Chapter-1

DEFENSIVE MECHANISM OF HOST

Immunity: *Immunity is defined as resistance offered by the body against invading disease producing microorganism or their products.* Our environment contains a large variety of invading pathogenic microorganisms, against which an effective protective cover is given by the immune system.

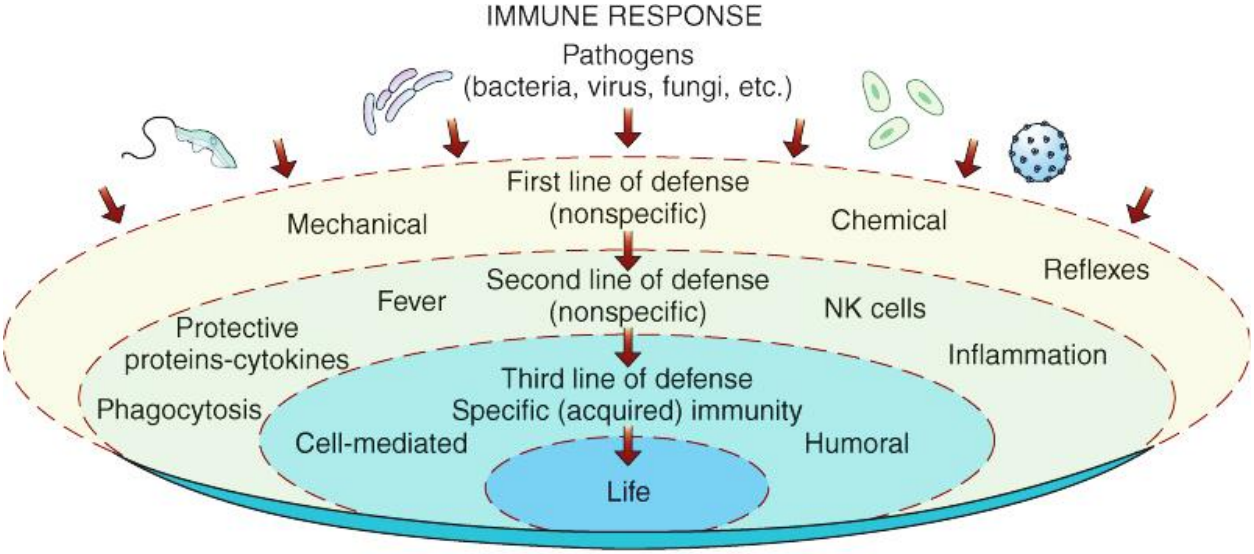


Figure1.1: Levels of defiance of human host body

The term immune system is derived from the Latin word immunis meaning exempt from charges (i.e. taxes and expenses). Although the concept of immunity existed since 430BC when ***Thucydides-*** described about plaque in Athens wherein he wrote that only people who recovered after a plaque infection could nurse the sick as they would not harbor the disease for a second time, it was almost two thousand years for making it medically acceptable and successful.

The immune system is remarkably adaptive defense system which is able to generate a variety of cells and molecules capable of specifically recognizing and eliminating a variety of limitless foreign invaders into the system is capable of recognizing and distinguishing one foreign pathogen from the other. Once it recognizes the foreign organism invaders it work out effectively to either eliminate or neutralize it.

TYPES OF IMMUNITY:

The defense pattern of an individual is categorized into two different classes, namely-

A. Nonspecific or innate immunity

B. Specific or Acquired immunity or adaptive immunity.

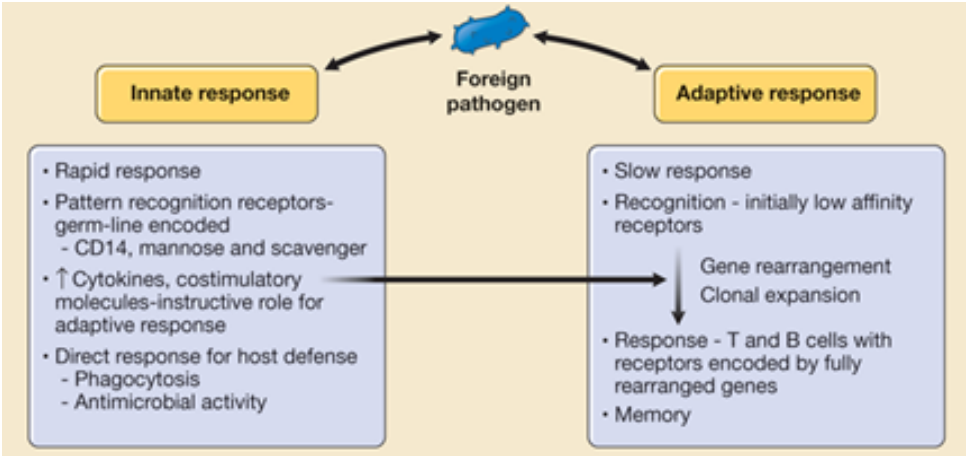


Figure1.2 :Immune reaction of human host body

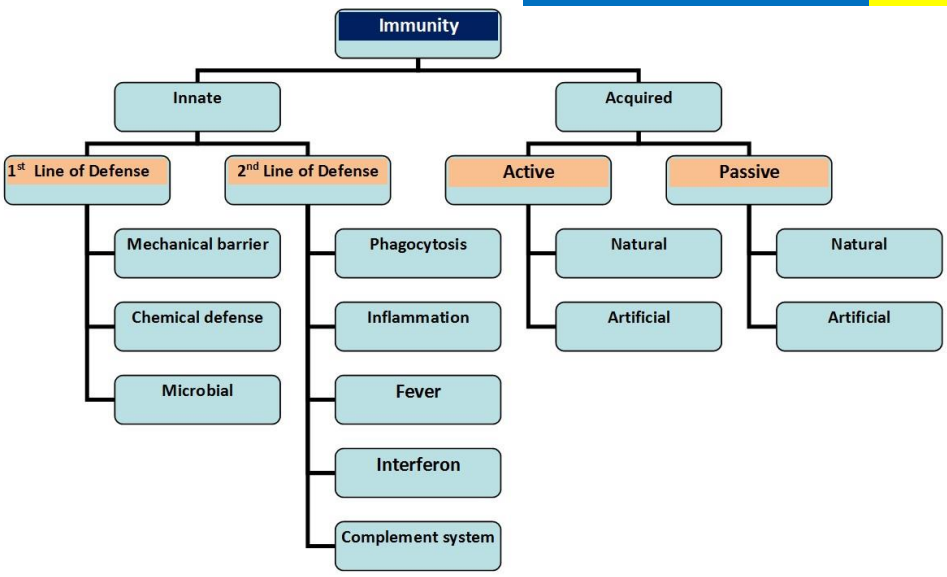


Figure1.3: Types of immunity

1.1 NON-SPECIFIC DEFENSES OF THE HOST (Innate immunity):

Innate immunity is a nonspecific defenseoffered by host body that against invading pathogen, regardless of pathogen species. This nonspecific defense is due to different barriers, nonspecific cells, nonspecifically acting proteins and some physiological reactions.

As this immunity is by birth immunity and no previous antigenic exposure is required to develop this immunity but it is gain by an individual by virtue of its genetic constitution, this immunity is termed as innate immunity.***This immunity is also called inherent immunity.***

Depending upon line of resistance offered this non specific defense is further categorized into two classes - include a first line of defense and a second line of defense.This immunity doesn't having specificity and immunological memorydevelopment, but providing antigen independent, immediate maximal response against invading pathogen.

1.1.1 Levels of innate immunity:

a. Species immunity: It is resistance to a pathogenic infection shown by all the members of particular species.

Exa: 1) Humans are always resistant to plant pathogens, due to physiological and biochemical differences. Exa: 2) Plague a dreaded disease which attacks man, but fowls are normally resistant to plague.Exa. 3. Humans do not contract cattle plague, chicken cholera, hog cholera, infectious horse anemia. Exa. 4) Animals are not affected by many human diseases such as enteric fever, scarlet fever, syphilis, gonorrhea, measles, etc.

b. Racial immunity: In a species, there are various types of races. So the type of infection resistance pattern limited to particular race is termed as racial immunity.

Exa: 1 Sheep: In Algeria there is an Algerian sheep, these are resistant to disease anthrax but the Indian sheep are susceptible to the anthrax disease. This shows racial immunity in the Algerian sheep against anthrax.

Exa: 2 Humans: In humans, Negroes are highly resistant to yellow fever while the whites are highly susceptible.

Exa: 3 Humans: In humans, Negroes races more susceptible to T.B., where as Americans (whites) have less susceptibility due to their racial immunity against pathogen *(M. tuberculosis)*

Exa: 4 Humans: South Africans do not have malaria but other races have. It shows racial immunity of South Africans against material parasites.

c. Individual Immunity: Individual immunity is an immunity limited to a particular individual of a race of species but not in other, due to differential genetic constitution.Exa: 1. In homozygous twins,

if one has T.B. susceptibility then other has also chance of T.B. susceptibility but in case of heterozygous twins if one has T.B. susceptibility other either have T.B. susceptibility or resistance.So an immunity pattern present in a particular individual of a race of species is called as individual immunity.

d. Heard Immunity:

Herd immunity is a form of indirect protection from***infectious (contagious) disease***within a population that occurs when a sufficiently high percentage of a population has become immune to an infection, especially through vaccination,disease.Sothere is little opportunity for an outbreak thereby providing a measure of protection for individuals who are not immune.

Even those who are not eligible for certain vaccines—such as infants, pregnant women, or immunocompromised individuals—get some protection because the spread of contagious disease is contained. This is known as "community immunity." The principle of community immunity applies to control of a variety of contagious diseases, including influenza, measles, mumps, rotavirus, and pneumococcal disease.Tetanus, for example, is infectious but not contagious, so herd immunity does not apply.

"The level of vaccination needed to achieve herd immunity varies by disease".

1.1.2 Factors influencing Non Specific Immunity

Nonspecific immunity of an individual is affected by various factors, which includes:

1. Age
2. Sex
3. Hormonal influence
4. Nutritional influence.

a. Age of individual :

Age is a major factor which influencing non-specific immunity. During development embryo is susceptible to infection ToRCH. ToRCH is a group of some pathogens which are able to pass across the placental barrier and so they infect the developing embryo.

To: *Toxoplasma gondii*

R: Rubella virus

C: Cytomegallo virus

H: Herpessimplex virus

Mainly during embryonic condition, an individual is susceptible to much pathogenic attack due to undeveloped immune system. Afterwards with increase in age immunity increases and approach at a peak up to adult stage. Beyond adult age with increase in age immunity are decreases due to aging or ageing and reaching minimal level at old age.

So embryonic and old age persons are susceptible to many infections due to their lowered immune power. But adult are more resistant to pathogenic attack due to their well-developed and mature innate immune power.

b. Sex of individual :

Sex is an important factor for deciding non-specific (innate immunity). Most of gonococci infections are seen in female, but absent in males, due to differential physiochemical structure.

c. Hormonal Influence:

Hormones are one of the factors important in non-specific immunity of an individual it seems that females are more susceptible to genital tract infection by bacteria mostly by gonococci due to sex hormones.Endocrine hormone (Insulin) regulates immune response during staphylococcal infection. Pregnant women are highly susceptible to infections due to production of steroid hormones.So hormonal constitution of an individual also decides there degree of resistance or susceptibility towards a particular infection.

d. Nutritional Influence:

Nutrition also influencing the non-specific immune response. Due to deference in nourishment obtained by individual there is difference in immunity of individuals.Most of mal nourished individuals are susceptible to many infections, due to improper of mal development of physiochemical reactions leading to generation of improper innate immunity.

1.1.3 MECHANISM OF NON-SPECIFIC DEFENSES (Innate immunity) :

Nonspecific resistance refers to defenses that protects individual against any pathogen, regardless of its species and numbers of exposures. This immunity restricts entry and invasion of pathogen inside the host body. The nonspecific defense offered at two levels - first line of defense (barriers of skin and mucous membranes) and second line of defense (Phagocytosis, inflammation, fever and antimicrobial substance -complement and interferon).

These all factors which are responsible for non-specific type of immune potential of host body. In this immunity physical condition, chemicals or microbial film of Normal microbiota serve as barrier or act as an antimicrobial chemicals or antagonistic living population to restrict entry of pathogen inside the host body.

Similarly against invaded pathogens a category of non-specific defense acts and restricts invasion and systemic spreading of pathogen inside the host body is called second line of defense. In this second line of defense nonspecific cells (Leucocytes), nonspecific proteins and physiological reaction offering immunity.

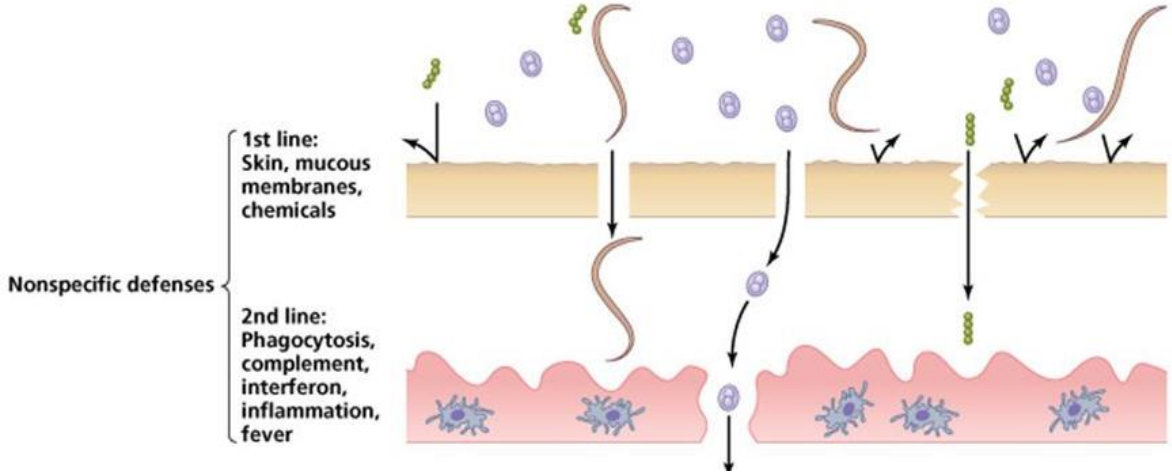


Figure1.4: mechanism of innate immunity

This non-specific resistance is primarily classified into two types, namely-

A. First line of defense
B. Second line of defense

A. FIRST LINE OF DEFENSE:

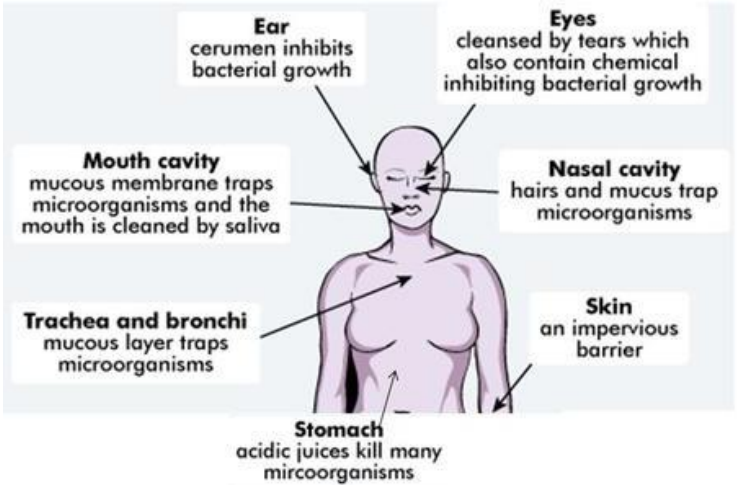


Figure1.5: Mechanism of first line of defense

For initiation and establishment of particular infection inside the host body pathogen need to be entered inside the host body through a particular entry point (portal of entry). These portals of entry includes, skin (dermal route), respiratory tract, Gastro intestinal tract, genito-urinary tract etc. At all the portals or route of pathogen entry the host body offers nonspecific resistance to invading pathogen

collectively called first line of defense. This first line of defense offered at portal of entry is due to mechanical or chemical property or due to normal microbial flora of that surface from which pathogen entering inside the host body. The all external surfaces of the body covered by skin and mucous membranes (mucosa) form an effective barrier between our cells/tissues and the external environment. These structures provide our first line of defense against infectious agents or potential pathogens.

Dry skin covers the **external surfaces** of our bodies and connects with mucous membranes in specific regions, e.g., the oral and anal openings of the gastrointestinal tract, urethral openings, vaginal openings, eyes, nostrils, etc., while **mucous membrane** covers the **internal surfaces**.

This first line of defense results from mechanical, chemical and biological factors. Mechanical factors include physical barriers to entry or processes that remove microbes from the body's surface, chemical factors include substances made by the body that inhibit microbial growth or destroy them. Normal microbiota serves as a biological factor imparting non-specific immunity against potential pathogens.

1. **Skin officering first line of defense**

Mechanical aspects of dry skin:

- The skin is multilayered, i.e., it includes an epidermis composed of epithelial cells supported by an underlying dermis, a layer of dense connective tissue.
- The epidermis is composed of stratified squamous epithelium, so is itself a multilayered structure, with flattened surface cells.
- Cells at the skin surface are highly keratinized, i.e., contain high levels of keratin proteins. These are tough, insoluble, fibrous proteins that interconnect to helpmake skin surfaces effective mechanical barriers.
- Cells at the skin surface are dead and constantly being shed, taking microorganisms with them. Many of the bacteria commonly found on air plates are inhabitants of human skin shed regularly by their human hosts.
- The dermis is composed of dense connective tissue, and forms a tough, leather-like barrier that is difficult to penetrate. Collagen, a long, fibrous protein with greattensile strength is one of the primary components. Note – Leather is typically madefrom animal hides (dermis layers) so accurately represents dermis structure.

Chemical aspects of dry skin:

- The skin surface is salty, due to the evaporation of water at the skin surface during thermoregulation and the natural salt content of sweat (perspiration). Salt in association with keratin makes the skin surface hypertonic, and inhospitable for many types of microorganisms.
- The skin surface is often acidic (pH around 5.5) and this also tends to inhibit microbial growth, as most bacteria prefer a pH around 7. This acidity is due primarily to the keratinization of epithelial cells as they move toward the skin surface.
- *Sebum* (Oils and waxes) produced by the sebaceous glands help waterproof the skin and prevent it from drying and cracking. Some of these also inhibit microbial growth. Sebum also forms a protective film over the surface of the skin. Sebum also contains unsaturated fatty acids, which inhibit the growth of certain pathogenic bacteria and fungi.

2. **Mucous membranes:**

Mechanical aspects of mucous membranes:

- Mucous membranes are multilayered, i.e., like dry skin they always include an epithelial layer supported by an underlying layer of connective tissue. The type of epithelium is variable.
- Mucous membranes are often covered with a thick, sticky material called mucus.
- Within the respiratory tract, mucus traps dust and microorganisms entering with inspired air and prevents them from reaching the lungs. Mucus moistens and lubricates the mouth and esophagus, allowing food materials to be readily masticated and swallowed. Within the stomach, mucus provides a protective layer preventing infection and damage to epithelial cells potentially caused by the acidic environment present. Cervical mucus also helps prevent infection.

- Within the respiratory system the epithelium is ciliated and the cilia sweep potential pathogens trapped in mucus, up and out of the airways. Since smoking causes damage to cilia, smokers are more likely to experience lung infections as bacteria are more likely to enter their lungs.
- Lachrymal apparatus of eye is responsible for production tears. Flushing action of tears acts as a physical barrier to protect eye ball from pathogenic attack. Cilia of eye lid also act as physical barrier.
- Saliva produced by salivary glands helps to dilute the numerous microorganisms and wash them from both the surface of the teeth and the mucous membrane of the mouth. This helps in prevention of colonization of microbes.
- Mucous membrane of nose also has mucus – coated hairs that filters inhaled air and trap microorganisms, dust and pollutants. Ciliary encapsulation also acts as physical barrier.
- The cleansing of the urethra by the flow of urine is another mechanical factor that prevents microbial colonization in the genitourinary tract. Vaginal secretions like wise move microorganisms out of the female body.

Chemical aspects of mucous membranes:

- Mucus and other secretions often associated with moist surfaces, e.g., tears and saliva, contain lysozyme enzymes.
- Lysozyme kills bacteria (especially Gram positive cells) by causing the hydrolysis of peptidoglycan, i.e., by breaking the covalent bonds linking N-acetyl muramic acid with N-acetylglucosamine.
- Lysozyme also acts as an opsonin, i.e., a substance causing opsonization (making particles more attractive to phagocytes). It can bind to bacteria, making them more readily engulfed by phagocytic WBCs.
- Mucous membranes and their secretions are commonly equipped with antibodies (immunoglobulins) in the isotype IgA, capable of binding with and sometimes immobilizing pathogens, causing some opsonization or neutralizing toxic bacterial products (IgA does not activate complement proteins).
- The pH in specific regions lined by mucous membranes is sometimes quite low (acidic). For example, within the stomach it may 1 or 2, while within the vagina it is commonly 3.8-4.5. These acidic conditions tend to inhibit bacterial growth because most bacteria prefer a more neutral environment.
- Gastric Juice: Gastric Juice is produced by the glands of the stomach. It is a mixture of hydrochloric acid, enzymes and mucus. The very high acidity of gastric juice (pH 1.2 – 3.0) is sufficient to destroy bacteria and bacterial toxins except those of *Clostridium botulinum* and *Staphylococcus aureus.*
- **Blood:** Blood contains antimicrobial chemicals like iron binding protein called transferring which inhibit bacterial growth by reducing the amount of available iron.
- **Other Chemicals:** Other substances which act as chemical barrier for non-specific immunity includes bacteriocins, spermine and spermidines, histamines, acute phase protein, cytochrome oxidases, interferon etc.

3. **Microbial defense as biological barrier:**

Normal microbiota is a microbial population includes a variety of different bacteria, fungi, protozoa and other organisms livingmostly on the surfaces of the human body. During birth host body is germ free. After short time as body exposed to environment the microbesgain access to the body (many passed from mother to infant) and colonize the various regions or habitats available. Most of the microbes living on surfaces of skin and mucosal linings of gastrointestinal, urinary, reproductive and respiratory tracts are not pathogenic.

These surface microbes normally slow growers, not showing penetration inside the host body so playing beneficial role.

This normal microbiota present over all the body surfaces, serve as biological barrier to restrict entry of pathogen inside the host body.

Microbiota serving defense by

- Competitive exclusion: Compition with pathogen for space, nutrients and moisture: prevent pathogens from colonizing the host.
- Blocking of receptor for pathogen binding: Most pathogens must bind with cellular surfaces in order to infect or cause damage. If the binding sites available on cellular surfaces are occupied by normal flora, pathogens can't bind.
- Antagonistic effect due to production of antimicrobial agents: They provide protection by producing chemical substances called **bacteriocins** that kill other closely related cells. Humans typically have *Escherichia coli* cells living within their guts, but do not normally carry highly pathogenic strains. The *E. coli* comprising the normal flora of most individuals form toxins called **colicins** that kill other, more pathogenic strains such as the *E. coli* O157:H7 responsible for causing hemolytic uremic syndrome.
- By altering conditions that affect the survival of the pathogens, such as pH and oxygen availability.The presence of normal micro biota in the female genitalia alters pH to prevent over-population by *Candida albicans*, a pathogenic yeast that cause vaginitis.In the large intestine, *E coli* produce bacteriocins that inhibit the growth of *Salmonella* and *Shigella.*

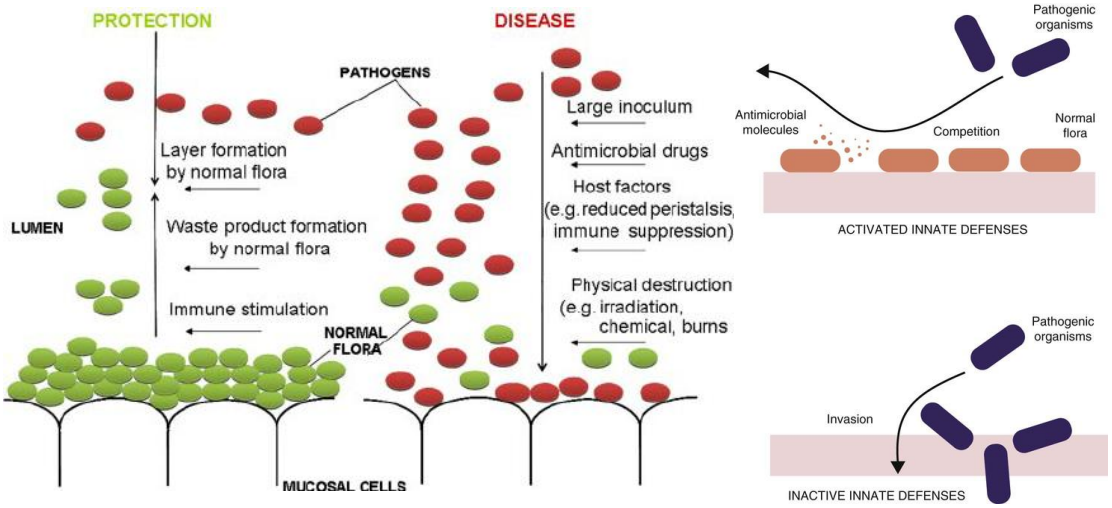


Figure1.6: Microbial defense as biological barrier:

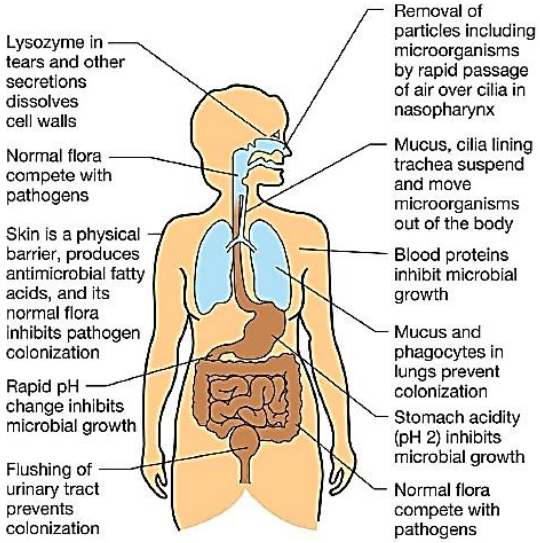


Figure1.7: Mechanism of first line of defense

B. SECOND LINE OF DEFENSE:

If the first line of defiance is unable to restrict entry of pathogen inside, then pathogen is entered inside the host body. The category of non-specific cell mediated, protein mediated or physiological defense offered by host body against invaded pathogen is called second line of defense.

Second line of defense given by host body is due to action of nonspecific cells like-Phagocytes, non phygocytic leucocytes, nonspecific proteins like interferon and complements as well as due to physiological reaction like fever.

Event of second line of defense categorized into different types based on defense imparting component.

A. Nonspecific cell mediated reaction:
   1. Phagocytosis
   2. Inflammation

B. Nonspecific Protein mediated reaction:
   1. Complement activation and action
   2. Interferon production and action

C. Nonspecific Physiological defense:
   1. Fever

PHAGOCYTOSIS

Phagocytosis is a nonspecific cell mediated second line of defense mechanism in which various nonspecific cells (phagocytes) engulf and destroy the invaded pathogens. Phagocytosis (word derived from Greek word phagein means to eat , cyte means cell and osis – a process) is one type of endocytosis, the general term for the uptake by a cell of material from its environment. The process of phagocytosis was discovered by ***Metchnikoff***. The term phagocytic denotes the engulfment and digestion of whole cells.

The major phagocyic cell types in the body which are associated with the engulfment and digestion of microorganism are the polymorphonuclear leucocytes (Neutrophils), tissue macrophages, monocytes and dendritic cells. Minor cell types are eosinophils.

The process of phagocytosis begins with **attachment** and **ingestion** of microbial particles into a bubble like organelle called a **phagosome.** Once inside the phagocyte, the phagosome containing the microorganism joins with a **lysosome**, which contributes enzymes. The fusion of phagosome and lysosome results in a **phagolysosome.** Microorganisms are destroyed within minutes, and the microbial debris is eliminated from the cell in the process of **egestion.** In the immune process, chemical portions of the microorganism called **antigenic determinants** are displayed on the surface of the phagocyte to stimulate the immune process.

Phagocytes:

The leukocyte cell population with phagocytic activity is called as phagocytes. This leukocyte with phagocytic activity includes**neutrophils, monocytes** and **macrophages.**

1. **Neutrophiles:** The neutrophils are granulocytes with phagocytic capability.The granules of neutrophiles stain pale lilac with a mixture of acidic and basic dyes. Neutrophiles are also commonly called polymorphonuclear leucocytes (PMN). Neutrophiles are highly phagocytic and motile and active in initial stages of an infection. They have the ability to leave the blood, enter an infected tissue and destroy microbes and foreign particles.
2. **Mononuclear Phagocytes:** The mononuclear phagocytic system consists of **monocytes**circulating in the blood and **macrophages** in the tissues.

Actions of Phagocytic Cells:

When an infection occurs both granulocytes and monocyte migrates to infected area. During this migration, monocyte enlarges and develops into actively phagocytic macrophages. These cells leave the blood and migrate into tissues where they enlarge and develop into macrophages. Some macrophages called fixed macrophages or histocytes are located in certain tissues and organs of the body. Other microphages are called wondering macrophages, which roam the tissues and gather at

sites of infection or inflammation. The various macrophage of the body constitute the mononuclear phagocytic system.

The Process of Phagocytosis

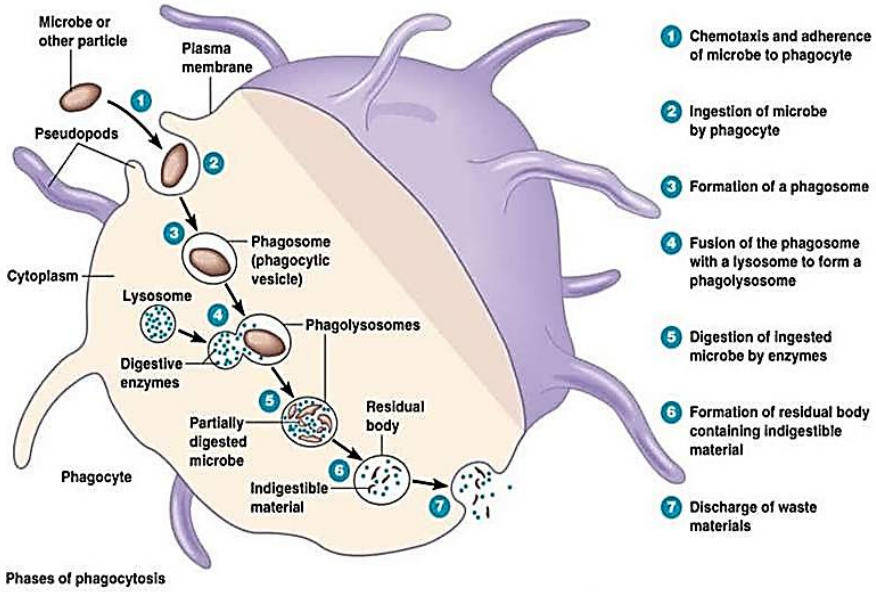


Figure1.8: The phages of phagocytosis

There are a number of distinct steps involved in phagocytosis:

1. **Production of phagocytes :** Bone marrow (14-day maturation time)
2. **Mobilization of phagocytes:** Release from bone marrow reserve. Most phagocytes in the body are found as fully mature cells held in reserve in the bone marrow. On infection, these cells can be rapidly mobilized to increase phagocyte concentrations in the blood and provide defense more quickly (4 to 12 hours, instead of 14 days).
3. **Activation of the phagocyte:** Resting phagocytes are activated by inflammatory mediators such as bacterial products, complement proteins, inflammatory cytokines and prostaglandins. As a result, the circulating phagocytes produce surface glycoprotein receptors that increase their ability to adhere to the inner surface of capillary walls, enabling them to squeeze out of the capillary and be attracted to the site of infection.
4. **Chemotaxis of phagocytes:** Chemotaxis is the movement of phagocytes toward a site of infection at which increasing concentration of some attractant such as bacterial factors (bacterial proteins, capsules, LPS, peptidoglycan, teichoic acids, etc.), complement proteins (C5a), chemokines (chemotactic cytokines such as interleukin-8 secreted by various cells), fibrin split products, kinins, and phospholipids released by injured host cells is reported. Some microbes, such as the influenza A viruses, *Mycobacterium tuberculosis*, blood invasive strains of *Neisseria gonorrhoeae* , and *Bordetella pertussis* have been shown to block chemotaxis.
5. **Attachment of the phagocyte to the microbe or cell:**
   The pathogen need is attached with pseudopod of phagocyte. Attachment of microorganisms is necessary for ingestion. Attachment may be unenhanced or enhanced.
   - Unenhanced attachment is the innate recognition of pathogen-associated molecular patterns or PAMPs –components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans common in microbial cell walls but not found on human cells - by means of endocytic pattern-recognition receptors, such as scavenger receptors and mannose receptors, on the surface of the phagocytes.
   - Enhanced attachment is the attachment of microbes to phagocytes by way of an antibody molecule called IgG, the complement proteins C3b and C4b produced during the complement

pathways, and acute phase proteins such as mannose-binding lectin (MBL) and C-reactive protein (CRP). Molecules such as IgG, C3b, and mannose-binding lectin (MBL) that promote enhanced attachment are called opsonins and the process are also known as **opsonization**. Enhanced attachment is much more specific and efficient than unenhanced.

- Extracellular trapping with NETs: In response to certain pathogen associated molecular patterns such as LPS, and certain cytokines such as IL-8, neutrophils release DNA and antimicrobial granular proteins. These neutrophil extracellular traps (NETs) bind to bacteria, prevent them from spreading, and kill them with antimicrobial proteins.

6. **Ingestion of the microbe or cell by the phagocyte**

After attachment of peudopod with pathogen, polymerization and then depolymerization of actin filaments resulting growth of pseudopods to engulf the microbe and form endocytic vesicle called a phagosome.

During this process, an electron pump brings protons (H^+) into the phagosome. This lowers the pH within the phagosome to 3.5 - 4.0 so that when a lysosome fuses with the phagosome, the pH is correct for the acid hydrolases to effectively break down cellular proteins. The acidification also releases defensins, cathelicidin, and bacterial permeability inducing protein (BPI), peptides and enzymes that can kill microbes, from a matrix and enabling their activation.

7. **Formation of phagolysosome**

Phagocytes contain membranous sacs called lysosomes produced by the Golgi apparatus that contain various digestive enzymes, microbicidal chemicals, and toxic oxygen radicals. The lysosomes travel along microtubules within the phagocyte and fuse with the phagosomes containing the ingested microbes to form phagolysosome. After formation of phagolysosome the microbes are destroyed.

8. **Destruction of the microbe or cell**

There are 2 destruction methods in neutrophils and macrophages: the oxygen-dependent system and the oxygen-independent system.

The oxygen-dependent system: production of reactive oxygen species (ROS)

- The cytoplasmic membrane of phagocytes contains the enzyme oxidase which converts oxygen into superoxide anion (O^{2-}). This can combine with water by way of the enzyme dismutase to form hydrogen peroxide (H_2O_2) and hydroxyl (OH) radicals.
- In the case of neutrophils, but not macrophages, the hydrogen peroxide can then combine with chloride (Cl^{2-}) ions by the action of the enzyme myeloperoxidase (MPO) to form hypochlorous acid (HOCl), and singlet oxygen.
- In macrophages, nitric oxide (NO) can combine with hydrogen peroxide to form peroxynitrite radicals.
- These compounds are very microbicidal because they are powerful oxidizing agentscollectively; these oxidizing free radicals are called reactive oxygen species (ROS).

Oxygen-independent system:

- Some lysosomes contain **defensins** , cationic peptides that alter cytoplasmic membranes;
- **Lysozyme** , an enzyme that breaks down peptidoglycan,
- **Lactoferrin**, a protein that deprives bacteria of needed iron;
- **Cathepsin G**: a protease that causes damage to microbial membranes;
- **Elastase**, a protease that kills many types of bacteria;
- **Cathelicidins**, proteins that upon cleavage are directly toxic to a variety of microorganisms;
- **Bactericidal permeability inducing protein** (BPI), proteins used by neutrophils to kill certain bacteria by damaging their membranes;
- **Collagenase**; and various other **digestive enzymes** that exhibit antimicrobial activity by breaking down proteins, RNA, phosphate compounds, lipids, and carbohydrates.

Table 1.1: Mediators of phagocytic destruction of pathogens

Sr. No	OXYGEN DEPENDENT KILLING	OXYGEN INDEPENDENT KILLING
1	**Reactive oxygen intermediate**	Defensin
	O_2^- (superoxide anion)	TNF-α (Tumor Necrosis Factor)
	OH^- (Hydroxide radical)	Lysozyme
	H_2O_2(Hydrogen Peroxide)	Hydrolytic enzyme
	$HClO^-$ (Hypochlorite anion)	
2	**Reactive Nitrogen intermediate**	
	NO (Nitric oxide)	
	NO_2 (Nitrogen dioxide)	
	HNO_2 (Nitrous acid	
	Other	
	NH_2Cl (monochlorane)	

9. **Exocytosis:**

It is a final stage in which debris of pathogen expelled by phagocytes out of the cell.

INFLAMMATION

- Inflammation (latin, inflammation, to set on fire) is an non-specific defense reaction that occur at the site of infection and injury caused by microbes.
- In response to tissue damage caused by a wound, mechanical injury or by an invading pathogenic a complex sequence of events collectively known as the **inflammatory response.**
- Roman physician Celsus described the "four cardinal signs of inflammation" as *rubor* (redness), *tumor* (swelling), *calor* (heat), and *dolor* (pain). Another physician, Galen, added a fifth sign: *functio laesa* (loss of function).
- The inflammation may result in triggering of a specific immune response to the invasion or clearance of the invading pathogen by components of the innate immune system.
- The inflammation can be classified into two classes based on time of persistence.
- An acute inflammatory response is an Inflammation which has a rapid persists for short time. Acute-phase response is characterized by a rapid alteration in the levels of several plasma proteins.
- A chronic inflammatory responseis an Inflammation which persists for long time. In some diseases persistent immune activation can result in chronic inflammation, which often has pathologic consequences.

Major events of an inflammatory response:

1. **Release of chemical mediators**

To trigger the inflammatory response a variety of chemical mediators are required. These mediators are derived from invading microorganisms, tissue injury, plasma enzyme systems and white blood cells participating in the inflammatory response.

Inflammatory chemicals

- Histamine - released from mast cells and basophils; promotes vasodilation of local arterioles and increased permeability of local capillaries
- Kinins - eg. bradykinin is plasma protein; promotes vasodilation of local arterioles, increases permeability of local capillaries, and induces chemotaxis
- Prostaglandins - made from fatty acids of cell membranes; amplify other inflammatory mediators
- Compliment - plasma protein.

2. **Vasodilation:**

At the site of injury, an increase in the diameter of blood vessels (arteries) carrying blood towards the site of infection and the blood vessels (veins) that carry blood away from the injured area constrict, resulting in blockage of the capillary network. The engorged capillaries are responsible for tissue redness (erythema) and an increase in tissue temperature.This increased temperature act as obstacle for microbial growth. This prevents spread and invasion of pathogen.

3. **An increase in capillary permeability:**

Vasodilation leads to an influx of fluid and immune cells from the engorged capillaries into the tissue. The fluid that accumulates (exudate) has a much higher protein content than fluid normally released from the vasculature. Accumulation of exudate contributes to tissue swelling (edema).

4. **Influx of phagocytes:**

Influx of phagocytes from the capillaries into the tissues is facilitated by the increased permeability of the capillaries. The emigration of phagocytes is a multistep process that includes

- Margination: Adherence of the cells to the endothelial wall of the blood vessels
- Diapedesis or Extravasation: emigration of cells between the capillary endothelial cells into the tissue
- Chemotaxis: Migration of cells through the tissue to the site of the invasion (chemotaxis).

As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus.

Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissue repair and regeneration of new tissue begins. Capillaries grow into the fibrin of a blood clot. New connective tissue cells, called fibroblasts, replace the fibrin as the clot dissolves. As fibroblasts and capillaries accumulate, scar tissue form.

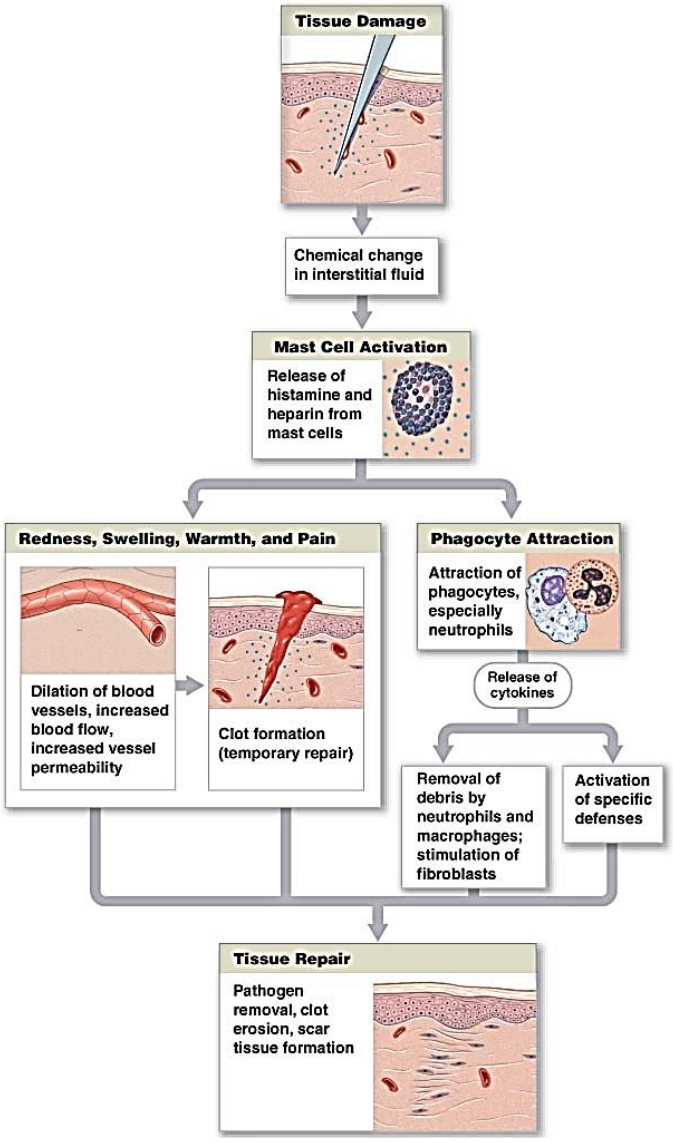


Figure1.9: Steps in inflammatory response

TYPES OF INFLAMMATORY RESPONSE

Infection or tissue injury induces a complex cascade of nonspecific events, known as the inflammatory response, which provides early protection by restricting the tissue damage to the site of infection or tissue injury. This response is either acute or chronic .The acute inflammatory response involves both localized and systemic responses.

ACUTE INFLAMMATORY RESPONSE:

Localized Inflammatory Response

- Within minutes after tissue injury, there is an increase in vascular diameter (vasodilation), resulting in an increase in the volume of blood in the area and a reduction in the flow of blood. The increased blood volume heats the tissue and causes it to redden. This results in an accumulation of fluid **(edema)** in the tissue and, in some instances, extravasation of leukocytes, contributing to the swelling and redness in the area.When fluid exudesfrom the bloodstream, the kinin, clotting, and fibrinolytic systemsare activated.
- Many of the vascularchanges that occur early in a local response are due to the direct effects of plasma enzyme mediators such as bradykinin and fibrino-peptides,which induce vasodilation and increased vascularpermeability.
- Some of the vascular changes are due to theindirect effects of the complement anaphylatoxins (C3a, C4a,and C5a), which induce local mast-cell degranulation with release of histamine. Histamine is a potent mediator of inflammation,causing vasodilation and smooth-muscle contraction.
- The prostaglandins can also contribute to the vasodilation and increased vascular permeability associated with the acute inflammatory response.

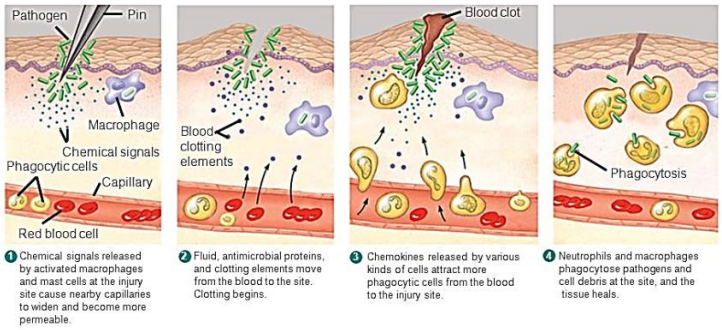


Figure1.10: Localized inflammatory response

Systemic Acute-Phase Response

- This response is marked by the induction of fever, increased synthesis of hormones such as ACTH and hydrocortisone, increased production of white blood cells (leukocytosis), and production of a large number of **acute-phaseproteins** in the liver.
- The increase in body temperature inhibits the growth of a number of pathogens and appears to enhance the immune response to the pathogen.

CHRONIC INFLAMMATION: DEVELOPS WHEN ANTIGEN PERSISTS

- Some microorganisms are able to evade clearance by the immune system, for example by possessing cell-wall components that enable them to resist phagocytosis. Such organisms often induce a chronic inflammatory response, resulting in significant tissue damage.
- Finally, chronic inflammation also contributes to the tissue damage and wasting associated with many types of cancer.
- The accumulation and activation of macrophages is the hallmark of chronic inflammation.
- Cytokines released by the chronically activated macrophages also stimulate fibroblast proliferation and collagen production. A type of scar tissue develops at sites of chronic inflammation by a process called fibrosis, a wound-healing reaction that can interfere with normal tissue function.

- Chronic inflammation may also lead to formation of a granuloma, a tumor-like mass consisting of a central area of activated macrophages surrounded by activated lymphocytes.

FEVER

Fever is a condition at which elevated body temp -triggered by microbial substance (e.g. LPS) or cytokines from activated phagocytes is seen.

A rise in temp in infected or injured tissues is one sign of an inflammatory rxn.

Fever a systemic increase in temp often accompanies inflammation.

Most often fever is caused by a substance called a pyrogen (i.e. toxins such as LPS or endotoxin from gram negative bacteria or certain cytokines).

Fever has several beneficial roles:

- Fever raises the body temp above the optimum temperature for growth of many pathogens. This slows their rate of growth, reducing the number of microorganism to be combated.
- Fever can heighten the level of the immune responses by increasing the rate of chemical reactions in the body.
- This results in a faster rate at which the body's defense mechanisms attack pathogens thus shortening the course of infection.
- Fever impedes the nutrition of bacteria by reducing the availability of iron.
- It has been demonstrated that during fever, the macrophages stop releasing their iron stores, which could retard several enzymatic reactions needed for bacterial growth.
- Fever makes a patient feel ill. In this condition the patient is more likely to rest preventing further damage to the body and allowing energy to be used to fight the infection.

Systemic response to infection associated with an abnormally high body temperature.

- WBCs and macrophages release chemicals (pyrogens) in response to pathogen exposure.
- Pyrogens act on the body's hypothalamic thermostat to raise body temperature.
- Mild increase in temp. accelerates WBC function; impairs bacterial metabolism; and causes the liver and spleen to sequester Zn and Fe, 2 minerals needed by bacteria.
- Major increase in temperature can result in protein denaturation and possible loss of life.

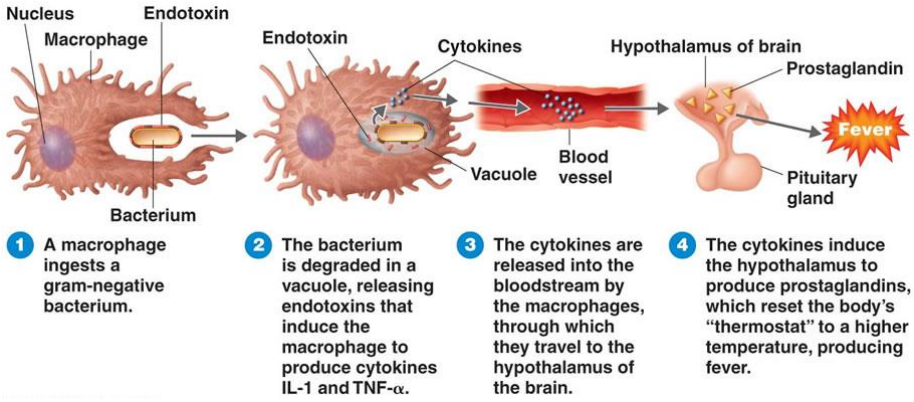


Figure1.11: Fever

COMPLEMENT ACTIVATION AND ACTION:

Complement is a group of serum proteins which can be activated (= "*fixed*") by antigen-antibody complexes or other substances, which may result in lysis of a microbial target, or a variety of other biological effects important in both innate and adaptive immunity. The term complement was coined by Paul Ehrlich.

Complement system consist of 30 heat labile, soluble and cell bound proteins mainly synthesized by liver and by blood monocytes.

Before activation these proteins circulate in the blood in an inactive state. Once activated, these proteins enhance both nonspecific and specific defense mechanisms. The effects of complement include the following:

- ***Destruction of pathogen:*** Complement can act directly by punching holes in a target cell's membrane, so that the cell is no longer able to maintain a constant internal environment.
- ***Enhancement of phagocytosis:*** Complement proteins attract macrophages and neutrophils to the site of infection to remove the foreign cells and enhances phagocytosis.
- ***Opsonization:*** One of the complement proteins act like opsonin, which binds to the surface of the microbe, making it easier for macrophages and neutrophils to "get a grip" on the intruder and devour it.
- ***Stimulation of inflammation:***Complement also causes blood vessels to widen and become more permeable. These changes provide increased blood flow to the area and increased access for white blood cells.
- ***Immune clearance:***Complementcausing removal of immune complexes from circulation and deposits.

The complement proteins are designated as c1 to c9 in addition to factor B, factor J, factor H, factor I, S protein, properdin, etc. There are three pathways by which complements are activated.

THREE PATHWAYS FOR COMPLEMENT FIXATION

The process of complement fixation requires specific *protein/protein interactions*, it involves *proteolytic cleavages* and conformational changes of proteins, and *new biologicalactivities* are generated as a result.

Three distinct (although related) mechanisms are known which can initiate the complement cascade, the **Classical Pathway**, the **Alternate Pathway**, and the more recently recognized **MB-Lectin Pathway**. The central event in all three of these modes of complement activation is the cleavage of component C3. The pathways differ only in the mechanism by which they achieve this cleavage, and we will consider them in turn.

- ***Classical pathway:***The classical pathway is initiated by antigen-antibody complexes (via complement components C_1, C_4, and C_2).IgM and some subclasses of IgG effectively activate this pathway.
- ***The alternate pathway:***The alternate pathway is activated by various cell surface components of microorganisms that are foreign to host without involvement of antibody. This pathway require to factor B, factor D and Properdin.
- ***The MB-lectin pathway:*** MB-lectin pathway does not depend on the antibody for its activation. It is activated by binding of Mannose Binding Lectins (MB-lectins) to mannose on glycoproteins on the surface of microorganisms.

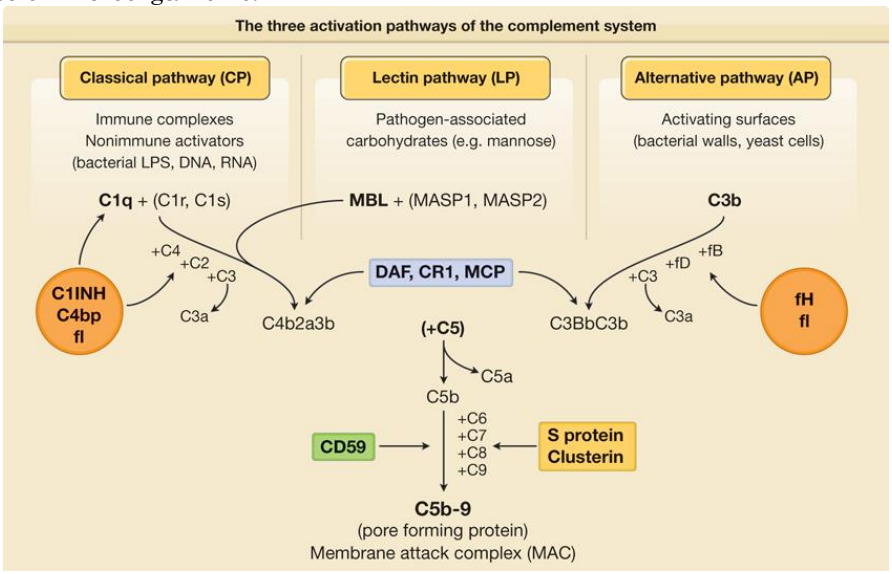


Figure1.12: Complement activation pathways

CLASSICAL PATHWAY: ANTIBODY-DEPENDENT COMPLEMENT FIXATION

This pathway is initiated by *antigen/antibody complexes* and requires **heat-sensitive** complement components. An outline of the components and events in complement fixationby the classical pathway is shown in Figure 1.13.

1) **Ag + Ab → AgAb** :***An antibody/antigen complex is formed.***
   Ca++
2) a) **C1q, C1r, C1s ----------→ C1qrs**(Spontaneous; occurs in serum without AgAb complex.)
   b) **Ag-Ab + C1qrs →)Ag-Ab C1qrs** Requires either a single IgM or 2 adjacent IgG's.
   The antigen-antibody complex binds C1qrs and activates the esterase activity associated with C1s.
3) **C1qrs + C4---------- → C1qrs/4b + C4a**
   New active complex (minimally vasoactive)
   C1s (in the complex) binds soluble C4 and cleaves it into C4a and C4b; C4b becomescovalently bound to the complex or to the nearby membrane; C4a is released, and while it is somewhat vasoactive, its low levelof activity is probably not biologically important.
4) **C1qrs/4b + C2----------→ C1qrs/4b2b + C2a**
new active complex *no known activity*
   The esterase activity of C1s acts again, this time to cleave C2 into C2b (which is bound by 4b in the complex) and C2a (which is released). At this point the presence of the AgAbC1qrs complex is no longer necessary for the cascade to continue.
5) **C4b2b + C3 ----------→ C4b2b3b + C3a**
"C3 convertase" *opsonizing (3b)* *vasoactive*
   The C4b2b complex has an enzymatic activity called **C3 convertase**, indicating it can cleave C3 into C3a and C3b. This is a *key step* in this pathway, involving *biologicalamplification* and the generation of molecules with *new biological activities*.
   Many hundreds of C3 molecules can be cleaved by a single C4b2b complex, resulting in considerable **amplification**. The resulting C3b molecules can either be *bound by the complex* or can be released and *bind elsewhere* on the membrane (C3b molecules which are *not* bound in one place or the other are rapidly destroyed.) **C3b has powerful opsonizing activity**, whether it is bound in the complex or to some other site on the membrane. **C3a is strongly vasoactive**. It is also chemotactic for mast cells.
6) **C4b2b3b + C5 → C4b2b3b5b + C5a** *Vasoactive- chemotactic for PMNs (and mast cells)*
   Cleavage of C5 results in a new complex and another biologically active molecule, C5a, which is both **vasoactive and chemotactic**.
7) **C4b2b3b5b + C6, C7 → C4b2b3b5b67→ C5b67** binds to nearby sites on the membrane,in soluble form is *chemotactic for PMNs*
8) The C5b67 complex may be released and bind to a nearby site in the membrane, and insoluble form also exhibits chemotactic activity. The resulting C4b2b3b complex leftbehind can then sequentially bind additional C5, C6 and C7 molecules, which results in *another important site of amplification in the complement cascade*
9) **C5b67 + C8 → C5b678***osmotic "holes", leakage*
10) **C5b678 + C9 → C5b6789***Membrane Attack Complex,"MAC", large "holes", lysis*
   The formation of large, open channels in target cell membranes results in the loss ofhemoglobin from erythrocytes (visible as HEMOLYSIS) or leakage of cytoplasmiccontents and death of nucleated cells (CYTOTOXICITY).

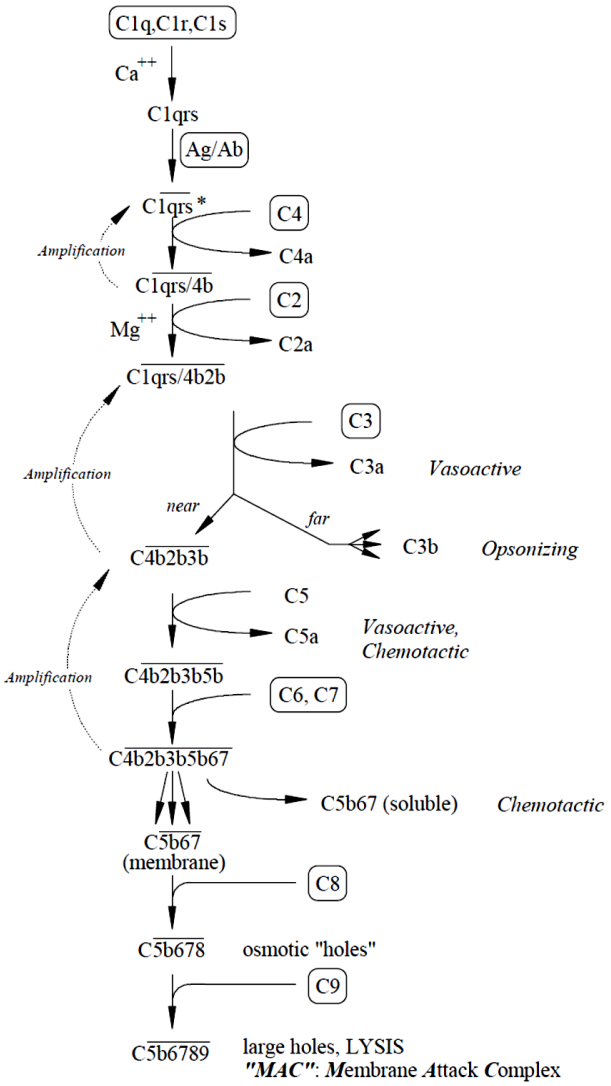


Figure1.13: Classical pathway of Complement activation pathways

ALTERNATE PATHWAY OF COMPLEMENT FIXATION

This pathway was discovered by the biochemist Louis Pillemer in the 1950. He discovered that complement fixation could be triggered by the yeast polysaccharide Zymosan in the *absence* of antibody. The initial steps of this process are differ from those of the classical pathway and involve several unique serum components, namely factor P (for "properdin"),factors B and factor D.

The initial step relies on the fact that *very small amounts of soluble C3bare normally present in serum*, due to low levels of spontaneous C3 cleavage , which may not be C4-dependent.

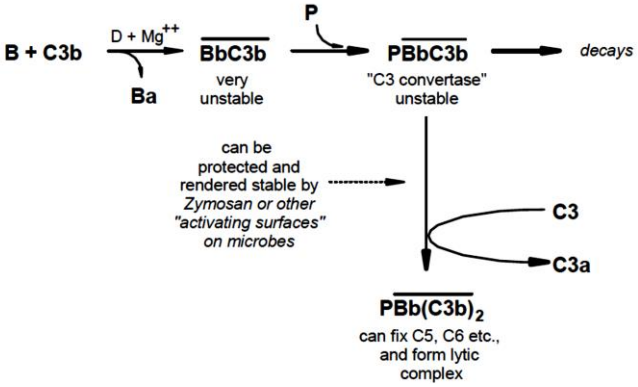


Figure1.14: Initial steps of alternate pathway of Complement activation pathways

- The initial steps of the alternate pathway result in the formation of an *unstable* complex which has "C3 convertase" activity (namely PBbC3b).
- This complex is formed continuously at low levels and rapidly degrades, but it may be bound and stabilized by "activating surfaces" such as *zymosan*-containing yeast cell walls, *LPS*-containing gram-negative bacteria, *teichoic acid*-containing gram-positive bacteria, and others.
- The result of stabilization is that *these C3-convertase complexes can carry out the remainder of thecomplement fixation cascade* in a manner identical to what we have outlined above for the classical pathway.

MB-Lectin COMPLEMENT PATHWAY

- A third mechanism for the initiation of complement fixation has been described which depends on the presence of another normal serum protein known as the ***m**annan-**b**indinglectin*, or MB-Lectin.
- This protein is capable of binding to microbial carbohydrates containing terminal mannose residues, and consequently binding two other proteins, MASP-1 and MASP-2 (**m**annan-binding lectin-**a**ssociated **s**erum **p**rotease-1 and -2).
- The resulting complex has C4-convertase activity (*i.e.* it can bind and cleave C4), and the remainder of the complement cascade (C2, C3, C5 *etc.*) is activated just as in the case of the classical and alternate pathways

BIOLOGICAL EFFECTS OF COMPLEMENT

1. **Cytolysis [C5b6789]** *(Note: the bar identifies an activated complex)* Destruction of target cells by lysis of the cell membrane. This is termed **cytotoxicity** inthe case of nucleated cells, **hemolysis** for red blood cells, or **bacteriolysis** in the case ofbacteria. (NOTE: *Not all bacterial and eukaryotic cells are susceptible to complement- dependent lysis*).
2. **Anaphylotoxin activity** (= "vasoactive" or "phlogistic") [**C3a, C5a**]: Stimulation of mast cells to release histamine and other substances, resulting in increased capillary permeability and local accumulation of fluid in the tissue.
3. **Chemotaxis** [**C5a, C5b67**]: Attraction of polymorphonuclear neutrophils (PMN's) to a local site of inflammation.
4. **Opsonization** (= "immune adherence") [C3b]: Facilitation of phagocytosis by macrophages or PMN's via cell-surface receptors specific for complement components ("complement receptors", or "CRs")

5. **Tissue damage [C5b6789; PMN's]**: Both the lytic complex and the inflammatory PMN's can cause considerable damage to normal tissues, for instance in an Arthus Reaction or in Immune Complex Disease.

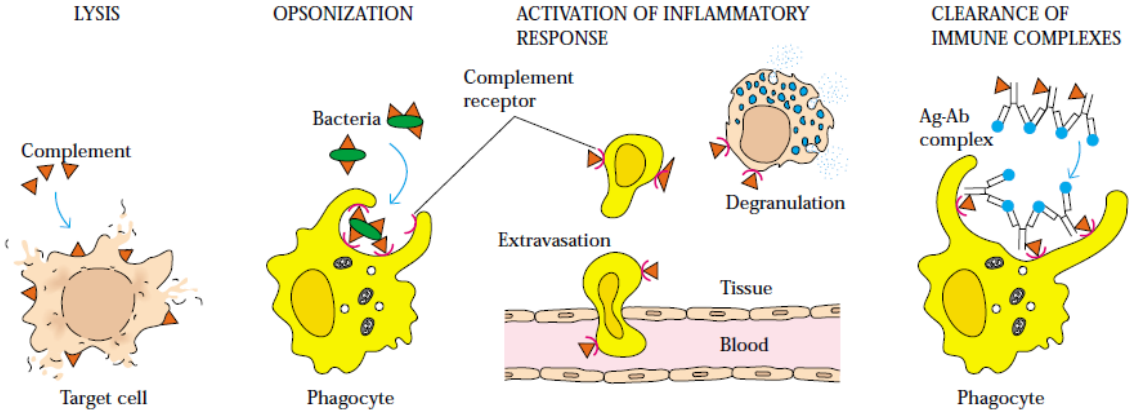


Figure1.15:biological effects of activated complements

INTERFERON

Interferon's (IFNs) are low molecular weight proteins produced by the cells of the immune system of most vertebrates in response to challenges by foreign agents such as viruses, parasites and tumor cells. Interferons belong to the large class of glycoproteins known as cytokines.

Types of interferon

There are three major types classes of Interferons ,IFN-α, IFN-ß and IFN-ω.

Natural function and synthesis

Interferon's in general having several effects in common.

- They are antiviral and possess anti-oncogenic properties, macrophage and natural killer lymphocyte activation, and enhancement of major histocompatibility complex glycoprotein classes I and II, and thus presentation of foreign (microbial) peptides to T cells.
- In a majority of cases, the production of Interferons is induced in response to microbes such as viruses as well as mitogens and other cytokines, for example interleukin 1, interleukin 2, interleukin-12, tumor necrosis factor and colony-stimulating factor, that are synthesized in the response to the appearance of various antigens in the body.
- The therapeutically used forms are denoted by Greek letters indicating their origin: leukocytes, fibroblasts, and lymphocytes for interferon-alpha, -beta and -gamma, respectively.

Viral induction of Interferon's

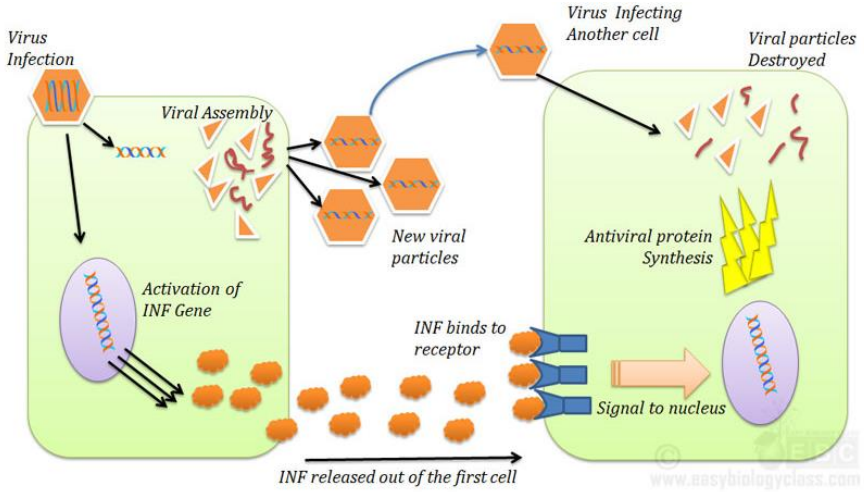


Figure1.16: Interferon synthesis and action

1.2 ACQUIRED OR SPECIFIC OR ADAPTIVE IMMUNITY:

The form of immunity that is mediated by lymphocytes and stimulated by exposure to infectious agents is adaptive immunity. It reflects the presence of a functional immune system that is capable of specifically recognizing and selectively eliminating foreign microorganism and molecules.

Specific immune response occurs when a particular antigen passes the body's passive defenses. It involves cells and proteins within the blood and lymph that attach, disarm, destroy and remove foreign bodies. The specific system gives a highly effective, long lasting immunity against anything the body recognize as foreign. It responds to specific microorganisms and enhances the activity of the non-specific system.

The central feature of the specific immune system is the ability to distinguish between self and non-self. Every cell has complex molecules (proteins and glycoproteins) on its surface membrane which act as recognition devices and have specific shapes. These molecules are called antigens or immunoglobins. The immune system is usually tolerant to the body's own antigens (self antigens) and does not attack against them. However, breakdown of the recognition system can lead to autoimmune disease such as AIDS and rheumatoid arthritis, which result in self-destruction of body parts.

When a foreign organism (bacteria, viruses or even another person's cells) enters the body, the foreign antigens on the invading cells activate an immune response. The foreign antigens are called non-self antigens. The immune system produces antibodies and specialized cells that attempt to destroy foreign cells and particles that have entered the body. There are two types of responses: Humoral (antibody) response (involving B cells) and cell mediated immunity (involving T cells).

It is characterized by four characteristics namely

- Antigenic specificity
- Diversity
- Immunologic memory
- Self / Non-self recognition

1. **Antigenic specificity:**

   A cardinal feature of the adaptive immunity, namely those immune responses are directed toward and able to distinguish between distinct antigens or small parts of macromolecular antigens. This fine specificity is attributed to lymphocyte antigen receptors that may bind to one molecule but not to another with only minor structural differences from the first. Antibodies can differentiate between two molecules that differ by only a single amino acid.

2. **Diversity:**

   Diversity is the fundamental property of the adaptive immunity and is the result of variability in the structure of the antigen binding sites of lymphocyte receptors for antigens. Example: Paratopes of antibodies and TCR of T-cells. Large number of lymphocytes with different antigenic specificities exists. Diversity allows adaptive immune system to specifically recognize billions of uniquely different structures on foreign antigens.

3. **Immunologic memory:**

   The property of the adaptive immune system to respond more rapidly with greater magnitude and more effectively to a repeated exposure to an antigen, compared with the response to the first exposure. Immunologic memory mediated by memory cells. Memory cells are clonally expanded progeny of T and B cells formed during the primary response following initial exposure to antigen. Memory cells are more easily activated than naive lymphocytes and mediate secondary response on subsequent exposure to antigen. They survive in a functionally quiescent state for many years after the antigen eliminated. Due to this attribute, the immune system can confer lifelong immunity to many infectious agents

4. **Self and Non-self recognition:**

   It is also an important property of adaptive immunity. Due to this ability, immune system was able to distinguish self from nonself antigen and respond only to nonself molecules. If immune system responds to self antigen then it results in auto immune diseases.

   Production of immune cells only against non-self molecules was achieved by selection procedure like positive selection and negative selection during maturation process of lymphocytes in bone marrow and thymus.

   Acquired immunity does not occur independently of innate immunity and vice versa. For example, the phagocytic cells crucial to nonspecific immune responses are intimately involved in activation of the specific immune response.

   Conversely, the soluble factors produced during a specific immune response, have been shown to augment the activity of these phagocytic cells.

   Acquired immunity on the basis of component involved in immunity classified in to two types namely Humoral immunity and Cell mediated immunity.

1.2.1 TYPES OF SPECIFIC IMMUNITY:

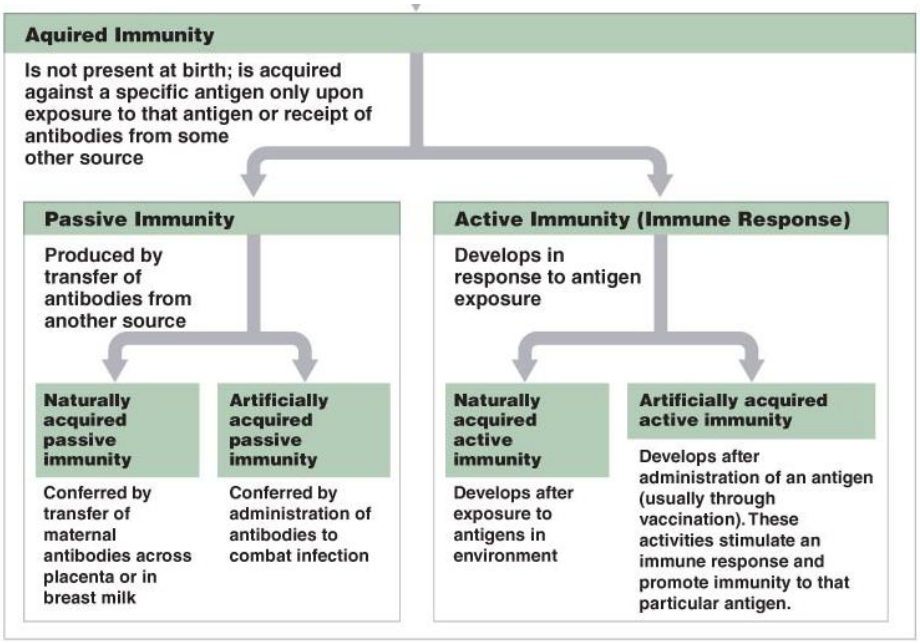


Figure1.17:types of specific immunity

1. **Natural passive immunity-**
   Antibodies made in one individual are passed into another individual of the same species. This only affords temporary protection, for, as the antibodies do their job, or are broken down by the body's natural processes, their number diminishes and protection is slowly lost. For example, antibodies from a mother can cross the placenta and enter her foetus. In this way they provide protection for the baby until its own immune system is fully functional. Passive immunity may also be conferred by colostrum (the mother's first milk), from which antibodies are absorbed from the intestines of the baby.
2. **Artificial passive immunity**
   Here, antibodies which have been made in one individual are extracted and then injected into the blood of another individual which may, or may not, be of the same species. For example, specific antibodies used for combating tetanus and hepatitis B are cultured in horses and later injected into Man. They act to prevent tetanus and hepatitis respectively. This type of immunity is again short-lived – a matter of weeks only.
3. **Natural active immunity**
   The body manufactures its own antibodies when exposed to an infectious agent. Since memory cells produced on exposure to the first infection are able to stimulate the production of massive quantities of antibody, when exposed to the same antigen again. This type of immunity is most effective and generally persists for a long time - sometimes even for life.
   When a bacterial infection occurs and an antigen is presented for the first time, time is taken for the B and T cells to proliferate. Once the B cells have differentiated into plasma cells, specific antibodies can be secreted.
   This primary response lasts several days or weeks and then the concentration of antibody decreases as the plasma cells stops secreting them. Once the infection is eradicated, plasma cells die, but B memory cells are left in the body.
   If another infection of the same pathogen occurs, then the same antigen is reintroduced. There is a more rapid response, called the secondary response. This is much faster because there are many more memory B-cells that can produce many plasma cells and the appropriate antibody. These destroy the pathogen before it has the chance to cause any symptoms to occur.
   Memory cells are the basis for immunological memory – they last for many years, often a lifetime. It is possible for suffer repeated infections from a single pathogen because pathogens occur in different form, each having minor changes in the shape of the antigen, due to a possible mutation, and therefore requiring a primary response.

4. **Acquired active immunity**
   This is achieved by injecting small amounts of antigen - the vaccine - into the body of an individual. The whole process is called vaccination orimmunization. The small dose of antigen is usually safe because the pathogen is either killed or attenuated (= crippled). This ensures that the individual does not contract the disease itself, but is stimulated to manufacture antibodies against the antigen. Often a second, booster, injection is given and this stimulates a much quicker production of antibody which is long lasting and which protects the individual from the disease for a considerable time. Several types of vaccine are currently in use.

VACCINATIONS:

Currently vaccines come in three forms:

- **Living attenuated microbes**: These are mutants of microbes that have lost the ability, either naturally or by treatment in the laboratory, to produce the dangerous, clinical disease. Some examples are the cowpox virus, measles, mumps and rubella (MMR vaccine) and polio vaccine virus. A vaccination consists of infecting you with a living microbe which then produces a limited infection. Because these attenuated strains are weak the immune system of normal healthy people quickly kill and eliminate them from the body. During this process the infection elicits a vigorous immune response that protects the host from infection by the related virulent, disease-producing form of the pathogen. Live vaccines produce the best immunization because they closely imitate the real thing. Immunity lasts for life.
- Dead Microbes: These vaccines consist of growing up cultures of the virulent, disease-producing microbial strains and killing them in such a way that they retain their ability to stimulate the body to produce an immunological response to the live form. Examples include anthrax and rabies vaccine. Immunity lasts several years.
- Virulence of Components of Pathogens: These vaccines consist of substances isolated from the virulent strains, such as polysaccharide material or proteins components. No whole organisms, living or dead are present in these vaccines. Examples include the toxins of diphtheria, tetanus and botulinum and the polysaccharide from virulent pneumococci.
- Vaccinations by eating: Experiments are underway to deliver vaccines through common foods like potatoes and bananas. Genes that make an antigen effective against a microbe are cloned into a common food. The food is eaten by the "patient" and the cloned-antigen stimulates the immune system.
- DNA Vaccines: Vaccines consisting of DNA fragments that can be transformed into host tissue. Once in the host tissue, the DNA is transcribed and translated and the protein produced is seen by the specific immune system as foreign material and an immune response is induced.

1.3 ORGANS OF IMMUNE SYSTEM

The organs of the immune system which are involved in defending the body against invading pathogens causing infections or spread of tumors is termed as Lymphoid organs. It includes bone marrow, blood vessels, lymph nodes, lymphatic vessels, thymus, spleen, and various other clusters of lymphoid tissue.

Lymphoid organs are the site of origin, maturation, and proliferation of lymphocytes. They exist as primary, secondary or tertiary and these are based on their stage of development and maturation. These organs consist of fluid connective tissues with different types of leukocytes or white blood cells. The highest percentage of Lymphocytes is present in the white blood cells or leukocytes.

Primary lymphoid organs: The primary lymphoid organs produce and allow the maturation of lymphocytes. It also serves by generating lymphocytes from immature progenitor cells. Therefore it is referred as the central lymphoid organs. Examples of primary lymphoid organs include thymus and the bone marrow.

Secondary lymphoid organs: The secondary lymphoid organs are referred to as the peripheral lymphoid organs as they are involved in promoting the sites for the interaction of lymphocytes with the antigen to become effector cells. They initiate an adaptive immune response. The secondary

lymphoid organs. Examples of secondary lymphoid organs spleen, tonsils, lymph nodes, appendix, etc. are secondary lymphoid organs.

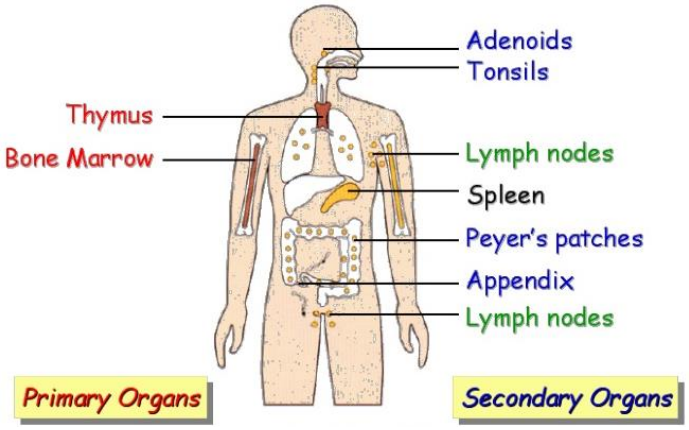


Figure1.18: Lymphoid organs in Human

1.3.1 CENTRAL OR PRIMARY LYMPHOID ORGANS:

Bursa of fabricus:

Anatomy:Bursa of fabricus s is a sac like lymphoepithelial structure arising as a dorsal diverticulum from the cloaca in birds. This organ was first described by Fabricius and hence the name Bursa of Fabricius. This organ originates from the hindgut epithelium of the chick embryo at about the 15th day of development. At the age of 4 months, it reaches the maximum size of about 3 cm in diameter after which it starts involuting. The major cells in bursa include lymphocytes, macrophages and plasma cells.

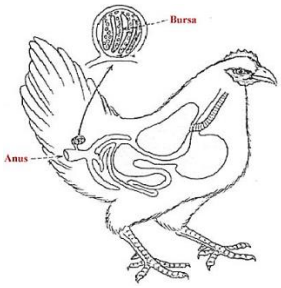


Figure1.19: PrimaryLymphoid organs in Bird: Bursa of fabricus

The function:The function of Bursa is identified through the bursectomy studies. In birds bursa of fabricus serve as a site of ***B cells maturation***. In bursa PreB cells enter and undergo rapid proliferation during which they mature. The mature B lymphocytes later migrate to the bursa dependent regions of the secondary lymphoid organs such as spleen and lymph node.

Bone marrow:

Anatomy: Bursa equivalent tissue in the mammalis the bone marrow. Bone marrow is the soft tissue present in the cavities of bones. Bone marrow serving as a site of hemopoesis so appear red. Due to its red colour it is called as red marrow. The red marrow consists of a sponge like reticular framework located between long trabeculae. The spaces in this framework are filled with fat cells, stromal fibroblasts and precursors of blood cells. These precursors mature and exit through the dense network of vascular sinuses to enter the vascular circulation.

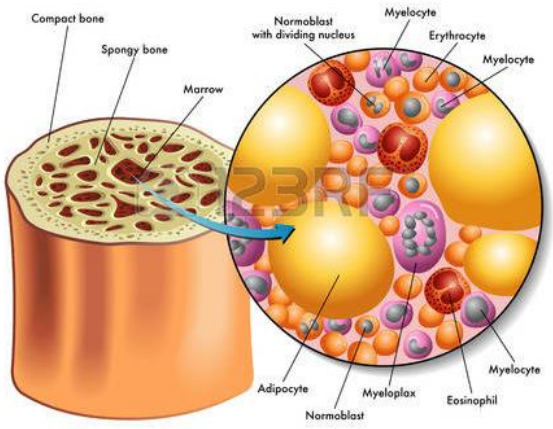


Figure1.20: Anatomy of Bone marrow

The function: In humans and mice, bone marrow is serving as the site of B-cell origin and development.

B-cell Maturation in bone marrow:

- The lymphoid progenitors are produced from pluripotent stem cells.
- These lymphoid progenitors develop into immature B cells.
- Immature B cells proliferate and differentiate within the bone marrow
- Stromal cells of bone marrow after interaction with the B cells, secrete various cytokines required for B cell development.
- A selection process within the bone marrow eliminates self-reactive clones of B cells.
- After maturation B cells exit the bone marrow and enter circulation.

Thymus:

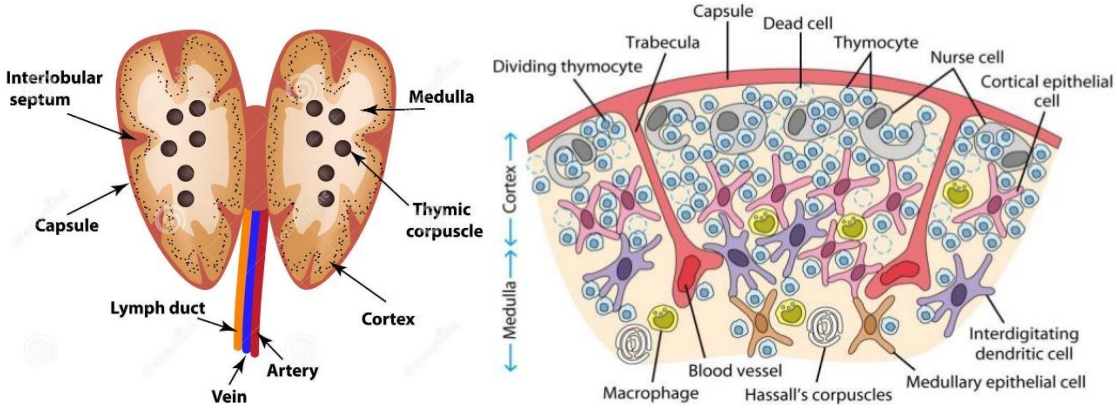


Figure1.21: Anatomy of thymus

Anatomy: Thymus is a primary lymphoid organ responsible for maturation of T cells,develops from the third pharyngeal pouch.

The thymus consists of two lateral lobes placed in close contact along the middle line situated partly in the thorax, resting in the chest beneath the neck. The two lobes differ slightly in size, may be united or separated, and may be broken down into smaller lobules. It is covered with a capsule of connective tissue that provides structural support.The lobule has outer cortex and inner medulla. The cortex is densely packed with rapidly dividing lymphocytes and medulla has most of resting cells, mature T cells. The organ enlarges during childhood into adolescence and begins to atrophy at puberty due to hormonal changes. After puberty, the thymus shrinks rapidly with age, eventually becoming almost indistinguishable from the surrounding fatty tissue.

The function:The thymus is the site of maturation of T cells.

- The thymus provides an environment for T cells to mature and proliferate.
- TheimmatureT cells generated in bone marrow enter in thymus through the post-capillary venules located at the juncture of the cortex and medulla.

- The lymphocytes in the thymus are called as thymocytes.
- After entrance in cortex region they migrate out into the cortex under the influence of some unknown chemotactic factor(s) for the next several days, then migrate into the medulla.
- During this time, thymocytes divide approximately every 12 hours.
- In influence of thymic hormones like thymopoeitin and thymosin, they gradually acquire the membrane and functional characteristics of mature T cells.
- Then the T cells are sorted by the thymus so that only T cells that express T-cell receptors (TcRs) and can bind to foreign MHC molecules will survive.
- The mature T cells then leave the thymus through the postcapillary venules and enter circulation.
- Central tolerance is another function of the thymus. (The thymus sorts T cells so that they will be inactive towards host molecules to limit an autoimmune disease).

1.3.2 SECONDARY OR PERIPHERAL LYMPHOID ORGANS:

The secondary lymphatic organs represent the location where the complete maturation and homing of lymphocytes takes place. Similarly it is a site where defense battles take place. In this location the T- and B-lymphocytes that have matured in the thymus and in the bone marrow develop further when they come into contact with antigens, leading to a clonal proliferation.

Specific proliferation zones form for the two lymphocyte groups. Thereby effector and regulator cells arise. The anlage material for the secondary lymphatic organs has a mesenchymal origin. It forms in connection with the differentiation of the lymph and vascular systems.

These secondary lymphoid organs include.

Lymph node:

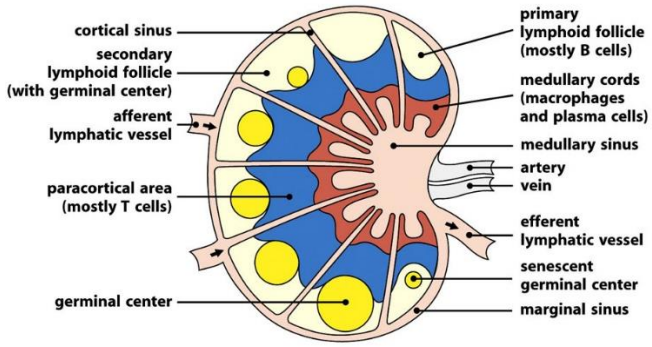


Figure1.22: Anatomy of lymph node

Anatomy:

Lymph nodes are small kidney or beanshaped organs, usually less than one inch (2.6 cm) in length. They are found in clusters of several nodes in many regions of the body, especially in the neck, armpits, trunk, and groin.

The tough exterior layer of a lymph node, known as the capsule, is made of dense irregular fibrous connective tissue containing many strong collagen fibers.The capsule provides a structural shell for the soft interior tissue. Many columns of fibrous tissue, known as trabeculae, extend from the capsule to the interior of the lymph node.

Inside the capsule and sinuses the lymph node is filled with lymphatic tissue, which can be further divided into the superficial region known as the cortex and a deep region known as the medulla.

The cortex is further divided into an inner cortex and outer cortex, each with distinct structures and functions.

The cortex is composed of an outer cortex of B cells organized into lymphoid follicles, and deep, or paracortical, areas made up mainly of T cells and dendritic cells. Beneath the subcapsular sinus, the outer cortex contains aggregates of cells called follicles. Some follicles contain central areas called germinal centers, which stain lightly with commonly used histologic stains.

Follicles without germinal centers are called primary follicles and those with germinal centers are secondary follicles. When an immune response is underway, some of the follicles contain central areas of intense B-cell proliferation called germinal centers and are known as secondary lymphoid follicles.

The function:

Lymph nodes play two major roles in the body: filtration of lymph and production of immune responses.

- Lymph draining from the extracellular spaces of the body carries antigens in phagocytic dendritic cells and macrophages from the tissues to the lymph node via the afferent lymphatics.
- Afferent lymphatic vessels drain fluid from the tissues and also carry antigen-bearing cells and antigens from infected tissues to the lymph nodes, where they are trapped.
- The medulla consists of strings of macro-phages and antibody-secreting plasma cells known as the medullary cords.
- In the lymph nodes, B lymphocytes are localized in follicles, with T cells more diffusely distributed in surrounding paracortical areas, also referred to as T-cell zones. Some of the B-cell follicles include germinal centers, where B cells are undergoing intense proliferation after encountering their specific antigen and their cooperating T cells.

Spleen:

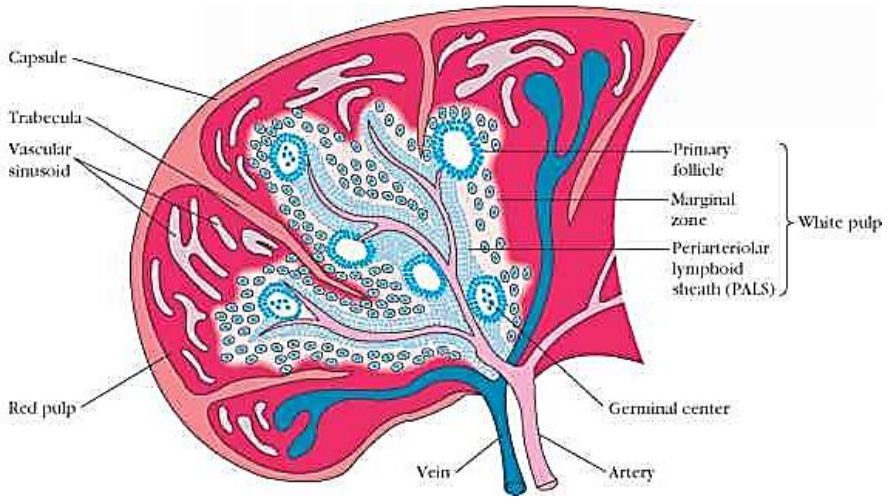


Figure1.23: Anatomy of Spleen

Anatomy:

The spleen is located in the left upper quadrant of the abdomen.The spleen is developed within the gut and is derived from mesenchymal tissue during embryonic development. However, it still shares the same blood supply as the foregut organs in the abdominal cavity.It is similar to an enlarged lymph node but is a bit more complex. The spleen possesses splenic artery provide its primary blood supply. The spleen plays a major role in mounting immune responses to antigens in the blood stream.

It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. The spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic infections. The spleen is not supplied by lymphatic vessels. Instead, blood-borne antigens and lymphocytes are carried into the spleen through the splenic artery.

The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure. The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone.

The splenic red pulp consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes; it is the site where old and defective red blood cells are destroyed and removed. Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin.

The splenic white pulp surrounds the branches of the splenic artery, forming a periarte-riolar lymphoid sheath (PALS) populated mainly by T lymphocytes. Primary lymphoid follicles are attached to thePALS. These follicles are rich in B cells and some of them contain germinal centers. The marginal zone, located peripheral to the PALS, is populated by lymphocytes and macrophages.

The function:

- Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone.
- In the marginal zone, antigen is trapped by interdigitating dendritic cells, which carry it to the PALS. Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS.
- The initial activation of B and T cells takes place in the T-cell-rich PALS.
- Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to TH cells.
- Once activated, these T_H cells can then activate B cells. The activated B cells, together with some T_H cells, then migrate to primary follicles in the marginal zone.
- Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers, where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.

MUCOSAL ASSOCIATED LYMPHOID TISSUE (MALT):

The mucous membranes of the digestive, respiratory and urogenital systems have a combined surface area of about 400 m^2. It is the major sites of entry for most pathogens. These membrane surfaces are defended by a group of organized lymphoid tissues collectively known as mucosal-associated lymphoid tissue (MALT). Structurally, these tissues range from loose, barely organized clusters of lymphoid cells in the lamina propria of intestinal villi to well-organized structures such as the familiar tonsils and appendix, as well as Peyer's patches, which are found within the submucosal layer of the intestinal lining.

These malty further categorized as GALT,BALT, SALT and NALT etc.

The function:The epithelial cells of mucous membranes play an important role in promoting the immune response by delivering small samples of foreign antigen from the lumina of the respiratory, digestive, and urogenital tracts to the underlying mucosal-associated lymphoid tissue.

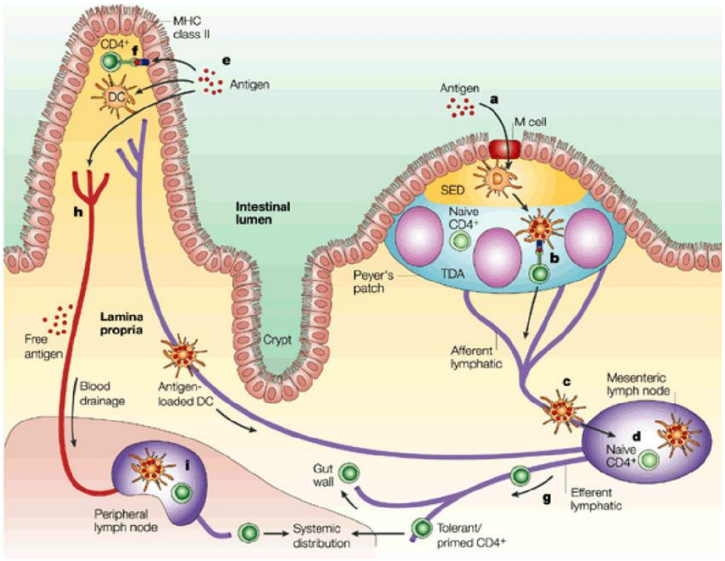


Figure1.24: Anatomy of GALT

GALT (Gut Associated Lymphoid Tissue):

The gut-associated lymphoid tissues (GALT), which include the tonsils, adenoids, appendix and specialized structures called Peyer's patches in the small intestine.

Tonsils:

Anatomy: The tonsils are found in three locations: lingual at the base of the tongue; palatine at the sides of the back of the mouth; and pharyngeal (adenoids) in the roof of the nasopharynx. All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and fibers interspersed with lymphocytes, macrophages, granulocytes, and mast cells.

The function: The B cells are organized into follicles and germinal centers. The germinal centers are surrounded by regions showing T-cell activity. The tonsils defend against antigens entering through the nasal and oral epithelial routes.

Adenoids:

Anatomy:

The adenoids are a mass of soft tissue behind the nasal cavity. Like lymph nodes, adenoids are part of the immune system and are made of the same type of tissue (lymphoid tissue). White blood cells circulate through the adenoids and other lymphoid tissue, reacting to foreign invaders in the body.

Appendix:

Anatomy:

Peyer's Patches:

Anatomy:The lamina propria, lies under the epithelial layer. It contains large numbers of B cells, plasma cells, activated T_H cells, and macrophages in loose clusters. Cross sections shows more than 15,000 lymphoid follicles within the intestinal lamina propria of a healthy child. The submucosal layer beneath the lamina propria contains Peyer's patches, nodules of 30–40 lymphoid follicles. Like lymphoid follicles in other sites, those that compose Peyer's patches can develop into secondary follicles with germinal centers.

The function:

In Peyer's patches the antigen is collected by specialized epithelial cells called multi-fenestrated or M cells. The lymphocytes form a follicle consisting of a large central dome of B lymphocytes surrounded by smaller numbers of T lymphocytes. The bulk of the GALT tissue is B cells, organized in a large and highly active domed follicle. T cells occupy the areas between follicles. The antigen enters across a specialized epithelium made up of so-called M cells.

BALT (Bronchial Associated Lymphoid Tissue):

Similar but more diffuse aggregates of lymphocytes protect the respiratory epithelium, where they are known as bronchialassociated lymphoid tissue (BALT).

CutaneousAssociated Lymphoid Tissue (CALT):

The epidermal (outer) layer of the skin is composed largely of specialized epithelial cells called keratinocytes. These cells secrete a number of cytokines that may function to induce a local inflammatory reaction. Scattered among the epithelial-cell matrix of the epidermis are Langerhans cells, a type of dendritic cell, which internalize antigen by phagocytosis or endocytosis. The Langerhans cells then migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells. These cells express high levels of class II MHC molecules and function as potent activators of naive T_H cells. The epidermis also contains so-called intraepidermal lymphocytes.

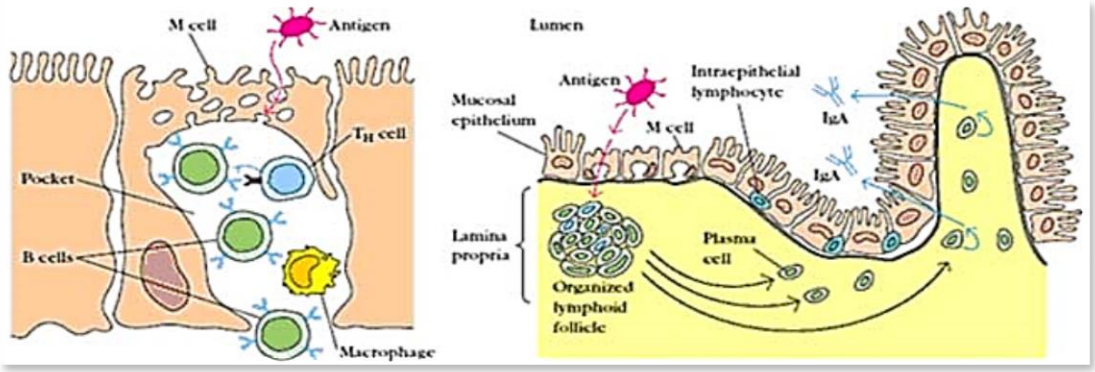


Figure1.25: Anatomy of GALT

1.4 LYMPHOCYTES CIRCULATION BETWEEN LYMPH AND BLOOD:

T-cell trafficking patterns: T cells originate from bone-marrow precursors that mature into naive T cells in the thymus. Naive T cells traffic to secondary lymphatic organs, including peripheral lymph nodes, Peyer's patches, mesenteric lymph nodes and the spleen, where they might encounter antigen and become polarized into T helper 1 (TH1), TH2 and other effector T cells, which are collected in efferent lymphatics and enter the circulation through the thoracic duct. Activated T cells traffic to extra-lymphoid organs, including the un-inflamed lungs, skin, central nervous system and gastrointestinal organs. Many activated T cells ultimately migrate to the liver to undergo apoptosis. Activated T cells can home to almost all inflamed organs and tissues (

- Small B and T lymphocytes that have matured in the bone marrow and thymus but have not yet encountered antigen are referred to as naive lymphocytes.
- These cells circulate continually from the blood into the peripheral lymphoid tissues, which they enter by squeezing between the cells of capillary walls.
- They are then returned to the blood via the lymphatic vessels or, in the case of the spleen, return directly to the blood.
- In the event of an infection, lymphocytes that recognize the infectious agent are arrested in the lymphoid tissue, where they proliferate and differentiate into effector cells capable of combating the infection.
- When an infection occurs in the periphery, for example, large amounts of antigen are taken up by dendritic cells which then travel from the site of infection through the afferent lymphatic vessels into the draining lymph nodes. In the lymph nodes, these cells display the antigen to recirculating T lymphocytes, which they also help to activate.
- B cells that encounter antigen as they migrate through the lymph node are also arrested and activated, with the help of some of the activated T cells.
- Once the antigen-specific lymphocytes have undergone a period of proliferation and differentiation, they leave the lymph nodes as effector cells through the efferent lymphatic vessel.
- Because they are involved in initiating adaptive immune responses, the peripherallymphoid tissues are not static structures but vary quite dramatically depending upon whether or not infection is present.
- The diffuse mucosal lymphoid tissues may appear in response to infection and then disappear, whereas the architecture of the organized tissues changes in a more defined way during an infection.
- For example, the B-cell follicles of the lymph nodes expand as B lymphocytes proliferate to form germinal centers, and the entire lymph node enlarges, a phenomenon familiarly known as swollen glands.

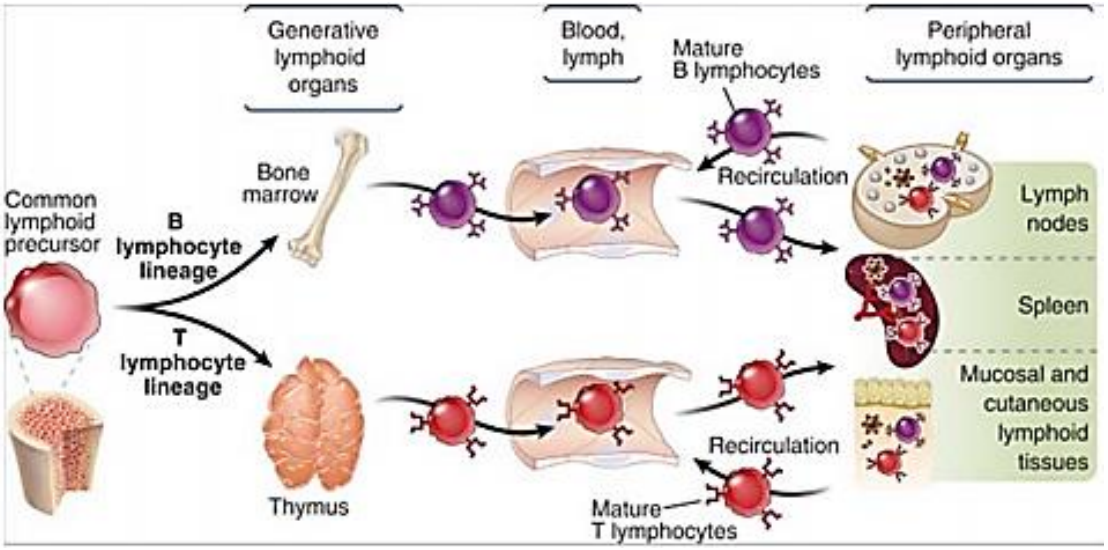


Figure1.26: Lymphocyte trafficking

DEFENSIVE MECHANISM OF HOST II

2.1 HAEMATOPOIESIS.

2.1.1 Site of Haematopoiesis

- All the cells of the immune system are derived from pluripotent stem cells in the bone marrow by a process called hematopoiesis.
- In the first few weeks of gestation the yolk sac is themain site of Haematopoiesis.
- These commonprecursors of endothelial and haemopoietic cells(haemangioblasts) are believed to seed the liver,spleen and bone marrow and from 6 weeks until6 – 7 months of fetal life the liver and spleen are themajor haemopoietic organs and continue to produceblood cells until about 2 weeks after birth.

Table 2.1: Site of Haematopoiesis

Sr. No.	Stage	Site of Haemopoesis
1	Fetus	0-2 months (Yolk sac)
		2-7 months (Liver , spleen)
		5-9 months (Bone marrow)
2	Infants	Bone marrow
3	Adults	Vertebrates,ribs, sternum, skill, sacrum and pelvis, proximal ends of femur.

- The bone marrow is the most important site from 5 to 9 months of fetal life.
- During normal childhood and adult life the marrow is the only source of new blood cells.
- The developing cells are situated outside the bone marrow sinuses; mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation.
- In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs.
- Even in these haemopoietic areas, approximately 50% of the marrow consists of fat.
- The remainingfatty marrow is capable of reversion to haemopoiesisand in many diseases there is also expansion ofhaemopoiesis down the long bones.
- Moreover, theliver and spleen can resume their fetal haemopoieticrole (‘extra-medullary haemopoiesis ’).

2.1.2 Process of Haematopoiesis

Haemopoietic stem and progenitor cells

- Haemopoiesis starts with a pluripotent stem cell that having potential of self renewal and differentiation. This ***haemopoietic stem cell*** is rare, perhaps 1 in every 20 million nucleated cells in bone marrow.
- There is considerableamplification in the system: one stem cellis capable of producing about 10^6 mature bloodcells after 20 cell divisions.
- On immunological testing it is $CD34^+$ $CD38^-$cell, negative for lineage markers (Lin –) and has the appearance of a small or medium - sized lymphocyte.

Synthesis of*haemopoietic* progenitors:

- Initially haemopoetic stem cell on treatment with IL-17 giving rises to formation of Common lymphoid progenitor.
- After treatment with IL-3, GM-CSF and M-CSF Common myeloid progenitor is produced.

Synthesis oflymphocytes from common lymphoid progenitors:

- After action of IL-2 and IL-7 Common lymphoid progenitors differentiated into T-lymphocytes in thymus.

- After action of IL-3, IL-5 and IL-7 Common lymphoid progenitors differentiated into B-lymphocytes in bone marrow.After encounter with antigen, B cells differentiate into antibody-secreting plasma cells.
- After action of IL-15 Common lymphoid progenitors differentiated into Natural killer cells.

Synthesis of erythrocyte and platelets:

- After action of IL-3, SCF and TPO Common myeloidprogenitors differentiated into Megakaryocyte, erythroid progenitors.
- Megakaryocyte, erythroid progenitors in presence of IL-11, TPO differentiated into platelets.
- Megakaryocyte, erythroid progenitors in presence of SCF differentiated into erythrocytes.

Synthesis of Granulocyte -Macrophages Progenitor:

- After action of GM-CSF Common myeloidprogenitors differentiated into granulocyte-Macrophages progenitors.

Synthesis of Granulocytes:

- Granulocyte-Macrophages progenitor on action of IL-11, TPO giving rise to myeloblasts.
- Myoloblast on further action of IL-3 ,GM-CSF and G-CSF giving rise to Basophil and mast cell.
- Myoloblast on further action of IL-3 ,GM-CSF and LI-5 giving rise to Eosinophil.
- Myoloblast on further action of G-CSF ,GM-CSF, IL-6 and SCF giving rise to Neutrophil.

Synthesis of monocytes macrophages:

- Granulocyte-Macrophages progenitor on action of GM-CSF and M-CSF giving rise to monocytes.
- Monocytes on action of G-CSF, IL-6 and SCF giving rise to macrophages.

2.1.3 Diagram of Haematopoiesis

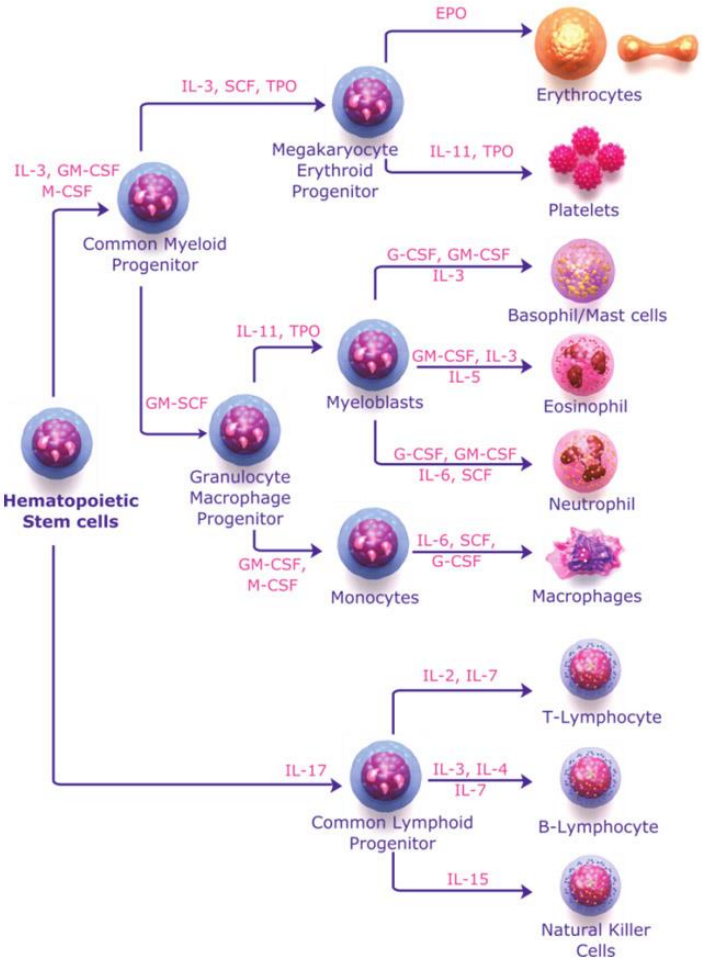


Figure: 2.1 Haemopoesis

2.2 CELLS OF IMMUNE SYSTEM:

The primary function of the immune system is to eliminate infectious agents that have breached the natural barriers. This is brought about by the integrated actions of different cells and molecules that, directly or indirectly, lead to recovery from infection. It is the effector cells and molecules that actually bring about the elimination of infectious agents.The different cells of the immune system can be classified according to overlapping principles. The most important classification is functional – what do they do? This relates to other aspects – their developmental origins and relationships, and their anatomical distribution in the body. Cells are also classified by their morphology, but this can be misleading – naive Band T cells look more or less the same under the light microscope– and it is more accurate to combine morphology with other approaches such as identification of the surface molecules that the cells express. Based on the these criteria we will now briefly introduce the major groups of cells involved in immune responses before discussing each in more detail. We will divide the cells into those primarily thought of as belonging to innate immunity and those of adaptive immunity, while emphasizing that in reality there is much overlap between the two groups.

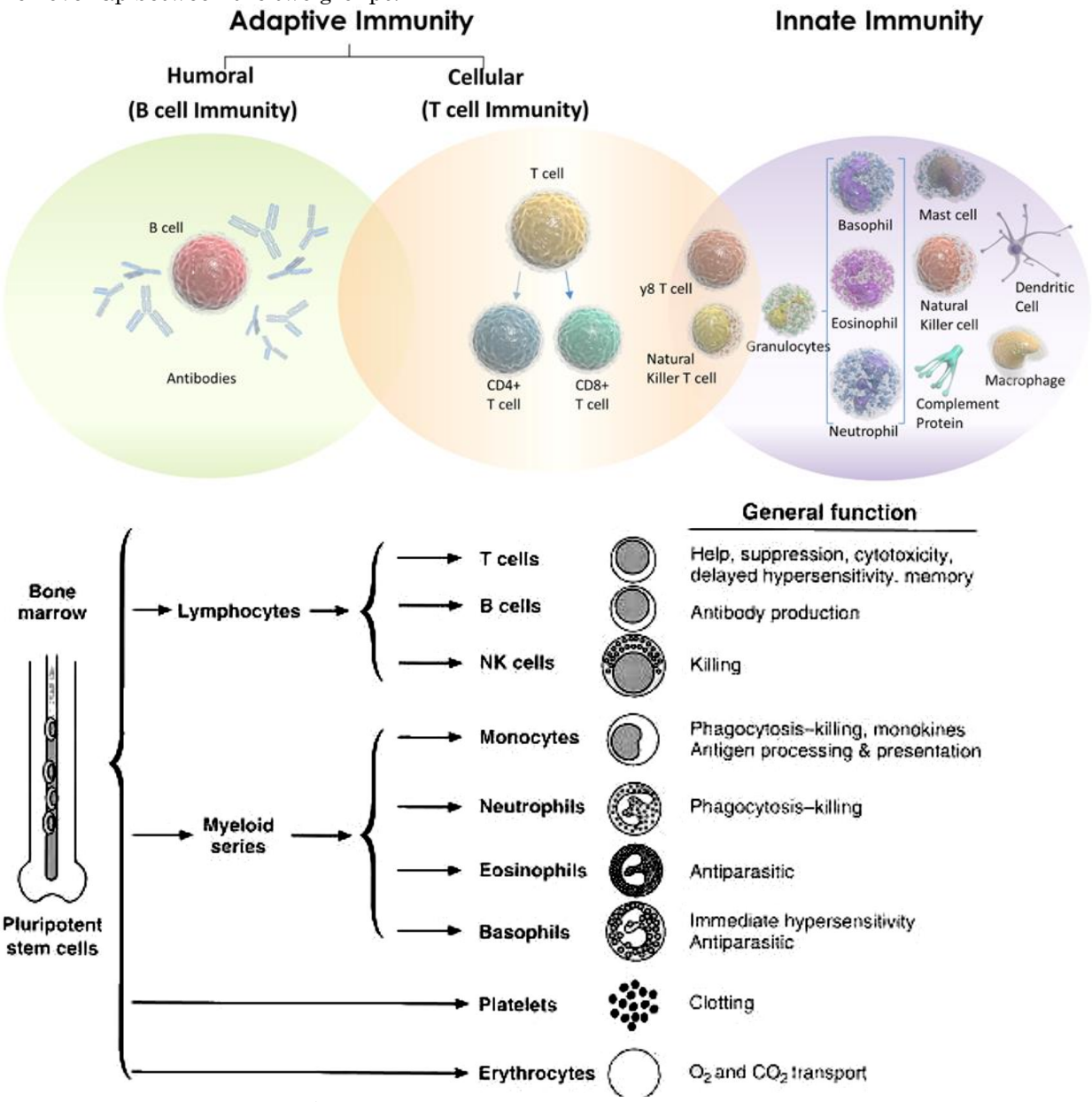


Figure: 2.2 Cells of Immune system

2.2.1 LYMPHOID CELLS (LYMPHOCYTES):

Lymphocytes are motile, non-phagocytic cells that cannot be distinguished morphologically. Based on their size lymphoid cells have been classified into two classes. These are

1. **Small lymphocytes:** Resting (non-dividing) lymphocytes, which are small (about 8 microns diameter), round shaped, having a large centrally located nucleus and a very thin rim of cytoplasm. They normally comprise from 20 - 40% of leukocytes in circulation. They become active, cycling cell only after interaction with an antigen or mitogen. After several rounds of cell division, lymphocytes differentiate into effector cells which act to eliminate or inactivate the invader.
2. **Large lymphocytes:** Other cells are important in the immune response primarily because of how they affect or are affected by lymphocytes and/or their products. Lymphocytes can be divided into two major like B – Lymphocyte and T- Lymphocyte and one minor Null cell functional subgroups. The major subgroups mature in different organs.

Small lymphocytes are categorized into two different classes

- B lymphocytes or B Cells
- T Lymhocytes or T cells

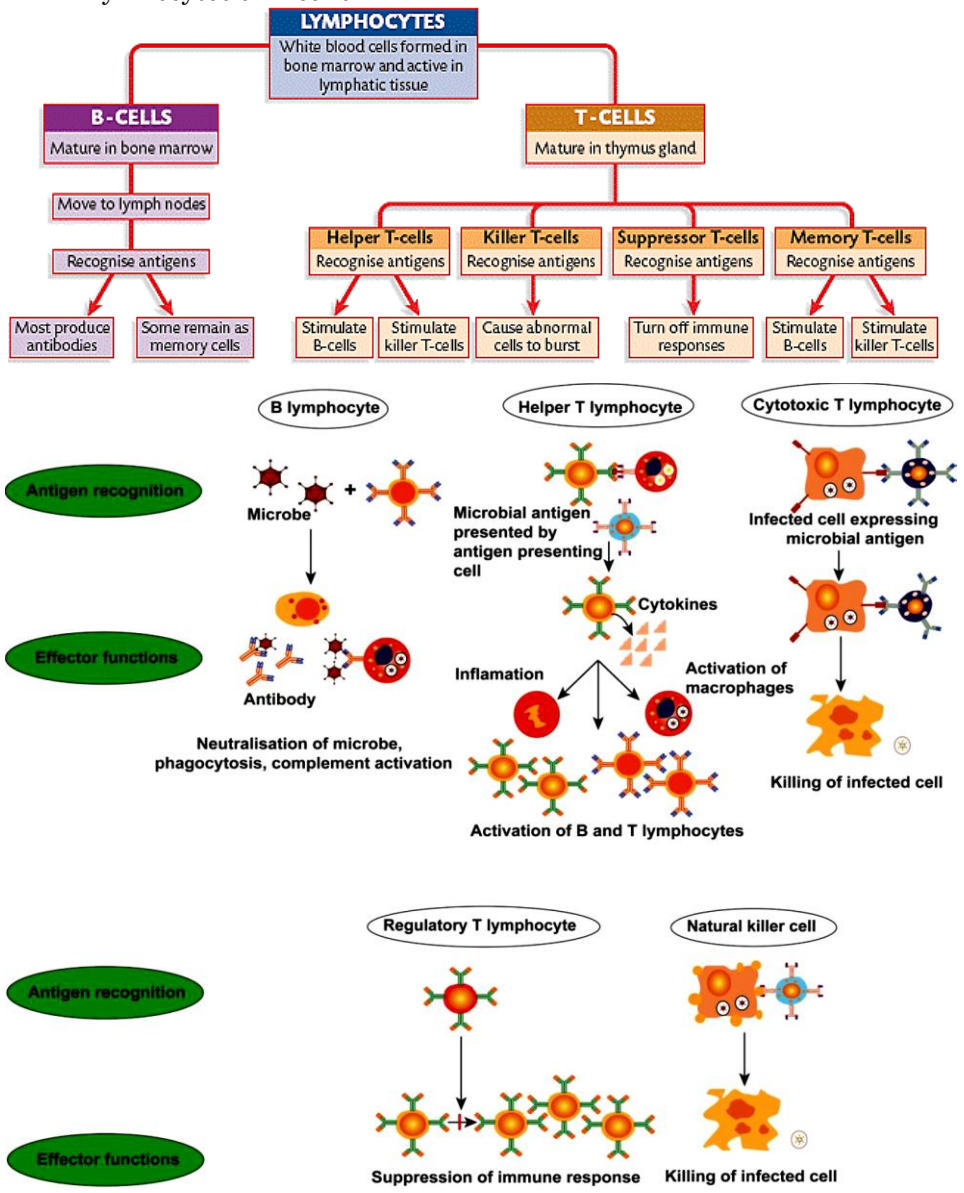


Figure: 2.3 Lymphocyte cell types

a. GENERAL CHARACTERS OF B CELL

- B cells develop from stem cells in the bursa of fabricus in bird and in bursa equivalent tissue (bone marrow) of mammals. At the youngest stages in the bone marrow, these cells bear antigen-specific surface antibody (IgM and IgD).
- A single B cell express approximately 1.5x10^{5}molecules of antibody with identical biding site for antigen.
- These B lymphocytes present in blood circulation, B cell areas of secondary lymphoid organs.
- It is important to know that each and every B cell has a different antibody on its surface.
- The B-cell antigen receptor (BCR) is a membrane-bound form of the antibody that the B cell will secrete after activation and differentiation to plasma cells. In the lymph nodes, naive B cells may encounter an antigen recognized by their surface antibodies.
- In addition, once a B cell makes surface IgM it can then make a different class of antibody which is known as class switching but all the antibodies made by that cell recognize the same antigen.
- These cells have short life span. (2-3 days).
- After encounter with extracellular pathogen B cell activated and differentiated into plasma cells and memory cells.
- Plasma cells are terminally differentiated cells having life span of 1-2 weeks and producing large quantity of pathogen specific soluble antibody.
- The memory cells maintains immunological memory for subsequent encounters of same pathogens. Memory cells has long life span.

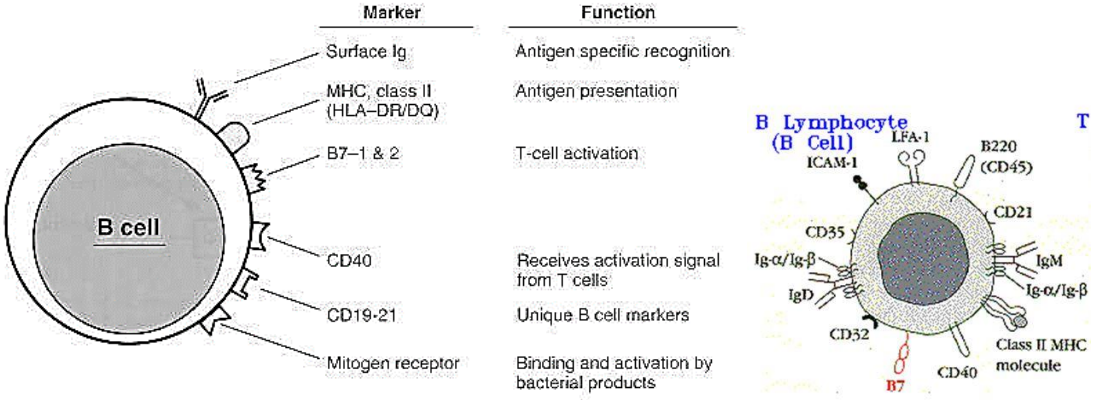


Figure: 2.4 Structure of B Cell

b. GENERAL CHARACTERS OF T CELL

T cells are so called because they develop, mature and proliferate in the thymus. The develop immune-competence in thymus but do not become functional. They become functional only when reach to T dependent areas of secondary lymphoid organs like lymph node, spleen etc. T cells bear TCR as an antigen receptor.

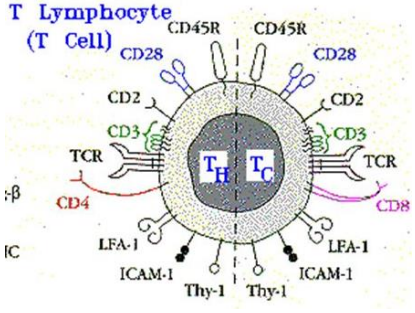


Figure: 2.5 Structure of T cell

Subsets of T Lymphocytes

On the basis of nature of TCR T lymphocytes are categorized into two main subsets. These are

1. TCR-1cells: Consist of γ and δ polypeptide
2. TCR-2 cells: Consist of α and β polypeptide

TCR-2 cells have two functional subsets of cells characterized by presence of surface marker molecule called as CD (Cluster of differentiation), that helps them to differentiate from one-another.These two classes are

- CD 4^+ cell or T_H cell
- CD 8^+ cell or T_C cell

The youngest T cells have a T cell receptor associated with a protein called CD3. In the thymus, the cells then develop both a CD4 and a CD8 marker. These cells are referred to as double positive. Eventually the cells lose either CD4 or CD8 to become one of the functional subsets. The cells with a CD4 marker are called helper T cells (T_H cells). The CD8 positive cells that develop are cytotoxic T cells (Tc cells). TH and Tc cells both have a TCR, but they perform very different functions in the immune system.

General characters of T_H cell

- The generation of both humoral and cell mediated immune response depends on the activation of T_H cells.
- Mature B cells that have already seen antigen require contact with a T cell in order to become plasma cells or memory cells (T-B cell interaction).
- The T cells provide signals to the B cell through contact of the TCR complex and MHC-antigen,
- In addition, the activated T cell produces cytokines such as IL-2 and IL4, 5, 6 or IFN α which stimulate B cell proliferation and differentiation into antibody secreting B cells.
- The type of cytokines produced by the T cells helps the plasma cells to produce different classes of antibodies.

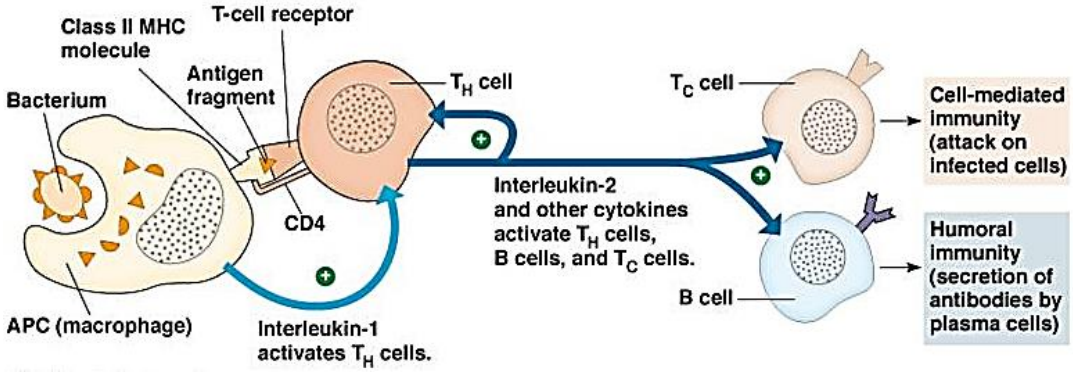


Figure: 2.6 Role of T_H Cell in Immunity

General characters of T_C cell

- Cytotoxic T cells (Tc) are derived from a lymphocyte stem cell matured in the thymus.
- These cells are characterized by the presence of the CD8 marker on their surface, and an antigen-specific T cell receptor which recognizes antigens in the context of MHC class I.
- The main role of the cytotoxic T cell, as the name suggests, is to kill other cells. The requirement for MHC class I on the target cell means that the Tc cell is very important in recognizing and destroying self-cells that have been altered or infected.
- The Tc cell must first become activated and mature into a cytotoxic T lymphocyte (CTL).
- Activation of the resting Tc cell is a two-step process.
- First, the TCR on the CD8+ cell must interact with an antigen-MHC (class I) on the surface of a target cell.
- Secondly, the CD8+ Tc cell must be stimulated by cytokines IL-2 especially. The IL-2 is probably supplied by activated T_H cells.
- Resting Tc does not express IL-2 receptors, but antigen stimulation increases the expression of IL-2 receptors on the surface on the Tc cell. This ensures that only the cells recognizing the antigen will become activated.
- **Cytotoxic T lymphocyte Activity:**Activated CD8+ T cells (CTL's) are very effective at destroying target cells, especially virus-infected cells and tumor cells. The killing happens in three steps:
  - Conjugate formation between the CTL and the target cell

- Membrane attack on the target cell
- Dissociation of the two cells and target cell death

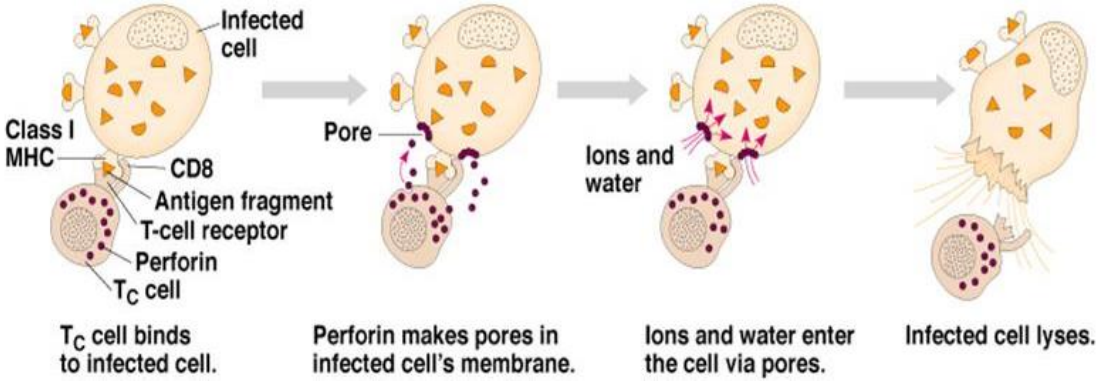


Figure: 2.7 Role of TC Cell in Immunity

General characters of Ts (Suppressor T) cell

- Another subpopulation of T cells (T suppressor cells or Ts cells) acts to inhibit T_H cells from function, thereby preventing the initiation of the response.
- They are CD4-8+ in contrast to T_H cells which are CD4+8-. Ts cells can also act directly on B cells or effector T cells to inhibit their activity, but this is probably less important than their inhibition of T_H cell activity. Some autoimmune diseases are thought to result from defects in Ts function.

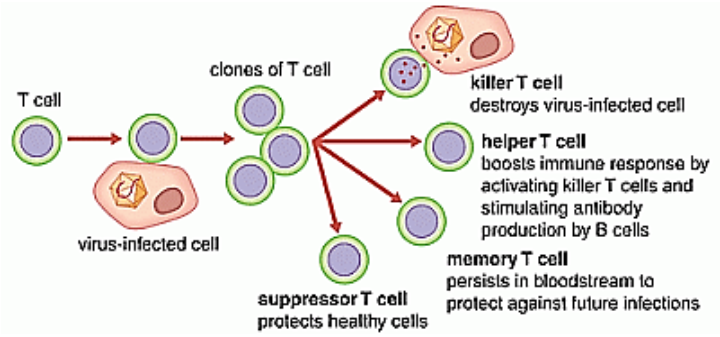


Figure: 2.8 Role of TS Cell in Immunity

General characters of T_{DTH}cell (Delayed type of hypersensitivity)

- The T helper cells (Th cells) are a type of T cell that play an important role in the immune system, particularly in the adaptive immune system.
- They help the activity of other immune cells by releasing T cell cytokines. These cells help suppress or regulate immune responses.

c. GENERAL CHARACTERS NULL CELL

- A small group of peripheral blood lymphocytes, called null cells, fail to express the membrane molecules that distinguish T and B cell lineages.
- These cells also fail to display antigen binding receptors of either the T or B cell lineage and therefore lack the attributes of immulogic specificity and memory.
- There are two sub populations of null cells namely Natural Killer cells (NK cells) and Killer cells (K cells).
- But in most of the books null cells referred as a single population of NK cell

General characters NK cell

- These cells are sometimes called large granular lymphocytes (LGL's).
- NK cells have some surface markers in common with T cells, and they are also functionally similar to cytotoxic T lymphocytes (CTL's).
- Like CTL's, NK cells are particularly important in the killing of cellular targets usually tumor cells.
- Unlike CTL's, however, the killing by NK cells is nonspecific; they do not need to recognize antigen/MHC on the target cell.

- NK cells do not have a T cell receptor and are not T cells. An NK cell kills a target cell by releasing perforin and other molecules which damages the target cell membrane leading to death. NK cells also cause death by inducing apoptosis in the target.
- The cytokine TNF alpha is released by the NK cells and may be involved in this process.
- They are able to recognize and kill some abnormal cells, for example some tumor cells and virus-infected cells, and are thought to be important in the innate immune defense against intracellular pathogens.
- The activation of NK cells basically depend upon the Killer cell Inhibitory Receptor (KIR) and Activation Receptor (AR). When both the receptors activated in the case of NK cell and normal cell interaction, there is no activation of NK cells where as activation of AR receptor alone in the case of NK cell and self altered or infected cell interaction results in the lysis of infected cell.

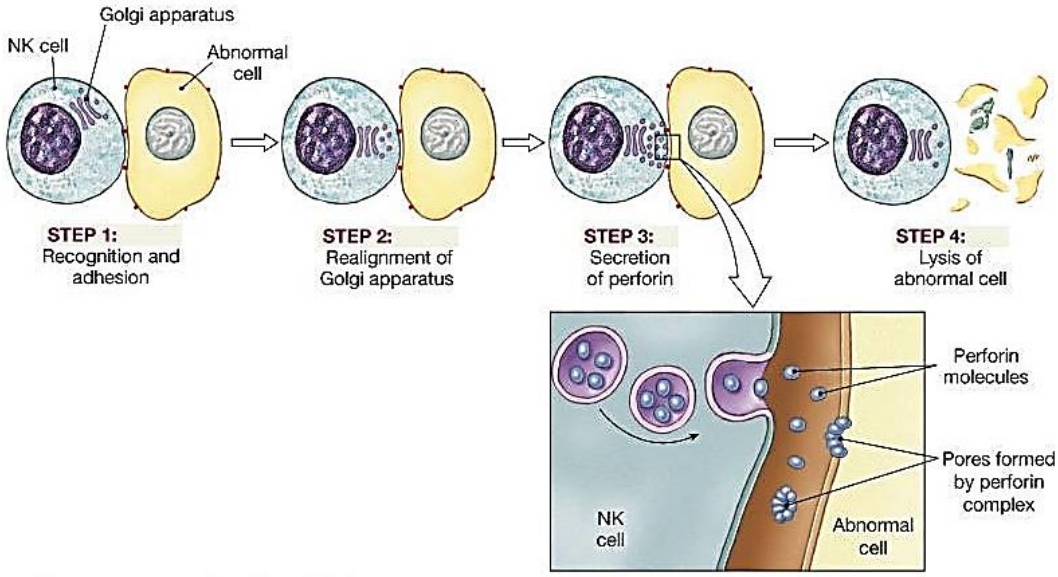


Figure: 2.8 Role of NK Cell in Immunity

General characters Killer cell (K cell)

- If NK-Cells possess Fc receptor for antibodies, then they kill the infecting agents specifically. They are referred as Antibody Dependent Cytotoxic Cells (ADCC). They are then called as Killer cells.

Table 2.2: Comparison of B cell and T cell

Characteristic	B Lymphocytes	T Lymphocytes
Site where processed	Bone marrow	Thymus
Type of immunity	Humoral (secretes antibodies)	Cell-mediated
Subpopulations	Memory cells and plasma cells	Cytotoxic (killer) T cells, helper cells, suppressor cells
Presence of surface antibodies	Yes—IgM or IgD	Not detectable
Receptors for antigens	Present—are surface antibodies	Present—are related to immunoglobulins
Life span	Short	Long
Tissue distribution	High in spleen, low in blood	High in blood and lymph
Percentage of blood lymphocytes	10%–15%	75%–80%
Transformed by antigens into	Plasma cells	Activated lymphocytes
Secretory product	Antibodies	Lymphokines
Immunity to viral infections	Enteroviruses, poliomyelitis	Most others
Immunity to bacterial infections	*Streptococcus, Staphylococcus,* many others	Tuberculosis, leprosy
Immunity to fungal infections	None known	Many
Immunity to parasitic infections	Trypanosomiasis, maybe to malaria	Most others

2.2.2 CELLS OF MYLOID LINEAGE:

a. Monocytes and Macrophages

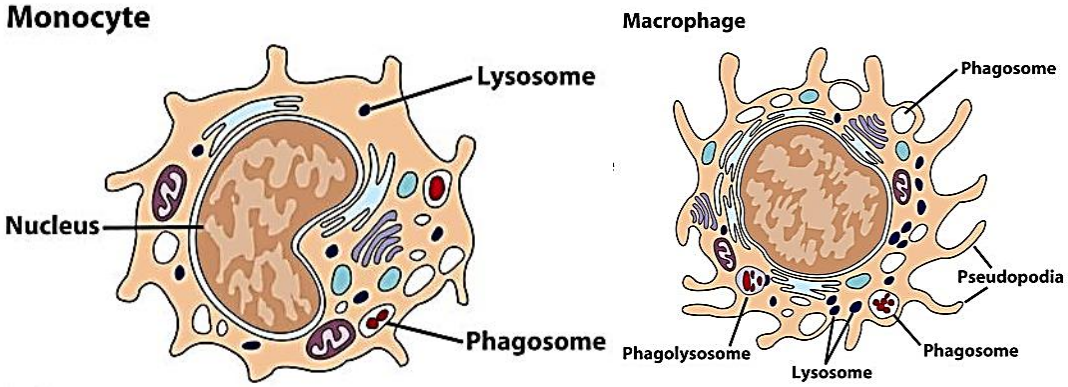


Figure: 2.8 Structure of Monocyte and macrophage

- Monocyte is agranulocytes circulate in the blood after leaving the bone marrow. Monocyte production and release from the bone marrow is increased during an immune response.
- Monocytes usually circulate in the blood for only a day or so before they enter the tissue to mature into macrophages.
- These fixed, resident macrophages play an important role in keeping the tissues clear of antigen and debris.
- When monocytes enter the tissues and become macrophages they undergo several changes.
- The cells enlarge and increase the amount of intracellular lysosome allowing greater phagocytosis.
- In the tissues, macrophages live for months or years and may be motile. Macrophages move with amoeboid movements using pseudopods.
- Macrophages are usually in the resting state unless activated during an immune response. Phagocytosis of antigens may also stimulate activation.
- Fixed macrophages serve different functions in different tissues and are named to reflect their tissue location like alveolar macrophage in the lung, Histiocytes in connective tissues, Kupffer cells in liver, Mesangial cells in the kidney and Microgilial cells in the brain.
- ***Role of Monocytes :*** Monocyte serve as antigen presenting cell
- ***Role of Macrophages:***
  1. Macrophage serve as antigen presenting cell as well as phagocytic cell.
  2. Activated macrophages have an important role in phagocytosis.
  3. Finally, macrophages have an important secretory role.
  4. After activation cells secrete factors that up-regulate the inflammatory response.

b. Neutrophils

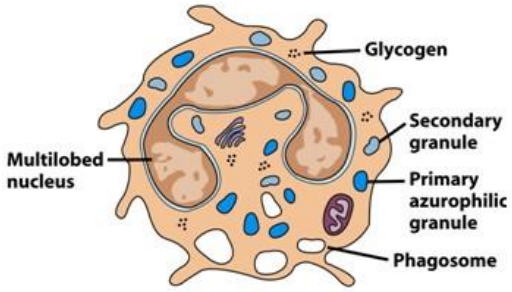


Figure: 2.9 Structure of Neutrophil

- Neutrophils are granulocytes, produced in the bone marrow from the granulocyte-monocyte progenitor cell. These cells have nucleus with multiple morphological forms so are often called polymorphonuclear cells (PMN's).
- It is constitute about 50 – 70 % of leucocyte population in blood.
- It consists of two types of granules. The larger, denser primary granules are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes. The smaller secondary granules contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules

fuse with phagosomes, whose contents are then digested and eliminated much as they are in macrophages.

- ***Role of Neutrophils:***
  1. The neutrophil's main role is in inflammation. They are the first cells to arrive at the site of inflammation.
  2. In the tissues, neutrophils are active phagocytic cells, like macrophages.
  3. Neutrophils, however, do not act as antigen presenting cells.
  4. Neutrophils are most effective phagocytic cell, killing ingested microorganisms by oxygen dependent or independent pathways.

c. Eosinophils

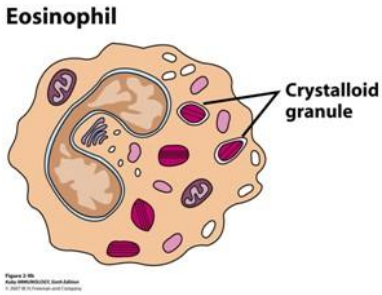


Figure: 2.10 Structure of Eosinophil

- Eosinophils are granulocytes stained staining with 'eosin'. Eosinophils make up about 1-3% of leukocytes in circulation.
- Eosinophils typically have a bilobed nucleus and contain many basic crystal granules in their cytoplasm.
- These basic granules take up eosin. The granules contain hydrolytic enzymes, peroxidase, and catalyze. About half of the material in the granules is major basic protein
- ***Role of eosinophil:***
  1. Eosinophils may help limit the inflammation induced by basophils and mast cells in that they contain histaminase.
  2. Eosinophil has basic proteins and aryl sulfatase. These compounds are toxic to parasitic worms (helminthes). So eosinophil eosinophils appear to be the major host defense against these helminthes. During parasitic infection a sharp rise in eosinophils reported. This condition is called eosinophilia.
  3. Eosinophilia may also be reported in allergic disease like asthma, hay fever etc.

d. Basophiles

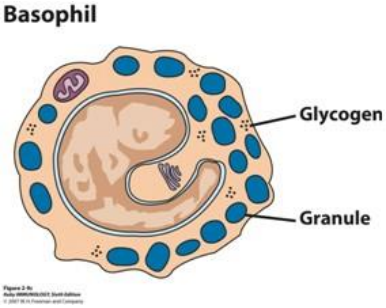


Figure: 2.11 Structure of Basophil

- Basophils are granulocytes found in circulation where they comprise about 0.2% of the leukocytes. They are rounded cells about 8-10 in diameter. They have an elongated nucleus usually with two constrictions which is sometimes folded into an S shape.
- Basophiles contain large numbers of cytoplasmic granules that take up basic dyes.They are so numerous that they obscure other cell organelles.
- These granules contain large amounts of heparin and eosinophil chemotactic factor of anaphylaxis (ECF-A) and lesser amounts of histamine, serotonin, and precursors of prostaglandins and leukotrienes.
- Basophils and mast cells have receptors for both C3a and C5a and for the Fc piece of IgE.

- ***Role of basophil:*** Basophil involve in allergic reaction

e. Mast cells

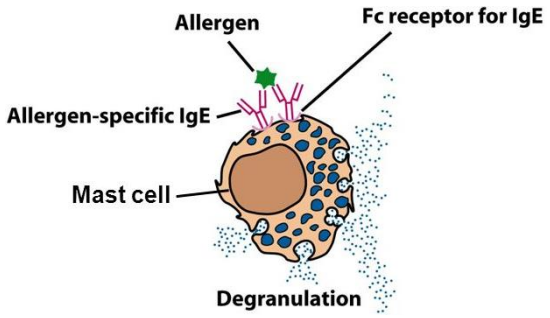


Figure: 2.12 Structure of Mast cell

- Mast cells are often elongated. Both mast cells and basophils contain large numbers of cytoplasmic granules that take up basic dyes. They are so numerous that they obscure other cell organelles.
- These granules contain large amounts of heparin and eosinophil chemotactic factor of anaphylaxis (ECF-A) and lesser amounts of histamine, serotonin, and precursors of prostaglandins and leukotrienes.
- Mast cellsalso have receptors for both C3a and C5a and for the Fc piece of IgE.
- Binding of C3a and C5a or cross-linking of membrane bound IgE by allergens induces release 60% - 80% of the granules.
- Mast cells are formed in the tissue from undifferentiated precursor cells released into the blood from the bone marrow.There are found either in the connective tissues or in mucosal sites.
- Both types contain numerous granules with preformed mediators which can be released from mast cells after stimulation.
- The preformed mediators include histamine and other pharmacologically active substances.
- Stimulation also results in the production of newly formed mediators by mast cells such as prostaglandins and leukotrienes.
- **Role of Mast cell:**
  1. Produce factors cause contraction of endothelial cells and vasodilation of capillaries resulting in the redness, warmth and fluid accumulation in tissues characteristic of inflammation. Systemic release can cause anaphylaxis.
  2. Release of ECF-A attracts eosinophils to the area which seems to be of particular importance in combating parasitic infestations.
  3. They have a similar importance in allergic reactions and are only found in tissues.

f. Dendritic cells

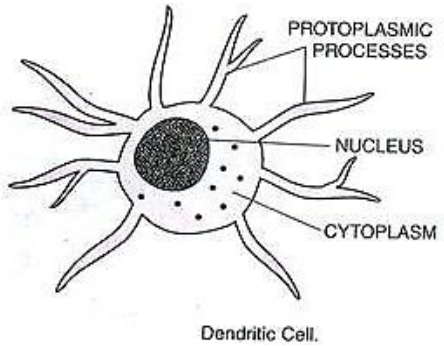


Figure: 2.13 Structure of Dendritic cell

- The name dendritic cell given to this cell because it is covered with a maze of long membrane processes resembling dendrites of nerve cells. Most dendritic cells are process and present antigen to T_H cells. All of these dendritic cells express high levels of both class II MHC molecules and the co-stimulatory B7 molecule.

- These cells can be classified based on their location.
- ***Langerhans cells*** found in the epidermis and mucous membrane. ***Interstitial dendritic cells*** which populate most organs like heart, lungs, liver, kidney and gastrointestinal tract.
- ***Circulating dendritic cells*** including those in the blood and those in the lymph which is otherwise known as veiled cells.
- ***Follicular dendritic cells (FDCs)*** are cells with membranous projections present in the germinal centers of lymphoid follicles in the lymph node, spleen and mucosal lymphoid tissues. FDCs do not express class II MHC molecules and therefore do not function as antigen presenting cells for TH cell activation
- **Role:**
  1. So they are more potent antigen presenting cells than macrophages and B cells.
  2. After capturing antigen in the tissues by phagocytosis or by endocytosis, dendritic cells migrate into the blood or lymph and circulate to various lymphoid organs where they present the antigen to T-lymphocytes.

2.2.3 CELLS OF ERYTHROID LINEAGE:

a. Platelets:

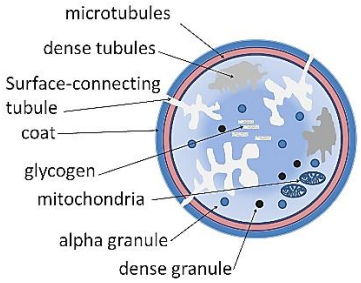


Figure: 2.14 Structure of Platelets

- These are nonnucleated cells of about 3 micron in diameter. They are also spherical and colorless. They are produced from stem cells of bone marrow.
- They have low affinity receptors for Ig E and Ig G and MHC – I molecules on its surface.
- They play vital role in blood clotting and inflammation. The clotting itself prevents invasion of pathogens through walling off reaction.
- They also contain granules which secrete histamine and serotonin and their release from the platelets contribute to immediate hypersensitivity reactions.

b. Erythrocytes (RBCS):

Erythrocytes plays vital role in oxygen transport but it also possess important immunological role. Erythrocytes removes antigen – antibody complex (immune complex) from circulation.

2.3 SPECIFIC IMMUNITY (adaptive immunity)

A Collective and coordinated response given by host body against invaded pathogen specifically against invaded pathogen, mediated by the cells and molecules of the immune system is referred as immune response. This specific immune response differ according to nature of invaded pathogen. Humoral immunity is a specific defense shown by host body against invaded extracellular pathogens. In humoral immunity B cell is a main effector cell and Antibody is a main effector molecule. Cell mediated immunity is a specific defense shown by host body against invaded Intracellular pathogens. In CMI, cytotoxic T Cell is a main effector cell.

Specific defense has following features

1. It is specific in nature
2. Immunological memory is developed.

Different stages of adaptive immune response

3. Step 1 Capture and display of antigens
4. Step 2 Recognition of antigen by lymphocytes

5. Step 3 Activation of T lymphocytes
6. Step 4 Activation of B lymphocytes
7. Step 5 Production of memory cells

Capture and display of antigens: Antigen presenting cells (APC) display the antigens to the B cell or CD4+ T cells to activate the antibody mediated (humoral) immune response and CD8+ T cells to activate cell mediated immune response. The APC contain MHC molecules that helps in the display of antigen to the cells of immune system.

Recognition of antigen by lymphocytes: Lymphocyte specific for an antigen is activated upon encountering with APCloaded with an antigen. The mechanism of activation of specific lymphocyte clone is explained by **clonal selection theory**.

Activation of T lymphocytes: Activated T helper cells proliferate and differentiate into effector cells with the help of cytokines. Interleukin-2, secreted by T-helper cells modulate the clonal expansion of activated T lymphocytes. Activated cytotoxic T cells kill the intracellular pathogens in the cytoplasm of infected cells.

Activation of B lymphocytes:B cells are activated with the help of CD4+ T lymphocyte and differentiated into antibody secreting plasma cells. Antibodies bind and prevent the pathogens, thus "neutralizing" the pathogens and block their ability to infect host cells.

Production of memory cells: Initial activation of T lymphocyte produces the long-lived memory cells that survive for many days following infection. Memory cells respond much faster than the naïve lymphocytes. Generations of long lasting memory cells are the major target for vaccine design against microbial pathogens.

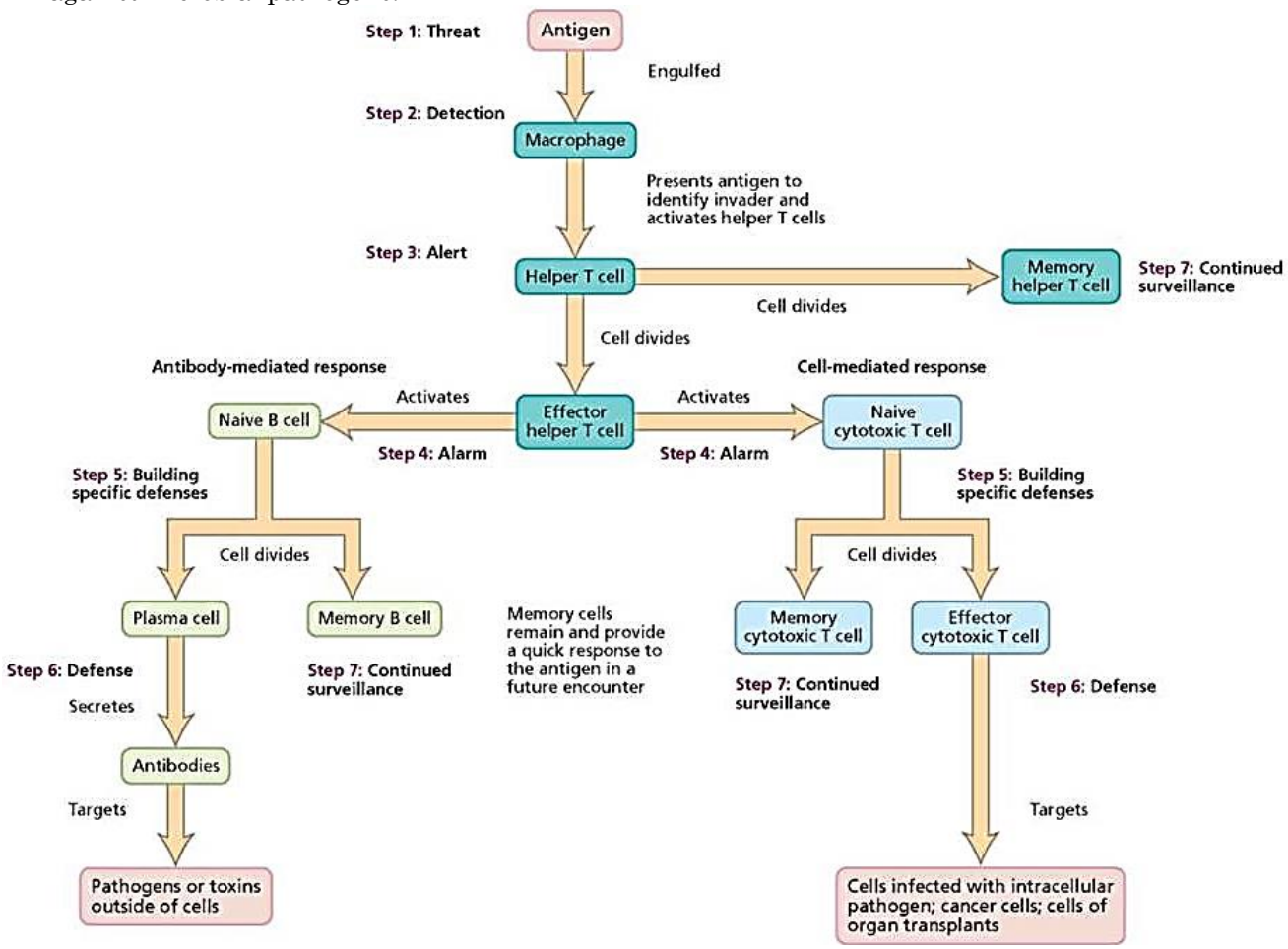


Figure: 2.15 Overview of Immune responses

2.3.1 Humoral immunity or Antibody mediated Immunity

Humoral immunity is a category of specific defense shown by host body against extracellular pathogens (microbes and microbial exotoxin), which is mediated by secreted antibodies and its physiological function.

The term humoral is derived from the Latin humour meaning "body fluid", thus humoral immunity refers to immunity that can be conferred on a non-immune individual by administration of serum antibodies from an immune individual.

In humoral immunity main effector cell is B lymphocyte and main effector molecule is antibody. So this immunity is also called as **Antibody mediated immunity**. Humoral immunity can be transferred to other individuals by the transfer of serum (antibodies).

Humoral immunity is the principal defense mechanism against extracellular microbes and their toxins because secreted antibodies can bind to these microbes and toxins and assist in their elimination.

- The humoral immune response is initiated by an **activation phase**. This is where macrophages (white blood cells) engulf and digest microbes (including their antigens) through a process of called phagocytosis.
- Some of the digested antigens are then displayed on the surfaces of the macrophages (called **epitopes**). This display provides other cells of the immune system with an opportunity to recognize the invader and become activated. This is called **antigen presentation.**
- During antigen presentation the macrophage selects T-helper cells and B-cells that have membrane receptors that are complementary in shape to the antigens exposed. This is known as **clonal selection.**
- T-helper cells recognize and bind to the displayed antigens. This then initiates the next phase of the humoral response (B and T cells).
- In the next phase, called the **effector phase**, activated T_h cells trigger specific B-cells to proliferate and release antibodies.
- These antibodies bind to the invader and fight infection.
- The effector phase involves specific lymphocytes (white blood cells) that mature in the bone marrow. These are called B lymphocytes (B-cells). B-cells can producea **specific antibody** in response to a particular antigen. An **antibody** is a type of globular protein that reacts with a specific antigen.
- When a B cell meets an antigen it will divide through mitosis and after several generations will differentiate into **plasma cells.** All plasma cells are formed from one type of B cell and will secrete the same antibody.
- Plasma B-cells can synthesize and secrete up to 2000 antibody molecules per second. The antibodies produced circulate in the blood and lymph or secrete antibodies onto the surfaces of mucous membranes, such as those found lining the lungs.
- Antibodies produced from plasma cells eliminate antigens.
- Whereas memory lymphocytes take up residence in normal tissues in preparation for next or future infection.

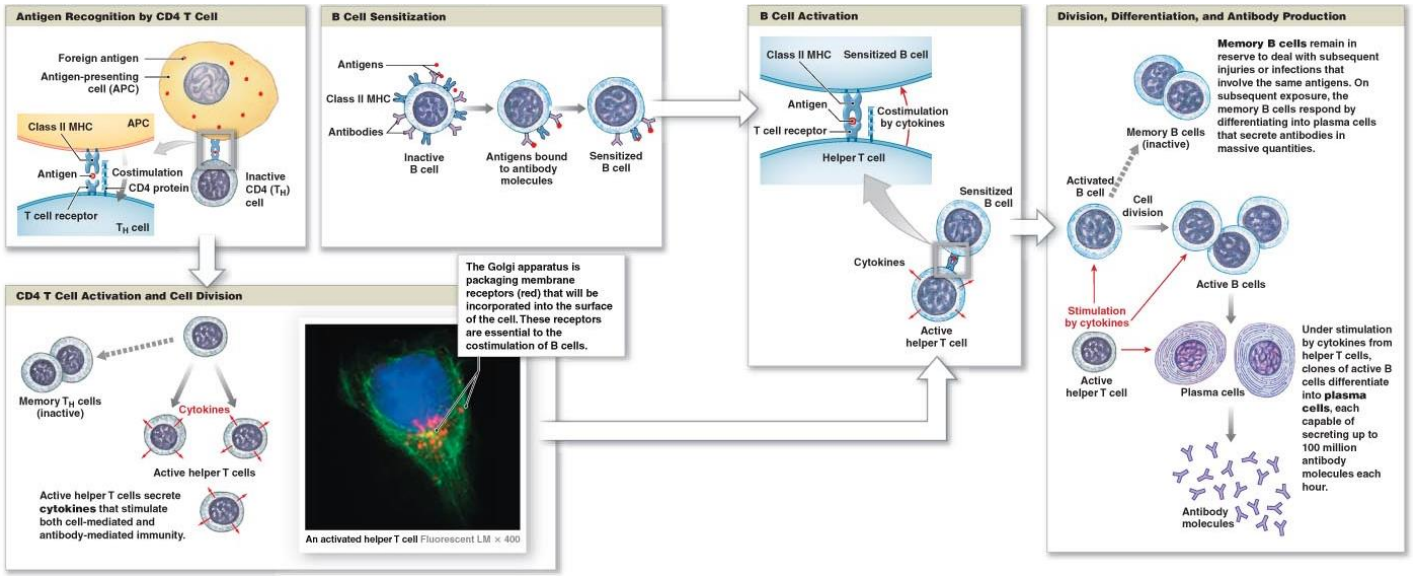


Figure: 2.16 Overview of Humoral Immunity

2.3.2 Primary and secondary immune response

Immune response is of two types namely Primary Immune Response and Secondary Immune Response.

A. **Primary Immune Response:** An adaptive immune response that occurs upon the first exposure of native lymphocytes with a foreign antigen is known as primary immune response. Primary responses are characterized by relatively slow kinetics and small magnitude compared with the response after a second or subsequent exposure. It has lag phase, log phase, effector phase and decline phase.

The initial phase of primary response has a lag of 5 – 7 days before antibody levels to start to rise. This lag time is the time required for activation of naïve B-cells by antigen and T_H cells and in log phase, subsequent proliferation and differentiation of the activated B cells into antibody secreting plasma cells and memory B cells occurs.

Antibody levels peak in the primary immune response at about day 14 and then begin to drop off as the plasma cells begin to die.

B. **Secondary Immune Response:** An adaptive immune response that occurs upon the second or subsequent encounter of primed lymphocytes with a given antigen is known as secondary immune response. A secondary response is characterized by more rapid kinetics and greater magnitude relative to the primary immune response which occurs on first encounter.

It has log phase, effector phase and decline phase. Absence of lag phase is significant in secondary immune response. Secondary immune response proves that immune system remembers the first encounter and is said to have memory.

In the secondary immune response, the lag is much shorter or even absent (1 – 2 days) and antibody levels are much higher and are sustained for much longer time. The secondary immune response reflects the response of the clonally expanded population of memory B cells.

These memory B cells respond to antigen more rapidly than naïve B cells. In addition, because there are many more memory cells than naïve B cells, larger number of plasma cells are generated during the secondary immune response and antibody levels are consequently 100 fold or 1000 fold higher

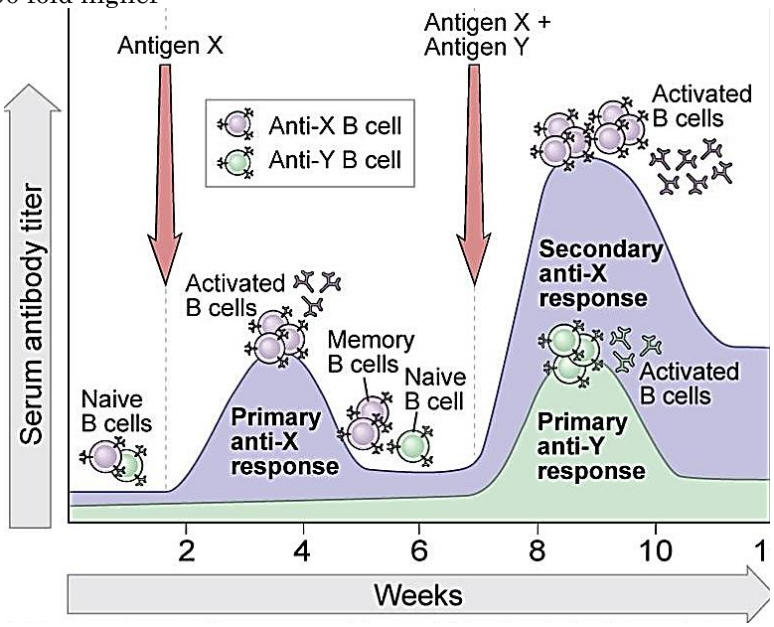


Figure: 2.17 Primary and secondary immune response

Example: Most of us were first exposed to the varicella-zoaster virus during childhood and came down with chickenpox. After this exposure we became immune to chickenpox, so subsequent exposure to the varicella-zoaster virus no longer results in illness. This immunologic memory or maintenance of the acquired immunity is often lifelong. The memory is specific for the original invader, in this case the varicella-zoaster virus and does not extend to unrelated invaders such as other viruses.

Table 2.3: Difference between Primary and secondary immune response

Sr. No	Features	Primary Immune Response	Secondary Immune Response
1	Time Lag after Immunization	5 – 7 days	1 – 2 days
2	Peak Response	Smaller	Larger
3	Antibody Isotype	Ig M > Ig G	Ig G, Ig A or Ig E
4	Antibody Affinity	Lower average affinity	Higher average affinity
5	Induced by	All immunogens	Only protein antigens
6	Required Immunization	High dose, Adjuvant needed	Low dose, Adjuvant not necessary

2.3.3 Regulation of immune response

A. Antibody-feedback: The simultaneous engagement of B cell immunoglobulin and Fcγ receptors by antigen-antibody complexes inhibit B cell activation. It is called antibody-feedback since the secreted IgG antibodies itself down regulates antibody production.

The simultaneous binding of the antigen-antibody complex to the surface Ig receptor as well as Fcγ receptor results in blocking of signals needed for B cell activation.

The lymphocytes are one of the classes of white blood cells, capable of responding to antigens in specific host defense mechanism. Under the microscope, all lymphocytes look like, differing only in size and other physiologically variable features, yet under this surface of morphological homogeneity, there is a hidden homogeneity.

B. Clonal selection: The theory was put forth first by **Neils Jerne** and explained further by **Burnet**. This theory says that antigen specific clones of lymphocytes exist even before the exposure of antigens and a large number of clones are generated during lymphocytic maturation to diversify the recognition of microbial antigens.

- Each newly generated lymphocyte expresses a single type of receptor on its surface. This receptor (antibody or TCR) has unique binding specificity, generated at random.
- When a lymphocyte receptors bind with complementary specific antigen, it triggers proliferation (clonal expansion) and differentiation of lymphocytes.
- The cells produced by clonal expansion have antigenic specificities identical to that of the parental cell.
- Newly generated cells carrying self-reactive specificities are deleted.
- A molecule that elicits antibody response is called and immunogen. The first injection of innumogen is followed after 2-4 weeks by the appearance of specific antibodies (primary response). A subsequent injection to the same immunogen is followed by a more rapid, specific response of higher titer than before (secondary response).
- The lag time represents a period of clonal expansion and differentiation of B cells to antibody secreting cells, called plasma cells.
- The rapidity, intensity and specificity of the secondary response (immunologic memory) are a direct result of the proliferation and differentiation of antigen specific B cell clones.
- There are five general classes of antibodies (IgM, D, G, A and E) ranging in size from 1,000,000 MW to 150,000 MW.
- All antibodies are constructed from a general, four polypeptide unit consisting of two heavy chains and two light chains. Each class of antibody has a distinctive heavy chain but there are only two types of light chains, κ and λ.
- Igs are comprised of variable regions and constant regions. The variable regions carry out recognition of foreign molecules. The constant regions engage various effector functions that eventually dispose of the bound material.
- ***A central tenet of the clonal selection theory is the existence of a generator of antigen receptor diversity.***

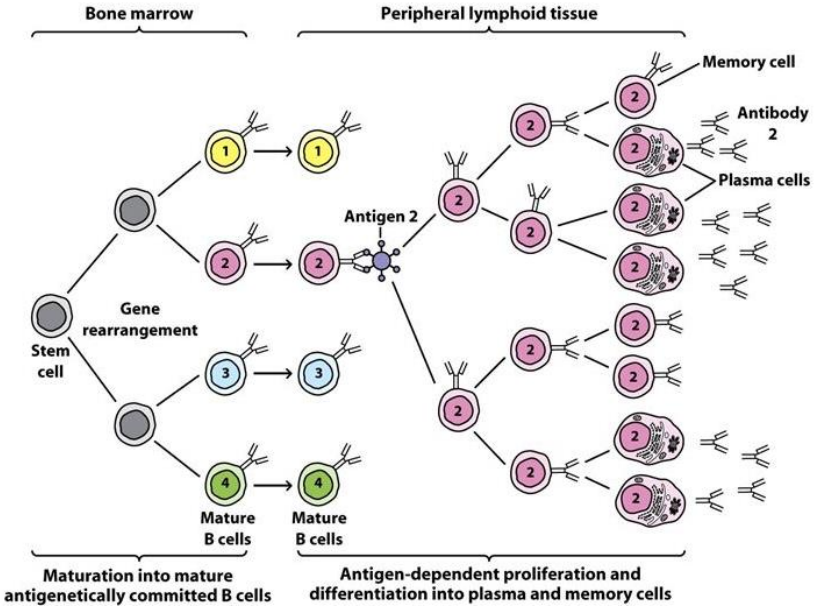


Figure: 2.18 clonal section

C. Clonal deletion:(immune tolerance)

Immunologic tolerance is the unresponsiveness of the adaptive immunity to antigens, as a result of inactivation or death of antigen specific lymphocytes, induced by exposure to antigens. Tolerance to self antigens is normal feature of the adaptive immune system, but tolerance to foreign antigens may be induced under certain conditions like hypersensitivity or transplantations.

An antigen which induces immune tolerance is known as tolerogens to distinguish from immunogens, which generate immunity.

The first example of immunological tolerance was the observation by Owen of erythrocyte chimerism in dizygotic cattle twins. Any antigen that comes into contact with the immunological system during embryonic life would be recognized as a self antigen and would not induce any immune response.

Self-tolerance: Immunological tolerance against self antigen was known as self-tolerance. Self-tolerance may be induced in generative lymphoid organs as a sequence of immature self-reactive lymphocytes recognizing self antigens, called central tolerance or in peripheral sites as a result of mature self-reactive lymphocytes encountering self-antigens under particular conditions, called peripheral tolerance.

As a result, different clones of immature lymphocytes may express receptors capable of recognizing various foreign antigens as well as self antigens. Potentially immunogenic self antigens are present on the cells and in the circulation of every individual. Individual lymphocytes may have free access to many of these self antigens yet they normal do not mount immune responses against the antigens. This is because of self tolerance.

It is due to self/non-self discrimination i.e the ability of the normal immune to recognize and respond to foreign antigen but not to self antigen. Failure of self tolerance results in immune reactions against self antigens. Such reactions are called as autoimmunity and the disease they cause are called as autoimmune disorders.

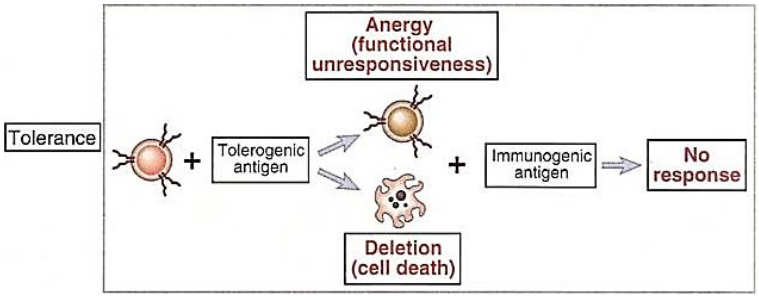


Figure: 2.19 Immune tolerance

Mechanism:

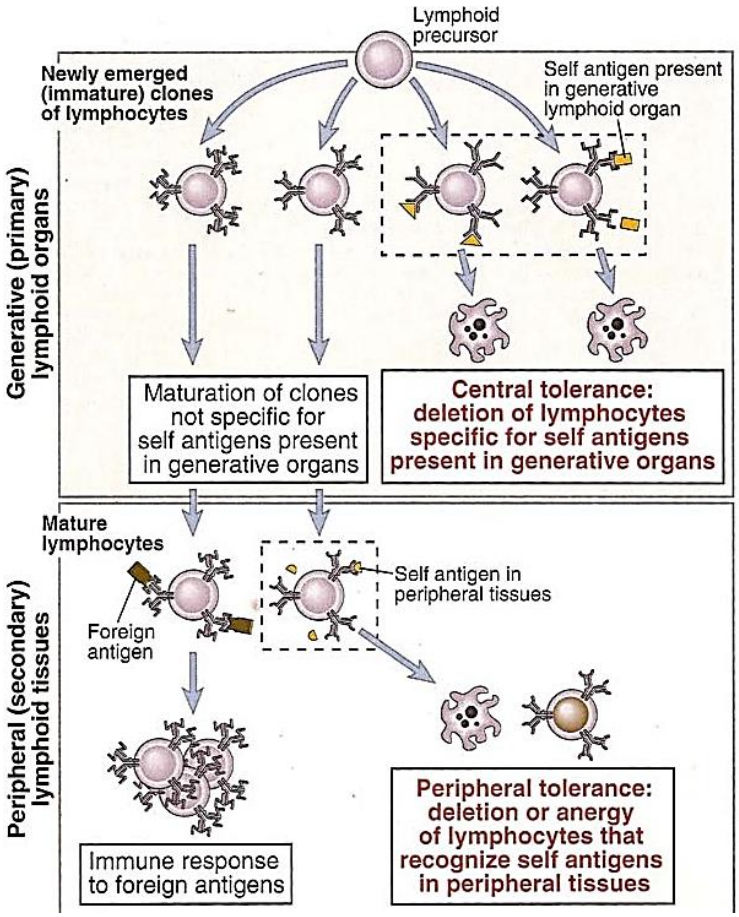


Figure: 2.20 Mechanism of Immune tolerance

Self-tolerance is of two types depending upon their place of occurrence namely central tolerance and peripheral tolerance.Central tolerance occurs during their maturation in the generative lymphoid organs. All lymphocytes pass through a stage which encounter with antigen leads to tolerance rather than activation. Usually only self antigens were available in high concentrations in generative lymphoid organs.

Therefore, in the generative lymphoid organs, immature lymphocytes normally encounter only self antigens at high concentration and clones of lymphocytes whose receptors recognize these self antigens with high affinity are killed. This process is called as **negative selection.** This eliminates self reactive lymphocytes. Mostly T-cells obtain central tolerance.

Peripheral tolerance occurs as a result of self-reactive lymphocytes encountering self antigens under particular conditions. It is induced when mature lymphocytes recognize antigens without adequate levels of the costimulators that are required for activation or as a result of persistent and repeated stimulation by self antigens in peripheral tissues. It is the most important for maintaining unresponsiveness to self antigens that are expressed in peripheral tissues and not in generative lymphoid organs. Mostly B-cells obtain peripheral tolerance.

The principal mechanisms of lymphocyte tolerance are apoptotic cell death, called as clonal deletion; functional inactivation without cell death called clonal anergy; and suppression of lymphocytes activation and effector functions by regulatory lymphocytes. Central tolerance is mainly due to deletion, whereas all three mechanisms contribute to peripheral tolerance.

Depending upon the cells involved in Tolerance, it is classified into T-cell tolerance and B-cell tolerance.

D. Class switching:

1. The first Ab class to be expressed during B cell development is IgM. These cells later give rise to mature, virgin B cells that express both IgM and IgD on their surfaces; on any given cell, these molecules have identical V region sequences and therefore identical binding specificities.
2. Later, after exposure to antigen, IgM+, IgD+ cells usually undergo further differentiation and begin to produce other classes of Ig; these new Ig classes nevertheless contain the same variable domain expressed by the parental cell.

2.3.4 Effector functions of Antibody

Although Ag specificity of Ab is determined by the variable regions, effector function is largely determined by the heavy chain constant regions.

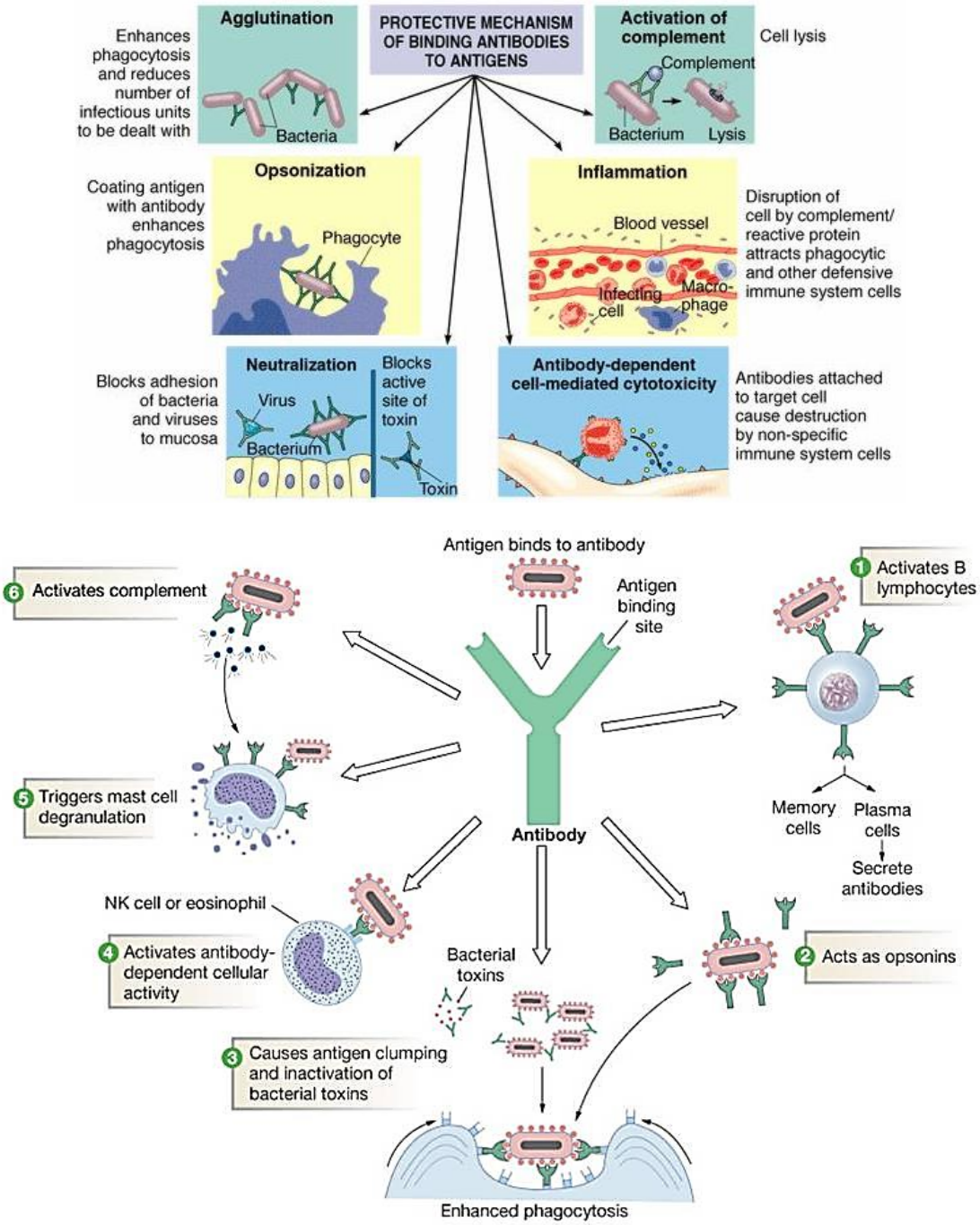


Figure: 2.21 functions of antibody in immunological reaction

A. **Mucosal immunity:** Antibodies that bathe the mucosal surface are synthesized locally and are not simply transported from the blood stream.

B. **Placental transport:** IgG antibodies are actively transported across the placenta

C. ***Virus neutralization:*** HA (rod shaped) and NA (mushroom shaped) are distinct glycoproteins that extend from the surface of viral envelope of influenza and aid in viral attachment to host cell. After a virus bind to the cell, it is uncoated.

Antibodies can neutralize viruses by:

- Inhibition of binding to target cells: Polymeric IgM can physically coat the surface of the virus and thereby block attachment to target cells.
- Blocking of attachment does not entirely explain the anti-viral effect of Ab . IgA can prevent infection at concentration of Ab that are too low to inhibit binding.
- Antibody can inhibit fusion with endocytic vesicle that is necessaryfor viral un-coating and translocation of viral nucleic acid into the nucleus of the infected cell.

D. ***Toxin neutralization:*** By analogous mechanism, high affinity IgA and IgG bind bacterial toxins and animal venoms, thereby preventing them from binding or entering host cell where they would otherwise interfere with cellular metabolism.

E. ***Opsonization:*** The rate of binding of phagocytes and subsequent ingestion is increased by an order of magnitude or more if IgG is bound to the polysaccharide capsule.

F. ***Complement activation:*** The complement system is composed of a group of plasma proteins that interact in a cascade. There are two pathways for initiation of this cascade: the classical pathway is activated by Ab-Ag complexes.

G. ***Activation of mast cells (allergy)*** . Its effector function depends on ability to bind to mast cells and basophils. These are highly specialized leukocytes whose cytoplasm contains large numbers of granules, making up as much as 50% of total cell volume.

H. ***Antibody-dependent cell-mediated cytoxicity (ADCC):*** NK cells are large granular non-B, non-T lymphocytes that have Fcγ receptors. NK cells are thus able to bind IgG fixed on the surface of a host cell infected by a viruses or the surface of a tumor cell.

2.3.5 T-cell dependent antibody response.

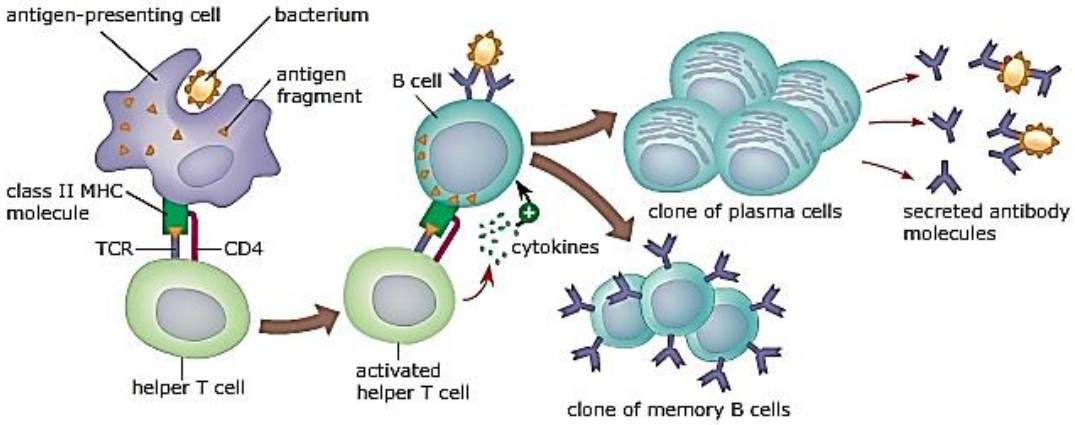


Figure: 2.22 T-cell dependent immune response

- Antibody response to protein antigen requires participation of both T cells and B cells.
- Those antigens which require participation of T cells for immune response are called T-dependent
- Since the CD_4 T lymphocytes stimulate B cells, they are called helper T cells.
- Helper T cell-dependent humoral immune responses to protein antigens generate antibodies of high affinity. This is because helper T cells, which recognize protein antigens, provide signals to B cells to produce high affinity antibodies.
- In contrast, antibodies to T-independent antigens are mainly of low-affinity.
- Antibody responses to T-independent antigens are simple and mainly consist of IgG and IgM
- Whereas helper T-cell dependent humoral response to protein antigen are highly specialized and consists of immunoglobulins of different classes and subclasses.

- Antibody responses to protein antigens require recognition of antigen by the helper T cells and co-operation between the antigen-specific B cells and T lymphocytes.
- The interaction between helper T cells and B cell sequentially involves antigen presentation by B cells to differentiated T cells, activation of helper T cells and expression of membrane and secreted molecules by the helper T cells that bind to and activate the B cells.
- The net result is the stimulation of B cell clonal expansion, isotype switching, affinity maturation and differentiation into memory cells.

Mechanism of T Dependent immunity:

- Antigen-specific B cells bind to native antigen to surface Ig receptors, internalize (receptor mediated endocytosis) and process it in endosomal vesicles.
- The peptide fragment of the antigen is then presented along with MHC class II proteins on their surfaces.
- The antibodies that are subsequently formed are specific to conformational determinants of the antigen.
- A single B cell may bind and endocytose a protein and present multiple different peptides complexes with MHC class II proteins to different T cells, but the resultant antibody response remains specific for the native protein.
- Antigen binding to membrane Ig enhances the expression of co-stimulators on the surface. As the internalized antigen is being processed, the B cell also expresses B7-1 and B7-2.
- Helper T cell that recognizes MHC-peptide complex on B cell also binds to B7 molecule with its CD28 and gets stimulated to proliferate.
- Once activated by antigen recognition and co-stimulation, T cells express a surface molecule CD40L that binds to CD40 on the B cell surface.
- This engagement results in initiation of enzyme cascades that leads to transcription of several genes. Engagement of B cell CD40 to helper T cell CD40L also leads to enhanced expression of B7 molecules on B cell, resulting in more T cell activation.
- Antigen recognition byB cells enhances the expression of receptors for cytokines.
- Activated helper T cell secretes cytokines that stimulate B cell proliferation. All the stimuli that B cell receives activate transcription of immunoglobulin genes. Some of the B cells that have proliferated differentiate into effector cells that actively secrete antibodies.

2.3.6 T-cell independent antibody response.

Many non-protein antigens such as polysaccharides and lipids stimulate antibody production in the absence of helper T cells, and these antigens are called T-independent antigens. Important TI antigens include polysaccharides, glycolipids, and nucleic acids. These antigens are not processed and presented along with MHC proteins and hence cannot be recognized by helper T cells. Most TI antigens are polyvalent, being composed of multiple identical epitopes. Such polyvalent antigens may induce cross-linking of surface Ig molecules on B cell. This leads to activation of B cell without the requirement of helper T cell.TI antigens are classifies into two types, TI-2 antigens are polysaccharides, glycolipids, and nucleic acids where as TI-1 antigen is lipopolysaccharide (LPS).

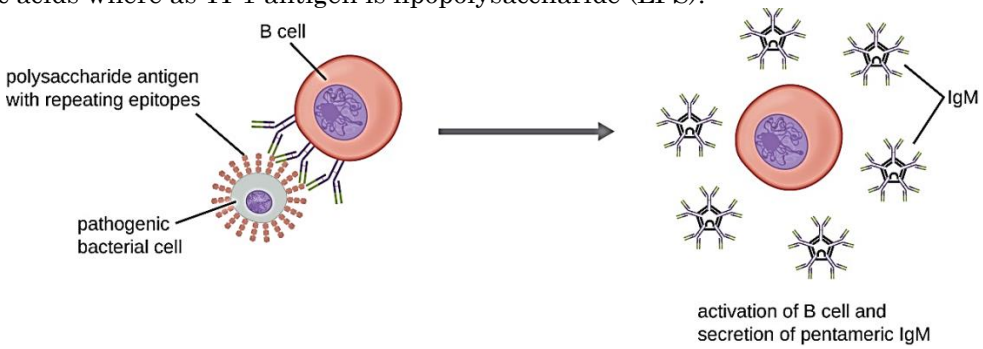


Figure: 2.23T-cellindependent immune response

TI-1 antigens can directly stimulate B cells without requirement of any other cell. At low concentration gram negative bacterial LPS stimulates specific antibody production, but at high levels it acts as a polyclonal B cell activator, stimulating growth and differentiation of most B cells without binding to the membrane receptors. LPS is a polyclonal activator in mice but not in humans.

In addition, many polysaccharides activate complement by alternate pathway and generate C3d, which binds to the antigen and provide second signal for B cell activation. Responses to TI antigens consist largely of IgM antibodies of low affinity and do not show significant heavy chain class switching, affinity maturation or memory. However, certain non-protein antigens such as penumococcal capsular polysaccharide can induce antibodies predominantly of IgG2 subclass.

Despite no participation from the helper T cells, certain polysaccharide vaccines provide long lasting immunity. The reason for this could be that the polysaccharides are not degraded readily and may persist for long periods in the lymphoid tissue, and continue to stimulate newly formed B cells. It is not known if memory cells are produced against these antigens, but subsequent exposure to the same antigens induces rapid and large secondary response.

2.3.7 Cell mediated Immunity.

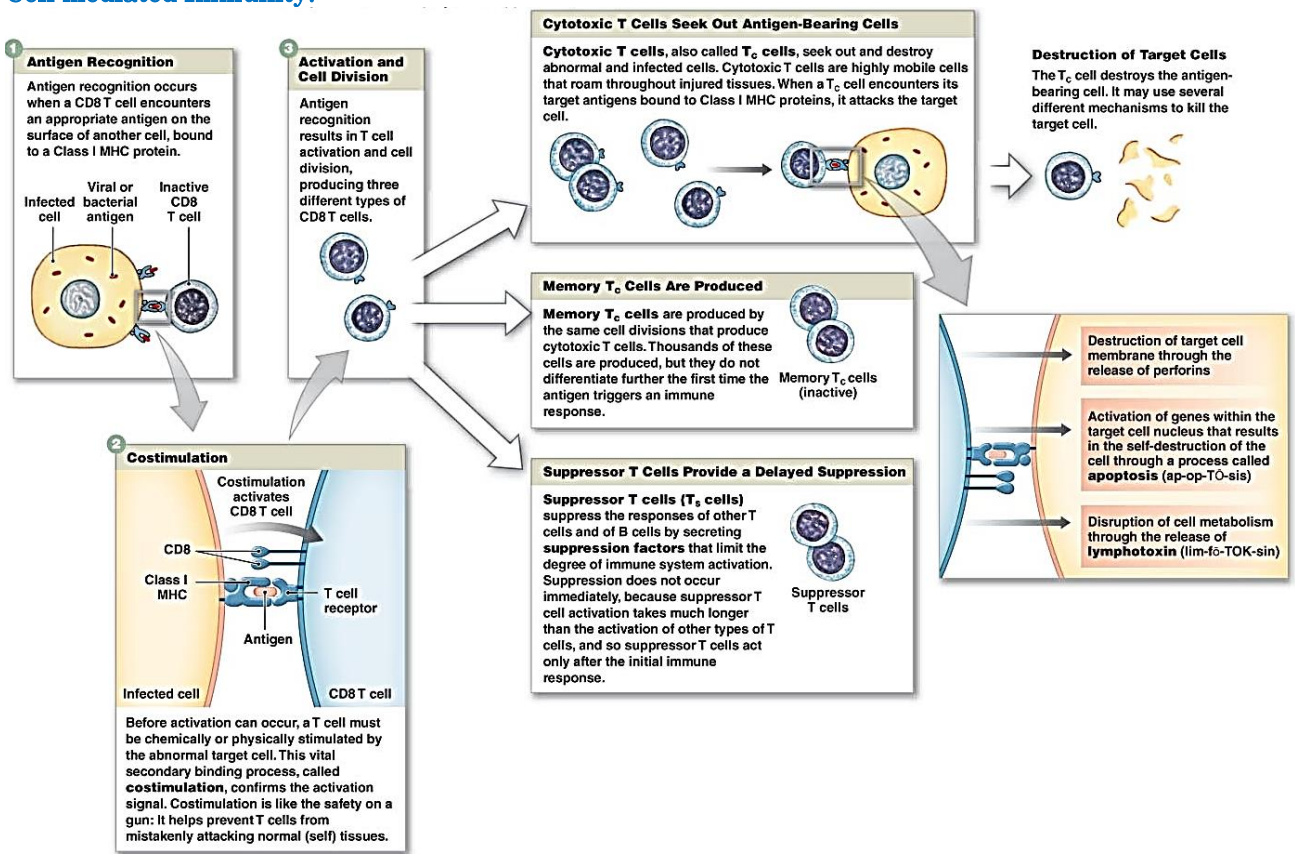


Figure: 2.24 Cell mediated immune response

Cell Mediated immunity also called as cellular immunity, is mediated by T lymphocytes or T cells. There are two types of T-cells mainly T_H and T_C, which differ by their surface marker CD4 and CD8 respectively. Effector T cells generated in response to antigen are responsible for cell mediated immunity. Both activated T_H cells and TC cells serve as effector cells in CMI.

Cytokines secreted by T_H cells can activate various phagocytic cells, enabling them to phagocytose and kill microorganisms more effectively. Cytotoxic T lymphocytes (CTL) participate in CMI by killing altered self cells.

They also play an important role in the killing of virus infected cells and tumor cells.Intracellular microbes, such as viruses and some bacteria, survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Defense against such infections is a function of cell mediated immunity.

Mechanism of Cell Mediated Response:

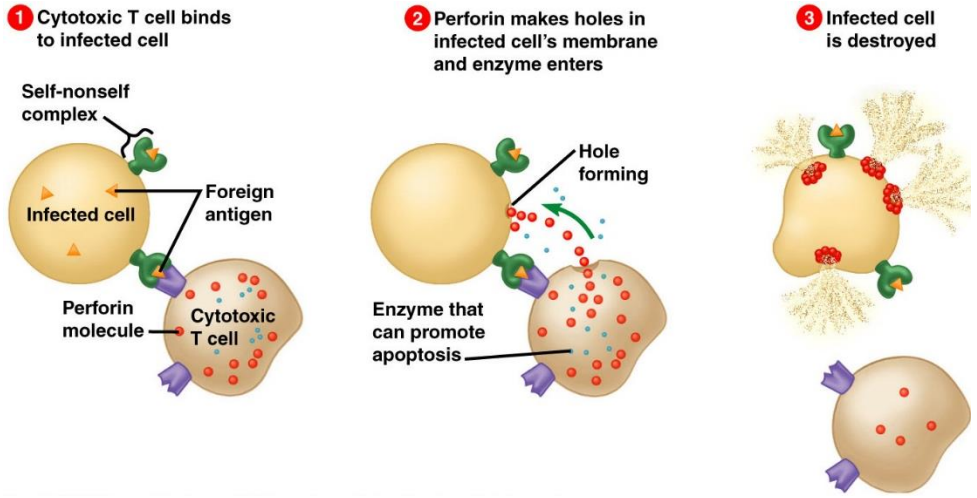


Figure: 2.25 Mechanism of Cell mediated immune response

The cell mediated response is generated by various subpopulations of T lymphocytes. After a T_H cells exposed to antigen through APCs, it's activated. Cytokines secreted by activated T_H cells help to activate various T effector cells responsible for cell mediated response.

- After a Tc cell binds to processed antigen associated with MHC-I molecules on the membrane of an altered self cell, IL-2 secreted by T_H cells stimulates and proliferation and differentiation of the T_c cells.
- This process generates cytotoxic T lymphocytes, which mediates membrane damage to the altered self cell leading to cell lysis as well as populations of memory T_H cells and T_c cells.
- Among the nonspecific effector cells involved in cell mediated immunity are NK cells and activated macrophages

- The Helper T-cells (T_H) recognize the non-self antigenthat the macrophages display on their outer surface. Lymphokines stimulate macrophages to engulf invading cells. The interlukin can stimulate cytotoxic T cells (Tc). Cytotoxic (killer) T cells attacks body cells that have been infected by virus, bacteria or fungus.
- A Tc cell identifies its antigen, where in this case a viral protein coat is left outside the infected cell, and kills the infected cell before the virus has time to replicate.Tccells kill the infected cells by secreting proteins (perforin) that punch holes in the membrane of the cell, and the contents ooze out.
- Tc cells cannot kill isolated virus particles, as they need the viral antigen before they become activated.
- Natural killer (Nk) cells have the same response as Tccells, however they may attack tumor and other cancerous cells.Once the Th and Tc cells are activated, they divide many times, where some of the cells become effector T cells, and others as memory cells, where they migrate to the lymph nodes to be activated quickly upon a second invasion.

2.3.8 Cluster of differentiation(CD)

Cluster of differentiation (CD) indicates a defined subset of cellular surface receptors (epitopes) that identify cell type and stage of differentiation, and which are recognized by antibodies. There are more than 250 identified clusters, each a different molecule, coating the surface of B lymphocytes and T lymphocytes. All T and B cells have about 105 molecules on their surface.

- B cells are coated with CD21, CD35, CD40, and CD45 together with non-CD molecules.
- T cells express CD2, CD3, CD4, CD8, CD28, CD45R, and other non-CD molecules.
- Many CDs are expressed on both B and T cells, including CD5, CD6, CD23, CD27, CD28, CD84.
- Dendritic cells also express CD4 and CD8.

2.3.9 T-cell receptor(TCR)

- Antigen-specific receptors on T cells are called t cell receptor for antigens.
- TCR is a member of the Ig superfamily, with Ig-like domains.
- Like Ig, each chain has a variable and a constant region; variable regions have CDR which define the antigen-binding specificity and framework residues.
- TCR is encoded in gene segments that undergo somatic recombination during T cell development to generate antigen-binding diversity.
- Each T cell bears a single specificity and a single allele of TCR.
- TCR is a heterodimer composed usually of a and b chains or, on a minority of T cells, g and d chains.
- The two chains are disulfide-bonded just outside the T cell plasma membrane in a short extended stretch of amino acids resembling the Ig hinge region; like Ig, TCR have very short cytoplasmic tails.
- Both chains of the TCR are glycosylated at sites on their V and C regions.
- Antigen-binding affinity is lower than that of Ig for native antigen, but MHC binding by the T cell membrane co-receptors CD4 (on helper T cells) or CD8 (on cytotoxic T cells) increases the binding avidity of the T cell for the antigen-MHC complex. CD4 and CD8 also signal the T cell to become activated.
- TCR is not secreted from the T cell. TCR is expressed on the T cell membrane with a signal transduction complex, CD3, also called the invariant TCR chains because CD3 molecules on all T cells are formed from identical subunits.

2.3.10 MHC molecules and antigen presentation.

Table 2.4: Different types of Antigen Presenting Cell

	Macrophage	**Dendritic Cell**	**B cell**
MHC-II Expression	Low levels. Induced by Bacteria and/or Cytokines	Always Expressed.	Always Expressed. Inducible upon Activation
Antigen type and presentation by MHC	Extracellular Antigens: presentation via MHC-**II**	Intracellular & Extracellular Antigens: presentation via MHC-**I & II**	Extracellular Antigen binds to specific Ig receptors: presentation via MHC-**II**
Co-Stimulation (B7 expression)	Low levels. Induced by Bacteria and/or Cytokines	Always expressed at high Levels	Low levels. Inducible upon Activation
Location	Lymphoid tissue Connective tissue Body Cavities	Lymphoid tissue Connective tissue Epithelium	Lymphoid tissues. Blood

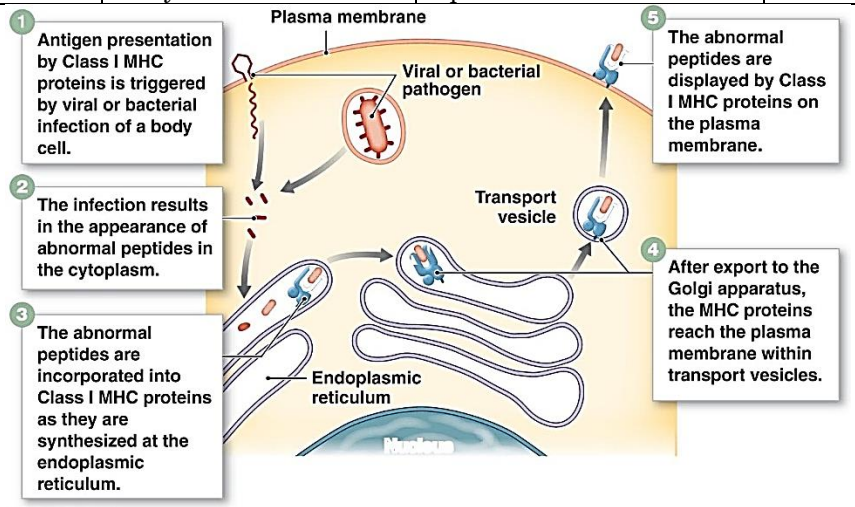


Figure: 2.26 The events in Antigen presentation of intracellular pathogens like Viruses.

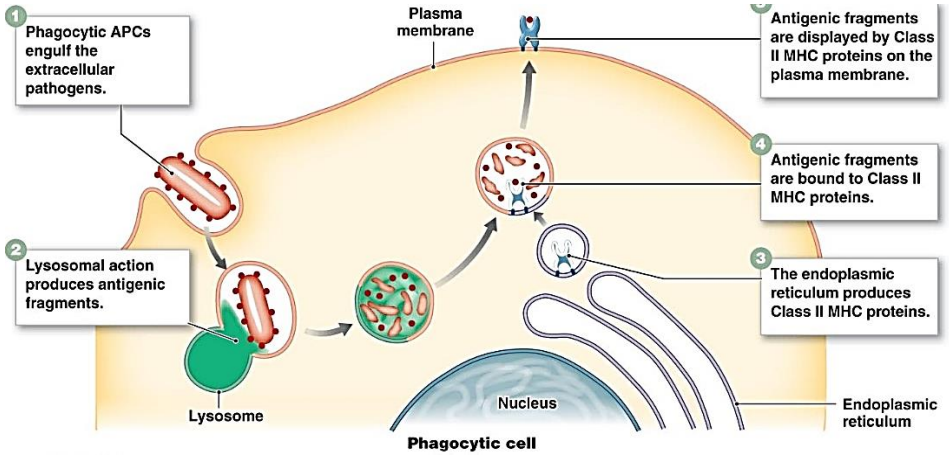


Figure: 2.27 The events in Antigen presentation of Extracellular pathogens like bacteria

2.3.11 Cytokines:

Cytokines are proteins produced by diverse cell types and they primarily interact with cells of the immune system in order to regulate the body's response to disease and infection. Cytokines also regulate several normal cellular processes in the body.

Cytokines are a diverse group of molecules:

- Colony Stimulating Factors (stimulate production of blood cells)
- Growth and differentiation factors (function primarily in development)
- Immunoregulatory and proinflammatory cytokines (interferons, interleukins and TNF-α that function in the immune system)

How Cytokines Work

Cytokines are released by various cell types cells into the circulation or directly into tissue. The cytokines locate target immune cells and bind to specific cell surface receptors on the target immune cells. The cytokine-cytokine receptor interaction activates specific signal transduction pathways leading to the modulation of activity of a number of proteins including transcription factors ultimately leading to distinct physiological responses.

Cytokines and their functions

- IL-1 activates Antigen Presenting Cell and CD4+ lymphocytes; affect the differentiation of the B-Cells and T-Cells and other immunocompetent cells and takes part in the regulation of productions of other cytokines and GMCSF (Granulocyte-Macrophage Colony-Stimulating Factor)
- IL-2 stimulates the proliferation and activation of B-Cells and T-Cells.
- IL-4 plays a role in the differentiation of TH2 (T Helper Type-2), in allergic responses, and in the switching of antibody types.
- IL-5 stimulates the production and maturation of eosinophils during inflammation.
- IL-8 acts as a chemotactic factor that attracts neutrophils, basophils and T-Cells to sites of inflammation.
- IL-12 is a critical linker between the innate immunity and adaptive immunity, capable of TH1 (T Helper Type-1) differentiation and IFN-Gamma release by T Cells and NK cells.
- IL-10 acts to repress secretion of pro-inflammatory cytokines.
- IL-3 is a poly potent activator of the hemopoietic cells. It stimulates NK-Cells and acts as a synergist with IL-4 during the induction of CD4+ lymphocyte activation process.
- IL-7 is known as the growth factor of the immature B-Cells and T-Cells. It induces apoptosis of tumor cells and causes differentiation of cells from a subgroup of acute myeloblastic leukemia.
- IL-9 stimulates the excretion of IL-2, IL-4, IL-6, IL-11, and takes part in a stimulation of cytotoxicity of T-killers and NK-Cells, inducing apoptosis.

- IL-11 is a pro-inflammative factor, which regulates the functions of B-Cells and T-Cells. It also takes part in the induction of various killer cells' activities and acts as an autocrine factor for the proliferation of megacaryocytes

The secretion of TNF-Alpha and TNF-Beta by TH1 cells activates macrophages, inhibits apoptosis of neutrophils and eosinophils, and induces vascular endothelial cells at the sites of infection to change the adhesion molecules they express so phagocytes circulating in the blood can bind to them IFN-Alpha, IFN-Beta and IFN-Gamma are produced in the area of infection during the early phase of immune response.

IFN-Alpha and IFN-Beta induce proliferation of NK-Cells and stimulate innate and adaptive immune responses that are specifically targeted to virus infections.

Upon activation, NK cells release IFN-Gamma, which activates macrophages to secrete cytokines that help to activate macrophages to secrete cytokines that help to activate T-Cells and promote the initiation of T-Cell responses.

Cytokines are key regulators of immune functions

They alter gene expression and promote cell growth They either boost (IL-2) or suppress (i.e., IL-10, and TGF ß1) immunity Type I IFN and TNFa are mediators of natural/innate immunity IL-2, IL-4, IL-5, IL-12, IL-1 are regulators of lymphocytic growth, activation and differentiation IFN g is an activator of inflammatory cells IL-3, GM-CSF, IL-7 stimulate hematopoiesis

Chapter-3
ANTIGEN-ANTIBODY REACTION I

3.1 ANTIGENS:

Any substance after entrance inside the host body able to able to sensitize immune system of the host and reacts with the products of specific immune response is called Antigen.

The word Antigen derived from two words i.e. ***Anti: Antibody; Gen: Genesis***. This word is originated from the notion that they can stimulate antibody generation. In short any substances which after entrance inside host body stimulate specific antibody production are called antigen.Ladislas Deutsch in 1899 named the hypothetical substances as antigen.

3.1.1 Definition

- **Complete antigen:** Complete Antigen (Ag) is a substance that sensitizes the immune system as well reacts with the products of a specific immune response. I.e complete antigen has ability of immune sensitization (Antigenicity) as well as immune reactivity. **Example: To Antigen –** Salmonella Somatic O antigen.
- **Hapten:** A Hapten is a substance which is non-immunogenic but on conjugation with carrier material (such as a protein) it becomes immunogenic. Haptens are usually molecules which are too small to be immunogenic. Free haptens, however, can react with products of the immune response. Haptens have the property of immunoreactivity but not immunogenicity. Also called as incomplete antigens or partial antigens.
- **Example:** Penicillin **is** ahapten which has a molecular weight of 320 Daltons (0.3 kDa).

  Haptens are partial antigens because:
  a. Haptens are **immunoreactive:** they can react with immune cell-lymphocytes or antibodies.
  b. However, haptens are **not immunogenic:** they cannot by themselves cause the production of immune lymphocytes or antibodies.

  Haptens include antibiotics, analgesics, and other low-molecular weight compounds.

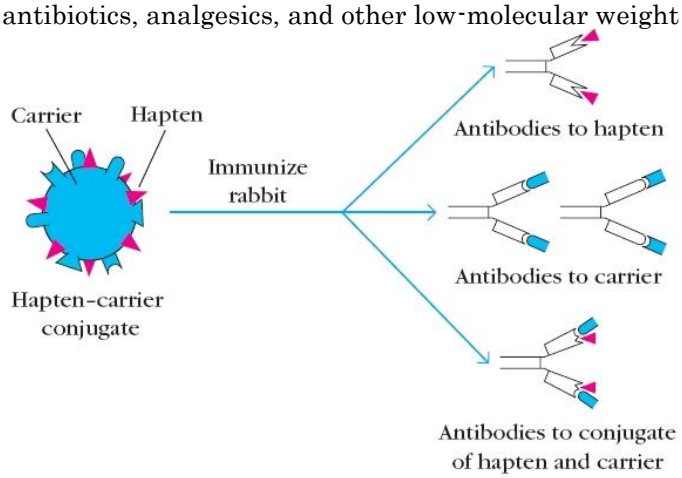


Figure: 3.1 The hapten

- **Carrier:** Carrier may be one which also does not elicit an immune response by itself .On their own they can never induce an immune response.

3.1.2 Antigenic determinants or epitope:

It is the regions of the antigen which recognize and specifically bind with receptor site of antibodies or TCR of T cells. Epitopes are also called "determinant groups or antigenic determinants". These are the sites either on or within the antigen with which antibodies or TCR react.

Physical properties

- Epitopes are very small (e.g., just four or five amino acid or monosaccharide residues).
- The epitopes on an antigen can be **linear** or **conformational**.
- Some epitopes are on the antigen's **surface** (topographic) and others are **internal**.
- Epitopes are immune-reactive only if their amino acids are spatially accessible due to tertiary protein structure.

Epitopes and antibody specificity

- Epitopes determine the specificity of the antigen molecule and are what induce the antibody response. Antibodies are specific for epitopes. Antigens are multivalent; that is, an antigen molecule carries a number of different epitopes. The **valence** of an antigen is equal to the total number of epitopes the antigen is having.

  Types of epitopes:

  There are two types of epitopes namely B cell epitope and T cell epitope.

  a. **B cell epitope:** The region of antigen, which recognized and specifically binds by antibodies. Size of this depends upon the paratope of antibodies. It is hydrophilic in nature. Even though antigen contain many number of B cell epitope. B cell epitope always present on surface of the antigen. B-cell epitopes tend to be located in flexible regions of an immunogen and display site mobility.

  b. **T cell epitope:** The region of antigen which recognized and specifically binds by TCR of T cells. The size of T cell epitope depends upon the type of MHC present the antigen i.e. if MHC-I presents the size of epitope is 8-12 amino acids and MHC-II is 12-25 amino acids.

3.1.3 **Antigenic valence:** Total number of determinants which can be bound by antibody or antigenic receptor of lymphocytes is called antigenic valence. The number of antigen binding sites possessed by an antibody molecule, two per immunoglobulin monomer, or the number of antigenic determinants possessed by an antigen, usually a large number **Most natural antigens are polyvalence antigen**.

For example: Glucagons contain 29 amino acid residues of which N-terminal region recognized by antibodies whereas C-terminal region is recognized by TCR of T cell.

3.1.4 ANTIGENICITY AND IMMUNOGENICITY:

Ability of an antigen to induce immune response is known as Antigenicity. Ability of immunogens to induce immune response is known as immunogenicity. These two terms used interchangeably.

Factors determining Antigenicity

These two terms used interchangeably.

Factors affecting Immunogenicity: These factors are mainly of two types i.e. factors contributed by Immunogens and factors contributed by host cells.

Factors by Immunogens: They are size, foreignness, chemical nature, complexity, heterogenicity and susceptibility for antigen processing.

- **Size:** Antigens with greater than 10,000 Daltons molecular weight, found to be effective immunogen and vice versa. However, in general, the larger the molecule the more immunogenic. **Example:** Hemoglobin is more immunogenic than penicillin.
- **Foreignness (Alien):**The immune system normally discriminates between self and non-self such that only foreign molecules are immunogenic.

  Example: Protein from gorilla found to have less immunogenic than the protein from fish to humans because fish is more distant from gorilla.
- **Chemical Nature:**In general, the more complex the substance is chemically the more immunogenic it will be. Depending upon the chemical nature immunogenicity varies. Proteins found to have greater immunogenicity than carbohydrates, lipids and nucleic acids. Solubility also plays an important role, less soluble antigens found to have more immunogenicity.
- ***Physical form***: In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.
- **Complexity:** As the complexity of the immunogens increases immunogenicity also increases. For example Primary structure of proteins found to have lesser immunogenicity than tertiary structure of same protein.
- **Heterogenicity:** In the case of multimeric proteins, hetero-multimeric proteins found to have more immunogenicity then homomultimeric proteins. This was mainly because of presence of different types of epitopes in heteromultimeric proteins.

- **Susceptibility for Processing and Presentation**: Those antigens which are found to be easily processed by APCs, found to have greater immunogenicity than those antigens which are not easily processed. For example: Horse RBCs found to have greater immunogenicity than the asbestos.

Factors by Host:

These factors include route, genotype and dose.

- **Route:** Route of entry is also important to provide immunogenicity. If a microorganism enters through unusual route it is degraded by immune mechanism so less immunogenic.
- For example: *Vibrio cholerae* when enters through circulatory system it won't cause diarrhea; but if it enters through GI tract it cause diarrhea.
- **Genotype:** The genetic constitution (genotype) of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response. Natures of human beings were protected humans from some diseases and also affected by some diseases. Infection by microbes depends upon the ability to infect the human cells. It purely depends upon the genotype of microorganism.
- **Dose:** To induce immunogenicity an optimum amount infecting agents are antigens required and this optimum amount is known as optimum does. When microbes enters above and below this optimum dose level fluctuation occurs in immunogenicity.

Table 3.1: Factors affecting Immunogenicity

S.No	Parameters	Increasing Immunogenicity	Decreasing Immunogenicity
1.	Size	High	Low
2.	Composition	Complex	Simple
3.	Similarity of self proteins	Multi different	Few different
4.	Form	Particulate and Denatured	Soluble and Native
5.	Dose	Intermediated	Low and High
6.	Route	Subcutaneous > Intra peritoneal cavity	Intravenous and Intragastric
7.	Adjuvant	Slow release with Bacteria	Rapid release with bacterial component

3.1.5 ANTIGENIC MOSAIC OF BACTERIA:

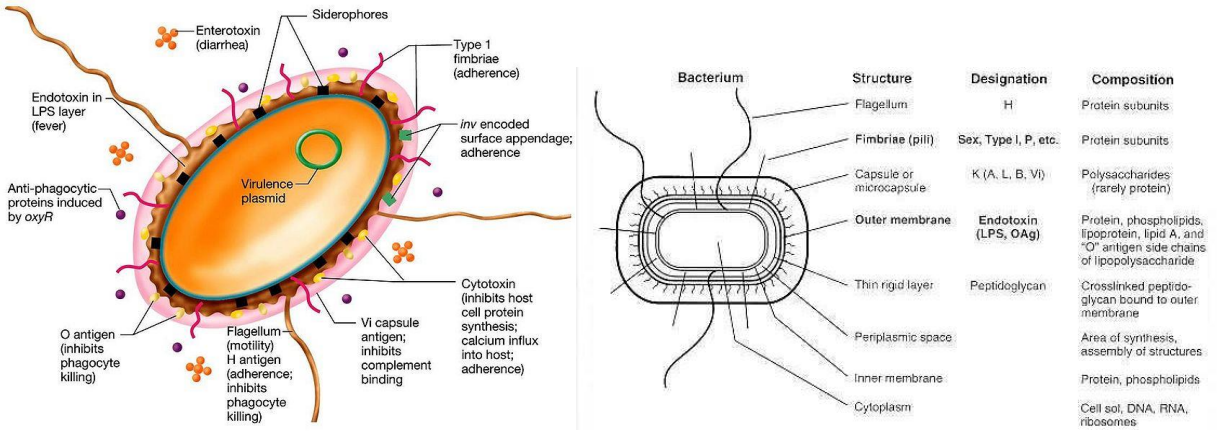


Figure: 3.2 Theantigenic Mossic of bacteria

Structure and Antigens

The generalized structure and antigenic composition of bacteria are denoted in **Figure 3-2.**The major antigens of bacteria are H, K, and O antigens.

- The H antigen determinants are flagellar proteins. Escherichia coli, Enterobacter, Serratia, Citrobacter, and Proteus organisms are peritrichous. Klebsiella species are nonmotile and nonflagellated and thus have no H antigens.
- Some strains of coliform and Proteus species have pili (fimbriae). Pili are associated with adhesive properties and, in some cases, are correlated with virulence. Different pilial colonization factors generally are detectable as hemagglutinins that can be distinguished by the type of erythrocyte agglutinated and by the susceptibility of the hemagglutination to inhibition by the sugar mannose. Sex pili, which have receptors for "male" specific bacterial viruses and are genetically determined by extrachromosomal plasmids, are important in coliform ecology and in the epidemiology of diseases produced by coliforms and Proteus species in that sex pili are involved in genetic transfer by conjugation (e.g., chromosome-mediated and plasmid-mediated drug resistances or virulence factors).
- K antigens (capsule antigens) are components of the polysaccharide capsules. Certain K antigens (e.g., K88 and K99 of E coli) are pilus-like proteins. The K antigens often block agglutination by specific O antisera. In the past, K antigens routinely were differentiated into A, L, and B groups on the basis of differences in their lability to heat; however, these criteria are subject to difficulties that make the distinction tenuous.
- Some Citrobacter serotypes produce Vi (virulence) antigen, a K antigen also found in Salmonella typhi. Species of Proteus, Enterobacter, and Serratia apparently have no regular K antigens. However, the K antigens are important in the pathogenesis of some coliforms. A diffuse slime layer of variable thickness (the M antigen) also may be produced but, unlike the K antigens, it is nonspecific and is serologically cross-reactive among different organisms.
- The outer membrane of the bacterial cell wall of these species contains receptors for bacterial viruses and bacteriocins (plasmid-encoded, antibiotic like bactericidal proteins called colicins in E coli that are active against the same or closely related species).
- The outer membrane also contains lipopolysaccharide (LPS), of which the lipid A portion is endotoxic and the
- O (somatic) antigen is serotype specific. The serologic specificity of the O antigens is based on differences in sugar components, their linkages, and the presence or absence of substituted acetyl groups. Loss of the O antigen by mutation results in a smooth-to-rough transformation, which often involves changes in colony type and saline agglutination, as well as loss of virulence . Certain strains of P vulgaris (OX-19, OX-2, and OX-K) produce O antigens that are shared by some rickettsiae. These Proteus strains are used in an agglutination test (the Weil-Felix test) for serum antibodies produced against rickettsiae of the typhus and spotted fever groups

3.1.6 Antigens in relation to human being :

- **Species specific antigen:** An antigen that is common to members of a single species and that provides a means by which that species can be immunologically distinguished. **Example:** Serum albumin of horses is immunologically different from that of man, dogs, sheep
- **Iso-antigens:** A genetically determined antigen present in some but not all individuals of a species (as those of a particular blood group) and capable of inducing the production of an alloantibody by individuals which lack it—called also *isoantigen* **Example:** Antigens of red blood cell
- **Auto antigens (Rheumatoid arthritis):** An endogenous body constituent that stimulates the production of auto-antibodies and an autoimmune reaction. **Example:** An autoantigen associated with Addison`s disease has been identified as the enzyme 17 α-hydroxylas.
- **Organ Specific:** A heterogenetic antigen with organ specificity; e.g., in addition to species-specific antigen, kidney of one species contains antigen that is identical to that in kidney of other species. **Example:**
- **Heterophile or Heterogenic or Cross reactive antigen:** The antigens interact with antibodies produced against another antigen. These antigens are also called as cross reactive antigens.The phenomenon is known as cross reaction. The function of binding of antibody to an antigen to which it is not produced known as cross reactivity. **For example:** Forssman antigen.

3.1.7 Types of Antigens :

Antigens are based on origin initially categorized into two classes: These types are

1. **Exogenous Antigens:**
   The antigenic substances which are entered inside the host body from outside and able to cause immune sensitization are called exogenous antigens. These antigens are usually secretary products of microbes or soluble antigens.
   These exogenous antigens entered inside host body through inhalation, injection or injection in the form of Microorganisms, drugs, pollutants and even pollen.
   These antigens are taken into the antigen-presenting cells (APCs) by endocytosis or phagocytosis and processed into fragments. APCs then present the fragments to T helper cells $(CD4)^+$ by the use of class II histocompatibility molecules on their surface. **Example:** Bacterial exotoxins (diphtheria toxin from *Cornebacterium diptheriae)*
2. **Endogenous antigens:** The antigens which have been generated within a normal host cells as a normal cell metabolism or because of viral or intracellular bacterial infection.
   The fragments are then presented on the cell surface in the complex with MHC class I molecules. If activated cytotoxic $CD8^+$T cells recognize them, the T cells begin to secrete different toxins that cause the lysis or apoptosis of the infected cell. **Example:** Heterologous antigens (Xenogenis), Autologous, allogenic (homologous) and viral proteins.
3. **Auto-antigens:** An auto-antigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease. These antigens should under normal conditions not be the target of the immune system, but due to mainly genetic and environmental factors the normal immunological tolerance for such an antigen has been lost in these patients. **Example:** An autoantigen associated with **Addison's disease** has been identified as the enzyme 17 α-hydroxylase. Also called **self-antigen**
4. **T-independent:** T-independent antigens are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help In general, polysaccharides are T-independent antigens. The responses to these antigens differ from the responses to other antigens. **Example:**
   Properties of T-independent antigens:
   - **Polymeric structure:** These antigens are characterized by the same antigenic determinant repeated many times.
   - **Polyclonal activation of B cells:** Many of these antigens can activate B cell clones specific for other antigens (polyclonal activation). T-independent antigens can be subdivided into Type 1 and Type 2 based on their ability to polyclonally activate B cells. Type 1 T-independent antigens are polyclonal activators while Type 2 is not.
   - **Resistance to degradation:** T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.
5. **T-dependent:** T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are characterized by a few copies of many different antigenic determinants.
   Example: *Bacterial toxins*

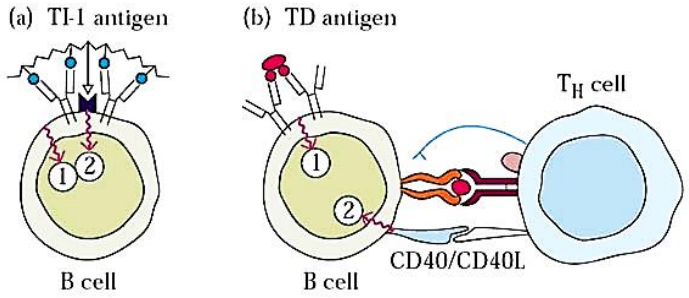


Figure: 3.3 The T dependent and T independent antigens.

6. **Immunogen:** Immunogen is a substance that induces a specific immune response inside a host body when administered through a specific route. The property of a substance is called immunogenicity. Immunogens are usually proteins or polysaccharides.
   Example: This includes parts (coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, and other microorganisms.
7. **Tolerogen:** Tolerogen is an antigen that invokes a specific immune non-responsiveness due to its molecular form. If its molecular form is changed a tolerogen can become an immunogen.
   Example:----------
8. **Allergen:** An allergen is a substance that triggers the allergic reaction. The reaction may result after exposure via ingestion, inhalation, injection or contact with skin. Cells present their antigens to the immune system via a histocompatibility molecule. Depending on the antigen presented and the type of the histocompatibility molecule, several types of immune cells can become activated. **Example:** Pollen antigen, egg proteins.
9. **Mitogens:** Differ to immunogen which activate only lymphocytes bearing specific receptors, **mitogens activate many clones of T or B cells to undergo cell division irrespective of their antigen specificity**. Because of this ability, mitogens are known as polyclonal-activators. Mitogen causes cancer due to inducing cell division. **Example:** Concanavalin – A (CON A), Phyto-hemagglutinin (PHA) Pokeweed mitogen (PWM).
10. **Super Antigens:** Super antigen is a class of antigen which causes non specific activation of T cell. **Example:** Staphylococcal enterotoxins: Bind on the outside of the antigen binding cleft. So super-antigen crosslink's a T cell to a class II MHC molecule in an antigen independent manner resulting in T cell activation. They are of two types namely exogenous and endogenous super-antigens.
    - ***Exogenous super antigens:*** They are capable of activating T cell – APC complex exogenously. They bound to surface of APC cells and activate T cells when they interact with APC cells. These antigens are not processed. **Example:** (Toxic shock syndrome toxin – 1) produced from staphylococcus.

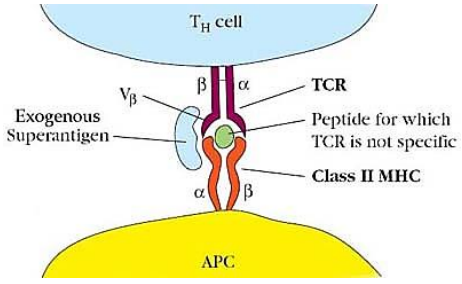


Figure: 3.4 Exogenous super antigens

- ***Endogenous super antigens:*** They are called endogenous because they are processed and presented by APC cells in irregular manner i.e. super antigens are not presented through

MHC molecules but they are present outside the MHC molecules. But they activate T_H cells but interacting α chain of MHC and V- region of α chain of TCR of T cells. **Example:** Mouse mammary tumor viral (MMTV) antigens.

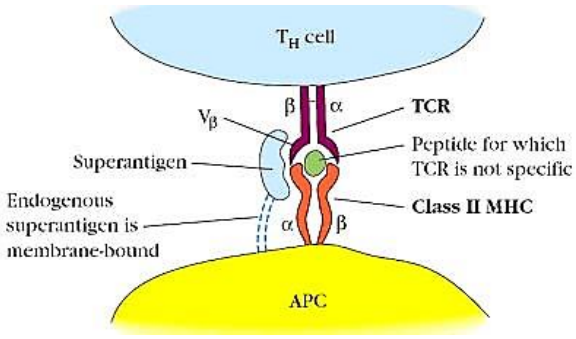


Figure: 3.5 Endogenous super antigens

11. **Sequestered antigens:** The antigens which were entrapped in coat like capsule, so not exposed to immune system are called **Sequestered antigens.** Due to breakage of coat when they exposed, they elicit immune response. **Example:** Lens proteins and sperm proteins.
12. **Neo-antigens:** Neo-antigens are the newly produced antigens formed due to the change in the chemical, physical and biological status of the agent's normal biomolecule. **Example:**.....
13. **Tumor Specific Antigens:** Tumor specific Ag (TSA) are the antigens which Only expressed on the tumor cells but not on normal cells. **Example: AFP**(alpha-fetoprotein): over-expression in liver cancer. **CEA** (carcino-embryonic antigen): over-expression in carcinoma of colon , pancreas, stomach ,and breast.
14. **Xenoantigens**: Xenoantigen is found in more than one species. An antigen is something that is capable of inducing an immune response. The prefix "xeno-" means foreign or other. It comes from the Greek "xenos" meaning stranger, guest, or host.Examples: **Pathogens:** bacteria, virus, fungi, parasite**Self antigens:** They are not originally antigens, they are normal cell surface components and proteins of normal host. But they can be converted to antigens like autogens. **Example:**----------
15. **Adjuvants:** Adjuvants are substances which have an ability to enhance antigenic response of antigen. These substances enhance the immunogenicity of molecules without altering their chemical composition. The **mechanisms** by which adjuvants exert their biological effects are multiple.
    - Adjuvants may increase the efficiency of macrophage processing of antigens.
    - Adjuvants can act as depots and prolong the period of exposure to the immunogen.
    - Adjuvants may amplify the proliferation of immunologically committed lymphocytes by enhancing the release or the action of lymphokines

Enhancing property achieved by any one or more of the following ways.

1. Increasing antigen persistence
2. Co-stimulatory signal activation
3. Granuloma formation and
4. Inducing T cell proliferation

Example: Alum: Alum acts as adjuvant by increasing the availability of antigen by the process of precipitation.

3.2 ANTIBODY:

Antibody is a protein substance in the blood produced by the immune system or tissues in response to a specific antigen that destroys or weakens bacteria and neutralizes organic poisons, thus forming the basis of immunity. An immunoglobulin molecule produced by B-lymphoid cells that combine specifically with an immunogen or antigen. Antibodies may be present naturally, their specificity is determined through gene rearrangement.

The specific humoral (fluid-borne) factors that provide 'humoral immunity' to the host against the offending antigens are collectively known as 'antibodies' (Abs).

3.2.1Introduction:

- Antibodies were first detected in the sera of animals injected with bacterial exotoxins, such as tetanus toxin and diphtheria toxin, by Emil von Behring and Shibasaburo Kitasato in the year 1886 A.D.
- Antibodies are products of antigen-specific B lymphocyte clones that are responsible for adaptive humoral immunity in vertebrate hosts.
- They are produced mainly in secondary lymphoid organs and tissues, such as lymph nodes, spleen, mucosa-associated lymphoid tissues, etc. After stimulation by the antigen, specific B cell clones undergo multiple cycles of cell division and terminally differentiate into plasma cells that secrete specific Abs in large amounts in lymphoid tissues.
- From lymphoid tissues, Antibodies are disseminated via peripheral blood circulation to entire body, tissue fluids and secretions.
- IgM and IgA are multimers of the basic structure and are held together by J chain.
- IgA in secretions also has secretory component which stabilizes and protects it from digestion.
- Antibodies protect the host against potential parasites by:
  1. Directly inhibiting binding sites of viruses or various enzymes and toxins produced by bacteria
  2. Agglutination
  3. Opsonization which facilitates removal by phagocytes
  4. Lysis of susceptible organisms via complement fixation,
  5. Inducing inflammation.

Table 3.2: Biological defense by Antibody

Sr. No	Antibody	Biological defense
1	IgG	High concentration, High longevity, Opsonic activity, ubiquity and ability to activate complement.
2	IgM	Activating complement and aggregation of targets
3	IgD	Antigen receptor for "virgin" B cells.
4	IgA	Extracorporeal fluids and acts mainly by aggregating the potential pathogen.
5	IgE	Antibody of allergic responses but is also an important agent of host defense against parasites.

3.2.2 Determination of structure of Antibody

- In 1950- 1960 experiments by Rodney Porter and by Gerald Edelman elucidated the basic structure of immunoglobulin. For this the awarded with Nobel Prize in 1972.
- Porter fragmented antibody molecule by treating with digestive enzymes
- Edelman dissociated the molecule by reducing the inter-chain disulfide bonds.
- The result obtained by these two experiments is complementary with each other.
- These results are base of Antibody structure elucidation.
- Using ultracentrifugation, both Porter and Edelman first separated the γ-globin fraction of serum into a high molecular weight fraction with a sedimentation constant of **19S** and a low molecular weight fraction with a sedimentation constant of **7S.**
- They used the 7S fraction containing a 1, 50,000 mw γ-globin designated as IgG for their studies.

- Porter subjected IgG to brief digestion with the enzyme papain and separated the fragments. It produced two identical fragments called Fab fragments nand one fragment called the Fc fragment.
- Brief pepsin digestion generated a single 1,00,000 mw fragment composed of two Fab-like fragments and designated $F(ab')_2$. Like the Fab fragments, the $F(ab')_2$ fragment was also able to visibly precipitate antigens. However, after pepsin digestion, the Fc fragment was not recovered because it had been digested into multiple fragments.
- Porter subjected IgG to mercaptoethanol reduction and alkylation, a chemical treatment that irreversibly cleaves disulfide bonds.
- The sample was then chromatographed on a column that separates molecules on the basis of size.
- This experiment revealed that the 1,50,000 mw IgG molecule was composed of two 50,000 mw polypeptide chains, designated as heavy (H) chains and two 25,000 mw chains, designated as light (L) chains.
- The remaining puzzle was to determine how the enzyme digestion products Fab, $F(ab')_2$ and Fc were related to the heavy chain and light chain reduction products.
- Porter answered this question by using antisera from goats that had been immunized with the Fab fragments and Fc fragments of rabbit IgG.
- He found that antibody to the Fab fragment could react with both the H and L chains whereas antibody to the Fc fragment reacted only with the H chain.
- These observations led to the conclusion that Fab consists of portions of a heavy and a light chain and that Fc contains only heavy chain components.
- Based on these results, Porter and Edelman proposed the prototype structure for IgG.

3.2.3General structure.

- Antibody is glycoproteins of heterogeneous size, charge, amino acid composition and antigenic nature. Each Antibody is however basically made up of four polypeptide chains: two identical smaller polypeptide chains designated as L (light) chain and the H (heavy) chain.
- L chain is composed of about 220 amino acids (AAs), whereas H chains have about 440 or 550 AAs in them. Only the H chains are glycosylated.
- In the basic monomeric form of Antibody, the polypeptide chains are joined together by inter-chain disulphide bonds between cysteine residues and number of non-covalent linkages such as hydrogen bonds, salt bridges and hydrophobic bonds in a manner that a structure that may be written as [L-H-H-L] is obtained.
- The basic structure of antibody was proposed by Porter and Edelman in 1950.

Light Chain (L):

- The smaller polypeptide chains of about 25-30 kDa size is designated as L (light) chain.
- L chain is composed of about 220 amino acids (AAs). This region is called variable (V_L) region. The carboxylic terminal of the amino acid chain showed constant sequence hence called constant (C_L) region.
- There are two types of light chains Kappa ( ) chain and Lambda ( ). In human 40% lambda and 60% Kappa chain present. A single antibody contain only 1 type of light chain i.e either kappa or lambda but never both.
- The sequencing of amino acid of this chain reveled that about 100-110 amino acid at amino terminal shows lots of variation among different antibodies.

Heavy Chain (H):

- The bigger polypeptide chains of 50-70 kDa size is designated as H (heavy) chain. H chains have about 440 or 550 AAs and glycosylated.
- Heavy chain consisting of 440 amino acids. Similar to light chain amino terminal of heavy chain has 100-110 amino acids with variation among different antibodies. This region is called as

Variable region (V_H). Carboxyl terminal showed constant sequences and therefore called constant (C_H) region. The length of constant reion is about 330 amino acids.

- Heavy chain is present in five different forms like-mu (), delta (), alpha (), and epsilon ().

Immunoglobulin domain:

- In 1969, Edelmen and Gall proposed that each homologous region of light and heavy chain is folded into a compact globular structure called as domain or immunoglobulin fold.
- These domains consist of about 110 amino acids and within them interchain disulphide bond form a loop of about 60 amino acids.
- The light chain consists of one variable domain (V_L) and one constant domain (C_L).
- The Heavy chain contain one variable (V_H) and three or four constant domains (C_{H1}, C_{H2}, C_{H3} and C_{H4})
- Each chain has the amino acid sequence, accounts for the variable and constant regions.

Complementarily determining region (CDR):

- V_L and V_H domains revealed that the sequence variation is concentrated in a few discrete regions of these domains called CDR. These regions were originally called hyper-variable regions in recognition of their high variability. Hyper variable regions form the antigen binding site of the antibody molecule. Because the antigen binding site is complementary to the structure of the epitope, these areas are now more widely called complementarily determining regions (CDRs).

Hinge region:

- The, λ, and ß heavy chains contain an extended peptide sequence between the C_{H1} and C_{H2} domains that has no homology with the other domains.
- This region, called the hinge region**,** is rich in proline residues and is flexible, giving IgG, IgD, and IgA segmental flexibility.
- As a result, the two Fab arms can assume various angles to each other when antigen is bound.

3.2.4 Classes of Immunoglobulin's and their functions.

Antibodies are classified mainly in two ways namely on the basis of constant region of heavy chain and antigenic determinants of antibody.

1. **Classification on the Basis of Constant region of Heavy chain:**
   - The heavy chains determine the class and are named with the Greek letter corresponding to the class.
   - Subclasses of immunoglobulin's can be distinguished on the basis of allotypes, which are single amino acid mutations in the constant region. There are 5 classes of immunoglobulins:
     1. IgG-Model
     2. IgM
     3. IgA
     4. IgD
     5. IgE
   - **Classes and Subclasses**: Igs can be subdivided on the basis of physical and functional properties into five classes, named for historical reasons IgG, IgM, IgA, IgD and IgE.
   - IgG has four subclasses numbered 1 – 4.
   - The different classes and subclasses are determined by differences between their heavy chains.

Immunoglobulin G:

- IgG was the first immunoglobulin to have its primary and secondary structures elucidated; therefore, its structure is considered the model for all immunoglobulin's.

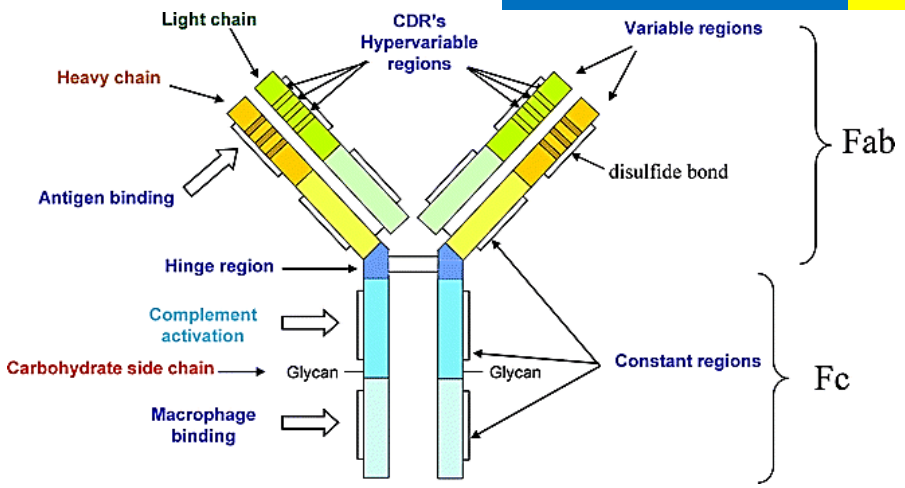


Figure: 3.6 Structure of IgG

- IgG is a major serum immunoglobulin. 80 % fraction of serum immunoglobulin is IgG.
- In serum it exists as monomer. It has a molecular weight of 1, 46,000 daltons.
- It is bilaterally symmetrical tetramer composed of two identical gamma heavy chains (with molecular weight 50,000 daltons) and two identical kappa or lambda light chains (with molecular weight 25,000 daltons).
- These chains are held together by disulfide bonds and by hydrophobic interactions.
- The human IgG has four subclasses based on heavy chain type; these subclasses are IgG_1, IgG_2, IgG_3 and IgG_4.
- The number of disulfide bonds between the heavy chains and their exact location varies with the class and subclass of immunoglobulin.

Function:

- The major antibody produced during secondary response.
- The IgG is the only immunoglobulin which can pass the placenta.
- IgG_3 involved in activation of complement.
- It act as opsonin and involved in opsonization.

Immunoglobulin -M

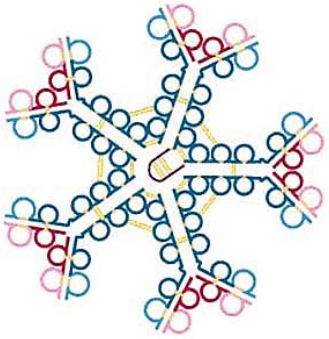


Figure: 3.7 Structure of IgM

- IgM as a pentameric immunoglobulin found in serum which accounts for 5-10% of total serum immunoglobulin. It has no hinge region.
- Monomeric IgM is expresszed as membrane bound antibody on B cells surface and has a molecular weight of 1, 80,000 Dalton.
- It is also present in the serum in the pentameric form, in which five monomer units are held together by disulphide bonds that link their carboxyl terminal Domains of heavy chains.
- In addition, its heavy chain ( ) is larger than the chain because it has four constant domains.

- The Fc region of each monomer is linked with called J chain. J chain is a peptide of about 15,000 Daltons which is rich in cysteine that are available to form disulfide bonds.
- In the pentameric form IgM molecule able to bind 10 small hapten molecules but it only able to bind five large antigens due to stearic hindrance phenomenon.

Function:

- IgM is a predominant immunoglobulin in primary immune response.
- It is the first antibody synthesized by newborn.
- It is present in intravascular pool.
- It is able to agglutinating the antigens as well as it can neutralize the viral particles.
- It is also able to activate complement by classical pathway.
- One of the benefits of the inflammatory response is it lets IgM escape into the tissue spaces at the site where it may be needed to bind potential pathogens.

Immunoglobulin A:

- Serum IgA is similar in structure to IgG. It constitute about 10-15% of total serum immunoglobulin.
- IgA consist of two alpha heavy chains and two kappa or two lambda light chains.
- It occurs in two subclasses IgA_1 and IgA_2. In serum more than 80% of IgA occur as monomer but can also occur in polymeric forms.
- IgA is also called as secretary antibody as it predominant in seromucous secretions, such as breast milk, saliva, tears, and mucous of bronchial, genitourinary and digestive tract.
- Usually it is dimer with molecular weight of 3, 85,000 Dalton.
- It has two additional polypeptide chains called secreatory component. This secretory component is derived from the poly –Ig receptor that helps in the transport of polymeric IgA across the membrane.
- **Function:**
- It serves as first line of defense against microbial invasion at the site of infection (mucosal surfaces).
- It inhibits viral infection and bacterial colonization.
- Protect newborn against infection during first month of life
- IgA is actively secreted into external body fluids such as saliva where it acts to inhibit the entry of potential pathogens into the body.

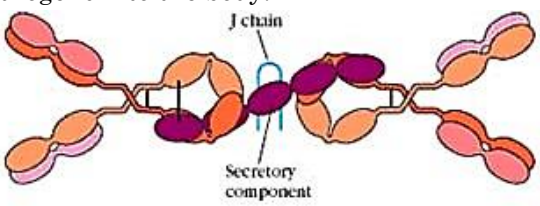


Figure: 3.8 Structure of IgA

Immunoglobulin D:

- IgD is an immunoglobulin which accounts for 0.2% of total serum immunoglobulin.
- It consists of two delta heavy chains and two kappa or two lambda light chains.
- It occurs as monomer with a single disulphide bond between the two chains .
- Its heavy chain has a much longer hinge region resulting in a molecular weight of about 60,000 Daltons.
- Because of this extended hinge region, it is very susceptible to being split apart by serum enzymes.
- IgD is similar to the structure of IgG.

Function:

- It is present on the surface of B cell as antigen receptor.
- IgD is part of the antigen-recognition/signaling mechanism on "virgin" B cells.

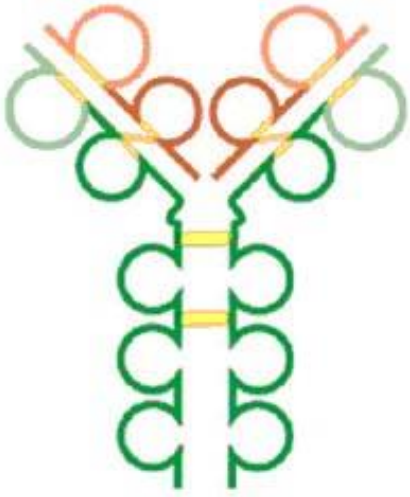


Figure: 3.9 Structure of IgD **Figure: 3.10** Structure of IgE

Immunoglobulin E:

- IgE is an immunoglobulin which accounts for 0.004 % 0f total serum immunoglobulin.
- It consists of two epsilon heavy chains and two kappa or lambda light chains.
- It occurs as monomer with molecular weight of 1, 88,000 Dalton.
- It has no hinge region hence least flexible.
- It has four C domains.
- The H chains are held together by two sets of intra-chain disulfide bonds.
- It was the last to be discovered and characterized because it is present in such small amounts in serum (0.3 g/ml).

Function:

- IgE is the immunoglobulin responsible for allergic reactions.

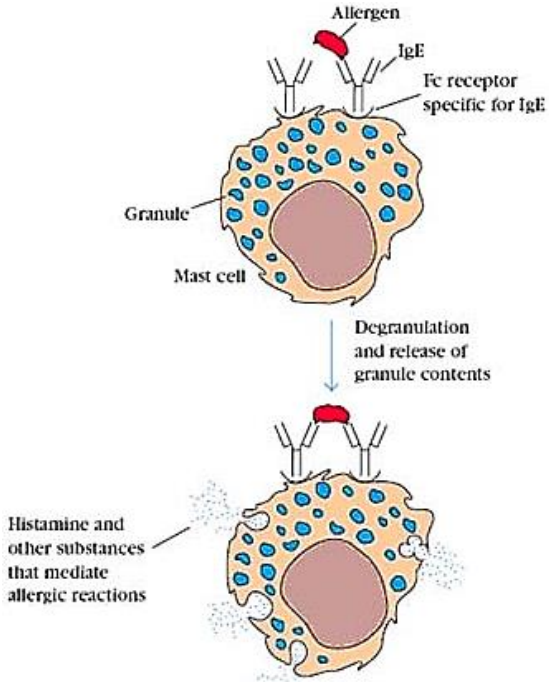


Figure: 3.11 Function of IgE

3.2.5 CLASSIFICATION BASED ON ANTIGENIC DETERMINANTS:

- Since antibodies are glycoproteins, they can themselves function as potent Immunogens to induce an antibody response.
- Such anti-Ig antibodies are powerful tools for the study of B-cell development and humoral immune responses.
- The antigenic determinants, or epitopes, on immunoglobulin molecules fall into three major categories: isotypic, allotypic, and idiotypic determinants.

Isotype: Isotypic determinants are constant-region determinants that collectively define each heavy-chain class and subclass and each light-chain type and subtype within a species. Each isotype is encoded by a separate constant region gene, and all members of a species carry the same constant-region genes (which may include multiple alleles

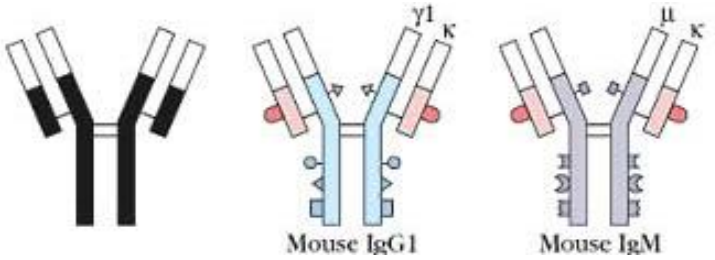


Figure: 3.12 Isotypes of Antibody

Allotype: Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes. These alleles encode subtle amino acid differences, called allotypic determinants, that occur in some, but not all, members of a species.

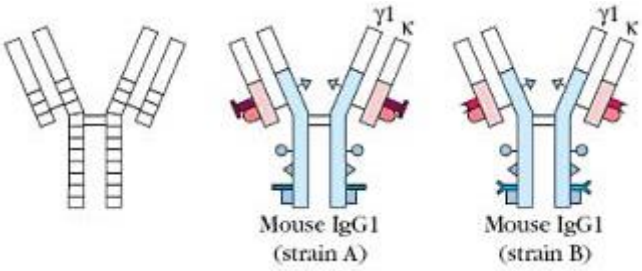


Figure: 3.13 Allotypes of Antibody

Idiotype:

The idiotypic determinants arise from the sequence of the heavy- and light-chain variable regions. Each individual antigenic determinant of the variable region is referred to as an idiotope. In some cases an idiotope may be the actual antigen-binding site, and in some cases an idiotope may comprise variable-region sequences outside of the antigen binding site.

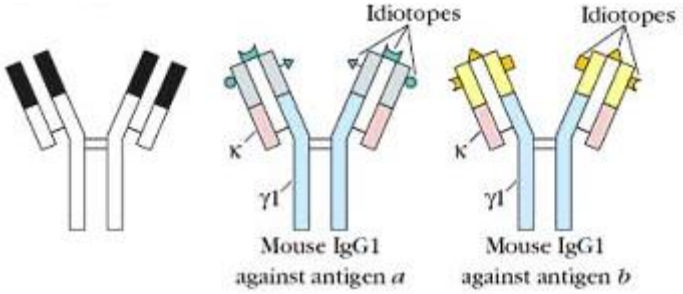


Figure: 3.14 Idiotypes of Antibody

3.2.6 MECHANISM OF ACTION OF IMMUNOGLOBULIN'S.

Immunoglobulin protective activities are expressed via several mechanisms.

1. **Direct action:** They may bind to the active site of enzymes secreted by various bacteria which help them spread (e.g., collagenase) or to the binding site on viruses which would prevent them from attaching to their target. Which limit spread of infection.
2. **Agglutination or aggregation:** Since all antibodies have at least two antigen binding sites, they can potentially bind to two organisms at the sometime. Eventually, a large number of organisms could be clumped together in this fashion. These aggregated organisms would be less free to move, would have more trouble penetrating physical barriers and membranes, and usually would be engulfed more readily by macrophages which are not very effective at phagocytizing very small bacteria and all viruses. As would be expected, the polymeric immunoglobulins are more effective at agglutination than the others.

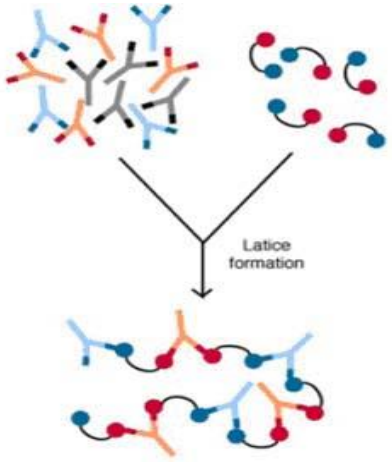


Figure: 3.15 Agglutination by Antibody

3. **Opsonization:** Macrophages and Neutrophils (PMNs) have surface receptors that recognize and bind to the Fc region of IgGl & IgG3. Therefore, anything coated with either of these antibodies will be bound by macrophages or PMNs and engulfed. One of the split products of complement, C3b, is also an opsonin, so IgM may indirectly contribute to opsonization via complement fixation.

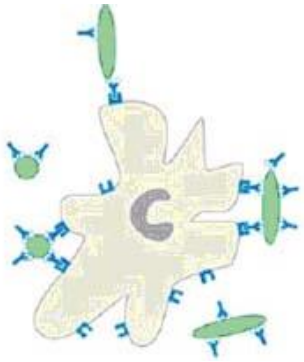


Figure: 3.16 Antibody as opsonin

4. **Lysis:** Complement activation can result in lysis of animal cells and gram negative organisms. Thus, complement fixing antibodies may indirectly cause lysis of susceptible targets.
5. **Inflammation:** IgE stimulates an inflammatory response which is an important host defense mechanism. It is only with prolonged or extensive inflammation that the destructive effects outweigh the beneficial effects.

3.3 AG-AB REACTIONS(DIAGNOSTIC IMMUNOLOGY) :

The interaction between antigen and antibody is a bimolecular association which does not lead to an irreversible chemical alteration in either the antibody or the antigen. The association between an antigen and antibody involves various non-covalent interactions between the antigenic determinant (epitope) of the antigen and the CDR segment of variable-region (V_H/V_L) domain of the antibody molecule. The specific interaction of antigens and antibodies is dependent on various weak and non-covalent linkages like hydrogen bonds, hydrophobic interactions, electrostatic forces, and van der Walls interactions. The large number of such weak interactions is required to form a strong antigen-antibody (Ag-Ab) interaction.

3.3.1 Properties of antigen-antibody interaction

Affinity: The combined strength of the non-covalent interactions between a single antigen-binding site on an antibody and a single epitope of antigen is the affinity of the antibody for that epitope. Affinity is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody. There exists a weak association between low-affinity antibodies and antigen, which dissociates easily whereas high-affinity antibodies bind antigen more tightly and remain bound longer. The binding of an antibody (Ab) to its antigen (Ag) is reversible, so the binding reaction can be expressed as:

Ag + Ab ↔ Ag-Ab .....

Avidity: When complex antigens containing multiple repeating antigenic determinants are mixed with antibodies containing multiple binding sites, the interaction of an antibody with an antigen at one site will increase the probability of reaction between those two molecules at a second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the avidity. Avidity is more than the sum of the individual affinities. **Specificity:** Specificity of an antigen-antibody reaction refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen. Antigen-antibody reactions are highly specific in nature, that means one antibody can react with its own antigen. A strong antigen-antibody interaction depends on a very close fit between the antigen and antibody which requires high degree of specificity.

Cross reactivity: The most important feature of the antigen–antibody interaction is its high specificity and affinity. But in some cases, antibody specific for one antigen can cross react with an unrelated antigen, which is known as cross-reactivity. Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen. Cross-reactivity is often observed among polysaccharide antigens that contain similar oligosaccharide residues. The ABO blood group antigens are the glycoproteins expressed on red blood cells. Subtle differences in the terminal residues of the sugars attached to these surface proteins distinguish the A and B blood group antigens. A number of viral and bacterial antigens elicit antibody that cross reacts with the host-cell components, which results in a tissue damaging reaction. Cross-reactivity is also exhibited by some vaccines.

These are reactions are studied under two different headings namely in vivo and in vitro reactions.

1. **In vivo Ag-Ab reactions:**

These reactions refer to the Ag-Ab reactions occurring inside the host. In the body, they form the basis of antibody mediated immunity in infectious diseases or of tissue injury in some types of hypersensitivity and autoimmune diseases. Functions of antibodies are also included under this category. They are

a. Agglutination
b. Neutralization
c. Opsonization (IgG1 and 3)
d. Complement fixation (IgM and IgG)
e. Induction of inflammation (IgE)

2. **In vitro Ag-Ab reactions:**
   - These reactions refer to the Ag-Ab reactions occurring outside host.
   - In the laboratory, they help in the diagnosis of infections, in epidemiological surveys, in the identification of infectious agents and of noninfectious antigens such as enzymes.
   - Antigen-antibody reactions in vitro are known as serological reactions.
   - The reactions between antigens and antibodies occur in three stages.
   - **The primary stage** is the initial interaction between the two, without any visible effects. This reaction is rapid, occurs even at low temperatures and obeys the general laws of physical chemistry and thermodynamics. The reaction is reversible, the combination between antigen and antibody molecules being affected by the weaker intermolecular forces such as Van der Waal's forces, ionic bonds and hydrogen bonding, rather than by the firmer covalent bonding. The primary reaction can be detected by estimating free and bound antigen or antibody separately in the reaction mixture by a number of physical and chemical methods, including the use of markers such as radioactive isotopes, fluorescent dyes or ferritin.
   - **Secondary stage:** In most instances, but not all, the primary stage is followed by the secondary stage leading to demonstrable events such as precipitation, agglutination, lysis of cells, killing of live antigens, neutralization of toxins and other biologically active antigens, fixation of complement, immobilization of motile organisms and enhancement of phagocytosis.
   - When such reactions were discovered one by one, it was believed that a different type of antibody was responsible for each type of reaction and the antibodies came to be designated by the reactions they were thought to produce. Thus, the antibody causing agglutination was called agglutinin, that causing precipitation precipitin, etc., and the corresponding antigen, agglutinogen, precipitinogen, etc., By the 1920s, this view was replaced by Zinsser's Unitarian hypothesis which held that an antigen gave rise to only one antibody, which was capable of producing all the different reactions depending on the nature of the antigen and the conditions of the reaction. Both these extreme views are fallacious. While it is true that a single antibody can cause precipitation, agglutination and most of the other serological reactions, it is also true that an antigen can stimulate the production of different classes of immunoglobulin which differ in their reaction capacities as well as in other properties.
   - **Tertiary stage:** Some antigen-antibody reactions occurring in vivo initiate chain reactions that lead to neutralization of destruction of injury antigens or to tissue damage. These are the tertiary reactions and include humoral immunity against infectious disease as well as clinical allergy and other immunological disease.

3.3.2 History

1. **Landsteiner** : The remarkable scientist who discovered the **blood group** antigens and their corresponding agglutinins in **1900** , which make blood transfusion possible without creating immunological reactions. Also he developed the concept of **antigenic determinants**, and introduced the idea of the **specificity** of antibodies for chemically defined **haptens**. Landsteiner was succeeded in producing an infinite number of different antibodies to various antigens, which initiated Landsteiner to **reject Ehrilich's side chain theory**.
2. **Index Medicus** in **1910** was the first which introduced the word "**immunology"**, and in **1916** the **Journal of Immunology** was published, which was concentrated on the study of serological reaction over the period of thirty years. The book "Specificity of Serological reactions" by Landsteiner was published in different languages German, English, etc…….
3. **Tiselius and Kabat** in **1939** had indicated that the antibodies were **gamma globulins.** One year later **Pauling** introduced the variable-folding theory of antibody formation.

4. **Immunochemistry** was the normally existed as the consequences of the development in the chemical approach to immunology, such as the discovery of the natural composition of the antibodies, and the concept of antigenic determinants by Landsteiner, the **quantitative precipitin test** was developed by **Heidelberger, Kendall, and Kabat** which used for the studying of the structure of polysaccharide antigens.
5. **Coons and Landsteiner** in **1941 and 1942** each had introduced a discovery but all was related to the **cell**, which brought back the idea of the involvement of the cell in the immune response activities. Coons indicated the presence of antigens and antibodies inside the cell, and he used the **immunofluorescence technique,** while Landsteiner noted that, the delayed hypersensitivity could be transferred by cells and not by serum. The positive role of the cell in the life was confirmed by the discovery of the role of DNA in the transfer of hereditary trains in bacteria by MacCleod**, and MacCarty in 1943.** Five years later, **1948, *Fagraeus** reported that, **antibodies, and** were **made** in.
6. **Burnet and Fenner in 1949** has proposed formally the **"self-marker"** concept, which was the first explanation of self tolerance. In **1953 Billingham, Brent, and Medawar** demonstrated acquired immunologic tolerance in bone marrow chimeras in mice injected with allogeneic bone marrow at or before birth. In the same year **1953**, **Watson and Crick** described the double helix of DNA. These two findings had indicated the close association in the development of immunology and molecular biology. **Natural, randomly diversified cell "selection theory of antibodies"** in **1955** was introduced by **Jerne;** this theory had explained the immunologic memory and the logarithmic rate of rise of antibody. But this theory was not compatible with the new concepts of cellular and molecular biology.
7. Thirty years later the **clonal selection theory** by **Burnet** substituted the theory of Jerne. Burnet indicated that, the clonal selection was randomly diversified **gamma globulin molecules** rather than randomly diversified **Cell.** This theory was confirmed the discovery of the technique of producing monoclonal antibodies in **1975 by Kohler and Milstein.** .
8. By the discovery of the cooperation between B and T cells in **1966** by **Claman, Chaperon, and Triplett,** cellular immunology reached its maximum peak, thus was given by immunologist's great attention and excessive work done on the development, specificity, and activation of B and T cells.
9. **Molecular Immunology:**The analysis of the antibody molecules was possible by the development of the protein chemistry in **1959.** The immunoglobulin molecule was splits into three fragments 2 Fab and one Fc by **Porter**, and the heavy and light chains was separated by **Edelman.** The discovery of the common and variable regions of the immunoglobulin in **1965** by **Putnam, Hilschmann, and Craig** has given a strong support to the molecular immunology. Four years later **1969**, **Edelman** has introduced the complete amino acid sequence of an immunoglobulin.
10. In the period between **1960s** and **1970s** many important molecules of the immune system were separated, identified, and characterized such as interleukins, complement components, and cell receptors.
11. **Gorer in 1936** discovered the major histocompatibility antigens. But it was fully characterized in **1968** by **McKevitt and Tyan** when they showed that, immune response genes were linked to the genes of the major histocompatibility complex. **Doherty and Zinkernagel in 1974** reported the recognition of antigen by T cells is restricted by the major histocompatibility complex. The development of the recombinant DNA, then allowed the demonstration of immunoglobulin gene rearrangement by **Tonegawa in 1978. Davis in 1984** identified the genes of the T cell receptor.
12. Since that time until now a lot of developments were added to the science of immunology. Immunology has always dependent on the application of technology, such as the use of the microscopy, electrophoresis, radiolabeling, immunofluorescence, and recombinant DNA.

Immunology has not become an inbred discipline but has maintained close associations with many other fields of medical science. Immunology has spread out into all of the clinical departments.

3.3.3 Terminologies:

- **Serology** - is the science of measuring antibody or antigen in body fluids. The immune reaction is the production of antibody (substances) that protect the body against the antigen. There are times, however, when antibodies are not protective (e.g. Hay fever, rash).
- **Serologic reactions:** Serologic reactions are in vitro Antigen-antibody reactions provide methods for the diagnosis of disease and for the identification and quantitation of antigens and antibodies. Some examples of these tests are the agglutination reactions, and precipitation reactions.
- **Antibody titre:** The term titre is used to denote the highest dilution of the serum at which antibody activity is demonstrable, which is usually expressed as the reciprocal of the dilution of the serum, e.g. 64, when antibody was detected at a final serum dilution of 1 in 64. The antigens and antibodies taking part in the reaction are also measured by their mass weight, e.g. mg nitrogen. The level of antibody in serum, can be measured by using known antigens. Antibody titers can be of diagnostic and prognostic importance. For example, a rise in the antibody titer between serum taken during the acute phase of an illness and during the convalescent phase (acute and convalescent serums) can be diagnostic for that illness.
  - **Rising antibody titre**
  - **Paired sera**
- **Environmental factors** can profoundly affect the forces involved in antigen-antibody interactions for example:

Physiological pH and salt concentration promote optimal union. Forces of attraction tend to be weaker in conditions that are acid (below pH 4.0) or alkaline (above pH 10.0).

Temperature, the higher the temperature (up to a maximum of 50C), the greater the kinetic motion of the reactions and therefore the more rapid the rate of reaction.

- The serologic identification of antibody types and types of antigen-antibody reactions are based on the physical state of antigen:
  - Agglutinins are antibodies that aggregate cellular antigens.
  - Lysins are antibodies that cause dissolution of cell membranes.
  - Precipitins are antibodies that form precipitates with soluble antigens.
  - Antitoxins are antibodies that neutralize toxins.

The relative sensitivity of the various tests for antigens and antibodies are presented in Table-3.3

Table 3.3: Relative Sensitivity of Tests Measuring Antibody and Antigen

Test	Approximate Detectable Amount (µg/ml)	
Precipitation	20.0	1.0
Immunoelectorophoresis	20.0	….
Double diffusion in agar gel	1.0	….
Complement fixation	0.5	….
Radial immunodiffusion	0.05	0.5
Bacterial agglutination	0.01	….
Hemolysis	0.01	….
Passive hemagglutination	0.01	….
Hemagglutination inhibition	…..	0.001
Antitoxin neutralization	0.01	……
Radioimmunoassay (RIA)	0.0005	0.000005
Enzyme-linked immunosorbent assay (ELISA)	0.0005	0.000005
Virus neutralization	0.000005	……

- Antibody molecules combine reversibly with antigens to form immune complexes. The detection and measurements of these reactions form the basis of serology, a sub discipline of immunology.

3.3.4 Application of serology tests

Antigen tests: Antigen tests often enable an early diagnosis or presumptive diagnosis of an infectious disease through: Identification of a pathogen that has been isolated by culture. Identification of pathogens in different samples of the patients, etc

Antibody tests: These tests are used mainly to diagnose a microbial disease when the pathogen or microbial antigen is not present in routine specimen or if present is not easily isolated and identified by other available techniques.To screen donor blood for different infectious diseases. To monitor the effectiveness of a given treatment by measuring antibody liter .To diagnose autoimmune disorders, etc.

Secondary binding tests: Secondary binding tests are tests that detect and measure the consequences (secondary effect) of antigen-antibody interaction.These consequences include:

- Precipitation of soluble antigens
- Clumping (agglutination) of particulate antigens
- Neutralization of bacteria, viruses, or toxins; and
- Activation of the complement system.
- They are usually less sensitive than primary binding tests, but may be easier to perform

Table : 3.4 Antigen-Antibody reactions

Receptor	Immunoglobulin			Modified tests
	IgG	IgM	IgA	
Precipitation	S	W	V	Immunoelectrophoresis, Immunoprecipitation
Agglutination	W	S	M	Latex agglutination, Indirect haemagglutination, Coagglutination, Antiglobulin (Coombs' test).
Complement fixation	S	W	N	Conglutination.
Neutralisation	S	W	N	Plaque assay, Measurement of lethal dose (LD).
Immunofluorescence	S	W	N	Indirect immunofluorescence, Immunofluorimetric assay.
Enzyme immunoassay	S	W	N	Enzyme-linked immunosorbent assay.
Radioimmunoassay	S	W	N	Immunoradiometric assay.

S = Strong, W = Weak, V = Variable, N = Negative, M = Moderate.

3.3.5 General features of antigen-antibody reactions.

Antigen-antibody reactions have the following general characteristics:

- The reaction is specific, an antigen combining only with its homologous antibody and vice versa. The specificity, however, is not absolute and cross reactions may occur due to antigenic similarity or relatedness. The phenomenon was known as cross reactivity.
- Entire molecules react and not fragment. When an antigenic determinants present in a large molecule or on a carrier particle reacts with its antibody, whole molecules or particles are agglutinated.
- There is no denaturation of the antigen or the antibody during the reaction.
- The combination occurs at the surface. Therefore, it is the surface antigens that are immunologically relevant. Antibodies to the surface antigens of infectious agents are generally protective.
- The combination is firm but reversible. The firmness of the union is influenced by the affinity and avidity of the reaction. Affinity refers to the intensity of attraction between single epitope of the antigen and paratope of antibody molecules. It is a function of the closeness of fit between an epitope and the antigen combining region of antibody. Avidity is the strength of the bond after the formation of the antigen antibody complexes. It reflects the overall combining property of the various antibody molecules in an antiserum, possessing different affinity constants with the multiple epitopes of the antigen. Generally IgG possess greater affinity and IgM possess higher avidity and vice versa.
- Both antigen and antibodies participate in the formation of agglutinates or precipitates.
- Antigens and antibodies can combine in varying proportions, unlike chemicals with fixed valencies. Both antigens and antibodies are multivalent. Antibodies are generally bivalent, though IgM molecules may have five or ten combining sites. Antigens may have valencies up to hundreds.

3.3.6 Measurement of antigen-antibody:

Many methods are available for the measurement of antigens and antibodies participating in the primary, secondary or tertiary reactions. Measurement may be in terms of mass or more commonly as units or titre. Antigens may also be titrated against sera. Two important parameters of serological tests are sensitivity and specificity. Sensitivity refers to the ability of the test to detect even very minute quantities of antigen or antibody. When a test is highly sensitive, false negative results will be absent or minimal. Specificity refers to the ability of the test to detect reactions between homologous antigens and antibodies only and with no other. In a highly specific test, false positive reactions will be absent or minimal. In general, sensitivity and specificity of a test will be in inverse proportion.

Techniques used to measure antigen-antibody called as immunotechniques, which broadly classified into two categories namely primary techniques and secondary techniques.

Primary techniques utilized the physical changes occurring during antigen antibody interaction for estimation whereas secondary techniques utilize labels or markers to estimate antigen and antibodies.

3.3.7 PRECIPITATION:

Precipitation reaction is the reaction, in which a soluble antibody reacts with a soluble antigen to give an insoluble product or the precipitate. Soluble antibodies that aggregate soluble antigens are called precipitins. Soluble antigen that induces the formation of a specific precipitin is called a precipitinogen. The antigens, which have two or more epitopes per molecule are cross-linked by the bivalent antibodies, a lattice formation occurs which ultimately develops into a visible precipitate. For the lattice to be formed, the bivalent antibody will bind to epitopes on two different antigens so that the complex is formed. The complex continues to grow and when it is sufficiently large, it becomes insoluble and can be visible as a precipitate. Precipitation reaction can occur using polyclonal antibodies or mixture of monoclonal antibodies. There is a marked effect on the precipitation reaction by changing the concentration of the antigen.

A. Lattice hypothesis

Marrack in 1934 proposed the *lattice hypothesis* to explain the prozone phenomenon. Marrack's hypothesis is based on the assumptions that each antibody molecule must have at least two binding sites, and antigen must be multivalent. In the zone of equivalence where optimum precipitation occurs, the number of multivalent sites of antigen and antibody are approximately equal. In this zone, precipitation occurs as a result of random, reversible reactions whereby each anti-body binds to more than one antigen and vice versa, forming a stable network or lattice.

As they combine, it results in a multimolecular lattice that increases in size until it precipitates out of solution. The prozone and postzone phenomena are taken into consideration in the interpretation of serological tests, because false negative reactions can occur in either of these conditions. A false negative reaction suspected to be due to prozone phe-nomenon can be rectified by diluting out the antibody and performing the test. In the postzone phenomenon, excess anti-gen may obscure the presence of small amount of antibodies.

Typically, such a test is repeated with an additional patient specimen taken about a week later. This would give time for the further production of antibodies. If the test is negative on this occasion, it is unlikely that the patient has that particular antibody.

A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigens to the tubes. Plotting the amount of precipitate against increasing antigen concentrations yields a precipitation curve.

A precipitation curve for a system of antigen-antibody in the figure shows three zones, among which the first one is the zone of antibody excess or prozone, in which the antigen concentration is very low and that of the antibody is relatively high, as a result of which precipitation is inhibited, formation of small complexes occur and residual antibodies will remain in the supernatant.

The second zone is the equivalence zone, also the zone of maximal precipitation in which antigen and antibody form large insoluble complexes and there is neither antigen nor antibody present in the supernatant. The third zone is the zone of antigen excess or postzone, in which the antigen concentration is very high, and therefore with increasing the amounts of antigen, the lattice size becomes too small to precipitate as a result of which precipitation is inhibited and binding of antigen-antibody is absent in the supernatant.

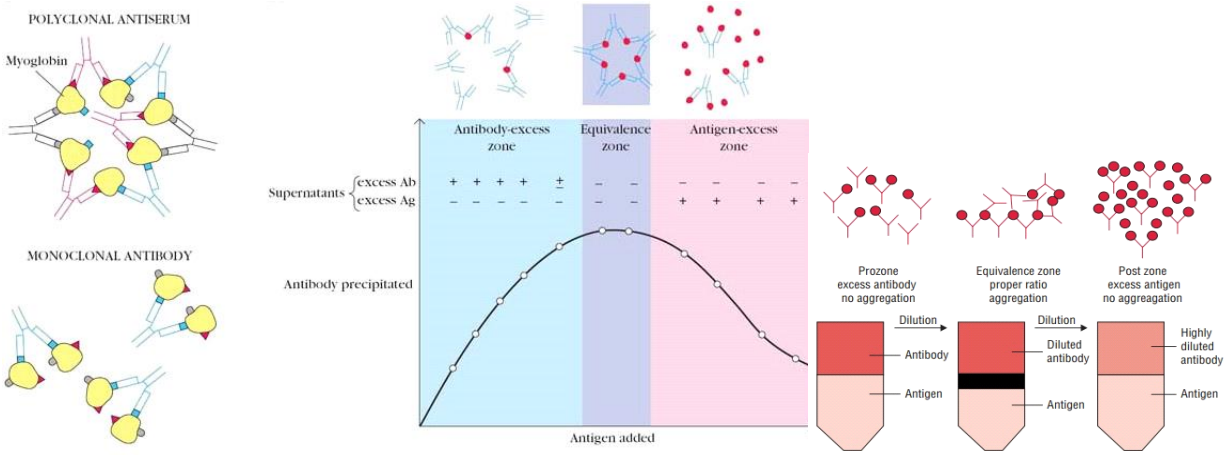


Figure: 3.17 Marracks lattice hypothesis

Table: 3.6 Precipitation tests

Test	Procedure	Diffusion	Uses
Simple precipitation (Precipitation in liquid medium)			
1. Ring test (Fig. 9.1)	Ag solution layered over a column of whole serum (Ab) in a narrow test tube	A ring of ppt. forms at junction of two	Ascoli's thermoprecipitin test, streptococcal grouping
2. Slide test	A drop each of Ag solution and serum placed on a slide, mixed by shaking	Floccules formed	VDRL
3. Tube flocculation	Serial dilutions of Ag made in test tube to which fixed amount of serum added	Amount of Ag that flocculates the serum is called Lf dose	Kahn test
Immunodiffusion (Precipitation in gel)			
1. Oudin [Fig. 9.2(i)]	Serum (Ab) incorporated in agar gel in a test tube and Ag solution layered over it	Antigen diffuses downward forming line of ppt	Single diffusion in one dimension
2. Oakley Fulthorpe variation of Oudin [Fig. 9.2(ii)]	Ab incorporated in gel, above which is placed a column of agar and Ag is layered on surface of plain agar	Ag and Ab move towards each other through plain agar and forms a band of ppt	Double diffusion in one dimension — used in Elek test
3. Radial (Fig. 9.3)	Ab incorporated in agar gel in Petri dish or slide, Ag kept in well cut out gel	Ag diffuses, concentric bands of precipitin form around the well	Single diffusion in two dimensions — used in complement components detection and their assay
4. Ouchterlony (Fig. 9.4)	Agar gel poured on a slide, wells are cut using a template. Ab placed in central well and Ags in surrounding wells	When adjacent Ags are identical, lines of ppt fuse; if unrelated, lines of ppt cross each other	Double diffusion in two dimensions — used in detection of Ags and their relatedness
5. Immunoelectrophoresis (Fig. 9.5)	(i) Ag placed in a well in agar gel in a slide. Electric current passed. Ags get separated. (ii) A trough is then cut in agar and filled with Ab.	By diffusion lines of ppt formed with each separated Ag	Detection of myeloma band
Counter immunoelectrophoresis (Fig. 9.6)			
	Simultaneous electrophoresis of Ag and Ab in gel is done in opposite direction	Ppt occurs at a point between the two	

B. Precipitation in liquids

A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. At one time this method was used to measure the amount of antigen or antibody present in a sample of interest. After the precipitate forms, each tube is centrifuged to pellet the precipitate, the supernatant is poured off, and the amount of precipitate is measured.

The following types of precipitation tests are in common use:

- **Ring test**: This, the simplest type of precipitation test, consists of layering the antigen solution over a column of antiserum in a narrow tube. A precipitate forms at the junction of the two liquids. Ring tests have only a few clinical applications now. Examples are Ascoli's thermoprecipitin test and the grouping of streptococci by the Lancefield technique.

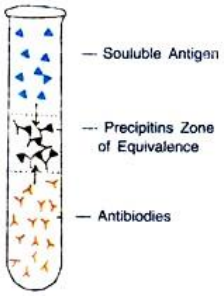


Figure: 3.18 The precipitation ring test (Ascoli test)

- **Slide test**: When a drop, each of the antigen and antiserum are placed on a slide and mixed by shaking flocculates appear. **Example**: The VDRL (Venereal Disease Research Laboratory) test for syphilis (*Treponema pallidum).* This test will detect antibodies against *Treponema pallidum,* and these antibodies appear in 4 to 6 weeks. VDRL is a nontreponemal test which detects reagin and antibodies that act against cardiolipin as an antigen. VDRL may show the negative result is late syphilis.

Figure: 3.19 VDRL test

- **Tube test**: The kahn test for syphilis is an example of a tube flocculation test. A quantitative tube flocculation test is employed for the standardization of toxins and toxoids. Serial dilutions of the toxin/toxoid are added to the tubes containing a fixed quantity of the antitoxin. The amount of toxin or toxoid that flocculates optimally with one unit of the antitoxin is defined as Lf dose.

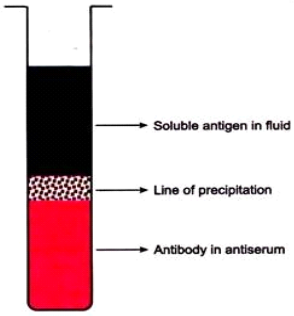


Figure: 3.20 Tube precipitation test

Limitation of precipitation in fluids: The reaction is not visible as a distinct band of precipitation. Band is not stable and can not be stained for preservation.

C. Precipitation in gel:

There are several advantages in allowing precipitation to occur in a gel rather than in a liquid medium. The reaction is visible as a distinct band of precipitation, which is stable and can be stained for preservation, if necessary. As each antigen-antibody reaction gives rise to a line of precipitation, the number of different antigens in the reacting mixture can be readily observed. Immunodiffusion also indicates identity, cross reaction and nonidentity between different antigens. Immunodiffusion is usually performed in a soft 1% agar gel. Immunodiffusion divided into two type namely simple immunodiffusion and electroimmunodiffusion.

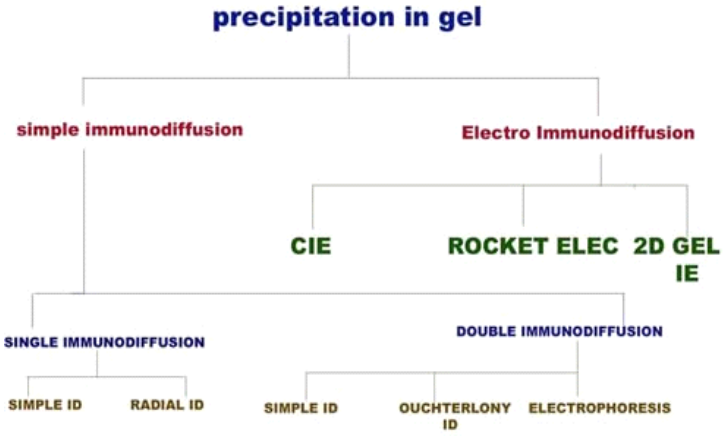


Figure: 3.21 types of precipitation in gel

a. **SIMPLE IMMUNODIFFUSION:**

Simple Immunodiffusion refers to the diffusion of antigens and antibodies without any external forces. This technique further divided into two types namely Single immunodiffusion and double immunodiffusion.

Single immunodiffusion: In this type of immunodiffusion, only antigen or antibody diffuses in gel.

- ***Single diffusion in one dimension (Oudin Procedure):*** The antibody is incorporated in agar gel in a test tube and the antigen solution is layered over it. The antigen diffuses downward through the agar gel, forming a line of precipitation that appears to move downwards. This is due to the precipitation formed at the advancing front of the antigen and is dissolved as the concentration of antigen at the site increases due to diffusion. The number of bands indicates the number of different antigens present.

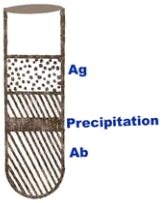


Figure: 3.21 Oudin procedure of single diffusion in one direction

- **Single diffusion in two dimensions (Radial immunodiffusion):**

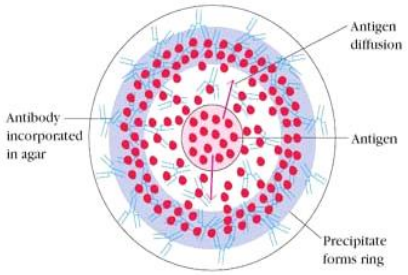


Figure: 3.22 Mancini procedure of single diffusion in two direction (Radial Immunodiffusion)

Here the antiserum is incorporated in agar gel poured on a flat surface of slide or Petri dish. The antigen is added to the wells cut on the surface of the gel. The antigen diffuses radially from the well and forms ring shaped bands of precipitation (halos) concentrically around the well. The diameter of the halo gives an estimate of the concentration of the antigen. This method has been employed for the estimation of the immunoglobulin classes in sera. It has also been used for screening sera for antibodies to influenza viruses. This method is also otherwise known as Mancini method.

The Mancini method is routinely used to quantitate serum levels of IgM, IgG and IgA by incorporating class-specific anti-isotype antibody into the agar. The technique is also applied to determine the concentrations of complement components in serum. The Mancini method cannot detect antigens present in concentrations below 5-10ug/ml; this moderate sensitivity is the major limitation of the radial immunodiffusion method. This method is used to determine alpha fetoprotein occurring in certain liver tumor.

- **Double immunodiffusion:**

In double immunodiffusion, double refers to the diffusion of both antigens and antibodies. Under this heading, simple double immunodiffusion, Ouchterlony procedure and immunoelectrophoresis are studied.

❖ **Simple double immunodiffusion (Oakley Fulthorpe procedure):**

It is double immunodiffusion in one dimension. Here, the antibody is incorporated in gel, above which is placed a column of plain agar. The antigen is layered on top of this. The antigen and antibody move towards each other through the intervening column of plain agar and form a band of precipitate where they meet at optimum proportion. This technique usually used to identify the presence of either antigen or antibody.

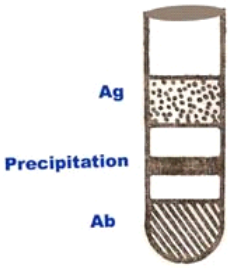


Figure: 3.23 Oakley Fulthorpe procedure of double diffusion in one dimension

❖ **Ouchterlony Procedure:**

It is double diffusion in two dimensions. This is the immunodiffusion method most widely employed and helps to compare different antigens and antisera directly. Agar gel is poured on a slide and wells are cut using a template. The antiserum is placed in the central well and different antigens in the surrounding well. If two adjacent antigens are identical, the lines of precipitate formed by them will fuse and prove "V" shaped curve. If they are unrelated, the lines will cross each other and provide "X" shaped curve. Cross reaction or partial identiy is indicated by spur formation and provide "Y" shaped curve. This method was a routine technique for the diagnosis of smallpox. When extracts of smallpox lesions are tested against the antiserum, precipitation lines can be seen within 2-6 hours. A special variety of double diffusion in two dimensions is the Elk test for toxigenicity in diphtheria bacilli. When diphtheria bacilli are streaked at right angles to a filter paper strip carrying the antitoxin implanted on a plate of suitable medium, arrowhead shaped lines of precipitation appear on incubation, if the bacillus is toxigenic.

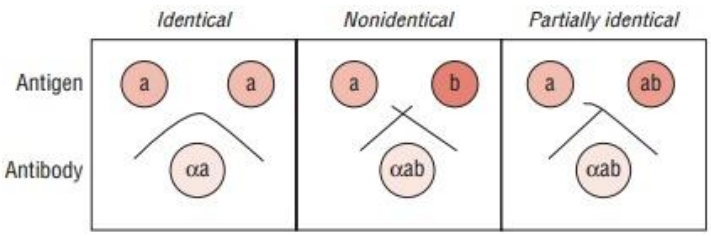


Figure: 3.24 Ouchterlony procedure of double diffusion in two direction

❖ **Immunoelectrophoresis:**

Immunoelectrophoresis, the antigen mixture is first electrophoresed to separate its components by charge. Troughs are then cut into the agar gel parallel to the direction of the electric field, and antiserum is added to the troughs. Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropriate proportions. Immunoelectrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum. A sample of serum is electrophoresed, and the individual serum components are identified with antisera specific for a given protein or immunoglobulin class.

This technique is useful in determining whether a patient produces abnormally low amounts of one or more isotypes, characteristic of certain immunodeficiency diseases. It can also show whether a patient overproduces some serum protein, such as albumin, immunoglobulin, or transferrin. The immunoelectrophoretic pattern of serum from patients with multiple myeloma, for example, shows a heavy distorted arc caused by the large amount of myeloma protein, which is monoclonal Ig and therefore uniformly charged.

Because immunoelectrophoresis is a strictly *qualitative* technique that only detects relatively high antibody concentrations (greater than several hundred ug/ml), it utility is limited to the detection of quantitative abnormalities only when the departure from normal is striking, as in immunodeficiency states and immunoproliferative disorders. Over 30 different proteins can be identified by this method in human serum. This is useful for testing normal and abnormal proteins in serum and urine. It is used for the identification of myeloma proteins.

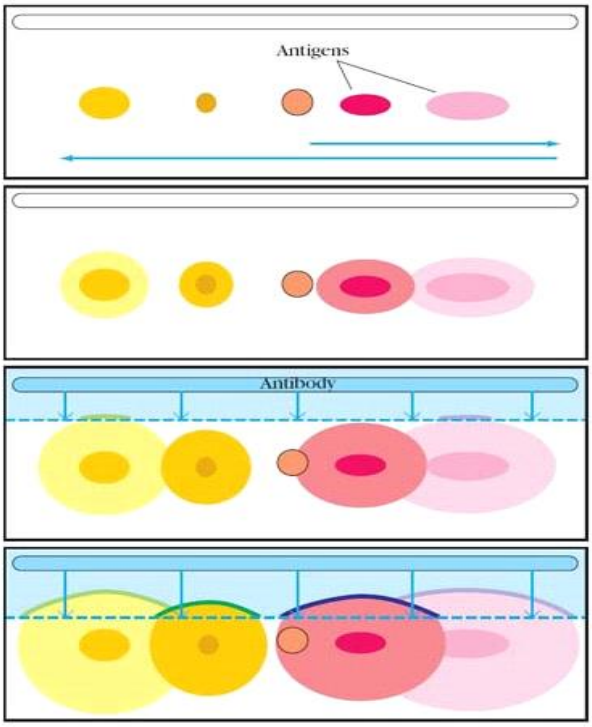


Figure: 3.25 Immuno electrophoresis

b. ELECTROIMMUNODIFFUSION:

The development of precipitin lines can be speeded up by electrically driving the antigen and antibody. Various methods have been described combining electrophoresis with diffusion. In this technique, charge of antibody and antigen plays vital role. Of these, one dimensional double electroimmunodiffusion (Counter currnet Immunoelectrophoresis), one dimensional single electrodiffusion (rocket electrophoresis) and two dimensional immunoelectrophoresis are used frequently in the clinical laboratory.

❖ **Countercurrent Immunoelectrophoresis (CIE):**

This involves simultaneous electrophoresis of the antigen and antibody in gel in opposite directions resulting in precipitation at a point between them. This method produces visible precipitation lines within thirty minutes and is ten times more sensitive than the standard double diffusion techniques. The clinical applications are for detecting various antigens such as alpha fetoprotein in serum and specific antigens of cryptococccus and meningococcus in cerebrospinal fluid.

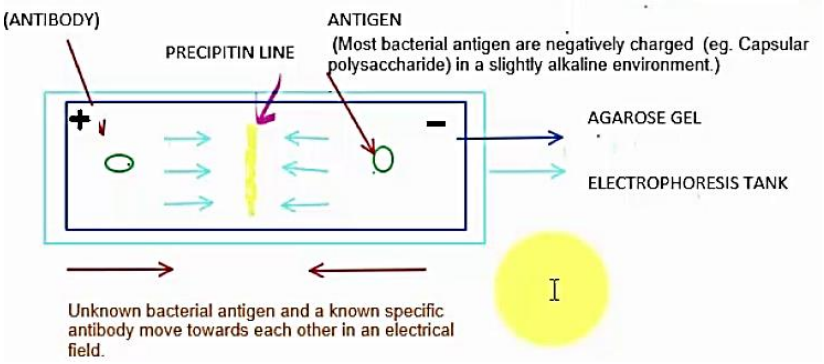


Figure: 3.26 Countercurrent Immunoelectrophoresis (CIE):

This method is used to determine anti-nuclear factor in systemic lupus erythromatosus. It is also used to determine hepatitis B antigen or antibody present in eh serum.

❖ **Rocket electrophoresis:**

The antiserum to the antigen to be quantified is incorporated in agarose and gelled on the glass slide. The antigen, in increasing concentrations, is placed in wells punched in the set gel. The antigen is then electrophoresed into the antibody containing agarose. The pattern of immunoprecipitation resembles a rocket and hence the name. The main application of this technique is for quantitative estimation of antigens. This method is useful in the quantitative determination of proteins such as albumin, transferrin, etc.; one limitation of rocketelectrophoresis is the need for the antigen to be negatively charged for electrophoretic movement within the agar matrix.

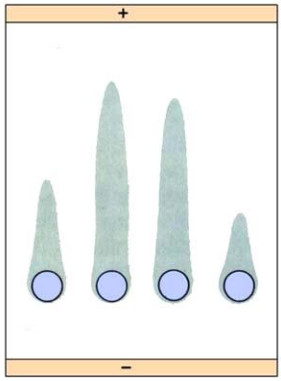


Figure: 3.27 Rocket Immunoelectrophoresis (CIE)

❖ **Two dimensional immunoelectrophoresis:**

Several antigens in a complex mixture can be quantitated simultaneously with a modification of rocket electrophoresis called two-dimensional immunoelectrophoresis. In this technique antigen is first separated into components by electrophoresis. The gel is then laid over another agar gel containing antiserum and electrophoresis is repeated at right angles to the first direction, forming precipitin peaks similar to those obtained with rocket electrophoresis. Measurement of the size of the peaks allows quantification of a number of proteins in a complex antigen mixture.

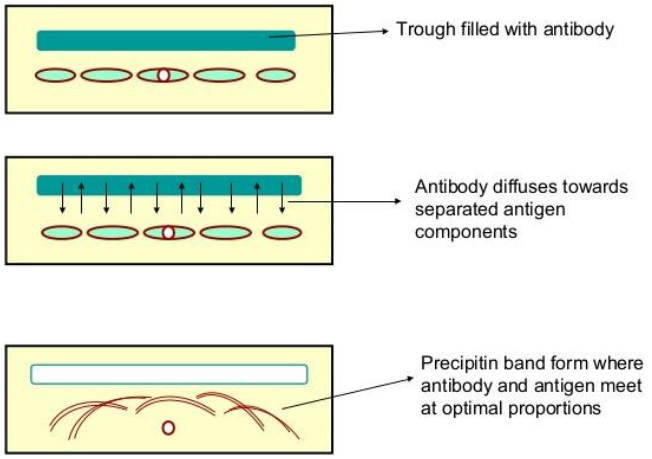


Figure: 3.28 Two dimensional immunoelectrophoresis

3.3.8 Agglutination:

The interaction between antibody and a particulate antigen results in visible clumping, called agglutination. The general term agglutinin is used to describe antibodies that agglutinate particulate antigens. Agglutination is a serological reaction and is very similar to the precipitation reaction. Both reactions are highly specific because they depend on the specific antibody and antigen pair. As an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions, this inhibition is known as prozone effect. The main difference between these two reactions is the size of antigens. For precipitation, antigens are soluble molecules, and for agglutination, antigens are large, easily sedimented particles. Agglutination reactions can be used to type blood cells for transfusion, to identify bacterial cultures, and to detect the presence and relative amount of specific antibody in a patient's serum.

Types of agglutination reactions

- **Quantitative agglutination (Bacterial agglutination):**

Agglutination has been commonly used to determine whether a patient had or has a bacterial infection. This type of agglutination reaction is also known as quantitative agglutination test as here the measurement of level of antibodies to particulate antigens is done. The presence of serum antibodies in a person specific for surface antigens on the bacterial cells can be detected by bacterial agglutination reactions. If a patient is suspected of having typhoid fever, the patient's serum is mixed with a culture of *Salmonella typhi*. If an agglutination reaction occurs, shown as clumping of the bacteria, the patient either had or has an *S. typhi* infection. Agglutination reactions also help to type bacteria. Example : Widal test and The Weil Felix reaction: for serodiagnosis of typhus fever

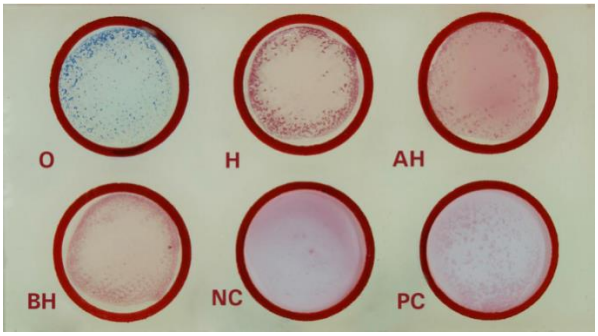


Figure: 3.29 Widal test

- **Haemagglutination (slide agglutination):**

When RBC bound allowed to interact specifically with complementary antibody, they agglutinate, forming a visible clump on the slide. At neutral pH, red blood cells are surrounded by a negative ion cloud that makes the cells repel one another this repulsive force is called zeta potential. Because of its size and pentameric in nature, IgM can overcome the zeta potential and cross link red blood cells, leading to agglutination. The smaller size and bivalency of IgG makes it less able to overcome the zeta potential. For this reason, IgM is more effective than IgG in agglutinating red blood cells.

Example: Blood grouping: In typing for the ABO antigens, R:BCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions.

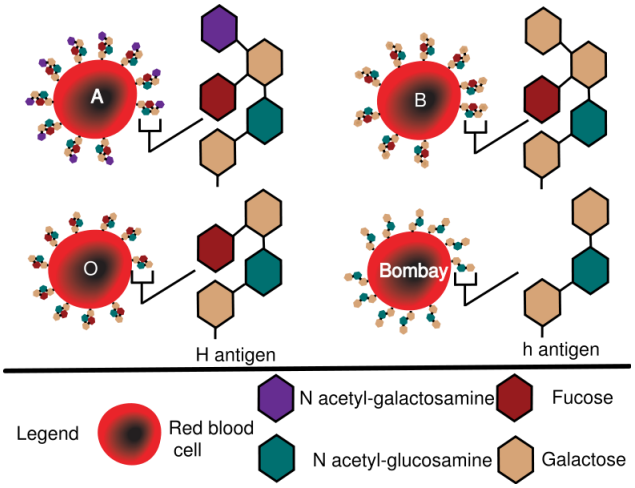


Figure: 3.30 Blood group antigens

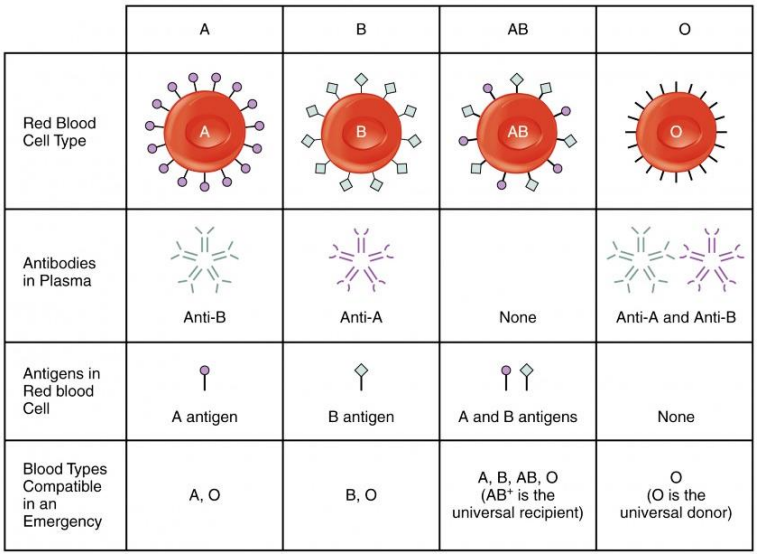


Figure: 3.31 Blood grouping test

❖ **Passive agglutination:**

Passive agglutination is like agglutination reaction but performed with soluble antigens. It is defined as the agglutination of particles that have been coated with soluble antigen, by antiserum specific for the adsorbed antigen. Passive hemagglutination is a kind of passive agglutination in which erythrocytes, usually modified by mild treatment with tannic acid or chromium chloride, is used to adsorb soluble antigen onto their surface, and which then agglutinate in the presence of antiserum

specific for the adsorbed antigen. Passive agglutination can be performed with tanned erythrocytes or synthetic particles, such as latex beads. The use of synthetic beads offers the advantages of consistency, uniformity and stability.

Example: Latex agglutination test (Latex fixation test) are widely employed in the clinical laboratory for the detection of hepatitis B, ASO, CRP, RA factor, HCG and many other antigens. A special type of passive hemagglutination test is the Rose-Waaler test.

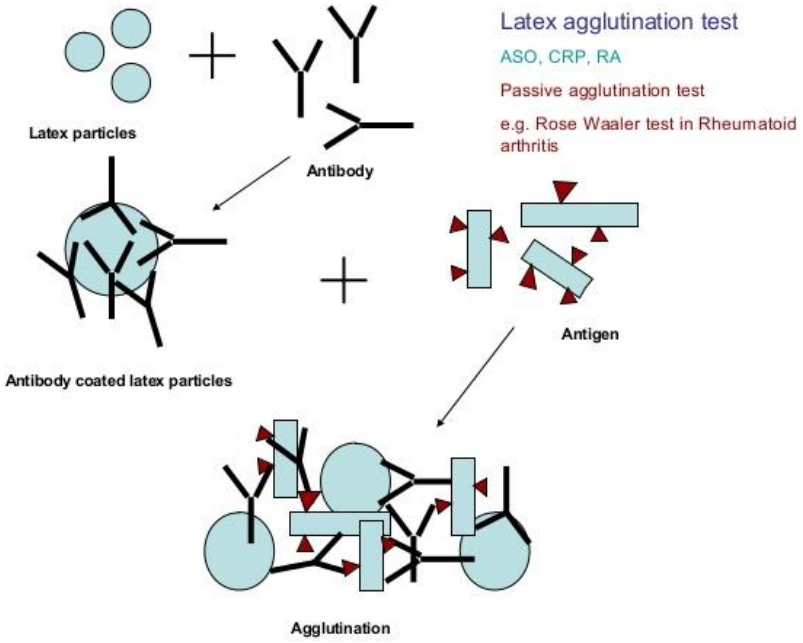


Figure: 3.32 Latest agglutination test

❖ **Agglutination inhibition:**

Agglutination inhibition is the modification of the agglutination reaction. If the antibody is incubated with antigen prior to mixing with latex, agglutination is inhibited; this is because free antibodies are not available for agglutination. In agglutination inhibition, the absence of agglutination is diagnostic of antigen, provides a high sensitive assay for small quantities of antigen. A agglutination inhibitionprovides a highly sensitive assay for small quantities of an antigen.

For example, one of the early types of home pregnancy test kits included latex particles coated with human chorionic gonadotropin (HCG) and antibody to HCG.

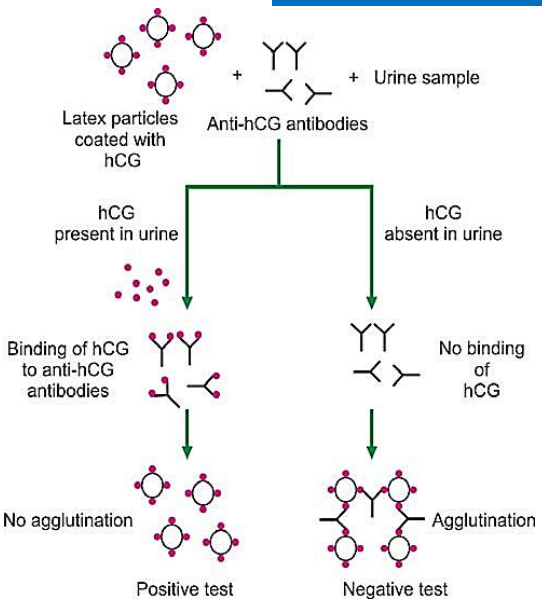


Figure: 3.33 Pregnancy test based on agglutination inhibition

❖ **Antiglobulin Test (Coombs Test):**

The antiglobulin test was devised by Coombs, Mourant and Race for the detection of anti-Rh antibodies that do not agglutinate Rh positive erythrocytes in saline. When sera containing incomplete anti-Rh antibodies are mixed with Rh positive red cells, the antibody globulin coats the surface of the erythrocytes, though they are not agglutinated. When such erythrocytes coated with the antibody globulin are washed free of all unattached protein and treated with a rabbit antiserum against human gammaglobulin (antiglobulin or Coombs serum), the cells are agglutinated. The Coombs test may be of the direct or the indirect type.

In the direct Coombs test, the sensitization of the erythrocytes with incomplete antibodies takes place in vitro, as in the hemolytic disease of the newborn due to Rh incompatibility. When the red cells of erythroblastotic infants are washed free of unattached protein and then mixed with a drop of Coombs serum, agglutination results. For unknown reasons, the direct Coombs test is often negative in hemolytic disease due to ABO incompatibility.In the indirect Coombs test, sensitization of red cells with the antibody globulin is performed in vitro. Originally employed for detection of anti-Rh antibodies, the Coombs test is useful for the demonstrating any type of incomplete or non-agglutinating antibody as for example, in brucellosis.

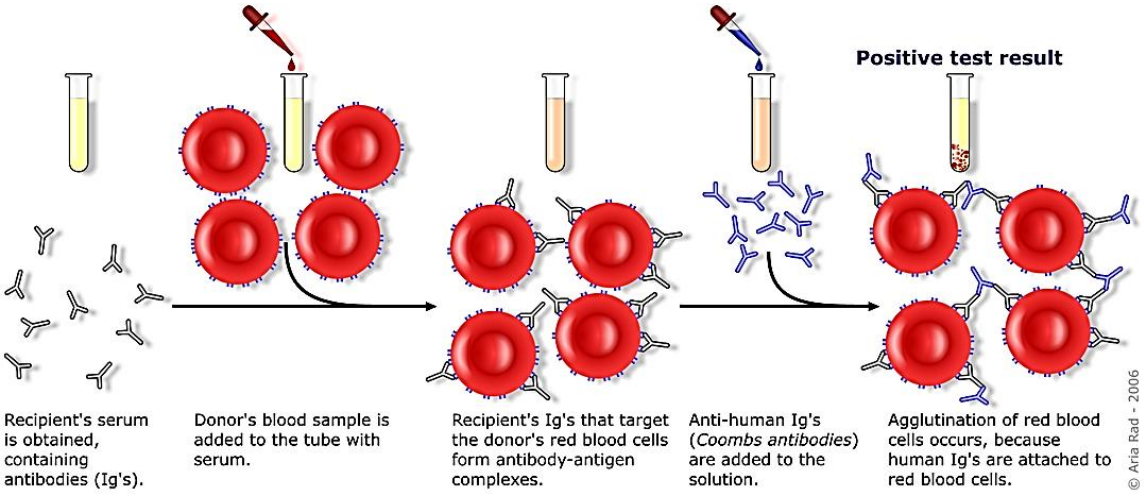


Figure: 3.34 Indirect Coombs test / Indirect Antiglobulin test

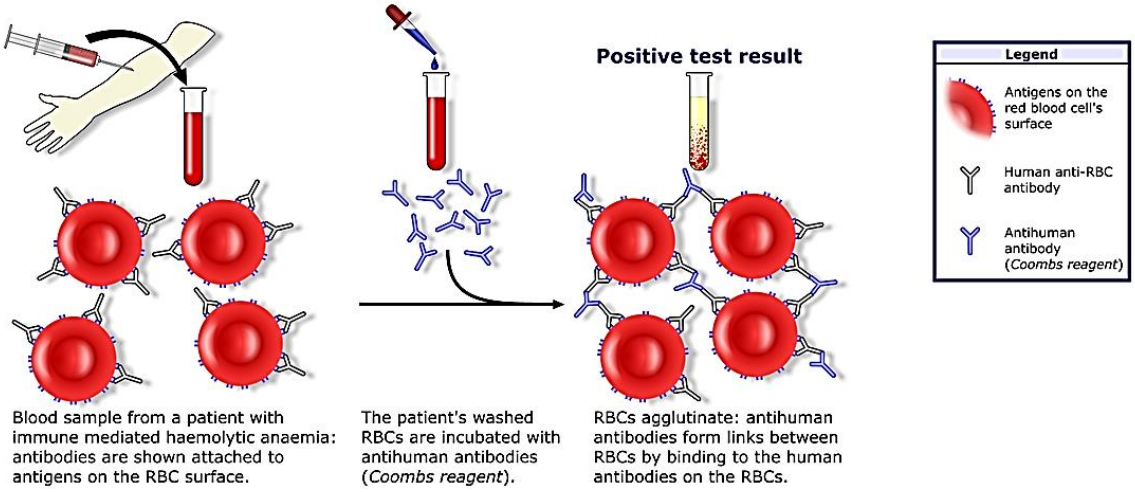


Figure: 3.35 Direct Coombs test / Direct Antiglobulin test

Table 3.1. Mechanisms of Select Antibody-Antigen Assays

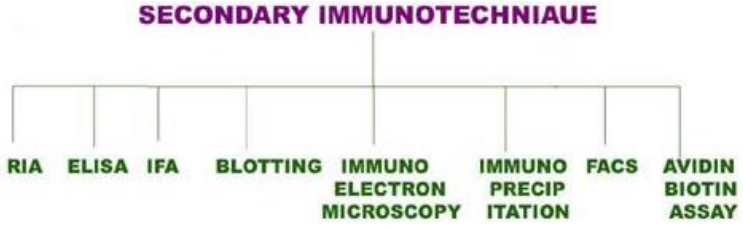
Type of Assay	Mechanism	Example
Agglutination	Direct: Antibody is used to clump bacterial cells or other large structures	Serotyping bacteria
	Indirect: Latex beads are coupled with antigen or antibody to look for antibody or antigen, respectively, in patient serum	Confirming the presence of rheumatoid factor (IgM-binding Ig) in patient serum
Hemagglutination	Direct: Some bacteria and viruses cross-link red blood cells and clump them together	Diagnosing influenza, mumps, and measles
	Direct Coombs' test (DAT): Detects nonagglutinating antibodies or complement proteins on red blood cells *in vivo*	Checking for maternal antibodies binding to neonatal red blood cells
	Indirect Coombs' test (IAT): Screens an individual for antibodies against red blood cell antigens (other than the A and B antigens) that are unbound in a patient's serum *in vitro*	Performing pretransfusion blood testing
	Viral hemagglutination inhibition: Uses antibodies from a patient to inhibit viral agglutination	Diagnosing various viral diseases by the presence of patient antibodies against the virus
	Blood typing and cross-matching: Detects ABO, Rh, and minor antigens in the blood	Matches donor blood to recipient immune requirements

Chapter-4

ANTIGEN-ANTIBODY REACTION II

4.1 Tagged antibody test:

In these techniques, antigen-antibody complexes are identified by using labels like enzyme, radioisotopes, fluorescent substances etc.



4.1.1 Enzyme immuno assay (EIA):

Enzymes labeled conjugates were introduced first in 1966 for localization of antigens in tissues, as an alternative for fluorescent conjugates. In 1971, enzyme labeled antigens and antibodies were developed as serological reagents for assay of antibodies and antigens. Their versatility, sensitivity, simplicity, economy and absence of radio-hazard have made EIA the most widely used procedure in clinical serology.

In this technique, enzyme become active only when it binds to its target molecule and that is why there is no need to separate enzyme and reaction mixture. The major type of EIA is enzyme linked immunosorbent assay (ELISA).

Enzyme Linked Immunosorbent Assay (ELISA):

Enzyme-linked immunosorbent assay, commonly known as ELISA, is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts

with a colorless substrate to generate a colored reaction product. Such a substrate is called a chromogenic substrate. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and ß-galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly. There are three types of ELISA, namely Indirect, Sandwitch and Competitive ELISA.

A. Direct ELISA:

This is the simplest form of ELISA (Figure 1). Here an antigen is attached passively to a plastic solid phase by a period of incubation. After a simple washing step, antigen is detected by the addition of an antibody that is linked covalently to an enzyme. After incubation and washing, the test is developed by the addition of a chromogen/substrate whereby enzyme activity produces a color change. Color development is read after a defined time or after enzyme activity is stopped by chemical means at a defined time. Color is read in a spectrophotometer.

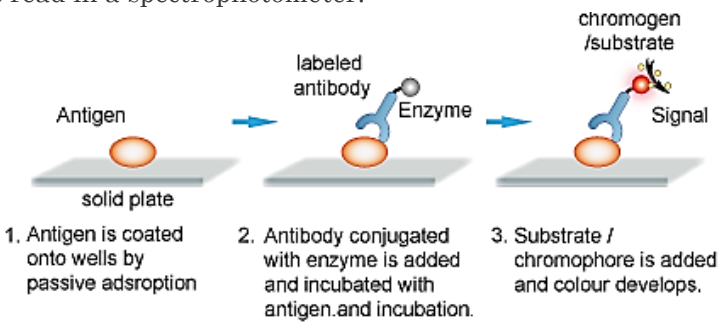


Figure: 4.1 Direct ELISA

B. INDIRECT ELISA: Antibody can be detected or quantitatively determined with an indirect ELISA. Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds. Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay, recombinant envelope and core proteins of HIV are adsorbed solid-phase antigens to microtiter wells. Individuals infected with HIV will produce serum antibodies to epitopes on these viral proteins. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection.

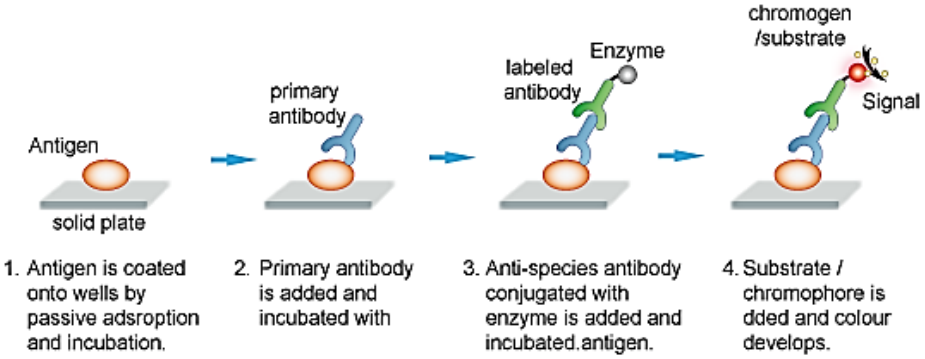


Figure: 4.2 Indirect ELISA

C. SANDWICH ELISA

Antigen can be detected or measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed; a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

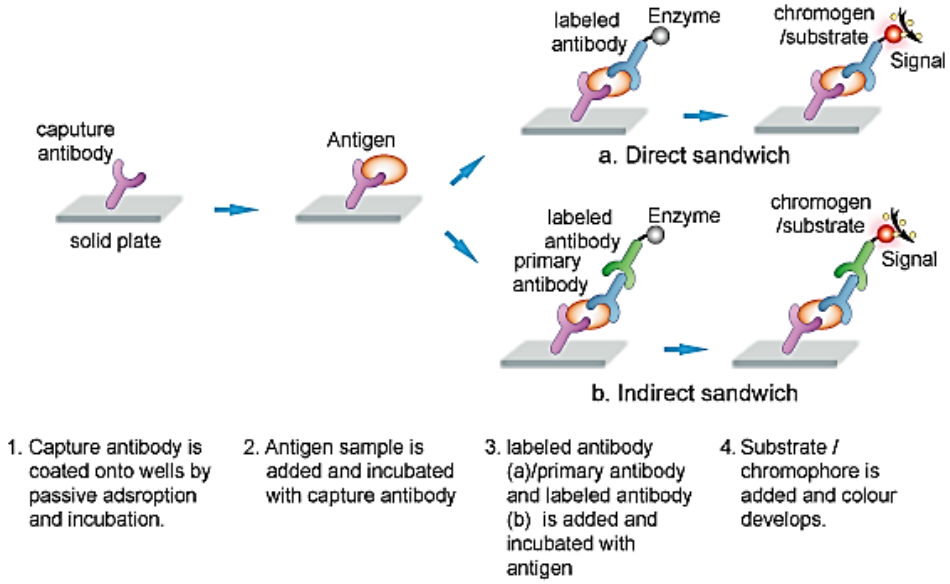


Figure: 4.3 Sandwich ELISA

D. COMPETITIVE ELISA

Another variation for measuring amounts of antigen is competitive ELISA. In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen coated microtiter well. The more antigens present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.

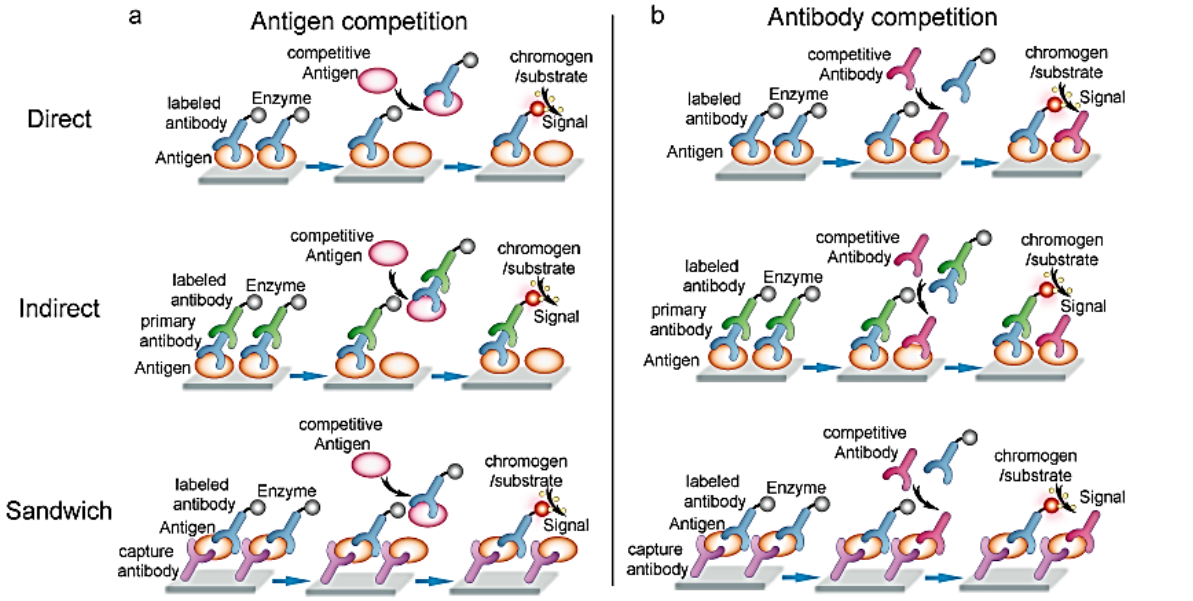


Figure: 4.4 The flowchart of antigen competition ELISA (a) and antibody competition ELISA (b)

E. CASSETTE ELISA: A simple modification of ELISA which has found wide application for testing one or a few samples of sera at a time is the cylinder or cassette ELISA. Here each specimen is tested in a separate disposable cassette. The test is rapid, taking only about 10 minutes as compared to 2-4hours which are taken for microplate ELISA. There is no need for microplate washers or readers. The result is read visually. Inbuilt positive and negative controls are usually provided for validation of the test procedure. An example of cassette ELISA is the one used for the detection of HIV type 1 and 2 antibodies. Specific type 1 and 2 antigens are immobilized at separate fixed sites on the nitrocellulose membrane in the cassette. Test serum is added on the membrane and allowed to filter into absorbent material placed below it in the cassette base. Antibody, if present in the serum will bind to the appropriate antigen. After washing to remove unbound antibody, enzyme labeled antihuman immunoglobulin antibody is added. After additional washing to remove unbound conjugate, a substrate yielding a colored product is added. A positive result is indicated by a colored spot developing at the site of the antigen against which antibody is present in the serum. Human immunoglobulin immobilized at a spot on the membrane acts as a control for the test procedure, as shown by the development of the color at the site.

Table 4.1 Comparison of Direct and Indirect ELISA

	Advantages	Limitations
Direct ELISA	Quick, only one antibody and fewer steps are used. No cross-reactivity of secondary antibody	Immune reactivity of the primary antibody might be adversely affected by labeling. No flexibility in choice of primary antibody label from one experiment to another. Minimal signal amplification.
Indirect ELISA	Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection. Maximum immune reactivity of the primary antibody is retained because it is not labeled. Sensitivity is increased because each	Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal. An extra incubation step is required in the procedure.

	primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification.	

4.1.2 Immunofluroscence

Fluorescence is the property of absorbing light rays of one particular wavelength and emitting rays with a different wavelength. Fluorescent dyes show up brightly under ultraviolet light as they convert ultraviolet into visible light. Coons and his colleagues (1942) showed that fluorescent dyes can be conjugated to antibodies and that such labeled antibodies can be used to locate and identify antigens in tissue. Because of this reason, immunofluorescence technique is also a type of immunohistochemistry assay.

The most commonly used fluorescent dyes are fluorescein and rhodamine but other highly fluorescent substances such as phycoerythrin and phycobiliproteins have also been used. Dyes can be conjugated to the Fc region of an antibody molecule without affecting the specificity of antibody. Fluorescein, an organic dye that is the most widely used label for immunofluorescence procedures absorb blue light (490nm) and emits an intense yellow green fluorescence (517nm). Rhodamine, another organic dye absorb in the yellow green range (515nm) and emits a deep red fluorescence (546nm).

Fluorescent-antibody staining of cell membrane molecules or tissue sections can be direct or indirect. In direct staining, the specific antibody (the primary antibody) is directly conjugated with fluorescein; in indirect staining, the primary antibody is unlabeled and is detected with an additional fluorochrome-labeled reagent. A number of reagents have been developed for indirect staining. The most common is a fluorochrome-Labeled secondary antibody raised in one species against antibodies of another species, such as fluorescein-labeled goat anti-mouse immunoglobulin.

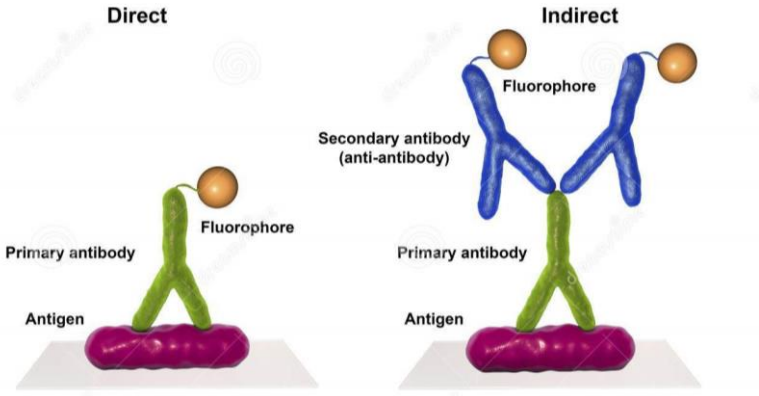


Figure: 4.5 Direct and Indirect Immunofluorescence

Indirect immunofluorescence staining has two advantages over direct staining. First, the primary antibody does not need to be conjugated with a fluorochrome. Because the supply of primary antibody is often a limiting factor, indirect methods avoid the loss of antibody that usually occurs during the conjugation reaction. Second, indirect methods increase the sensitivity of staining because multiple molecules of the fluorochrome reagent bind to each primary antibody molecule, increasing the amount of light emitted at the location of each primary antibody molecule. In indirect staining procedure, Protein A molecule labeled with flurochrome used to detect the primary antibodies. The protein A is obtained from cell wall of staphylococcus aureus. Its ability to bind with Fc region of immunoglobulins utilized in this assay procedure. It might also used in other techniques like RIA and ELISA instead of secondary antibodies. Immunofluorescence has been applied to identify a number of subpopulations of lymphocytes, notably the CD4 and CD8 T-cell subpopulations. The technique is also suitable for

identifying bacterial species, detecting Ag-Ab complexes in autoimmune disease, detecting complement components in tissues, and localizing hormones and other cellular products stained in situ. Indeed, a major application of the fluorescent-antibody technique is the localization of antigens in tissue sections or in subcellular compartments. Because it can be used to map the actual location of target antigens, fluorescence microscopy is a powerful tool for relating the molecular architecture of tissues and organs to their overall gross anatomy.

Table 4.2 Direct and Indirect Immunofluorescence

	Direct	Indirect
Time	Protocols for direct IF are usually shorter as they only require one labeling step.	The fact that you have to use a conjugated secondary antibody to detect the primary antibody results in additional steps.
Cost	Conjugated primary antibodies are usually more expensive than their unconjugated counterparts.	Secondary antibodies are relatively inexpensive compared to primary antibodies. Further cost savings may be made by using the same conjugated secondary antibody to detect different primary antibodies.
Complexity	Fewer steps in the protocol simplify direct methods.	Added complexity in indirect methods may result from having to select the appropriate secondary antibody. This is particularly relevant in multiplex experiments where several secondary antibodies, each targeting a different species and conjugated to different dyes, are needed.
Flexibility	Commercially available pre-conjugated primary antibodies limit your flexibility.	The possibility of using different conjugated secondary antibodies adds greater flexibility.
Sensitivity	The signal obtained in direct methods may seem weak when compared to indirect methods as signal amplification provided by the use of secondary antibodies does not occur.	Several secondary antibodies will bind to the primary antibody resulting in an amplified signal.
Species cross-reactivity	Species cross-reactivity is minimized in direct methods as the fluorophore is already conjugated to the primary antibody.	Secondary antibodies may cross-react with species other than the target. The use of pre-adsorbed secondary antibodies can prevent cross-reactivity.
Background	Non-specific binding is reduced through the use of conjugated primary antibodies.	Samples with endogenous immunoglobulins may exhibit a high background with indirect methods.