Immunotechnology, is an important arm of biotechnology, constituting the industrial scale application of immunological procedures to produce vaccines, for mass immunisation to prevent prevalent diseases and/or producing immunological therapeutic agents to cure the afflicted. Production of protein vaccines has been in large-scale use for a long time and the current trend is to develop the more specific DNA vaccines.

IMMUNOLOGICAL DEFENSIVE RESPONSE

When an infecting organism gains entry into the mammalian system for the first time, the immune system of the mammal reacts, mainly in response to the proteins of the invading organism, generally called **antigens**, by producing a special class of proteins called **antibodies**. This first encounter is very important because the system gains a '**memory**' template of the three dimensional structural configuration of the **epitopes** of the antigens. This memory may last for a few hours or days (common colds), a few months (cholera, tetanus) or the lifetime (smallpox) of the individual.

Antigens, the molecules that trigger antibody response, fall into three categories, called **antigens, immunogens or haptens**, which are sourced in the protein coats of viruses, cell walls of bacteria, secretions of pathogenic organisms, proteins from plant or animal parts that were injected, ingested or inhaled, or introduced into the system by some means.

When the same antigen gains entry a second and the subsequent times, the immune system recognises the foreign entity and produces antibodies specific to the antigen, basing on the memory, developed on the first encounter. This is **immune response**, which results in a) the production of antibodies, b) antibody-bearing cells or c) cell mediated hypersensitivity reaction (**allergy**).

Each re-entry of the exogenous entities triggers enhanced production of the corresponding antibodies (**booster reaction**).

The antibodies recognise their antigens, bind with them, and neutralise them, before they can cause harm to the individual or

cause the disease specific to them. This is **immunological defence**.

The antigen-antibody recognition is a highly specific phenomenon of **biorecognition** at the molecular level. Such a high degree of specificity is also found between the enzymes and their substrates, and lectins and their specific carbohydrates.

Immune response is a selective reaction of a mammalian body to substances that are foreign (**exogenous**) to it or those that the immune system identifies as foreign. The three important aspects are:

a) **Memory**: the primary response of the formation of the memory template at the first encounter,

b) **Distinction between self- and non-self**: distinction between the organism's endogenous proteins and those that are foreign (exogenous), and

c) **Specificity**: the secondary response of production of antibodies very specific to each foreign agent.

Other animals and even plants have defence mechanisms against diseases, but they are not identical to the mammalian immunological defences. For this reason, immunology is a mammal centred area of biology. Mice, rats, rabbits, dogs, horses and monkeys have been instrumental in the advancement of the field of immunology. Mammalian systems produce a highly specific antibody to each of the pathogens and even their different strains. The antibody recognises the antigen and binds with it forming an **antigen-antibody complex**, and neutralise the pathogen's potential to cause the disease. The antigen-antibody complex is scavenged by the body, mainly through the lymphatic system and often seen as **pus**, in dermal eruptions in the form of pustules. Pus, the fluid from pustules, contains serum, antigen-antibody complexes, expended white blood cells, dead and live pathogens, and debris of tissue.

Serum: The liquid part of blood, without the cells and the coagulating factors, but containing antigens and antibodies; it is the storehouse and means of transport of immunological components.

Lymphatic system: is parallel to the blood conducting system and is constituted of the lymph, lymphocytes, lymph vessels, lymph nodes and lymph glands. The lymph is a watery, transparent or slightly yellow, fluid conducted through the lymph vessels. Lymph contains only one kind of cells, the lymphocytes, unlike blood that contains several different kinds of cells including lymphocytes. The blood and the lymphatic systems come into a sort of confluence in the lymph nodes and the tissues. The lymph cells that secrete lymph are aggregated into lymphatic tissue in the form of glands or occur in small groups of cells in different parts of the body.

Haemopoiesis (haematopoiesis): The formation of the cellular components of blood, originating very early in the yolk sac of the egg. In the foetus the liver performs this function and later the bone marrow takes it over and continues throughout life. Haemopoiesis originates with the **stem cells** in the bone marrow.

Stem cells: Stem cells are the basic cell type with potential to develop different cell components of the mammalian body system. Stem cells from the foetus are totipotent and can form almost any organ. The stem cells from the bone marrow are multipotent and form the cellular elements of both the blood and lymphatic systems in addition to the formation of new stem cells. The stem cells migrate to the **thymus** and differentiate into **T-lymphocytes**, in the microenvironment of the thymus.

Erythrocytes: The enucleate discoid cells in the blood with membrane bound haemoglobin, to which oxygen binds reversibly (red blood corpuscles). Erythrocytes bear antigens on their surfaces that are responsible for the human blood groups in the ABO system. Blood group antigens also circulate in the blood and hence are responsible for the rejection of transplanted tissues/organs.

Leucocytes: All the different kinds of cells in the blood (the so-called white blood corpuscles), including the **lymphocytes**, but with the exception of the erythrocytes.

Lymphatic tissue/cells: As explained above, the lymphatic system is also composed of cells and tissue.

Lymphocytes: The cells of the lymphatic system (lymphoid group) which play the main role in immune responses.

Role of lymphocytes: The lymphocytes have an important role to play both in humoural and cell-mediated immunity. The lymphocytes re-circulate in the blood, lymph nodes, spleen and other tissues and back to blood by the lymphatic vessels.

When rats were depleted of lymphocytes, their ability to show the primary response to antigens or to reject skin grafts was very much impaired. Immunological responses were restored in these rats when lymphocytes from another rat were injected. This adequately shows the importance of lymphocytes in mounting immune response.

Kinds of lymphocytes:

a) **T-lymphocytes**, of four subclasses and the **B-lymphocytes** (T-cells and B-cells) basing on origin, and

b) Three kinds of lymphocytes, large, medium and small, basing on size.

When lymphocytes are incubated at 37°C for 24 h, the large and medium lymphocytes are killed. The remaining small lymphocytes can restore immune responses when injected into rats that were previously drained of lymphocytes.

The small lymphocytes are necessary for the primary response to an antigen and they can become

a) Antibody synthesising cells called **plasma cells**, or
b) **Effector cells** called **lymphoblasts**.

The lymphoblasts, along with blood group antigens, are responsible for immunological tissue rejection reactions in transplantations. The small lymphocytes also carry the memory of the first contact with an antigen. Without this memory mechanism, there can be no secondary response and so no immunological defence.

Hybridoma: A synthetic cell line (such a myeloma cell and a spleen cell) that can grow in a culture indefinitely, at the same time producing antibodies.

Thymus: A gland lying behind the breastbone and extending up to the thyroid gland. The thymus is well developed in the infancy (about 40 g) and reaches its greatest size at about puberty (100 to 120 g) but is reduced by about 50 years of age (about 20 g), as it is progressively replaced by fatty tissue.

So long as it is occurring, the thymus mediates the differentiation of the T-lymphocytes, which are concerned mostly with **cell-mediated immunity**.

When the thymus was removed from mice at birth, they showed a decrease in lymphocyte count, their ability to reject tissue grafts was severely affected, their humoural antibody response was restricted and they soon died. When mice without the thymus were grown under germ free conditions, they survived showing that the ability to fight infection was impaired due to the removal of the thymus. When mice were subjected to x-rays, their lymphocytes failed to multiply. When these mice were injected with bone marrow cells, their lymphocyte count normalised but not in mice without the thymus. These studies emphasise that the bone marrow cells develop into lymphocytes and that the thymus is necessary for this process.

Children with abnormalities of the thymus suffer from immunological disorders.

Distinguishing T- and B-cells: It is very difficult to distinguish between the T- and B-lymphocytes using a light microscope or even an electron microscope but certain tests ensure this. One of the common methods used to recognise human T-cells is to mix them with the red blood corpuscles of sheep when the two kinds of cells form rosettes (formations resembling roses). The B-cells are recognised by using fluorescent dyes along with anti-immunoglobulins (**antithetic antibodies**).

Modified T- and B-cells: The populations of both the T- and B-cells are stimulated to proliferate and undergo morphological changes by antigens. The T-cells become lymphoblasts and participate in cell-mediate reactions. The B-cells become the **plasma cells** participating in the humoural antibody synthesis.

There is co-operation between the two populations of lymphocytes. The mature plasma cell actively synthesises and secretes the antibody. There are no antibodies in, or secreted by, the T-lymphocytes.

T-cell dependence of B-lymphocytes: Certain of the B-lymphocytes in mammals are dependent upon the T-lymphocytes for their function (T-cell dependent) while the others are independent of the T-lymphocytes (T-cell independent).

Monocytes, macrophages and phagocytes: Monocytes, originate from stem cells, have a single nucleus and develop into macrophages--the phagocytic cells, which engulf particulate matter, in a non-specific defence mechanism.

Mast cells: Mast cells occur in the skin and epithelial layers. They contain histamine in the form of granules bound to membranes. Explosive de-granulation results in the release of histamine, which increases the permeability of the blood vessels, causing **inflammatory reactions**. Mast cells have a key role in **allergy**.

Eosinophils: These are cells with granules in the cytoplasm (one kind of granulocytes), also known as polymorphonuclear leucocytes, stainable with the reddish biological stain eosine. The mast cells and eosinophils have an important role in allergy.

Antigen: a substance, usually a protein, that stimulates the immune system to produce a set of specific antibodies and that combines with an antibody specific to itself, at a specific binding site; differs from immunogen in that it is not involved in eliciting cellular response and in that it can complex with antibodies.

Immunogen: a substance, usually a protein, that elicits a cellular immune response, and/or antibody production; differs from antigen in that it mainly elicits cellular response but does not complex with an antibody.

Hapten: a low-molecular weight non-protein molecule which contains an antigenic determinant but which is not itself antigenic unless it complexes with an antigenic carrier, such as a protein; once an antibody is available, it can readily recognise the hapten, even

without the carrier, and bind with it. To be antigenic, the hapten must bind to an exogenous protein carrier.

Epitope: a part of a protein molecule that acts as an immunogenic/antigenic determinant, and so determines specificities; a macromolecule, such as a protein, may contain many different epitopes, each capable of stimulating the production of specific antibodies, each with a correspondingly specific binding site.

Antibodies: Globulin (roughly spherical in shape and extractable in saline solutions) glycoproteins (proteins with a carbohydrate content ranging from 3 to 13%), produced by the immune system of an organism in response to exposure to a foreign molecule and characterised by its specific binding to a site, related to an epitope of that molecule; induced response proteins.

The antibodies, like all proteins, are formed of chains of amino acids, which undergo very complex packing, giving the proteins a specific and functionally significant final shape (tertiary configuration), which determines most of the characteristics of the protein.

As globulin proteins are involved in immune reactions, antibodies are called immunoglobulins (abbreviated to Ig).

Antiserum: Blood serum containing antibodies arising out of immunisation or after an infectious disease.

Polyclonal antibodies: antibodies produced by molecules with several different antigenic determinants (epitopes) and/or several different cell populations.

Monoclonal antibodies: antibodies produced against a single antigenic determinant (epitope) and/or by a single cell population; hence are very specific.

Vaccine: An agent containing antigens/immunogens produced from killed, attenuated or lives pathogenic microorganisms, synthetic peptides, by recombinant organisms or DNA, used for stimulating the immune system of the recipient to produce specific antibodies providing active immunity and/or passive immunity.