

# **A TEXT BOOK OF IMMUNOLOGY**

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## 1. SCOPE OF IMMUNOLOGY

Immunology is a biologic science devoted to studying the development and function of the **cellular** and **humoral** components of the immune system by which the body reacts to expel, destroy, or neutralize foreign substances and organisms, including pathogens. It is concerned with the processes by which these components interact as well as the mechanisms that control their function in health and disease.

Immunology consists of three major subdivisions:

1. **Immunochemistry** is dedicated to studying the chemical nature of antigens, antibodies, and their interactions.
2. **Immunobiology** includes those aspects of immunology related to the biologic functions of the cell and tissue components of the immune system.
3. **Serology** is the study of immune reactions mediated by antibodies or immunoglobulins present in the serum. The most important serologic reactions described are agglutination, precipitation, complement fixation, and cytotoxicity. Serologic reactions have had wide applications in blood grouping, tissue typing, diagnostic microbiology, and medicine.

Immunologic techniques and reactivity have diverse application in biology and medicine.

1. **Diagnostic microbiology and clinical medicine.** Diagnostic microbiology uses a variety of immunologic methods and reactions to study the host's immune responses to microbial pathogens. It is useful in diagnosing bacterial infections such as typhoid fever and brucellosis, as well as viral, fungal, and spirochete (syphilis) infections. Immune reactions are also used to diagnose a variety of clinical disorders such as plasma cell neoplasms (i.e., multiple myeloma) and immunodeficiencies (congenital or acquired). Furthermore, antibodies are used extensively for measuring hormones and drugs in serum and urine by radioimmunoassay.
2. **Blood transfusion serology:** This specialty represents the study and characterization of red cell antigen-antibody systems (ABO, Rh) and the application of immunologic methodology for blood typing, performance of compatibility (cross-matching) tests, and diagnosis of hemolytic disorders (congenital or acquired).
3. **Tissue typing and histocompatibility testing:** Various serologic techniques are used to detect and characterize antigenic determinants coded by *histocompatibility* series. Such testing is important for donor selection, especially in kidney and bone marrow transplantation. Other diagnostic uses involve diseases influenced by genes linked to the major immunogene complex.
4. **Forensic medicine:** Immunologic tools are frequently used in forensic science laboratories to assist in solving cases of doubtful paternity (blood grouping), stain identification (e.g., blood and semen stains), milk and meat testing (e.g., to distinguish horse meat from other meats), and so on.
5. **Clinical laboratory immunology:** Immune-based therapies are being introduced at an accelerating rate and assessments of complex immune-based therapies are having an increasing impact on the responsibilities of the clinical immunology laboratory. Protein chemistry contributed electrophoresis. Immunologists contributed immunoelectrophoresis and monoclonal antibodies. Immunogeneticists and collaborating engineers brought forth flow cytometry. Many contributed to the huge advances in quantitative immunochemical methodologies. Autoimmune diseases, allergy and asthma, organ and bone marrow transplantation, lymphoid and plasma cell malignancies, and primary and secondary immune deficiencies, have all provided challenges and opportunities to advance clinical laboratory immunology.

## 2. IMMUNITY

The ability of an organism to resist disease, either through the activities of specialized blood cells or antibodies produced by them in response to natural exposure or inoculation (active immunity) or by the injection of antiserum or the transfer of antibodies from a mother to her baby via the placenta or breast milk (passive immunity).

### 2.1. RECOGNITION OF SELF AND NON SELF IMMUNITY

The key to a healthy immune system is its remarkable **ability to distinguish between the body's own cells, recognized as "self," and foreign cells, or "nonself."** The body's immune defenses normally coexist peacefully with cells that carry distinctive **"self" marker molecules**. But when immune defenders encounter **foreign cells or organisms carrying markers that say "nonself,"** they quickly launch an attack.

#### Nonself Markers

Anything that can trigger this immune response is called an **antigen**. An antigen can be a **microbe** such as a virus, or a part of a microbe such as a molecule. Tissues or cells from another person (except an identical twin) also carry nonself markers and act as foreign antigens. The body's immune defenses do not normally attack tissues that carry a self marker (Figure 1).

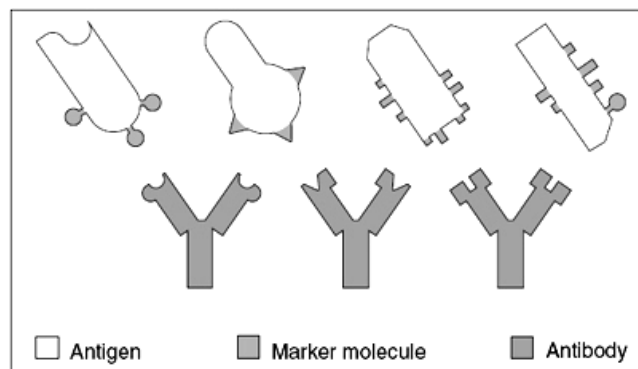


Figure 1. Indicates the Marker Molecule.

Rather, immune cells and other body cells coexist peacefully in a state known as **self-tolerance**. But when immune defenders encounter cells or organisms carrying molecules that say **"foreign,"** the immune troops move quickly to eliminate the intruders. This explains why tissue **transplants may be rejected**.

An antigen announces its **foreignness** by means of intricate and characteristic shapes called **epitopes**, which protrude from its surface. Most antigens, even the simplest microbes, carry several different kinds of epitopes on their surface; some may carry several hundred. However, some epitopes will be more effective than others at stimulating an immune response.

#### Autoimmune Diseases (self)

In abnormal situations, the immune system can mistake self for nonself and launch an attack against the body's own cells or tissues. The result is called an autoimmune disease. Some forms of diabetes, rheumatoid arthritis and systemic lupus erythematosus are autoimmune diseases.

#### Allergen

In some people, an apparently harmless substance such as ragweed **pollen or cat hair** can provoke the immune system to set off the inappropriate and harmful response known as allergy; in these cases the antigens are known as allergens.

## 2.2. TYPES OF IMMUNITY

Immunity against infectious diseases falls into two categories: (A) Innate/Native (nonspecific) Immunity and (B) Acquired/Adaptive Immunity. The Acquired immunity again subdivided into Artificial active immunity passive and active immunity).

### 2.2. 1. Innate Immunity

Innate immunity is the **resistance to infections** which an individual possesses by virtue of his **genetic and constitutional make up**. It does not depend on prior contact with microorganisms or immunization.

It may be **non specific**, when it indicates a degree of resistance to infections in general or specific, when resistance to a particular pathogen is concerned. **Innate immunity is present at birth** and changes life throughout the life of the individual. The cells and molecules of this innate system are mainly responsible for the **first stages expulsion of the microbe and may give rise to inflammation**. **Phagocytes are important cells in the innate immune system** since they ingest and kill microbes.

**Natural barriers:** Animals anatomy have variety physical (natural) and biochemical barriers (Figure 2) and substances which help to prevent infections with micro-organisms and parasites. The **skin** and **mucus-containing secretions** act as barriers and **proteolytic enzymes** (digestive enzymes which break down protein) present in body fluids have the ability to destroy some invading organisms.

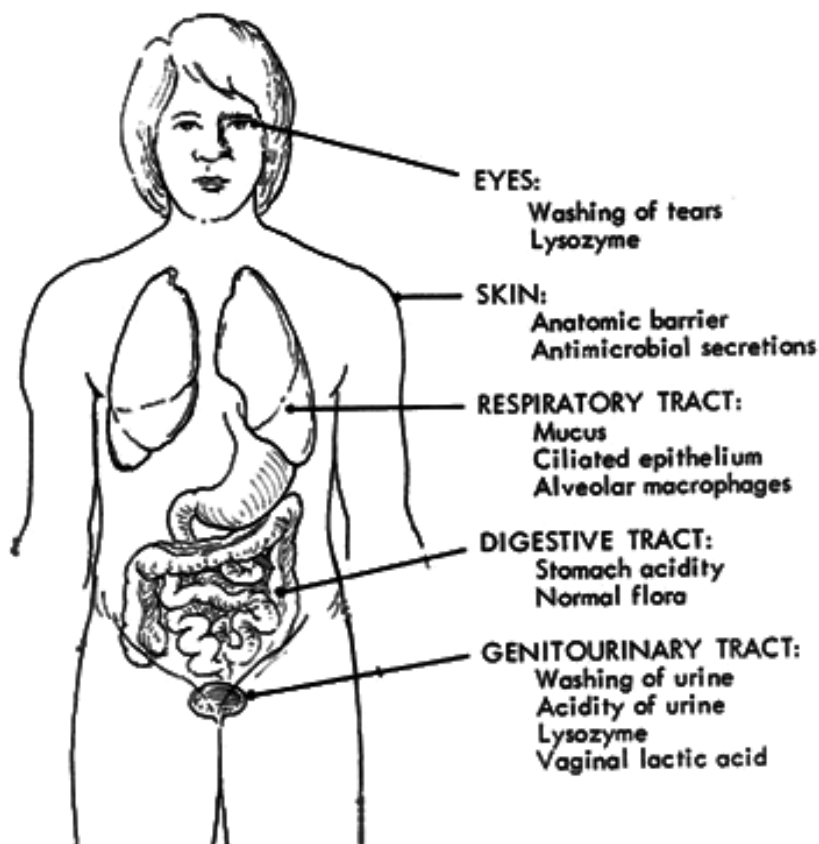


Figure 2. The physical (natural) and biochemical barriers of innate immunity in a body.

**Cells:** In addition to this, cells with specific innate immune functions rapidly respond to invading organisms to destroy them. These cells are primarily of two types: monocytes (especially macrophages) and polymorphonuclear leucocytes. Both of these can ingest micro-organisms by a process known as “phagocytosis” and destroy them. They also synthesize and secrete many substances, including cytokines and enzymes, which protect against infections and promote the development of the immune response.

**Polymorphonuclear leucocytes** circulate in the blood, but can migrate rapidly into tissues in response to stimuli provoked by unwanted foreign bodies and substances. Circulating monocytes also migrate from blood into tissues, while macrophages are usually present in all body tissues.

**Macrophage:** Primitive animals as well as higher species can produce innate immune responses; early work by the Russian immunologist Metchnikoff showed that starfish larvae can respond to foreign material by macrophage-mediated processes.

**Inflammatory response:** The inflammatory response that often accompanies the immune response is also important in defence against infection or protection against unwanted materials; this inflammation is induced by cells of the immune and other systems.

**complement system:** The complement system is also important in protection against some micro-organisms and can play a part in innate immunity.

**First-line defence:** Innate immunity is relatively non-specific, although normally it clearly discriminates between self and non-self. It reacts rapidly and provides a quick **first-line defence against unwanted invasion and infection.**

#### **2.4. 2. Acquired Immunity**

The resistance that an individual acquires during life, is known as **acquired immunity**. The antigens of the invading microorganisms come in contact with cells of the immune system (macrophages and lymphocytes) which lead to the initiation of an adaptive immune response specific for the inducing agent. The adaptive response improves with each successive encounter with the same pathogen: in effect the adaptive immune system remembers the infectious agent and can prevent it from causing disease later. **For example,** diseases such as **measles and diphtheria** induce adaptive immune responses which generate a life-long immunity following an infection. The key features of the adaptive immune response are thus **specificity and memory.**

The ability to respond specifically to non-self is acquired after interaction with antigens (foreign substances potentially dangerous to the body) present in such organisms. Non-vertebrate animals show little if any ability to respond in a genuinely specific manner; acquired immunity is most developed in mammals and birds. Amphibians and fish show some acquired immunity and primitive vertebrates such as lampreys and hagfish are able to respond to some antigens, although their response is usually weak and relatively simple.

Acquired immunity relies on the activity of two systems: **humoral immunity and cell-mediated immunity.**

**Humoral immunity:** Humoral immunity is mediated by soluble proteins called immunoglobulins or antibodies. Mammals produce five different classes of immunoglobulin molecules known as immunoglobulins G, M, A, D, and E. Parts of the structure of these relatively large globular proteins consist of short sequences which are positioned closely together on the surface of the molecule and interact specifically with the antigen. Other parts of the antibody molecules mediate immunobiological functions such as complement activation interaction with macrophages and other cells.

All classes of immunoglobulins are present in blood but immunoglobulin G (IgG) is the predominant class and is a major serum protein (serum from normal human beings contains 8 to 16 mg/ml IgG). IgM antibodies are induced in blood early in the immune response and IgA is secreted in gastric and lung fluids, sweat, and saliva. IgE mediates anaphylactic (histamine-releasing) and allergic responses and can be important for protection against parasites. The function of circulating IgD is unclear. Immunoglobulins are produced and secreted by B-lymphocytes.

Antibodies bind specifically to non-self organisms and substances. This often results in inactivation of undesired properties. Antigen-antibody complexes are removed from the body by various processes and antibody-coated micro-organisms are particularly susceptible

to phagocytosis by macrophages and other cells. After interacting with an antigen, antibodies trigger a range of immunobiological mechanisms which protect against infections and other undesirable effects.

**Cell-mediated immunity:** Cell-mediated immunity is mediated by T-lymphocytes. Antigen-specific T-cells are produced, which interact with antigens to mediate a number of immunobiological functions. One example of this is the production of cytotoxic cells that specifically “kill” unwanted micro-organisms or cells. A third class of lymphocyte, known as large granular lymphocytes or natural killer (NK) cells, also destroy non-self cells and micro-organisms. The acquired immune response complements the innate response to provide a specific, very efficient defence system.

### **Types of acquired immunity**

Acquired immunity may be of two types Passive immunity and Active immunity.

#### **2.2. 2.1. PASSIVE IMMUNITY**

This type of immunity is attained simply by the transfer of actively formed antibodies from one person (or animal) to another, the recipient thereby receiving preformed antibodies. There is no latent period in passive immunity, protection being effective immediately following passive immunization. This immunity is transient *i.e.*, not long lasting. It is less effective and provides an immunity inferior to that produced by active immunization.

Passive immunity is of two types:

##### **I. Natural passive immunity**

Natural passive immunity is an important source of protection for the **infant** during the first few months of life, when his own antibody synthesizing capacity is relatively poor. **The antibodies pass from the mother to the foetus during pregnancy across the placenta.**

##### **ii. Artificial passive immunity**

Artificial passive immunity is achieved by **administration of antibodies**. It involves the use of various animals-rabbit, sheep, horse, guinea pig that may be infected and allowed to manufacture specific antibodies against some infectious agent. **Blood serum** of these animals, containing the antibodies, may then be used to protect man against the same infectious agent. **Diphtheria antitoxin is an example of an Ig** which confers passive immunity. Similarly **pooled gamma globulin** is given to a person suffering from certain infections such as **measles, hepatitis or tetanus**. The protection afforded by this passive transfer of antibodies is relatively short lived, usually lasting only a few weeks.

Both Active and Passive Immunity is produced against Diphtheria and Tetanus.

#### **2.2. 2.2. Active immunity.**

The immunity generated by the production of antibodies by the body when it is exposed to a specific infection or an antigen through vaccination into the tissues is called **active immunity**.

The actual material (antigen) injected may be small quantities of living or weakened microbes, e.g., polio vaccine, small quantities of toxins or harmless antigenic materials derived from the microorganism or its toxin. This results in the active functioning of the person's immune apparatus leading to the **synthesis of antibodies** and/or the production of immunologically active cells. Active immunity sets in only after a latent period which is required for the immunological machinery to be set in motion. Active immunity is of two types:

**Life time immunity** : It is caused by infections, such as diphtheria, whooping cough, small pox and mumps.

**Short time immunity:** Infections such as common cold, influenza, pneumococcal pneumonia and bacillary dysentery confer immunity for a shorter time.

Active immunity is **two types**, namely natural and artificial immunity.

### **I. NATURAL ACTIVE IMMUNITY**

**Natural active immunity** results from either a clinical or in apparent infection with the parasite. A person who has recovered from an attack of **small pox** develops natural active immunity.

### **II. ARTIFICIAL ACTIVE IMMUNITY**

**Artificial active immunity** is the resistance induced by **vaccines**, which are preparations of live or killed microorganisms, or their products used for immunization. Some example are:

**Small-pox vaccine** was introduced by an English Physican Edward Jenner in 1796.

**Salk vaccine** against Polio prepared by Jonas Salk in 1953 from killed polio virus. **Sabin oral vaccine** contains live but tamed (weak) virus.

**Rabies** : 14 day series of injections known as **Pasteur Treatment** development by Louis Pasteur in 1885.

**Typhoid And Cholera** : T.A.B. Cholera Vaccine containing *Salmonella typhi*, *Salm. Paratyphae A* and B and *vibrio cholerae*. Initial dose 0.5 ml, 2<sup>nd</sup> dose 1 ml.

**Tuberculosis** : BCG Vaccine prepared from a culture of living bacteria, named **Bacillis Calmette Guerine** after 2 french bacteriologists, who made it.

**Diphtheria, Pertussis** (whooping cough) and **Tetanus\*** DPT vaccine.

In our country, The **Haffkine Institute** at Bombay, the **Virus Institute** at Poona and the **Pasteur Institute** at Coonoor produce several kinds of vaccines.

### 3. PRIMARY AND SECONDARY LYMPHOID STRUCTURES AND ORGANS

1. Structure and functions of bone marrow,
2. Structure and functions Thymus,
3. Structure and functions Bursa of Fabricius,
4. Structure and functions Spleen,
5. Structure and functions GALT,
6. Structure and functions BALT and
7. Structure and functions Lymph nodes.

The organs involved in immune system can be divided into two types based on their function as primary and secondary lymphoid organs (Figure 3).

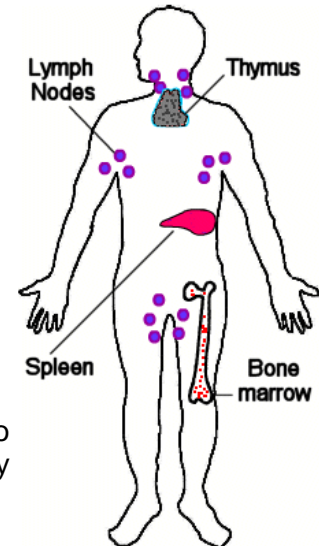


Figure 3. Lymphoid organs.

#### PRIMARY LYMPHOID ORGANS

The primary (or central) lymphoid organs are thymus and bone marrow in case of mammals. They provide appropriate microenvironment for the development and maturation of lymphocytes (a type of white blood cells/leukocytes). They constitute the primary (or central) lymphoid organs. Once the lymphocytes mature in the primary lymphoid organ, they are released into blood and lymphatic system.

##### 3.1. Structure and functions of bone marrow

Bone marrow is actually a tissue (Figure 4), rather than an organ and indeed in the medical literature is often referred to as *myeloid* or *medullary* tissue. It consists of various hematopoietic stem cells and adipocytes embedded in a spongy matrix of reticular **cells** and blood vessels. It is found in cavities of almost all bones of the body, including **skull**, ribs, collarbone, and so on, but is most abundant in the long bones such as the femur, and in the sternum and spine. The average adult human volume of bone marrow is on the order of 2 to 3 liters, comprising anywhere from 2 to 5 percent of total body weight.

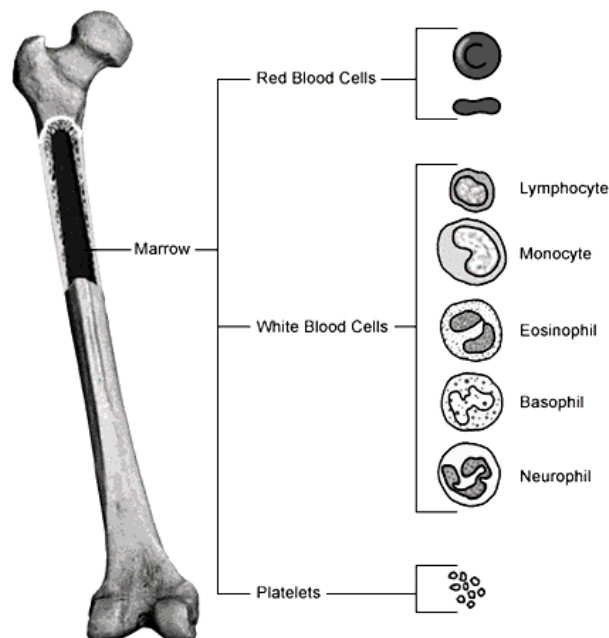


Figure 4. Bone marrow.

## Function

1. The major function of the bone marrow in adults is the generation of all of the cellular elements of the blood, both erythroid and lymphoid, and as such it is the **principal** repository of hematopoietic stem cells.
2. Haemopoietic **stem cells** arise in the bone marrow.
3. From the haemopoietic stem cells of bone marrow all the different types of **blood cells**, namely RBCs, WBCs (granulocytes, lymphocytes, and monocytes), reticular cells and platelets are developed.
4. During secondary immune response, large number of **plasma cells** are produced in the bone marrow. They secrete large amount of antibodies. So, bone marrow is a source of antibody synthesis.
5. Bone marrow functions both as a **primary lymphoid** organ and as a **secondary lymphoid** organ.

### 3. 2. Structure and functions of thymus

The thymus (so called because in shape it resembles the leaf of the thyme plant) is situated above the heart. The structure of the thymus is shown schematically in Figure 5.

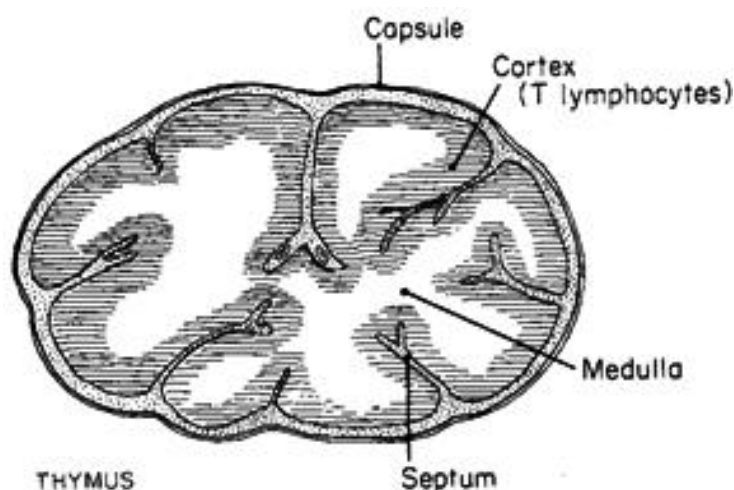


Figure 5. The structure of the thymus.

It is bilobed structure. Each lobe is divided by septa of connective tissue into a series of lobular such compartments. These septa or *trabeculae* provide the major supporting framework for the thymic tissues. On close histological examination, major portions of each thymic lobule can be distinguished as outer region of densely packed; rapidly dividing lymphocytes called the cortex and a central area of more loosely arranged lymphoid and epithelial cells called the **medulla**. The thymus is internally zoned into many lobules, which are separated from each other by connective tissue strands called trabeculae. Each lobule consists of central medulla and the outer cortex. The medulla is sparsely populated by thymocytes, whereas the cortex is densely packed with immature T cells called thymocytes. It is believed that the progenitor T cells enter the thymus and start proliferating rapidly within the cortex (Fig. 4).

The rapid proliferation of thymocytes is coupled with apoptosis (cell death), then a small proportion of surviving T cells migrates to medulla where they continue to mature and finally leave the thymus. Some studies have shown that a small population of thymocytes mature in the cortex itself and leave the thymus without ever entering the medulla.

Both the cortex and medulla of the thymus is composed of network of stromal cells, epithelial cells, interdigitating dendritic cells and the macrophages. These cells contribute for the maturation of thymocytes. For ex. the developing thymocytes and the stromal cells physically interact. The thymic epithelial cells act as nurse cells by having long membrane processes that surround as many as 50 thymocytes, resulting in the formation of multicellular complexes.

### Function

1. T cells functions in cellular immunity of the body. Improper functioning of thymus results in decreased circulating T cells and cellular immunity with an increase in the infectious disease.
2. It is the site of maturation of a major subclass of lymphocytes called T cells. T-cell precursors migrate into the thymus from the yolk sac, in early embryos, and from the bone marrow, at later stages of embryonic development and in the adult, via the bloodstream. Mature T cells also leave the thymus via the bloodstream.
3. The epithelial elements of the thymus produce a hormone, called **thymosin**, which appears to be involved in T-cell maturation and integrity both within the thymus and in other lymphoid organs.
4. Removal of the thymus in adult animals compromises long range immune function somewhat, but generally not with fatal results, However, thymectomy at birth has a drastic effect on the subsequent ability of the organism to mount an immune response.

### 3.3. Structure and functions of bursa of fabricius

In mammals the bone marrow acts as primary, lymphoid organ, whereas in birds a specialized organ called bursa of Fabricius (a gut associated lymphoid tissue) helps in maturation, division and diversification of B cells. B cells produce various types of antibodies and functions in the immunity of the body.

The bursa is an epithelial and lymphoid organ that is found only in birds, although possible structural analogs have been suggested in some reptiles. The bursa arises from the epithelium of the gut in the proctocleal region of the cloaca (Figure 6).

The bursa develops as an outgrowth of the hindgut epithelium very early in ontogeny. The finger-like epithelial buds become lymphoid in character much later in development. Each bud or lobe then has a dense outer cortex of rapidly dividing large lymphocytes and a more thinly populated medulla of mostly small.

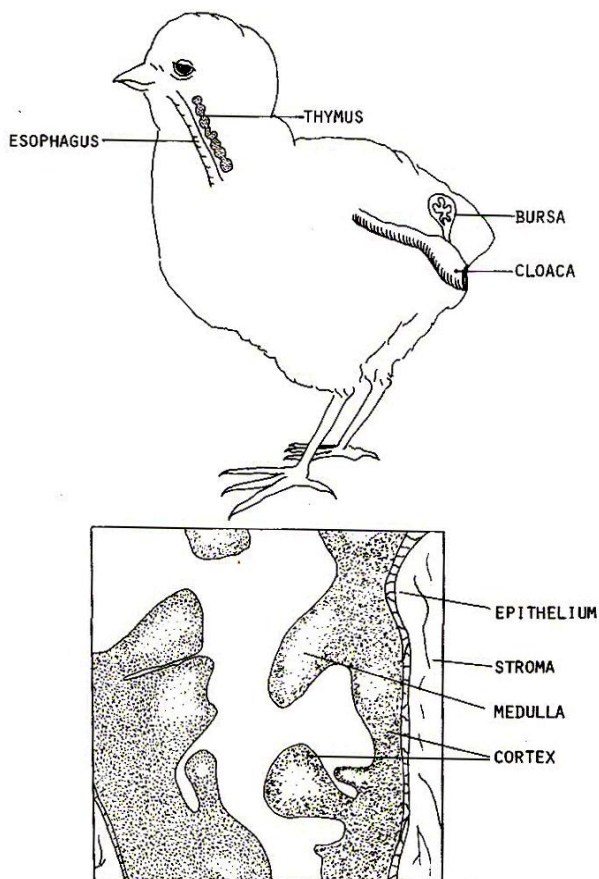


Figure 6. Bursa of fabricius.

The **structure** of the bursa is very similar to that of the thymus. It is composed of numerous lobes, each of which has a cortical and a medullary area. The cortex contains mostly large, undifferentiated lymphoid cells, whereas the medulla contains small, apparently mature cells (Fig. 5). However, in the bursa the cortical and medullary regions are separated

by a layer of epithelial cells. Like the thymus, the bursa gradually involutes and atrophies after the onset of sexual maturity.

**Function:** The functional characterization of the bursa played a key role in the **identification of lymphocyte subpopulations**. The bursa in birds is important for the proper functioning of B lymphocytes, which **become plasma cells and synthesize and secrete antibody**.

**Removal of the bursa** at or before hatching results in a failure of centers to develop in the lymph nodes and spleen in response to immunization and in a total inability of the bursectomized bird to produce antibodies.

**A direct analog of the bursa** has not been identified in mammals. Numerous gut associated organs have been proposed, but unconvincingly. It is now clear that in adult mammals the bone marrow itself is the site of B-cell development: in mammalian embryos, the fetal liver appears to play a crucial role in the maturation of B lymphocytes.

### SECONDARY LYMPHOID ORGANS

The secondary (or peripheral) lymphoid organs are spleen, tonsil, lymph nodes and Payer's Patches. The secondary lymphoid organs help in trapping the antigen and provide site for mature lymphocytes to interact with that antigen. Lymph nodes and the spleen are the most highly organized secondary lymphoid organs.

#### 3. 4. Structure and functions of spleen

The spleen (Figure 7) is a bright red organ. It is a secondary lymphoid organ, oval in shape, situated in the left abdominal cavity below the pancreas. Like the thymus, it is encapsulated by a tough coating of connective tissue, from which arise numerous inward projections or trabeculae that form a general structural matrix for the spleen.

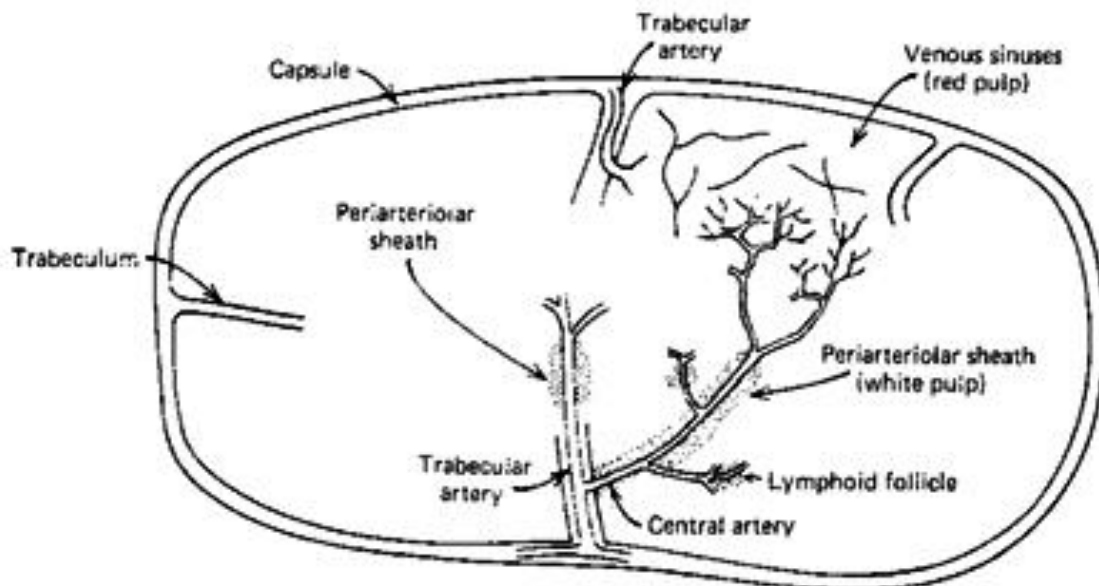


Figure 7. Spleen showing internal zonation of red pulp and white pulp area.

The red pulp consists of network of sinusoids enriched with macrophages and RBCs. It is the site where aged and defective RBCs are destroyed and removed. The white pulp surrounds the arteries, forming periaarterial lymphatic sheath (PALS) and mainly consists of T lymphocytes. The marginal zone located next to the PALS is made up of B cells organized into lymphoid follicles. The initial activation and B and T cell takes place in the T cell-rich PALS. This region consists of interdigitating dendritic cells, which capture the antigen and present it to TH cells on their class II MHC. This leads to activation of T cells, which further leads to activation of B cells. The activated T and B cells then migrate to primary follicles in the marginal zone. These ordinary follicles transform themselves into secondary follicles

upon antigenic stimulation. These secondary follicles resemble the germinal centers of lymph node, where rapidly dividing B cells and dense clusters of concentrically arranged lymphocytes surround plasma cells.

The spleen is not supplied by the afferent lymphatics as found in lymph node, instead, blood passing through the spleen will carry the antigens and empties it in the marginal zone. Antigens that enter marginal zone are trapped by interdigitating dendritic cells, which carry the antigen to the periarteriolar lymphoid sheath. Lymphocytes from the blood also enter the spleen in the marginal zone and then migrate to periarteriolar lymphoid sheath.

### Functions

The spleen has a dual function; it is **the major site for removal** and destruction of dead red blood cells, and it is an important organ of the immune system,

Spleen responds to systemic infection, because it filters the blood and traps the blood borne antigens, unlike the lymph nodes, which trap localized antigens from the regional tissue spaces.

### 3. 5. Structure and functions GALT,

The digestive tract's immune system is often referred to as GALT (Gut-Associated Lymphoid Tissue) and works to protect the body from invasion. GALT is an example of mucosa-associated lymphoid tissue.

#### Structure

Lymphoid tissue in the gut, the gut part of **MALT**, which is comprised of the following:

- **Tonsils and Adenoids** (Waldeyer's ring): The tonsils and adenoids are made of lymph tissue (Figure 9) and help to fight off infection. Largest in childhood and gradually shrink throughout life.
- **Peyer's patches:** Lymphoid aggregates in the appendix and large intestine.
- Lymphoid tissue accumulating with age in the stomach.
- Small lymphoid aggregates in the oesophagus.
- Diffusely distributed lymphoid cells and plasma cells in the lamina propria of the gut.

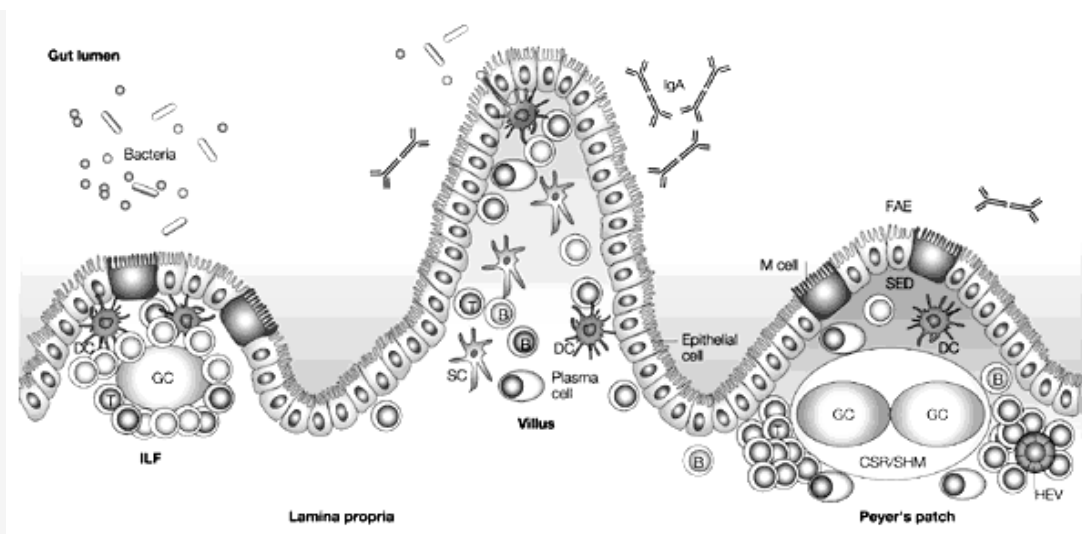


Figure 9. Structure of GALT.

Schematic representation of gut-associated lymphoid tissue (GALT), with organized lymphoid structures — Peyer's patches and isolated lymphoid follicles (ILFs) — and diffuse tissue of the epithelium and the lamina propria. Peyer's patches and ILFs are composed of a specialized follicle-associated epithelium (FAE) containing M cells, a subepithelial dome

(SED) rich in dendritic cells (DCs), and B-cell follicle(s) that contain germinal centres (GCs) (Figure 10), where follicular B cells efficiently undergo class-switch recombination (CSR) and somatic hypermutation (SHM). Migration of B cells into the mucosa takes place through high endothelial venules (HEVs), located in the interfollicular regions of Peyer's patches, which contain mostly T cells. The diffuse tissues of the lamina propria contain a large number of immunoglobulin A (IgA)+ plasma cells, T and B cells, macrophages, dendritic cells (DCs) and stromal cells (SCs). Lamina-propria DCs take up antigens from the lumen and present them directly to T cells and B cells, which can induce IgA class-switching and differentiation *in situ*. Secreted IgA is transported across the epithelium, where it serves as a first line of defence against pathogens and for the maintenance of gut-flora homeostasis. IgA+ B cells and plasma cells are shown in red, IgG+ cells in blue and IgM+ cells in pink.

### Functions

1. The role of GALT is to block normal flora bacteria (bacteria that are normally present in the gastrointestinal tract to aid digestion) from penetrating into other tissues or the BLOOD circulation.
2. GALT also helps prevent gastrointestinal viruses from causing infection. The presence of GALT in the lining of the STOMACH increases with aging.

GALT also includes the small node like lymphoid structures called Peyer's patches that pepper the small intestine. Peyer's patches intensify the presence of the immune system and are the sites of much antibody activity from B-lymphocytes.

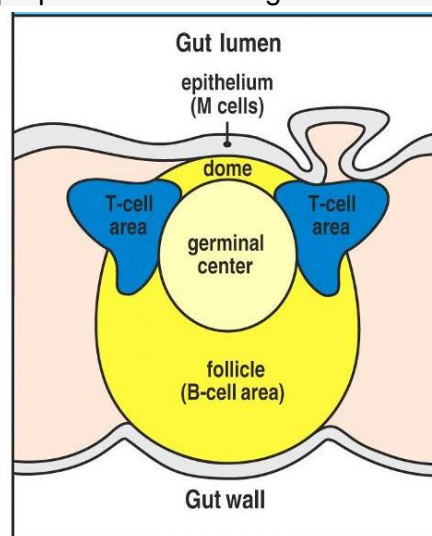


Figure 10. Peyer's patches.

### 3. 6. Bronchus-associated lymphoid tissue (BALT)

It is aggregations of B and T lymphocytes in the lower respiratory tract. An accumulation of lymphoid cells with a typical localisation of B lymphocytes (preferentially in a follicle), T lymphocytes (more peripherally around HEV in the wall of bronchi) is called bronchus-associated lymphoid tissue BALT. A further structural component is a cap-like accumulation of lymphoid cells partly bulging into the lumen of the bronchus, called dome area. The epithelium covering the dome lacks goblet cells, is infiltrated by lymphocytes and contains cells specialised for antigen uptake – M cells. BALT is not present in all species and age groups and can be classified as a tertiary lymphoid organ.

BALT disappears as children get older. In young children BALT may act as an entry site for antigens to initiate an immune response, as is well documented for the gut-associated lymphoid system.

Inducible BALT (iBALT), is an ectopic lymphoid tissue that is formed upon inflammation or infection in both mice and humans and can be found throughout the lung. Both BALT and iBALT acquire antigens from the airways and initiate local immune responses and maintain memory cells in the lungs.

Bronchus-associated lymphoid tissue (BALT) was first described in the lungs of rabbits and differs greatly between species. It is part of the integrated mucosal immune system. In healthy humans, BALT can only be found in the lungs of children and adolescents. BALT is part of the integrated mucosal-associated lymphoid system (MALT).

### Functions

Primary B- and T-cell responses to influenza, which seem to be initiated at sites of induced BALT (iBALT). Areas of iBALT have distinct B-cell follicles and T-cell areas, and

support T and B-cell proliferation (Figure 11). Thus, iBALT functions as an inducible secondary lymphoid tissue for respiratory immune responses.

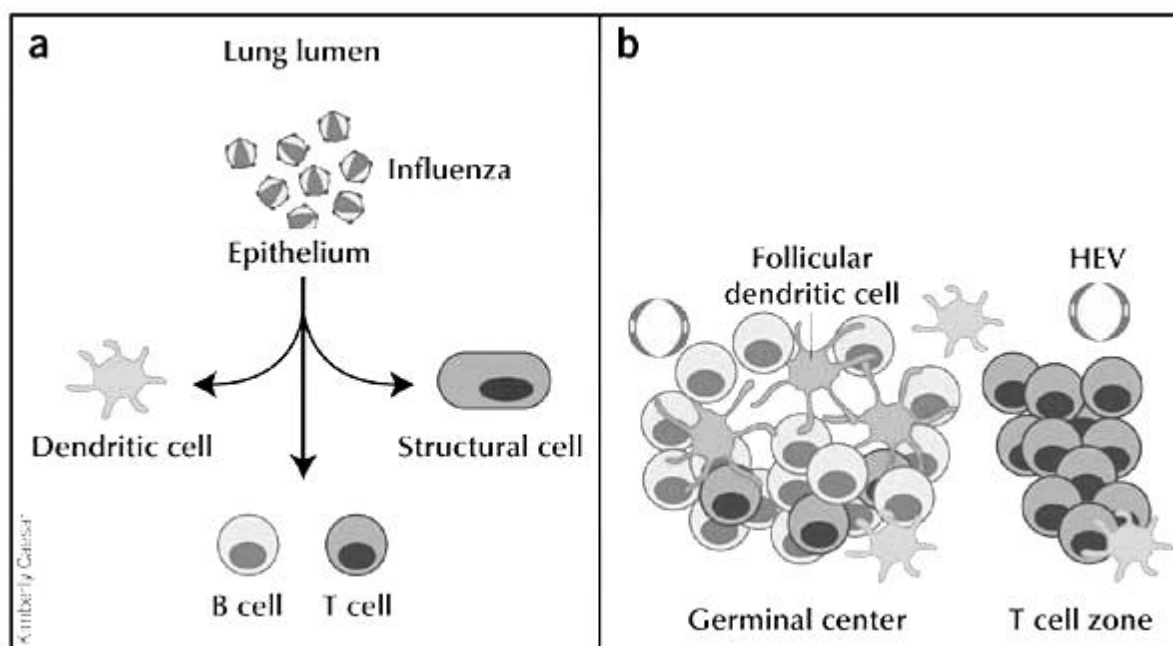


Figure 11. Induced secondary lymphoid tissue formation in the lung upon influenza infection.

(a) Pulmonary infection generates bronchus-associated lymphoid tissue (BALT) *de novo*, independently of  $LT\alpha$ . Undefined mediators, possibly originating from the epithelium, act on resident lung cells, such as macrophages, dendritic cells, lymphocytes and structural cells (fibroblasts, endothelial and mesenchymal cells), to coordinate BALT formation. (b) Once formed, BALT sustains adaptive immune responses. Entering the BALT through high endothelial venules (HEV) expressing PNA $d$  and the lymphocyte attractant CCL21, naive B and T lymphocytes encounter their cognate antigens and segregate into distinct B and T cell rich zones. Within these structures, proliferating CD4, CD8 and B cells are observed. Once activated and clonally expanded, lymphocytes exit the BALT through efferent lymphatics.

### 3. 7. Structure and functions of lymph node

The lymph nodes are small (less than 1 cm unless inflamed), bean-shaped structures distributed throughout the entire body and linked together by an equally extensive circulatory system of lymphatic vessels. They are encapsulated by connective tissue and enclose within them a reticular pattern with macrophages, lymphocytes and dendritic cells.

Each node is surrounded by a dense connective tissue capsule and is, composed principally of lymphocytes embedded in a reticulum cell network. A lymph node internally consists of a microenvironment of its own with three zones-cortex, paracortex and medulla (Figure 12). **The outer most layer of the lymph node is called cortex**, which consists of B lymphocytes, macrophages and follicular dendritic cells arranged in primary follicles. Antigenic challenge, leads to enlargement of primary follicles into secondary follicles, each containing a germinal center. In the germinal center, intense activation and differentiation of B cells occurs. **Paracortex zone** is beneath the cortex, which is richly populated by T lymphocytes and interdigitating dendritic cells. The interdigitating dendritic cells express high levels of class II MHC molecules, which are necessary for antigen presentation to TH cells. **The innermost zone is called medulla** is sparsely populated by lymphocytes, but many of them are plasma cells, which actively secrete antibody molecules.

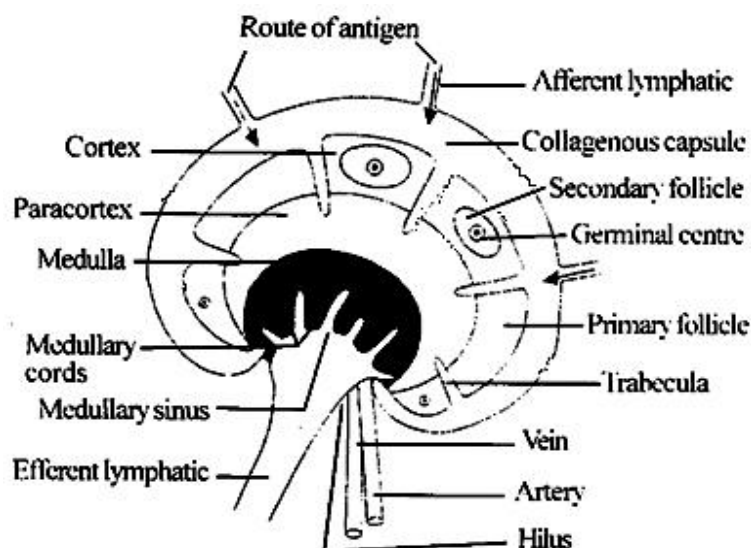


Figure 12. Diagrammatic sketch of internal structure of lymph node.

### Functions

The lymph nodes are served by both lymph and blood circulation. In fact, it is in the lymph nodes that lymphocytes pass from blood to lymph circulation. They play their unique role in trapping by the phagocytic cells, follicular and interdigitating dendritic cells and processing foreign antigen from extravascular tissue spaces.

**The principal functions** of the lymph nodes are to entrap foreign matter in the lymph circulation and to act as a site for the antigen driven maturation of lymphocytes.

As the antigen enters the regional lymph node through the lymph, it is processed and presented on class II MHC molecule by the interdigitating dendritic cells to the TH cells, resulting in TH cell activation in paracortex area. The initial activation of B cell also occurs in the T cell rich area of paracortex. Activated B cells, plasma cells and start secreting IgG and IgM.

Antigen trapped on the membrane of these cells is responsible for activation of B cells. The follicular dendritic cells on trapping the antigen on their membrane surface get self activated and start producing certain growth factors, which play an important role in activation of B cells. The activated B cells in the germinal center divide rapidly and undergo differentiation to form plasma cells or memory B cells. Some self-reactive B cells go through a process of selection and undergo apoptosis. The plasma cells migrate from the germinal center into medulla and start secreting antibody.

As the lymph enters the lymphatic capsule through the afferent lymphatic vessels into the subcapsular area of the lymph node, it slowly percolates through the cortex, paracortex and medulla, giving sufficient chance for the phagocytic cells and dendritic cells to trap the antigen brought in by the lymph. The lymph leaving a node through its single efferent lymphatic vessel will carry enriched antibodies secreted by the medullary plasma cells against the antigens that entered the lymph node. Efferent lymph also carries lymphocytes, which leave the lymph node due to excessive proliferation in the lymph node on challenge with antigen. Some times visible swelling of the node occurs due to active immune response and the increased concentration of lymphocytes within the lymph nodes. Antigenic stimulation within the node, also known to increase the migration of lymphocytes as an indication of active immune response.

#### 4. CELLS OF IMMUNE SYSTEM

The immune system is operated by cells. The following are the important cells of immune system.

1. B cells.
2. T lymphocytes.
3. Null cells (a. Killer cells, b. Natural killer cells).
4. Macrophages.
5. Eosinophils
6. Basophils
7. Neutrophils
8. Antigen presenting cells.
9. Mast cells
10. Platelets.

##### 1. B-cells

B cells constitute about 27% of the total lymphocytes. The B lymphocytes mature in Burse of Fabricius (in Aves) or in bone marrow (in mammals). The B lymphocytes produce antibodies (Figure 13) and hence they responsible for humoral immunity. The B cells kill bacteria, virus etc.

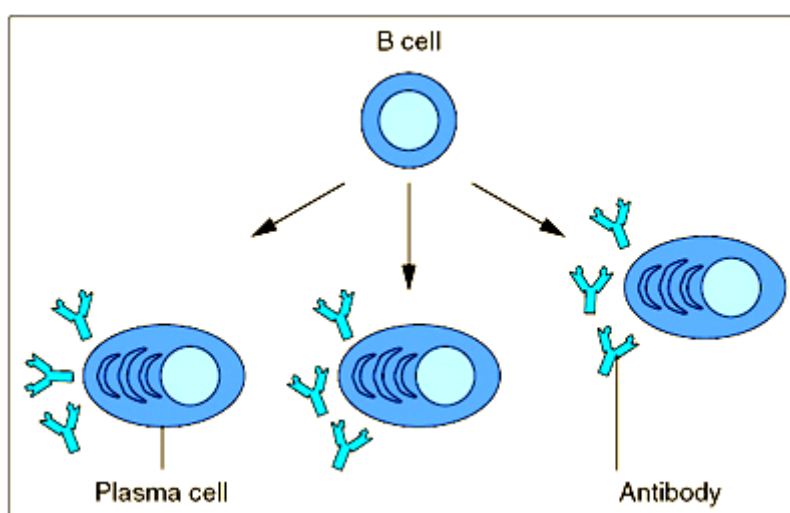


Figure 13. B cells mature into plasma cells that produce antibodies.

##### 2 T-cells

T-cells belong to a group of white blood cells (WBCs) called lymphocytes. The T cells constitute about 70 % of the total lymphocytes. The T lymphocytes mature in the thymus under the influence of thymic hormones. They involve in cell mediated immunity. The main job of T-cells is to fight infection.

The main job of T-cells is to fight infection. There are a number of different types of T-cells that act in many ways to identify, directly attack and destroy infectious agents. Along with other WBCs, they play a major role in the immune system, which guards the body against infection. They are responsible for the killing of cancer cells, killing viral infected cells, allograft rejection etc.

T cells respond to antigens by producing cytokines, not antibodies. When activated (Figure 14), some T cells become *cytotoxic* (CD8) and kill tumor cells or infected cells. Cytokines that promote inflammation include certain *interleukins*, tumor necrosis factor, and *interferon gamma*. Other cytokines, like transforming growth factor, are immunosuppressants. So T cells may either enhance or suppress inflammation.

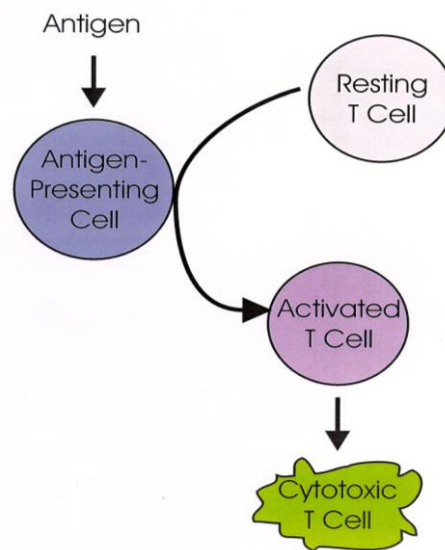


Figure 14. T-cells.

T cells only recognize antigen presented to them by other cells called *antigen-presenting cells* (APCs). In the blood, these are often macrophages or B cells; in the brain, microglia. Unlike natural killer cells, which have innate ability to recognize and destroy tumor cells or infected cells, T cells must follow a tightly regulated protocol involving the *major histocompatibility complex* (MHC I or II).

MHC proteins allow T cells to distinguish "self" from "non-self." When class II MHC proteins present antigens, they are detected by different T cells (T-helper) than when class I MHC proteins are involved (T-cytotoxic). Non-self antigens are targeted for destruction.

### 3. Null cells

The null cells constitute about 3% of the total lymphocytes. They form a third population of lymphocytes. They are intermediate between T and B cells. They have cytotoxic property.

**There are two types of null cells namely,**

- a. natural killer cells.
- b. killer cells

#### a. Natural Killer Cells (NK cells)

T Natural killer cells (also known as NK cells, K cells, and killer cells) are a type of lymphocyte (a white blood cell) and a component of innate immune system. The NK cells have 2 or 3 large granules in the cytoplasm. Hence they are also called large granular lymphocytes (LGL). They have a kidney shaped nucleus. They kill the target cells (virus

infected cells) without the aid of antibody or complement. So they are antibody independent. They kill the cells infected with herpes and mumps virus.

Natural Killer (NK) cells are yet another type of lethal lymphocyte. Like cytotoxic T cells, they contain granules filled with potent chemicals. They are called "natural" killers because they, unlike cytotoxic T cells, do not need to recognize a specific antigen before swinging into action. They target tumor cells and protect against a wide variety of infectious microbes. In several immunodeficiency diseases, including AIDS, natural killer cell function is abnormal. Natural killer cells may also contribute to immunoregulation by secreting high levels of influential lymphokines. NK cells also secrete cytokines (TNF) which modulate functions of cells of the lymphocytes (Figure 15). NK cells kill tumour cells through mechanisms that involve: antibody (Ab)-dependent cellular cytotoxicity (ADCC), in which the Fc portion of an Ab bound to antigen (Ag) on the tumour cell surface binds to Fc receptor (FcR) on the NK cell; Fas (CD95)–Fas ligand (CD95L) interaction; and release of perforin and granzyme B molecules, which cause apoptosis/necrosis of the tumour cell.

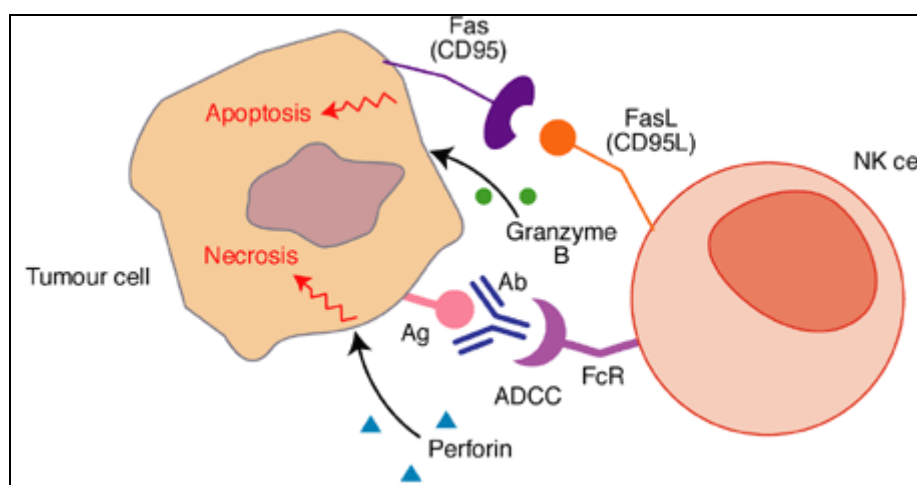


Figure 15. The natural killer (NK)-cell response to tumour cells.

NK cells play a major role in the host-rejection of both tumours and virally infected cells. NK cells are cytotoxic; small granules in their cytoplasm contain special proteins such as perforin and proteases known as granzymes. Upon release in close proximity to a cell slated for killing, perforin forms pores in the cell membrane of the target cell through which the granzymes and associated molecules can enter, inducing apoptosis.

## b. Killer cells

Killer T cells are a sub-group of T cells that kill cells that are infected with viruses (and other pathogens), or are otherwise damaged or dysfunctional. As with B cells, each type of T cell recognises a different antigen. Killer T cells are activated when their T cell receptor (TCR) binds to this specific antigen in a complex with the MHC Class I receptor of another cell. Recognition of this MHC:antigen complex is aided by a co-receptor on the T cell, called CD8. The T cell then travels throughout the body in search of cells where the MHC I receptors bear this antigen. When an activated T cell contacts such cells, it releases cytotoxins, such as perforin, which form pores in the target cell's plasma membrane, allowing ions, water and toxins to enter. The entry of another toxin called granulysin (a protease) induces the target cell to undergo apoptosis.

These cells can combine with specific antibody when it is in complex with antigen. If the complex is on the surface of a target cell, these lymphocytes become activated, destroying the target cell.

By this cytotoxic property. The K cells can kill a variety of cells (Figure 16) such as tumour cells, bacteria, viruses, fungi and parasites.

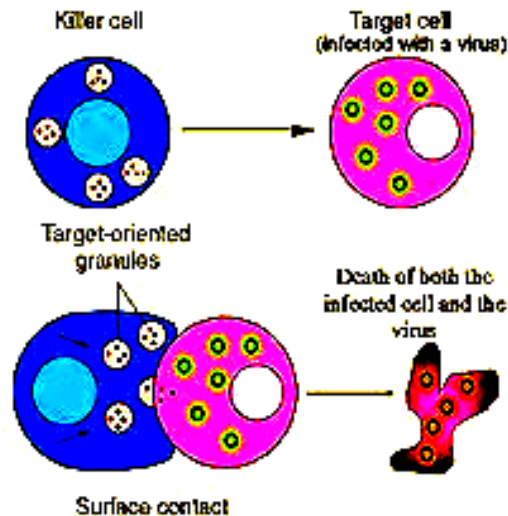


Figure 16. Killer T cells directly attack other cells carrying foreign or abnormal antigens.

#### 4. Macrophages.

Macrophages are white blood cells within tissues, produced by the division of monocytes. Human macrophages are about in diameter that crawl around in the extracellular fluids of your body and gobble up microbes and other foreign material. They ingest these microbes by phagocytosis ("cell eating"). Parts of the cell surround the particle to be eaten, then the macrophage's membrane flows together and the particle ends up inside. They stimulate lymphocytes and other immune cells to respond to the pathogen.

Monocytes and macrophages are phagocytes (Figure 17), acting in both non-specific defense (innate immunity) as well as to help initiate specific defense mechanisms (adaptive immunity) of vertebrate animals. Macrophages help destroy bacteria, protozoa, and tumor cells. They also release substances that stimulate other cells of the immune system. And they are involved in antigen presentation. To do this, they carry the antigen on their surface and present it to a T cells.

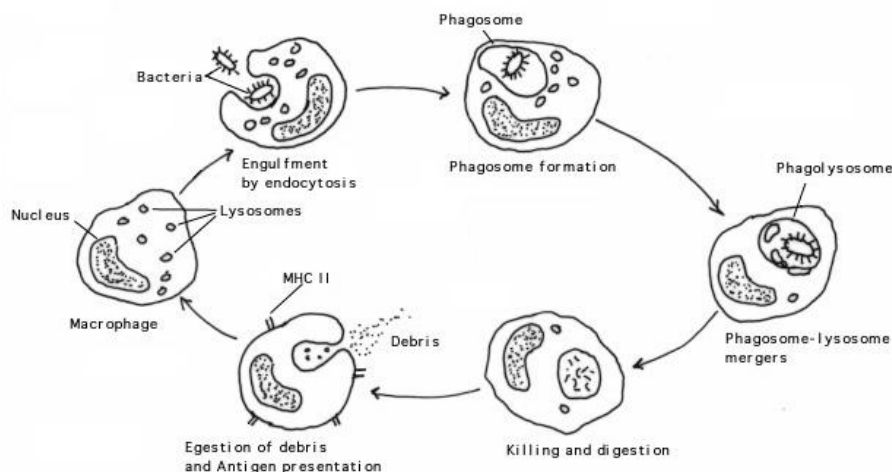


Figure 17. phagocytic activity of macrophages.

**5. Eosinophils** carry receptors for IgE, are involved in the destruction of IgE coated parasites, such as helminths, and contribute to the response to allergens.

**6. Basophils** are the circulating counterpart of tissue **mast cells**. They express high affinity receptors for IgE and are stimulated to secrete the chemicals responsible for **immediate hypersensitivity** following antigen induced aggregation of these receptors.

**7. Neutrophils** also known as polymorphonuclear leukocytes, express receptors for immunoglobulin and complement and are involved in the acute inflammatory response.

## 8. Antigen presenting cells

An antigen-presenting cell (APC) or accessory cell is a cell that displays foreign antigen complexed with MHC on its surface (Figure 18). T-cells may recognize this complex using their T-cell receptor (TCR). Antigen presentation stimulates T cells to become either "cytotoxic" or "helper" cells.

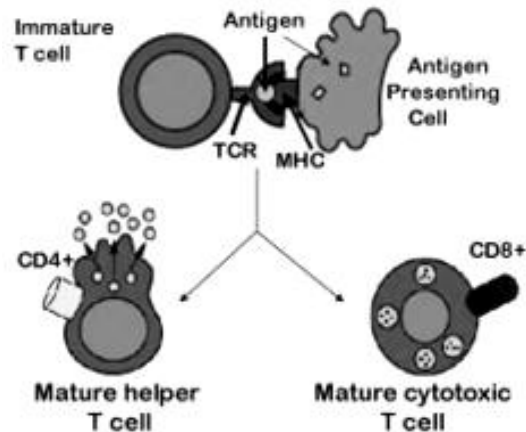


Figure 18. Antigen presenting cells.

## 9. Mast cells

A mast cell (also known as *mastocyte* and *labrocyte*) is a resident cell of several types of tissues and contains many granules rich in histamine and heparin (Figure 19). Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing and defense against pathogens

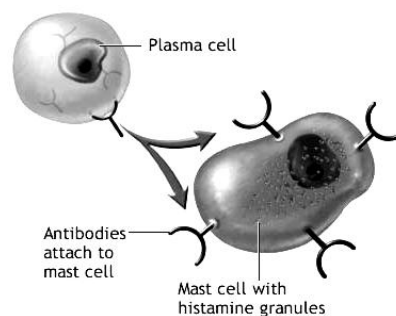


Figure 19. Mast cell contains many granules rich in histamine

## 10. Platelets

Platelets **are** only about 20% of the diameter of red blood cells, the most numerous cell of the blood. Platelets are irregular, disc-shaped element in the blood that assists in blood clotting. During normal blood clotting, the platelets clump together (aggregate), which slows or stops bleeding. Although platelets are often classed as blood cells, they are actually fragments of large bone marrow cells called megakaryocytes.

They contain proteins on their surface that allow them to stick to breaks in the blood vessel wall and also to stick to each other. They contain granules that can secrete other proteins required for creating a firm plug to seal blood vessel breaks. Also platelets contain proteins similar to muscle proteins that allow them to change shape when they become sticky.



#### 4. 1. ORIGIN AND DIFFERENTIATION OF IMMUNE CELLS

T-cells and B-cells and macrophages are involved in immune system (Figure 21). These cells develop from undifferentiated embryonic stem cells.

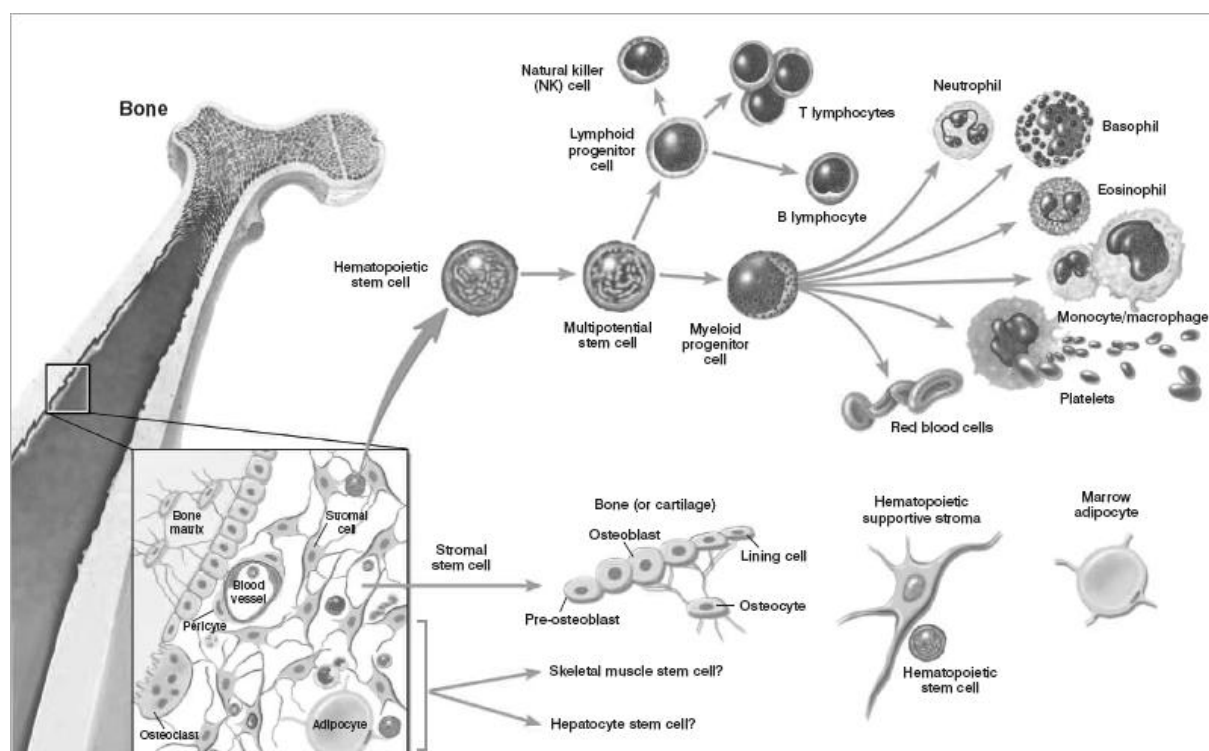


Figure 21. Cells of immune System.

#### 4. 2. ORIGIN AND DIFFERENTIATION OF T CELLS

The stem cells are located in the **bone marrow**. However, in the early embryo they originate in the liver. In the adult they reach the bone marrow. They stem cells develop into blood cells through a process called haemopoiesis. They are also called as haemocyto blasts or haemopoietic stem cells. The **haemopoietic stem cells** develop into **lymphoid progenitor cell**, which produces **T lymphocytes**. T lymphocytes arise in bone marrow but mature in the **thymus**.

T-cell differentiation takes place in the medullar regions of thymus. Thymus epithelial cells produce a series of peptide hormones which mostly promote T-cell differentiation. T-cells have surface receptors structurally related to Ig and recognizes processed antigen.

#### 4. 3. ORIGIN AND DIFFERENTIATION OF B CELLS

The stem cells are located in the **bone marrow**. However, in the early embryo they originate in the liver. In the adult they reach the bone marrow. They stem cells develop into blood cells through a process called haemopoiesis. They are also called as haemocyto blasts or haemopoietic stem cells. The **haemopoietic stem cells** develop into **lymphoid progenitor cell**, which produces **B-lymphocyte**.

The stem cells are located in the bone marrow. However, in the early embryo they originate in the liver. In the adult they reach the bone marrow. The B-lymphocyte precursors, pro-B-cells are present among the islands of hematopoietic cells in fetal liver by 8-9 weeks of gestation in man. Production of B-cells wanes and is taken over by the bone marrow for the remainder of life.

B-cells differentiation takes place from B-lymphocyte precursors, pro-B-cells are present among the islands of hematopoietic cells in the bone marrow and peripheral lymphoid tissues such as the spleen. **B lymphocytes** produce antibodies and some soluble mediators called cytokines.

### Origin and differentiation of macrophage

The stem cells are located in the bone marrow. However, in the early embryo they originate in the liver. In the adult they reach the bone marrow. They stem cells develop into **myeloid progenitor**, which develop into **monocytes**.

**Monocytes** are the precursors of Macrophages. (Figure 22) macrophages are white blood cells produced by the **differentiation of monocytes** in bone marrow tissues.

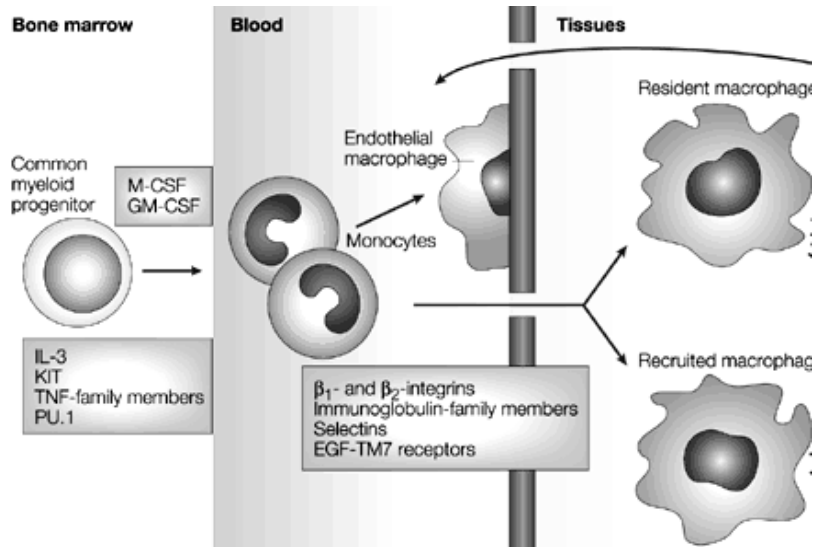


Figure 22. Origin and differentiation of macrophage.

## 5. ANTIGENS

An **antigen** is a molecule recognized by the immune system or reacts with the products of a specific immune response. Originally the term came from **antibody generator** and was a molecule that binds specifically to an antibody, but the term now also refers to any molecule or molecular fragment that can be bound by a major histocompatibility complex (MHC) and presented to a T-cell receptor. "Self" antigens are usually tolerated by the immune system; whereas "Non-self" antigens are identified as intruders and attacked by the immune system. Autoimmune disorders arise from the immune system reacting to its own antigens.

Antigens are usually proteins or polysaccharides. This includes parts (coats, capsules, cell walls, flagella, fimbriae, and toxins) of bacteria, viruses, and other microorganisms. Lipids and nucleic acids are antigenic only when combined with proteins and polysaccharides. Non-microbial exogenous (non-self) antigens can include pollen, egg white, and proteins from transplanted tissues and organs or on the surface of transfused blood cells.

Similarly, an **immunogen** is a specific type of antigen. An immunogen is defined as a substance that is able to provoke an adaptive immune response if injected on its own. Said another way, an immunogen is able to induce an immune response, while an antigen is able to combine with the products of an immune response once they are made.

Haptens are low molecular weight molecules that can bind to the antigen recognition site (Figure 23), but need a "carrier" (a larger molecule they can associate with) to stimulate a response.

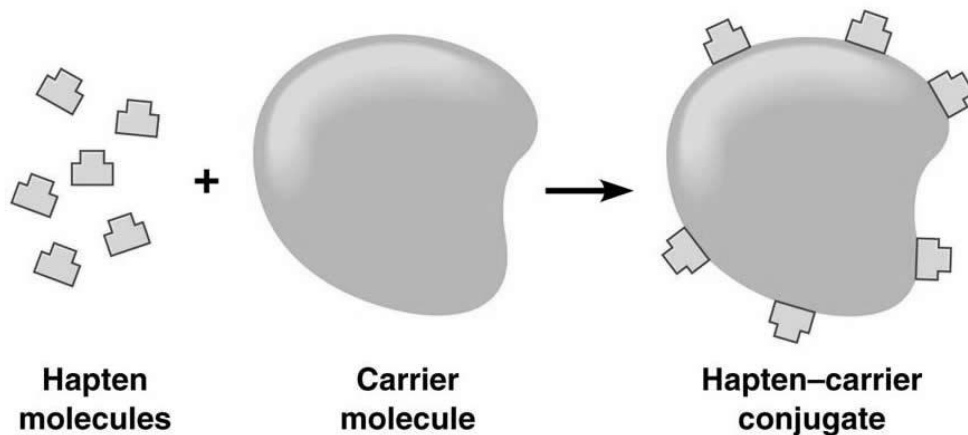


Figure 23. Hapten and carrier molecule.

### 5.1. CLASS DETERMINANTS

That portion of an antigen that combines with the products of a specific immune response is called Epitope or antigenic determinant (Figure 24). The distinct molecular surface features of an antigen capable of being bound by an antibody (a.k.a. *antigenic determinant*). Antigenic molecules, normally being "large" biological polymers, usually present several surface features that can act as points of interaction for specific antibodies. Any such distinct molecular feature constitutes an epitope. Most antigens therefore have the potential to be bound by several distinct antibodies, each of which is specific to a particular epitope. Using the "lock and key" metaphor, the antigen itself can be seen as a string of keys - any epitope being a "key" - each of which can match a different lock. Different antibody **idiotypes**, each having distinctly formed complementarity determining regions, correspond to the various "locks" that can match "the keys" (epitopes) presented on the antigen molecule.

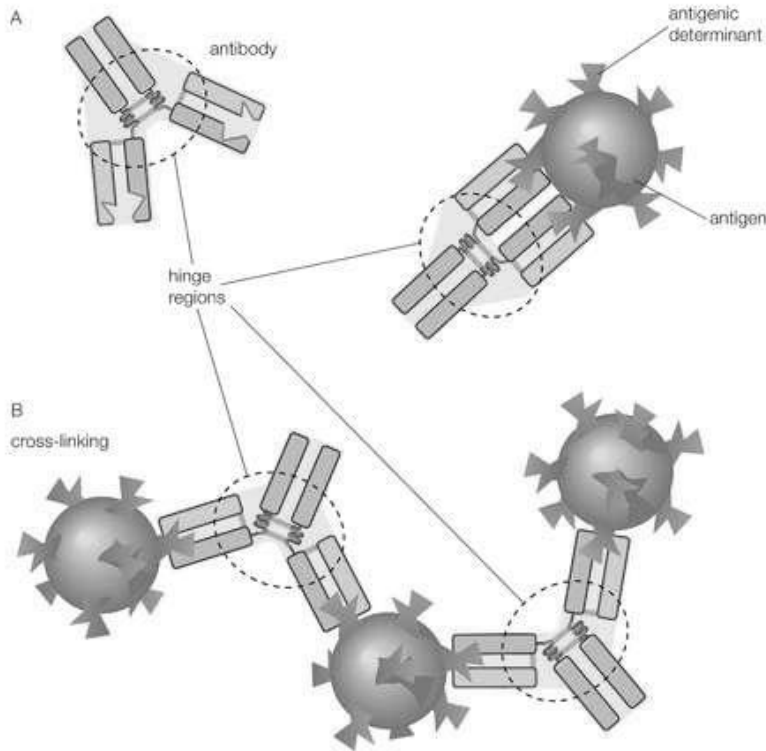


Figure 24. Illustrations antibody antibodies hinge flexibility antigenic determinant cross-linking antigen-antibody binding region complexes molecule antigen-binding.

## 5.2. REACTIVE SITES AND RECEPTOR SITES

The antigen contains class determinants, which are reactive sites. The antibody has binding sites, which is receptor sites for antigen binding (Figure 25).

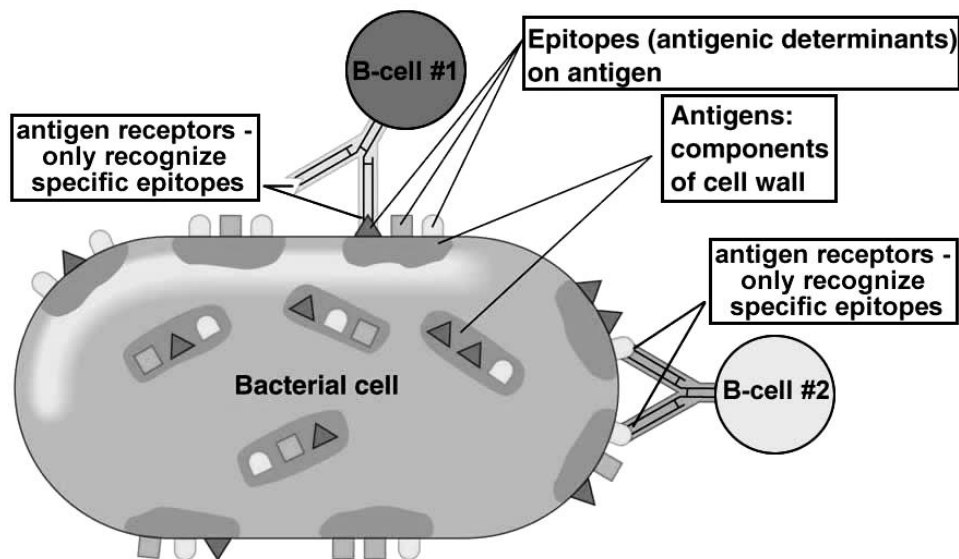


Figure 25. Both B cells and T cells have surface receptors for antigen. Each cell has thousands of receptors of a **single specificity**; that is, with a binding site for a particular epitope.

### T cell receptor

The **T cell receptor** or **TCR** is a molecule found on the surface of T lymphocytes (or T cells) that is, in general, responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules (Figure 26). T-cell receptors enable the cell to bind to and, if additional signals are present, to be activated by and respond to an epitope presented by another cell called the **antigen-presenting cell** or **APC**. T cells can recognize disease-causing microorganisms and rally other immune cells to attack the invaders, or kill the invaders themselves

### B-cell receptors

B-cell receptors (**BCRs**) enable the cell to bind to and, if additional signals are present, to be activated by and respond to an epitope on molecules of a **soluble antigen** (Figure 27). The response ends with descendants of the B cell secreting vast numbers of a soluble form of its receptors. These are **antibodies**.

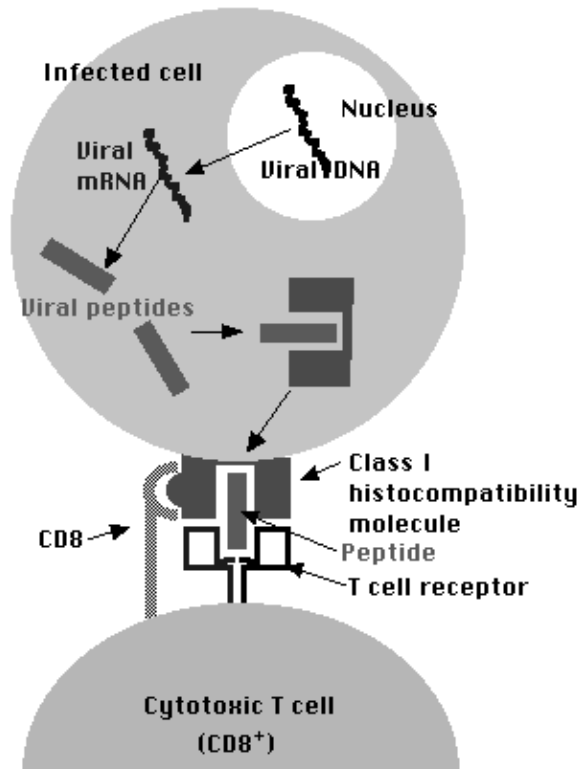


Figure 26. T cell receptor.

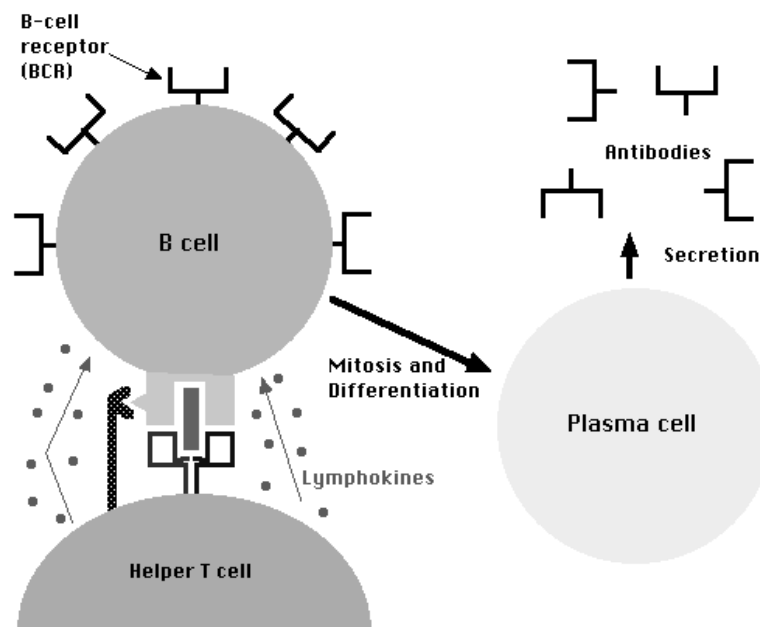


Figure 27. B cell receptor.

BCRs bind soluble antigens (like diphtheria toxoid, the protein introduced into your body in the DTP vaccine). The bound antigen molecules are engulfed into the B cell by receptor-mediated endocytosis. The antigen is digested into fragments which are then displayed at the cell surface nestled inside a class II histocompatibility molecule. Helper T cells specific for this structure (i.e., with complementary TCRs) bind the B cell and secrete **lymphokines** that stimulate the B cell to enter the cell cycle and develop, by repeated mitosis, into a **clone** of cells with identical BCRs.

## 6. VACCINES AND TOXOIDS

### A. VACCINES

A vaccine is an antigenic preparation of microorganisms such as bacteria, viruses or rickettsiae administered for prevention treatment of infectious diseases.

#### Preparation of Vaccines

Vaccines are prepared from the microbial agents or their products responsible for infectious diseases. For example cholera vaccine is prepared from the cholera producing bacterium *Vibrio cholerae*.

Active vaccination (immunization) may be performed with either killed or live attenuated vaccines and toxoids.

Since the process of attenuation is cumbersome and time consuming several newer approaches to vaccine development are being researched.

#### Synthesis of sub-unit vaccines

Sub unit vaccines are being designed which use only the relevant immunogenic portions and radio labeling techniques. Surface projections of the influenza virus, the measles virus and the rabies virus elicit neutralization antibody and can be exploited for this purpose.

#### Biosynthesis of immunogenic proteins

Specific immunogenic surface proteins need to be available in large quantities for vaccine preparation. The problem of isolating and characterizing antigenic protein moieties has been overcome by cloning genes that code for these proteins in bacterial or eukaryotic (Yeast) cells or in the vaccinia virus.

In 1986, the first recombinant (cloned) viral vaccines was licensed for use. The hepatitis B surface antigen (HBsAg) is the immunogen that stimulates protective immunity. It is prepared from yeast cells containing the recombinant gene.

#### Synthetic peptide vaccines

A limited number of sites on an organism are involved in evoking an immune response. If these sites, consisting mainly of peptide fragments, can be synthesized they provide a possible means of obtaining chemical polypeptides as vaccines.

#### Efficiency of vaccination

Vaccination provides effective immunity by establishing adequate levels of antibody and primed population of memory cells which can rapidly expand on renewed contact with antigen and so provide protection against infection.

The effectiveness of the vaccine depends on the following factors. (Table1).

Table1. Factors required for a successful vaccine.

S.No	Factors	Requirements
1.	Effectiveness	Must evoke protective levels of immunity at the appropriate site of relevant nature (Ab, Tc,Th) of adequate duration.
2.	Availability	Readily cultured in bulk or accessible source of subunit.
3.	Stability	Stable under extreme climatic conditions preferably not requiring refrigeration.
4.	Cheapness	What is cheap in the west may be expensive in developing countries but WHO tries to help.
5.	Safty	Eliminate any pathgenicity.

## **Commonly used killed vaccines**

### **1. Bacterial Vaccines**

Typhoid  
Cholera  
Pertussis  
Plague

### **2. Viral Vaccines**

Rabies  
Poliomyelitis  
Hepatitis B  
Influenza.

## **Example for Live attenuated Vaccines**

### **Bacterial**

BCG- a live attenuated mycobacterium boris for tuberculosis.

Ty2la – live oral attenuated typhoid bacillus.

### **Viral**

Live vaccinia virus for small box, rubella, measles, mumps, polio (the sabin vaccine) yellow fever.

### **Besides these, there are toxoid vaccines .**

1. Diphtheria and tetanus.
2. Cholera toxin B subunit in combination with killed whole vibrios is gaining favour over the relatively ineffective killed cholera vaccine.

## **Killed organisms as vaccines**

The simplest way to destroy the ability of microbes to cause disease yet maintain their antigenic constitution is to prevent their replication by killing in an appropriate manner. Many bacteria and viruses are extremely easy to grow up and manufactured in bulk. The inactivated (killed) micro organisms have generally provide safe antigens for immunizations. Examples are typhoid, cholera, poliomyelitis, vaccines.

## **Live attenuated organisms have many advantages as vaccines**

The objective of attenuation is to produce a modified organism which mimics the natural behaviour of the original microbe without causing significant disease. Immunity conferred by live organisms is better than killed vaccines even when with adjuvants.

This must be partly because of living microbes confronts the host with larger and more sustained dose of antigen. Multiplication of viruses or infected cells required for the establishment of good cytotoxic T-cell memory.

Another significant advantage of using live organisms is that the immune response takes place largely at the site of the natural infection. This is well illustrated by the nasopharyngeal IgA response to immunization with polio vaccine. In contrast with the ineffectiveness of parenteral injection of killed vaccine, intranasal administration evoked a good local antibody response, however whereas this decline over a period of months or so after oral immunization with live attenuated virus established a persistently high IgA antibody level (Fig.)

### **Classical methods of attenuation**

The objective of attenuation, that of producing an organism which cause only a very mild form of the natural disease, can be equally will attained if one can identify heterologous strains which are virulent for another species, but avirulent in man. The best example of this was Jenner's seminal demonstration that cowpox would protect against smallpox.

Attenuation itself can be achieved by modifying the conditions under which an organism grows. Pasteur first achieved the production of live but nonvirulent forms of chicken cholera bacillus and anthrax it was achieved by culture of organisms in higher temperatures.

A virulent strain of mycobacterium tuberculosis become attenuated by adding bile to the culture medium. The same organism, BCG (Bacille, Calmette, Guerin) is widely used today for tuberculin negative individuals.

Attenuation by cold adaptation of influenza and other respiratory virus seems hopeful.

Genetic recombination is being used to develop various attenuated strains of viruses such as influenza with lower virulence for man. The virulence genes have been identified and modified by genetic engineering in salmonella and virbrio cholerae.

### **Advantages of using Vaccines**

Live attenuated vaccines have many advantages. Attenuation mimics the natural behaviour of the organism without cause disease. The immunity conferred with live attenuated vaccines is superior because actively multiplying organism provide a sustained antigen supply. The immune responses take place largely at the site of natural infection as in the case of the live polio vaccine and the oral typhoid vaccine producing an obviously advantages local secretory IgA response.

### **TOXOIDS**

A toxoid is a bacterial toxin whose toxicity has been weakened or suppressed while other properties, typically immunogenicity, are maintained. Toxoids are used in vaccines as they induce an immune response to the original toxin or increase the response to another antigen. toxoids can be given in large quantities with no risk of tissue damage, they have superseded the highly poisonous toxins as immunizing agents against such diseases as diphtheria and tetanus.

In many bacterial diseases, the bacteria produce a toxin that causes the disease manifestations. Heating the toxin or treating it chemically converts it into a harmless toxoid that can be injected into a human or a nonhuman animal to confer immunity from subsequent infection. The vaccines for tetanus and diphtheria are toxoids. . Eg. Diphtheria vaccine, Cholera and Tetanus vaccines.

## 6.1. TYPES OF VACCINES

Scientists take many approaches to designing vaccines against a microbe. These choices are typically based on fundamental information about the microbe, such as how it infects cells and how the immune system responds to it, as well as practical considerations, such as regions of the world where the vaccine would be used. The following are some of the options that researchers might pursue:

1. **Live, attenuated vaccines**
2. **Inactivated vaccines**
3. **Subunit vaccines**
4. **Toxoid vaccines**
5. **Conjugate vaccines**
6. **DNA vaccines**
7. **Recombinant vector vaccines**

### 1. Live, Attenuated Vaccines

Live, attenuated vaccines contain a version of the living microbe that has been weakened in the lab so it can't cause disease. Because a live, attenuated vaccine is the closest thing to a natural infection, these vaccines are good "teachers" of the immune system: They elicit strong cellular and antibody responses and often confer lifelong immunity with only one or two doses.

Despite the advantages of live, attenuated vaccines, there are some downsides. It is the nature of living things to change, or mutate, and the organisms used in live, attenuated vaccines are no different. The remote possibility exists that an attenuated microbe in the vaccine could revert to a virulent form and cause disease. Also, not everyone can safely receive live, attenuated vaccines. For their own protection, people who have damaged or weakened immune systems—because they've undergone chemotherapy or have HIV, for example—cannot be given live vaccines.

Another limitation is that live, attenuated vaccines usually need to be refrigerated to stay potent. If the vaccine needs to be shipped overseas and stored by health care workers in developing countries that lack widespread refrigeration, a live vaccine may not be the best choice.

Live, attenuated vaccines are relatively easy to create for certain viruses. Vaccines against measles, mumps, and chickenpox, for example, are made by this method. Viruses are simple microbes containing a small number of genes, and scientists can therefore more readily control their characteristics. Viruses often are attenuated through a method of growing generations of them in cells in which they do not reproduce very well. This hostile environment takes the fight out of viruses: As they evolve to adapt to the new environment, they become weaker with respect to their natural host, human beings.

Live, attenuated vaccines are more difficult to create for bacteria. Bacteria have thousands of genes and thus are much harder to control. Scientists working on a live vaccine for a bacterium, however, might be able to use recombinant DNA technology to remove several key genes. This approach has been used to create a vaccine against the bacterium that causes cholera, *Vibrio cholerae*, although the live cholera vaccine has not been licensed in the United States.

### 2. Inactivated Vaccines

Scientists produce inactivated vaccines by killing the disease-causing microbe with chemicals, heat, or radiation. Such vaccines are more stable and safer than live vaccines: The dead microbes can't mutate back to their disease-causing state. Inactivated vaccines

usually don't require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them accessible to people in developing countries.

Most inactivated vaccines, however, stimulate a weaker immune system response than do live vaccines. So it would likely take several additional doses, or booster shots, to maintain a person's immunity. This could be a drawback in areas where people don't have regular access to health care and can't get booster shots on time.

### **3. Subunit Vaccines**

Instead of the entire microbe, subunit vaccines include only the antigens that best stimulate the immune system. In some cases, these vaccines use epitopes—the very specific parts of the antigen that antibodies or T cells recognize and bind to. Because subunit vaccines contain only the essential antigens and not all the other molecules that make up the microbe, the chances of adverse reactions to the vaccine are lower.

Subunit vaccines can contain anywhere from 1 to 20 or more antigens. Of course, identifying which antigens best stimulate the immune system is a tricky, time-consuming process. Once scientists do that, however, they can make subunit vaccines in one of two ways:

- They can grow the microbe in the laboratory and then use chemicals to break it apart and gather the important antigens.
- They can manufacture the antigen molecules from the microbe using recombinant DNA technology. Vaccines produced this way are called “recombinant subunit vaccines.”

A recombinant subunit vaccine has been made for the hepatitis B virus. Scientists inserted hepatitis B genes that code for important antigens into common baker's yeast. The yeast then produced the antigens, which the scientists collected and purified for use in the vaccine. Research is continuing on a recombinant subunit vaccine against hepatitis C virus.

### **4. Toxoid Vaccines**

For bacteria that secrete toxins, or harmful chemicals, a toxoid vaccine might be the answer. These vaccines are used when a bacterial toxin is the main cause of illness. Scientists have found that they can inactivate toxins by treating them with formalin, a solution of formaldehyde and sterilized water. Such “detoxified” toxins, called toxoids, are safe for use in vaccines.

When the immune system receives a vaccine containing a harmless toxoid, it learns how to fight off the natural toxin. The immune system produces antibodies that lock onto and block the toxin. Vaccines against diphtheria and tetanus are examples of toxoid vaccines.

### **5. Conjugate Vaccines**

If a bacterium possesses an outer coating of sugar molecules called polysaccharides, as many harmful bacteria do, researchers may try making a conjugate vaccine for it. Polysaccharide coatings disguise a bacterium's antigens so that the immature immune systems of infants and younger children can't recognize or respond to them. Conjugate vaccines, a special type of subunit vaccine, get around this problem.

When making a conjugate vaccine, scientists link antigens or toxoids from a microbe that an infant's immune system can recognize to the polysaccharides. The linkage helps the immature immune system react to polysaccharide coatings and defend against the disease-causing bacterium.

The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.

## **6. DNA Vaccines**

Once the genes from a microbe have been analyzed, scientists could attempt to create a DNA vaccine against it.

Still in the experimental stages, these vaccines show great promise, and several types are being tested in humans. DNA vaccines take immunization to a new technological level. These vaccines dispense with both the whole organism and its parts and get right down to the essentials: the microbe's genetic material. In particular, DNA vaccines use the genes that code for those all-important antigens.

Researchers have found that when the genes for a microbe's antigens are introduced into the body, some cells will take up that DNA. The DNA then instructs those cells to make the antigen molecules. The cells secrete the antigens and display them on their surfaces. In other words, the body's own cells become vaccine-making factories, creating the antigens necessary to stimulate the immune system.

A DNA vaccine against a microbe would evoke a strong antibody response to the free-floating antigen secreted by cells, and the vaccine also would stimulate a strong cellular response against the microbial antigens displayed on cell surfaces. The DNA vaccine couldn't cause the disease because it wouldn't contain the microbe, just copies of a few of its genes. In addition, DNA vaccines are relatively easy and inexpensive to design and produce.

So-called naked DNA vaccines consist of DNA that is administered directly into the body. These vaccines can be administered with a needle and syringe or with a needle-less device that uses high-pressure gas to shoot microscopic gold particles coated with DNA directly into cells. Sometimes, the DNA is mixed with molecules that facilitate its uptake by the body's cells. Naked DNA vaccines being tested in humans include those against the viruses that cause influenza and herpes.

## **7. Recombinant Vector Vaccines**

Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector" refers to the virus or bacterium used as the carrier.

In nature, viruses latch on to cells and inject their genetic material into them. In the lab, scientists have taken advantage of this process. They have figured out how to take the roomy genomes of certain harmless or attenuated viruses and insert portions of the genetic material from other microbes into them. The carrier viruses then ferry that microbial DNA to cells. Recombinant vector vaccines closely mimic a natural infection and therefore do a good job of stimulating the immune system.

## **6.2. VACCINATION SCHEDULE**

A **vaccination schedule** is a recommended series of vaccinations including the suggested timing of all doses. A vaccine is an antigenic preparation used to produce active immunity to a disease, in order to prevent or reduce the effects of infection by any natural or 'wild' pathogen. Many vaccines require multiple doses for maximum effectiveness, either to produce sufficient initial immune response or to boost response that fades over time. Vaccine schedules are developed by governmental agencies or physicians groups to achieve maximum effectiveness using required and recommended vaccines for a locality while minimizing the number of health care system interactions. Over the past two decades, the recommended vaccination schedule has grown rapidly and become more complicated as many new vaccines have been developed.

### **Types of Vaccination schedule**

Vaccination carried out in 2 ways namely routine vaccination and vaccination under special circumstances.

## 1. Routine Vaccinization

Routine vaccinization is the fixed and regular way of doing immunization. The children are protected from diseases like polio, tetanus, whooping caough, small pox etc., by subjected to routine immunization. The WHO (World Health Organisation) in the year 1970 has given a schedule of immunization for children. This schedule is carried out in all the countries (Table 2).

Table 2. Immunization schedule for children.

S.No.	Age	Vaccination
1.	0-1 Month	Smallpox and BCG vaccinations
2.	3 Month	DPT (adjesvanted) oral Polio (1st dose) (Small pox vaccination if not given at 0-1 Month)
3.	4 Month	Oral Polio (2 <sup>nd</sup> dose)
4.	5 Month	Oral Polio (3 <sup>rd</sup> dose) (2 <sup>nd</sup> dose) (BCG vaccination, if not given at 0-1 Month)
5.	1 Year	Oral Polio – booster SmallPox revaccination (if given at 0-1 Month)
6.	2 years	Booster DPT and Oral Polio Vaccine
7.	5-6 Years	Typhoid vaccine ( one dose) Booster tetanus toxoid (combined) BCG revaccination.
8.	11-12 Years (primary school leaving)	Small pox revaccination Booster tetanus toxoid and Typhoid vaccine (Combined).

## 2. Vaccination on special circumstances:

Sudden outbreaks of certain diseases prevented by mass vaccination in the affected areas. For example during flood, there is possibility for the outbreak of cholera. In such cases cholera vaccines are given.

Similarly vaccinations for plague, typhoid fever, influenza, yellow fever, etc. given on certain occasion.

When mass camps are conducted, the participants are given vaccinations against infections diseases.

People are traveling by air, sea and land to all parts of the world for commercial cultural and recreational purposes. These international travelers are usually immunized against diseases, like small box, yellow fever, cholera and plague.

## 6.3. VACCINATION AND SEROTHERAPY

### VACCINATION

Vaccination is the injection of vaccines into the body to produce immunity and to protect against diseases. Jenner introduced vaccination in 1776 using cow pox to protect against small pox.

### SEROTHERAPY

The treatment of infectious disease by the injection of serum obtained from an immunized animal or antitoxin.

### Serum Antibody Products

Serum antibody products as the name implies, derived from serum, the fraction of whole blood that contains the disease-fighting proteins known as antibodies. Antibodies are produced by normal animals in response to an antigen (in our case, bacteria or bacterial toxins) and are very specific for that antigen. An antigen, in the presence of its specific antibodies is destroyed or neutralized. The end result is an animal immune to that antigen.

One way to produce antibodies (immunity) is to vaccinate, but that takes time. An alternate method to produce immunity is to provide the actual antibodies in a ready-to-use format, or a serum antibody product.

Like the cavalry unit that storms over the hill to save the day, a serum antibody product provides immediate treatment or prevention of a specific threat, like bacteria or a toxin capable of causing disease in the animal. This type of immunity is known as passive immunity. Colostrum is another example of passive immunity as opposed to active immunity produced by vaccines that require a minimum of two to four weeks to produce an effective immunity. Active immunity is like the soldiers holding the fort and always ready to mobilize against an enemy threat.

Advantages of a serum antibody product are of course its immediate response. It can be used for treatment or prevention (vaccines are for prevention). Bacterial resistance is not an issue and there are no drug residues. It will not interfere with antibiotic therapy. Derived from live animals, it is a natural product (thimerosal and phenol are added as preservatives).

Serum antibody products can be used as an immediate, but temporary prevention of a disease or as a treatment of a current disease. It can be used to supplement inadequate consumption or quality of colostrums. A producer can supplement or support an antibiotic treatment or treat animals that are not responding to treatment. They can also be used in the face of an epizootic (animal version of an outbreak).

Remember the protection will be short-term, lasting only about ten to fourteen days. For long-term protection, vaccination should follow about two to three weeks after the use of a serum antibody product. As always, read and follow the label directions.

At Colorado Serum Company, we provide four serum antibody products.

### **Bovi-Serum Sera Antibodies**

- For the treatment or immediate, temporary prevention of respiratory (pneumonia, shipping fever) and enteric (scours) conditions caused by *Actinomyces pyogenes*, *Escherichia coli* (including K99), *Mannheimia haemolytica* Types 1 & 2, *Pasteurella multocida* Type A, and *Salmonella typhimurium*.
- Licensed for use in cattle and sheep.
- For subcutaneous or intramuscular administration.
- Available in 20 ml, 250 ml, and 1000 ml sizes.
- Available in 20 ml, 250 ml, and 1000 ml sizes

**INDICATIONS:** For use as an aid in the prevention and treatment of enteric and respiratory conditions caused by the micro-organisms named.

**DIRECTIONS:** Store at 2° to 7° C. Do not freeze. Shake well before use. Use entire contents when first opened. Do not vaccinate within 21 days before slaughter.

**PRECAUTIONS:** Anaphylactoid reaction may occur following administration of products of this nature. If noted, administer adrenalin or equivalent.

**DOSAGE AND ADMINISTRATION:** Inject subcutaneously or intramuscularly and repeat according to judgment of user. Administer at 12-24 hour intervals until improvement is noted. Use multiple sites or IV for large doses.

It is recommended to limit injections to no more than 10ml per injection site.

	CALVES	CATTLE	SHEEP	
For Prevention:	20 to 40 ml	50 to 75 ml	10 to 15 ml	Provides immediate and short-term protection lasting 7-21 days
For Treatment:	40 to 100 ml	75 to 150 ml	20 to 40 ml	Can be used in conjunction with antibiotic treatments

**Veterinary vaccination-Respiragen Serum Antibodies**

- For the treatment or immediate, temporary prevention of respiratory (pneumonia, shipping fever) and enteric (scours) conditions caused by *Actinomyces pyogenes*, *Mannheimia haemolytica* Types 1 & 2, *Pasteurella multocida* Type A, and *Salmonella typhimurium*.
- Licensed for use in cattle and sheep.
- For subcutaneous or intramuscular administration.
- Available in 100ml and 250 ml sizes.

INDICATIONS: For use as an aid in the prevention and treatment of diseases in calves, cattle, and sheep due to the organisms named in the formulation which are often associated with Shipping Fever, Hemorrhagic Septicemia, and Pasteurellosis.

Donor cattle are hyperimmunized with *Arcanobacterium pyogenes*, *Mannheimia haemolytica* Types 1 and 2, and *Pasteurella multocida* Type A (bovine). They also received repeated injections of Bovine rhinotracheitis, Virus diarrhea and Parainfluenza<sub>3</sub> viruses.

DIRECTIONS: Store at 2° to 7° C. Do not freeze. Shake well before use. Use entire contents when first opened. Do not vaccinate within 21 days before slaughter.

PRECAUTIONS: Anaphylactoid reaction may occur following administration of products of this nature. If noted, administer adrenalin or equivalent.

Cattle should not be vaccinated with injectable Bovine rhinotracheitis, Virus diarrhea, or Parainfluenza<sub>3</sub> for 21 days after administration of Respiragen. DOSAGE AND ADMINISTRATION: Inject subcutaneously or intramuscularly. Dosage may be repeated according to the judgment of the user.

Use multiple sites or IV for large doses. It is recommended to limit injections to no more than 10 ml per injection site.

Prevention: Provides immediate and short-term protection lasting 7-21 days	
Calves	20-40 ml as soon after birth as possible
Cattle	50-75 ml
Sheep	10-15 ml

## 7. ANTIBODY

Antibody is otherwise called as immunoglobulin. It is abbreviated Ig. It is present in blood. It is a protein and it is formed in response to an antigen when it enters the body. For example, proteins extracted from a mouse are injected into another animal, say a rabbit. The rabbit will recognize these proteins as foreign and produce specific antibodies to protect itself. Antibodies are produced by vertebrates only. They are synthesized by lymphocytes and plasma cells.

Immunoglobulins generally assume one of two roles: immunoglobulins may act as i) plasma membrane bound antigen receptors on the surface of a B-cell or ii) as antibodies free in cellular fluids functioning to intercept and eliminate antigenic determinants. In either role, antibody function is intimately related to its structure and this page will introduce immunoglobulins (antibodies) and relate their structure to their function in host defense.

### 7.1 PRIMARY STRUCTURE OF IMMUNOGLOBULIN

Rodney Porter (1962) proposed the basic structure of immunoglobulin. Molecular weight is 150,000. Immunoglobulin is a glycoprotein. It is Y-shaped. It is made up of 4 polypeptide chains. Of these two chains are short they are called light chains (L-chains) and they are identical. The other two chains are longer. They are called heavy chains (H-chains) and they are identical (Figure 28).

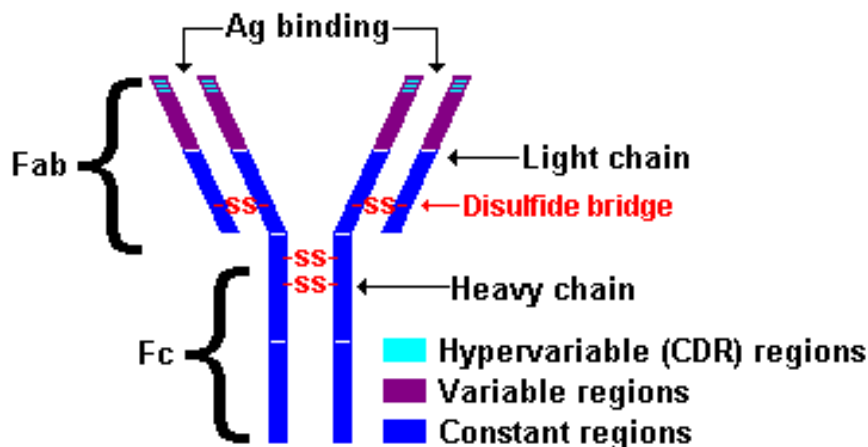


Figure 28. Basic structure of immunoglobulin

Immunoglobulins are composed of four polypeptide chains: two "light" chains (lambda or kappa), and two "heavy" chains (alpha, delta, gamma, epsilon or mu). The type of heavy chain determines the immunoglobulin isotype (IgA, IgD, IgG, IgE, IgM, respectively).

**Light chains** are composed of 220 amino acid residues while heavy chains are composed of 440-550 amino acids. Each chain has "constant" and "variable" regions as shown in the figure. Each light chain is linked to a heavy chain by a single interchain disulfide bond.

**Variable regions** are contained within the amino (NH<sub>2</sub>) terminal end of the polypeptide chain (amino acids 1-110). When comparing one antibody to another, these amino acid sequences are quite distinct.

**Constant regions**, comprising amino acids 111-220 (or 440-550), are rather uniform, in comparison, from one antibody to another, within the same isotype.

**"Hypervariable" regions**, or "Complementarity Determining Regions" (CDRs) are found within the variable regions of both the heavy and light chains. These regions serve to recognize and bind specifically to antigen. The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds. The **tertiary structure** of antibodies brings the 3

hypervariable regions of **both** the L and the H chains together. Together they construct the **antigen binding site** against which the epitope fits. For this reason, the hypervariable regions are also called **complementarity determining regions**

The variable regions contain highly variable zones called hypervariable regions (HV) or hot spots. Three such regions exist in each VL domain and four in each VH domain. These regions are actually involved in the **antigen binding site** and they are called the **idiotypic regions**.

## 7.2. CLASSES OF ANTIBODY

There are five classes or isotypes of immunoglobulins in the human serum and they are named based on the nature of heavy chains they have. They are IgG, IgM, IgD, IgA and IgE (Figure 29). They carry out different functions and are directed to different parts of the body.

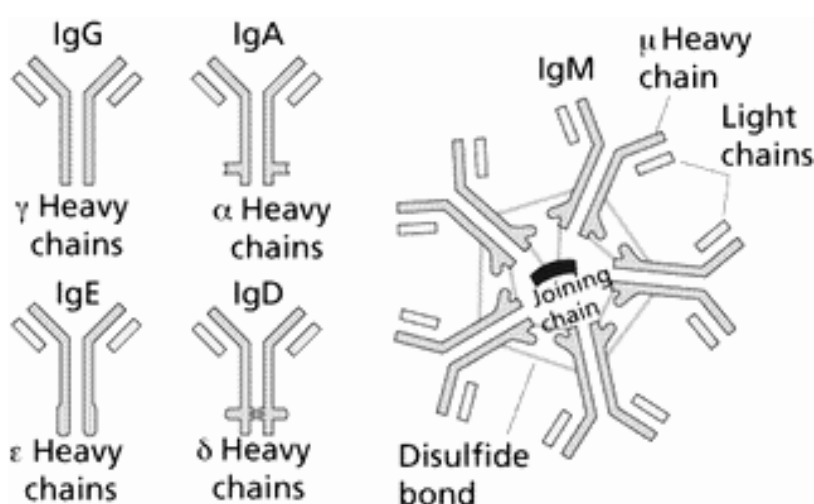


Figure 29. Diagram of different classes of antibodies.

**IgM** – The first antibody that is produced in the immune response and it activates complement. It also has low affinity which means the strength of binding between the antibody and antigen is low. B cell may switch to other Ig classes later that will carry out other functions.

**IgG** – It is the major class of Ig in the blood and it can cross the placenta to provide passive immunity to the fetus. There are 4 subclasses of IgG. IgG 1-4 are involved in neutralisation. IgG1 is involved in opsonisation and IgG 3 is able to activate complement.

**IgA** – It protects mucosal surfaces (eg: respiratory tract). It is also most abundant Ig. It is found in tears, mucous, saliva, sweat and breastmilk.

**IgE** – It has low concentrations in serum and binds to basophils, eosinophils and mast cells through their FcR. When these cells are activated, inflammation will occur. IgE and mast cells are involved in allergic reactions.

**IgD** – It is found on cell membrane of naive B cells (B cells that have not encountered antigen), co-expressed with IgM. Nevertheless, its function is unknown and barely found in blood.

Properties and functions of different classes of immunoglobulin are given in Table 3.

Table 3. Properties and functions of different classes of immunoglobulin.

Class	H chain	L chain	Subunits	mg/ml	Molecular weight	Functions
IgG	Gamma ( $\gamma$ )	kappa or lambda	$H_2L_2$	6–13	150000	Involved in opsonization,, Neutelize toxins Transferred across placenta
IgM	Mu ( $\mu$ )	kappa or lambda	$(H_2L_2)_5$	0.5–3	950000	first antibodies to appear after immunization, Bacterio lysis.
IgA	Alpha ( $\alpha$ )	kappa or lambda	$(H_2L_2)_2$	0.6–3	160000- 50000	Promote bacterolytic activity & phagocytosis
IgD	Delta ( $\delta$ )	kappa or lambda	$H_2L_2$	<0.14	180000	Act as antigen receptor on the B-cell surface.
IgE	Epsilon ( $\epsilon$ )	kappa or lambda	$H_2L_2$	<0.0004	190000	Involved in allergic reactions

### 7.3. FUNCTIONS OF IMMUNOGLOBULIN

The function of immunoglobulin can be understood by the study of a number of process.

Antibody molecules have two main functions to perform:

- recognize and bind to an epitope on an antigen
- trigger a useful response to the antigen

The division of labor is:

- V regions are responsible for epitope recognition
- C regions are responsible for triggering a useful response

So, V regions finger the culprit; the C regions take action.

Antibodies function in a variety of ways designed to eliminate the antigen that elicited their production. For example, an antibody might bind to a toxin and prevent that toxin from entering host cells where its biological effects would be activated. Similarly, a different antibody might bind to the surface of a virus and prevent that virus from entering its host cell. In contrast, other antibody functions are dependent upon the immunoglobulin class (isotype). These functions are contained within the constant regions of the molecule. For example, only IgG and IgM antibodies have the ability to interact with and initiate the complement cascade. Likewise, only IgG molecules can bind to the surface of macrophages via Fc receptors to promote and enhance phagocytosis. The following table summarizes some immunoglobulin properties.

They can prevent pathogens from entering or damaging cells by binding to them; they can stimulate removal of a pathogen by macrophages and other cells by coating the pathogen; and they can trigger direct pathogen destruction by stimulating other immune responses such as the complement pathway.

#### 1. Agglutination

It is an interaction of antigen binding sites of immunoglobulin molecule with the antigen determinants. This reaction leads to clumping of particulate antigen by antibody.

Of all the immunoglobulin molecules the IgM with its ten efficient antigens binding sites. (5X2 Fabs) becomes more effective in agglutinating antigens. The IgM produced against a particular bacterial antigen has been found to have more capacity to combine with bacteria. The appearance of clear agglutinates proves this character IgM antibody is larger. Therefore, it cannot pass into the tissue spaces and thereby it can stay for longer time in the circulation. Due to this it gets an important role in fighting against infecting agents and preventing microbial diseases.

## **2. Detoxification:**

The IgG molecules play an important role in detoxifying or neutralizing toxins produced by certain species like diphtheria and tetanus and polioviruses.

## **3. Opsonization:**

Opsonins are certain substances which enhance the process of phagocytosis. It is now known that immunoglobulin can combine with antigens present on the surface of the bacteria and act as opsonin to make the phagocytic process as an enhancing process. This process called as opsonization.

## **4. complement activation:**

Complement is a group of serum proteins that is activated by antibody antigen interaction and results in lysis of bacteria. In addition to agglutination and opsonization, the IgM molecules play an efficient role in activating the complement system. In the IgG molecule the FC part brings about the complement activation.

## **5. Selective Transport:**

Of the five major classes of immunoglobulins, the IgG and IgA have the ability to move selectively from one environment to the other. The FC portion in the IgG molecule is responsible for the selective transfer from mother to foetus across the placenta.

## **6. Antibody Avidity**

This term relates to how tightly an antibody binds to its target antigen. This is a very important functional characteristic of an antibody as antibody of high avidity is likely to be more effective than antibody of low avidity.

## **7.4. SYNTHESIS OF IMMUNOGLOBULIN**

### **(Cellular, Sub Cellular and Molecular)**

Antibody is an immunological missile produced against an antigen. It is and of serum protein called globulin. As the globulin is involved in immunological reactions the antibody is called on immunoglobulin.

Antibodies are synthesized in the secondary lymphoid organs such as spleen, lymph nodes. Bone-marrow and gut-associated lymphoid tissues.

### **Humoral mediated Immunity**

The body fights against the antigen by producing antibodies which are present in the fluids of the body namely plasma, lymph and tissue fluids, hence the immunity is called the antibody mediated immunity.

It involves the production of specific antibodies in response to the specific antigen that enters the body (Fig.1) The cellular basis for the synthesis effector and memory antibody forming cells.

### **Primary Immune Response**

The immune response produced by the body following its first encounter with an antigen is called the primary immune response, which has 4 phases (Figure 30).

### Lag phase

The phase immediately following the injection of an antigen during which no antibody is produced. It is also called latent or induction period. Duration – it varies from hours to days depending on many factors. Eg. 2-3 weeks with antigens such as diphtheria toxoid and with pneumococcal polysaccharide. It is only a few hrs.

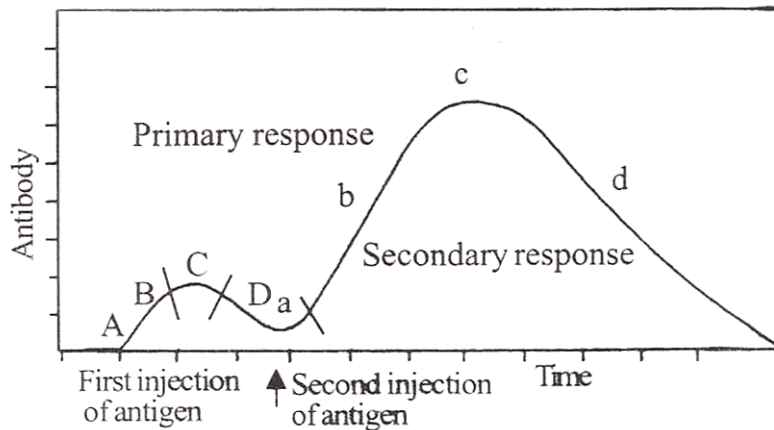


Figure 30. Primary and secondary responses.

### Log phase

When the antibody titre rises steadily it is known as log phase.

### Plateau phase

This is a period in which the antibody titre is stable i.e., without any increase or decrease.

### Decline phase

A phase in which the catabolism of antibodies exceeds that of production so that there is a fall in the antibody titre.

### Secondary Immune Response

If the same animal is subsequently exposed to the same antigen, the immune response produced differs from the primary immune response both qualitatively and quantitatively and it is called the secondary immune response.

The animal response is very quick; there is a very short lag phase. Following this, there is a marked increase in the antibody content of the blood, and ultimately the antibody level in the secondary response exceeds that of the primary. The sharp rise in the antibody titre is followed by a much extended plateau phase. This is followed by a decline phase.

### Synthesis of antibody by B cells (Cellular and subcellular basis of antibody synthesis)

The increase in the number of antibody titre of immunized animals shows B cells produced by bone marrow. They occur in lymphoid organs, spleen, B-cells enlarge and produce. The activated B cells may differ, that there is an increase in the number of cells engaged in the synthesis of antibody. An analysis of antibody production shows that there are at least a million different types of preformed B lymphocytes that are capable of forming the highly specific antibodies. Each one of these preformed B-lymphocytes is capable of forming only one type of antibody and can be activated by a specific antigen which can react with it and activate it. Once that specific B lymphocyte is activated by the specific antigen, it divides widely, forming a tremendous number of similar lymphocytes. This progeny of B lymphocytes will eventually secrete the same antibody. All these lymphocytes that are capable of forming one specific antibody are called a clone of lymphocytes, and immunization leads to the selective expansion of a clone of cells capable of producing a specific antibody cells (Figure 31).

The B cell destined to become an antibody forming cells (AFC) carries surface immunoglobulin determinants. These determinants serve as receptors for recognition and binding with a specific antigen. The sequence of changes that occur from the encounter of a B cell with the specific antigen up to the formation of specific antibody forming plasma cells and memory cells are as follows:

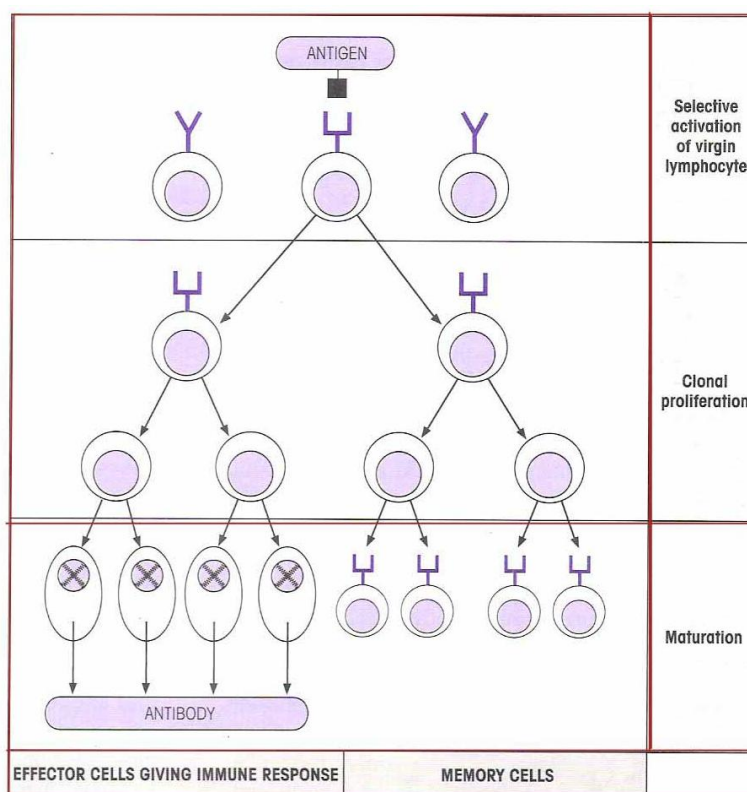


Figure 31. The cellular basis for the synthesis effector and memory antibody forming cells.

Processing and presentation of antigen by macrophages.

1. Triggering of the B cell.
2. Clonal proliferation
3. Differentiation and maturation into antibody forming plasma cell production of memory cells.

### Molecular events in activation of B cells

The B cells directly produce antibodies when exposed to large number of antigens. In this method bacterial lipopolysaccharides (TI-1; **Thymus-independent antigen 1**) or bacterial flagellin/ cell wall polysaccharides (TI-2) acts as an activator for B cells and both the signals are produced. Here T cells will not help the B cells hence these antigens are referred as thymus- independent antigens. In case of **thymus dependent** activation first signal produced by cross linking of surface immunoglobulins on surface of B cells by thymus dependent (TD) antigen and second one by the interaction with T helper cells through CD40 (on the B cell) and CD40L (on TH cells) with assistance by cytokines. Then the B cells directly produce antibodies.

Antibody synthesis occurs in plasma cells. It involves two steps namely transcription and translation.

During transcription, the message from DNA is transcribed to mRNA. The message from the mRNA is translated into polypeptide chain in the ribosome.

Two heavy polypeptide chains and two light polypeptide chains are assembled together to form an antibody molecule. After the linking of polypeptide chains, carbohydrate residues are attached to the antibody molecule.

The fully formed antibody molecules are released into the body fluid by the plasma cells. The released antibodies may remain free in the body fluid or bound to the surface of lymphocytes, polymorphs, monocytes, macrophages, mast cells and T cells.

## **8. MONOCLONAL ANTIBODIES AND THEIR APPLICATIONS**

The antibodies derived from a clone of cells are homogenous and they are called monoclonal antibodies. The two main sources of monoclonal antibodies are myelomas and hybridomas.

**Myelomas** are naturally occurring cancerous cells. They secrete antibodies of unknown specificity. They are derived from malignant B-lymphocytes.

**Hybridomas** are voluntarily produced from somatic hybrid cells. They secrete antibodies of known specificity. They are produced by fusing together malignant myeloma cells and B-cells.

Monoclonal antibodies are pure antibodies with a single specificity to a given antigen. They are monospecific.

Monoclonal antibodies are synthesized on a large scale by a hybrid cell called hybridoma. Hybridoma is produced by the fusion of a B cell and a myeloma cell. The hybridoma can produce a specific antibody continuously. The production of monoclonal antibodies by hybridomas is called **hybridoma technology**.

**Polyclonal Antibodies:** Antibodies derived from immunized man or animal are heterogenous and these are known as polyclonal antibodies. Immunization with serum albumin, a multivalent globular protein antigen, usually results in the production of antibodies with differing specificity.

### **Monoclonal Antibodies Production Technique**

Monoclonal antibodies can be obtained by fusing individual antibody forming cells with a B cells tumor to produce a constantly dividing clone of cells dedicated to making the one antibody.

A fantastic technological breakthrough was achieved by Milstein & Kohler who devised a technique for the production of "immortal" clones of cells making single antibody specificities by fusing normal antibody forming cells with an appropriate B cells tumor line. These so called "hybridomas" are selected out in a tissue culture medium which fails to support growth of the parental cell types and by successive dilutions or by plating out, single clone can be established (Figure 4).

It appears as antibodies in the serum. The spleen is removed and the individual cells fused (i.e. immortal) B tumor cells which are unable to secrete Ig. The resulting cells are distributed into micro well plates in HAT (hypoxanthine, aminopterin, thymidine) medium which kills off the fusion partners, at such a dilution that on average each well contains less than one hybridoma cell. Each hybridoma the fusion product of a single antibody-forming cell and a tumor cell will have the ability of the former to secrete a single species of antibody and the immortality of the latter enabling it to proliferate continuously. Thus clonal progeny can provide an unending supply of antibody with a single specificity the monoclonal antibody.

These clones can be grown up in the ascitic form in mice when quite prodigious titers of monoclonal antibody can be attained, but bearing in mind the imperative to avoid using animals wherever feasible, propagation in large-scale culture is to be preferred. Remember that even in a good antiserum, over 90% of the Ig molecules have little or no avidity for the antigen, and the 'specific antibodies' themselves represent a whole spectrum of molecules with different determinants on the antigen. What contrast is provided by the

monoclonal antibodies where all the molecules produced by a given hybridoma are identical they have the same Ig class and allotype, affinity and specificity for a given epitome.

The objective of fusing human B cells to make hybridomas (Figure 32) is still appealing. However, large numbers of human monoclonal have been established. Many human monoclonal are awaiting the go ahead for clinical use, one can cite IgG anti-RhD and highly potent monoclonal for protection against varicella Zoster, cytomegalovirus group B streptococci and lipopolysaccharide endotoxins of Gram-negative bacteria.

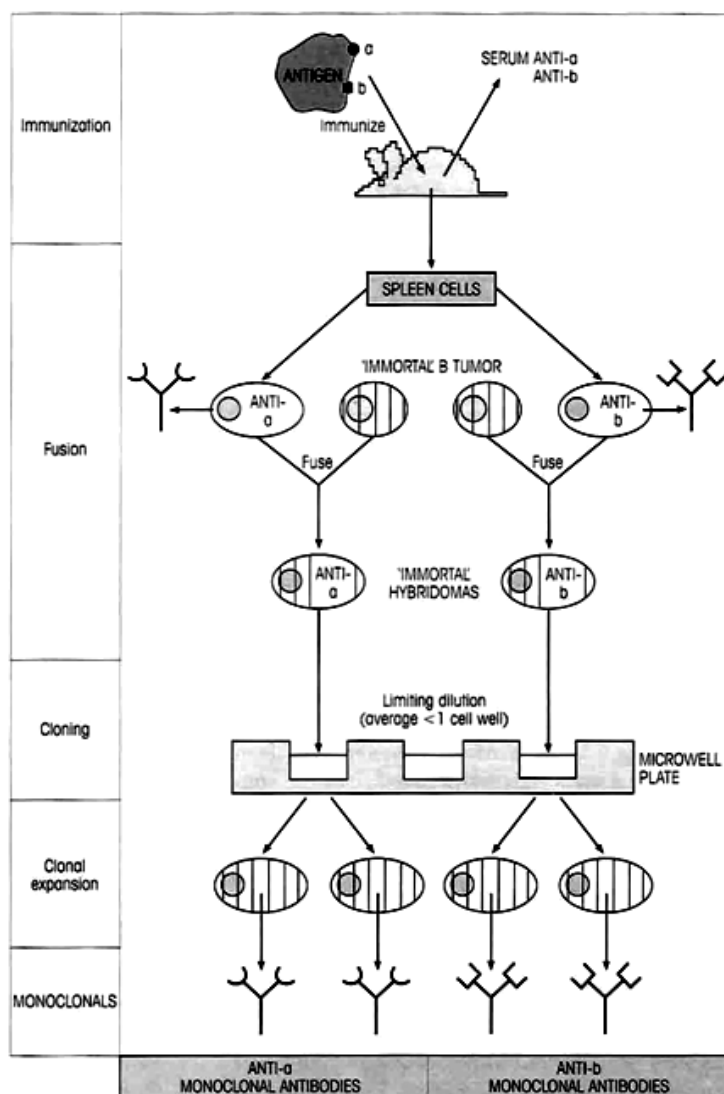


Figure 32. Production of monoclonal antibodies.

### Application of Monoclonal Antibody

1. They help in the diagnosis, treatment and prophylaxis of various clinical disorders.
2. They provide powerful means for the study of pathogenesis of many diseases specially those related to autoimmune and immune deficiency.
3. They are used to study the antigen-antibody specificity, antigen-antibody reactions etc.
4. They are of much use in diagnostic and research techniques.

## 9. GENETIC MECHANISMS IN GENERATION OF ANTIBODY DIVERSITY

The immune system has the capacity to recognise and respond to about  $10^7$  different antigens. This extreme diversity can be generated in at least three possible ways:

1. Multiple genes in the germ line DNA.
2. Variable recombination during the differentiation of germ line cells into B-cells.
3. Mutation during the differentiation of germ line cells into B-cells.

It is known that all three of these possibilities take place to produce antibody diversity. The following figures illustrate (Figure 33).

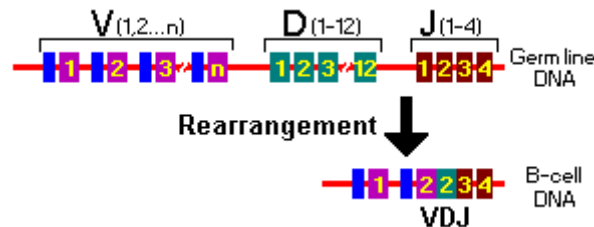


Figure 33. Multiple genes in the germ line DNA.

### Multiple genes

The figure 1 shows the genetic makeup of a germ line cell and a mature B-cell at the loci controlling heavy chain production. Germ line DNA has many (up to 200) different variable (V) region genes, in addition to 12 diversity (D) region genes and four joining (J) region genes. During differentiation of this cell into the B-cell, rearrangement of the DNA occurs. This rearrangement aligns one of the many V genes with one of the D genes and one of the J genes, producing a functional VDJ recombinant gene. Since any of the genes may recombine with any others, this rearrangement has the potential to generate  $200 \times 12 \times 4 = 9600$  different possible combinations. The same type of event occurs in the genes encoding the immunoglobulin light chains where about 200 different V regions may recombine with about 5 different J regions giving rise to  $200 \times 5 = 1000$  possible light chains. Since in any particular B-cell, any light chain combination can occur along with any heavy chain combination, the total possible immunoglobulin combinations approaches  $10^7$  ( $9600 \times 1000$ ).

### Recombination

A second way that diversity can result is through a process of variable or "inaccurate" recombination. The Figure 34 illustrates three possible recombination events between the variable (V) and joining (J) regions of an immunoglobulin light chain. In the first event, a proline-tryptophan dipeptide sequence is produced in the resulting protein. However, in the second and third events, differential recombination places proline-arginine or proline-proline sequences into the resulting immunoglobulin. These types of events may also occur between the V and D regions and the D and J regions of the heavy chain DNA sequence.

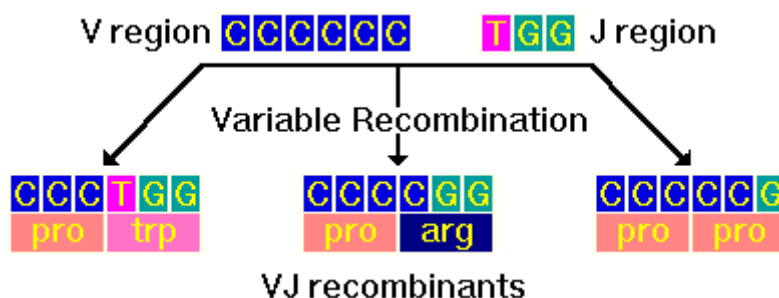


Figure 34. Variable recombination during the differentiation of germ line cells into B-cells.

## Mutation

A third way that diversity can result is through a process of mutation. This process simply involves changes in DNA sequence that occur during differentiation of the B-cell. The figure 35 illustrates how an A:T to G:C transition mutation could change a serine residue into a glycine residue in the resulting immunoglobulin. This process may, in part, explain the diversity observed in hypervariable (CDR) regions.

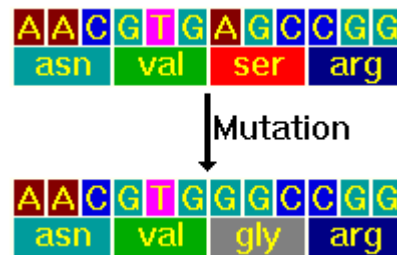


Figure 35. Mutation during the differentiation of germ line cells into B-cells.

## IMMUNOGLOBULIN PRODUCTION

The production of immunoglobulins by B-cells or plasma cells occurs in different stages. During differentiation of the B-cells from precursor stem cells, rearrangement, recombination and mutation of the immunoglobulin V, D, and J regions occurs to produce functional VJ (light chain) and VDJ (heavy chain) genes. At this point, the antigen specificity of the mature B-cell has been determined. Each cell can make only one heavy chain and one light chain, although the isotype of the heavy chain may change. Initially, a mature B-cell will produce primarily IgD (and some membrane IgM) that will migrate to the cell surface to act as the antigen receptor. Upon stimulation by antigen, the B-cell will differentiate into a plasma cell expressing large amounts of secreted IgM. Some cells will undergo a "class switch" during which a rearrangement of the DNA will occur, placing the VDJ gene next to the genes encoding the IgG, IgE or IgA constant regions. Upon secondary induction (i.e. the secondary response), these B-cells will differentiate into plasma cells expressing the new isotype. Most commonly, this results in a switch from IgM (primary response) to IgG (secondary response). The factors that lead to production of IgE or IgA instead of IgG are not well understood.

## 10. SOMATIC MUTATION

The passage of time after immunization there is usually a progressive increase in the affinity of the antibodies produced against the immunizing antigen. This phenomenon, known as affinity maturation, is unique to antibodies and is due to the accumulation of **point mutations** specifically in both heavy- and light-chain V-region coding sequences. These mutations occur long after the coding regions have been assembled, when **B cells are stimulated** by antigen and helper T cells **to generate memory cells** in the activated center (so-called *germinal center*) of a lymphoid follicle in secondary lymphoid organs (Figure 36).

The point mutations occur at the rate of about one mutation per V-region coding sequence per cell generation, which is about a million times greater than the spontaneous mutation rate in other genes; hence, the process is called somatic hypermutation. The mechanism that allows the nucleotide changes to be targeted to the DNA of a precisely specified part of the genome in this way is not known.

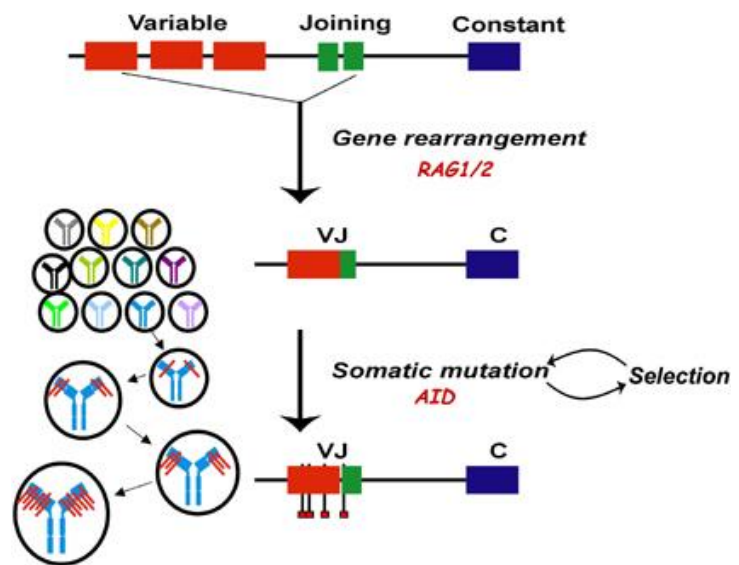


Figure 36 . Somatic mutation.

Only a small minority of these point mutations will result in antigen receptors that have an increased affinity for the antigen. The few B cells expressing these high-affinity receptors, however, will be preferentially stimulated by antigen to survive and proliferate, while the other B cells will undergo programmed cell death. Thus, as a result of repeated cycles of somatic hypermutation followed by antigen-driven selection, antibodies of increasingly higher affinity are produced during the course of an immune response, providing progressively better protection against harmful antigens.

Mutations that occur in V genes of heavy and/or light chains during the lifetime of a B cell also increase the variety of antibodies produced by the B cell population. Generally, an antibody of low affinity is produced in the primary response to antigen. DNA and polypeptide sequencing of antibodies formed in the primary response indicate that the sequences closely match the sequences encoded by germ line DNA. As the response matures, however, and especially after secondary stimulation by the antigen, the affinity for antigen of the antibodies synthesized increases, and the amino acid sequences of these antibodies diverge from those coded for in the germ line DNA.

The mechanism that generates antibody diversity occurs in the mature B cell. Following activation with antigen, B cells begin to proliferate rapidly. In these rapidly dividing cells, the genes encoding the variable domains of the heavy and light chains undergo a high rate of point mutation, by a process called **somatic hypermutation (SHM)**.

This divergence results predominantly from point mutations in the VDJ recombined unit of antibody V genes, which result in changes in individual amino acids. This phenomenon is referred to as **somatic hypermutation** because it occurs at a rate at least 10,000 - fold higher than the normal rate of mutation. Somatic hypermutation results in the observed increased affinity of antibodies for antigen in the secondary response. As a consequence of this fine-tuning of the immune response, somatic hypermutation increases the variety of antibodies produced by the B cell population. The evidence suggests that there is a narrow window for somatic hypermutation to occur—that is, after antigenic stimulation in the germinal centers of spleen and lymph node.

## 11. CLASS SWITCHING

During B cell development many B cells switch from making one class of antibody to making another - a process called class switching. All B cells begin their antibody-synthesizing lives by making IgM molecules and inserting them into the plasma membrane as receptors for antigen. Before they have interacted with antigen, many B cells then switch and make both IgM and IgD molecules as membrane-bound antigen receptors. Upon stimulation by antigen, some of these cells are activated to secrete IgM antibodies, which dominate the primary antibody response. Other antigen-stimulated cells switch to making IgG, IgE, or IgA antibodies; memory cells express one of these three classes of molecules on their surface, while activated B cells secrete them. The IgG, IgE, and IgA molecules are collectively referred to as *secondary* classes of antibodies because they are thought to be produced only after antigen stimulation and because they dominate secondary antibody responses. As we saw earlier, these different classes of antibodies are each specialized to attack microorganisms in different ways and in different sites.

Since the class of an antibody is determined by the constant region of its heavy chain, the fact that B cells can switch the class of antibody they make without changing the antigen-binding site implies that the same assembled  $V_H$ -region coding sequence (which specifies the antigen-binding part of the heavy chain) can sequentially associate with different  $C_H$  gene segments. This has important functional implications. It means that in an individual animal a particular antigen-binding site that has been selected by environmental antigens can be distributed among the various classes of immunoglobulin and thereby acquire the different biological properties characteristic of each class.

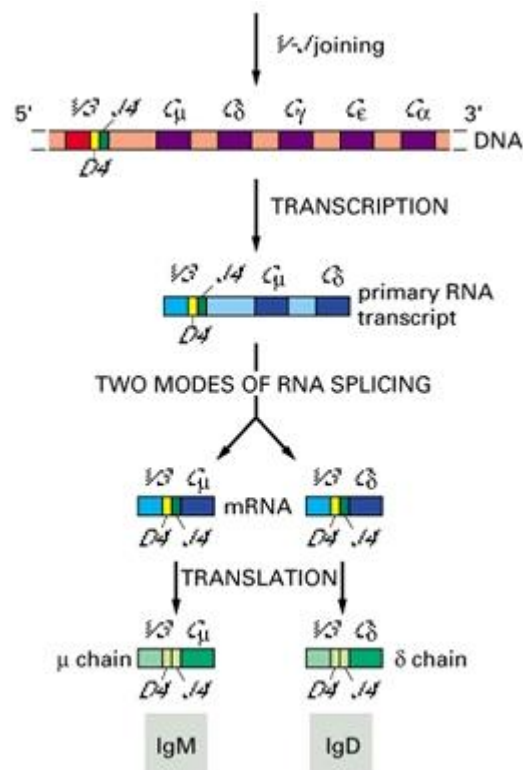


Figure 37. Simultaneous synthesis of IgM and IgD

Class switching occurs by at least two distinct molecular mechanisms. When virgin B cells change from making membrane-bound IgM alone to the simultaneous production of membrane-bound IgM and IgD, the switch is thought to be due to a change in RNA processing. The cells produce large primary RNA transcripts that contain the assembled  $V_H$ -region coding sequence along with both the  $C_\mu$  and  $C_\delta$  sequences; IgM and IgD molecules are then produced by differential splicing of these RNA transcripts (Figure 37).

By contrast, terminal maturation to an activated B cell that secretes one of the secondary classes of antibody is accompanied by an irreversible change at the DNA level - a process called *class switch recombination*. It entails deletion of all the  $C_H$  gene segments upstream (that is, on the 5' side as measured on the coding strand) of the particular  $C_H$  segment the cell is destined to express. Evidence that this step in class switching involves DNA deletion comes from experiments on myeloma cells: myeloma cells that secrete IgG lack the DNA coding for  $C_{\mu}$  and  $C_{\delta}$  regions, and those that secrete IgA lack the DNA coding for all of the other classes of heavy-chain C regions.

Isotype or class switching is a biological process occurring after activation of the B cell, which allows the cell to produce different classes of antibody (IgA, IgE, or IgG). The different classes of antibody, and thus effector functions, are defined by the constant (C) regions of the immunoglobulin heavy chain. Initially, native B cells express only cell-surface IgM and IgD with identical antigen binding regions. Each isotype is adapted for a distinct function, therefore, after activation, an antibody with a IgG, IgA, or IgE effector function might be required to effectively eliminate an antigen. Class switching allows different daughter cells from the same activated B cell to produce antibodies of different isotypes. Only the constant region of the antibody heavy chain changes during class switching; the variable regions, and therefore antigen specificity, remain unchanged. Thus the progeny of a single B cell can produce antibodies, all specific for the same antigen, but with the ability to produce the effector function appropriate for each antigenic challenge. Class switching is triggered by cytokines; the isotype generated depends on which cytokines are present in the B cell environment.

Class switching occurs in the heavy chain gene locus by a mechanism called class switch recombination (CSR). This mechanism relies on conserved nucleotide motifs, called *switch (S) regions*, found in DNA upstream of each constant region gene (except in the  $\delta$ -chain). The DNA strand is broken by the activity of a series of enzymes at two selected S-regions. The variable domain exon is rejoined through a process called non-homologous end joining (NHEJ) to the desired constant region ( $\gamma$ ,  $\alpha$  or  $\epsilon$ ). This process results in an immunoglobulin gene that encodes an antibody of a different isotype.

As we have described above, one B cell makes antibody of just a single specificity that is fixed by the nature of VJ (L chain) and VDJ (H chain) rearrangements. These rearrangements occur in the absence of antigen in the early stages of B cell differentiation. We have also described how a single B cell can synthesize IgM and IgD with the same antigenic specificity. In the paragraphs that follow, we show how an individual B cell can switch to make a different class of antibody, such as IgG, IgE, or IgA. This phenomenon is known as **class or isotype switching**. Class switching changes the effector function of the B cell but does not change the cell's antigenic specificity.

Class switching occurs in antigen-stimulated mature B cells synthesizing IgM and IgD and involves further DNA rearrangement, juxtaposing the rearranged VDJ genes with a different heavy-chain C region gene (Figure 38).

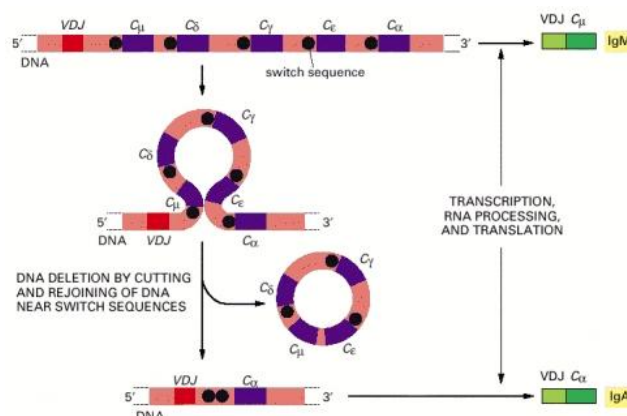


Figure 38. A schematic diagram of class switching in the of heavy chain genes.

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

A B cell making an IgM antibody from an assembled VDJ DNA sequence is stimulated by antigen and the cytokines made by helper T cells to switch to making an IgA antibody. In the process, it deletes the DNA between the VDJ sequence and the C<sub>α</sub>-coding sequence. Specific DNA sequences (switch sequences) located upstream of each C<sub>H</sub>-coding sequence recombine with each other to delete the intervening DNA. Class switch recombination is thought to be mediated by a switch recombinase, which is directed to the appropriate switch sequences when these become accessible under the influence of cytokines, as we discuss later.

In addition to antigen, class switching is dependent on the presence of factors known as **cytokines** released by T cells. There is little or no class switching by B cells in the absence of such T cell-derived cytokines.

The cytokines that affect class switching induce further rearrangement of B cell DNA and produce switching to other Ig classes in a downstream progression (e.g., to IgG<sub>4</sub> or IgE). Thus a single B cell with a unique specificity is capable of making an antibody of all possible classes, depending on the switches occurring in the DNA coding for its H chain.

The mechanism by which mature B cells undergo class switching is shown in Figure At H chain C region (C<sub>H</sub>) gene, apart from C<sub>δ</sub>, is a stretch of repeating base sequences called a **switch (S) region**. This S region permits any of the C<sub>H</sub> genes (other than C<sub>δ</sub>) to associate with the VDJ unit; in the figure, only the C<sub>H</sub> genes λ<sub>1</sub>, λ<sub>2</sub> and α<sub>2</sub> are shown, but other C<sub>H</sub> genes may also be used. Under the stimulating influence of antigen and T cell-derived cytokines a B cell with a VDJ unit linked to C<sub>μ</sub> and C<sub>δ</sub> further rearranges its DNA to link the VDJ to an S region in front of another C<sub>H</sub> region gene (γ<sub>1</sub>, in Fig.). After a primary RNA transcript is made from the rearranged DNA, the introns are spliced out to give a mRNA coding for the IgG i H chain. In so doing, the intervening C region DNA is removed. Thus, at this stage. The cell loses its ability to revert to making a class of antibody whose C region gene has been deleted (IgM, IgD, or IgG<sub>3</sub>, in this example).

Class switching is a mechanism unique to Ig H chains of B cells. It allows an antibody with a single antigenic specificity to associate with a variety of different constant region chains and thus have different effector functions. For example, an antibody with a VDJ unit specific for a bacterial antigen may be linked to C<sub>γ</sub> to produce an IgG molecule; this IgG antibody interacts with cells such as macrophages that express receptors for Fc<sub>γ</sub>. Alternatively, the same VDJ unit may be linked to C<sub>ε</sub> to produce an IgE molecule, IgE antibody interacts with cells such as mast cells that express receptors for Fc<sub>ε</sub>.

Cytokines present when antigen activates B cells play a key role in CH gene selection during isotype switching. For example, in the presence of the cytokine interferon-γ, the B cell can rearrange its VDJ to the C<sub>γ2</sub> heavy chain, and the cell switches to IgG<sub>2</sub> synthesis. By contrast, in the presence of the cytokine interleukin-4 (IL-4), a B cell can rearrange its VDJ C<sub>γ4</sub>, and the cell switches to IgG<sub>4</sub> or IgE synthesis, respectively. Each cytokine is thought to loosen the structure of the DNA double helix at only certain points along the Ig locus, allowing an enzyme known as a "switch recombinase" to recognize DNA coding for specific C regions.

## 12. ALLELIC EXCLUSION

Allelic exclusion is a process by which only one allele of a gene is expressed while the other allele is silenced.

For most genes, the individual inherits one copy of each gene from each parent. Each copy of the gene is called an allele.

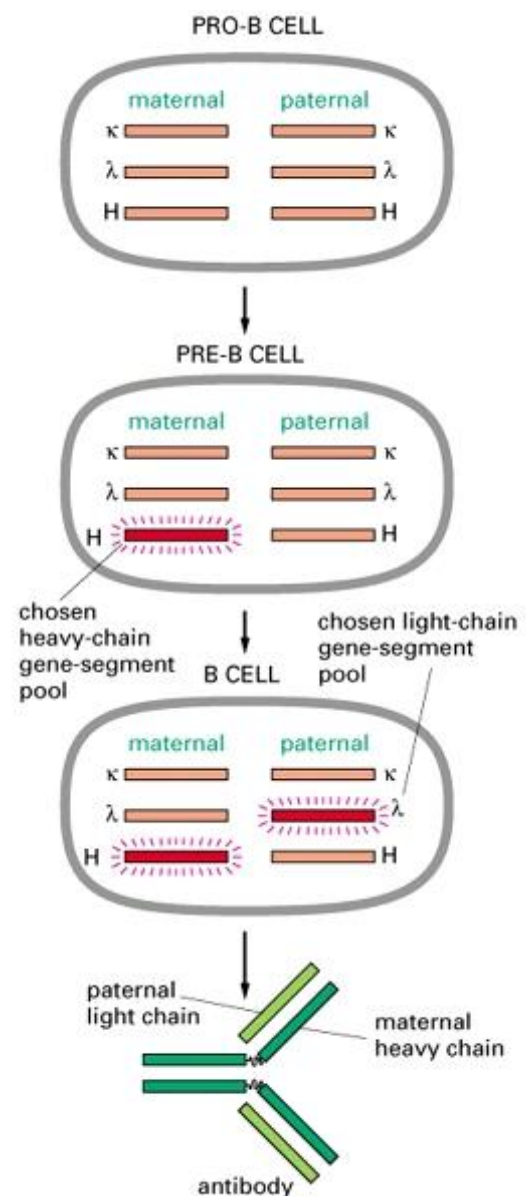
Allelic exclusion has been observed most often in genes for cell surface receptors and has been extensively studied in immune cells such as B lymphocytes. In B lymphocytes, successful heavy chain gene rearrangement on one chromosome results in the shutting down of rearrangement on the second chromosome. If no successful rearrangement occurs, rearrangement takes place on the second chromosome. If no successful rearrangement occurs on either chromosome, the cell dies. As a result of allelic exclusion, all the antigen receptors on an individually lymphocyte will have the same amino acid sequence in the variable domain of the heavy chain protein. As the specificity of the antigen receptor is modulated by the variable domain of the light chain encoded by one of the immunoglobulin light chain loci, the specificities of B cells containing the same heavy chain recombination event can differ according to their light chain recombination event.

The mechanism by which allelic exclusion occurs is not fully understood.

At least two distinct selection events can lead to allelic exclusion. On one hand, one allele of the gene can be transcriptionally silent, which would result in the expression of only the second allele. On the other hand, both alleles can be transcribed, in which case posttranscriptional and posttranslational mechanisms will lead to the elimination of the protein product of one allele.

Theoretically, any one B cell has many genes from which to choose to synthesize an Ig molecule: multiple V, D, and J genes to form the variable regions, and different genes for the light chains, K and  $\lambda$ . In reality, each B cell uses only one set of VDJ genes and one type of light chain. As a result, a single B cell produces an Ig only one antigenic specificity.

Furthermore, a given B cell has two sets of chromosomes, one set from each parent. So theoretically, Ig genes located on both chromosomes could synthesize Ig molecules. This does not occur. In contrast to almost all other gene products, which are derived from genes from both parental chromosomes, Ig chains are coded for by only one set of genes, either from the maternal or the paternal chromosome (Figure 39). For example, the H chain may be coded for by genes on the paternal chromosome and the L chain (either K or X) by genes on the maternal chromosome. This phenomenon of using genes from only one parental chromosome is known as **allelic exclusion**. Figure 39. Rearrangement of V, D, and J gene DNA occurs on one of the parental chromosomes during allelic exclusion.



The steps in rearrangement, allelic exclusion and hence the synthesis of a complete Ig molecule are very tightly controlled, although all the controlling mechanisms are not yet completely clear. If a successful or productive rearrangement of V, D, and J gene DNA occurs on one of the parental chromosomes, and an H chain polypeptide is produced, the other parental H chain DNA stops rearranging as a result of some kind of suppressive mechanism (Figure). If the attempt to rearrange the V, D, and J genes on the first parental chromosome is unsuccessful (i.e., if it fails to produce a polypeptide chain), then the second parental chromosome continues H chain rearrangement. The same process then occurs with the L chain, first with the K and then with the chain genes. Productive rearrangement resulting from the joining of a V segment and J segment of any one of these genes causes the others to remain in germ line form. In this way, the cell progresses through some or all of its chromosomal copies until they have successfully completed the productive rearrangement of genes for one H and one L chain. These chains then become the basis of the antibody specificity of that particular cell.

### 13. REGULATION OF ANTIBODY

#### Antigen is a major factor in control

The acquired immune response evolved so that it would come into play when contact with an infectious agent was first made. The appropriate antigen-specific cells expand, the effectors eliminate the antigen and then the response quiets down and leaves room for reaction to other infections. Feedback mechanisms must operate to limit antibody production, otherwise, after antigenic stimulation, we would become overwhelmed by the responding clones of antibody-forming cells and their products -obviously an unwelcome state of affairs, as may be clearly seen in multiple myeloma where control over lymphocyte proliferation is lost. It makes sense for antigen to be a major regulation, factor and for antibody production to be driven by the presence of antigen, falling off in intensity as the antigen concentration drops (Figure 40). There is abundant evidence to support this view. Antigens can stimulate lymphocytes through their surface receptors directly, as witnessed by proliferation of T-cell clones presented with antigen *bi vitro* and formation of a clone of antibody-forming cells from a single B-cell precursor cultured with antigen and T-cell soluble factors under limiting-dilution conditions. Furthermore, clearance of antigen by injection of excess

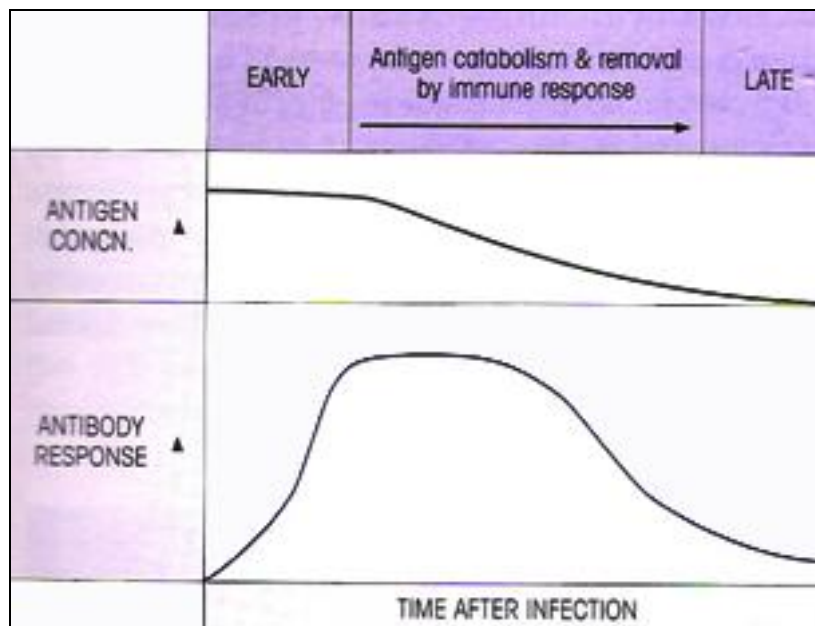


Figure 40. Antigen drives the immune response. As the antigen concentration falls due to catabolism and elimination by antibody, the intensity of the immune response declines but is maintained for some time at a lower level by antigen trapped on the follicular dendritic cells of the germinal centers.

antibody during the course of an immune response leads to a dramatic drop in antibody synthesis and the number of antibody-secreting cells.

#### Antigens can interfere with each other

The presence of one antigen in a mixture of antigens can drastically diminish the immune response to the others. This is true even for epitopes within a given molecule; for example the response to epitopes on the Fab fragment of IgG is far greater when the Fab rather than whole IgG is used for immunization due to the inhibitory nature of the Fc region. The mechanism of this effect lies at the level of competition of processed antigenic peptides for the MHC groove. There is a clear hierarchy of epitopes with respect to this competitive binding based on differential accessibility to proteases as the molecule unfolds, and the presence or absence of particular amino acid sequences which facilitate breakdown to yield peptides in high abundance and with relatively high affinity for the MHC groove. Thus, Sercarz envisages **dominant epitopes** which bag the lion's share of the available MHC

grooves, **subdominant epitopes which** are less successful, and **cryptic epitopes** which generate miserably low concentrations of MHC complex which are ignored by potentially reactive naive T-cells.

Clearly the possibility that certain antigens in a mixture, or particular epitopes in a given antigen, may block a desired protective immune response has obvious implications for vaccine design. Contrariwise, the identification of inhibitory peptides with a predatory affinity for the MHC groove(S) Should provide therapeutic agents to squash unwanted hypersensitivity reactions.

### **Antibody exerts feedback control**

A useful control mechanism is to arrange for the product of a reaction to be an inhibitor and this type of negative feedback is seen with antibody. Thus we see examples of antibody diverting antigen to immunogenically, inoffensive sites in the body to prevent primary sensitization in the protection against rhesus immunization afforded by administration of anti-D to mothers at risk and the inhibitory effect of maternal antibody on the peak titers obtained on vaccinating infants. Removal of circulating antibody by plasma-pheresis during an on-going response leads to an increase in synthesis, whereas injection of preformed I-G antibody markedly hastens the fall in the number of antibody-forming cells consistent with feedback control on overall synthesis. It is unlikely that this is achieved by simple neutralization of antigen since whole IgG is so much more effective than its F(ab) 2 fragment in switching off the reaction, even allowing for the longer half-life of the complete immunoglobulin. A- clue to the underlying mechanism comes from the finding that cross-linking of IgM on the resting B-cell surface by an IgG anti-p has no discernible effect, whereas the F(ab)2 fragment of this anti-.Lt induces proliferation The current view is that a complex of antigen with IgG antibody could block the productive phase of the T-dependent B-cell response by cross-linking the **antigen and Fcy** surface receptors.

In complete contrast, injection of IgM antibodies enhances the response (figure 11.3), presumably by **cross-linking antigen bound to the Fcy** receptors without activating the Fcy inhibitory receptor. Since antibodies of this isotype appear at an early stage after antigen challenge, they would be useful in boosting the initial response.

## **T-CELL REGULATION**

### **T-helper cells**

We have deliberated at length on the role of T-helper cells in the facilitation of cytotoxic T-cell and B-cell responses, and in the production of class-switching and memory responses. We should perhaps note that the T-helpers do not expand B-cell and Tc-cell clone sizes indefinitely since the maturation factors inhibit the action of the proliferative lymphokines. Different T-cells have surface receptors for the Fc regions of the various Ig classes, and a role for these in isotype-specific help has been sought. CD4 Th2 helpers can use their Fc $\mu$  receptors to bind to the surface IgM on B-cells, so contributing to the formation of antigen-specific MHC restricted T-B cytoconjugates i.e. they act as additional accessory adhesion molecules to target IgM-positive B-cells and maybe augment activation of the helper cell. T-cell lines from Peyer's patches produced many more IgA-producing B-cells from Peyer's patch precursors than did splenic T-cell lines. However, the bias was for help to IgA-precommitted B-cells rather than inducing a class-switch to IgA since Peyer's patch T-cells did not markedly enhance IgA production by splenic B-cells. Evidence for the production of IgE-binding factors from Fc  $\gamma$  receptor bearing T-cells which enhance B-cell secretion of IgE has been obtained; intriguingly, lipocortin produced by CD8 T-cells under the influence of steroids, or following immunization with mycobacteria is said to block glycosylation of the IgE-binding factor so that it now becomes a suppressor of IgE synthesis.

### **T-cell suppression**

We have now raised the question of suppression as distinct from help and perhaps it is inevitable that Nature, having evolved a functional set of T-cells which promote immune responses, should also develop a regulatory set whose job would be to prevent the helpers

from getting out of hand. T-cell suppression was first brought to the serious attention of the immunological fraternity by a phenomenon colorfully named by its discoverer, 'infectious tolerance'. Quite surprisingly it was shown that if mice were made unresponsive by injection of a high dose of sheep red cells, their T-cells would suppress specific antibody formation in normal recipients to which they had been transferred (figure 11.5). It may not be apparent to the reader why this result was at all surprising, but at that time antigen-induced tolerance was regarded essentially as a negative phenomenon involving the depletion or silencing of clones rather than a state of active suppression. Over the years, T-cell suppression has been shown to modulate a variety of humoral and cellular responses, the latter including delayed-type hypersensitivity, cytolytic T-cells and antigen-specific T-cell proliferation. However, the existence of dedicated professional-suppressor cell is a question which has generated a great deal of heat.

### **Suppressor and helper epitopes can be discrete**

Detailed analysis of murine responses to antigens such as hen egg-white lysozyme tells us that certain determinants can evoke very strong suppressor rather than helper responses depending on the mouse strain, and also, that T-suppressors directed to one determinant can switch off helper and antibody responses to other determinants on the same molecule. Thus mice of H-2<sup>b</sup> haplotype respond poorly to lysozyme because they develop dominant suppression; however, if the three N-terminal amino acids are removed from the antigen, H-2<sup>b</sup> mice now make a splendid response showing that the T-suppression directed against the determinant associated with the N-terminal region had switched off the response to the remaining determinants on the antigen. Similar results have been obtained in several other systems. This must imply that the antigen itself must act as a form of bridge to allow communication between T-suppressor and cells reacting to the other determinants, as might occur through these cells binding to an antigen presenting cell expressing several different processed determinants of the same antigen on its surface.

## 14. COMPLEMENTS

Complement refers to a group of enzymatic proteins found in the serum and body fluids and it completes the antigen antibody reaction lysis and phagocytosis. Antibody alone cannot destroy a bacterium although it can opsonize it for more efficient phagocytosis. Although an antibody can combine with soluble foreign molecules, it is not in itself very efficient in triggering the elimination of foreign matter from the blood stream. Many of the biological functions of antibodies require the participation of a group of proteins (Known collectively as complement). It was discovered by Bordet in 1895 and he named it as alexin. The term complement was coined by Ehrlich.

Complement is characterized by the following salient features.

1. Complement is a collective term for a series of complex proteins found in normal serum.
2. They are B globulins. They are neither antigens nor antibodies.
3. They exist as proenzymes and circulate as inactive forms.
4. on activation, they become active, acquire enzymatic or esterase activity. They activated components are shown by putting a bar over them. Eg. C3̄, C2̄ etc. Inactive forms of complement components are indicated by suffix. Eg C4i, C3i.
5. They are thermolabile and are destroyed on exposure to room temperature or to a temperature of 56C.
6. Complement proteins are designated by letter C and are numbered C1 to C9.
7. Complement C1 consists of three subunits namely C1q, C1r and C1s.
8. Other component include B, D, P, H and I.
9. These complement components function in a cascade pattern.
10. When complement become activated a number of biological events are initiated which facilitate the destructions of foreign materials by the cells of immune systems.

### **Biosynthesis of Complements**

Complement components are synthesized in various sites in the body. C1 is synthesized in the intestinal epithelium, C2 and C4 are synthesized from Macrophages, C3, C6 and C9 are synthesized in the liver. The site of synthesis of C7 and C8 are not known.

### **Complement Activation**

The complement proteins remain in an inactive form in the blood plasma. The complement components are made active by certain substances called activating substances or activating agents. They include antigen antibody complexes, Gram negative bacteria, animal viruses aggregated antibodies like IgG or IgA endotoxin yeast etc. When one component is activated other components are triggered in a sequence in of a cascade pattern to bring about a biological activity such as lysis, phagocytosis, etc.

When a complement is activated, it acquires the following unique properties.

1. The activated complement binds on to the biological membrane of a bacterium.
2. It generates enzyme activity.
3. It activates the next complement protein in order to participate in a chain reactions.

When the complement system is activate it passes through two distinct phases. They are

1. Classical path way
2. Alternative path way

## 14.1. CLASSICAL PATHWAY

1. Classical pathway is a simple step-wise reaction brought about by the activation of complement system (Figure 41).

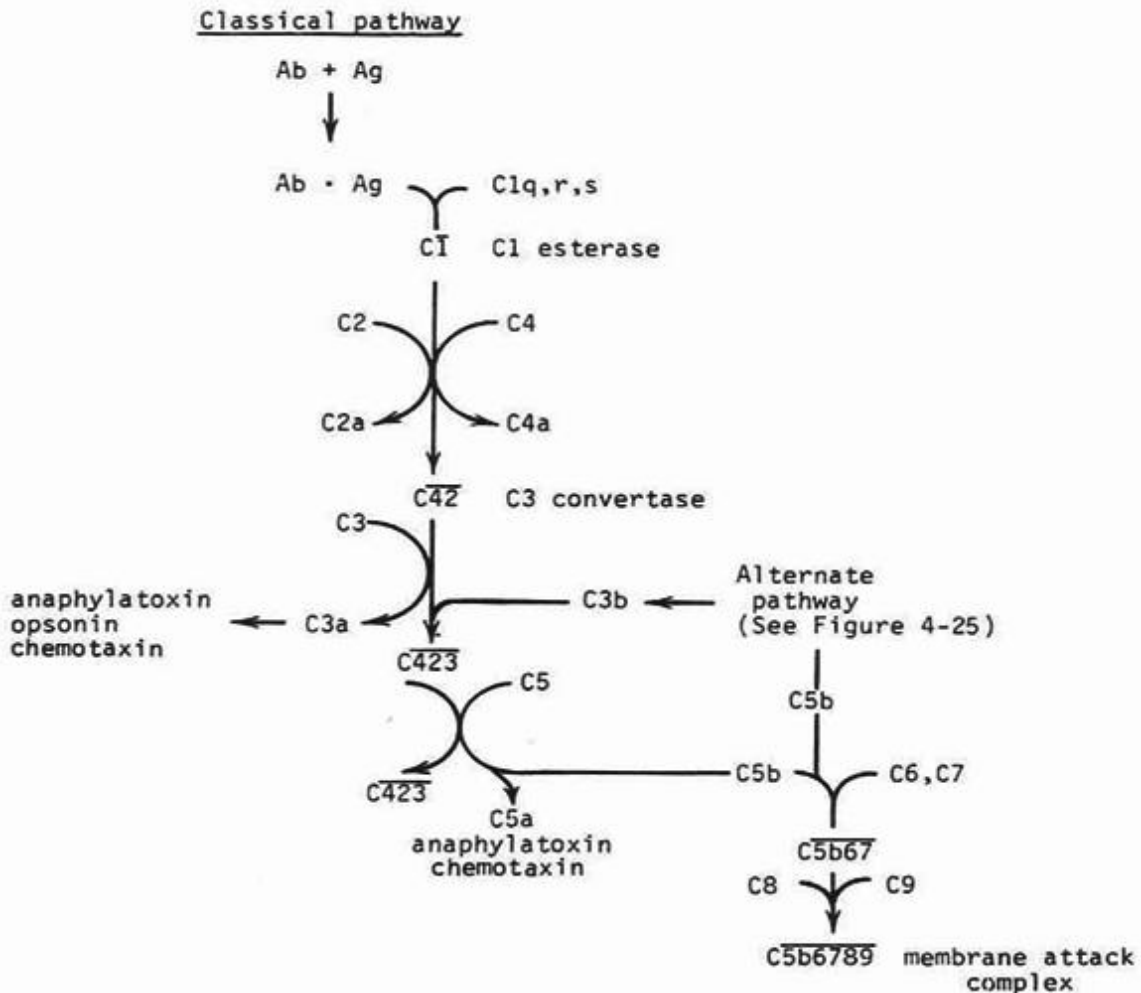


Figure 41. The classical pathway of complement activation.

2. The classical pathway is activated by antigen antibody complex, Gram negative bacteria and animal viruses.
3. The complement components involved in the classical pathway are 11 components.
4. They react in the following segments.  
C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8, C9.
5. The activating agent such as antigen-antibody complex interacts with first component of the complement C1q. Actually the C1q recognizes the antigen antibody complex and binds with Fc portion of the antibody. This process of binding of complement to the antibody is called complement fixation.  $Ca^{++}$  are essential for effective C1q binding.
6. Attachment of C1q to the immune complex activates the C1r. activated C1r functions as serine histidine esterase and it activates C1s. C1s activates the complements C4 and C2 in the presence of  $Mg^{++}$ .
7. The complement C4 on activation into small fragment called C4a and a large fragment called C4b. The C4b becomes activated and this activated C4b becomes attached either to the antibody C1 complex or to the surface of the microbe.

The activated C2 is also split into a small fragment called C2a and a large fragment called C2b. C2b reacts with C4b to form C4b2b complex. The C4b2b complex functions C3 convertase enzyme and it activates C3 component. The activated C3 component is cleaved into a small fragment. C3 and a large fragment C3b. The C3b binds with C4b2b complex to form C4b2b3b complex.

The 3b has binding sites for macrophages and phagocytes and hence the C4b2b3b complex is adhered to macrophages or phagocytes along with the microbe.

The C3b does the following functions:

- a. it facilitates the adherence of bacteria and viruses to macrophages
- b. It facilitates the ingestion of certain bacteria by macrophages and phagocytes.
- c. It arrangements IgG-induced phagocytosis and IgG- mediated cell cytotoxicity.

The C4b2b3b complex activates the next complement C5. The activated C5 splits into a small fragment called C5a and a large fragment called C5b.

C5b has two combining sites. One site is to bind it to the antigen (Microbe) and the other site is to bind with the complement factors C6 and C7. This trimolecular complex called C5b67 complex. It can remain in solution or bend to the surface of the microbe. When it is bound to the microbe, it is stable. When it is in free solution C5b67 has a half life of only 0.1 sec.

C5b67 complex binds with C8 to form a tetramolecular complex called C5b678 complex. The C5b678 complex has a binding site for C9. it binds with C9 to form C5b6789 complex.

This complex causes a disruption of the lipid bilayer of the membrane of the microbe. As a result a hole is made on the microbe.

Through this hole the contents of the cell are released and the cell is lysed. This phenomenon is called lysis. When the lysed cell is a bacterium, the lysis is called bacteriolysis. When the lysed cells is an RBC the lysis.

### **Significance of Classical Pathway**

When a microbe enters the body, the body responds by producing antibody. The antibody binds with the microbe to form a antigen-antibody complex is formed, the complement system is activated to enhance the removal of the microbe. This is done in the following ways.

- a. The complement system helps to adhere the microbe to the neutrophils and macrophages.
- b. It facilitates the ingestion of microbes by neutrophils and macrophages through phagocytosis.
- c. It brings about the lysis of the microbes.
- d. It arrangement the IgG mediated cell cytotoxicity.

## **14.2. ALTERNATIVE PATHWAY**

The activation of complement C3 without the prior participation of C12 is called alternative pathway.

The alternative pathway was discovered by Pillemer in 1954. The alternative pathway is a step-wise reaction of the complement system to bring about the destruction of microbes in the body (Figure 42).

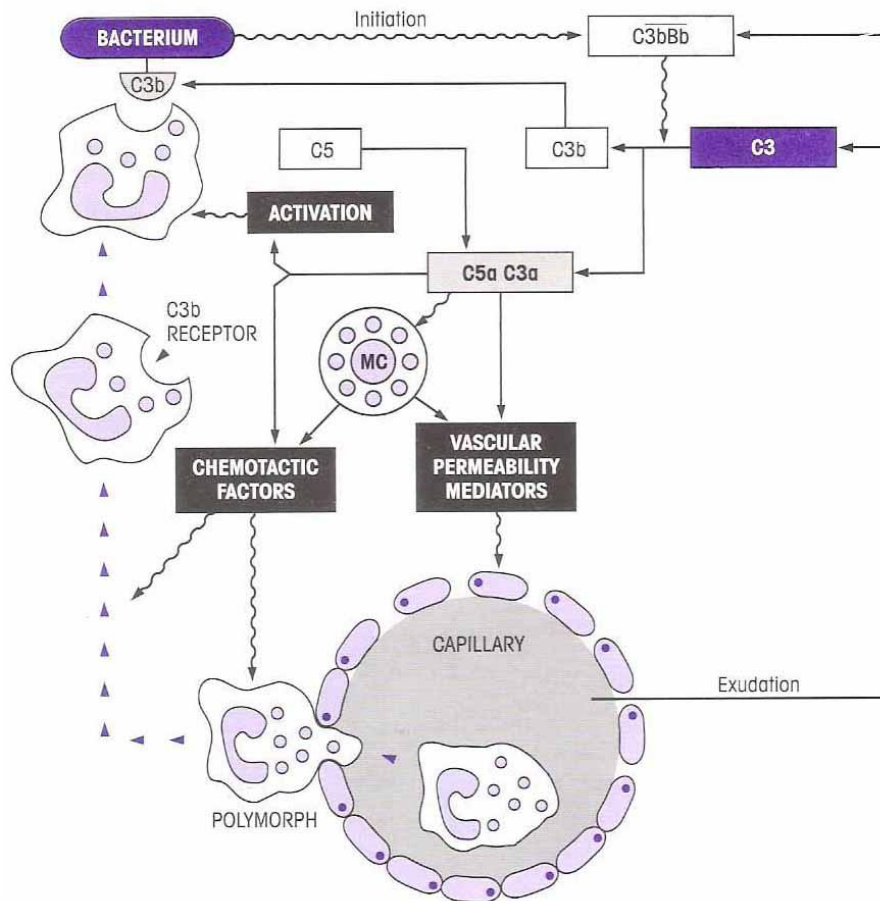


Figure 42. The alternative pathway of complement activation.

The alternative pathway is activated by aggregated antibodies IgG or IgA, lipopolysaccharide bacterial endotoxin and yeast.

About 6 complement components are involved in the alternative pathway. They are C3, B, D, P, H and I.

In alternative pathway C3 is activated by PZ complex formed by the reaction of properdin a normal serum protein with zymosan. The PZ complex functions as a proteinase enzyme and it cleaves C3 into a small fragment C3a and a large fragment C3b. C3b is coated on the surface of the microbe.

C3b is coated on the surface of the microbe. Then factor B binds with C3b to form a complex called C3bB complex. The C3bB complex is a weak bridge and it is stabilized by factor P called properdin.

Activated factor D activates and cleaves the C3bB complex releasing C3 cleavage enzyme C3bBb called C3 convertase.

The activated C3bBb splits C3 into C3a and C3b. C3b formed can be used in several ways. It can be deposited on the nearby microbes and interact with factors B and D. Once C3b is formed a cycle of activation occurs which maintains C3b formation without the original activation agents.

The C3b may activate the C5 and enter classical pathway and produce C5b6789 complex in sequence resulting in lysis of the microbe.

### **14.3. BIOLOGICAL SIGNIFICANCE OF COMPLIMENTS**

**OR**

#### **FUNCTIONS OF COMPLEMENT SYSTEM**

Complement is one of the most important effector systems in the body. Antibodies alone do relatively little harm to invading bacteria; most of the damage is either caused or induced by various components of the so called complement system

##### **1. Immune Adherence**

The complement components facilitate the binding of microbes to phagocytic cells. This is possible because the phagocytic cells have receptors for C3b component and the microbes coated with C3b are adhered to phagocytes.

##### **2. Chemotaxis**

Complement bound by Ag-Ab complexes release chemotactic factors and attract leucocytes which in turn release lysosomal enzymes. This helps in the localization and inactivation of infectious agents or may enhance tissue injury.

##### **3. Opsonization**

Opsonization is the rendering of bacteria and other cells to phagocytosis. During opsonization the complement component binds with the Ag-ab complex. The complement bound to the Ag-ab complex has a binding site to phagocytes. Complement system facilitates ingestion of microbes by macrophages and neutrophils.

##### **4. Phagocytosis**

Complement system facilitates ingestion of microbes by macrophages and neutrophils.

##### **5. Lysis**

Attachment of complement factors to the cell membrane of the microbe causes disruption of the lipid bilayer of the membrane of the microbe. As a result a hole is made on the microbe. Through the hole, the contents of the cell are released and the cell dies. This is called lysis. When a body cell, such as a tumor cell, is lysed the lysis is called cytolysis. If a bacterial cell is lysed, the lysis is called bacteriolysis. When RBC is lysed, the lysis is called haemolysis.

##### **6. Anaphylactoid Reaction**

The complements C3a and C5a are considered as anaphylatoxins. They cause the release of histamine from the mast cells. These factors cause increased vascular permeability, oedema, constriction of smooth muscles, vasodilatation, collection of inflammatory cells at the site of activation and increased phagocytic activity. C5a in addition switches on neutrophil production of leucotrienes-B<sub>4</sub> which prolongs increased permeability phase induced by C5a. It appears that the whole series of reactions are directed towards the destruction of infective agents.

The release of histamine from mast cells causes hypersensitivity (Anaphylaxis).

##### **7. Coagulation**

Complement system brings about the coagulation of blood. The blood platelets adhered to activated C3 are lysed. The lysed platelets release a factor which converts prothrombin into thrombin. Thrombin brings about coagulation.

## 27. MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) AND ITS PRODUCTS IN MAN (HLA)

### MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

Major histocompatibility complex refers to a cluster of genes responsible for immune reactions transplantation antigens and proteins of complement system. It is abbreviated as MHC. It was discovered by Gorer in 1930. MHC complexes in mice and humans and in all species of birds and mammals analyzed to date contain genes coding for antigens that are expressed on the surface of almost every cell in the body. Class I antigens are recognized by the immune system during rejection of a foreign tissue transplant and are what Gorer was detecting in his earliest studies.

During 1960, a second class of genes and antigens, was described that turned out to be associated with MHC. These antigens, called class II antigens, are present principally on the surface of lymphocytes and reticular cells. They were originally defined by their ability to control the antibody response to particular antigens. That is, depending on the type of class II molecules a given mouse display on its, lymphocytes, it might respond well poorly, or not at all to antigen.

The class III MHC genes code for certain complements components like C2 and C4 both in man and mouse.

All mammals have these MHC. The MHC of mouse called H-2 and in human it is called HLA.

### HUMAN LEUCOCYTE ANTIGEN (HLA)

The major histocompatibility complex of man is called HLA (human leucocyte antigen). It is a cluster of structural genes responsible for the production of antigens located on the nucleated cells and components of complements.

The HLA complex of man is located in the short arm of chromosome number 6 (Figure 43) it has six loci and they are named A,B,C,D,S and T1a. The locus D is further sub divided into three loci, namely DR, DQ and DP. T1a is located adjacent to A and S is located between B and D. Locus S is further subdivided into three loci, namely C<sub>2</sub>, C<sub>4</sub> and Bf (factor B).

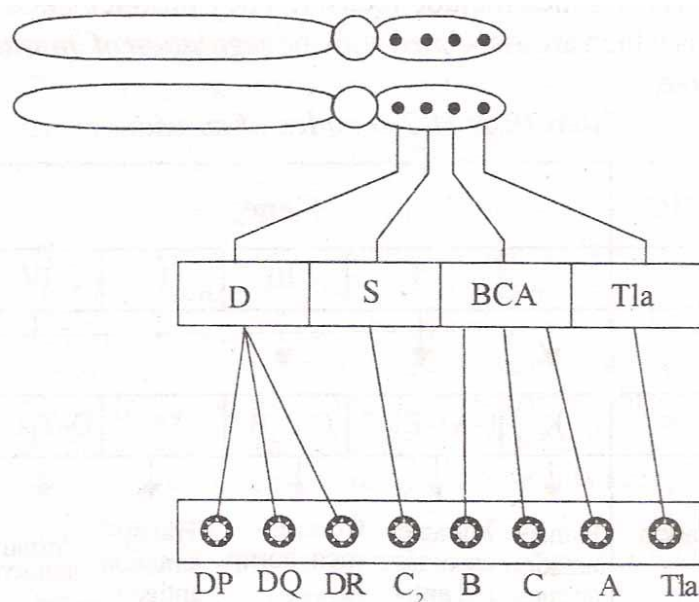


Figure 43. The HLA complex of man is located in the short arm of chromosome number.

### **MHC CLASS:**

Major histocompatibility molecules clusters of genes. They are classified into 4 classes.

#### **Class I gene:**

Loci A, B and C are called class I genes. They are responsible for the production of antigens located on surface of nucleated cells and are causing transplantation reactions. Hence these antigens are called transplantation antigens.

#### **Class II genes:**

Loci D<sub>r</sub>, D<sub>q</sub> and D<sub>p</sub> belong to class II genes. They produce antigens called immune associated antigen (I<sub>g</sub>). These antigens are associated with the regulation of immune response. These antigens are present on the surface of B-Cells, macrophages, monocytes, antigen presenting cells and activated T-cells.

#### **Class III genes:**

Locus S is called class III genes. They are responsible for the levels of complements C4 and C2 and factor B.

#### **Class IV genes:**

Locus T<sub>1a</sub> is called class IV genes. It is located adjacent to A and is associated with antigens present on T-cells of leukemia (T<sub>1a</sub>).

### **H2-COMPLEX OF MOUSE**

The major histocompatibility complex (MHC) of mouse called H-2 complex H-2 complex is a cluster of genes responsible for the production of antigens located on the membrane of nucleated cells and complement components. This complex is located in the short arm of the chromosome number 17. It consists of a set of structural genes. The genes that make up a given histocompatibility complex are called haplotypes.

#### **Class I**

These are 6 loci in the complex. They are K, I, S, D, L and T. They are grouped into four groups of genes namely class I genes, Class II genes, Class 3 genes and class 4 genes. The under indicates the class I genes include loci K, D and L. They produce class I molecules called transplantation antigens responsible for graft rejection.

#### **Class II**

Class II genes include I. They produce class antigens which are associated with the regulation of immune response.

#### **Class III**

Class III genes include locus S and they control the components of the complement system.

#### **Class IV**

Class IV genes include locus T. They control primitive antigens present on T cells of leukaemic (T<sub>1a</sub>) as well as on immature thymocytes which loci T<sub>1a</sub> during differentiation into mature T-cells.

### **Major Histocompatibility Complex (MHC)**

An individual cell will express many different kinds of MHC molecule. Half of your repertoire of MHC molecules (an MHC haplotype) is derived from one of your mother's 2 copies of chromosome 6 and the other haplotype is on one of your father's 2 copies of chromosome 6.

Each MHC molecule has a cleft in its surface, which can bind with certain general types of peptide fragments. For example it may be able to bind amino-acid strings 20 amino-acids long with hydrophobic amino acids at the centre. Any string of amino-acids conforming to such characteristics may be bound so that the limits for what an MHC molecule can bind are less rigid than those governing what can bind to a T cell receptor or an Ig.

If a cell is digesting a protein the resulting peptides are "tried out" by the cell's MHC molecules. If the cell has an MHC molecule which can accept a peptide fragment from a protein being processed within the cell then the MHC molecule/peptide complex appears at the cell surface. This dependence on the MHC system for presenting antigens to T lymphocytes is probably the basis for the relationship between HLA type and many autoimmune diseases.

### **There are 2 major subtypes of MHC molecules.**

#### **MHC I**

This is present on almost all cells and can present peptides derived from protein which have their origin within the cell, which is endogenously synthesized proteins. Every cell in the body expresses several different MHC I molecules. (MHC I molecules in humans are also called the CLASS I HLA antigen group called HLA-A, HLA-B and HLA-C).

#### **MHC II**

These are present mostly on phagocytes; B lymphocytes and dendritic cells and can present peptides which originate from outside the cell that is exogenously synthesized proteins. (MHC II is also present on NK cells and certain other cell types including T lymphocytes and epithelial cells when those cell types are activated. MHC II molecules in humans are also called class II HLA antigen group called HLA-DR and HLA-DQ).

### **FUNCTIONS OF MHC**

The MHC performs the following functions.

#### **Production of HLA**

The MHC genes II control the production of antigens located on the nucleated cells such as lymphocytes. These antigens are called human leucocyte antigens or graft rejection antigens or transplantation antigens. These antigens are bound by helper T-cells (TH). The B-cells then recognize antigen and the helper factor by their surface immunoglobulins and Ia (MHC antigen). The interleukins are produced by the antigen presenting cell and they activate the B-cells.

The MHC genes control the production of immune associated antigens (Ia) located on the surface of B-cells, macrophages, monocytes, antigen presenting cells and activated T-cells.

#### **Control of the level of complement components**

The MHC genes III are responsible for the control of the levels of complements like C4 and C2 of the classical pathway and factor B of alternative pathway.

#### **HLA Complex Help in T-Cell Recognition**

T-cells recognize foreign antigen only in the presence of MHC class I molecules as well as MHC class II molecules. The process of recognition of antigen along with MHC antigen by T-lymphocyte is called MHC restriction. Class I and II MHC antigens are involved in MHC restriction.

Cytotoxic T lymphocytes usually respond to foreign antigen in association with class I MHC antigens, whereas T helper cells respond to foreign antigen association with class II (Ic) antigen.

### **Graft Rejection**

Graft rejection is an immunological reaction. It is mediated by lymphocytes. The MHC is responsible for intense graft reactions. This reaction occurs when genetically dissimilar lymphocytes interact.

During graft rejection the foreign MHC and antigen on the grafted tissue activate T-cells of the host. Lymphokines are released by activated T-cells of the host. These lymphokines attract T cells to the place of the graft and damage the graft.

### **Role of MHC to Kill Virally Infected Cells**

The virally infected cells are killed by cytotoxic cells (TC). When a cell is infected by virus, the viral antigen is processed and presented on the surface of the infected cell. The viral antigen is presented on the surface of the virally infected cell along with the MHC 2 antigen. The viral antigen along with the MHC 2 antigens is recognized by the T-helper cell (TH). Then the T-helper cell gives help to cytotoxic cell (TC) and this triggers the cytotoxic cell to kill the target (the virally infected cell).

## 16. DISEASES AND IMMUNE RESPONSE

### 16.1. IMMUNE RESPONSES TO VIRAL INFECTIONS

Virus causes many diseases in human beings. Influenza virus, Rhinovirus, (common cold) poliomyelitis virus, measles virus, B virus, HIV, adenovirus, oncovirus, chicken pox, small pox, vesicular stomatitis virus are diseases in human beings. They are able to escape from immune response by change and shed their antigen on the membrane, they are able to grow within the cell there by reducing their antigenicity. All the class I antigens produce negative cytotoxicity protected from immune attack. B cells, antibodies, T-cells, macrophages, monocytes, interferon are playing important roles in viral destructions.

The antibody molecule can neutralize viruses by a variety of means. It may stereospecifically inhibit combination with the receptor site on cells, thereby preventing penetration and subsequent intracellular multiplication.

Antibodies prevent entry and binding of virus into the cell and spread of virus from cell to cell by antibodies blocking the fusion antigen. Antibody may destroy a free virus particle directly through activation of the classical complement pathway or produce aggregation, enhanced phagocytosis and intracellular death.

#### Viruses

The viruses cause the following infectious diseases.

Measles, Mumps, Influenza, Common cold, Small pox, Chicken pox, Herpes simplex, Poliomyelitis, Rabies, Hepatitis, etc.

#### Interferon against Virus

The rapid production of interferon is the most significant mechanism used to counter the viral infection such as influenza and common cold.

Experimental studies certainly indicate that after an early peak of interferon production there is a rapid fall in the titer of live virus in the lungs of mice infected with influenza.

#### Elevation of Antibodies

Recent investigations have shown that antibody levels may be elevated in the local fluids bathing the infected surfaces, e.g. nasal mucosa and lung, despite low serum titers and it is in the production of antiviral antibody (most prominently IgA) which is of major importance for the prevention of subsequent infection.

Local or systemic antibodies can block the spread of cytolytic viruses which are released from host cell (eg. Oncogenic virus, Influenza virus, mumps virus, measles virus, rabies virus, adenovirus, polyomavirus etc.);

#### Cell mediated immunity response to viral infection

##### Production of lymphokines:

Viral antigen stimulates the T-cell to release of lymphokines such as IFN- $\gamma$ . The IFN- $\gamma$  activates the macrophage or monocytes and NK cells for infected cells. They destroy the virally infected cells.

### 16.2. IMMUNE RESPONSES TO BACTERIAL INFECTIONS

Bacteria causes many diseases in human beings. They are *Streptococcus*, *Salmonella*, *Pneumonia Staphylococcus* and *Vibrio cholera*.

#### Complement

Complement refers to a series of enzymatic proteins present in non-natal serum that combines with antigen-antibody complex. They cause lysis of cells and destruction of bacteria.

## **Properdin**

Properdin is a serum protein. It is involved in the resistance to infection. In the presence of C3 and Mg<sup>+</sup>, it acts non-specifically against Gram-negative bacteria and viruses and plays a role in lysis of erythrocytes.

## **Antimicrobial Substances**

The bacterial flora of the human gut live as commensals. They secrete *colicin* and *acids*. These chemicals function as antimicrobial substances and they prevent the entry of other microorganisms into the body.

## **Phagocytosis**

Phagocytosis refers to *the engulfing* of microorganism or other cells or foreign particles by phagocytes. It is a process of cell eating

The cells involved in phagocytosis are called phagocytes. They are of two types, namely *microphages* and *macrophages*.

The microphages are nothing but polymorphonuclear leucocytes, a kind of WBC present in the blood.

The macrophages are derived from the monocytes of the blood. They remain in the blood only for 24 hours. They migrate into the mononuclear phagocyte system become macrophages.

Phagocytes are usually found engulfing bacteria, viruses and protozoans. Phagocytosis occurs in the following steps

1. The phagocyte migrates to the site of infection chemotaxis.
2. The phagocyte gets attached to the microbe.
3. The cell membrane of the phagocyte produces pseudopodia around the microbe, As a result the microbe completely enclosed in a vacuole called phagosome.
4. The phagosome soon fuses with the lysosome the phagocytic cell resulting in the formation of phagolysosome or secondary lysosome.
- 5 The phagolysosome secretes antimicrobial substances. These substances include lysozyme, hydrogen oxide, myeloperoxide, etc. These **substances kill** the microbe in the phagolysosome.
6. The killed microbe is digested by the hydrolyzymes of the lysosomes.
7. The digested material of the microbe is expelled out of the cell.

## **Natural Killer Cells (NK Cells)**

Natural killer cells are a special group of **leucocytes** which kill virus infected cells. The natural killer cells containspecial **receptors** on the surface. These receptors recognise the **glycoproteins** (product of virus) present on the surface of the virus-infected cells. This recognition brings the NK cells closer to the virus infected cells. After recognition the NK cells release cytotoxic substances such as perforin or **cytolysin**. These substances cause the lysis of the virus infected cell and thus cause the death of the virus.

### **1. Humoral Immunity**

*Humoral immunity refers to the resistance developed by man against microbes by the production of chemical substances called antibodies. Hence it is called antibody mediated immunity, Here the antibodies are produced in the humors (body fluids) and hence this immunity is called humoral immunity.*

When a microbe enters the body, the microbe functions as the antigen. The human body responds to the microbe and produces specific antibody. The antibody combines with

the microbe (antigen) resulting in the clumping of the microbe. The toxicity of the microbe is neutralised and the microbes are allowed for phagocytosis.

The antibody produced may act on the whole microbe or on the toxin produced by the microbe.

## **2. Anti-toxin Immunity**

The bacteria, after entry into the human body, release *toxins*. The toxins are of two types, namely *endotoxins* and *exotoxins*.

Endotoxins are released as a result of the death of Gram negative bacteria. They are *lipopolysaccharides*. The exo toxins are released by living Gram-positive bacteria. They are composed of proteins.

Both endotoxins and exotoxins are *antigenic* and the function as antigens. In response to these antigens, the human body produces antibodies. These antibodies are called *anti-toxin antibodies*. The antitoxin antibodies neutralise the toxins. The *diphtheria* and *tetanus* infections are prevented by the action of antitoxin antibodies,

## **3. Anti-bacterial Immunity**

The whole bacteria entering the body are clumped by specific antibodies produced against the bacterium. These antibodies are called **and bacterial antibodies**. These antibodies attach with the bacteria and the bacteria are clumped and immobilized.

## **4. Opsonization**

Opsonization is a phenomenon of coating an antigen with substances such as Opsonization of a bacterium. The bacterium is coated with IgG type antibody and is rendered to phagocytosis.

**Opsonin** is an antibody which renders bacteria and other cells susceptible to phagocytosis. The antibody, which functions as the opsonin belongs to the class of IgG.

When the bacteria enter the human body IgG, antibodies are produced. These antibodies coat the surface of bacteria. The phagocytes have receptors for IgG antibodies. So when the bacteria are coated with IgG antibodies, the phagocytes migrate towards the antibody-coated bacteria and are eaten by phagocytosis.

Bacteria with a capsule are disposed by the method of opsonization. Eg. *Streptococcus aureus*, *Bacillus anthrax*, *Haemophilus influenzae*, etc.

## **Bacterial Lysis**

Lysis is the dissolution of a cell or particle. Bacterial lysis is dissolution, degradation and decomposition of a bacterial cell.

Certain bacteria such as *E. coli* are lysed by the production of a special type of antibody called IgA antibody, IgA antibodies are called *copro-antibodies*. These antibodies are selectively and predominantly produced by lymphoid tissues in the mucous membrane of the respiratory and intestinal tracts.

## **6. Cell-mediated Immunity**

The resistance against infection produced by the sensitised lymphocytes is called *cell-mediated immunity*. Here antibodies are not produced. But specific lymphocytes are triggered against microbes and the microbes are disposed. In cell-mediated immunity the participation of *T-lymphocytes* and *macrophages* is predominant.

Cell-mediated immunity gives adequate protection, against viruses, fungi, Protozoa and certain bacteria. It does not give protection against cocci.

When a microbe enters the body, it is processed by macrophage. While processing, macrophage prepares the cell for immunogenicity and it retains antigen (of the microbe) on

its surface membrane. The macrophage then presents the microbe to the T-cell. The T-cell recognizes the microbe, when a cell to cell contact between T-cell and antigen-macrophage complex is made.

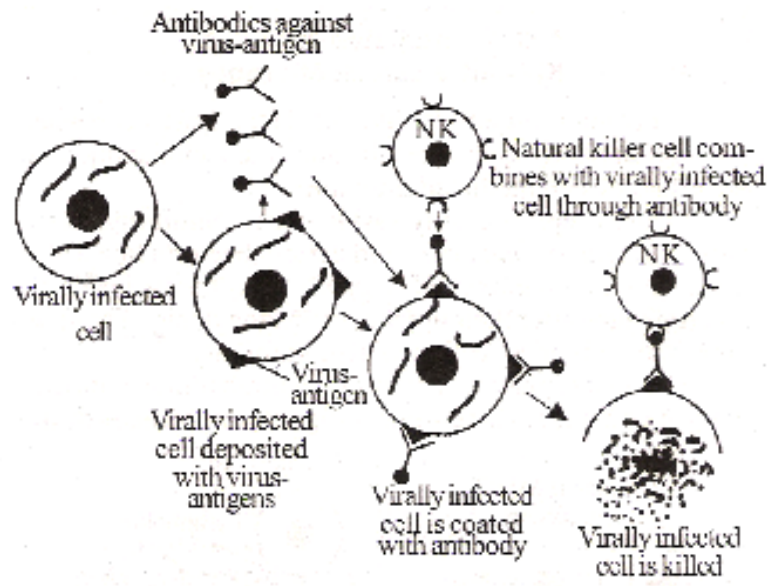


Figure 44. Cell-mediated Immune response to virus.

This recognition activates a population of T-lymphocytes. These activated T-cells release certain chemical substances called **lymphokines**. The lymphokines mediate the immune response.

The lymphokines recruit and draw macrophages to the site of infection and the microbe is destroyed by the macrophages.

Many microorganisms live inside the host cells where humoral antibody cannot reach. Such intracellular microbes are killed by cell mediated immunity.

### 16.3. IMMUNE RESPONSE TO PARASITIC INFECTIONS

Even though parasites are very antigenic, not all of the responses that they induce are effective. This is mainly due to the fact that they produce different antigens during the different stages of their cycles. The immunological fight against parasitic diseases is still very limited.

The first line of action against parasites is induced by macrophages, neutrophils and cytokines of the innate response (IL 1, IL 6, IL 12 and TNF).

We can conclude that the phagocytosis mechanism is related to the first stages of the parasitic infection. During the adaptive response, antibodies and cytotoxic mechanisms will be the most important ones. Antibodies will have a direct effect and will also activate phagocytosis and the classical pathway of the complement activation, as well as ADCC mechanisms. The activation of CD 8+ cells is very important as a defensive mechanism, as well as several cytokines related to inflammation, especially during the blood stage.

People living in **malaria** endemic areas tend to be infected repeatedly. Over time, they gradually acquire immunity to malaria. This immunity includes the development of mechanisms that can kill parasites or inhibit the replication of parasites.

Innate immune responses mediate synchronization between the replication cycles of parasites in different red blood cells which is reflected in periodic fevers. Helminth parasites are a large group of multicellular organisms that affect vast numbers of humans and are a major cause of disease. T helper and other effector cells that can mediate immunopathology and protection in response to infection with these important pathogens.

## 17. TUMOUR IMMUNOLOGY

The immune response to foreign antigens consists of humoral (eg, antibodies) and cellular mechanisms. Most humoral responses cannot prevent tumor growth. However, effector cells, such as T cells, macrophages, and natural killer cells, have relatively effective tumoricidal abilities. Effector cell activity is induced by antigen-presenting cells and is supported by cytokines (eg, interleukins, interferons—see Biology of the Immune System: Cytokines). Despite the activity of effector cells, host immunoreactivity may fail to control tumor occurrence and growth.

### Cellular Immunity

The T cell is the primary cell responsible for direct recognition and killing of tumor cells. T cells carry out immunologic surveillance, then proliferate and destroy newly transformed tumor cells after recognizing tumor-associated antigens (TAAs). The T-cell response to tumors is modulated by other cells of the immune system; some require the presence of humoral antibodies directed against the tumor cells (antibody-dependent cellular cytotoxicity) to initiate the interactions that lead to the death of tumor cells. In contrast, suppressor T cells inhibit the immune response against tumors.

Cytotoxic T lymphocytes (CTLs) recognize antigens on target cells and lyse these cells. These antigens may be cell surface proteins or may be intracellular proteins (eg, TAAs) that are expressed on the surface in combination with class I major histocompatibility complex (MHC) molecules. Tumor-specific CTLs have been found with neuroblastomas, malignant melanomas, sarcomas, and carcinomas of the colon, breast, cervix, endometrium, ovary, testis, nasopharynx, and kidney.

Natural killer (NK) cells are another population of effector cells with tumoricidal activity. In contrast to CTLs, NK cells lack the receptor for antigen detection but can still recognize normal cells infected with viruses or tumor cells. Their tumoricidal activity is termed “natural” because it is not induced by a specific antigen. The mechanism by which NK cells discriminate between normal and abnormal cells is under study. Evidence suggests that class I MHC molecules on the surface of normal cells inhibit NK cells and prevent lysis. Thus, the decreased level of class I molecule expression characteristic of many tumor cells may allow activation of NK cells and subsequent tumor lysis.

Macrophages can kill specific tumor cells when activated by a combination of factors, including lymphokines (soluble factors) produced by T cells and interferon. They are less effective than T-cell-mediated cytotoxic mechanisms. Under certain circumstances, macrophages may present TAAs to T cells and stimulate tumor-specific immune response.

Dendritic cells are dedicated antigen-presenting cells present in barrier tissues (eg, skin, lymph nodes). They play a central role in initiation of tumor-specific immune response. These cells take up tumor-associated proteins, process them, and present TAAs to T cells to stimulate the CTL response against tumor. The presence of dendritic cells in tumor tissues correlates with improved prognosis.

Lymphokines produced by immune cells stimulate growth or induce activities of other immune cells. Such lymphokines include IL-2, also known as T-cell growth factor, and the interferons. IL-12 is produced by dendritic cells and specifically induces CTLs, thereby enhancing antitumor immune responses.

### Humoral Immunity

In contrast to T-cell cytotoxic immunity, humoral antibodies do not appear to confer significant protection against tumor growth. Most antibodies cannot recognize TAAs. Regardless, humoral antibodies that react with tumor cells in vitro have been detected in the sera of patients with various neoplastic processes, including Burkitt's lymphoma; malignant melanoma; osteosarcoma; neuroblastoma; and lung, breast, and GI carcinomas.

Cytotoxic antibodies are directed against surface antigens of tumor cells. These antibodies can exert anti-tumor effects through complement fixation or by serving as a flag for destruction of tumor cells by T cells (antibody-dependent cell-mediated cytotoxicity). Another population of humoral antibodies, called enhancing antibodies (blocking antibodies), may actually favor rather than inhibit tumor growth. The mechanisms and relative importance of such immunologic enhancement are not well understood.

### **Failure of Host Defenses**

Although many tumors are eliminated by the immune system (and thus are never detected), others continue to grow despite the presence of TAAs. Several mechanisms have been proposed to explain this deficient host response to the TAA, including specific immunologic tolerance to TAAs in a process that involves antigen-presenting cells and suppressor T cells, possibly secondary to prenatal exposure to the antigen; suppression of immune response by chemical, physical, or viral agents (eg, helper T-cell destruction by HIV); suppression of the immune response by cytotoxic drugs and radiation; and suppression of the immune response (decreased T, B, and antigen-presenting cell function, decreased IL-2 production, increased circulating soluble IL-2 receptors) by the tumor itself through various complex and largely uncharacterized mechanisms. The immune response to foreign antigens consists of humoral (eg, antibodies) and cellular mechanisms. Most humoral responses cannot prevent tumor growth. However, effector cells, such as T cells, macrophages, and natural killer cells, have relatively effective tumoricidal abilities. Effector cell activity is induced by antigen-presenting cells and is supported by cytokines (eg, interleukins, interferons). Despite the activity of effector cells, host immunoreactivity may fail to control tumor occurrence and growth.

### **Cellular Immunity**

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## **18. IMMUNE DEFICIENCY DISEASES – AIDS.**

**Immunodeficiency** (or **immune deficiency**) is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent. Most cases of immunodeficiency are acquired ("secondary") but some people are born with defects in the immune system, or primary immunodeficiency. Transplant patients take medications to suppress their immune system as an anti-rejection measure, as do some patients suffering from an over-active immune system. A person who has an immunodeficiency of any kind is said to be **immunocompromised**. An immunocompromised person may be particularly vulnerable to opportunistic infections, in addition to normal infections that could affect everyone.

### **Primary immunodeficiency (PID)**

A number of rare diseases feature a heightened susceptibility to infections from childhood onward. Many of these disorders are hereditary and are autosomal recessive or X-linked. There are over 80 recognised primary immunodeficiency syndromes; they are generally grouped by the part of the immune system that is malfunctioning, such as lymphocytes or granulocytes.<sup>[1]</sup>

The treatment of primary immunodeficiencies depends on the nature of the defect, and may involve antibody infusions, long-term antibiotics and (in some cases) stem cell transplantation.

### **Acquired immunodeficiency**

Immune deficiency may also be the result of particular external processes or diseases; the resultant state is called "secondary" or "acquired" immunodeficiency. Common causes for secondary immunodeficiency are malnutrition, aging and particular medications (e.g. chemotherapy, disease-modifying antirheumatic drugs, immunosuppressive drugs after organ transplants, glucocorticoids).

Many specific diseases directly or indirectly impair the immune system. This includes many types of cancer, particularly those of the bone marrow and blood cells (leukemia, lymphoma, multiple myeloma), and certain chronic infections. Immunodeficiency is also the hallmark of acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV). HIV directly infects a small number of T helper cells, and also impairs other immune system responses indirectly.

## **IMMUNODEFICIENCY**

Immunodeficiency refers to a group of disorders in which the immune system does not function normally. Our bodies' immune cells attack and kill what they see as foreign invaders, usually bacteria, viruses, and fungi. When the immune system does not work properly, a person is more likely to suffer from frequent and longer lasting infections, often from organisms that don't normally make most people sick. Congenital immunodeficiency is present at birth. Another type of immunodeficiency disorder is called acquired immunodeficiency, which develops later in life.

### ***Affected cases***

Infants can inherit immunodeficiency disorders. Children with chronic diseases are more likely to develop acquired immunodeficiency disorders. Children without a spleen or with spleen problems, or without a thymus or an underdeveloped thymus, are also likely to have immunodeficiency disorders.

### ***Causes***

Different immunodeficiency diseases involve different components of the immune system. The immune system is composed of white blood cells (macrophages, neutrophils, and lymphocytes), as well as substances called antibodies. Congenital immunodeficiencies are hereditary, and may occur as a result of defects in B lymphocytes, which make

antibodies; T lymphocytes, which help identify foreign substances; or both, called severe combined immunodeficiency disease (SCID). Or, immunodeficiencies can be caused by an antibody defect. There are five classes of antibodies (IgM, IgG, IgA, IgE, and IgD) and each type has a different function. Antibodies are also called immunoglobulins. When immunodeficiency is caused by problems with the thymus, it is called DiGeorge syndrome. Newborns can also be infected with the human immunodeficiency virus (HIV) during childbirth if their mothers are infected. Acquired immunodeficiency is most commonly caused by disease, such as malnutrition, sickle cell anemia, cancers, and diabetes; infections, such as measles, bacterial and fungal infections, and cytomegalovirus; or as a side effect of certain drugs and therapies used in the treatment of chronic disease. Burn trauma can also affect the function of the immune system.

### **Symptoms**

Children with immunodeficiency disorders have frequent infections, such as recurrent respiratory infections. A simple sore throat or cold will lead to severe bacterial infections, most commonly bronchitis, sinusitis, and ear infections (otitis). Chronic bronchitis can develop into pneumonia. It is common for children with immunodeficiencies to develop thrush, a fungal infection of the mouth, and other infections of the skin and mucous membranes in the eyes, mouth, and genital area. Gastrointestinal infections cause diarrhea, weight loss, and failure to thrive. Other symptoms in children with immunodeficiency disorders include hair loss, eczema, and areas of broken blood vessels near the surface of the skin, enlarged lymph nodes, distended stomachs, and unnaturally pale skin.

### **Diagnosis**

Congenital immunodeficiency is suspected in children with recurring severe infections, especially when there is a family history of recurring infections. He or she will look for a history of adverse reactions to immunizations; surgeries, especially those involving the spleen, tonsils, or adenoids; or radiation therapy, particularly to the thymus. Immunodeficiency disorders are diagnosed through blood tests, which are studied for the presence of immune components such as antibodies, lymphocytes, and phagocytes. The doctor will also look at the blood cell count and appearance. The results of these tests will determine which additional tests are needed. Your doctor will also look at the type of infections the child has had to help determine the type of immunodeficiency disorder. Delayed hypersensitivity skin tests are performed after the age of two to determine how the body reacts to common infectious organisms, which are injected under the skin.

### **Treatment**

There is currently no cure for immunodeficiency disorders. The goal of treatment is to control infections and maintain the patient's quality of life. Infections are treated with antibiotics, sometimes on a regular basis as a preventive treatment. Children with low antibody levels may be given injections of gamma globulin to increase antibodies. Children with severe DiGeorge syndrome may benefit from a bone marrow transplant or thymus transplant. Bone marrow transplant is the recommended treatment for severe combined immunodeficiency disease (SCID). Children with HIV infection, which causes AIDS, are treated with drugs called antivirals. Improving the child's general nutrition can reverse immunodeficiency caused by malnutrition. Discontinuing treatments that affect the immune system, such as chemo or radiation therapy, also improves immune function.

### **Self-care tips**

Although there is no way to prevent congenital immunodeficiency disorders, you can help improve your child's health by making sure he or she follows a healthy diet, avoids situations where he or she will be exposed to infection, avoids eating undercooked food that can contain bacteria, and practices good personal hygiene. Because mouth infections are so common in someone who is immunodeficient, good dental care is especially important. Someone with a history of congenital immunodeficiency disorder may want to seek genetic counseling before deciding to start a family.

### **Congenital Immunodeficiency Disorders**

Congenital immunodeficiency is present at the time of birth and is the result of genetic defects. These immunodeficiency disorders are also called primary immunodeficiencies. Even though more than 70 different types of congenital immunodeficiency disorders have been identified, they rarely occur. Congenital immunodeficiencies may occur as a result of defects in B lymphocytes, T lymphocytes, or both. They also can occur in the innate immune system.

### **HUMORAL IMMUNITY DISORDERS**

The congenital immunodeficiency disorder, Bruton's agammaglobulinemia, also known as X-linked agammaglobulinemia, results in a decrease or absence of B lymphocytes and, therefore, a decreased ability to make antibodies. People with this disorder are particularly susceptible to infections of the throat, skin, middle ear, and lungs. It is seen only in males because it is caused by a genetic defect on the X chromosome. Since males have only one X chromosome, they always have the defect if the gene is present. Females can have the defective gene, but since they have two X chromosomes, there will be a normal gene on the other X chromosome to counter it. Women may pass the defective gene on to their sons.

### **B LYMPHOCYTE DEFICIENCIES**

If there is an abnormality in either the development or function of B lymphocytes, the ability to make antibodies will be impaired. This deficit makes the body susceptible to recurrent infections.

A type of B lymphocyte deficiency involves a group of disorders called selective immunoglobulin deficiency syndromes. The five different types of immunoglobulins are called IgA, IgG, IgM, IgD, and IgE. The most common type of immunoglobulin deficiency is selective IgA deficiency, occurring in about one in every 500 white persons. The amounts of the other antibody types are normal. Some patients with selective IgA deficiency experience no symptoms, while others have occasional lung infections and diarrhea. In another immunoglobulin disorder, IgG and IgA antibodies are deficient, and there is increased IgM. People with this disorder tend to get severe bacterial infections.

Common variable immunodeficiency (CVID) is another type of B lymphocyte deficiency. In this disorder, the production of one or more of the immunoglobulin types is decreased, and the antibody response to infections is impaired. It generally develops in people between the ages of ten and 20. The symptoms vary among affected people. Most people with this disorder have frequent infections, and some also experience auto-immune phenomena, such as autoimmune hemolytic anemia or rheumatoid arthritis. Persons with CVID develop cancer at a higher rate than the general population, particularly lymphomas.

### **T LYMPHOCYTE DEFICIENCIES:**

Severe defects in the ability of T lymphocytes to mature result in impaired immune responses to infections with viruses, fungi, and certain types of bacteria. These infections are usually severe and can be fatal.

DiGeorge syndrome is a genetic syndrome most frequently associated with a chromosomal deletion). This syndrome is often associated with T lymphocyte deficiencies. Children with DiGeorge syndrome either do not have a thymus or have an underdeveloped thymus. Since the thymus is a major organ that directs the production of T lymphocytes, these patients have low numbers of T lymphocytes. If the T cell count is very low the patients are susceptible to recurrent infections. The syndrome can be associated with other physical abnormalities. For example, these individuals may have distinctive facial features such as thin upper lip and flattened nasal bridge, and they may have low calcium from hypoparathyroidism or cardiac defects. If the entire syndrome is not present (as is the usual case), the syndrome is called incomplete DiGeorge, and if all elements are present

and the thymus is absent, the syndrome is called complete. Children with complete DiGeorge are particularly susceptible to viral and fungal infections.

In some cases, no treatment is required for DiGeorge syndrome because T lymphocyte production improves. Either an underdeveloped thymus begins to produce more T lymphocytes, or organ sites other than the thymus compensate by producing more T lymphocytes.

### **COMBINED IMMUNODEFICIENCIES**

Some types of immunodeficiency disorders affect both B lymphocytes and T lymphocytes. For example, severe combined immunodeficiency disease (SCID) is caused by the defective development or function of these two types of lymphocytes. It results in impaired humoral and cellular immune responses. SCID usually is recognized during the first year of life. It tends to cause fungal infections, including severe thrush that does not respond to usual treatment; severe diarrhea; and serious bacterial infections. If the deficiency is not treated (usually by bone marrow transplant), a person with SCID usually dies from infection before the age of two years. The most common form of SCID is X-linked, i.e. the defect is on the X chromosome and, therefore, occurs only in boys. In the early 2000s new genetic defects leading to SCID are being identified each year.

### **Disorders of Innate Immunity**

Disorders of innate immunity affect phagocytes or the complement system. These disorders also result in recurrent infections.

### **AIDS (Acquired Immunodeficiency Disorders)**

Acquired immunodeficiency is more common than congenital immunodeficiency. It is the result of an infectious process or other disease. For example, the human immunodeficiency virus (HIV) is the virus that causes acquired immunodeficiency syndrome (**AIDS**). HIV, however, is not the most common cause of acquired immunodeficiency.

Acquired immunodeficiency often occurs as a complication of other conditions and diseases. For example, the most common causes of acquired immunodeficiency are malnutrition, some types of cancer, and infections. People who weigh less than 70 percent of the average weight of persons of the same age and gender are considered to be malnourished. are chicken pox, Examples of types of infections that can lead to immunodeficiency cytomegalovirus, German measles (rubella), measles, tuberculosis, infectious mononucleosis (Epstein-Barr virus), chronic hepatitis, lupus, and bacterial and fungal infections.

In some cases, acquired immunodeficiency is brought on by drugs used to treat another condition. For example, patients who have an organ transplant are given drugs to suppress the immune system so the body will not reject the organ. Also, some chemotherapy drugs that are given to treat cancer have the side effect of killing cells of the immune system. During the period of time that these drugs are being taken, the risk of infection increases. It usually returns to normal after the person stops taking the drugs.

### **Causes and Symptoms**

Congenital immunodeficiency is caused by genetic defects that generally occur while the fetus is developing in the uterus. These defects affect the development and/or function of one or more of the components of the immune system. Acquired immunodeficiency is the result of a disease process, and it occurs later in life. The causes can be diseases, infections, or the side effects of drugs given to treat other conditions.

People with an immunodeficiency disorder tend to become infected by organisms that do not usually cause disease in healthy persons. The major symptoms of most immunodeficiency disorders are repeated infections that heal slowly. These chronic infections cause symptoms that persist for long periods of time. People with chronic infection

tend to be pale and thin. They may have skin rashes. Their lymph nodes may be absent or larger than usual, and in some types of immune deficiency the spleen and liver may be enlarged. (The lymph nodes are small organs that house antibodies and lymphocytes.) This can result in black-and-blue marks in the skin. The person may lose hair from their head. Sometimes, a red inflammation of the lining of the eye (conjunctivitis) is present. They may have a crusty appearance in and on the nose from chronic nasal dripping.

### **Diagnosis**

Usually, the first sign that individuals may have an immunodeficiency disorder is that they do not improve rapidly when given antibiotics to treat an infection. An immunodeficiency disorder is likely to be present when rare diseases occur or the patient gets ill from organisms that do not normally cause diseases, especially if the patient gets repeatedly infected. When this happens in very young children, a genetic defect may be causing an immunodeficiency disorder. When this situation occurs in older children or young adults, their medical history may indicate that childhood diseases may have caused an immunodeficiency disorder. Other possibilities also exist, such as recently acquired infections (e.g. HIV, hepatitis, tuberculosis, etc.).

Laboratory tests are used to determine the exact nature of the immunodeficiency. Most tests are performed on blood samples. Blood contains antibodies, lymphocytes, phagocytes, and complement components, all of the major immune components that might cause immunodeficiency. A blood cell count determines if the number of phagocytic cells or lymphocytes is below normal. Lower than normal counts of either of these two cell types correlates with immunodeficiency. The blood cells also are checked for their appearance. Sometimes a person may have normal cell counts, but the cells are structurally defective. If the lymphocyte cell count is low, further testing is usually done to determine whether any particular type of lymphocyte is lower than normal. A lymphocyte proliferation test is done to determine if the lymphocytes can respond to stimuli. The failure to respond to stimulants correlates with immunodeficiency. Antibody levels can be measured. Complement levels can be determined by immunodiagnostic tests.

### **Treatment**

There is no cure for congenital immunodeficiency disorders. Therapy is aimed at controlling infections (such as with antibiotics) and, for some disorders, replacing defective or absent components.

Patients with Bruton's agammaglobulinemia must be given periodic infusions of pooled immunoglobulin from multiple donors. The product is called intravenous immunoglobulin (IVIG). The infusions are given approximately once a month for life to compensate for the patients' inability to make these proteins.

Common variable immunodeficiency also is treated with periodic infusions of IVIG throughout life. Additionally, antibiotics are given when necessary to treat infections.

Patients with selective IgA deficiency usually do not require any treatment. Antibiotics can be given for frequent infections.

In some cases, no treatment is required for DiGeorge syndrome because T lymphocyte production improves on its own. In some severe cases, a bone marrow transplant or thymus transplant can be performed to correct the problem.

For most patients with SCID, bone marrow transplantation is necessary. In this procedure, healthy bone marrow from a donor who has a similar type of tissue (usually a relative, such as a brother or sister) is removed. The bone marrow, a substance that is found in the cavity of bones, is the factory that produces blood cells, including some of the white blood cells that make up the immune system. The bone marrow of the person receiving the transplant is destroyed and is then replaced with marrow from the donor. One type of SCID called adenosine deaminase (ADA) deficiency is treated with infusion of the deficient enzyme on a regular basis, and another type of SCID due to an absence of an interleukin, a protein

that is important in directing the immune response, is also treated by infusions of the missing protein.

Treatment of the HIV infection that causes AIDS consists of drugs called antiretrovirals. These drugs interrupt the virus replication cycle and, therefore, spare the T cells. Several of these drugs used in various combinations with one another can treat but not cure the disease. Decreasing the viral in the blood to very low levels allows the immune system to remain intact. Other treatments for people with AIDS are aimed at the particular infections and conditions that arise as a result of the impaired immune system.

For people being treated for cancer, periodic relief from chemotherapy drugs can restore the function of the immune system. In some cases, IVIG is utilized to boost the immune system.

### **Alternative Treatment**

For some individuals, alternative treatments such as acupuncture therapy to ease infection-related symptoms or homeopathic medicines to boost immunity may be used in conjunction with traditional medicine as part of a patient's treatment plan.

### **Nutritional Concerns**

In most cases, immunodeficiency caused by malnutrition is reversible. The health of the immune system is directly linked to the nutritional status of the patient. Among the essential nutrients required by the immune system are proteins, vitamins, iron, and zinc.

### **Prognosis**

The prognosis depends on the type of immunodeficiency disorder. People with Bruton's agammaglobulinemia who are given IVIG infusions generally live into their 30s or 40s. They often die from chronic infections, usually of the lung. People with selective IgA deficiency generally live normal lives. They may experience problems if given a blood transfusion, and therefore they should wear a Medic Alert bracelet or have some other way of alerting any physician who treats them that they have this disorder.

SCID is the most serious of the immunodeficiency disorders. If a bone marrow transplant is not successfully performed, the child usually may not live beyond two years of age.

People with HIV/AIDS are living longer than in the past because of the antiretroviral drugs that became available in the mid-1990s. In the early 2000s HIV is still a potentially fatal illness, but medications have changed the face of the disease for those who have access to them. If medical treatment is timely and successful, T cells do not become depleted and opportunistic infections do not occur.

### **Prevention**

Primary or congenital immunodeficiencies are genetic and are not preventable by avoidance of exposures or by dietary measures. However, someone with a congenital immunodeficiency disorder might want to consider getting genetic counseling before having children in order to find out if there is a chance they will pass the defect on to their children.

Some of the infections associated with acquired immunodeficiency can be prevented or treated before they cause problems. For example, there are effective treatments for tuberculosis and most bacterial and fungal infections. HIV infection can be prevented by practicing safe sex (e.g. using a condom) and by not using illegal intravenous drugs. These are the primary routes of transmitting the virus.

### **Nutritional Concerns**

In general, people with immunodeficiency disorders should maintain a healthy diet because malnutrition can aggravate immunodeficiencies. People can prevent malnutrition by getting adequate nutrition. They also should avoid being near others who have colds or are

sick because they can easily acquire new infections. For the same reason, they should practice good personal hygiene, especially dental care. People with immunodeficiency disorders also should avoid eating undercooked food because it might contain bacteria that could cause infection. While this food might not cause infection in others, it is a potential source of infectious organisms for someone with an immunodeficiency.

### **Parental Concerns**

If a child has been diagnosed with an immunodeficiency disorder, the parents may be instructed to refrain from having normal childhood vaccinations that contain live viruses, since even weakened versions of the virus may cause serious disease. In some cases, the immuno-deficient child needs to be encouraged to wear a mask when in public or around family members who are sick in order to reduce the risk of developing an infection.

HIV stands for human immunodeficiency virus. It kills or damages the body's immune system cells. AIDS stands for acquired immunodeficiency syndrome. It is the most advanced stage of infection with HIV.

HIV most often spreads through unprotected sex with an infected person. It may also spread by sharing drug needles or through contact with the blood of an infected person. Women can give it to their babies during pregnancy or childbirth.

The first signs of HIV infection may be swollen glands and flu-like symptoms. These may come and go a month or two after infection. Severe symptoms may not appear until months or years later.

A blood test can tell if you have HIV infection. Your health care provider can perform

## **19. AUTOIMMUNE DISEASES – EXAMPLES, CONCEPT AND MECHANISMS**

**Autoimmunity** is the failure of an organism to recognize its own constituent parts as *self*, which allows an immune response against its own cells and tissues. Any disease that results from such an aberrant immune response is termed an autoimmune disease. Autoimmunity is often caused by a lack of germ development of a target body and as such the immune response acts against its own cells and tissues.

### **EXAMPLES OF AUTOIMMUNE DISEASES**

#### **Rheumatoid Arthritis**

In people with rheumatoid arthritis, the immune system predominantly targets the lining (synovium) that covers various joints. Inflammation of the synovium is usually symmetrical (occurring equally on both sides of the body) and causes pain, swelling, and stiffness of the joints. These features distinguish rheumatoid arthritis from osteoarthritis, which is a more common and degenerative "wear-and-tear" arthritis.

Currently available therapy focuses on reducing inflammation of the joints with anti-inflammatory or immunosuppressive medications. Sometimes, the immune system may also target the lung, blood vessels, or eye; occasionally patients may also develop symptoms of other autoimmune diseases such as Sjogren's the inflammation, itching, and scaling. For more severe cases, oral medications are used. Psoriasis is common and may affect more than 2 out of 100 Americans. Psoriasis often runs in families.

#### **Multiple Sclerosis**

Multiple sclerosis is a disease in which the immune system targets nerve tissues of the central nervous system. Most commonly, damage to the central nervous system occurs intermittently, allowing a person to lead a fairly normal life. At the other extreme, the symptoms may become constant, resulting in a progressive disease with possible blindness, paralysis, and premature death. Some medications such as beta interferon are helpful to people with the intermittent form of multiple sclerosis.

In young adults, multiple sclerosis is the most common disabling disease of the nervous system. Multiple sclerosis afflicts 1 in 700 people in this country. Researchers continue to search for triggers of the disease.

#### **Immune-Mediated or Type 1 Diabetes Mellitus**

Type 1 diabetes mellitus results from autoimmune destruction of the insulin-producing cells of the pancreas. Insulin is required by the body to keep the blood sugar (glucose) level under control. High levels of glucose are responsible for the symptoms and the complications of the disease. However, most of the insulin-producing cells are destroyed before the patient develops symptoms of diabetes. Symptoms include fatigue, frequent urination, increased thirst, and possible sudden confusion.

Type 1 diabetes mellitus is usually diagnosed before the age of 30 and may be diagnosed as early as the first month of life. Together with Type 2 diabetes (not considered an autoimmune disease), diabetes mellitus is the leading cause of kidney damage, loss of eyesight, and leg amputation. Close control of sugar levels decreases the rate at which these events occur. There is a genetic predisposition to Type 1 diabetes, which occurs in 1 out of 800 people in the United States. Among individuals who have a close relative with Type 1 diabetes, those at high risk for developing disease can be identified. Efforts are now under way to evaluate prevention strategies for these family members at risk.

## **Inflammatory Bowel Diseases**

This medical term is used for both Crohn's disease and ulcerative colitis, two diseases in which the immune system attacks the gut (intestine). Patients may have diarrhea, nausea, vomiting, abdominal cramps, and pain that can be difficult to control. Illness in afflicted individuals can result from intestinal inflammation and from side effects of the drugs used for the disease. For example, daily use of high-dose corticosteroid (prednisone) therapy, which is needed to control severe symptoms of Crohn's disease, can predispose patients to infections, bone thinning (osteoporosis), and fractures. For patients with ulcerative colitis, surgical removal of the lower intestine (colon) will eliminate the disease and their increased risk for colon cancer. More than 1 in 500 Americans has some type of inflammatory bowel disease.

## **Systemic Lupus Erythematosus**

Patients with systemic lupus erythematosus most commonly experience profound fatigue, rashes, and joint pains. In severe cases, the immune system may attack and damage several organs such as the kidney, brain, or lung. For many individuals, symptoms and damage from the disease can be controlled with available anti-inflammatory medications. However, if a patient is not closely monitored, the side effects from the medications can be quite serious. Lupus occurs in 1 out of 2,000 Americans and in as many as 1 in 250 young, African-American women.

## **Psoriasis**

Psoriasis is an immune system disorder that affects the skin, and occasionally the eyes, nails, and joints. Psoriasis may affect very small areas of skin or cover the entire body with a buildup of red scales called plaques. The plaques are of different sizes, shapes, and severity and may be painful as well as unattractive. Bacterial infections and pressure or trauma to the skin can aggravate psoriasis. Most treatments focus on topical skin care to relieve the inflammation, itching, and scaling. For more severe cases, oral medications are used. Psoriasis is common and may affect more than 2 out of 100 Americans. Psoriasis often runs in families.

## **Scleroderma**

This autoimmune disease results in thickening of the skin and blood vessels. Almost every patient with scleroderma has Raynaud's, which is a spasm of the blood vessels of the fingers and toes. Symptoms of Raynaud's include increased sensitivity of the fingers and toes to the cold, changes in skin color, pain, and occasionally ulcers of the fingertips or toes. In people with scleroderma, thickening of skin and blood vessels can result in loss of movement and shortness of breath or, more rarely, in kidney, heart, or lung failure. The estimated number of people with any type of scleroderma varies from study to study but may range from 1 to 4 affected individuals for every 10,000 Americans (or as many as 1 out of 2500 individuals).

## **Autoimmune Thyroid Diseases**

Hashimoto's thyroiditis and Grave's disease result from immune system destruction or stimulation of thyroid tissue. Symptoms of low (hypo-) or overactive (hyper-) thyroid function are nonspecific and can develop slowly or suddenly; these include fatigue, nervousness, cold or heat intolerance, weakness, changes in hair texture or amount, and weight gain or loss. The diagnosis of thyroid disease is readily made with appropriate laboratory tests. The symptoms of hypothyroidism are controlled with replacement thyroid hormone pills; however, complications from over- or under-replacement of the hormone can occur. Conventional treatment of hyperthyroidism requires long-term anti-thyroid drug therapy or destruction of the thyroid gland with radioactive iodine or surgery. Both of these treatment approaches carry certain risks and long-term side effects. Thyroid diseases are estimated to affect as many as 10 percent of the population, and affect women seven times

more often than men. They are frequently found in families where there are other autoimmune diseases.

### **CONCEPT**

Autoimmune disorders are diseases caused by the body producing an inappropriate immune response against its own tissues. Sometimes the immune system will cease to recognize one or more of the body's normal constituents as "self" and will create autoantibodies – antibodies that attack its own cells, tissues, and/or organs. This causes inflammation and damage and it leads to autoimmune disorders.

The cause of autoimmune diseases is unknown, but it appears that there is an inherited predisposition to develop autoimmune disease in many cases. In a few types of autoimmune disease (such as rheumatic fever), a bacteria or virus triggers an immune response, and the antibodies or T-cells attack normal cells because they have some part of their structure that resembles a part of the structure of the infecting microorganism.

Autoimmune disorders fall into two general types: those that damage many organs (systemic autoimmune diseases) and those where only a single organ or tissue is directly damaged by the autoimmune process (localized). However, the distinctions become blurred as the effect of localized autoimmune disorders frequently extends beyond the targeted tissues, indirectly affecting other body organs and systems.

### **MECHANISMS**

Researchers don't appear to have come to any definite conclusions regarding the mechanism's of immune dysfunction behind autoimmune diseases. This is not surprising since the immune system itself is a new frontier of study.

The following are some examples of credible possibilities put forth by a variety of researchers. It's an intricate area and experts don't necessarily agree. There may also be some overlapping. Some theories are more widely held than others.

#### **T Suppressor Cells**

The function of T suppressor cells in the immune system is to stop the immune response; the foe is destroyed! T suppressor cells can "damp down" an immune response at the appropriate time.

It is a widely held belief that the seemingly unregulated autoantibody production in autoimmune disease is a result of inadequate T suppressor cell function. T suppressor cells also are thought to be responsible for distinguishing between "self" and foreign tissue and thus prevent autoimmunity.

"Decreased numbers and activity of T suppressor cells in patients with virtually all types of autoimmune disorders have been reported." Drs. Isenberg and Morrow, *Friendly Fire*

#### **T Helper Cells**

The Helper T cell is the "quarterback" of the immune system. They're the organizers of immune activity; aiding, abetting and directing virtually every facet of the immune system.

Almost everything your immune system can do is dependent on the T Helper cell. It directs the activity through the secretion of protein molecules called cytokines.

Interleukin 2 and Gamma Interferon are examples of cytokines. Cytokines are information molecules and are produced by other cells as well.

T Helper cells produce Th1 and Th2 cytokine profiles, among others, based upon the cytokine environment (information/cytokines from other cells). Th1 cytokines are great for promoting a defense against a viral or bacterial attack.

Th2 cytokines organize defenses against parasites and mucosal infections but will shut down the activity of Th1 in the process. Some researchers believe that some viruses, bacteria and mycoplasma make proteins that mimic a cytokine that effectively turns on the Th2 cytokines thereby turning off the Th1 cytokines actually needed to promote killing them.

This leaves the immune system, or part of it, in a state not fully functional for the task at hand.

Dr. Lauren Sompayrac states that by “secreting the appropriate set of cytokines, Th cells can help produce an immune response that is appropriate to a given invader – so that the punishment fits the crime.”

## 20. TYPES OF HYPERSENSITIVITY

Hypersensitivity is defined as the violent reaction of the immune system leading to severe symptoms and even death in a sensitised animal when it is re-exposed to the same antigen for the second time. It is nothing but **allergy**.

The immune system is devised by nature primarily to protect the human body against pathogens. Sometimes this system becomes overenthusiastic, and brings discomfort. This phenomenon is similar to the overenthusiastic servant who tried to kill the fly sitting on the king's nose with a strong sharp knife.

Immune response is always directed towards the protection of host. But in hypersensitivity the immune response becomes injurious to the host. Hence the immune response becomes a destructive process in hypersensitivity.

In protective immune response, the antigen or bacterium or virus is killed or neutralised. But in hypersensitivity, the cells of the host are killed or the host itself is damaged or killed.

Hypersensitivity is the changed reactivity of the immune system. It is a beneficial protective system gone out of order.

Hypersensitivity does not occur in all human beings. Only about 10% of human population suffers from hypersensitivity.

The factors causing hypersensitivity are called **allergens**.

In clinical terms, hypersensitivity is called allergy.

A familiar example for hypersensitivity is the allergy caused by penicillin injection. Sometimes, in the hospitals a patient dies immediately after penicillin injection. This is due to allergy. The allergy caused by drugs is called drug allergy.

Hypersensitivity can bring its symptoms either immediately within minutes or in a delayed state, after 24 to 48 hours.

### Factors Causing Hypersensitivity

Hypersensitivity is caused by numerous factors. These factors causing allergy are called **allergens**. They may be **extrinsic factors** (introduced into the body from outside) or **intrinsic factors** (factors which remain within the body). They are the following:

1. Drugs such as penicillin, sulphamide, aspirine, etc.
2. Airborne particles such as pollen grains, house dust mite, spores, animal danders (scales and feathers of animals), etc.
3. Food stuffs such as shell fish, strawberries, brinjal, etc.
4. Infectious organisms like bacteria, viruses, fungi, parasite, etc.
5. Blood transfusion of mismatched blood.

### Common Hypersensitivity Reactions

Hypersensitivity produces a variety of reactions. The reactions may manifest immediately within minutes or in a delayed state after 24 to 48 hours.

The reactions caused in hypersensitivity may be local reactions or systemic reactions. When the symptoms appear in a restricted area or areas, the reaction is called local. When the symptoms affect all the organ systems of the body, the reaction is called systemic.

The **common hypersensitivity reactions** are Anaphylaxis, Transfusion reactions, Erythroblastosis foetalis, Arthus reaction, Serum sickness, Mantoux reaction, Contact dermatitis and Graves disease.

## Types of Hypersensitivity

Hypersensitivity is classified in two ways. They are, 1. Based on the time taken for the reaction. 2. Based on the different mechanisms of pathogenesis.

### 1. Classification Based on the Time Taken for Reactions

Based on the time required for a sensitised host to develop clinical reactions upon reexposure to the antigen, hypersensitivity is classified into two categories. They are immediate hypersensitivity and delayed hypersensitivity.

#### 1. Immediate Hypersensitivity

When the immune reactions manifest in a short duration of time, within minutes, the hypersensitivity is called immediate type.

Most of the hypersensitivities to drugs like penicillin belong to this category. Hayfever is another example. Immediate hypersensitivity is characterised by the following salient features:

1. This immune reaction appears and disappears rapidly.
2. Inflammatory response occurs in a few minutes.
3. It produces urticaria, wheal and granulocyte accumulation.
4. It involves the interaction of antigen and antibody.
5. It is handled by B-cells by the production of antibodies. Hence immediate hypersensitivity is antibody-mediated.
6. It can be passively transferred from one host to another by the transfer of serum.
7. Desensitization can be easily done by small allergen.
8. It is inhibited by antihistamine drugs except Arthus reaction.

Classification of hypersensitivity is given in the Figure 45.

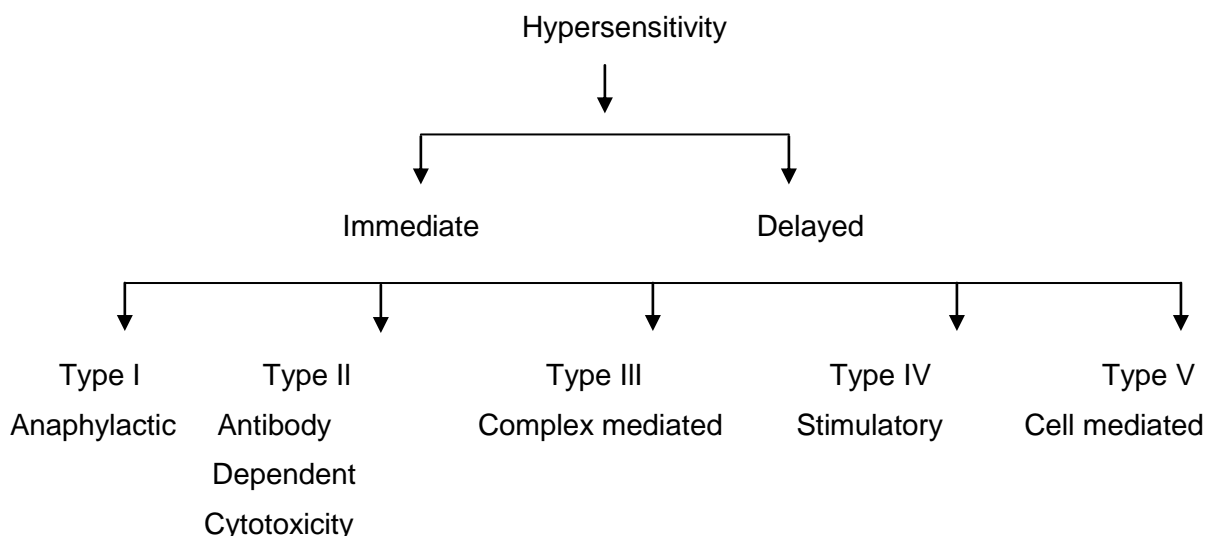


Figure 45. Classification of hypersensitivity.

#### 2. Delayed Hypersensitivity

When the immune reactions manifest slowly from 24 hours to 72 hours, the hypersensitivity is called **delayed type**. A familiar example for delayed hypersensitivity is Mantoux reaction (Figure 46) obtained by injection of tuberculin the skin of an individual in whom previous infection with mycobacterium had induced a cell mediated immunity reaction appears after 24 to 48 hours. The reaction is characterized by **erythema and induration**.

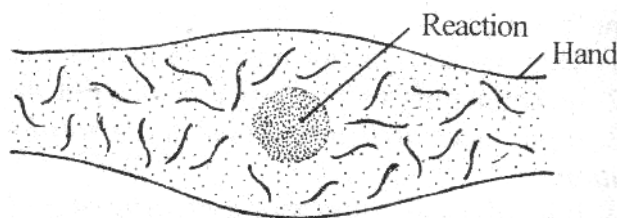


Figure 46. Mantoux reaction

Delayed hypersensitivity is characterized by the following salient features:

1. This immune response appears slowly and lasts longer.
2. Inflammatory response manifests only after 24 hours.
3. It produces erythema, induration and lymphocyte infiltration.
4. It involves the reaction between antigens and T- cells.
5. As it is handled by T-cells, it is a cell-mediated immunity.
6. It cannot be transferred from the sensitised host to another by the transfer of serum.  
But it can be transferred by the transfer of T-cells.
7. Desensitization cannot be easily done by drugs.
8. It is suppressed by corticosteroids.

## II Classification Based on the Different Mechanisms of Pathogenesis

Coombs and Gell (1963) proposed 5 types of hypersensitivity based on the different mechanisms of pathogenesis. They are the following:

1. Type I : Anaphylactic hypersensitivity
2. Type II : Antibody-dependent cytotoxic hypersensitivity.
3. Type III: Immune complex - mediated hypersensitivity.
4. Type IV: Cell - mediated hypersensitivity.
5. Type V : Stimulatory hypersensitivity.

Of these 5 types, type IV alone is delayed type of hypersensitivity and the other types are immediate type.

### Type 1: Anaphylactic Hypersensitivity (Anaphylaxis)

Anaphylaxis is defined as an allergic reaction of an organism to a foreign substance to which it has previously become sensitised resulting from the release of histamine, serotonin and other vasoactive substances,

The term anaphylaxis was coined by Richet in 1902 and it means without protection (ana = without, phylaxis = protection). Anaphylaxis is immediate type hypersensitivity. In anaphylaxis, antibodies are fixed on the surface of tissue cell (mast cell and basophils) in sensitised host. The antigen combines with the cell fixed antibody. Leading to release of pharmacologically active substances (vasoactive amines) which produces the clinical reaction.

### Factors causing Anaphylaxis

Anaphylactic reactions are caused when the sensitised animals receive allergens for the second time. The factors causing anaphylaxis are certain drugs, the faeces from house dust, mite, animal danders, pollen grains, food allergens, etc.

## **Symptoms of Anaphylaxis**

Anaphylactic reactions cause the following symptoms:

1. **Death:** When penicillin injection is given to a sensitive person, he shows anaphylactic reactions. There is intense constriction of bronchi and bronchioles. Smooth muscles contract and the capillaries dilate. The person begins to wheeze\* and within minutes, he dies due to asphyxia\*.

Similar reactions also occur rarely, when a man is stung by bees or wasps.

2. **Diarrhoea and Vomiting:** These are caused by food allergens.

3. **Urticaria:** Urticaria is a vascular reaction of the skin marked by slightly elevated patches which are reddish or paler than the surrounding skin. It is often attended severe itching. It is caused by certain foods or drugs.

4. **Atopy:** Atopy literally means out of place or strangeness. The term atopy was coined by Coca (1923). This term is used to refer to naturally occurring familiar hypersensitivities such as hayfever and asthma.

The antigens responsible for atopy are called atopens. They may be inhalants (pollens, house dust, etc.) or ingestants (egg, milk, etc.) or contactants. They elicit the production of antibodies of the type IgE. These antibodies are called **reagin antibodies**.

The tendency to develop atopy is genetically determined. Atopy therefore runs in families. All human beings are capable of forming reagin antibody in small amount, but in atopics, the reagin antibodies are produced in large amount. About 10% of persons have this tendency to over produce reagin. It has been reported that bottle feed babies tend to develop atopy in later life more often than breast feed babies.

The atopic allergy is due to the production of IgE antibodies which in turn is due to the deficiency of IgA antibodies.

Thus IgA deficiency may predispose to atopy.

**The symptoms of atopy** include conjunctivitis in the rhinitis in the respiratory tract, dermatitis in the skin, urticaria, vomiting, diarrhoea, etc.

## **Mechanism of Anaphylaxis**

The culprit of anaphylactic hypersensitivity is one type of Immunoglobulin called IgE antibody. When a person receives the allergens (antigens) for the first time, the allergens get attached to the B cells. The allergens stimulate the B cells to change into plasma cells. The plasma cells make IgE antibodies.

The IgE antibodies are called reagins and they are made by people who are allergic. The reaginic antibodies have a strong affinity for fixation to mast cells or basophils in skin or mucous surfaces.

The IgE antibodies produced for the first time, get attached to the surface receptors of mast cells with the help of their Fc segment. This reaction would not harm the person and the person is now \*said to be immunised or sensitised that particular antigen.

As this initial contact with antigen leads to the priming, the B-cells. This is known as sensitising or priming dose. Subsequent contact with the allergen causes manifestations of hypersensitivity. This is known as shock - dose.

When the animal is exposed to the same antigen for the second time the animal would be in danger. The IgE antibodies attached to the surface of mast cells, bind the antigens. The allergens cross link the IgE antibodies attached to the mast cells.

This cross linking of IgE antibodies triggers the mast cells and a series of enzymatic reactions occurs inside mast cells. As a result the mast cells release granules. The phenomenon of releasing of granules from mast cells is called degranulation. These granules contain substances like histamine, serotonin, heparin, etc. These substances are the **primary cause for anaphylaxis** (Figure 47).

The manifestations of anaphylaxis are due to the mediators. The mediators are of two types, namely primary mediators and secondary mediators. The primary mediators include histamine, serotonin, heparin, etc. They are released from the granules of mast cells and basophils. They bring about their action quickly and immediately.

The secondary mediators are produced by leucocytes upon the stimulation of mast cells. They act slowly. They include slow reacting substance of anaphylaxis (SRS-A), prostaglandins, leukotrienes, platelet activating factor (PAF), etc.

In addition to the above mediators, complement activation releases anaphylatoxins.

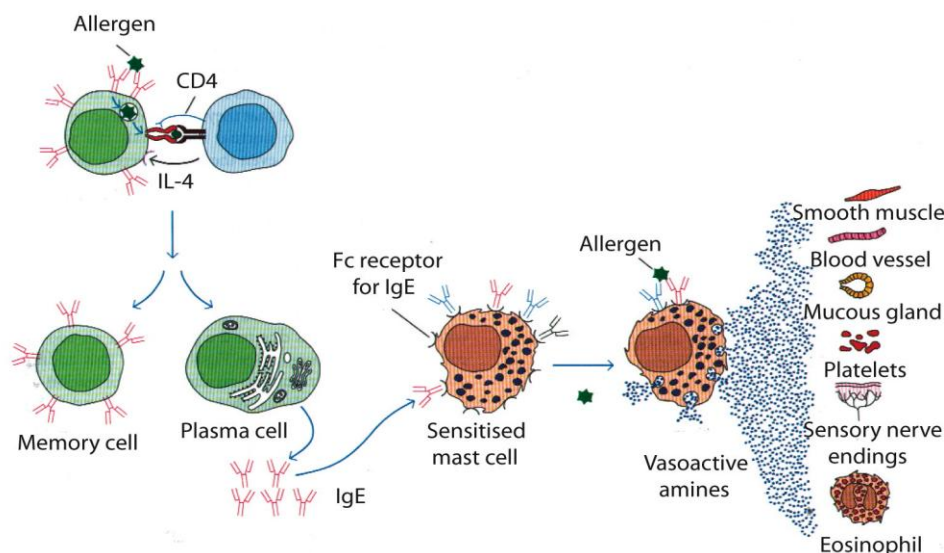


Figure 47. The processes underlying type I hypersensitivity reactions.

These mediators bring about the anaphylactic reactions such as burning and itching sensations, vasodilatation, capillary permeability, oedema, smooth muscle contraction, vasoconstriction, bronchoconstrictions and platelet adhesion and aggregation.

### Prevention and Treatment

1. Anaphylactic hypersensitivity can be prevented and treated in the following methods:
  1. **Avoiding Contact with Allergens:** Anaphylaxis can be prevented by avoiding contact with allergens.
  2. **Desensitization:** The hypersensitivity can be prevented by the administration of graded doses of allergen.
  3. **Hyposensitivity:** Anaphylactic reactions are caused by IgE antibodies. However IgG antibody blocks the IgE antibody. The IgG antibodies divert the allergen from contact with mast cells - IgE antibody and thus prevents allergy.
  4. **Stabilizing Mast Cells:** The anaphylactic reactions are caused by the triggering of mast cells. Mast cell triggering can be prevented by giving drugs like isoprenaline and sodium chromoglycate. Isoprenaline binds to the surface receptors of mast cells and thus the mast cells are stabilized.

Sodium Chromoglycate can stabilize the lysosomal membrane of mast cells even during cross-linking of antigen and IgE.

5. **Inhibition of Histamine Receptors:** Antihistamines suppress anaphylaxis. Antihistamines do not prevent the release of histamines from mast cells. But they inhibit the receptor for histamine on cell surface.
6. **Blocking the Release of Histamines :** The derivatives of adrenergic drugs and theophyllin derivatives block the release of amines from mast cells indirectly. They influence the mast cells to stimulate adenocyclase and raise cAMP and thereby stabilize lysosomal membrane and block the release of amines. These drugs also influence the smooth muscles for relaxation, a reverse of anaphylactic reactions.
7. **Steroids and chromoglycate** also inhibit the release of amines from mast cells. Both these drugs stabilize the lysosomal membrane and inhibit the release of amines from mast cells.

## **Type II: Antibody Dependent Cytotoxic Hypersensitivity**

Type II hypersensitivity is due to the interactions of antibodies and cell associated antigens. When antibodies attach to the antigens located on the surface of cells, the cells become cytotoxic. Hence the name cytotoxic reaction. eg. Agglutination and lysis of blood cells due to mismatched blood groups, erythroblastosis foetalis, autoimmune haemolytic anaemia, etc.

### **Types of Cytotoxic Hypersensitivity**

Based upon the type of antigen involved in the reaction the cytotoxic hypersensitivity is classified into two types, namely isoimmune reactions and autoimmune reactions.

#### **1. Isoimmune Reactions**

Isoimmune reaction is due to the involvement of isoantigens and antibodies. Isoantigens are the antigens of the same species differing in their antigenetic properties from one individual to another. The reactions brought about by the antigen and antibody of two individuals belonging to the same species are called isoimmune reactions. The blood group antigens and leucocyte antigens are isoantigens. Isoimmune reactions include,

1. Transfusion reactions.
2. Erythroblastosis foetalis.
3. Transplant rejection reactions.

#### **1. Transfusion Reaction**

Transfusion is the introduction of the blood of a donar into the blood stream of a recipient person. If the blood of the donor and the recipient are not properly matched, the blood cell agglutination or lysis takes place. The agglutination or lysis of recipient blood due to mismatched bloodgroup is called **transfusion reaction**. It occurs in ABO blood groups as well as Rh blood group.

The ABO blood group system has four groups of human beings, namely group A, group B, group AB and group O. This grouping is based on the presence of ABO blood group antigens and antibodies (Table 4).

There are two types of ABO group antigens, namely antigen A and antigen B. They occur on the surface of RBC. Similarly there are two types of antibodies, namely anti-a and anti- b. They occur in the blood serum.

A group persons contain antigen A and anti - b. B group persons contain antigen B and anti-AB group persons contain both antigen A and antigen B; but there is no antibody. O group person contains no antigens; but both, anti-a and anti-b are present.

This pattern of distribution of antigen and antibody clearly shows that antigen A and anti-a will not co-exist in the same blood. Similarly antigen B and anti-b will not coexist in the same blood. If they co-exist in the same blood, agglutination or lysis takes place.

Table 4. Distribution of antigens and antibodies in ABO blood group system.

Group	Antigen	Antibody
A	A	Anti-b
B	B	Anti-a
AB	AB	NIL
O	NIL	Anti-a and Anti-b

There will not be any reaction when A group person receives blood from another A group person; B group person receives from another- B group; AB group person receives blood from another AB group person: and O group person receives blood from another O group person. This is because here both donor and recipient have the same type of antigens and antibodies. For example, A group donor and A recipient have the antigens A and anti-b; they will not cross react.

When transfusion takes place between A group person and B group person, there will be transfusion reaction (Figure 48), For example, when A group person donates blood to a B group person, antigen A is coated with anti-a of B group person and, the RBCs are agglutinated or lysed.

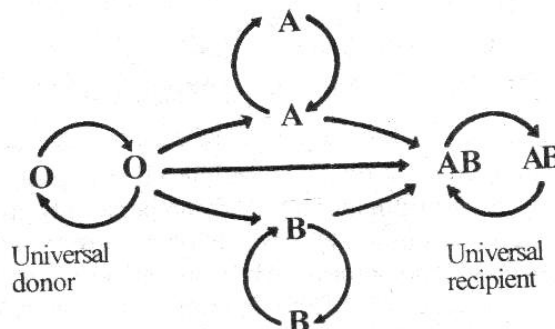


Figure 48. Blood transfusion.

Similarly when AB group person donates blood to O group person, there will be transfusion reaction because antigen A and antigen B will react with anti-a and anti-b of O group persons.

AB group person has no antibodies in his blood. So he can receive blood from any group person. So AB group person is called universal recipient.

Similarly, the O group person has no antigens in his blood. So he can donate blood to any group. So O group person is called universal donor.

Similarly, the O group person has no antigens in his blood. So, he can donate blood to any group. So O group person is called universal donor.

The antibodies involved in transfusion reaction belong to a class of IgM. These are called isohaemagglutinins and the reaction is an isoimmune reaction.

### Erythroblastosis Foetalis

Erythroblastosis foetalis is a haemolytic disease caused by the reaction of Rh antigen and Rh antibody. It occurs in the Rh<sup>+</sup> baby developing in an Rh<sup>-</sup> mother. The Rh antibody involved in this reaction belongs of IgG type. It is an isoimmune reaction.

Landsteiner (1940) found an antigen in Rhesus monkey and it is called Rh antigen or antigen D. Some human beings possess this antigen on their RBC and others do not contain it. The persons containing antigen D on their RBC are called Rh positive or Rh<sup>+</sup>. The persons who do not contain antigen D are called Rh negative or Rh<sup>-</sup>.

In India 93.5% is Rh<sup>+</sup> and 7.5% is Rh<sup>-</sup>. In China 99.5% is Rh<sup>+</sup> and only 0.5% is Rh<sup>-</sup>. The Rh blood group system has no natural antibody. But when a Rh<sup>-</sup> person receives blood, either 1 ml or more from a Rh<sup>+</sup> person, the blood of Rh<sup>-</sup> person responds and produces Rh antibody called anti-D.

The anti-D remains in his blood and it does not do any harm as such to the possessor, Now he is sensitised to antigen D. When he receives Rh<sup>+</sup> blood for the second time, antigen D and anti - D cross react with each other leading to is **haemolysis**.

Such a type of haemolysis occurs in the Rh<sup>+</sup> foetus of a Rh<sup>-</sup> lady. When an Rh<sup>-</sup> lady marries an Rh<sup>+</sup> man, their foetus will be Rh<sup>+</sup>.

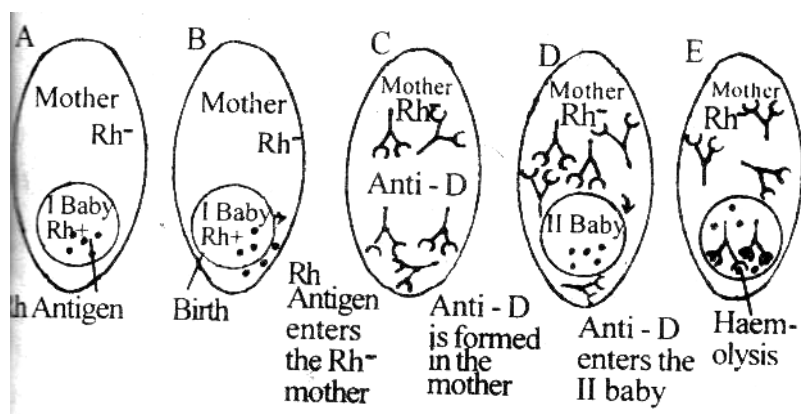


Figure 49. Blood transfusion.

The Rh<sup>+</sup> baby develops in the uterus of the mother. The RBC of Rh<sup>+</sup> baby contains antigen D. During delivery of this baby the placenta breaks with the rupture of some blood vessels connecting the foetus and the mother. As a result some antigen D present in the blood of the baby mixes with the blood of the mother. The blood of the Rh<sup>-</sup> mother responds and produces the anti-D. The anti-D formed now persists in the blood throughout her life.

The complication arises when she becomes pregnant for the second time. When the foetus makes contact with mother through the placenta, the anti - D from her blood passes into the blood of the foetus. The anti - D combines with Rh antigen present on the RBC of foetus. This binding causes the lysis of RBC of foetus. This haemolytic disease of the foetus is called **erythroblastosis foetalis**. The lysis of RBC leads to jaundice and the baby is killed. All subsequent babies will meet the same consequences.

Now this problem can be solved by therapy. The negative mother is given a dose of anti-D, within 72 hours soon after the first delivery. This step prevents Rh sensitization of the mother.

The Rh<sup>-</sup> mother lacking A and B group is not sensitized to D antigen perhaps as a result of quick distribution and elimination of antigen of the foetal RBC.

The antibody of the Rh blood group belongs to IgG type and the erythroblastosis foetalis is an **isoimmune reaction**.

### 3. Transplant Rejection Reaction

A transplant can produce antibodies to leucocyte antigens or transplantation antigens or HLA antigens. These antibodies are cytotoxic to graft tissues. Such damaged tissues are attacked by phagocytic and lymphoid cells. Platelets are also involved in such rejection phenomenon.

#### 4. Autoimmune Reaction

The reaction brought about by the interactions of antigen and antibody of the same individual is called autoimmune reaction. Autoimmune reaction is due to the interaction of autoantigens and antibodies. Autoantigens are the antigens of an individual. These antigens are not antigenic to the possessor. But antigens can stimulate immune system and give rise to immune reactions. The familiar examples of this reaction are the following:

1. Autoimmune haemolytic anaemia
2. Autoimmune thrombocytopenic purpura
3. Autoimmune thyroiditis
4. Autoimmune glomerulonephritis

#### Autoimmune Haemolytic Anaemia

The individual produces antibodies against his own RBC antigens. These antibodies become attached to antigens of RBCs. The red cells coated with antibodies are destroyed in the spleen and liver.

#### Mechanism of Cytotoxic Reaction

In cytotoxic reaction (Figure 50), the cell damage occurs in any one of the following methods:

##### 1. Phagocytosis

The antibodies are attached to the cell surface antigens. Then the macrophages bind to the antibody coated cells. The macrophage engulf the antibody coated cells.

##### 2. Lysis

The antibody coated cells bind to the phagocytic cells through their receptor for C3b. They may continue to fix in sequence upto the lytic C8 and C9 components to cause damage to the cells by a mechanism similar to that in lysis.

##### 3. Killing

The antibody coated cells may be attacked by cytotoxic killer cells which carry receptors for C3b and Fc portion of IgG.

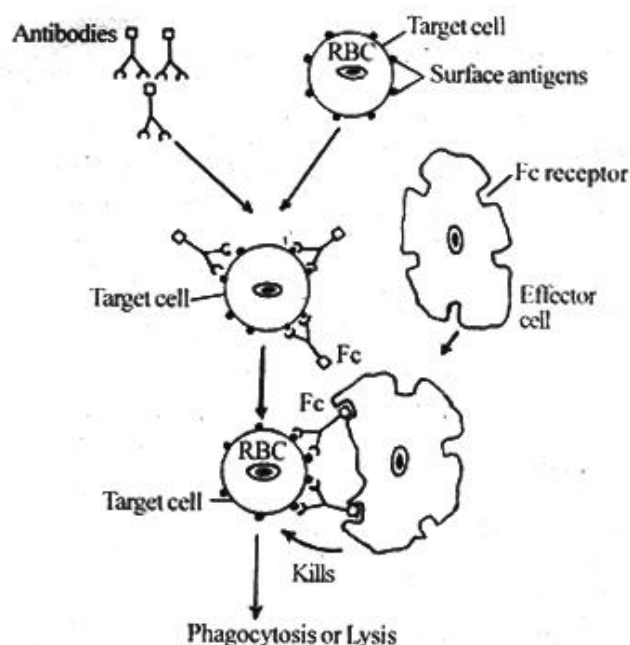


Figure 50. cytotoxic reaction.

### Type III: Immune Complex Mediated Hypersensitivity

The hypersensitivity produced by the antigen-antibody complex is called immune complex mediated hypersensitivity. In certain occasions, enormous amount of antigens enter the body. In response, the body produces higher concentration of antibodies. These antigens and antibodies combine together to form an insoluble precipitate called antigen antibody complex or immune complex.

These complexes get attached in and around minute blood vessels in the regions of glomerulus, synovium, skin, choroid plexus and tract. They cause tissue damage leading to hypersensitivity.

The antigens involved here are soluble antigens. The antibodies bodies involved in this reaction belong to the class of or IgM.

This hypersensitivity is caused by repeated infections to a microorganism or repeated contact with environmental agents and autoimmunity antibodies.

The familiar examples for immune complex mediated immunity are Arthus reaction, serum sickness, etc.

### Mechanism of Immune Complex Mediated Hypersensitivity

When enormous amount of soluble antigen enters the body, B cells produce large amount of antibodies of the type or IgM.

The antibodies bind with the antigens to form immune complexes (antigen - antibody complex).

These immune complexes get attached in and around minute capillaries (Figure 51).

These complexes bind to the complement.

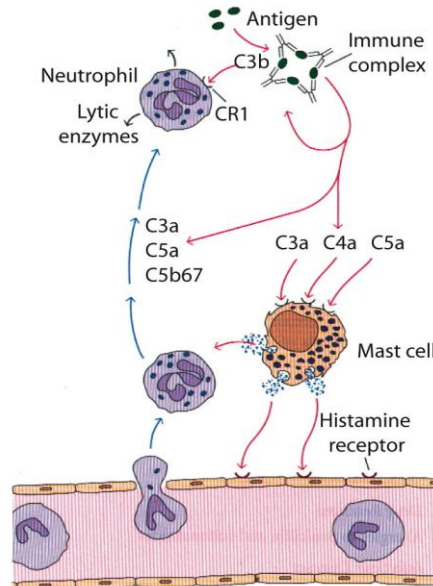


Figure 51. The processes underlying type III hypersensitivity reactions.

. This activates the classical pathway of the complement, The complement 3( $C_3$ ) and complement 5( $C_5$ ) produce active factors called **anaphylotoxin and chemotoxin**.

The anaphylotoxin triggers the mast cells to release vasoactive amines. The amines increase the tissue permeability facilitating the deposition of immune complexes and flow of accessory cells around the site of reaction.

The chemotoxin attracts the polymorphs and mast cells and promotes phagocytosis resulting in the release of hydrolytic enzymes from the granules which may lead to oedematous and haemorrhagic lesions.

### **Arthus Reaction**

1. Arthus reactions were first observed by Arthus in 1903 on rabbit by the repeated injection of horse serum.
2. It is a type III hypersensitivity reaction.
3. Arthus reaction is a local immune complex reaction produced on the skin by the intradermal injection of nontoxic foreign substances like horse serum, egg albumin, etc. into rabbits or guinea pigs.
4. The antibodies, produced in response to the foreign substances, are of IgG type and they combine with the antigen (foreign substance) to form immune complexes which get attached on the blood vessels.
5. The immune complexes activate complement components to produce toxins which in turn trigger polymorphs and mast cells to produce vasoactive amines resulting in severe reactions.
6. The reactions may cause erythema, induration, oedema, haemorrhage and necrosis. Leukocyte platelet thrombin are formed and they reduce blood supply and lead to tissue necrosis.
7. The reaction occurs in antibody excess.
8. It appears in 2 to 8 hours after injection and persists for about 12 to 24 hours.
9. Animal's genetically lacking one of the complement components may fail to demonstrate Arthus reaction.
10. Arthus reaction can be passively transferred from one animal to another by transferring large quantities of serum.
11. In human beings Arthus reaction is produced by the intradermal injection of respective antigens in patients with aspergillosis, farmer's lungs, bird fanciers disease, etc.

### **Serum Sickness**

1. Serum sickness is an immune complex disease caused by the enormous amount of foreign serum, such as antidiphtheria antiserum, antitetanus, antiserum.
2. Serum sickness was discovered by Von Pirquet and chick in 1905.
3. It is systemic (Affecting all organ systems of the body. Local form refers to a specific area) form of type III hypersensitivity
4. The clinical symptoms of serum sickness include fever, lymphadenopathy, splenomegaly, arthritis, glomerulonephritis, endocarditis, vasculitis, urticarial rashes, abdominal pain, nausea and vomiting.
5. The pathogenesis is caused by the formation of immune complexes by the combination of foreign serum and antibodies'
6. The antibodies involved are IgG type.
7. The complexes activate complements to produce anaphylotoxin and chemotoxin. These toxins trigger mast cells and polymorphs to release amines and enzymes.
8. The immune complexes get attached to the endothelial lining of the blood vessels.
9. Serum sickness differs from other types of hypersensitivity reactions in that a single injection can serve both sensitising and shocking dose.
10. The serum sickness appears 7 to 12 days after the injection. However, when subsequent injections are given, the symptoms manifest earlier.

## Type IV Cell-mediated Delayed Hypersensitivity

Type IV hypersensitivity is caused by the interaction between antigens and sensitised T cells. This reaction leads to inflammatory reaction and causes tissue damage.

Antibodies are not involved in type IV hypersensitivity. As T cells are involved in this reaction, it is called cell mediated hypersensitivity.

The symptoms on the skin appear only after 24 to 72 hours. As the reaction appears well later it is also called delayed type hypersensitivity,,

As it is a cell mediated reaction, it cannot be passively transferred from one animal to another by the transfer of antibodies. But it can be transferred by the transfer of T cells.

The T cells on contact with the antigen produce a soluble protein called lymphokine, which is responsible for type IV hypersensitivity.

Type IV hypersensitivity is caused by infectious pathogens like bacteria, viruses, fungi and parasites. It is also caused by contact to certain chemicals like nickel salts formed from jewellery, neomycin ointment, etc.

The common examples for type IV hypersensitivity are tuberculin reaction and contact dermatitis. Other examples are leprosy, small pox, measles, herpes, candidiasis, histoplasmosis, leishmaniasis, schistosomiasis, etc.

### Mechanism of Type IV Hypersensitivity

When T cells primed to an antigen (viral or bacterial) come in contact with the same antigen for the second time, the cells release soluble proteins called lymphokines (cytokines). The lymphokines activate macrophages (Figure 52) to kill intracellular bacteria like tubercle bacilli and lead to the formation of inflammatory cells like giant cells and epitheloid cells.

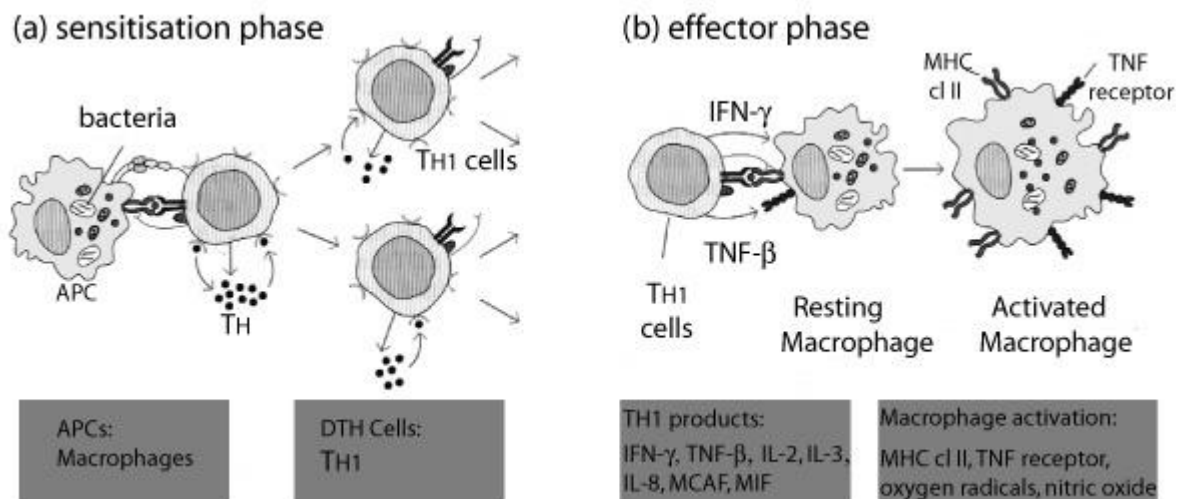


Figure 52. The processes underlying type IV hypersensitivity reactions.

### Tuberculin Reaction (Mantoux Reaction)

1. Tuberculin or mantoux reaction is a type IV hypersensitivity.
2. It is due to the interaction of sensitised T-cells and tuberculin bacterium.
3. This reaction is produced by T cells and not antibodies. Hence this reaction is called cell-mediated hypersensitivity.
4. The reactions manifest on the skin very late and only after 48 to 72 hours. Hence it is called delayed hypersensitivity.

5. When a small dose of tuberculin is injected intradermally in an individual already sensitised to tuberculo protein by prior infection, the reaction occurs. In unsensitised animals. tuberculin injection provokes no response. The mantoux reaction therefore indicates tuberculin infection in children.
6. The tuberculin reaction is characterized by local erythema and induration.
7. This reaction is caused by T cells. The T cells are activated by tuberculin and release lymphokines. The lymphokines trigger macrophages.

### **Contact Dermatitis**

Contact dermatitis is the inflammation of the skin due to contact with a substance to which the person is allergic.

The substances causing this allergy include cosmetics, disinfectants. Insecticides, rubber, leather plant and so on.

Dinitrochlorobenzene (DNCB) is a potent skin sensitizer. It evokes contact dermatitis in 95% of people who have T cell functions.

The reaction is characterised by Oedema of the epidermis.

It appears 12 to 15 hours after contact. So it is a delayed hypersensitivity. The reaction is due to the sensitization of T cell and not due to the production of antibodies. Hence it is a cell- mediated hypersensitivity. It is a type IV hypersensitivity.

Contact hypersensitivity commonly occurs in persons who use nickel jewellery clasps, neomycin ointment, etc.

### **Type V Stimulatory Hypersensitivity**

Type V hypersensitivity is caused by the interaction of antibodies with cell surface antigen leading to **stimulation of cells**. In type 11 hypersensitivity also the interaction between antibodies and cell surface antigens occur. But here, instead of stimulation destructions of cells occur.

This phenomenon of stimulation occurs in Grave's disease (thyroloicosis). Stimulation of thyroid cells by thyrold stimulating hormone is another example for type V hypersensitivity.

### **Thyrotoxicosis or Grave's Disease**

Thyrotoxicosis is a disease condition owing to the over activity of thyroid gland.

It is caused by an antibody called long acting thyroid stimulator (LA TS). It is an IgG type. It acts on the thyroid cell surface antigen which is basically a receptor for thyroid stimulating hormone produced by the pituitary gland.

This causes the release of thyroxine in higher dose from the thyroid cells. This causes the Grave's disease.