CHAPTER

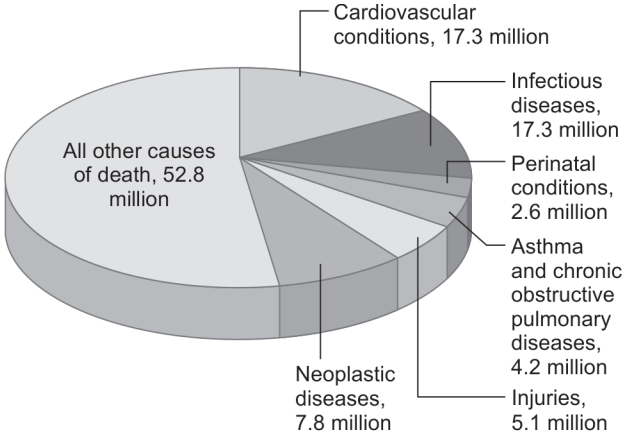
1

Introduction and Biological Significance of Immune System

1.1 INTRODUCTION

The immune system consists of a complicated network of various proteins and cells and keeps the record of all the invading foreign microorganisms and plays an essential role in the defending the body against infection as soon as microbes enter the body.

Our immune system is highly adaptive and protective in nature. The nature of recognition may range from a very small molecules of 30 nm including virus and large cells like *Dioctophyme renale*. But this diversity needs different recognition and destruction tools as immune mechanisms and network of cells.



Infectious diseases	Annual deaths (million)
Respiratory infection	3.53
Diarrheal diseases	2.46
HIV/AIDS	1.78
Tuberculosis	1.34
Malaria	0.83
Vaccine-preventable childhood diseases	0.45
Meningitis	0.34
Hepatitis B and C	0.20
Tropical parasitic diseases	0.14
STDs (other than HIV)	0.12
Dengue	0.02
Leprosy	0.01

Fig. 1.1: Comparison of annual deaths due to infectious diseases

Figure 1.1 shows that infectious diseases constitute a considerable proportion of all deaths worldwide. This drags the attention of various health agencies including World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) to guideline their prevention. Recently, the pandemic of Corona virus (Covid-19) results in many deaths throughout the world and the concept of immunity had become major research area.

IMPORTANT BARRIERS OF OUR BODY

To establish an infection by any pathogenic molecule/microbe in our body, it needs to cross many barriers that are studied under innate immune system. The two important barriers are epithelial cells available in the skin and intestinal gut. These barriers continuously work for preventing the entry of foreign microbes inside the body. Many chemicals secreted by skin epithelial cells that have the capacity to restrict the growth and entry of pathogens and also many degrading enzymes secreted by intestine and lower pH range again help in creating an environment unsuitable for pathogen entry.

Along with epithelial cell and gut, normal flora available at mucosal surface including GIT, genitourinary track and respiratory track has the capability to inhibit the attachment of pathogen to host cells.

ADAPTIVE IMMUNE RESPONSE

When the barriers for restricting the entry of pathogen in the host cells are breached, the next major system that comes into the picture is an adaptive immune response at the site of infection. This system is based on the functional mechanism of B lymphocytes and T lymphocytes. But the adaptive immune responses take much time to come at the picture but it is much more antigen-specific. The adaptive immune response is known for its better recognition, elimination and memorization property against the antigen.

Comparison of innate and adaptive immune responses

S. No.	Response	Innate immunity	Adaptive immunity
1.	Time	Minutes to hours	Days
2.	Specificity	Limited and unchanging	Highly diverse and it is adapted for improvement during the immune response
3.	Response to repeat infection	Similar every time	More rapid
4.	Major components	Barriers like skin, phagocytes, etc.	T and B lymphocytes, antigen-specific receptors and antibodies

1.2 CONCEPTULIZING IMMUNE SYSTEM

ANTIGEN

Antigens are the foreign substances that have the capability of stimulating the immune response in our body and activate lymphocytes to combat with the incoming threat. It could be a bacteria, virus, etc.

Antigens are high molecular weight molecules and made-up of proteins or polysaccharides. Sometimes, polypeptides, lipids, nuclear acids can also behave as an antigens. If the molecular size of antigen is small, it is called haptens. Haptens are combined with large carrier proteins like bovine serum albumin or synthetic matrices to make it immunogenic. For binding and recognition of antigen with the antibodies, antigen consists of epitope. Epitopes are readily available for binding with antibodies as shown in Figure 1.2.

CHARACTERISTICS OF A GOOD ANTIGEN

- It should have structural stability and also chemical complexity.
- Significant stretches lacking extensive repeating units.
- It should have molecular weight between 8,000–10,000 Daltons. Haptens have molecular weights less than 200 Daltons can be used in the combination with a carrier protein.
- It should have the ability to process and recognize by the immune system of the body.
- It should have immunogenic regions and are accessible to the antibody-forming cellular mechanisms.
- It should be foreign in nature, i.e. the structural elements of antigen should be different from the host.
- In case of peptide as an antigens, it should contain at least 30% of amino acids like lysine, glutamic acid, arginine, aspartic acid, etc. which are immunogenic in nature.

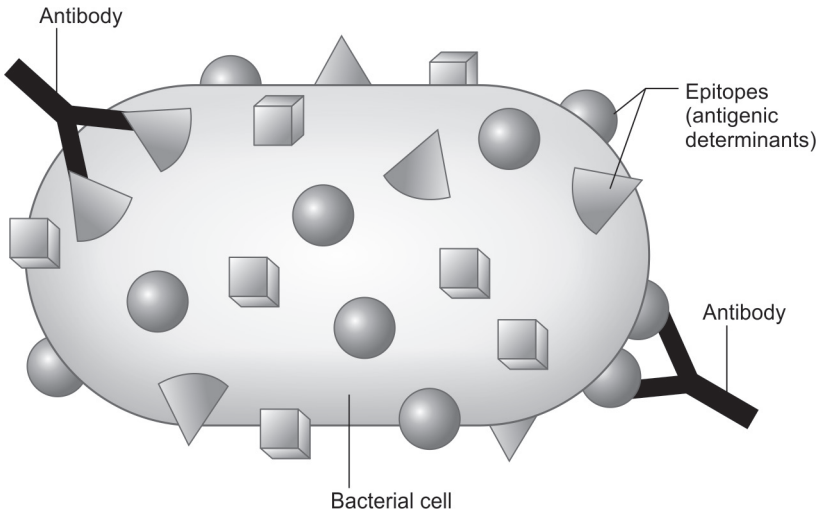


Fig. 1.2: Epitopes on the surface of bacterial cells

ANTIBODIES

Once, the antigen stimulates the immune system, in response to it, B lymphocytes secrete antibodies. Antibodies contain antigen-binding sites, more specifically, binding sites for epitopes in order to initiate a cascade of reaction that ends with the elimination of incoming antigen.

Structure of Antibodies

Antibodies have Y-shaped structure. It consists of four polypeptides and divided as two heavy chains and two light chains. Both the chains are linked by disulfide bond between the cystein residue along with noncovalent interaction between VH and VL, CH and CL (Fig. 1.3A).

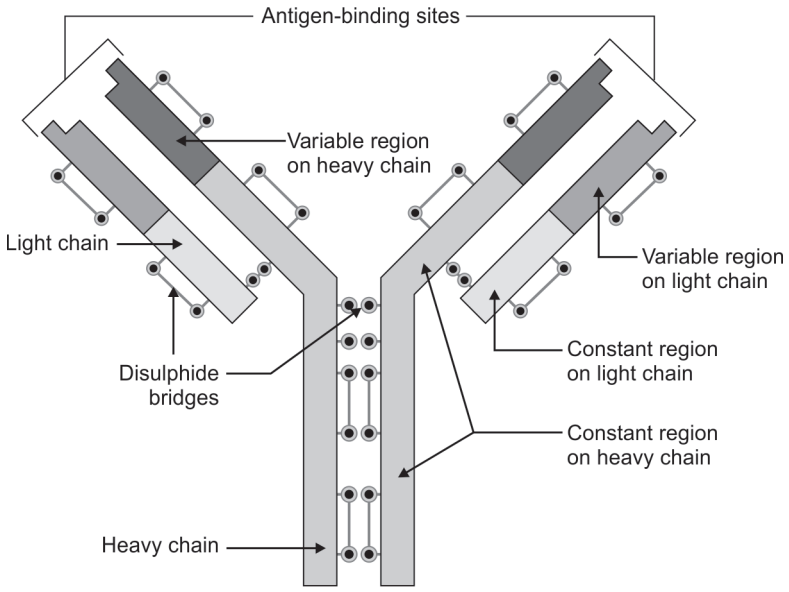


Fig. 1.3A: Structure of an antibody

Antibodies can also be divided into two regions as

A. Fab: Antigen-binding fragment
B. Fc: Crystallizable fragment

A. Fab fragment: It is a region on an antibody that particularly binds to epitopes of antigens. Fab fragment is composed of a constant region and a variable region of heavy and the light chains. Collectively, these domains form paratope or antigen-binding site.

B. Fc fragment: It is the region of antibody that interacts with cell surface receptors. These receptors are called Fc receptors along with some proteins of the complement system. This property allows antibodies to activate the immune system.

Types of Antibodies

On the basis of types of constant region of heavy chain and its amino acids sequence, the antibodies are classified into five major classes (Fig. 1.3B). The five basic sequences are named with Greek letters as mu (μ), delta (δ), gamma (γ), epsilon (ε) and alpha (α).

- **IgG antibody:** Immunoglobulin G (IgG) antibodies have gamma sequence and are large globular proteins. The molecular weight is 150 kDa. The four polypeptides are divided as two identical γ (gamma) heavy chains (50 kDa) and two identical light chains (25 kDa). IgG gives a long-term shield because it remains in our body for months and years as the antigen enters and triggers its production. IgG protects the body against many bacteria, viruses and also neutralizes bacterial toxins. The triggering of compliment protein systems along with binding of antigens also enhances the efficacy of phagocytosis.
- **IgM antibody:** Immunoglobulin M (IgM) antibodies have mu (μ) sequence and are constructed of five units of antibodies, i.e. they have pentamers structure. The pentameric structure is bounded with the help of J-chain. IgM is involved in the

ABO blood group antigens on the surface of RBCs. IgM enhances ingestions of cells by phagocytosis.

- **IgA antibody:** Immunoglobulin A (IgA) antibodies have alpha (α) sequence and consist of heavy (H) and light (L) chains. Each H chain is comprised of the constant region (Cα1, Cα2, Cα3), hinge region and the variable (V) region. Light chains consist of the CL and Vκ or Vλ elements. These antibodies are called secretary antibodies, since they are found in body secretion. The important function of IgA antibodies is to bind with an antigen before they invade tissues. They work by aggregating the antigens and stay the antigens in the secretions. When the secretion is expelled, the antigen can also take its exit from the body. IgA constitutes the defense mechanism of mucosal surfaces including intestines, nose, and lungs.

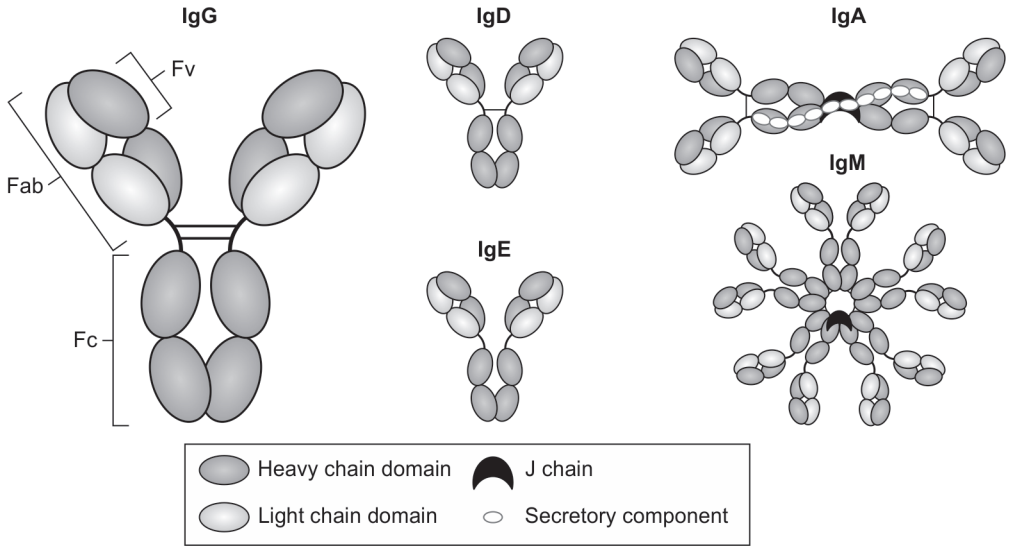


Fig. 1.3B: Types of antibodies

- **IgE antibody:** Immunoglobulin E (IgE) antibodies have epsilon (ε) sequence and found only in mammals. It is synthesized by plasma cells of the body. IgE consists of two heavy chains (ε chain) and two light chains, with the ε chain containing 4 Ig-like constant domains (Cε1–Cε4). IgE plays an important role in allergic reactions and binds to mast cells and basophils cells which participate in the immune response.
- **IgD antibody:** Immunoglobulin D (IgD) antibodies consists of delta (δ) sequence and are expressed in the plasma membranes of immature B lymphocytes. IgD is also produced in a secreted form that is found in small amounts in blood serum. IgD plays a role in the induction of antibody production.

1.3 CELLS OF IMMUNE SYSTEM

The cells of the immune system originate from the pluripotent stem cells in the bone marrow. The word pluripotent signifies that these cells can be differentiated into different types of tissue cells. The pluripotent stem cells can form myeloid stem cells and lymphoid stem cells. Further the myeloid stem cells generate monocytes, granulocytes

(neutrophils, eosinophils, and basophils) and macrophages. RBCs and blood platelets as lymphoid stem cells generate B lymphocytes or B cells, T lymphocytes or T cells and also natural killer (NK) cells.

1.4 CLASSIFICATION OF IMMUNITY

Immunity is broadly classified into two types:

1. Innate immunity
2. Adaptive immunity

INNATE IMMUNITY

The word innate refers to inborn, i.e. the immunity that is inheritated from the parents comes under this category. For examples, we (humans) are immune from diseases like distemper, which is considered as fetal disease of dogs. It is also called nonspecific immunity. The innate immunity protects our body by offering many barriers that hinder the entry of any pathogen. The barriers are again of many types and can be categorized as

1. Physical barriers
2. Physiological barriers
3. Cellular barriers
4. Cytokine barriers

Physical Barriers

The physical barriers are basically some mechanical barriers and include skin and mucous membrane that protect the body from exposure to antigens.

A. *Skin*: The outer lining of our body has tough statum corneum layer that acts as a physical barrier in the entry of pathogenic microorganisms.
B. *Mucous membrane:* Secretes mucous and entraps the microorganism rather make it immobile. For example, if pathogens enter our respiratory tract, are trapped in mucus and with the help of cilia, these pathogens are thrown into pharynx and finally swallowed and eliminated in faeces.

Physiological Barriers

The temperature of body, body secretions, pH of body fluids, etc. are few examples of physiological barriers. Considering the physiology of our body many such physiological barriers are available. The acidic pH of the stomach kills many microbes, bile inhibits the growth of bacteria, nasal hair creates a barrier to filter microbes entering through air, highly acidic vaginal secretions, sweat, presence of lysosomes kill bacteria by dissolving their cell wall, etc. are few classic examples.

Cellular Barriers

The cells of innate immunity also play an essential role in combating the pathogens. The cells like leucocytes including netrophiles and monocytes, macrophages, natural killer (NK) cells, etc. are the key players of cellular barriers. The possible mechanism of some cells like monocytes and macrophages is the engulfment of pathogen and its degradation though phagocytosis. Specially, macrophages are available in various locations of our body and named differently depending upon the location, for example,

kupffer cells present in liver, glomerular mesangial cells present in kidney, pulmonary alveolar cells in lungs, osteoclasts in bones, etc. Similarly, natural killer cells also constitute cellular barriers. NK cells release perforins, a chemical that is inserted inside the celluar membrane of pathogen and also creates the pores and make it weak and ready for cellular lysis. The pores generated by NK cells also make easy entry of water inside the pathogen and thus it swells and bursts and further the macrophages clear the cell debris. NK cells also perform apoptosis and present around 5–10% of total peripheral blood leucocytes (PBL) in humans.

Apart from macrophages and natural killer cells, complement proteins produced by liver, a group of 20 proteins available in serum and plasma membrane also help in defending the body in innate way and complement the fight against invaded pathogen along with other mechanisms like antigen–antibody interaction.

The complement proteins like NK cells also create pores in cell membrane of the pathogens. Due to which the entry of water took place inside the invader cells and finally it bursts and destroyed. In brief, proteins of the complement system kill the pathogen by cytolysis, inflammation and phagocytosis. The complement proteins also prevent damage of the host tissues (Fig. 1.4).

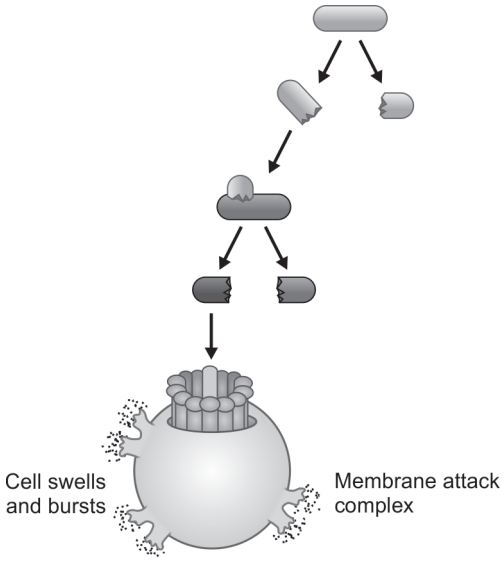


Fig. 1.4: The complement system

Inflammation and Fever

Inflammation is again a way to dispose the pathogen at the site of injury. The mechanism of inflammation helps to prevent the spread of infection to other tissues and also prepares the site for tissue repair. Inflammation is characterized by redness, heat, swelling and pain at the site of infection. Histamine is released by mast cell at the site and thus capillary dilation along with small blood vessels takes place and thus the area seems to be red and heated and also swelling is visible.

The pathogenic microorganisms produce toxins in body along with a protein known as pyrogen released by macrophages. When these pyrogens reach our brain, the thermostat of our body is reset to an elevated temperature that is recognized as a fever.

Mild fever also helps in stimulating the phagocytosis to initiate the innate responses and it also inhibits the growth of pathogens. But higher temperature is dangerous to our body and should be brought into notice by medical practitioners.

Cytokine Barriers

Cytokines are proteins that stimulate and inhibit the differentiation, proliferation or function of immune cells. They are involved in the cell-to-cell communication. Kinds of cytokines include interleukins produced by leucocytes, lymphocytes produced by lymphocytes, tumour necrosis factor and interferons (IFNs). Interferons protect against viral infection of cells.

ADAPTIVE IMMUNITY

It is also called specific immunity or acquired immunity. As the word suggests, it is the type of immunity that an individual develops or acquires after birth. It is specific in nature and both T lymphocytes and B lymphocytes work together to fight with the incoming antigen. This immunity also assures the prevention of attack by the same pathogen in the future by the formation of memory cells. So, it not only kills the prevalent pathogen but also makes our body ready in advance in case of any further exposure by the same microorganism.

Qualities of Adaptive Immunity

Specific: The adaptive immunity can differentiate various antigens and thus shows specific action against specific antigen.

Diverse: The diversified nature of adaptive immunity helps in the recognition of variety of antigens.

Self and nonself differentiation: It can easily recognize and discriminate antigen as foreign molecule and avoid any action against the self molecules or cells.

Memory creation: When for the first time an antigen attacks the body, the effector cells combat with it to assure its destruction but also it formulates memory cells so that when our body is exposed to the similar antigen the quick and effective response to neutralize it could be achieved.

Further Classification of Adaptive Immunity

The adaptive or acquired immunity is further classified into two major classes:

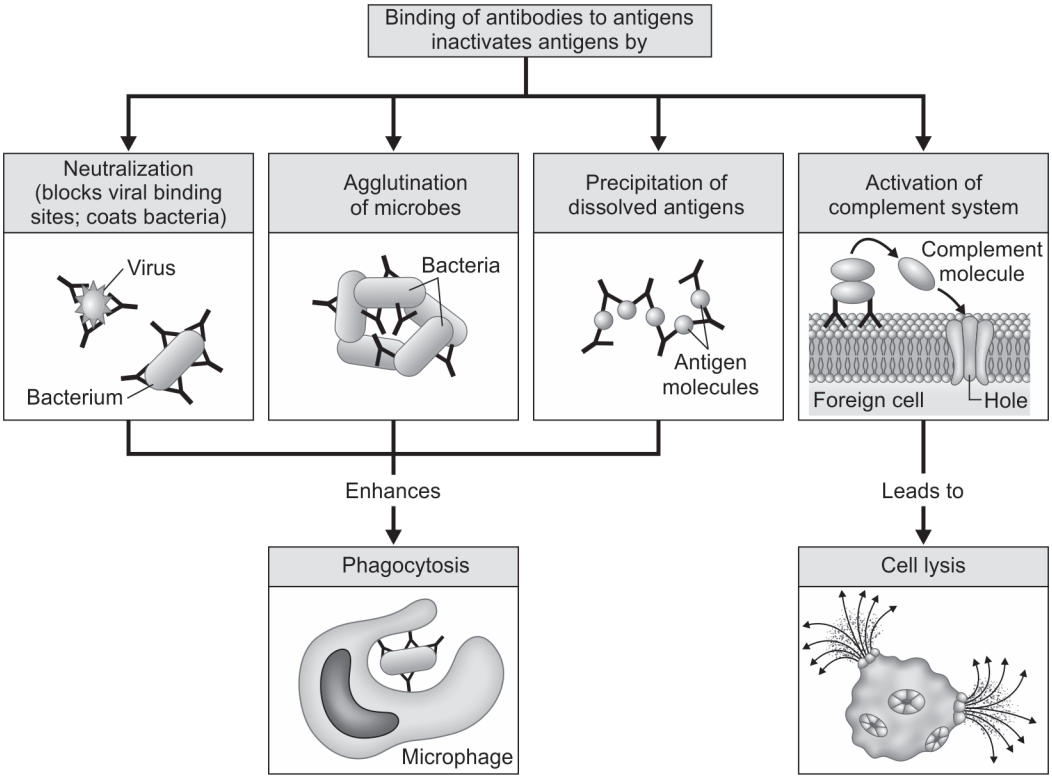
A. Humoral immunity
B. Cell-mediated immunity

Humoral Immunity

The word humoral comes from humor it means fluid. In this type of immunity, the B lymhocytes produce antibodies which circulate in the body fluid like plasma and lymph.

In humoral immunity, the invasion of antigen causes differentiation of B lymphocytes into plasma B cells. These B cells now produce antibodies and the free moving (outside the infecting cells) (Flowchart 1.1) antigen is neutralized and lysis takes place by the antibodies. This mechanism also pevents the spread of infection to intracellular level.

Flowchart 1.1: Role of antibodies in defense against antigen



The activation and differentiation of B lymphocytes into an antibody producing molecules requires a triggering from helper T cell (T_H). To infect any cell, the pathogen requires binding to the host cell. But when antigen–antibody interaction takes place, this binding is prohibited and said that antibody neutralizes antigen. This neutralization reaction also prevents the entry of bacterial toxins to enter to the host cells. Further the complex is injected by specialized phagocytotic cells in order to destroy ingested pathogen.

The specialized receptors are available on the surface of phagocytic cells and recognize Fc receptors. These receptors are complementary to constant region of antibodies. The binding of ag–ab complex on the surface of phagocytic cells facilitates opsonization reaction and thus engulfing and destruction of antigen take place. Alternatively, the antigen–antibody complex can activate the proteins of complement system. The activation of complement system helps in binding of the complement protein on the surface of pathogen. This results in the opsonization of pathogen by phagocytic cells. The other components of complement also appoint phagocytic cells to the site of infection.

These components can directly kill or lyse the pathogen by creating a pore in their membrane. When the process of antigen binding with antibody is initiated and formation of antibody by B cells took place, the clonal selection is again an important aspect that needs to be discussed. In this mechanism, the B cell is activated and divides, producing a clone of daughter B cells. These clones give rise to plasma B cells and memory B cells (Fig. 1.5).

Plasma B Cells or Effector B Cells

In order to fight with the free antigens, the activated B cells undergo enlargement and divide and differentiated into various clones of plasma cells recognized as plasma B cells or effector B cells. The life of these effector cells is only few days but in this period also they produce a large quantity of antibodies.

Memory B Cells

Some activated B cells do not differentiate into plasma cells. These cells remain as memory cells. They have a longer lifespan. As the name suggests, the memory cells play an important role when the body encounters the same antigen and they remain dormant until activated by new quantity of the same antigen.

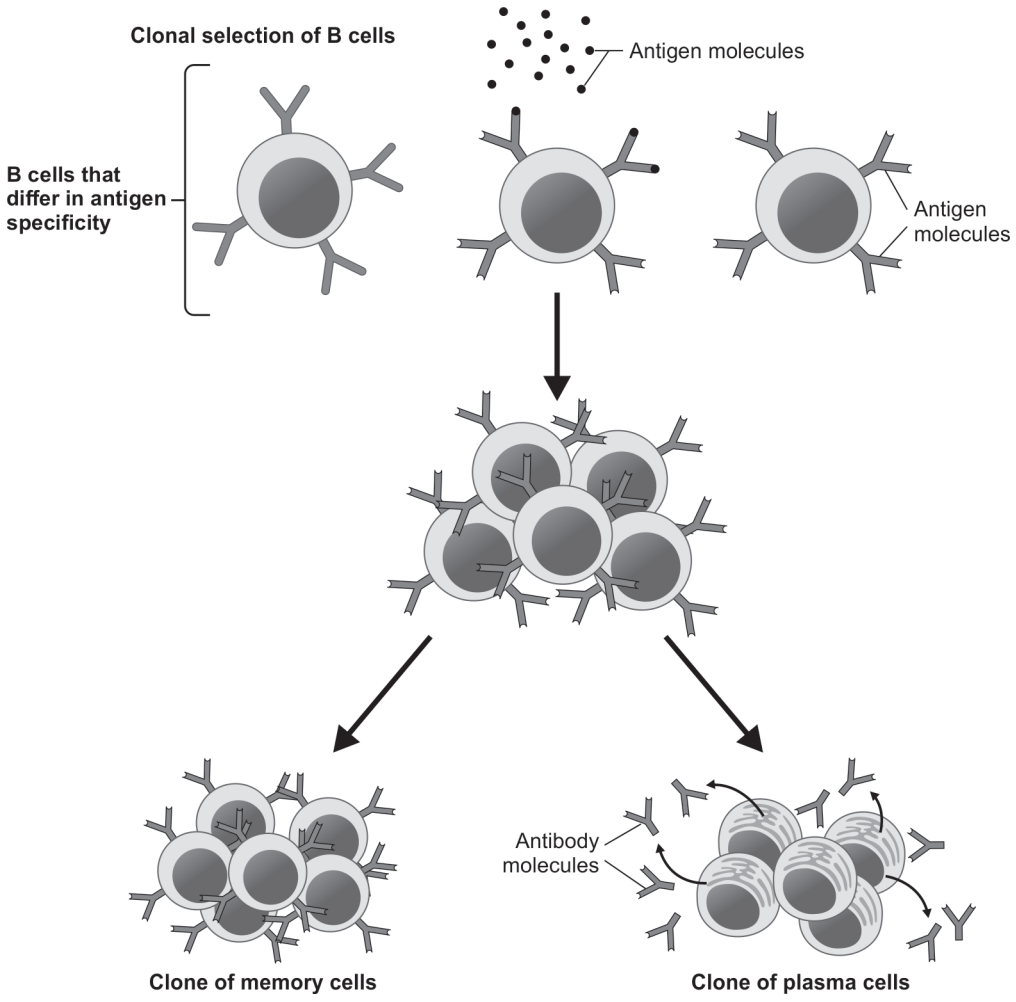


Fig. 1.5: Clonal selection of B lymphocytes

Cell-mediated Immunity

It is also called T-cell immunity. As the name suggests this immunity is coordinated by T lymphocytes. The T and B lymphocytes are produced by bone marrow by the

process of haematopoisis and T cells mature in thymus. Here, the T lymphocytes have two important responsibilities. First as an effector cells that cause pathogenic cell lysis and second as an regulatory cells in order to increase and suppress other lymphocytes and accessory cells of immunity.

Types of T cells

T cells are divided into many types as

1. Helper T cells (T_H)
2. Cytotoxic T cells (T_C)
3. Suppressor T cells (T_S)

Helper T Cells (T_H)

They are highest in number amongst all T cells. They are known to release chemical mediators that stimulate proliferation and differentiation of B lymphocytes and also the formation of antibodies by plasma B cells. They are also responsible for enhancing the activity of T_C cells.

Cytotoxic T Cells (T_C)

These cells are also called killer cells as they can directly destroy the pathogen. The antigen receptors on the surfaces of the cytotoxic cells cause specific binding with antigens present on the surface of foreign cell and coordinate its destruction.

Suppressor Cells (T_S)

These cells are capable of suppressing the functions of T_C and T_H cells. They also inhibit the immune system from attacking the body's own cells. It is believed that suppressor cells regulate the activities of the other cells. For this reason, the suppressor cells are classified as regulatory T cells.

Types of Adaptive Immunity

Adaptive or acquired immunity is divided into two classes as active immunity and passive immunity (Flowchart 1.2).

Active Immunity

In active immunity, the cells of our body produce antibodies as a result of response to clinical infection or with help of vaccines. The process is slow and takes a long time for the generation of antibodies. But at the same time it is long lasting and harmless. It may be further divided as natural or artificial.

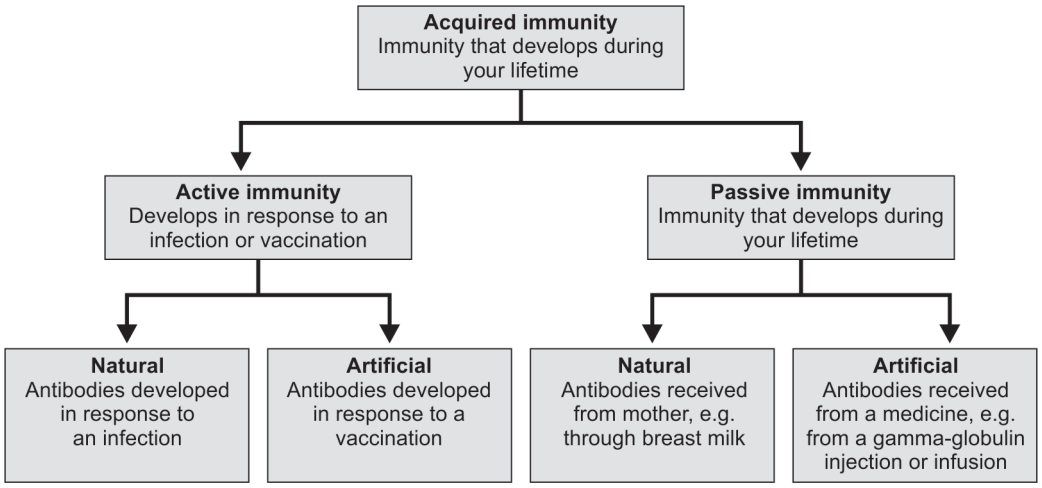
a. As a result of clinical infection when a person is recovered it can develop naturally acquired active immunity. For example, after attack of smallpox, measles and mumps.
b. Artificially acquired active immunity includes the resistance induced by the use of vaccines. Vaccines contain an agent that is similar to disease-causing pathogen and is prepared from weakened or killed forms of the pathogen or toxins, or surface proteins available over pathogenic microorganisms. Common examples are MMR vaccine for measles, mumps, rubella, live-Sabin vaccine for poliomyelitis, BCG vaccine for tuberculosis, etc.

Passive Immunity

When prepared antibodies are injected in a patient to provide a protection against foreign pathogen the immunity is said to be passive immunity. Passive immunity gives as immediate relief but it do not last for longer period of time. Passive immunity can be sub-divided as natural or artificial.

a. Natural acquired passive immunity: It is type of immunity that is transferred from mother to fetus through placenta. As we know that IgG class of antibody can easily cross placental barrier, so here it reaches the fetus to give passive protection against diseases. Even after the birth, the passive class of immunity naturally performs its function by passing immunoglobulins to the newborn through the breast milk. Human colostrum (mother's first milk) is enriched with IgA class of antibody and protects the infant by the age of 3 months.
b. Artificial acquired passive immunity: It is the resistance transferred to a patient by administration of prepared antibodies in the form of hyper-immune sera of man or animals. Serum contains antibodies. For example, anti-tetanus serum (ATS) is prepared in horses by active immunization of horse with tetanus toxoid. The serum is further separated. ATS is used for passive immunization against tetanus.

Flowchart 1.2: Types of acquired immunity



Mechanism of Cell-mediated Immunity

The process of cellular immunity starts with the recognition of foreign molecule as an antigen by some specialized cells of our body called antigen presenting cells (APC). The APC detect and stimulate adaptive immunity response and give an indication about the infection. These cells upon recognition of antigen phagocyte them and cause fragmentation of antigen, further these fragments are loaded into a complex called (major hitocompatibility complex-II (MHC-II) transported on the surface of APC and starts its display on the cell surface. The dendritic cells available on skin, lining of nose, intestine, etc. can act as an APC. Macrophages and B cells before differentiation can also act as an APC in our body. The important point to be noted here is the T lymphocyte or cellular immune response is not activated or give any response until and unless the presentation of antigen on the surface of APC takes place (Fig. 1.6).

After the processing and presentation of antigen, it is the T-helper cells that respond to the complex. Now, the T_H cells are having a molecule on its surface called CD4+. The CD4+ carrying T_H cells interacts with the processed APC that contains antigen complex as shown in Figure 1.7. The interaction of APC with its MCH-II molecule-loaded antigen with T_H cells that contain CD4+ molecule stimulate T_H cells to release chemical mediator known as cytokines.

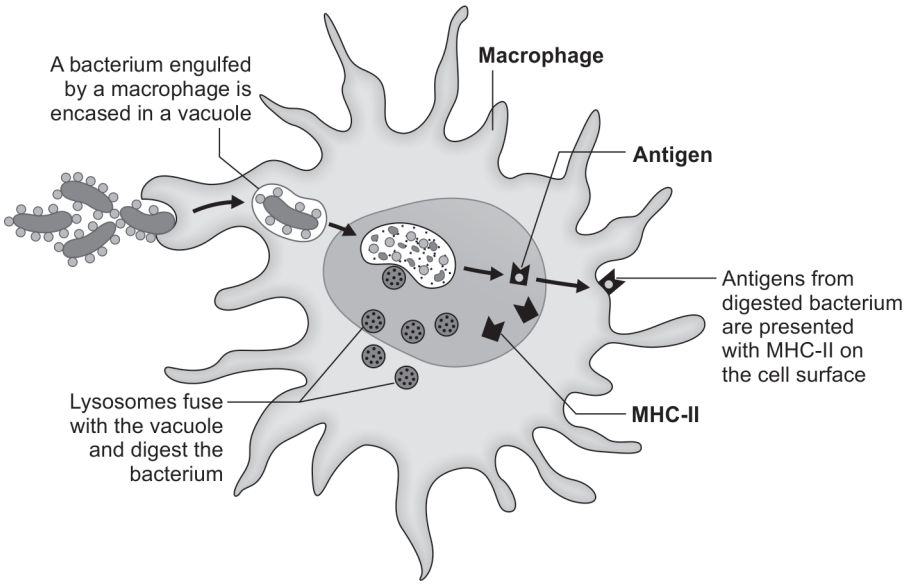


Fig. 1.6: Processing and presentation of antigen by APC

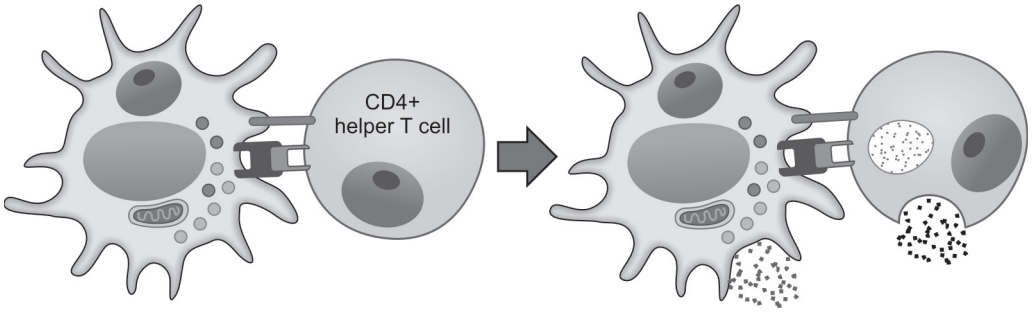


Fig. 1.7: Release of cytokines by T_H cells after interacting with antigen-loaded APC

The release of cytokines is an stimulating factor for various cells of immunity including T_C cells, macrophages and B-cells. Actually, T_H cells are again divided into 2 populations as T_H1 and T_H2. The T_H1 cells are responsible for release of cytokines and activation of macrophages and other T cells including T_C cells. Whereas, T_H2 cells are responsible for activation of B cells.

MACROPHAGES AND PHAGOCYTOSIS

The activation of macrophages by the release of cytokines by T_H cells is associated with phagocytosis. Recalling that the frontline defenses of macrophages involved in the innate immune response.

Phagocytosis is an essential part of immune system. Briefly, it is the process in which phagocytic cells bind with the antigen through specialized phagocytic receptors and engulf it inside and undergoes destruction of antigen. Once, the antigen is engulfed by the macrophages, the lysosomes present in the macrophages constitute phagolysosome by merging with the engulfed antigen. The foreign antigen is killed by variety of microbicidal actions like reactive oxygen intermediates, toxic peptides, action of hydrolases, etc. The destructed and fragmented antigen is further released by the macrophages (Fig. 1.8).

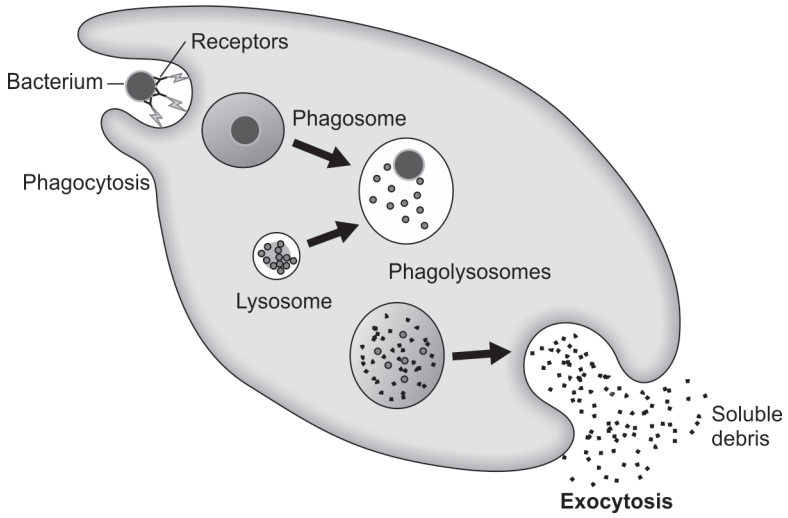


Fig. 1.8: Phagocytosis mechanism

CYTOTOXIC T LYMPHOCYTES (CTLS)

T_C cells are mainly esponsible for destroying the infected cells which also can be called self-altered cells. They plays an important role, especially in protecting against viruses, since virus replicates inside the host cell and gets protected from recognition by circulating antibodies. The CTLs help in preventing further spread of infection in body and try to halt the process of pathogen multiplication inside the infected cells. T_c cells directly interact with MHC-I embedded antigen (infected cells or self-altered cells). Such interaction results into release of degrading enzymes as perforins and granzymes that induce apoptosis of infected cells as shown in Figure. 1.9.

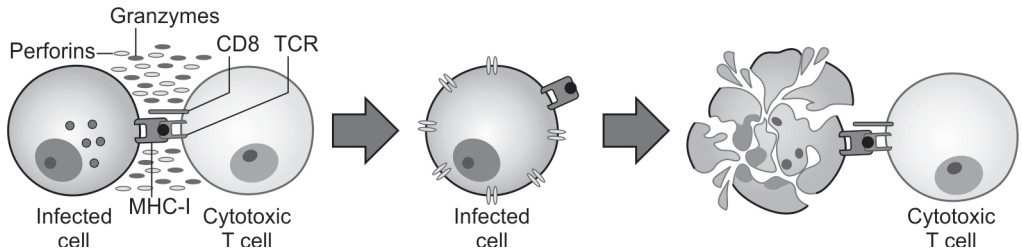


Fig. 1.9: Mechanism of T_c cell and destruction of infected cell

The natural killer (NK) cells also work by the similar mechanism. Here, we can say that the function of T_c cells and NK cells is almost complementary. If the infected cells lack the receptors which are recognized by T_c cells, than the role of NK cells become significant and kills the infected cells as shown in Figure 1.10.

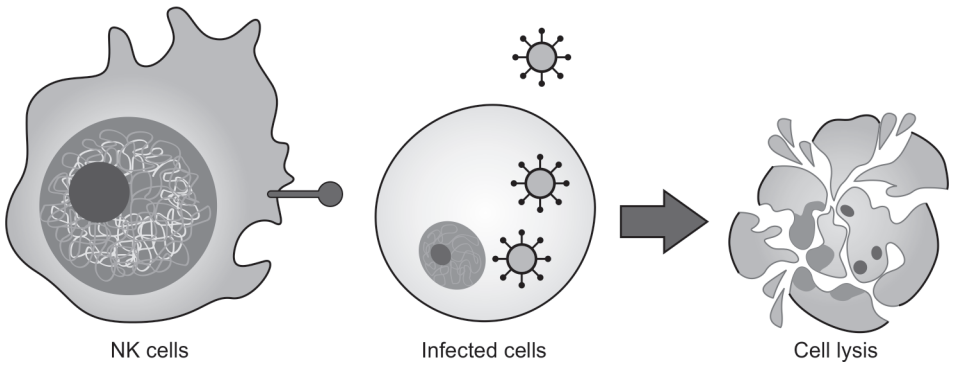


Fig. 1.10: Infected cell lysis by NK cells

B-cell Activation by Cytokines

When B cells are activated by T_H2 cells, they start differentiating into antibody secreting plasma B cells. The structure, type and function of antibodies are explained in humoral immunity.

1.5 IMMUNOLOGICAL MEMORY

One of the classic features of adaptive immunity is the creation of immunological memory. This helps in the efficient and response by cells of adaptive immunity to react at the time of re-invasion by the same antigen.

When for the first time body is invaded by the pathogen both the B and T lymphocytes initiate their responses for the first time, B cells release antibodies through plasma cells and T cells differentiate and trigger cellular immune response. At this time, B and T cells are mature into effector cells and give their immunological actions.

But at the same time, a subset of B and T cells also differentiated into memory B and T cells. These memory cells are those cells which do not become effector at the time of primary immune response. But as soon as the body is exposed to same antigen or reinfection takes place, they automatically are converted into effector population. At the end of the primary response, the effector cells are no longer needed and hence undergo apoptosis and memory cells remain in the circulation.

If our body does not encounter the same antigen still the memory B and T cells remain circulating in our body for several decades and then gradually die. But, if the reinfection or re-exposure takes place, the memory cells will soon be converted to plasma cells and CTL. This response does not require the processing by APC or cytokine release by T_H cells as in case of primary immune response. So, if we compare the time requirement for activation, than definitely the primary immune response in adaptive immunity is more and at the time of reinfection the basic steps are skipped and more rapid immune defense is achieved. And if we look at the production of antibodies at the time of reinfection, it is ten to hundred times more as compared to primary response as shown in

Figure 1.11. The rapid antibody response can hinder the infection even before it is established and the individual even may not apprehend that they had been exposed.

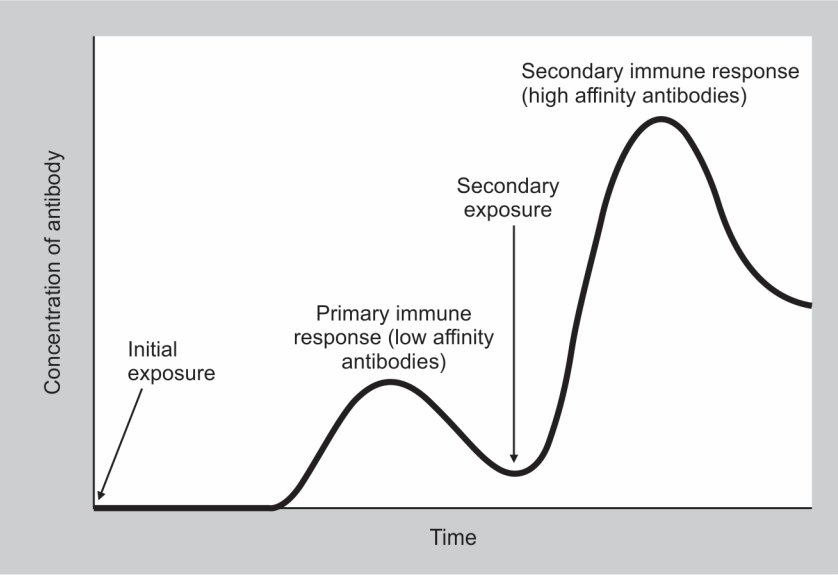


Fig. 1.11: Concentration of antibody at the time of primary and secondary immune response