Immune Response:-

The defensive reaction of the immune system of an organism to pathogens or other foreign substances is called **immune response** . The immune response can be antibody- mediated (humoral), cell- mediated (cellular), or both.

There are two main sites where pathogens may reside in an infected host—extracellularly in tissue spaces or intracellularly within a host cell; the immune system has different ways of dealing with pathogens at these sites.

Humoral immunity acts mainly against extracellular pathogens,while cell-mediated immunity (CMI) acts against intracellular pathogens.

Factors causing immune response:

The immune response is caused by the following factors:

Bacteria , bacterial toxins , viruses, fungi, protozoa, worms, vaccines, tumour cells, etc.

Humoral Immunity

Humoral immunity is based on the action of antibodies and complement. It is directed primarily against:

■ Extracellular bacteria, in particular exotoxin-producing b acteria, such as *Corynebacterium diphtheriae*, *Clostridium tetani*, etc.

■ Bacteria whose virulence is due to polysaccharide capsules

(e.g., *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus*

pneumoniae, etc.), and

■ Certain viruses that cause infection through respiratory or intestinal tract.

The humoral immunity also participates in the pathogenesis of hypersensitivity reactions and certain autoimmune diseases.

Humoral immune response is of two types: primary and secondary.

Primary Response

During the primary response, when an individual encounters an antigen for the first time, antibody response to that antigen is detectable in the serum after a longer lag period than occurs in the secondary response. The serum antibody concentration continues to rise for several weeks and then declines; it may drop to very low levels. During this primary response, a small clone of B cells and plasma cells specific for the antigen are formed.

The lag period is typically of 7–10 days duration but can be longer, even for weeks, depending on the nature of the antigen.

For example, the lag phase may be as long as 2–3 weeks with some antigens, such as diphtheria toxoid, while it may be a short as a few hours with pneumococcal polysaccharide. The lag period also depends on dose of the antigen and the route of administration whether oral or parenteral.

IgM is the first antibody to be formed, followed by IgG, IgA, or both. IgM levels tend to decline sooner as compared to IgG levels.

Secondary Response

The antibody response is typically more rapid in the secondary response, due to second encounter with the same antigen, or a closely related “cross-reacting” antigen, months or years after the primary response. The lag period is typically very short (only 3–5 days). The level of antibody is also much higher than that during the primary response. These changes in secondary response are attributed to the persistence of antigen-specific “memory cells” following the first contact with the antigen. These memory cells proliferate in large numbers to produce large clones of specific B cells and plasma cells that mediate the secondary response.

In the secondary response:

■ The amount of IgM produced is qualitatively similar to that produced after the first contact with the antigen; however, much more IgG is produced and the level of IgG tends to persist much longer than in the primary response.

■ Furthermore, such antibody tends to bind antigen more firmly (i.e., to have higher affinity) and thus to dissociate less easily. Improved antibody binding is due to mutations that occur in the DNA that encodes the antigen-binding site.

This process is called ***somatic hypermutation***.

Fate of Antigen in Tissues

Route of administration of antigen affects the site of localization of these antigens in the body. For example, most of the antigens introduced subcutaneously are localized mainly in the draining lymph nodes and only a small amount is there in the spleen. On the other hand, most of the antigens introduced intravenously are localized in the spleen, liver, bone marrow, kidney, and lungs but not in lymph nodes. Approximately, three-fourths of these antigens are broken down by reticuloepithelial cells and are excreted out in the urine.

Production of Antibodies

Synthesis and production of antibodies typically is dependent on complex interaction of three cells: (*a*) macrophages, (*b*) helper T cells, and (*c*) B cells.

Antigens are presented to immunocompetent cells by antigen presenting cells (APCs), such as macrophages and dendritic cells. Processing by macrophages appears to be a prerequisite for formation of antibodies against many T-cell-dependent antigens, such as proteins and erythrocytes. However, antibody production does not require macrophage participation in case of T-cell-independent antigens. Both the macrophages and dendritic cells present the antigen either native or processed at the cell surface. Macrophages play a key role by modulating the optimum dose of antigen presented to lymphocytes to induce the immune responses.

After processing of antigens by a macrophage, fragments of antigen appear on surfaces of macrophages in association with class II MHC proteins. The antigen-class II MHC protein complex binds to specific receptors present on the surface of helper T cells. Subsequently, these helper T cells produce cytokines that activate B cells, producing antibodies that are specific for that antigen. The activated cytokines are interleukin-2 (T-cell growth factor), interleukin-4 (B-cell growth factor), and interleukin- 5 (B-cell differentiation factor). The activated B cells undergo clonal proliferation and differentiate to form plasma cells, which then produce specific immunoglobulins (antibodies).

Major host defense functions of antibodies include neutralization of toxins and viruses and opsonization (coating) of the pathogen, which aids its uptake by phagocytic cells.

Although helper T cells play a key role in the formation of antibodies, certain substances (e.g., polysaccharides) can activate B cells directly without the help of T cells. Such substances are called T-cell-independent antigens. These antigens, however, induce only the production of IgM antibodies but not other antibodies by B cells. This is because B cells require interleukins 4 and 5 to switch classes to produce IgG, IgA, and IgE.

These interleukins 4 and 5 are produced by T helper cells only.

B cells perform two important functions: First, they recognize antigens with their surface IgM that acts as an antigen receptor;second, they present epitopes to helper T cells in association with class II MHC proteins. IgM antigen receptor on the B cells recognizes foreign proteins as well as lipids, carbohydrates, DNA, RNA, etc. On the other hand, class II MHC proteins present protein fragments to the helper T cells. The IgM antigen receptor binds with this wide variety of molecules that stimulate B cells to produce antibodies against all the molecules possible.

‣ Theories of antibody formation

There are two sets of theories of antibody formation. These are instructive theory and selective theories.

Instructive theory

Instructive theory suggests that an immunocompetent cell is capable of synthesizing antibodies of all specificity. The antigen directs the immunocompetent cell to produce complementary antibodies. Two instructive theories are postulated as follows:

Direct template theory: This theory was first postulated by Breinl and Haurowitz (1930). They suggested that a particular antigen or antigenic determinants would serve as a template against which antibodies would fold. The antibody molecule would thereby assume a configuration complementary to antigen template.

Indirect template theory: This theory was first postulated by Burnet and Fenner (1949). They suggested that the entry of antigenic determinants into the antibody-producing cells induced a heritable change in these cells. A genocopy of the antigenic determinant was incorporated in genome and transmitted to the progeny cells. However, this theory that tried to explain specificity and secondary responses is no longer accepted.

Selective theories

Three selective theories were postulated as follows:

Side chain theory: This theory was proposed by Ehrlich (1898). According to this theory, immunocompetent cells have surface receptors that are capable of reacting with antigens, which have complementary side chains. When antigens are introduced into host, they combine with those cell receptors that have a complementary fit. This inactivates the receptors. There is an overproduction of the same type of receptors that circulate as antibodies, as a compensatory mechanism.

Natural selection theory: This theory was proposed by Jerne (1955). According to this theory, during the embryonic life, millions of globulin molecules were formed against all possible range of antigens. The antigen when introduced to the host combines selectively with the globulin molecule that has the nearest complementary fit. The globulin with the combined antigen stimulates antibody-forming cells to produce the same type of antibody.

Clonal selection theory: Burnet (1957) suggested that immunological

specificity existed in the cell but not in the serum and proposed the most acceptable clonal selection theory. According to this theory, a large number of clones of immunological competent cells bearing specific antibody patterns are produced during fetal development by a process of somatic mutations of immunological competent cells (ICCs) against all possible antigens.

This theory suggests that an individual ICC expresses membrane

receptors that are specific for a distinct antigen. This

unique receptor specificity is determined before the lymphocyte

is exposed to antigen. Binding of antigen to its specific

receptor activates the cell and leads to cellular proliferation to

form clones, synthesizing the antibody.

The clonal selection theory is most widely accepted and provides

a framework for better understanding of the specificity,

immunological memory, and the property of recognition of

self and nonself by adoptive immunity.

- **Factors affecting production of antibodies**

Many factors affect the production of antibodies. These factors

are discussed below:

Genetic factors

Genetic factors influence the response of the host to antigen.

Persons responding to antigens are called responders, while

persons not responding are called nonresponders. These differences are controlled genetically and are being controlled by immune response (Ir) gene located in the short arm of the 6th chromosome.

Age

The embryo and the infant, at birth, are not fully immunologically competent. Full competence is achieved by about the age of 5–7 years for IgG and 10–15 years for IgA by the development of lymphoid organs.

Nutritional status

Malnutrition affects both the humoral and cell-mediated immunities. Deficiencies of amino acid and vitamins have shown to decrease the production of antibodies.

Route of antigen

Induction of immune response in a host depends on the route of administration of the antigen. Parenteral administration of the antigen induces a better immune response than the oral or nasal routes.

Dose of antigen

A minimum critical dose of antigen is essential to elicit an optimum immunological response. A very high or small dose fails to stimulate the immune system. This phenomenon is referred to as ***immunological paralysis***.

Multiple antigens

Antibody responses vary when two or more antigens are administered simultaneously. Antibody responses to one or more of them may be diminished due to antigenic competition, or enhanced as seen after vaccination with triple vaccine (diphtheria, pertussis, and tetanus), or may be similar. Hence, the nature and relative proportions of different antigens should be carefully adjusted for optimal effect.

Adjuvants

Adjuvants are the substances that enhance the immunogenicity of an antigen. The adjuvants delay the release of an antigen from the site of injection and prolong the antigenic stimulus. The substances that are used as adjuvants include:

(a) Freund's incomplete adjuvant (protein antigen incorporated in water phase of water in oil emulsion);

(b) Freund's complete adjuvant (incomplete adjuvant along with suspension of killed tubercle bacilli);

(c) Aluminum salts both phosphate and hydroxide; and

(d) Others, such as silica particles, beryllium sulfate, endotoxin, etc. ***Immunosuppressive agents***

Immunosuppressive agents are those that suppress immune response. They are used in transplantation surgery and in situations that require suppression of host immunity. The agents are as follows:

X-irradiation: Sublethal dose of irradiation is toxic to replicating cells and is used to suppress antibody formation. Antibody production ceases after 24 hours of receiving irradiation.

Radiometric drugs: These include alkylating agents (such as cyclophosphamide, nitrogen mustard, etc.), which suppress antibody production. Cyclophosphamide, given for 3 days, completely suppresses the antibody response. It selectively prevents replication of B cells.

Corticosteroids: Corticosteroids are anti-inflammatory drugs that diminish the responsiveness of both B and T cells. They alter maturation of activated cells by suppressing the production of interleukins. They suppress delayed hypersensitivity, but in therapeutic doses for a short period, they have little effect on the production of antibodies.

Antimetabolites: These include folic acid antagonists (such as methotrexate); analogs of purine (6-mercaptopurine and azathioprine); and analogs of cytosine (cytosine arabinose); and uracil (5-fluorouracil). These substances inhibit DNA and RNA synthesis, thereby inhibiting the cell division and differentiation, which is essential for cellular and humoral immune responses. These are usually used for prevention of graft rejection.

Antilymphocyte serum: Antilymphocyte serum (ALS) is a heterogeneous antiserum raised against T lymphocytes. The ALS acts mainly against circulating lymphocytes but not

against lymphocytes in lymphoid organs. It is mainly used to prevent graft rejection in transplantation surgery.

Monoclonal Antibodies

Antibodies that arise from a single clone of cells (e.g., myeloma) are homogenous and are called monoclonal antibodies. For example, in multiple myeloma, antibodies are produced by a single clone of plasma cells against a single antigenic determinant, and hence antibodies are monoclonal. The monoclonal antibodies differ from polyclonal antibodies, which are heterologous and are formed by several different clones of plasma cells in response to antigen.

- **Method of production of monoclonal antibodies**

Kohler and Milstein (1975) were the first to describe a method for production of monoclonal antibodies against a desired antigen for which they were awarded Nobel Prize in 1984. Monoclonal antibodies are produced by fusion of myeloma cells with antibody-producing cells, resulting in production of hybridomas. Such hybridomas produce virtually unlimited quantities of antibodies that are useful in research and diagnostics. In this procedure, mouse splenic lymphocytes are first fused with mouse myeloma cells to produce hybrid cells or hybridomas. Myeloma cell provides the hybrid cell immortality, whereas splenic plasma cell provides the antibody- producing capacity. These hybridomas can be maintained indefinitely

in culture and continue to produce monoclonal antibodies.

Hybridoma cells are prepared in following ways (Fig. 17-2):

■ First, an animal (e.g., mouse) is immunized with the a ntigen of interest.

■ Spleen cells (lymphocytes) are then fused with mouse myeloma cells and grown in culture, which are deficient in the enzyme hypoxanthine phosphoribosyl transferase (HPRT).

■ Fusion of the cells is facilitated by addition of certain chemicals, such as polyethylene glycol. The fused cells are grown in a special culture medium (HAT medium) that supports the growth of the fused hybrid cells but not of the parent cells.

■ Finally, the resulting clones of cells are screened for the production of antibody to the antigen of interest.

■ These clones are then selected for continuous cultivation.

The hybridomas can be maintained indefinitely and will continue to produce monoclonal antibodies.

Human monoclonal antibodies, such as chimeric antibodies, have been produced with modification of the original technique for therapeutic use, since mouse monoclonal antibodies are not suitable. The chimeric antibodies consisting of human constant regions and mouse variable regions are being prepared for use in treatment of leukemia. Chimeric antibodies are also used to kill tumor cells either by delivering toxins, such as diphtheria to tumor cells, or by killing tumor cells through

complement-mediated cytotoxicity.

Key Points

■ Monoclonal antibodies are now used widely in research and diagnostics.

■ They are used in various clinical situations, such as treatment of cancer and autoimmune diseases.

■ They are used in inducing immune suppression in transplant surgery, and in the prevention of infectious diseases.

Function of Antibodies

Antibodies are the primary defense against infectious patho gens or their products. Antibodies can be induced in the host actively by use of vaccines or acquired passively for conferring immediate protection against the pathogen. For example, hyperimmunized sera containing readymade antitoxins against toxins of tetanus, botulism, or diphtheria are given to neutralize the actions of these toxins immediately in the body. Also, hyperimmune sera containing high titer of specific antibodies are given to inhibit attachment and replication of rabies and hepatitis A and B viruses early during the period of incubation. The functions of the antibodies can be summarized as follows:

Neutralization: By binding to the pathogen or foreign substance, antibodies can block the binding of the pathogen with their targets. For example, antibodies to bacterial toxins can prevent the binding of the toxin to host cells, thereby rendering

the toxin ineffective. Similarly, antibody binding to a virus or bacterial pathogen can block the attachment of the

pathogen to its target cell, thereby preventing infection or

colonization.

Opsonization: Antibody binding to a pathogen or foreign

substance can opsonize the material and facilitate its uptake

and destruction by phagocytic cells. The Fc region of other

antibody interacts with Fc receptors on phagocytic cells,

rendering the pathogen more readily phagocytosed.

Complement activation: Activation of the complement

cascade by antibody can result in lysis of certain bacteria and

viruses. In addition, some components of the complement

cascade (e.g., C3b) opsonize pathogens and facilitate their

uptake via complement receptors on phagocytic cells.

Tests for Detection of Humoral Immunity

The measurement of IgG, IgM, and IgA in the patient's serum

is the primary method for detection of humoral immunity.

Radial immunodiffusion and immunoelectrophoresis

are the methods frequently employed for measurement of

antibodies.

Cell-Mediated Immunity

Cell-mediated immunity (CMI) is a specific type of acquired

immune response not mediated by antibodies but by sensitized

T cells. This form of immunity is transferred from donor to

recipient, not with antisera but with intact lymphocytes; hence

it is called cell-mediated immune reaction. CMI performs the following immunological functions:

1. It confers immunity in diseases caused by obligate intracellular bacteria (*Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Brucella*, etc.), viruses (small pox, measles, mumps, etc.), fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, etc.), and parasites (*Toxoplasma gondii*, *Leishmania donovani*, etc.).

2. It participates in immunological surveillance and immunity against cancer.

3. It plays an important role in pathogenesis of delayed hypersensitivity reactions and in pathogenesis of certain autoimmune diseases, such as autoimmune thyroiditis, encephalitis, etc.

Induction of CMI

Antigen processing and presentation are the means by which antigens become associated with self-MHC molecules for presentation to T cells with appropriate receptors. Proteins from exogenous antigens, such as bacteria, are internalized via endocytic vesicles into APCs, such as macrophages. Then, they are exposed to cellular proteases in intracellular vesicles. Peptides, approximately 10–30 amino acid residues in length, are generated in endosomal vesicles. The endosomal vesicles can then fuse with exocytic vesicles containing class II MHC molecules.

Induction of CMI involves sequence of events, which is explained below.

- **Presentation of foreign antigen by APCs to T lymphocytes**

Induction of CMI begins with presentation of foreign antigen by APCs to T lymphocytes. T-cell receptors (TCRs), which are antigen recognition receptors, are present on T lymphocytes, and recognize foreign antigen and a self-MHC molecule on the surface of APCs. Subsequently, the sensitized T lymphocytes undergo blast transformation, clonal proliferation, and differentiation into memory cells and effector cells, such as Th, Tc, Td, and Ts. Finally, the lymphokines, which are biologically active products responsible for various manifestations of CMI, are released by the activated lymphocytes.

- **Recognition of antigen by T cells**

T cells recognize antigens only when presented with MHC molecules. The combination of foreign antigen and class I MHC molecule is recognized by CD8_ cells. These CD8_ cells after recognition differentiate into Tc and Ts lymphocytes. On the other hand, CD4_ cells recognize the combination of antigen and class II MHC antigen, after which they are differentiated into Th and Td cells. The class II MHC molecules are synthesized, as for other membrane glycoprotein, in the rough endoplasmic reticulum and then proceed out through the Golgi

apparatus. A third polypeptide, the invariant chain (Ii), protects the binding site of the class II dimer until the lowered pH of the compartment created after fusion with an endosomal vesicle causes a dissociation of the Ii chain. The MHC class II peptide antigen complex is then transported to the cell surface for display and recognition by a TCR of a CD4 T cell.

The lymphocyte recognizes antigen and class I MHC molecule and gets attached to the target cells. Endogenous antigens such as cytosolic viral proteins synthesized in an infected cell are processed for presentation by class I MHC molecule. In brief, cytosolic proteins are broken down by a peptidase complex known as the proteasome. The cytosolic peptides gain access to nascent MHC class I molecules in the rough endoplasmic reticulum via peptide transporter systems (transporters associated with antigen processing; TAPs). The TAP genes are also encoded in the MHC.

The binding groove of the class I molecule is more constrained than that of the class II molecule; for that reason, shorter peptides are found in class I than in class II MHC molecules.

- **Release of cytokines by Tc lymphocytes**

This stimulates Tc lymphocytes to release cytokines, resulting in the lysis of the target cells. The T cells then detach from the target cells and attach with other target cells, and the same process

is repeated. Interferon-gamma synthesized and secreted by Tc lymphocytes possibly also contributes for macrophage activation in some way.

Cytokines

Cytokines are biologically active substances secreted by monocytes, lymphocytes, and other cells and are actively involved in innate immunity, adoptive immunity, and inflammation. They actively take part in a wide range of biological activities varying from chemotaxis to activation of specific cells.

Cytokines were initially identified as products of immune cells that act as mediators and regulators of immune processes. Many cytokines are now known to be produced by cells other than immune cells, and they can have effects on nonimmune cells as well. Cytokines are currently being used clinically as biological response modifiers for the treatment of various disorders.

Cytokines are not typically stored as preformed proteins. Rather their synthesis is initiated by gene transcription and their mRNAs are short-lived. They are produced as needed in immune responses. Many individual cytokines are produced by many cell types and act on many cell types (i.e., they are pleiotropic), and in many cases cytokines have similar actions (i.e., they are redundant). Redundancy is due to the nature of the cytokine receptors.

- **Categories of cytokines**

Cytokines can be grouped into different categories based on their

functions or their source, but it is noteworthy that because they can be produced by many different cells and act on many different cells (Table 17-1), any attempt to categorize them will be subject to limitations. Cytokines may be categorized as follows:

1. Mediators affecting lymphocytes.

2. Mediators affecting macrophages and monocytes.

3. Mediators affecting polymorphonuclear leukocytes.

4. Mediators affecting stem cells.

5. Mediators produced by macrophages that affect other cells.

Mediators affecting lymphocytes

Interleukin-1 (IL-1): It is a protein produced mainly by activated macrophages and monocytes. Its production is stimulated by antigens, toxins, and inflammatory processes but inhibited by cyclosporine and corticosteroids. It is an important interleukin, which mediates a wide range of metabolic, physiological, inflammatory, and hematological activities. It has many important functions, which are given below:

■ It activates a wide range of target cells including T and B lymphocytes, neutrophils, epithelial cells, and fibroblasts to proliferate, differentiate, or synthesize specific products. For example, it stimulates helper T cells to produce IL-2, and stimulates B cells to proliferate and synthesize antibodies, etc.

■ It acts on the hypothalamus to cause fever associated with infections and other inflammatory reactions.

Interleukin-2 (IL-2): IL-2 is a protein produced mainly by helper T cells. It is a major T-cell growth factor. It stimulates both helper and cytotoxic T cells to grow. It also promotes the growth of B cells and can activate natural killer (NK) cells and monocytes.

IL-2 acts on T cells in an autocrine fashion. Activation of T cells results in expression of IL-2R and the production of IL-2. The IL-2 binds to the IL-R and promotes cell division. When the T cells are no longer being stimulated by antigen, the IL-2R will eventually decay and the proliferative phase ends.

Interleukin-4 (IL-4): It is a protein produced mainly by helper T cells and macrophages. It stimulates the development of Th-2 cells, the subset of helper T cells that produces IL-4 and IL-5 and enhances humoral immunity by producing antibodies.

It is also required for class (isotype) switching from one class of antibodies to another within antibody-producing cells.

Interleukin-5 (IL-5): It is a protein produced by helper T cells. It promotes the growth and differentiation of B cells and eosinophils. It enhances the synthesis of IgA and also stimulates the production and activation of eosinophils.

Interleukin-6 (IL-6): It is a protein produced by helper T cells and macrophages. It stimulates the production of acute phase proteins by the liver. It also acts on the hypothalamus to cause fever.

Other interleukins: IL-10, IL-12, and IL-13 are the other

interleukins that affect lymphocytes. IL-10 is produced by activated macrophages and Th-2 cells. It is predominantly an inhibitory cytokine. It inhibits production of type I interferon. It inhibits production of interferon-gamma by Th-1 cells, which shifts immune responses toward a Th-2 type. It also inhibits cytokine production by activated macrophages and the expression of class II MHC and costimulatory molecules on macrophages, resulting in a depression of immune responses.

IL-12 is produced by activated macrophages and dendritic cells. It stimulates the production of interferon-gamma and induces the differentiation of Th cells to become Th-1 cells. In addition, it enhances the cytolytic functions of Tc and NK cells.

IL-13 is produced by Th-2 cells. It is associated with pathogenesis of allergic airway disease (asthma). It is involved in the occurrence of hyper-responsiveness seen in asthma.

Transforming growth factor-beta (TGF-_): It is produced by T cells and many other cell types. It is primarily an inhibitory cytokine. It inhibits the proliferation of T cells and the activation of macrophages. It also acts on polymorphonuclear leukocytes and endothelial cells to block the effects of proinflammatory cytokines. In essence, it suppresses the immune response when it is not required after an infection, and thereby it promotes the healing process.

Mediators affecting macrophages and monocytes

Chemokines are a subtype of cytokines of low molecular weight and with a characteristic structural pattern. More than 50 chemokines varying in size from 68 to 120 amino acids have been identified. The alpha-chemokines, such as IL-8 are produced by activated mononuclear cells, which attract neutrophils. The beta-chemokines, such as RANTES (regulated upon activation, normal T-cell expressed and secreted) and MCAF (monocyte chemotactic and activating factor), are produced by activated T cells and attract macrophages and monocytes.

Chemokines are produced by endothelial cells, resident macrophages and various cells present at the site of infection:

■ They attract either macrophages or neutrophils to the site of infection, hence are involved in chemical-induced migration of leukocytes—a process called chemotaxis. Specific receptors for chemokines are present on the surface of monocytes and neutrophils.

■ They also facilitate migration of white cells into the tissue to reach the infected area. They do so by activating integrins on the surface of neutrophils and macrophages that bind to the intercellular adhesion molecule (ICAM) proteins on the surface of the endothelium.