms, which included the external and internal mechanisms. We shall next, study about the specific defence mechanisms.

Check Your Progress Exercise 1

1) Explain the following terms:

   a) Immunology

   .....

   .....

   b) Chemokines

   .....

   .....

   c) Phagosome

   .....

   .....

   .....

2) What is an immune system? List the components of immune system.

   .....

   .....

   .....

3) What are the different defence mechanisms operating in our body? Briefly discuss the types of non-specific defence mechanisms.

   .....

   .....

   .....

4) How does the non-specific defence mechanism operates in our body?

   .....

   .....

   .....

5) List a few important functions of:

   a) Macrophages

   .....

   .....

   b) Lymphocytes

   .....

   .....

   c) Complement system

   .....

   .....

3.4 SPECIFIC DEFENCE MECHANISM

In the section on white blood cells, we learnt that whenever a germ or infection enters our body, the WBCs snap to attention and destroy the culprit. How does a white blood cell know what to attack and what to leave alone? Why doesn't a white blood cell attack every cell in the body? The answer to these questions lies in the discussion below.

The specific defence mechanism provides protection against specific foreign materials. The important characteristic is that its cells (lymphocytes) have an ability to recognize body's own cells and macro molecules (self) from those which are foreign invaders (non self). It tolerates the *'self'* but destroys the *'non self'*. The lymphocytes bearing receptors specific for self i.e. molecules already present in the body, are either made non-functional or destroyed by programmed cell-death known as *apoptosis*. The lymphocytes that react to non self i.e. foreign molecules are left to function in immune responses.

So, you realize that there is a system built into all of the cells in our body that marks the cells in our body as "self". Anything that the immune system finds that does not have these markings (or that has the wrong markings) is definitely "not self" and is therefore a fair game. This system is called the *Major Histocompatibility Complex* (MHC). Let us learn about this specific mechanism next.

3.4.1 Major Histocompatability Complex (MHC)

Major histocompatability complex is also known as the Human Leukocyte Antigen (HLA). MHC molecules are important components of the immune response. They allow cells that have been invaded by an infectious organism to be detected by the cells of the immune system called *T-lymphocytes* or *T-cells*. The MHC molecules do this by presenting fragments of proteins (peptides) belonging to the invader on the surface of the cell. The T-cell recognizes the foreign peptide attached to the MHC molecule and binds to it, an action that stimulates the T-cell to either destroy or cure the infected cell.

The MHC, therefore, is a set of genes that code for cell surface glycoproteins. These glycoproteins mark the body cells as 'self' and are divided into two main classes – *class I MHC* molecules are found on the surface of almost every cell of the body. *Class II MHC* molecules are confined to a few specialized cell types, such as macrophages, B-cells and activated T-cells. Each MHC antigen combination forms a unique complex that is recognized by specific antigen receptors on T-cells as 'self'.

T-cell surface proteins CD4 and CD8 greatly enhance the interaction between an antigen presenting cell (APC) and killer T-cell and between APC and helper T-cell, respectively.

The primary immunological function of MHC molecules, therefore, is to bind and "present" antigenic peptides on the surfaces of cells for recognition (binding) by the antigen-specific T-cell receptors (TCRs) of lymphocytes.

The other specific defence mechanism in our body is the antibodies. Let us learn about their role in the immune system and their interaction with antigens.

3.4.2 Antibodies

Antibodies (also referred to as immunoglobulins and gammaglobulins) are produced by plasma cells. They are Y-shaped proteins (as illustrated in Figure 3.4) that each respond to a specific *antigen*. What is an antigen? *The foreign matter (bacteria, virus or toxin) that enters the body and elicits a specific immune response by lymphocytes is called an antigen or immunogen.* It stimulates the immune system to produce protective chemicals or special cells to destroy the antigens. These protective chemicals are called *antibodies*.

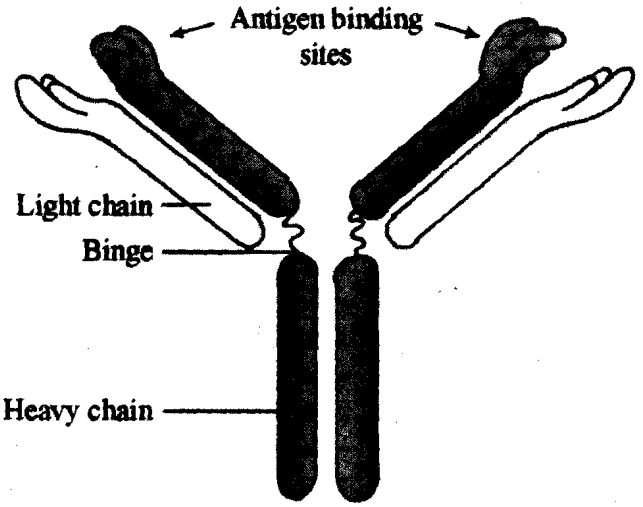

Figure 3.4: Structure of antibody molecule

The molecule of antibody has 2 pairs of peptide chains linked by disulphide bonds. The 2 longer chains are called as *heavy chains* and the 2 shorter chains are called *light chains* as shown in Figure 3.4. The 2 long and short chains are identical. The antigen (Ag) binding site is at the N-terminal end of the polypeptide chain. The site of complement fixation is towards C-terminal. The difference in structure forms the basis of the antigen specificity of each antibody. The structure of the N-terminal varies from one antibody to another. Whereas long part of the peptide chain towards the C-terminal end are relatively constant. Within the variable region of the molecule, there are some selected amino acid sequences which are more variable than the rest, these are known as *hyper variable regions*. Splitting of the molecule is done by proteolytic enzyme.

The antigen entering the body may be molecules on the surface of viruses, bacteria, fungi, protozoan or worms. The protein molecule present on the surface of the foreign material act as antigens, but some carbohydrates and lipids also can act as antigens. Each antigen causes formation of a specific antibody. Antigens (Ag) and antibodies (Ab) have complementary reactive sites that fit together in a lock-and-key fashion, forming an antigen-antibody complex as shown in Figure 3.5.

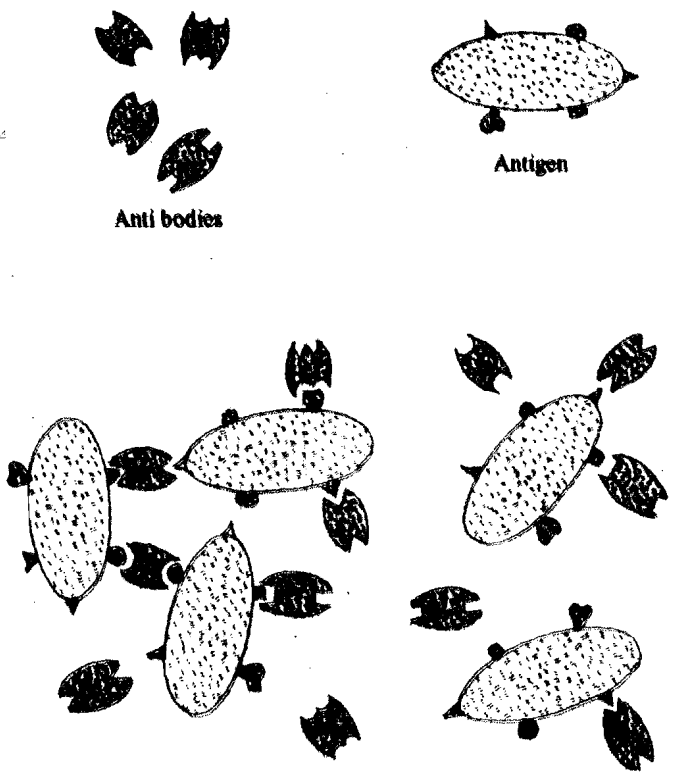

Figure 3.5: Antigen-Antibody reaction

Antibodies react with antigens and make them inactive or harmless. The differences between antigens and antibodies are highlighted in Table 3.3.

Table 3.3 : Differences between antigens and antibodies

Antigens (immunogens)	Antibodies (immunoglobulins)
1. It is a foreign material, elicits antibody formation.	1. It is a molecule synthesized by the organism to combat foreign material.
2. It is a protein or polysaccharide molecule.	2. Each antibody is a immunoglobulin
3. It may occur on the surface of a microbe or as a free molecule.	3. It occurs on the surface of a plasma cell and also in the body fluids.
4. It binds to macrophages to reach a helper T cell to initiate immune response.	4. It directly joins an antigen to destroy the latter.

Other than bacteria, virus, the other antigens which enter the body include pollen, egg white, feathers, some fruits, vegetables, chicken, blood cells from other persons, transplanted tissues, organs, certain medicines, toxins such as snake poison.

Antibodies, on the other hand, are classified in five classes. These are:

- *Immunoglobulin A (IgA):* present in secretions, protects mucosal surfaces. It is found in tears, saliva, gastrointestinal secretion, respiratory and genitourinary tract. Secreted in combination with peptide – forms secretory piece is protected from proteolytic digestion.
- *Immunoglobulin D (IgD):* present on surface of B lymphocytes, along with IgM plays a role in Ag recognition.
- *Immunoglobulin E (IgE):* protects against organisms which escaped IgA, major deference against helminthes, mediates allergy. On coming in contact with specific Ag, it releases mast cell granules. These granules contain a chemical which leads to an inflammatory reaction and also chemotactic migration of granulocytes.
- *Immunoglobulin G (IgG):* major defence against bacteria and their toxins. It is the major Ig – 80% in the body, can cross the capillary wall very easily. It can cross placenta, secreted into milk, thus transfers immunity from mother to child.
- *Immunoglobulin M (IgM):* protects against bacteria, gives an early immune response, present on surface of lymphocytes. It stays confined to blood stream and is involved in Ag recognition.

The antigen-antibody reaction, about which you have studied above, is also useful in detecting infections. We will look at this aspect later in the unit.

In the sections above we have studied about the non-specific and the specific defence mechanisms in our body. It must be clear to you by now that our body is equipped with multiple defence mechanisms. These are generally known as *immune mechanisms*. Immunity, you would realize, is of 2 types – *innate* and *acquired*. In the next two sections, we shall get to know about the innate and acquired immunity.

Check Your Progress Exercise 2

1) What is the important characteristic of specific defence mechanism?

.....

.....

.....

2) What do you understand by the term 'MHC'? What are its two types?

.....

.....

.....

3) What is an immunogen? How are immunoglobulins (antibodies) classified?

.....

.....

.....

4) List any 3 differences between immunogens and immunoglobulins.

.....

.....

.....

3.5 INNATE IMMUNITY

The term *'innate immunity'* refers to *the basic resistance to disease that a species possesses – the first line of defence against infection.* In fact, innate immunity refers to antigen non-specific defence mechanisms that a host uses immediately or within several hours after exposure to almost any antigen. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

In our discussion above we have seen that the best way to avoid pathogens/infections is to create a barrier. This is a very sensible, desirable and effective way to protect the body from many infections. They are physical and chemical barriers, about which we learnt earlier. E.g. the skin is an impermeable covering. Here fatty acids and low pH sebaceous secretions prevent bacterial growth. On the other hand, the surface which cannot be covered by skin will have special barriers. E.g. eyes with tears, mouth with saliva, stomach with acids, air passage with mucous and cilia.

Potential pathogens are encountered routinely, but only rarely cause disease. The vast majority of microorganisms are destroyed within minutes or hours by innate defences. The acquired specific immune response comes into play only if these innate defences are breached. The efficacy of innate immunity improves in us after the pathogen enters in us and then we develop resistance. Unlike innate immunity, acquired immunity develops as a result of exposure to a pathogen which is very specific.

The characteristics of the innate immune response, therefore, include the following:

- Responses are broad-spectrum (non-specific)
- There is no memory or lasting protective immunity
- There is a limited repertoire (stock) of recognition molecules
- The responses are phylogenetically ancient

Pathogens have involved on earth before our studies on defence mechanism. The best way to drive them away is to have a need based defence mechanism and the system be activated by the invader itself. This can be achieved by initiating phagocytotic process. In fact, the innate immune responses involve:

- phagocytic cells (neutrophils, monocytes and macrophages),
- cells that release inflammatory mediators (basophils, mast cells and eosinophils),
- natural killer cells (NK cells), and
- molecules such as complement proteins, acute phase proteins and cytokines.

Let us get to know about these innate responses next.

3.5.1 Phagocytosis

Phagocytosis involves the ingestion of particulate material including whole pathogenic microorganisms. The plasma membrane expands around the particulate material to form large vesicles called *phagosomes.* It is carried out either by neutrophils or macrophages. Neutrophils are highly effective against pus forming (pyogenic) bacteria. We already know that macrophages are tissue phagocytes and are derived from circulating monocytes. These monocytes and macrophages together contribute to the phagocytic system (reticulo endothelial system). Macrophages are most effective against those microorganisms which live within the cells.

What is the mechanism of phagocytosis?

Figure 2.7 in Unit 2 illustrates phagocytosis. In phagocytosis, the organism is entrapped into vacuoles known as "Phagosome". The cytoplasmic granules, organelles or lysosomes fuse with the phagosome releasing powerful microbicidal substance. These substances are divided into:

a) those which are dependent on O_2 mechanism, and

b) those which are O_2 independent mechanisms

Example for O_2 dependent mechanism is superoxide anion, singlet O_2 hydroxyl free radicals. The O_2 independent ones are lysosomes, proteolytic enzymes and several other hydrolytic enzymes.

The next innate response includes the complement system.

3.5.2 The Complement System

To remove a microorganism by phagocytosis requires phagocytes at the right place in the right time. The organism should adhere to the phagocyte. Many of these organisms may not be viable to this type of a situation. To deal with such a situation, therefore, one more non-specific defence mechanism is found in plasma. This is known as the *complement system.* You may recall reading about this earlier in section 3.3. Complement system contains about 20 proteins, when triggered can enter into *cascade reaction* (occurs one after the other sequentially, with a number of factors). The system helps to handle microbial invasion basically by 3 mechanisms.

1) Some components of a complement system coat microorganisms. These organisms with such a coat can be easily phagocytosed because of the receptors, which are similar for the same complement components which coat the microorganisms.

2) Some component of the system stimulates the lethal mechanisms of phagocytes released earlier. They also release histamine and several other useful substances from mast cells and basophil granules. The major effects of granule release are vasodilatation and chemotactic migration of neutrophils and eosinophils to the site of infection.

3) The complement pathway leads to the formation of membrane attack complex which stabs a hole into the microbial cell wall which would lead to the entry of water and Na^+ ions into microbial cells leading to its lysis.

3.5.3 Humoral Mechanisms

Besides the complement system, there are some other humoral mechanisms which help in innate immunity. These include:

1) *Acute phase proteins:* Following any infection, there is a rise in concentration of several plasma proteins. The best known amongst them are *C– reactive proteins* (CRP) which adheres to the surface of a number of microorganisms. These CRP coated organisms can activate complement system. This, in turn, facilitates phagocytosis.

2) *Interferons:* Interferons are soluble proteins secreted naturally when cells become infected by foreign bodies. Infected lymphocytes release interferons alpha which is one out of the 14 sub types known. Other cell types when infected by some viruses release interferon beta. These interferons are released into extra cellular fluids, where they diffuse to form a protective ring of uninfectable cells, thereby limiting the spread of infection.

   Besides this, they inhibit protein synthesis by interfering with the process of translation and promote degradation of mRNA.

   Since viruses depend partly on nucleic acids of the host for protein synthesis, interferons have a marked inhibitory effect on the replication of viruses.

3) *Natural killer cells (NK cells):* They are large sized lymphocytes, specifically equipped to kill *virally infected cells*, some of the *virally infected cells* acquire a special glycoprotein on their surface which signals their neighbouring cells that they are in trouble and they need help.

   The NK cells are especially receptive to this cry for help because they have surface proteins which are very similar to the ones which have glycoprotein coatings. When the 2 components come together in contact, the NK cells would release lethal substances which lead to the death of infected cell. The infected cell is killed and it has a chance to multiply. The major drawback of this process is that the host cell gets sacrificed.

4) *Contribution of eosinophils:* They are specifically equipped to deal with a lot of parasites (all helminth worms). The coating of helminthes and some complement components facilitates its adherence to the eosinophils. This releases several lethal particles from the eosinophil granules, which causes death.

   Having studied about the innate immune response, we move on to specific acquired immunity.

3.6 SPECIFIC ACQUIRED IMMUNITY

Body in the long run develops specific immune mechanisms for each species. These mechanisms are not innate but are acquired after exposure to the specific organism. Acquired (adaptive) immunity, therefore, refers to *antigen-specific defence mechanisms that take several days to become protective and are designed to react with and remove a specific antigen.* This is the immunity one develops throughout life.

Adaptive immunity usually improves upon repeated exposure to a given infection and involves:

- antigen-presenting cells (APCs) such as macrophages
- the activation and proliferation of antigen-specific B-lymphocytes

- the activation and proliferation of antigen-specific T-lymphocytes, and
- the production of antibody molecules, cytotoxic T-lymphocytes (CTLs) and cytokines.

The human body can respond to antigens in many different ways. These fall into two major categories:

- *Antibody Mediated Immune System (AMIS)* - by circulating antibodies (Ab), also known as humoral immunity, about which you have learnt above, and
- *Cell Mediated Immune System (CMIS)* - by sensitized cells known as cell mediated immunity.

Both the systems need antigens to come into action and they respond in different ways. The B-cells and T-cells of the lymphocytes recognize the specific antigens by means of antigen receptors bound to their plasma membrane. Ag-receptors on a B-cell are simply antibody molecules that move from the cytoplasm onto the plasma membrane. These are called *membrane antibodies*. The T-cell receptors are structurally related to membrane antibodies.

What is the scene of action?

Primary lymphoid organs, as you already know, are thymus and bone marrow. Secondary organs are lymph nodes, spleen and lymphoid tissue. When an Ag microorganisms enter the body, one of the secondary lymphoid organs traps it and mounts an immune response. An exact organ involved depends at the site of entry. Let us then get to know what are the sites of entry.

Sites of entry

- Tissue – traps in draining lymph node
- Blood stream – trapped in spleen
- Mucosal surface – reponses in mucosal associated lymphoid tissue.

Irrespective of the above mentioned situation, the agent is trapped by phagocytic cells which degrade it, coat their surface with some chemical fragment derived from the agent. These fragments help in recognizing the phagocyte by immuno competent cells which finally leads to the production of effectors. Effectors in humoral immunity are specific Ab. In CMI, the effectors are lymphokinesis and cytotoxic lymphocytes.

Let us now get to know how the two acquired immunity system works. We start with AMIS.

3.6.1 Antibody Mediated Immune System (AMIS)

In antibody-mediated immunity, the antibodies dissolved in blood, lymph and other body fluids bind the antigen and trigger a response to it. AMIS, therefore, involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes. This form of immunity is also called *humoral immunity*, as you may recall reading above.

What is the specific role of AMIS?

The AMIS defends the body against free viruses, bacteria with polysaccharide capsules and toxins that enter the body fluids (blood and lymph).

How are the antibodies formed?

The process of antibody formation is illustrated in Figure 3.6. When membrane antibodies on B-cells surface bind antigens, the B-cell is activated and divides producing a clone of daughter B-cells. The daughter B-cells are of two types – *Plasma B-cells* and *Memory B-cells*.

Plasma B-cells are antibody factories. They secrete antibodies on stimulation of T-helper cells. The antibodies pass into and circulate in the lymph to dispose off the antigens. Each person makes about 10^7 - 10^8 different kinds of Ab molecules so that there is an Ab on a B cell to fit any antigen. Thus, there are numerous antigen specific-B lymphocytes in the body. The plasma membrane of each B-cell should be sensitized by contact with a specific antigen for the release of antibodies. The plasma cells do not migrate to the site of infection and act through lymph. Hence, they are said to form humoral system (fluid mediated). The B-cells are short lived and are replaced every few days from bone marrow.

Memory B-cells live for a long time and serve to quickly dispose off the antigens, in case reinfection of the same virus or bacterium occurs.

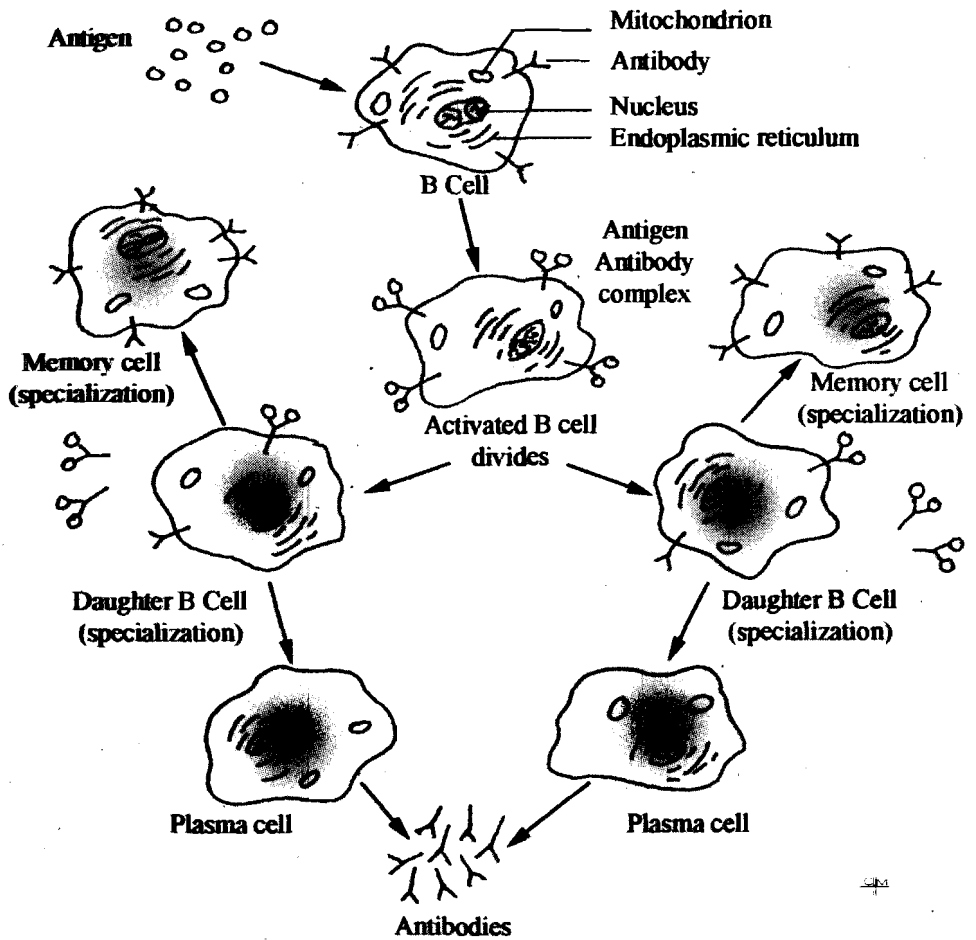

Figure 3.6: Formation of plasma cells and memory cells from B-cells

How do antibodies act?

The antibodies bind to their specific antigens and inactivate the invading microorganisms or foreign molecules, so that these are conveniently disposed off by the phagocytes.

How are the antigens disposed?

We have seen earlier that there are 5 types of antibodies – IgG, IgA, IgM, IgD, and IgE. They fight the antigens in 5 different ways. These include:

a) *Neutralization:* Since antibodies neutralize the antigens (viral or bacterial toxins), make them ineffective, they are called as *antitoxins*. The phagocytes dispose off the antigen - antibody complexes.

b) *Agglutination:* Certain antibodies cause the particulate antigens (bacteria, red corpuscles) to stick together in clumps, thus immobilizing them for easy disposal by the phagocytes through ingestion. They are termed as *agglutinins*.

c) *Precipitation:* Other antibodies combine with the antigens to form precipitates that are easily ingested by phagocytes. They are known as *precipitins*.

d) *Opsonization:* Some antibodies coat the surface of the microbes and make them more susceptible to phagocytosis, such antibodies are known as *opsonins*.

e) *Complement activation:* Antibody-antigen complex activates complement proteins, which may:

   i) lyse walls of bacteria, causing their disintegration,

   ii) initiate inflammatory response,

   iii) ospomise antigens, and

   iv) attract phagocytes to areas of infection.

Next, let us learn about the second arm of the immune response, referred to as cell mediated immune system.

3.6.2 Cell Mediated Immune System (CMIS)

Cell-mediated immunity is an immune response that does not involve antibodies, but rather involves the production of T-lymphocytes, activated macrophages, activated NK cells and cytokines in response to an antigen and is mediated by T-lymphocytes. Thus in CMIS, T-cells (lymphocytes) bind to the surface of other cells that display the antigen and trigger a response.

What is the specific role of CMIS?

The CMIS defends the body against viruses and bacteria, which have entered the host cells, and also against protozoan, fungi and parasitic worms. Its defensive cells cannot deal with free antigens present in body fluids. It reacts with foreign tissue transplants and also against body's own cells which become cancerous. The cancer cells are perceived as foreign cells.

Cellular immunity, therefore, protects the body by:

1) activating antigen-specific cytotoxic T-lymphocytes (CTLs) that are able to lyse body cells displaying epitopes (sites on the surface of the antigen molecule to which a single antibody molecule binds) of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens

2) activating macrophages and NK cells, enabling them to destroy intracellular pathogens, and

3) stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

What is the mode of action?

The cellular immune response is given by T-cells, unlike the B-cells in AMIS. There are separate T-cells for each type of antigen that invades the body. T-cells have a life span of 4-5 years or even longer. They acquire the ability to recognize particular 'non self' cell surfaces and molecules in thymus.

On stimulation by contact with antigens, the T-lymphocytes produce a clone of T-cells by division - the lymphoblasts. The T-cells comprising the clone are committed T-cells having specific functions. They are of four types. They are morphologically similar but functionally different. These include:

a) Cytotoxic or killer T-cells (Tc)

b) Helper T-cells (Th)

c) Suppressor T-cells (Ts)

d) Memory T-cells (Tm)

Let us get to know them.

a) *Killer T-cells:* They migrate to the site of infection. They have a surface peptide that acts as receptors for foreign antigens. When a T-cell encounters 'non self' cell, its surface receptors draw the two cells into a physical contact. Killer T-cells secrete a protein called *perforin,* which can puncture invader cell membrane. Take a look at Figure 3.7, here you can see how water and ions flow into the non-self cell, which swells up and finally lyses the infected cell. Death of infected cell helps the host in two ways: it deprives the pathogen of a place to multiply and expose the pathogen to circulating antibodies for disposal. Killer cell further proceeds to kill another non-self cell. Killer T-cells are guided by their receptors to the body cells covered with viruses. They destroy the body cells before the viruses can enter and multiply to spread infection. They can also destroy cancer cells. Since the killer cells must be present on the spot to play their role, they are said to form cell mediated immune system.

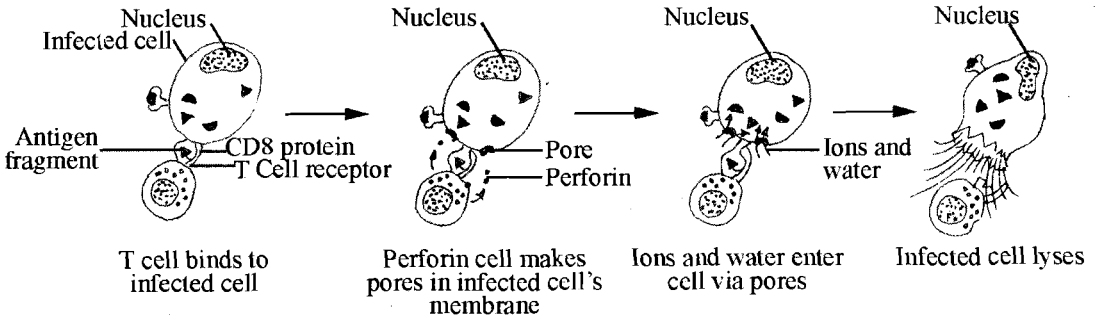

Figure 3.7: Function of killer T-cells

b) *Helper T-cells(Th)*: These cells stimulate the B-cells to produce antibodies. They stimulate killer T-cells to destroy the non-self cells. Refer to Figure 3.8 for understanding the interaction of Helper T-cells with an infected cell (A) and macrophage (B).

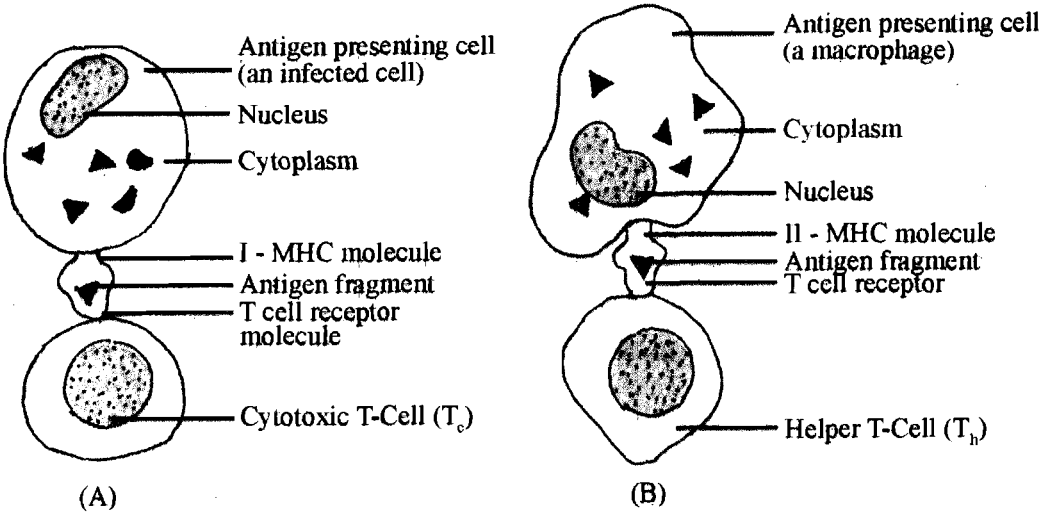

Figure 3.8: Interaction of T-Cells with MHC molecules on an infected cell (A) and on a macrophage (B)

c) *Suppressor T-cells (Ts):* These cells inhibit the immune response of both T and B lymphocytes to foreign antigens when infections are controlled. They also inhibit the immune system from attacking the body's own cells.

d) *Memory T-cells:* These cells keep ready to mount a rapid and vigorous attack as soon as the same pathogens infect the body again.

In the discussion above, you may have realized that the helper T-cells (Th) and the suppressor T-cells (Ts) have a dual role to play. This role is highlighted next.

Dual role of Th and Ts cells

The killer cells are the effector cells of the CMIS, whereas helper T- cells and suppressor T-cells are the regulatory cells of both AMIS and CMIS. Both Th and Ts cells secrete a protein known as *interleukin*-2, which stimulates proliferation of activated B-cells and T-cells.

The discussion above would have helped you to understand the innate and adaptive immune response in our body. While talking about the immune mechanisms, we must re-emphasize the role of macrophages in the immune system. You may recall reading about the functions of macrophages earlier in section 3.3. Here we will focus on their role in the immune system, per se.

Role of macrophages in immune system

Macrophages, you already know, are white blood cells that continually search for foreign (non-self) antigenic molecules, viruses, or microbes. When found, the macrophages engulf and destroy them as shown in the Figure 3.9.

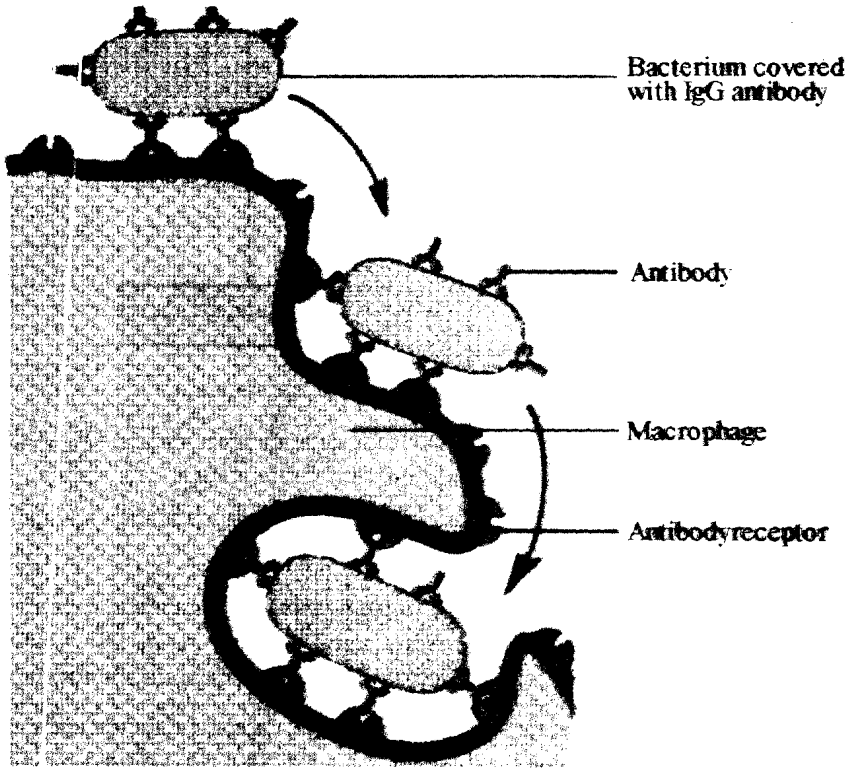

Figure 3.9: Action of macrophages on foreign bodies

The process involved includes:

1) Macrophages respond to infection.

2) They engulf microbes and display the antigen to alert the lymphocytes.

3) The antigen is held onto the surface of macrophage by a protein which marks the macrophage as 'self' (as own part).

4) With the antigen flagged on it, macrophage moves to nearest lymph node, meets various types of lymphocytes.

5) A helper T-cell recognizes the macrophages antigen flag and joins it. This initiates immune reaction.

6) Simultaneously macrophages secrete endogenous pyrogen, which:

   a) activates T and B cells to grow and proliferate

   b) causes fever, microbial action slows down.

All these steps are indicated in the flow chart given in Figure 3.10.

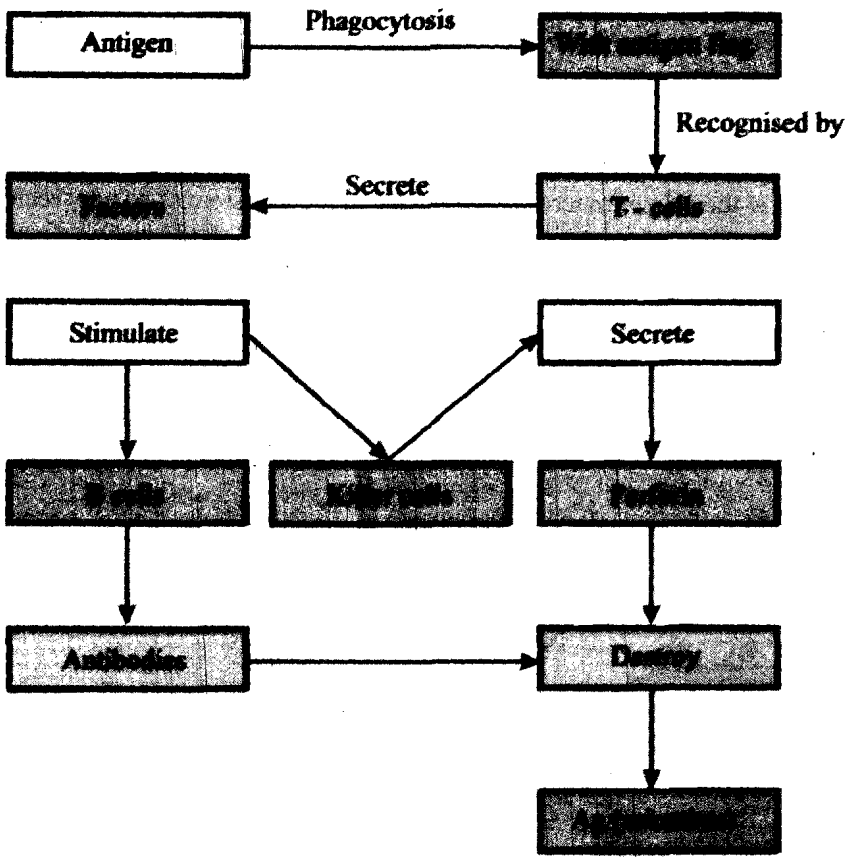

Figure 3.10: Role of macrophages in immune system

From the discussion above, it is clear that the macrophages, B-cells and T-cells interact to produce immune responses. What is the outcome?

- Infection disappears due to Ag-Ab interaction along with killer T-cells and non-self cells interaction.
- Some of the specific lymphocytes remain in the lymphatic tissue as "memory or primed cell". They produce killer cells if the same antigens reappear. Hence, a second attack of infections and disease elicits quick, abundant antibody formation.

Memory cells give rise to more effector cells and memory cells (effector cells have only a few days life-span). The memory cells live long, some even for whole life. Hence diseases which attack in childhood, do not attack again. Memory cells are stored in the spleen and lymph nodes.

Check Your Progress Exercise 3

1) What is innate immunity?

.....

.....

2) What is 'phagocytosis'? Discuss its mechanism of action.

.....

.....

.....

3) How does a complement system functions?

.....

.....

.....

4) Give the specific roles of:

a) Antibody Mediated Immune System (AMIS)

.....

.....

b) Cell Mediated Immune System (CMIS)

.....

.....

5) Give the role of macrophages in immune system.

.....

.....

.....

3.7 THE LEUKOCYTES: DEVELOPMENT AND REGULATION

Since leukocytes play such a major role in the immune system, it is important that we learn about the development and regulation of leukocytes in our body.

In sub-section 3.3.2, we learnt that leukocytes or WBCs are classified as *granulocytes* and *agranulocytes*. Let us get to know how the granulocytes develop.

3.7.1 Development of Granulocytes

Granulocytes are formed in the bone marrow from the *totipotent haemopoietic stem cell (myeloid series)* (blood forming stem cells in the bone marrow). Following are the stages of granulocyte development:

1) *Myeloblast:* The first identifiable cell of the granulocyte series is the myeloblast. It has a large nucleus, 2-5 nucleoli, no granules in cytoplasm, capable of cell division and has poor motility.

2) *Promycelocyte:* Division and maturation of myeloblast yields promyelocyte (myelocyte A). This stage has primary granules in cytoplasm.

3) *Myelocytes (myelocyte B):* After promyelocyte division and maturation, the next stage is the myelocyte. In this stage, the nucleus is flattened on one side; primary and secondary granules in the cytoplasm are seen.

4) *Metamyelocyte (myelocyte C):* Metamyelocytes no longer divide or produce granules (capacity for mitosis is lost). Myelocytes are the last cells to undergo cell division. In this stage, the nucleus changes shape from round/oval to indented and nucleoli is absent. More secondary granules seen in the cytoplasm and fewer primary granules seen in the cytoplasm. Amoebid movements appear.

5) *Band cell (juvenile granulocyte*): Band cells are later stages of metamyelocyte. By this stage, the nucleus is deeply indented (sausage shaped) so that it resembles a hair band. The cytoplasm is filled with specific granules.

6) *Mature granulocyte:* Completely developed cells with a lobed nucleus.

Neutrophils are abundant among granulocytes. *Granulopoiesis, therefore, generally refers to the formation of neutrophils*. Now that we know how the granulocytes are formed, let us briefly look at the factors which influence granulopoiesis.

A detailed discussion on these growth factors is present in sub-section 3.7.2. You will find this information a bit technical and advance. Do not panic. This is additional information for those of you who would like to know more on this topic. You will not be tested for this knowledge in the term end examination.

3.7.2 Growth Factors which Affect Granulopoiesis

The factors which influence granulopoiesis include:

1) *Interleukin-I:* Interleukin is a cytokine found in WBCs that stimulates to light infections. The structure of human IL-1 is illustrated in Figure 3.12, Interleukin is a protein factor which is produced by *T lymphocytes* and *macrophages* (a type of white blood cell) in the presence of *antigens*. They cause the T lymphocytes to activate and proliferate. Interleukin acts on relatively early progenitor cells to stimulate their proliferation. It also enhances the effectors function of all types of leukocytes. Since macrophages are activated more during an injury, IL-1 seems to act as a general signal for intercellular dialogue which speaks in a language, which a wide variety of cells can understand.

Figure 3.11: Structure of human Interleukin

The cells that respond to IL-1 produce growth factors which are specific in their action. It also directly releases mature neutrophils from bone narrow and stimulates cytotoxic effects of macrophages themselves.

2) *Tumour Necrosis Factor (TNF):* The name itself is because of its inhibitory effect on some of the tumors cells. It is produced by macrophages and its effects are known to be similar to that of IL-1. IL-1 and TNF stimulate granulopoietic activity by involving a pathway through granulocyte macrophages colony stimulating factors (GM-CSF).

3) *Granulocyte macrophage colony stimulating factor (GM-CSF):* Stem cells obtained from the bone marrow are capable of giving rise to countable colonies of daughter cells in spleen. The growth factors were identified on the basis of their ability for colonial proliferation. Thus the name was assigned.

   GM-CSF stimulates the proliferation of committed cells to form granulocyte and macrophages. It also stimulates proliferation for precursors of eosinophils, megakaryocytes and erythrocytes. In addition, it acts on the mature neutrophils, eosinophils, macrophages to enhance their effector responses.

4) *Interleukin – 3 (IL – 3) or Multi CSF :* It is produced by T-lymphocytes and stimulates proliferation of precursors for neutrophils, eosinophils, basophils, and mast cells.

   In addition, it also stimulates effectors responses of mature eosinophils and T-lymphocytes. It can activate many cells other than IL-1.

5) *Gametocyte colony stimulating factor (G-CSF):*It is produced by monocytes, fibroblasts and endothelial cells due to the stimulation of IL-1. It stimulates proliferation of processors which are very specific – granulocytes. It also enhances effector response of these cells. Further, it can specifically stimulate:

   - neutrophil proliferation
   - their phagocyoticability
   - produces cytototic free radicals such as the super oxide components, thus activates the immune system.

6) *Macrophages – CSF (M-CSF):* It is produced by macrophages, fibroblasts and endothelial cells. They specifically stimulate precursors of macrophages. It also enhances effector response of mature macrophages.

7) *Inhibitory Factors:* Haemopoiesis may be inhibited by a decrease in the level of stimulatory growth factors. Generally there is a competition. No particular substance can actively inhibit this process. But now it is known that tissue specific locally produced inhibitor of cell proliferation are *chalones*. A granulocytic chalone is produced by mature / immature granulocyte. It inhibits DNA synthesis granulocyte precursor cells, thereby, decreasing the number of mitosis and cell differentiation. They have specific factor to inhibit haemopoesis wherein neutrophil level increases. Some other inhibitory factors are small peptides "Fe" string proteins etc. They are not cytotoxic generally.

The above regulation of haemopoesis generalizes to a statement that proliferation of stem cells and most immature committed cells is stimulated by one set of growth factors. These factors can stimulate specific factors which can act for both differentiated precursors and can promote production of one specific type of blood cell.

The discussion above focused on the formation and factors influencing the formation of granulocytes. How are agranulocytes formed? Let's get to know about this mechanism, next.

3.7.3 Development of Agranulocytes

Agranulocytes, we know, are the *monocytes* and *lymphocytes*. Agranulocytes develop in the bone marrow and thymus. They start as *stem cells*. They are primary lymphoid organs where pluripotent stem cells (cells which cannot grow into a whole organism) form lymphoblasts. They proliferate and differentiate into immature lymphocytes. The ones which mature in bone marrow are called *B-lymphocytes*. Another group which matures in the thymus is called *T-lymphocytes*. These lymphocytes travel to secondary lymphoid organs such as lymph nodes and spleen, where lymphopoiesis continues. They become unique among blood cells since their production is not limited to bone marrow alone.

With this, we end our discussion on development and regulation of leukocytes. We shall end our study of the immune system by presenting a review about the antigen-antibody relationship and the various methods of their in-vitro detection.

3.8 IN-VITRO DETECTION OF ANTIGEN-ANTIBODY INTERACTION

Any infection caused, requires a proper treatment. This can be achieved only if the correct organism is identified. Sometime organisms like viruses cannot be detected or an infection in the inaccessible part of the body can occur. During such situations it becomes necessary to depend on presence of specific antibodies in the blood as an indirect evidence of an infection. This is obtained by specific antigen-antibody interaction (Ag-Ab). They can be detected through various laboratory tests. Some common tests are as follows:

1) *Precipitation:* If Ag-Ab are present in an appropriate ratio, they form a precipitate in blood. Turbidity is developed, this can be measured optically.

2) *Agglutination:* If Ag is present on the surface of cells, or can be made to coat these surfaces of cells, these would form a clump in the presence of antibody. This process is known as *agglutination.* If no agglutination occurs that means Ab probably is absent.

3) *Immunoassay:* This helps us to measure concentration of Ag derived from infectious organism or to measure specific Ab bound to it. Here specific antibodies are tagged. This can be further quantitatively estimated which gives a clue of the concentration of Ag-Ab complexes.

4) *Immunoblotting (Western blots):* In this technique, different Ag (which may be viral proteins) are separated by electrophoresis in a solid phase. They are subjected to electrophoresis again on nitrocellulose sheets. This is known as *blotting.* The antigens are stained with radio labeled Ab. The technique is highly specific in detection of AIDS.

5) *Immunohistochemistry:* Here the Ag is tagged with a fluorescent dye (e.g. rhodamine). Inspite of a tag, the Ab binds to the Ag. In the section of a tissue, the Ag with tag bounded to Ab can be detected in a fluorescence microscope. The location of the Ag gives the clue of the site of infection.

Check Your Progress Exercise 4

1) Enumerate the stages of granulocyte development.

.....

.....

.....

2) Explain any three growth factors involved in granulopoiesis.

.....

.....

3) Give some common tests for in-vitro detection of antigen-antibody interaction.

.....

.....

3.9 LET US SUM UP

In this unit, we learnt about the immune system. We were introduced to the various components of the immune system. We learnt about the defence mechanisms – non-specific and specific – functioning in our body.

Going through the unit we realized that our body is equipped with multiple defence mechanisms. These are generally known as immune mechanisms. Immunity is of two types – innate and acquired. Innate immunity gets activated by invasion but is non-specific. Acquired immunity is the most highly evolved defence mechanism. The struggle for survival is never ending with us.

The unit also focused on the antigen - antibody interaction and how it helps in maintaining immunity. The different immunoglobulins and their functional role in the body was highlighted. Finally, we studied about different methods available to detect the presence of Ag-Ab complexes.

3.10 GLOSSARY

Agglutinins : the antibodies corresponding to the blood groups antigens.

Antibodies : protective chemicals or special cells produced by the immune system.

Antigen : a protein which is released as a specific immune response elicited on the entry of foreign matter in the body.

Antitoxins : antigens that sense the antibodies, neutralize these and make them ineffective.

Chalones : a tissue-specific locally produced inhibitor of cell proliferation.

Diapedesis : the amoeboid movement of leukocytes into intercellular spaces in case of an infection.

Endogenous Pyrogen : a protein which is released in response to toxins produced by pathogens and causes the body temperature to rise.

Fibrin : a product of an activated coagulation system.

Granulopoiesis : process of formation of neutrophils.

Hemopoiesis : formation of blood cells.

Immunology : study of body's defence mechanisms against invading pathogens.

Myeloblast : a cell committed to differentiate into a granulocyte.

Opsonins : substances in the plasma which strongly stimulate phagocytosis.

Perforin : protein secreted by killer T-cells which can puncture the invader cell membrane.

Phagosome : a vacuole in which a bacterium is enclosed on account of invagination of the cell membrane of a phagocyte.

Phagocytosis : the process of engulfing the digesting the micro-organisms infecting the body tissues by neutrophils.

Precipitins : antibodies which combine with the antigens to form precipitates that are easily ingested by phagocytes.

3.10 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) a) Immunology is the study of body's defence mechanisms against invading pathogens.

   b) Tissues and cells damaged by the invading microbes release certain chemicals which attracts neutrophils from blood.These chemicals are referred to as chemokines.

   c) In the phagocytosis process, the cell membrane of phagocyte invaginates and encloses a bacterium in a vacuole known as phagosome.

2) A protective mechanism functioning inside the body is called the immune system. It is designed to protect us from the millions of the microbes, toxins, parasites. The components of immune system are:

   - Spleen
   - Thymus
   - Lymph system
   - Bone marrow
   - White blood cells
   - Antibodies
   - Complement system
   - Hormones

3) The defence mechanism operating in our body can be of two types:

   - Non-specific defence mechanisms which can be further subdivided into first line of defence and second line of defence.
   - Specific defence mechanism

   The non-specific defence mechanism is implemented for different types of infections. It resists infection by blocking entry of pathogens by destroying the microbes through means other than antibodies. It can be subdivided into:

   — First line of defence or external defence –

   Physical bariers: skin, mucous, membranes, eyes and internal tracts

   Chemical barriers: skin secretins, saliva, gut secretions, bile, tears, nasal secretions, cerumen vaginal bacteria

   — Second line of defence of internal defence-

   WBCs, macrophages, inflammatory reactions, fever, interferons, complement system and natural killer cells.

4) The non-specific defence mechanism is implemented for different types of infections. It resists infection in two ways, first, by blocking the entry of pathogens into the body i.e. through external mechanism and secondly by destroying the microbes, if they enter the body through means other than the antibodies.

5) a) The functions of macrophages include

   - phagocytose microorganisms and inert particles
   - secrete IL-1, TNF, G-CSF and M-CSF

- process and present the antigen to immuno competent cells, and
- destroy old RBCs and initiates catabolism of Hb.

b) The lymphocytes handle most of the bacterial and viral infection. Lymphocytes are directly involved with specific acquired immunity. T-Lymphocytes for cell mediated and B-Lymphocytes for humoral immunity.

c) They cause lysing (bursting) of cells and signal to phagocytes that a cell needs to be removed.

Check Your Progress Exercise 2

1) The important characteristic of specific defence mechanism is that the lymphocytes have an ability to recognize body's own cells and macromolecules (self) from those which are foreign invaders (non-self). It tolerates the self but destroys the non-self.

2) MHC or Major Histocompatability complex is a set of genes that code for cell surface glycoproteins. Its two types are class I MHC molecules, which are found on the surface of almost every cell of the body and Class II MHC molecules which are confined to a few specialized cell types such as macrophages, B-Cells and activated T-cells.

3) The foreign matter that enters the body and elicits a specific immune response by lymphocytes is referred to as immunogen. It stimulates the immune system to produce protective chemicals – antibodies. It is classified as follows:

- Immunoglobulin A (IgA) – present in secretions, protects mucosal surfaces. It is found in tears, saliva, gastrointestinal secretion, respiratory, genitourinary tract. Secreted in combination with peptide – forms secretory piece is protected from proteolytic digestion.
- Immunoglobulin D (IgD) – present on surface of B lymphocytes, along with IgM plays a role in Ag recognition.
- Immunoglobulin E (IgE) – protects against organisms which escaped IgA, major deference against helminthes, mediates allergy. On coming in contact with specific Ag, it releases mast cell granules. These granules contain a chemical which leads to an inflammatory reaction and also chemotactic migration of granulocytes.
- Immunoglobulin G (IgG) – major defence against bacteria and their toxins. It is the major Ig – 80% in the body, can cross the capillary wall very easily. It can cross placenta, secreted into milk, thus transfers immunity from mother to child.
- Immunoglobulin M (IgM) – protect against bacteria, gives an early immune response, present on surface of lymphocytes. It stays confined to blood stream and is involved in Ag recognition.

4) Any three of the following:

Antigens (immunogens)	Antibodies (immunoglobulins)
1) It is a foreign material, elicits antibody formation.	1) It is a molecule synthesized by the organism to combat foreign material.
2) It is a protein or polysaccharide molecule.	2) Each antibody is a immunoglobulin.
3) It may occur on the surface of a microbe or as a free molecule.	3) It occurs on the surface of a plasma cell and also in the body fluids.
4) It binds to macrophages to reach a helper T-cell to initiate immune response.	4) It directly joins an antigen to destroy the latter.

Check Your Progress Exercise 3

1) Innate immunity is the resistance which is developed and improved in our body after the entry of pathogens.

2) Phagocytosis involves the ingestion of particulate material including whole pathogenic microorganisms.

   In this process, the organism is entrapped into vacuoles known as phagosome. The cytoplasmic granules organelles or lysosomes fuse with the phagosome releasing powerful microbicidal substance. These substances are divided into two:

   a) those which are dependent on O_2 mechanism, and

   b) those which are O_2 independent mechanisms

3) Complement system handles microbial invasion by 3 mechanisms:

   - Some components of a complement system coat microorganisms which can be easily phagocytosed.
   - Some component of the system stimulates the lethal mechanisms of phagocytes released earlier. They also release histamine.
   - Leads to the formation of membrane-attack complex which stabs a hole into the microbial cell wall leading to the entry of water and sodium ions into microbial cells.

4) a) AMIS defends the body against free viruses, bacteria with polysaccharide capsules and toxins that enter the body fluids.

   b) CMIS defends the body against viruses and bacteria which have entered the host cells, and also against protozoa, fungi and parasitic worms. It reacts with foreign tissue transplants and cancerous cells.

5) The role of macrophages in the immune system are as follows:

   - Response to infection
   - Engulfs microbes and displays the antigen to alert the lymphocytes
   - Marks the macrophage as self, which moves to the nearest lymph node and meets lymphocytes which further initiates an immune reaction.
   - Alongwith B-cells and T-cells, it produces immune responses.
   - Acts as a memory or primed cell.

Check Your Progress Exercise 4

1) The stages of granulocyte development are:

   - Myeloblast: It is the first identifiable cell of the granulocyte series
   - Promycelocyte: Division and maturation of myeloblast yields promycelocyte (myelocyte A). This stage has primary granules in cytoplasm.
   - Myelocytes (myelocyte B): In this stage, the nucleus is flattened on one side, primary and secondary granules in the cytoplasm is seen.
   - Metamyelocyte (myelocyte C): Metamyelocytes no longer divide or produce granules (capacity for mitosis lost). Myelocytes are the last cells to undergo cell division.
   - Band cell (juvenile granulocyte): Band cells are later stages of metamyelocyte. By this stage the nucleus is deeply indented (sausage shaped) so that it resembles a hair band. The cytoplasm is filled with specific granules.
   - Mature granulocyte : develops completely with a lobed nucleus.

2)
- Interleukin-(IL-I): It is produced by macrophages under the stimulation of lipopolysaccharides and acts on relatively early progenitor cells to stimulate their proliferation. It also enhances the effector function of all types of leukocytes.
- Tumor Necrosis Factor: It has inhibitory effects on some of the tumour cells. IT is produced by macrophages and stimulates granulopoietic activity by involving a pathway through granulocyte macrophages colony stimulating factors (GM-CSF).
- GM-CSF: stimulates the proliferation for the precursors of eosinophily megakaryocytes and erythrocytes. It stimulates the proliferation of committed cells to form granulocytes and macrophages. It acts on the mature neutrophils, eosinophils and macrophages to enhance their effect or responses.

3) Precipitation, Agglutination, Immunoassay, Immunoblotting and Immuno-histochemistry are the tests for in-vitro detection of antigen-antibody interaction.