UNIT –3 Immunity

Vertebrates (including humans) are continuously exposed to microorganisms and their metabolic products that can cause disease. Fortunately these animals are equipped with an immune system that protects them against adverse consequences of this exposure.

- The **immune system** is composed of widely distributed cells, tissues, and organs that recognize foreign substances and microorganisms and act to neutralize or destroy them.
- **Immunity** [Latin *immunis,* free of burden] refers to the general ability of a host to resist a particular infection or disease.
- **Immunology** is the science that is concerned with immune responses to the foreign challenge and how these responses are used to resist infection. It includes the distinction between "self" and "nonself" and all the biological, chemical, and physical aspects of the immune response.

There are two fundamentally different types of immune responses to invading microorganisms and foreign material. The **nonspecific immune response** is also known as **nonspecific resistance or innate** or **natural immunity** and other is **specific immune response** also known as **acquired** or **specific immunity.**

The nonspecific resistance offers resistance to any microorganism or foreign material encountered by the vertebrate host. It includes general mechanisms inherited as part of the innate structure and function of each animal, and acts as a first line of defense. The nonspecific immune response lacks immunological memory—that is, nonspecific responses occur to the same extent each time a microorganism or foreign body is encountered.

In contrast, the **specific immune responses** also known as **acquired** or **specific immunity** resists a particular foreign agent or microorganism; moreover, specific immune responses improve on repeated exposure to foreign agents such as viruses, bacteria, and toxins.

Substances that are recognized as foreign and provoke immune responses are called **antigens**. The antigens cause specific cells to produce proteins called **antibodies**. Antibodies bind to and inactivate a specific antigen. Other cells destroy virus-infected cells. The nonspecific and specific responses usually work together to eliminate pathogenic microorganisms and other foreign agents.

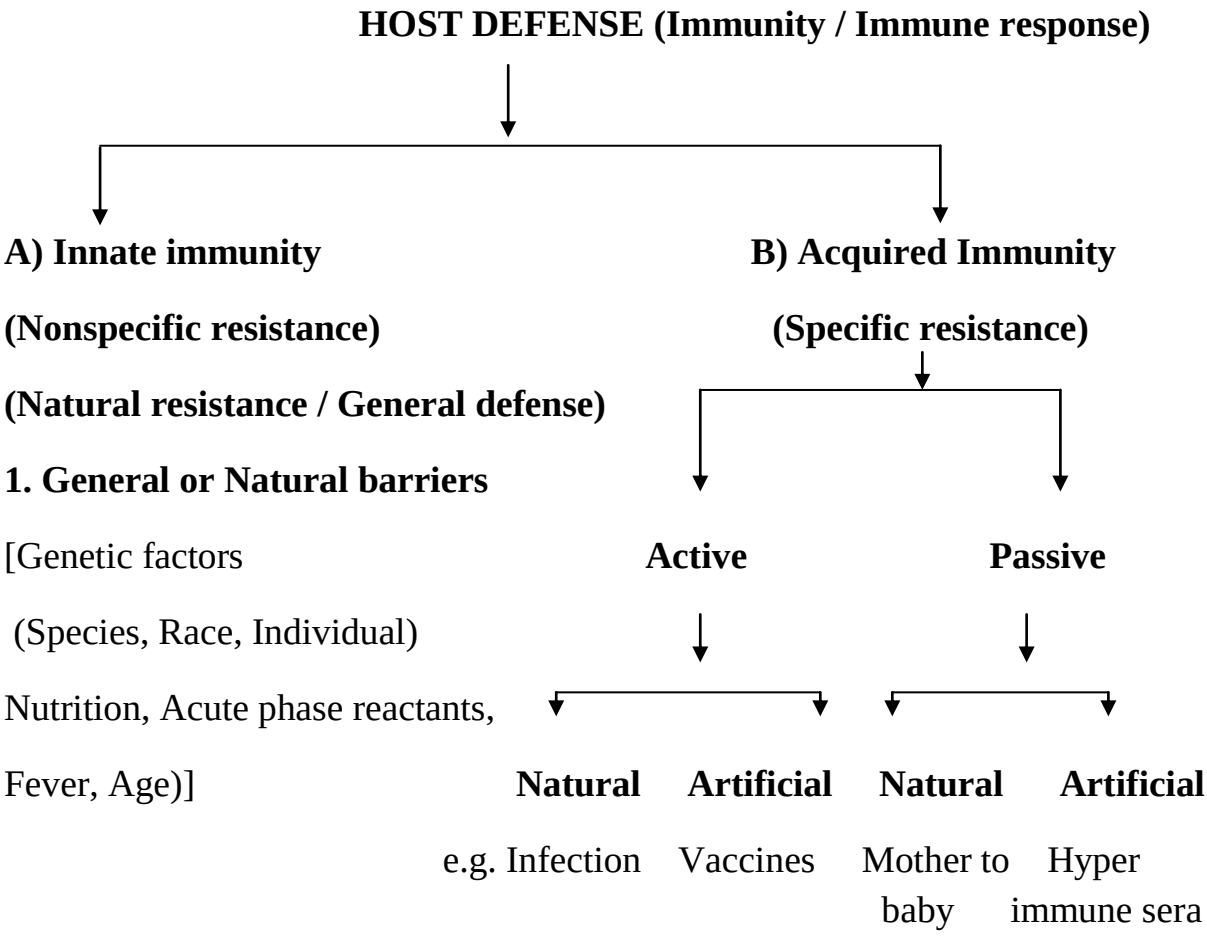
*** Immunity: -**

In Latin immunis means free of burden.

It refers to the general ability of a host to resist a particular infection or disease. There are mainly two types of immunity

A) Innate immunity or Native immunity or Natural resistance or Nonspecific defense mechanisms or General defense mechanisms.

B) Acquired immunity or specific resistance



2. Physical barriers

(Skin & mucous membranes,

Respiratory system, Intestinal tract,

Genitourinary tract, Eye)

3. Chemical barriers

(Complement, Interferon, Fibronectin,

Hormones, β-Lysin & other polypeptides,

Tumors Necrosis factor Alpha,

Bacteriocins, Cytokines)

4. Biological barriers

(Normal indigenous microbiota,

Phagocytosis, Inflammation,

Natural killer cells)

A) Innate immunity / Nonspecific resistance (Natural resistance / General defense)

It is the resistance to infections, which an individual possesses due to his genetic and constitutional make up. Innate immunity does not depend on contact with microorganisms or on immunization. It is considered at 4 levels as follows-

1. General or Natural barriers: -

a) Genetic factors

b) Nutrition

c) Acute phase reactants

d) Fever

e) Age

a) <u>Genetic factors</u>: -

Innate immunity may be considered at the level of the Species, Race or Individual.

- **Species innate immunity: -**

It is the total resistance to a pathogen, shown by all members of a species.

E. g. All human beings are totally insusceptible to plant pathogens & many animal pathogens. The mechanism of species immunity is not understood, but may be due to physiological & biochemical differences between tissues of the different species, which determine whether or not a pathogen can multiply in them.

- **Race innate immunity: -**

Within a species, different races may show differences in susceptibility to infections. This is known as racial immunity.

e. g. **Algerian sheep** are resistance to anthrax while other sheep are susceptible to anthrax. **Negroes** in the USA are more susceptible to tuberculosis than **Whites. Brahman** cattle are resistant to the protozoan parasite responsible for tick fever than other breeds of cattle.

Such racial differences are known to be genetic origin. It is possible to develop such resistances by selection and inbreeding.

- **Individual innate immunity: -**

The difference in innate immunity exhibited by different individuals in a race is known as individual immunity. Such individual resistance is due to a combination of both natural and adaptive resistance factors. Age is important, as young individuals are susceptible to 'children's diseases'. Conversely, the aged are susceptible to diseases as a result of a decline of immune functions with age. Certain individuals have genetic defects which result in selective or general immunodeficiency. Other factors include nutrition, personal hygiene, sex, the nature of workplace, the opportunity for contacts with infected individuals and the individual's hormonal & endocrine balance.

b) <u>Nutrition</u>: -

In general, the more malnourished the host, the greater will be its susceptibility to, and the severity of infections. This is especially true for very young hosts.

c) <u>Acute phase reactants</u>: -

These are the qualitative and quantitative changes that occur in the host's blood plasma during an acute infection. These changes can decrease the virulence of the pathogen and increase the overall general defense of the host.

e. g. The virulence of many microorganisms is enhanced with increased iron availability.

e. g. Gonococci spread most often during menstruation.

d) <u>Fever</u>: -

From a physiological point of view, fever results from disturbances in hypothalamic thermoregulatory activity, leading to an increase of the thermal "set point." In adult humans, **fever** is defined as an oral temperature above 98.6°F (37°C) or a rectal temperature above 99.5°F (37.5°C).

The most common cause of a fever is a viral or bacterial infection (or bacterial toxins). In almost every instance there are specific constituents, the endogenous pyrogens, which directly trigger fever production. Examples of these pyrogens include interleukin-1, IL-6, and tissue necrosis factor (TNF) that are produced by host macrophages in response to pathogenic microorganisms. After their release, these pyrogens circulate towards the hypothalamus and induce neurons to secrete **prostaglandins**. Prostaglandins reset the hypothalamic thermostat at a higher temperature, and temperature-regulating reflex mechanisms then act to bring the core body temperature up to this new setting.

The fever induced by a microorganism augments the host's defenses by three complementary pathways

(a) it stimulates leukocytes so that they can destroy the microorganism,

(b) it enhances the specific activity of the immune system, and

(c) it enhances microbiostasis (growth inhibition) by decreasing available iron to the microorganism.

Evidence suggests that some hosts are able to redistribute the iron during a fever in an attempt to withhold it (**hypoferremia**) from the microorganism. Conversely, the virulence of many microorganisms is enhanced with increased iron availability (**hyperferremia**). Gonococci, for example, spread most often during menstruation, a time in which there is an increased concentration of free iron available to these bacteria.

e) <u>Age</u>: -

Generally, when the host is very young or very old, susceptibility to infection increases. Babies are at a particular risk after their maternal immunity has disappeared and before their own immune system has matured. In very old persons, there is a decline in the immune system and in the homeostatic functioning of many organs that reduces host defenses.

2. Physical barriers (Mechanical barriers) [First line of defense]: -

a) **Skin and mucous membranes**
b) **Respiratory system**
c) **Intestinal tract**
d) **Genitourinary tract**
e) **Eye**

(Physical or mechanical barriers, along with the host's secretions [flushing mechanisms] are the First Line of Defense against organisms.)

a) **<u>Skin and mucous membranes</u>: -**

The intact skin forms a very effective mechanical barrier to parasitic invasion. There are several reasons for this—

→ Its outer layer consists of thick closely packed keratinized cells (components of hair, nails & outer skin cells) that organisms cannot enzymatically attack.

→ Continuous shedding of the outer squamous epithelial cells (desquamation) removes those organisms that do manage to adhere.

→ Relative dryness of the skin slows microbial growth.

→ Mild acidity (pH 5 to 6, due to the breakdown of lipids into fatty acids by the normal skin microbiota) inhibits the growth of many microorganisms.

→ The normal skin microbiota acts antagonistically against many pathogens; it also occupies attachment sites and competes for nutrients.

→ Sebum liberated from the oil glands (sebaceous) forms a protective film over the surface of the skin.

→ Normal washing (by humans) continuously removes organisms.

→ The mucous membranes of the respiratory, digestive, and urinogenital systems resist parasitic organisms because the intact stratified squamous epithelium and mucous secretions form a protective covering that resists penetration and traps many microorganisms. Furthermore, many mucosal surfaces are bathed in specific antaparasitic secretions.

e. g. cervical mucous, prostatic fluid and tears are toxic to many bacteria. One antibacterial substance is 'Lysozyme' (muramidase), an enzyme that lyses bacteria by hydrolyzing the β (1- 4) bond connecting N- acetylmuramic acid N-acetylglucosamine in the bacterial cell wall peptidoglycan. These mucous secretions also contain specific proteins that help to prevent the attachment of organisms and significant amounts of iron binding proteins (lactoferrin) that sequester iron away from these organisms.

b) <u>Respiratory system</u>: -

It has air filtration system at the upper and lower respiratory tracts. Organisms are deposited on the moist, sticky mucosal surfaces. The cilia in the nasal cavity beat towards the pharynx, so that mucus with its trapped microorganisms is moved towards the mouth and expelled. Humidification of the air by the nasal turbinate causes many hygroscopic organisms to swell and helps the phagocytic process.

Organisms less than 10 µm in diameter are transported by ciliary action away from the lungs and those larger than 10 µm are trapped by hairs and cilia lining the nasal cavity. Coughing and sneezing reflexes clear the respiratory system by expelling air forcefully from the lungs through the mouth and nose. Salivation also washes organisms from the mouth and nasopharyngeal areas into the stomach.

c) <u>Intestinal tract</u>: -

Once parasitic organisms reach the stomach, many are killed by its gastric juice (a mixture of hydrochloric acid, enzymes and mucous). The very high acidity of gastric juice (pH 2 to 3) is usually sufficient to destroy most organisms and their toxins. (except protozoan cysts, clostridium and staphylococcus toxin). However, many organisms are protected by food particles and reach the small intestine.

Once in the small intestine, pathogens often are damaged by various pancreatic enzymes, bile, enzymes in intestinal secretions and secretary Ig A antibody. Peristalsis and normal loss of columnar epithelial cells remove intestinal microorganisms.

In addition, the normal microbiota of the large intestine is extremely important in preventing the establishment of pathogenic organisms by producing inhibitory substances (fatty acids) and by competing for attachment sites and for nutrients.

d) <u>Genitourinary tract</u>: -

Urine kills some bacteria due to its low pH and the presence of urea and other metabolic end products (uric acid, hippuric acid, indicant, fatty acids, mucin, and enzymes). The kidney medulla is so hypertonic that few organisms can survive. The urinary tract is flushed with urine and some mucus 4 to 10 times a day, eliminating potential pathogens.

In males, the anatomical length of the urethra (20 cm) provides a distance barrier that excludes microorganisms from the urinary bladder. Conversely, the short urethra (5 cm) in females contains more microorganisms; this explains why general urinary tract infections are **14 times** more common in females than in males.

The vagina has another unique defense. Under the influence of estrogens, the vaginal epithelium produces increased amounts of **glycogen** that acid tolerant *Lactobacillus acidophilus* species called *Doderlein's bacilli* degrade to form lactic acid. Normal vaginal secretions contain up to 10^8 *Doderlein's bacilli* per ml. Thus, an acidic environment is established. Cervical mucous also has some antibacterial activity.

e) <u>Eye</u>: -

The conjunctiva is specialized mucus secreting epithelial membrane that lines the interior surface of each eyelid and the exposed surface of the eyeball. It is kept moist by the continuous flushing action of tears (lacrimal fluid) from the lacrimal glands. Tears contain large amounts of lysozyme and other antimicrobial substances.

3. Chemical barriers: -

a) **Complement System**
b) **Interferons**
c) **Fibronectin**
d) **Hormones**
e) **β – Lysin & other polypeptides**
f) **Tumor Necrosis factor Alpha**
g) **Bacteriocins**
h) **Cytokines**

Mammalian hosts have a chemical arsenal with which to combat the continuous onslaught of parasitic organisms.

a) Complement System: -

Complement was discovered many years ago as a heat-labile component of human blood plasma that augments (helps) opsonization of bacteria by antibodies and helps other antibodies to kill bacteria. This activity was said to "complement" the antibacterial activity of antibody; hence, the name complement. It is now known that the **complement system** is composed of a large number of serum proteins that play a major role in the animal's defensive immune response. For example, complement proteins can lyse antibody-coated eukaryotic cells and bacteria (cytolysis).

Complement can mediate inflammation which attracts and activates phagocytic cells. Generally, complement proteins amplify the effects of antibodies (e.g., lysis of cells).

[The complement cascade is made up of at least 20 complement proteins designated C1 (which has three protein subcomponents) through C9 in addition to Factor B, Factor D, Factor H, Factor I, C4b binding protein, C1 INH complex, S protein, and properdin).

The complement system acts in a cascade fashion, the activation of one component resulting in the activation of the next. Collectively the complement proteins make up much of the globulin fraction of serum. Within plasma and other body fluids, complement proteins are in an inactive state.]

b) Interferons: -

Interferons (IFNs) are a group of related low molecular weight, regulatory cytokines produced by many eucaryotic cells in response to numerous inducers: a virus infection, double stranded RNA, endotoxins, antigenic stimuli, mitogenic (stimulating mitosis) agents, and many pathogenic organisms capable of intracellular growth (*Listeria monocytogenes,* chlamydiae, rickettsias, and protozoa). Interferons usually are species specific but virus nonspecific. Several classes of interferons are recognized: INF-α is a family of 20 different molecules that can be synthesized by virus-infected leukocytes. INF-β is derived from virus-infected fibroblasts; and INF-γ is produced by antigen-stimulated T cells. Probably many other virus-infected cells can synthesize INF-α and INF-β.

c) Fibronectin: -

It is a high molecular weight **glycoprotein** that can interact with certain bacteria. E. g. it binds to surface components of *Staphylococcus aureus* and groups of A, C, & G *Streptococci.* It helps in the nonspecific clearance of the bacteria from the body. Fibronectin also covers the receptors of certain epithelial cells to block the attachment of many bacteria.

d) Hormones: -

The effects of various mammalian hormones on host defense mechanisms are just beginning to know. The depressive effects of the corticosteroids on the inflammatory response and immune system are well known. **Estrogen's** effect on the microbiota of the vagina varies during the menstrual cycle, nonspecific resistance increases with rises in estrogen concentration. The activity of **testosterone & adrenal hormone** in *acne vulgaris* caused by *Propionibacterium acnes* has been proved.

e) Beta (β) Lysin & other polypeptides: -

Beta Lysin is a cationic polypeptide released from blood platelets. It can kill some Gram-positive bacteria by disrupting their plasma membranes. Other cationic polypeptides include leukins, plakins & phagocytin. A Zinc- containing polypeptide known as the *prostatic antibacterial factor* is an important antimicrobial substance secreted by the prostate gland in males.

f) Tumor Necrosis factor Alpha (TNF – Alpha α): -

It is released from **monocytes or macrophage cells** in response to lipopolysaccharides or bacteria such as *Mycobacterium tuberculosis.* It is an important **inflammatory mediator.** It affects a variety of cell types, including polymorphonuclear cells, endothelial cells, fibroblasts and macrophages.

g) <u>Bacteriocins</u>: -

Many of normal bacteria synthesize and release plasmid encoded toxic proteins (e.g., colicin, staphylococcin) called **bacteriocins** that are lethal to related species. Bacteriocins may give their producers an adaptive advantage against other bacteria. Sometimes they may increase bacterial virulence by damaging host cells such as mononuclear phagocytes. Most bacteriocins that have been identified are peptides or proteins and are produced by Gram-negative bacteria. (However, recently it has been discovered that some Gram-positive bacteria produce bacteriocin-like peptides).

For example, *E. coli* synthesizes bacteriocins called **colicins,** which are coded by several different plasmids (ColB, ColE1, ColE2, ColI, and ColV). Some colicins bind to specific receptors on the cell envelope of sensitive target bacteria and cause cell lysis, attack specific intracellular sites such as ribosomes, or disrupt energy production. It is now widely recognized that these antimicrobial peptides act as defensive effector molecules in the large intestine.

.h) Cytokines

Defense against viruses, microorganisms and their products, parasites, and cancer cells is mediated by both nonspecific and specific immunity. Cytokines are required for immunoregulation of both of these immune responses.

The term **cytokine** [Greek *cyto,* cell, and *kinesis,* movement] is a generic term for the soluble protein or glycoprotein released by one cell population that acts as an intercellular (between cells) mediator or signaling molecule.

- When released from mononuclear phagocytes, these proteins are called **monokines;**
- when released from T lymphocytes they are called **lymphokines;**
- when produced by a leukocyte and the action is on another leukocyte, they are **interleukins;** and
- if their effect is to stimulate the growth and differentiation of immature leukocytes in the bone marrow, they are called **colony-stimulating factors (CSFs).**
- Recently cytokines have been grouped into the following categories or families: chemokines, hematopoietins, interleukins, and members of the tumor necrosis factor (TNF) family.

Cytokines can affect the same cell responsible for their production (an autocrine function), nearby cells (a paracrine function), or can be distributed by the circulatory system to their target cells (an endocrine function). Their production is induced by nonspecific stimuli such as a viral, bacterial, or parasitic infection; cancer; inflammation; or the interaction between a T cell and antigen. Some cytokines also can induce the production of other cytokines.

4. Biological barriers (Second line of defense): -

Once organisms break the first line of defense, they come across the host's second line of defense: cells that can be mobilized against invading organisms to form a living barrier. This second line of defense involves cells initially derived from bone marrow cells. Most of these are **phagocytes** of the *Mononuclear Phagocyte System (MPS)* or *Reticuloendothelial System (RES).* This is the collection of *macrophages, monocytes, and associated cells* that are located in the liver, spleen, lymph nodes, and bone marrow. When pathogens in the blood or lymph pass by these cells, they are usually phagocytosed and destroyed.

<u>Biological barriers are</u> : -

a) **Normal indigenous microbiota**
b) **Phagocytosis**
c) **Inflammation**
d) **Natural killer cells**

a) <u>Normal indigenous microbiota</u>: -

Normal microbiota inhibit pathogens by following ways –

i) Producing bacteriocins toxic to other bacteria

ii) Competing with pathogens for space & nutrients.

iii) Preventing pathogens from attaching to host surfaces.

iv) Influencing specific clearing mechanism.

b) <u>Phagocytosis</u>: -

During their lifetimes, humans and other vertebrates encounter many microbial species, but only a few of these species can grow and cause serious disease in healthy hosts. Phagocytic cells (monocytes, tissue macrophages, and neutrophils) are important early defense against invading microorganisms. These phagocytic cells recognize, ingest, and kill many extracellular microbial species by the process called **phagocytosis**.

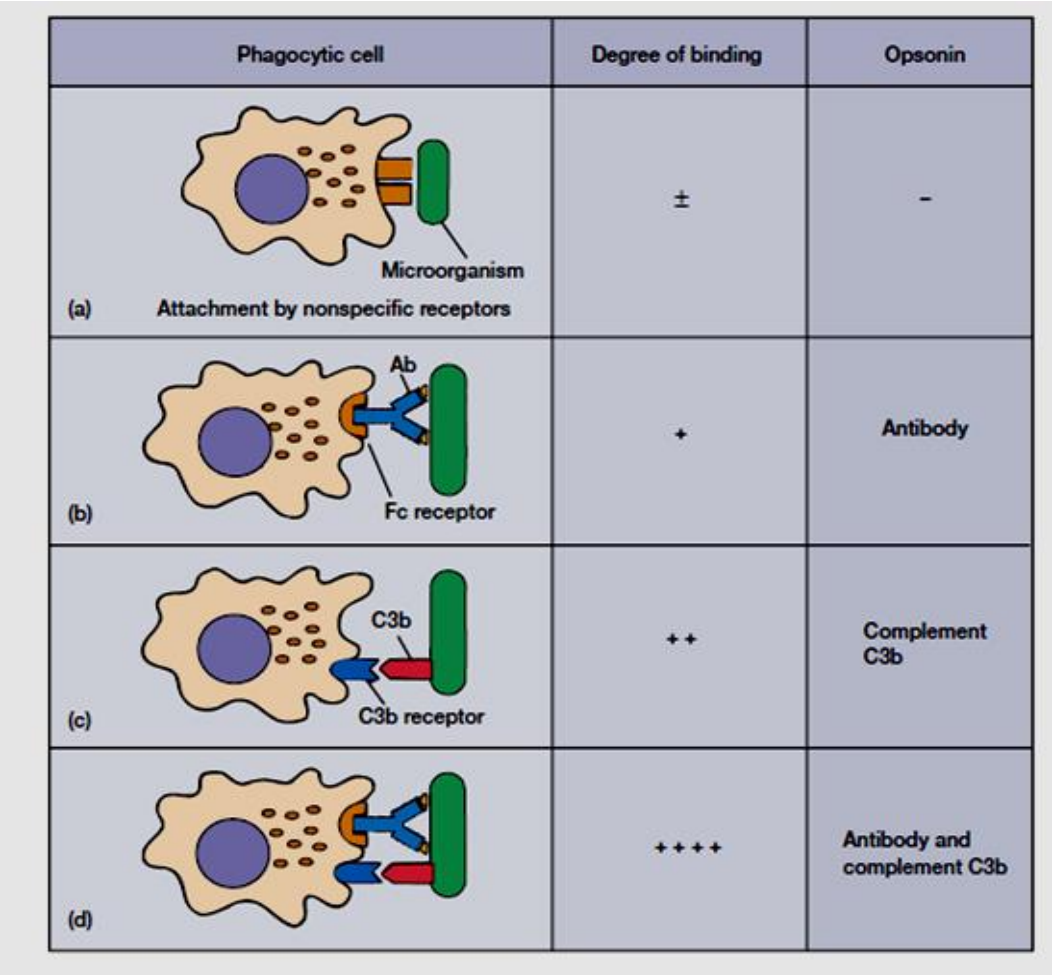
Phagocytic cells use two basic molecular mechanisms for the recognition of microorganisms: (1) opsonin-dependent (opsonic) and (2) opsonin-independent (nonopsonic) recognition. The phagocytic process can be greatly enhanced by opsonization.

Opsonization is a process in which microorganisms or other particles are coated by serum components (antibodies and/or complement C3b) thereby preparing them for recognition and ingestion by phagocytic cells. In the **opsonin-dependent** recognition mechanism, it is the serum components that function as a bridge between the microorganisms and the phagocyte. They act by binding to the surface of the microorganism at one end and to specific receptors on the phagocyte surface at the other**.**

The **opsonin-independent** mechanism does not require opsonins and uses other nonspecific and specific receptors on phagocytic cells that recognize structures expressed on the surface of different microorganisms.

Three main forms of recognition in opsonin-independent phagocytosis have been identified. **One mode,** termed lectin phagocytosis, is based on the recognition between surface lectins on one cell and surface carbohydrates on the opposing cell. The **second mode** is the result of protein-protein interactions between the Arg-Gly-Asp peptide sequence of microorganisms and macrophage receptors. **The third mode** is hydrophobic interactions between bacteria and phagocytic cells also promote phagocytosis. It should be noted that a particular

microbial species can express multiple adhesins, each recognized by a distinct receptor present on phagocytic cells.



Opsonization. (**a**) A phagocytic cell has some intrinsic ability to bind directly to a microorganism through nonspecific receptors. (**b**) This binding ability is enhanced if the microorganism elicits the formation of antibodies (Ab) that act as a bridge to attach the microorganism to the Fc receptor on the phagocytic cell. (**c**) If the microorganism has activated complement (C3b), the degree of binding is further enhanced by the C3b receptor. (**d**) If both antibody and C3b opsonize, binding is greatly enhanced.

Once ingested by phagocytosis, membrane-enveloped microorganisms are delivered to a lysosome by fusion of the phagocytic vacuole, called a **phagosome,** with the lysosome membrane, forming a new vacuole called a **phagolysosome**.

Oxygen independent enzymes: -Lysosomes contribute to the phagolysosome a variety of hydrolases such as lysozyme, phospholipaseA2, ribonuclease, deoxyribonuclease, and proteases. An acidic vacuolar pH favors the activity of

the hydrolases. Collectively these participate in the destruction of the entrapped microorganism.

Oxygen-dependent enzymes: - Besides these oxygen-independent lysosomal hydrolases, macrophage and neutrophil lysosomes contain oxygen-dependent enzymes that can produce toxic **Reactive Oxygen Intermediates (ROIs)** such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radical (OH^-). The NADPH required for this process is supplied by a large increase in pentose phosphate pathway activity. Neutrophils also contain myeloperoxidase and produce hypochlorous acid. Some reactions forming these toxic products are shown here.

Superoxide formation

$$NADPH + 2O_2 \xrightarrow{\text{NADPH oxidase}} 2O_2^{-}\cdot + H^+ + NADP^+$$

Hydrogen peroxide formation

$$2O_2^{-}\cdot + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

Hypochlorous acid formation

$$H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} HOCl + OH^-$$

Singlet oxygen formation

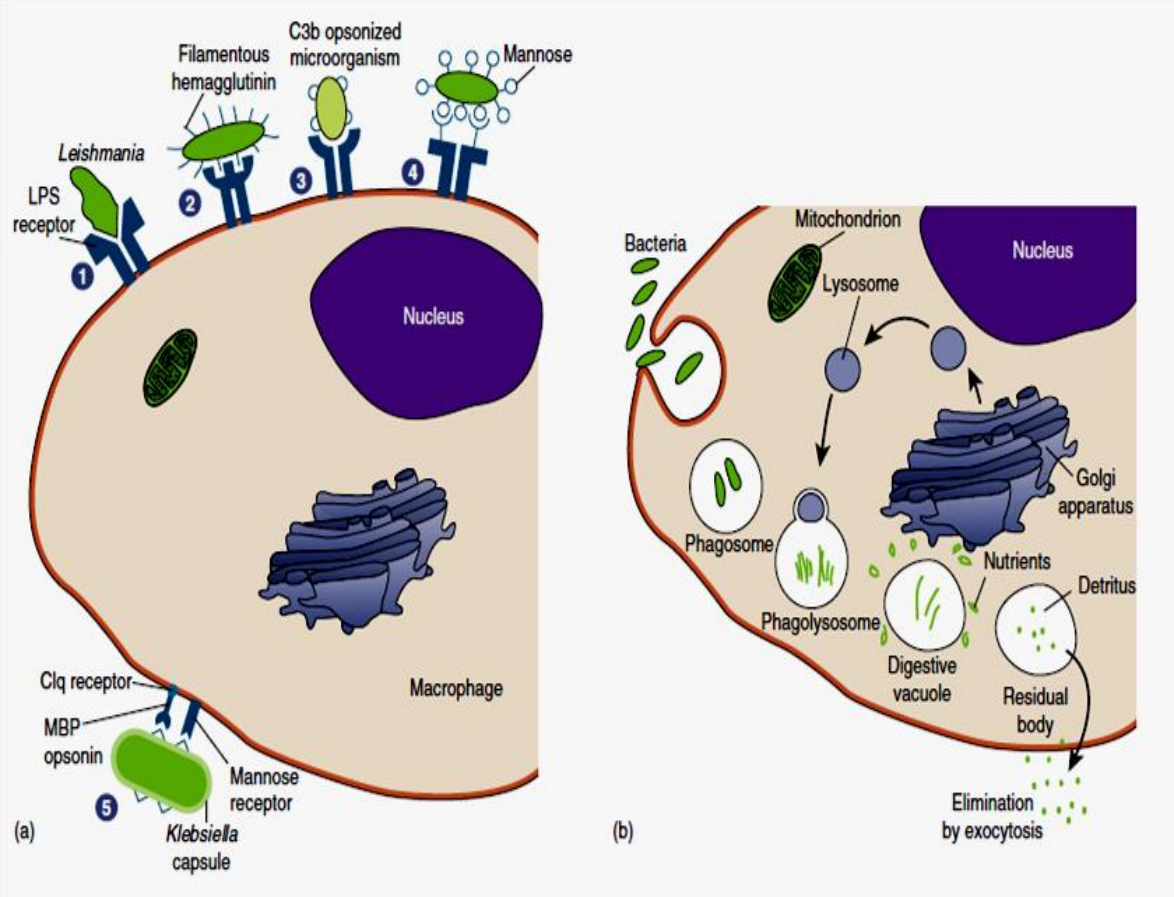
$$ClO^- + H_2O_2 \rightarrow {}^1O_2 + Cl^- + H_2O$$

Hydroxyl radical formation

$$O_2^{-}\cdot + H_2O_2 \rightarrow OH\cdot + OH^- + O_2$$

These reactions result from the **respiratory burst** that accompanies the increased oxygen consumption and ATP generation needed for phagocytosis. Because these reactions occur as soon as the phagosome is formed, lysosome fusion is not necessary for the respiratory burst. The toxic oxygen products produced are effective in killing invading microorganisms.

Phagocytic cells (Monocytes, Macrophages, Tissue macrophages, and Neutrophils) ingest microorganisms and destroy them.



Phagocytosis. (**a**) Drawing shows receptors on a phagocytic cell, such as a macrophage, and the corresponding adhesins on microbial surfaces participating in phagocytosis. (1) The LPS binding site for lipophosphoglycan of *Leishmania* spp; (2) filamentous hemagglutinin of *Bordetella pertussis;* (3) binding to C3b on an opsonized cell; (4) the mannose-containing oligosaccharide side chain for lectin phagocytosis mediated by type 1 fimbriated bacteria; and (5) the participation of capsular polysaccharide of *Klebsiella pneumoniae* in nonopsonic binding mediated by the mannose receptor. (**b**) Drawing of phagocytosis, showing ingestion, intracellular digestion, and exocytosis.

c) <u>Inflammation</u>: -

Inflammation is an important nonspecific defense reaction to tissue injury, such as that caused by a pathogen or wound. Acute inflammation is the immediate response of the body to injury or cell death. These signs include redness (*rubor*), warmth (*calor)*, pain (*dolor*), swelling (*tumor*), and altered function (*functiolaesa*). The acute inflammatory response begins when injured tissue cells release chemical signals (inflammatory mediators) that activate the inner lining (endothelium) of nearby capillaries. Within the capillaries **selectins**

(a family of cell adhesion molecules) are displayed on the activated endothelial cells—first P-selectin and then E-selectin. These adhesion molecules randomly attract and attach neutrophils to the endothelial cells, slow the neutrophils down, and cause them to roll along the endothelium.

As the neutrophils roll along the endothelium, they encounter the inflammatory mediators that act as activating signals. These signals activate **integrins** (adhesion receptors) on the neutrophils. The neutrophil integrins then tightly attach to endothelial adhesion molecules such as the intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). This causes the neutrophils to stick to the endothelium and stop rolling. The neutrophils now undergo dramatic shape changes, squeeze through the endothelial wall (extravasation) into the interstitial tissue fluid, migrate to the site of injury, and attack the pathogen or other cause of the tissue damage. Neutrophils and other leukocytes are attracted to the infection site by chemotactic factors or chemotaxins such as substances released by bacteria and mast cells, and tissue breakdown products. Depending on the severity and nature of tissue damage, other types of leukocytes (e.g., lymphocytes, monocytes, and macrophages) may follow the neutrophils.

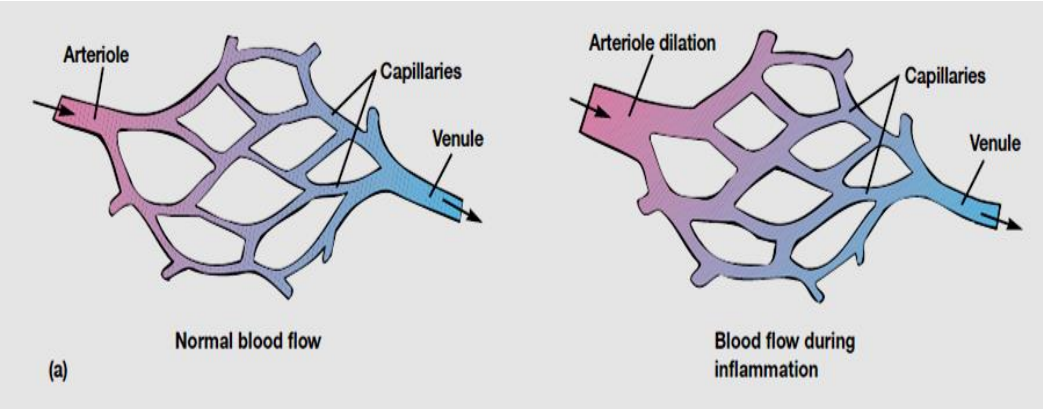
The inflammatory mediators that are released by the injured tissue cells also raise the acidity in the surrounding extracellular fluid. This decrease in pH activates the extracellular enzyme kallikrein, which splits bradykinin from its long precursor chain.

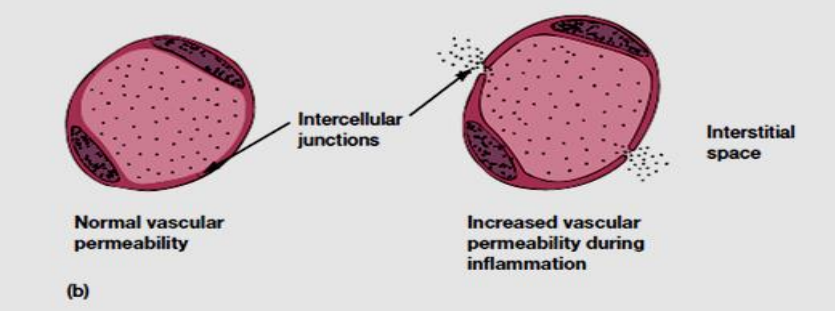
Bradykinin binds to receptors on the capillary wall, opening the junctions between cells and allowing fluid and infection-fighting leukocytes to leave the capillary and enter the infected tissue. Simultaneously bradykinin binds to mast cells in the connective tissue associated with most small blood vessels. This activates the mast cells by causing an influx of calcium ions, which leads to degranulation and release of preformed mediators such as histamine. If nerves in the infected area are damaged, they release substance P, which also binds to mast cells, boosting preformed-mediator release. Histamine in turn makes the intercellular junctions in the capillary wall wider so that more fluid, leukocytes, kallikrein, and bradykinin precursors move out, causing edema. Bradykinin then binds to nearby capillary cells and stimulates the production of prostaglandins to promote tissue swelling in the infected area. Prostaglandins also bind to free nerve endings, making them fire and start a pain impulse. The change in mast cell plasma membrane permeability associated with activation allows phospholipase A2 to release arachidonic acid. Arachidonic acid is then metabolized by the cyclooxygenase or lipoxygenase pathways, depending on

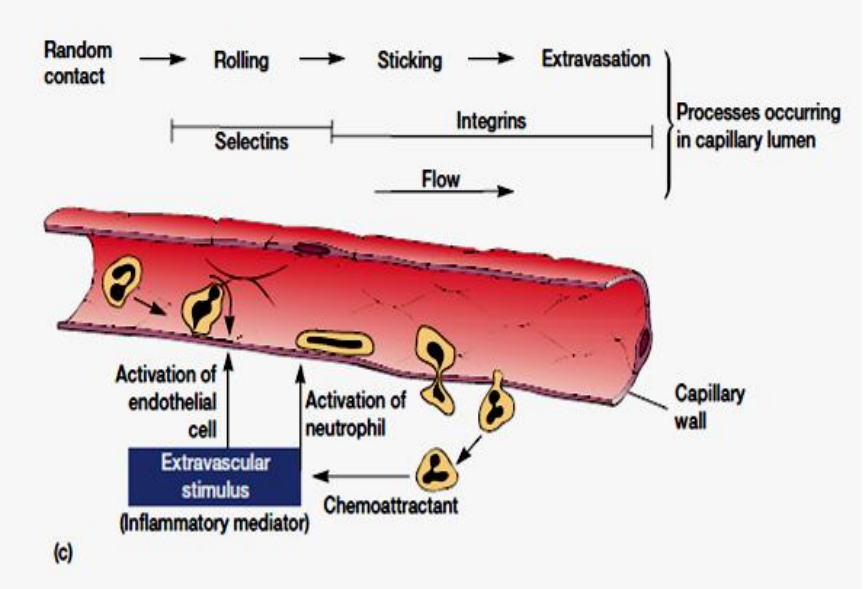
mast cell type. The newly synthesized mediators include prostaglandins, thromboxane, slow-reacting substance (SRS), and leukotrienes. All play various roles in the inflammatory response.

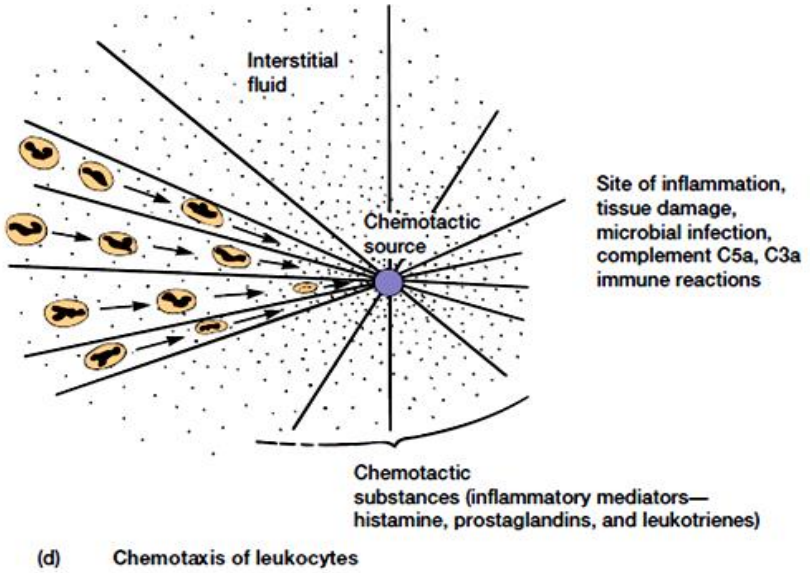
During acute inflammation, the offending pathogen is neutralized and eliminated by a series of events, the most important of which are the following:

1. The increase in blood flow and capillary dilation bring into the area more antimicrobial factors and leukocytes that destroy the pathogen. Dead cells also release antimicrobial factors.
2. The rise in temperature stimulates the inflammatory response and may inhibit microbial growth.
3. A fibrin clot often forms and may limit the spread of the invaders so that they remain localized.
4. Phagocytes collect in the inflamed area and phagocytose the pathogen. In addition, chemicals stimulate the bone marrow to release neutrophils and increase the rate of granulocyte production.









Physiological Events of the Acute Inflammatory Response.

(**a**) The diameter of the arteriole controls the normal blood flow into a capillary bed. Inflammation causes arteriole dilation and increased blood flow (hyperemia) into the affected tissue. The capillary becomes distended and produces the gross red-purple discoloration of tissue due to an increased blood content.

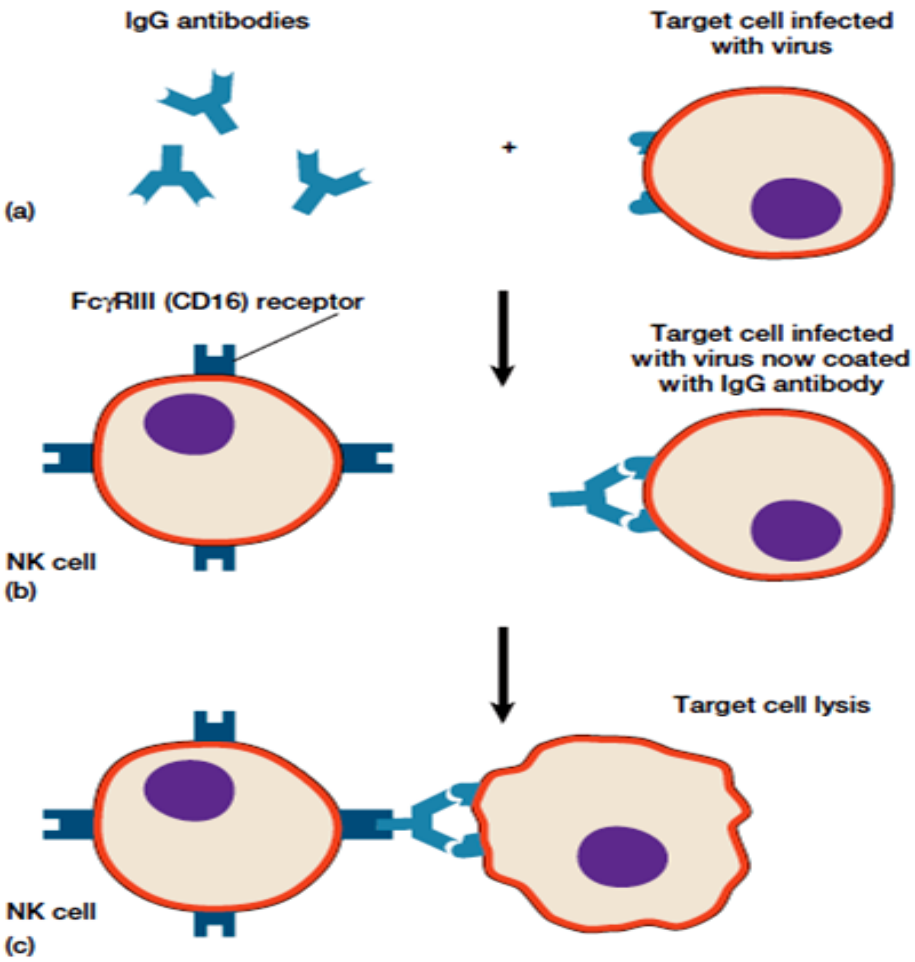
(**b**) The junctions between capillary endothelial cells are sufficiently tight to keep large molecules within the lumen. In inflammation, gaps are created that allow leakage of fluid and large molecules into the interstitial space. The fluid leakage produces swelling (edema).

(**c**) Normally leukocytes (such as neutrophils) are carried in the lumen of a capillary. During inflammation selectins and integrins cause leukocytes to adhere to the capillary wall, insert pseudopods between the endothelial cells, and squeeze through (extravasation) by a process called diapedesis.
(**d**) After leaving the capillary, the leukocytes are attracted (chemotaxis) to the source of the inflammation by various chemotactic or chemokinetic substances (extravascular stimulus). Once at the infection site, leukocytes phagocytose the microorganisms or dead tissue.

d) <u>Natural killer cells</u>: -

Natural killer (NK) cells are a small population of large nonphagocytic granular lymphocytes. Their major function is to destroy malignant cells and cells infected with microorganisms. They recognize their targets in one of two ways. Like many cells, they possess Fc receptors that bind IgG antibody. These receptors link the NK cells to IgG-coated target cells, which they kill by a process called **antibody-dependent cell-mediated cytotoxicity (ADCC).** The second way NK cells recognize infected cells and cancer cells relies on the killer-activating receptors and killer-inhibitory receptors of the NK cells.

The killer-activating receptors recognize a number of different molecules present on the surface of all nucleated cells. The killer inhibitory receptors recognize a surface marker known as the major histocompatibility complex (MHC) class I molecule that is also present on all nucleated cells and is an indicator of "self." If the killer activating receptors are engaged with a ubiquitous surface molecule, a "kill" instruction is issued to the NK cell. Conversely, when this "kill" signal is overridden by an inhibitory signal sent by the killer inhibitory receptor binding to the MHC class I molecule (which indicates "self "), no killing is done.



Antibody-Dependent Cell-Mediated Cytotoxicity (**a**) In this mechanism, IgG antibodies bind to a target cell infected with a virus. (**b**) NK cells have Fc_RIII (CD16) receptors on their surface. (**c**)When the NK cells encounter infected virus cells coated with IgG antibody, they kill the target cell by releasing cytotoxic mediators and/or membrane damage.

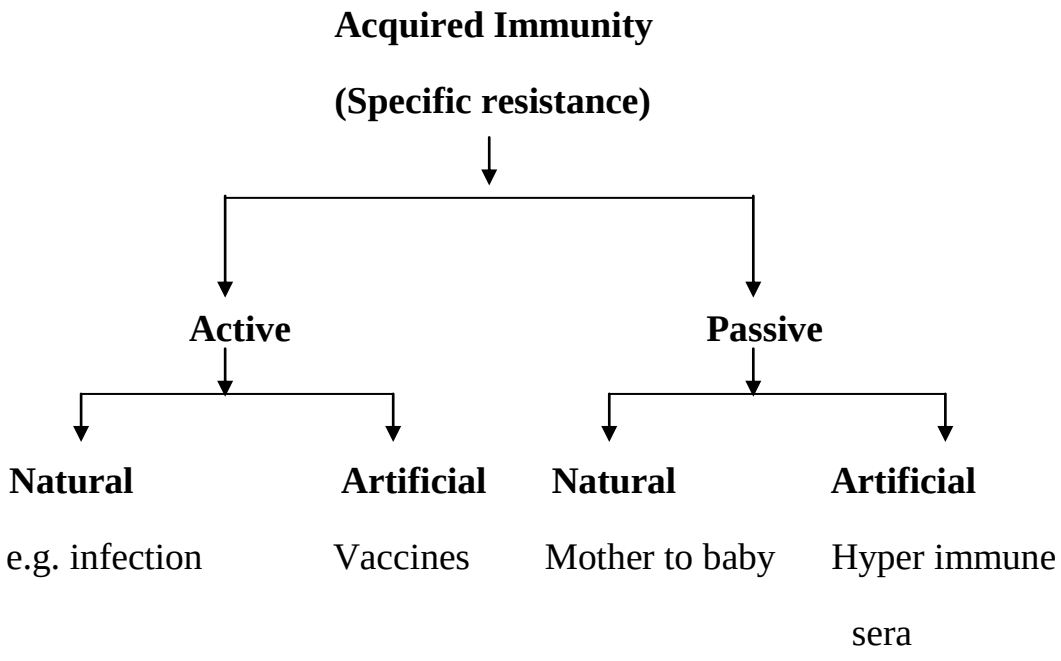
B) Acquired immunity (Specific resistance)

The specific immune system of vertebrates has three major functions:
i) to recognize anything that is foreign to the body ("nonself"),
ii) to respond to this foreign material, and
iii) to remember the foreign invader.

The recognition response is highly specific. The immune system is able to distinguish one pathogen from another, to identify cancer cells, and to recognize the body's own "self" proteins and cells as different from "nonself" proteins, cells, tissues, and organs. After recognition of an invader has occurred, the specific immune system responds by recruiting its defensive molecules and cells to attack the invader. This is called an **effector response.** The effector response either eliminates the foreign material or renders it harmless to the host, thus preventing disease. If the invader is encountered at a later time, the immune system remembers, and mounts a more intense and rapid memory or anamnestic response that eliminates the invader once again and protects the host from disease

The resistance that an individual acquires during life is known as acquired immunity. It is of two types-

I) Active immunity
II) **Passive immunity**



Types of Acquired Immunity

Acquired immunity refers to the type of specific immunity a host develops after exposure to a suitable antigen, or after transfer of antibodies or lymphocytes from an immune donor. Acquired immunity can be obtained by natural or artificial means and actively or passively

I) <u>Active immunity</u>: -

It is the resistance developed by an individual as a result of an antigenic stimulus. This leads to the synthesis of antibodies & action of cells (AMI & CMI). After development of active immunity, it is long lasting. If an individual who has been actively immunized against an antigen shows the immune response quick and abundant when the same antigen is given subsequently. This is known as secondary response.

Active immunity is of two types –

1) Natural active immunity
2) Artificial active immunity

1) Natural active immunity: -

Naturally acquired active immunity occurs when an individual's immune system contacts an antigenic stimulus such as an infection. The immune system responds by producing antibodies and activated lymphocytes that inactivate or destroy the antigen. The immunity produced can be either lifelong, as with measles or chickenpox, orlast for only a few years, as with tetanus.

2) Artificial active immunity: -

Artificially acquired active immunity results when an animal is given an antigen preparation to induce the formation of antibodies and activated lymphocytes. This preparation is called a vaccine and the procedure is vaccination (immunization). A vaccine consists of a preparation of killed microorganisms; living, weakened (attenuated) microorganisms; or inactivated bacterial toxins (toxoids) that are administered to an animal to induce immunity artificially.

e.g. a) Bacterial vaccines: -

i) Live – BCG for tuberculosis

ii) Killed – TAB for typhoid

b) Viral vaccines: -

i) Live – Oral poliomyelitis (Sabin)

ii) Killed – Salk vaccine for poliomyelitis

c) Bacterial products: - Toxoids for diphtheria and tetanus

.

II) Passive immunity

The resistance that is transmitted to a recipient in a 'readymade' form is known as passive immunity. Here immune system of recipient does not play active role. There is no antigenic stimulus instead preformed antibodies are administered. Protection effective immediately after passive immunization, but lasting for few days or weeks. No secondary type of response.

When a foreign antibody is administered second time, it is eliminated more rapidly than initially. It is less effective than active immunity. The main advantage of passive immunization is that it is immediate in action and therefore can be employed when 'instant' immunity is desired.

Passive immunity is also of two types—

1) **Natural passive immunity**
2) **Artificial passive immunity**

1) Natural passive immunity: -

Naturally acquired passive immunity involves the transfer of antibodies from one host to another. For example, some of a pregnant woman's antibodies pass across the placenta to her fetus. If the female is immune to diseases such as polio or diphtheria, this placental transfer also gives the fetus and newborn immunity to these diseases. Certain other antibodies can pass from the female to her offspring in the first secretions (called colostrum) from the mammary

glands. These maternal antibodies are essential for providing immunity to the newborn until its own immune system matures. Unfortunately naturally acquired passive immunity generally lasts only a short time (weeks or months at the most).

By active immunization of mothers during pregnancy, it is possible to improve the quality of passive immunity in the infants. Immunization of pregnant women with **tetanus toxoid** is recommended for this purpose in communities in which neonatal tetanus is common.

2) Artificial passive immunity: -

It is the resistance transferred passively to a recipient by administration of **antibodies.** The agents used for this purpose are **Hyper Immune Sera (HIS)** of animal or human. Hyper immune sera are prepared by active hyper immunization in horses using the appropriate antigen.

e.g. Anti Tetanus Serum, Anti Botulism Serum, Anti Venom Serum, Anti Rabies Serum.

e.g. **Anti Tetanus Serum (ATS)** used for passive immunization against tetanus is prepared by administrating a series of doses of tetanus toxoid to horses, bleeding them and separating the serum. The antibodies in the serum are concentrated & purified by fractionation and enzyme treatment. The preparation is standardized to contain an adequate number of units of **antitoxin per ml.**

ATS is administered parenterally and subcutaneously for prophylaxis and intravenously for treatment. ATS provides an immediate supply of the antitoxin in the recipient's circulation.

Passive immunization is indicated for providing immediate & temporary protection in a non-immunized host, when there is insufficient time for active immunization to take effect. It is also indicated for treatment of infections.

Comparison between Active & Passive Immunity

Sr. No.	Active Immunity	Sr. No.	Passive Immunity
1	Produced actively by the host's immune system.	**1**	Received passively by the host. No participation by the host's immune system.
2.	Induced by infection or by contact with antigens (Vaccines, Allergens etc).	**2.**	Conferred by introduction of readymade antibodies.
3.	Affords durable and effective protection.	**3.**	Protection short time & less effective.
4.	Immunity effective only after production of antibodies.	**4.**	Immunity effective immediately.
5.	Immunological memory present, subsequent challenge more effective (Booster dose effective)	**5.**	No immunological memory, subsequent administration of antibody is less effective due to 'immune elimination'.
6.	No applicable in immune deficient hosts.	**6.**	Applicable in immune deficient host.

- **Humoral and Cellular Immunity / Immune response**

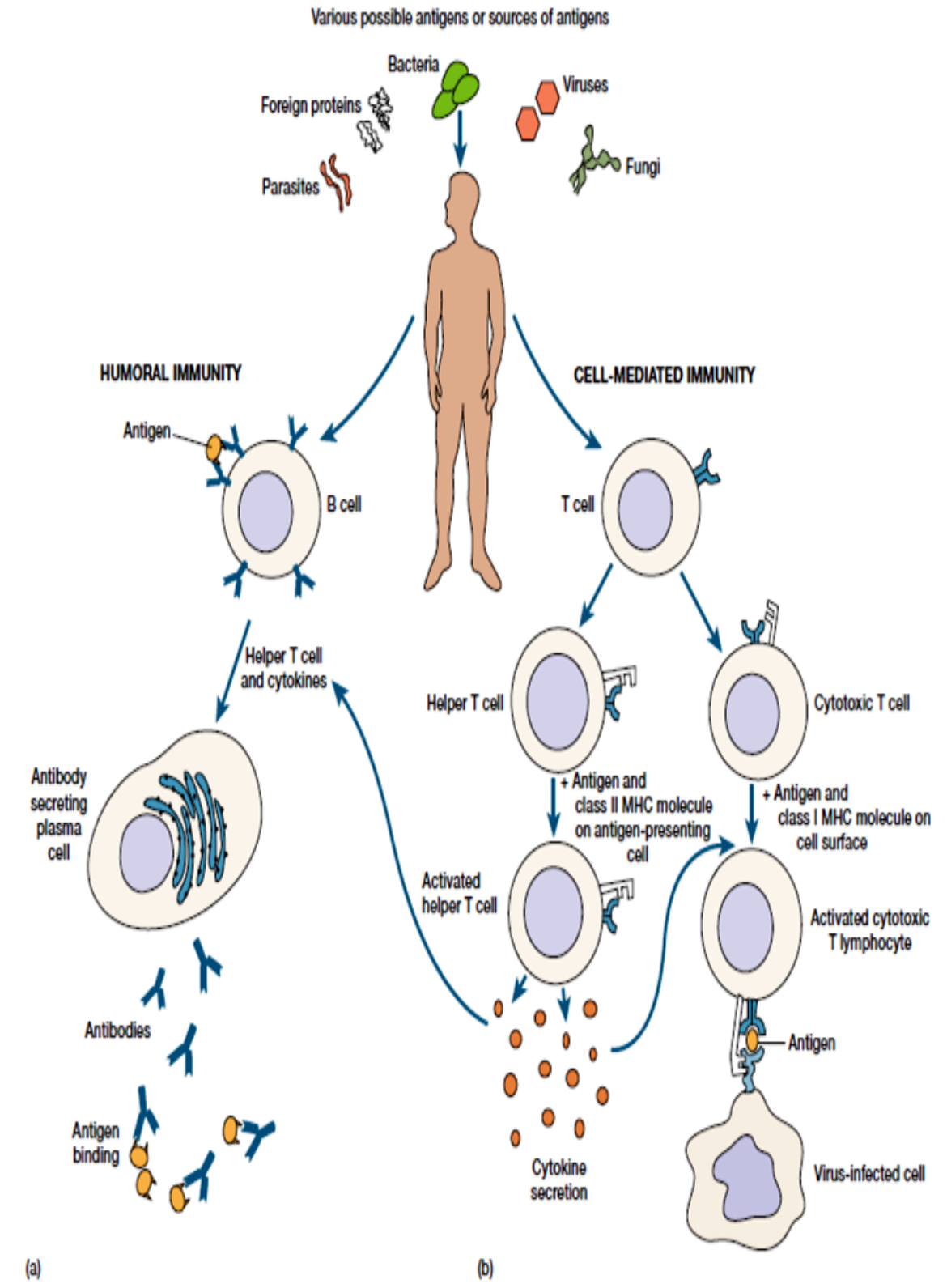
Two branches or arms of specific immunity are recognized: humoral (antibody-mediated) immunity (AMI) and cellular (cell-mediated) immunity (CMI).

Humoral (antibody-mediated) immunity, named for the fluids or "humors" of the body, is based on the action of soluble proteins called antibodies that occur in the body fluids and on the plasma membranes of B lymphocytes. Circulating antibodies bind to bacteria, toxins, and extracellular viruses, neutralizing them or "tagging or marking" them for destruction by mechanisms like **Toxin Neutralization, Viral Neutralization, Adherence Inhibition, Parasitic Infections control, Opsonization, Immune Complex Formation etc**

The antigen-antibody interaction is a bimolecular association that exhibits exquisite specificity. The in vivo interactions that occur in vertebrate animals are absolutely essential in protecting the animal against the continuous attack of viruses, microorganisms and their products, certain macromolecules, and cancer cells.

Cell mediated immunity (CMI)

During the time that B cells are actively responding to antigens, the T-cell portion of the immune system is equally active. The responses of T cells, however, are considered **cell-mediated immunities** (CMI). These reactions are among the most complex and diverse in the immune system. They involve several subsets of T cells that differ in the types of CD receptors and the precise actions they have against foreign antigens and cells. T cells are restricted. Before they can be activated, they must have the antigen offered by an MHC complex on an APC to ensure recognition of self. All produce cytokines that, working together, have a spectrum of biological effects and immune functions. T cells have other notable differences in function from B cells. Rather than releasing antibodies to control foreign antigens, the whole T cell reacts directly in contact with other cells rather than by secretion of molecules into circulation. T cells also stimulate other T cells, B cells, and phagocytes.


Various possible antigens or sources of antigens
Bacteria
Viruses
Foreign proteins
Fungi
Parasites
HUMORAL IMMUNITY
CELL-MEDIATED IMMUNITY
Antigen
B cell
T cell
Helper T cell and cytokines
Helper T cell
Cytotoxic T cell
Antibody secreting plasma cell
+ Antigen and class II MHC molecule on antigen-presenting cell
+ Antigen and class I MHC molecule on cell surface
Activated helper T cell
Activated cytotoxic T lymphocyte
Antibodies
Antigen
Antigen binding
Cytokine secretion
Virus-infected cell
(a)
(b)

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Antigens (Immunogens)

The immune system distinguishes between **'self'** and **'nonself'** through an highly structured recognition process. Prior to birth the body somehow makes an inventory of the proteins and various other large molecules present (self) and removes most T cells specific for self-determinants. Subsequently self-substances can be distinguished from non-self substances, and lymphocytes can produce specific immunologic reactions against the non-self, leading to their removal.

Non-self foreign substances, such as proteins, nucleoproteins, polysaccharides, and some glycoproteins, to which lymphocytes respond, are called **'Antigens'** (**Anti** --- Antibody, **Gen** --- Generator).

Antigens (sometimes called **immunogens**) provoke a highly specific immune response, which involves the formation of **antibodies (immunoglobulins)** or highly specialized **T cells**.

Antigen is defined as any substance, which stimulates the production of an antibody (AMI) and reacts specifically with it when injected into the body. Some antigens may not induce antibodies but may lead to cell mediated immunity (CMI).

Thus main functions of antigens are induction of an immune response and specific reaction with antibodies or sensitized cells.

The vast majority of antigens are ---

⟶ **Proteins**

⟶ **Nucleoproteins (**Nucleic acid + protein)

⟶ **Lipoproteins** (Lipid + protein)

⟶ **Glycoproteins** (Carbohydrate + protein) or

⟶ **Large polysaccharides**

These compounds are often components of invading microorganisms like—

⟶ **Capsules**

⟶ **Cell walls**

⟶ **Flagella**

⟶ **Fimbriae (pilli)**

⟶ **Toxins of bacteria**

⟶ **Coats of viruses**

⟶ **Surfaces of many other types of microbial cells**

Nonmicrobial antigens include—

⟶ **Pollen grains**

⟶ **Egg white**

⟶ **Blood cells**

⟶ **Serum proteins from other persons or species**

⟶ **Transplanted tissues and organs**

Based upon the ability of antigens to carry out functions, they may be classified as

1) Complete antigens
2) Haptens
3) Proantigens

1) Complete antigens: - These antigens are able to induce antibody formation and produce a specific and observable reaction with the antibody so produced.

2) Haptens: - These are the substances, which are not capable of inducing antibody formation by themselves but can react specifically with antibodies produced by complete antigens.

Haptens become capable of inducing antibodies on combination with larger molecules (carriers).

3) Proantigens: - These are low molecular weight substances, such as Picryl chloride and Dinitrochlorobenzene; which do not induce antibody formation but cause delayed hypersensitivity (CMI) when applied on skin.

- **Antigenic determinant (Epitope): -**

The smallest unit of antigenicity is known as the antigenic determinant. This is the small area on the antigen having a specific chemical structure and configuration. Antigens possess a number of determinant groups or sites. The

determinant group has a size of about 25 to 35 Angstrom and molecular weight of about 400 to 1000.

- **Properties important for a substance to be antigenic (Determinants of antigenicity): -**

1) **Size**
2) **Chemical nature**
3) **Susceptibility to tissue enzymes**
4) **Foreignness**
5) **Antigenic specificity**
    a) **Species specificity**
    b) **Iso specificity**
    c) **Auto specificity**
    d) **Organ specificity**
    e) **Heterogenetic specificity**

1) Size: -

Antigenicity depends on the molecular size. Very large molecules, such as **haemocyanins** (MW 6.7 million Daltons) are highly antigenic and particles with low molecular weight (less than 10,000 Daltons) are non-antigenic.

2) Chemical nature: -

Most antigens are proteins and polysaccharides. Lipids and Nucleic acids are less antigenic. Their antigenicity is enhanced by combination with proteins. However not all proteins are antigenic (Gelatin is a protein but not antigenic). It has been suggested that the presence of an aromatic radical is essential for antigenicity (e.g. Tyrosine amino acid).

3) Susceptibility to tissue enzymes: -

The substances, which are metabolized and are susceptible to the action of tissue enzymes, behave as antigens. Antigens introduced into the body are degraded by intracellular enzymes and phagocytes into fragments. These fragments contain antigenic determinants of appropriate size. The substances which are not susceptible to enzymes (e.g. Polysterene latex, polypeptide containing D-amino acids) are not antigenic. Polypeptides containing L-amino acids are antigenic which are metabolized.

4) Foreignness: -

Only antigens, which are 'foreign' to the body, induce an immune response. An individual does not normally mount an immune response against his own normal constituent antigens. Tolerance of self antigens is conditioned by contact with them during the development of the immune apparatus. Breakdown of this homeostatic mechanism results in autoimmune disease.

Antigens of one body induce an immune response into another body but there is no immune response against own antigens by the body. Thus antigens from other individuals of same species are less antigenic than those from other species.

5) Antigenic specificity: -

The basis for specificity is stereochemical. Thus the portion of antigenic determinant group in the antigen molecule is important e.g. Ortho, Meta or Para positions and Dextro, Leavo and Meso isomers. There are different categories of specificity as follows –

a) Species specificity: -

Tissues of all individuals in a species contain species-specific antigens. These are useful in the identification of the species of blood. It has been used in tracing evolutionary relationships between species. It also has forensic applications in the identification of the species of blood and of seminal stains.

b) Iso specificity: -

Iso antigens are the antigens found in some, but not in all members of a species. E.g. Human RBCs antigens based on which individuals can be classified into different blood groups **(A, B, AB, and O)**.

These are of clinical importance in blood transfusion and in isoimmunization during pregnancy. Also helpful in determining disputed paternity cases.

c) Auto specificity: -

Autologous or self-antigens are not actually antigens because they are not normally found free in circulation hence not recognized as self-antigens. Such antigens are called as **'Sequestrated antigens'** e.g. Lens protein of the eye, Sperms. Sequestrated antigens that are not normally found free in circulation or

tissue fluids (such as lens protein normally confined within its capsule) are not recognized as self-antigens. Similarly, antigens that are absent during embryonic life and develop later (such as sperms) are also not recognized as self-antigens. But when these are released into tissues or circulation due to some injury to lens or damage to testis, antibodies are produced against them.

d) Organ specificity: -

Some organs such as brain, kidney, and lens protein of different species have the same antigen called as organ specific antigens. These cause immune response when injected with some materials e.g. Antirabic Vaccine containing sheep brain tissue cause complications in man due to brain specific antigens of sheep and man.

The neuroparalytic complications (due to damage of nervous tissues) following antirabic vaccination using sheep brain vaccines are a consequence of brain specific antigens shared by sheep and human beings.

e) Hetergenic specificity: -

The same or closely related antigens may sometimes occur in different biological species, classes and kingdoms, which are called as heterogenic antigens. E.g. Forssman antigens (Lipid-Carbohydrate complex) is present in man, animals, birds, plants and bacteria.

Types of antigens

1) **Species Specific antigens**
2) **Iso antigens**
3) **Auto antigens**
4) **Organ specific antigens**
5) **Forssman antigens (Heterogenic / Shared, Common or Group)**
6) **Cellullar antigens (present in and on cells)**
    a) **Flagellar (H) antigens**
    b) **Fimbrial antigens**
    c) **Capsular**
    d) **K antigens**
    e) **O antigens**
    f) **Extracellular antigens**

1) Species Specific antigens: -

Tissues of all individuals in a species contain species specific antigens e.g. if we inject horse serum into a man, the man's tissue cells instantly recognize the horse serum proteins as nonself and they respond by producing antibodies and destroy and reject it.

2) Iso antigens: - Iso antigens are the antigens found in some but not in all members of species. E.g. Human RBCs antigens based on which individuals can be classified into different blood groups.

(A, B, AB and O) and Rh antigens.

A -- A antigen is present

B – B antigen is present

AB – Both A and B antigens are present

O – Neither A nor B antigen is present

Rh positive – Contains Rh antigen

Rh negative – No Rh antigen present

3) Auto antigens: -

Autologous or self-antigens are not actually antigenic because they are not normally found free in circulation hence not recognized as self-antigens. Such antigens are called as **'sequestrated antigens'** e.g. eye lens proteins, sperms, certain connective and nervous tissues, sperms. But when these are released into the tissue or in circulation due to some injury, antibodies are produced against them.

4) Organ specific antigens: -

Some organs such as brain, kidney and lens protein of different species have the same antigens called as organ specific antigens. These cause immune response when injected with some other material. E.g. Antirabic vaccine containing sheep brain tissue cause complication in man due to brain specific antigens of sheep and man.

5) Forssman antigens (Heterogenic / Shared, Common or Group): -

The same or closely related antigens may sometimes occur in different biological species, classes and kingdoms, called as heterogenic antigens. E.g. Forssman antigen is a lipid-carbohydrate complex, which is present in man, animals, birds, plants and bacteria.

e.g. Pneumococci and chickens contain antigens like human blood group A.

6) Celluar antigens present in and on cells: -

Some times complete cell behave as antigen. But a bacterial cell may contain several antigens. E.g.

---> Proteins of various enzymes

---> Nucleoproteins, ribosomes

---> Protein-polysaccharide complex of cell wall and capsule

a) Flagellar (H) antigen: -

Flagellin protein present in flagella serves as antigens in motile bacteria called as H antigens. 'H' is derived from German word 'Hauch' meaning motility. H antigens are described by boiling, alcohol and dilute acids. Thus each species or strain of flagellated bacteria has determinant groups located on the flagella. From the H antigens, serological typing of species is done.

b) Fimbrial antigens: -

Fimbriae occur on many species of enterobacteria. They are strongly antigenic. These are more resistant to heat, alcohol than H antigens.

c) Capsular antigens: -

Most capsules are heteropolymers of various simple sugars with glucosamine and other sugar derivatives. Capsules are conjugated with proteins. These capsules act as antigens. The polysaccharide antigens of the capsule are not all alike e.g. there are about 75 types of pneumococcus capsular antigens. Each antigen presents a different antigenic or serological type of pneumococcus. Similar capsular types are found in *Klebsiella, Haemophilus influenzae, Menigococci.*

In addition to antigenic specificity on the cell, capsules act as a protective coating against phagocytes, complements, and drying and other unfavourable conditions.

d) K antigens: -

K for Kapsel means capsule. These are like capsule antigens but are present inside the cell wall.

e) O antigens: -

'Ohne Hauch' German word meaning 'non-motile', non-flagellated. These antigens are components of the lipopolysaccharide portion of the cell wall of most Gram-negative bacteria. These are heat stable and are injured by alcohol and dilute acid.

f) Extracellular antigens: -

Extracellular metabolic products, exotoxins, behave as antigens, which are protein in nature.

Antibodies (Immunoglobulins)

[Structure & properties]

After the entry of an antigen, certain substances (Glycoproteins) called as **antibodies** or **Immunoglobulins** appear in the serum & tissue fluids, which react with antigen (in observable manner).

These immunoglobulins can recognize, bind to, and help the destruction of antigens. Antibodies are highly specific; they can interact with only one type of antigenic determinant on an antigen.

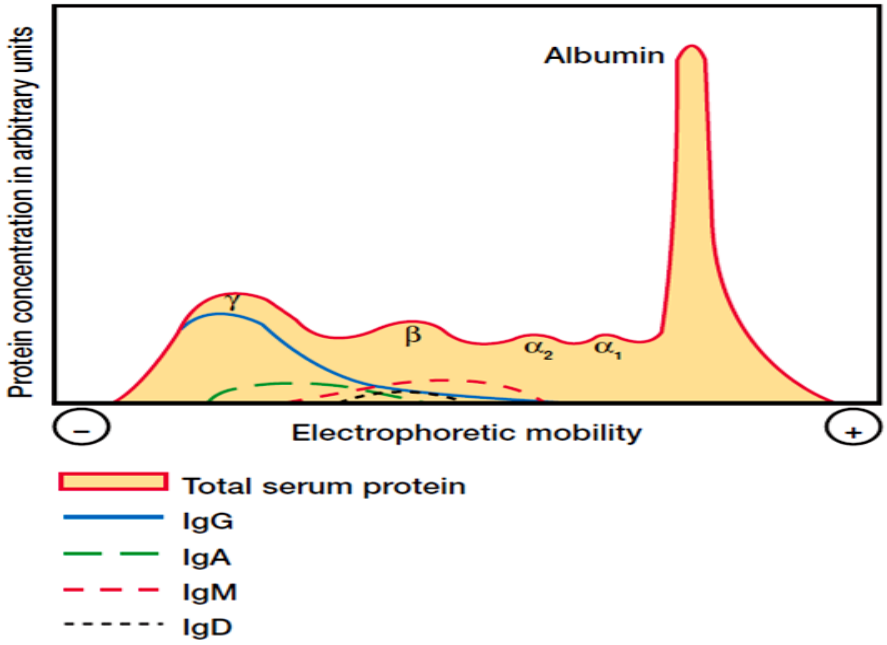
Tiselius (1937) separated the serum proteins based on their electrophoretic mobility into—

→ Albumin

→ α - Globulin

→ β - Globulin

→ γ - Globulin



Electrophoresis of Human Serum. Schematic representation of electrophoretic results illustrating the distribution of serum proteins and four major classes of immunoglobulins.

Tiselius showed that antibody activity is associated with γ - Globulin fraction. But later different scientists found that some antibodies, antitoxins are found in α and β Globulin and different scientists gave different names.

Then in 1964 WHO gave the name **'Immunoglobulin'** and intermittently accepted for—

'Proteins of serum with known antibody activity'.

Immunoglobulins constitute 20-25 % of total serum proteins. Based on physicochemical & antigenic differentiation, 5 classes of immunoglobulins are recognized.

1. Ig G
2. Ig A
3. Ig M
4. Ig D
5. Ig E

*** Structure of immunoglobulins**

1. Immunoglobulins are **Glycoproteins.**
2. Each molecule consists of 2 pairs of polypeptide chains of different sizes, which are held together by **'disulphide (-S-S-) bonds'.**
3. The smaller chains are called as **'Light' (L)** chains & larger are **'Heavy' (H)** chains.
4. **L chains: -** L chain has M.W. about 25,000 Daltons & is attached to the H chain by a disulphide bonds. There are 2 L chains in a single Ig molecule. These are similar in all classes of Ig. They occur in 2 varieties--

   ---- Kappa (k) &

   ---- Lambda (λ).

   Single molecule of Ig has either Kappa or Lambda chains but never both together.

6. **H chain: -** H chain has M.W. about 50,000. The 2 H chains are joined together by 1 to 5 –S-S- bonds, depending on the class of Ig.

   The H chains are structurally & antigenecally distinct for each class & are designated by the Greek letter corresponding to the Ig class.

Immunoglobulin Class	Designation of H chain
Ig G	γ (Gamma)
Ig A	α (Alpha)
Ig M	μ (Mau)
Ig D	δ (Delta)
Ig E	ε (Epsilon)

7. The 4-peptide chain structure of the Ig G molecule composed of 2H & 2L chains linked by interchains disulphide bonds. Loops formed by interchain disulphide bonds are **'Domains'.** Each chain has one domain in the variable region **(V_H & V_L).** Each L chain has one domain in the constant region **(C_L).** Each H chain has 3 domains in the constant region--

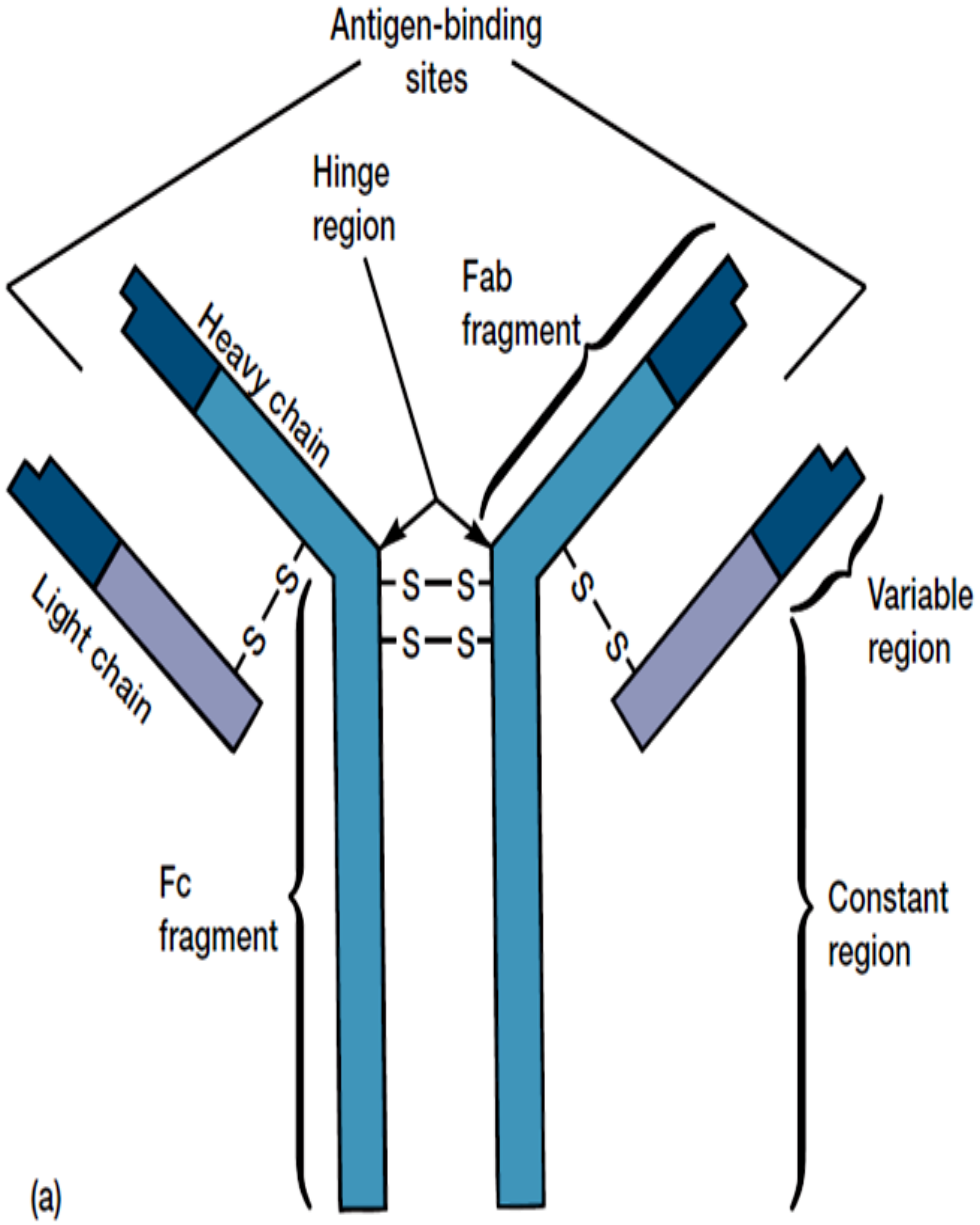
   (C_H1, C_H2 & C_H3).

   There is a **'Hinge region'** between **C_H1 & C_H2.**

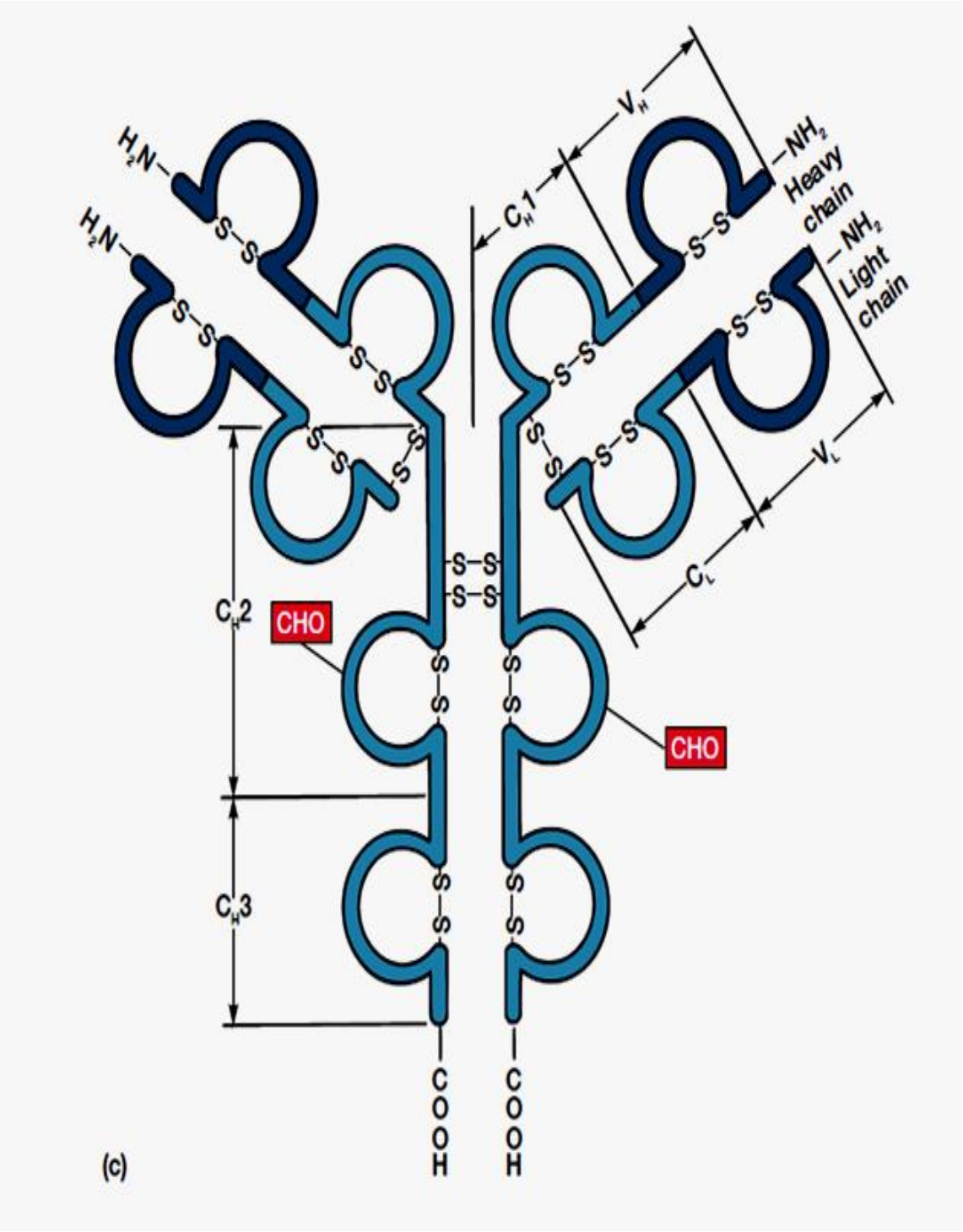
8. The antigen-combining site of the Ig molecule is its amino terminus. It is composed of both L & H chains. Each antibody has at least two identical sites that bind to antigenic determinants. These sites are known as **'antigen-binding' sites**. The number of antigen binding sites on an antibody determines the **valence** of that antibody. E.g. most human antibodies have two binding sites; therefore, they are **bivalent.** The amino acid sequence in the amino terminal half of the chain is highly variable hence called **'Variable region'.** This region determines the specificity of Ig.
9. Both L & H chain has constant carboxy terminus end & Variable amino terminus end.
10. The Fc fragment is composed of carboxy terminal portion of the H chain. It does not possess antigen-combining activity.
11. Fc portion is responsible for complement fixation etc.
12. H chain carries a carbohydrate portion, which is different for each class of Ig.
13. Each Ig peptide chain has internal disulphide links (-S-S-) & also has interchain disulphide bonds, which bind H & L chains.
14. The intrachain disulphide bonds form loops in the chain called as domain. Each domain has separate function. The variable region domains V_L & V_H are responsible for the formation of specific antigen binding sites. The

C_H2 domain binds complement. The C_H3 domain is responsible for adherence to the monocyte surface.

15. The area between C_H1 & C_H2 is called **'Hinge region'**, which is more flexible and attacked by enzymes and chemicals.



Immunoglobulin (Antibody) Structure. (**a**) An immunoglobulin molecule. The molecule consists of two identical light chains and two identical heavy chains held together by disulfide bonds.



(**c**)Within the immunoglobulin unit structure, intrachain disulfide bonds create loops that form domains. All light chains contain a single variable domain (VL) and a single constant domain (CL). Heavy chains contain a variable domain (VH) and either three or four constant domains (CH1, CH2, CH3, and CH4). The variable regions (VH, VL), when folded together in three-dimensions, form the antigen-binding sites.

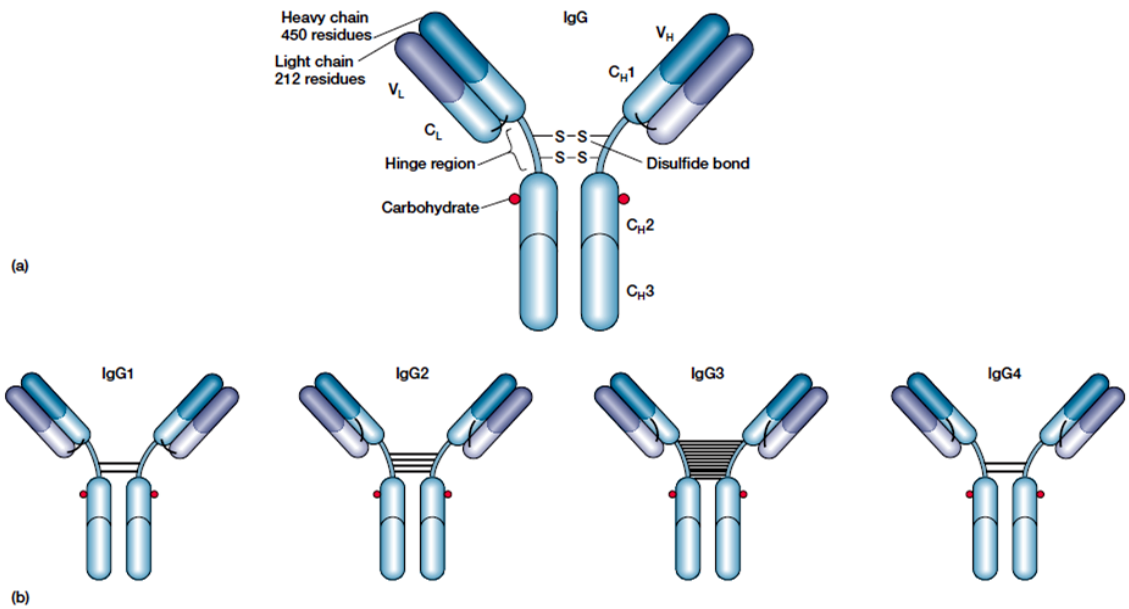
*** Immunoglobulin classes: -**

Human sera contains following immunoglobulins in order of descending concentration –

I. Ig G
II. Ig A
III. Ig M
IV. Ig D
V. Ig E

I. Ig G: -

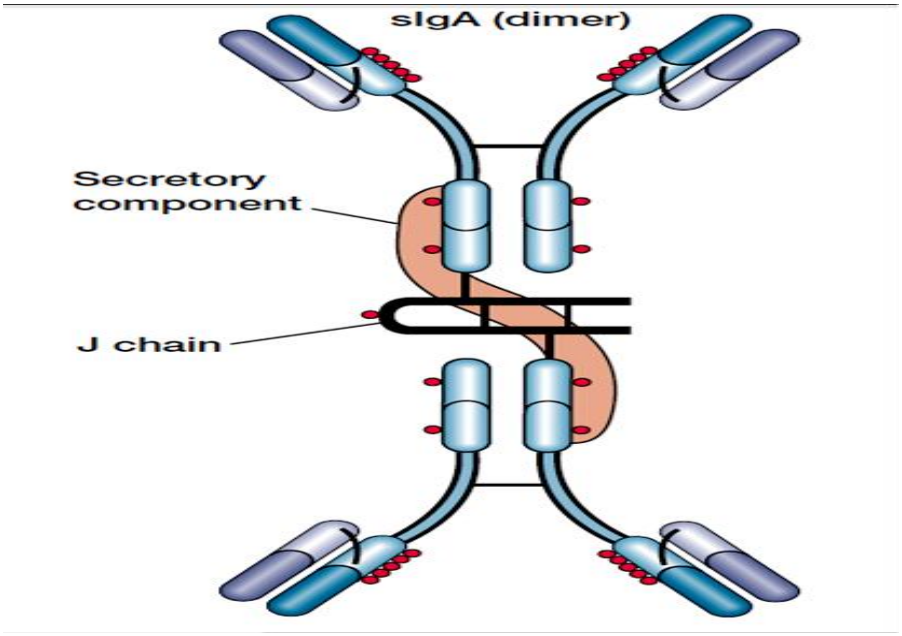
1. Major serum immunoglobulin constituting about 80 % of the total.
2. M.W. 150, 000 and Sedimentation coefficient 7S.
3. Distributed in intravascular and extra vascular components.
4. Contains less carbohydrate and has a half-life of approximately 23 days.
5. Normal serum concentration 8 to 16 mg / ml.
6. It is the only maternal Ig i.e. transported across the placenta from mother to foetus (provides natural passive immunity in the newborn).
7. IgG binds to microorganisms and enhances their phagocytosis.
8. IgG participates in the reactions such as Complement fixation, Precipitation and Neutralization of toxins and Viruses.
9. IgG is protective against blood and tissue infectious agents.
10. 4 classes of IgG have been recognized (IgG1, IgG2, IgG3, IgG4).



Immunoglobulin G. (**a**) The basic structure of human IgG. (**b**) The structure of the four human IgG subclasses. Note the arrangement and numbers of disulfide bonds (shown as thin black lines).

II) IgA

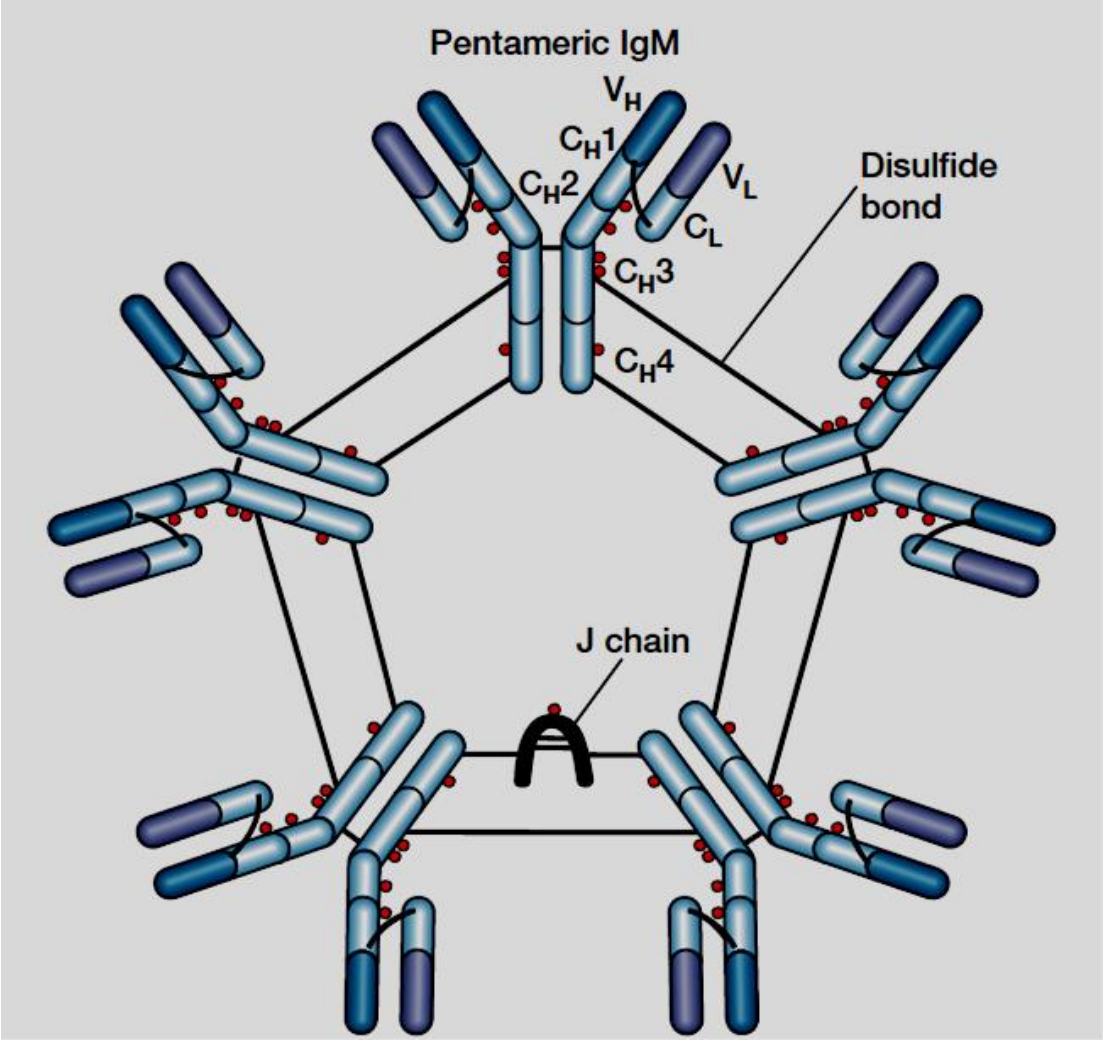
1. Second most abundant immunoglobulin constituting about 10 to 13 % of total Ig.
2. M.W. 160, 000 and Sedimentation coefficient 7S.
3. Found in **colostrums, saliva and tears** is selectively concentrated in secretions and on mucous surfaces and believed to play an important role in local immunity against respiratory and intestinal pathogens.
4. Half-life 6 to 8 days.
5. Normal serum concentration 0.6 to 4 mg / ml.
6. IgA is found in secretions and it contains an additional structural unit called as **'Transport (T)** or **'Secretary (S)'** piece. This piece is not synthesized in plasma cells, but in epithelial cells of glands, intestines and respiratory tract and is attached to the IgA molecules during transport.
7. T or S piece joins together 2 IgA molecules producing 11S dimer responsible for secretion.
8. Another polypeptide chain called **'J chain'** is present in IgA and IgM (Joining chain), which is synthesized by plasma cell responsible for joining 2 IgA.
9. IgA inhibits the adherence of microorganisms to the surface of mucosa cells by covering the organisms and prevent their entry into the body tissues.
10. IgA does not fix complement but can activate Alternate Complement Pathway.
11. It promotes phagocytosis and intracellular killing of microorganisms.
12. There are 2 IgA subclasses (IgA1 & IgA2).



Immunoglobulin A. The dimeric structure of human secretory IgA. Notice the secretory component (tan) wound around the IgA dimer and attached to the constant domain of each IgA monomer.

III) IgM

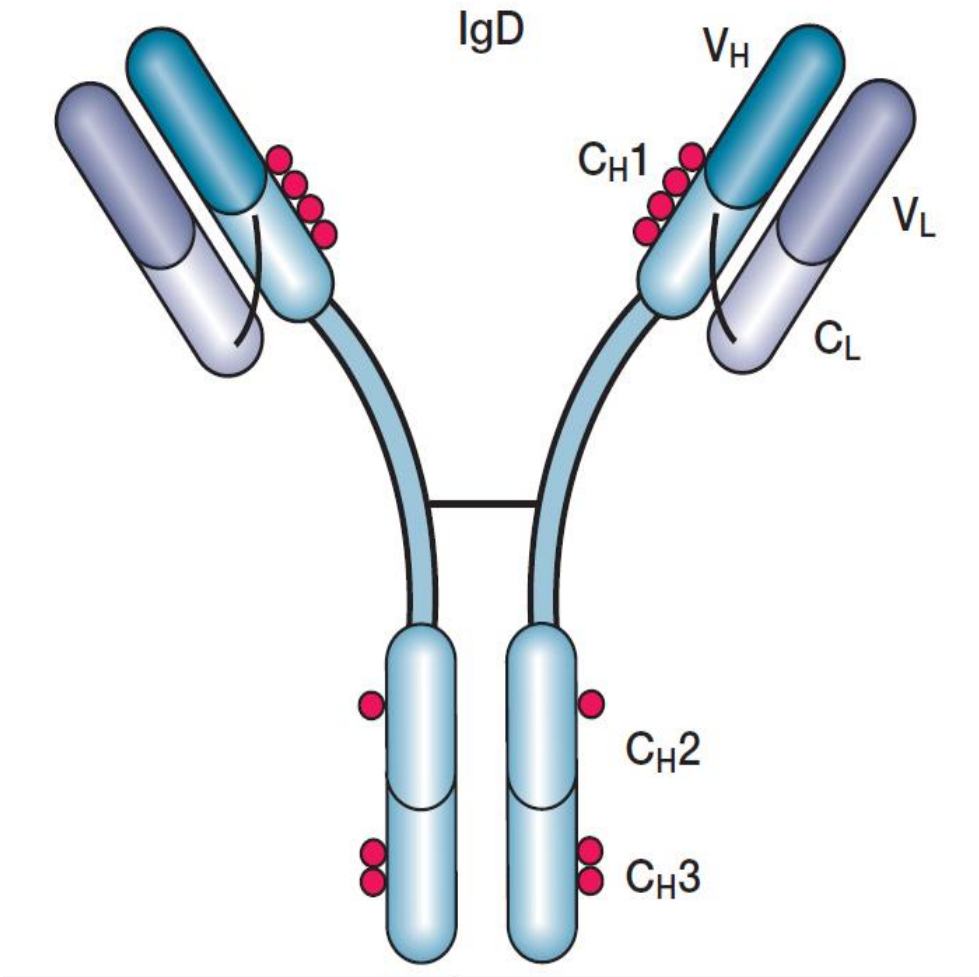
1. Constitutes 5 to 8 % of total Ig.
2. M.W. 900,000 and Sedimentation coefficient 19S.
3. Distributed mainly intravascularly.
4. Half-life 5 days.
5. Normal serum concentration 0.5 to 2 mg / ml.
6. It is a heavy molecule of 5 units of Ig joined together by J chain.
7. It is the earliest Ig to be synthesized by the foetus (In 5th month).
8. Not transported across placenta.
9. These Igs are relatively short lived and disappear earlier than IgG. Hence their demonstration in serum indicates recent infection.
10. Provides protection against microorganisms and large antigens.



Immunoglobulin M. The pentameric structure of human IgM. The disulfide bonds linking peptide chains are shown in black; carbohydrate side chains are in red. Note that 10 antigen-binding sites are present.

IV) IgD

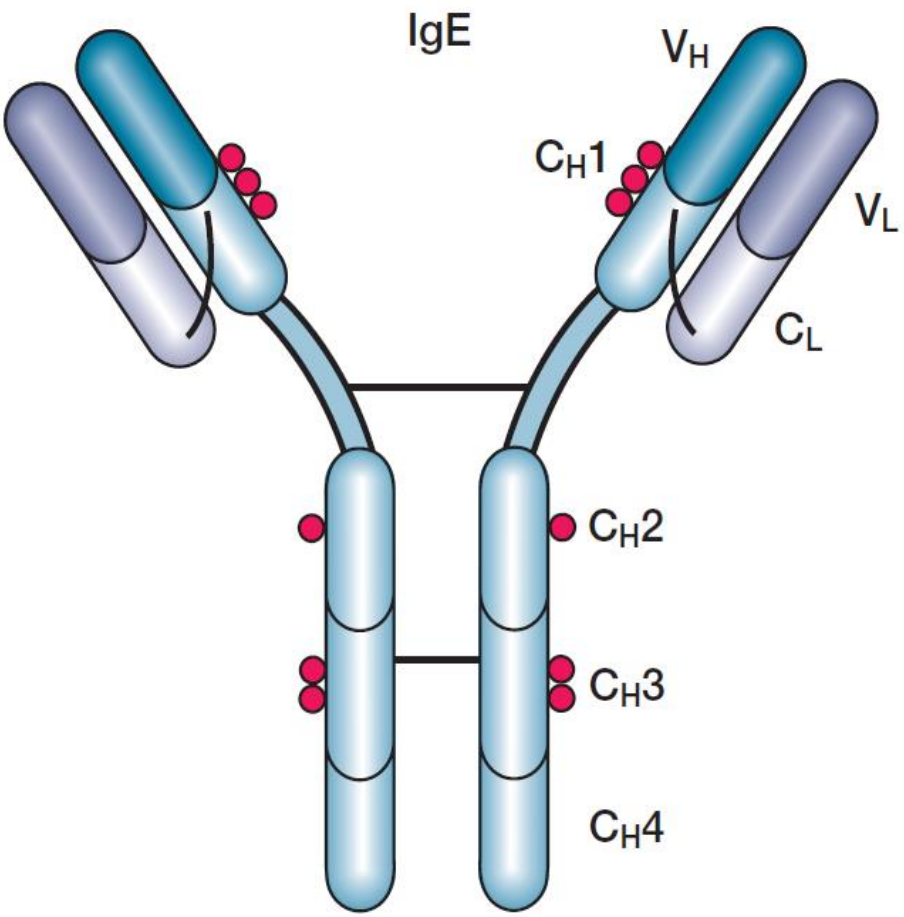
1. Constitutes 3 % of total Ig.
2. M.W. 180,000 and Sedimentation coefficient 7S.
3. Distributed mainly intravascularly.
4. Half-life 3 days.
5. Normal serum concentration 0.3 mg / ml.
6. IgD and IgM occur on the surface of unstimulated B-lymphocytes and serve as recognition receptors for antigens. When antigen binds to IgD, it leads to specific stimulation of the B cell to produce antibody (or suppression).
7. The structure of IgD is like IgG.



Immunoglobulin D. The structure of human IgD. The disulfide bonds linking protein chains are shown in black; carbohydrate side chains are in red.

V) IgE

1. Constitutes 0.004 % of total Ig.
2. M.W. 190,000 and Sedimentation coefficient 8S.
3. Chiefly produced in lining of respiratory and intestinal tract.
4. Half-life 2 days.
5. Normal serum concentration 0.00004 mg / ml.
6. Not transported across the placenta and not fix the complement.
7. The normal serum level is greatly enhanced in **asthma, hay fever & intestinal parasite infection.**
8. Responsible for the anaphylactic type of hypersensitivity.
9. No beneficial function of IgE is known but it may play important role in the defense against intestinal parasitic infection.
10. The structure of IgE is like IgG.



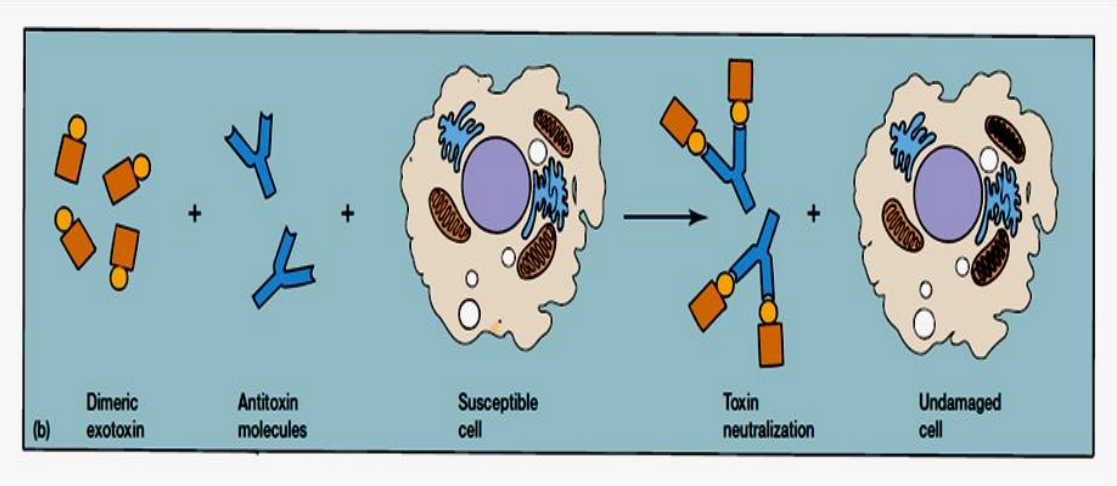
Immunoglobulin E. The structure of human IgE.

Sr No	Character	IgG	IgA	IgM	IgD	IgE
1	H - chain	γ	α	μ	δ	ε
2	L - chain	κ Or λ	κ Or λ	κ Or λ	κ Or λ	κ Or λ
3	Sediment Coefficient	7	7	19	7	8
4	Molecular Weight	150,000	160,000	900,000	180,000	190,000
5	Serum concentration (mg/ml)	12	2	1.2	0.03	0.00004
6	Percent. %	80	10-13	5-8	3	0.004
7	Half life (Days)	23	6	5	3	2
8	Carbohydrate %	3	8	12	13	12
9	Complement fixation Classical Alternative	++ --	-- +	+++ --	--	--
10	Placental transport	+	--	--	--	--
11	Selective secretion by glands	--	+	--	--	--
12	Functions	Provides passive immunity In foetus, enhances phagocytosis, neutralization of toxins & viruses	Inhibit adherence Of micro-organisms to tissue & prevent entry into tissue, promotes phagocytosis	Earliest Ig synthesized, protection against microorganisms & large antigens	Stimulate B cell to synthe-size anti-bodies	Respon-Sible for hypersensitivity (asthma, hay fever), defence against intestinal parasites

Types of antibodies

Antitoxin / Toxin Neutralizing

Immunity to a disease like diphtheria depends on the production of specific antibodies that inactivate the toxins produced by the bacteria. This process is termed **toxin neutralization.** Once neutralized, the toxin-antibody complex is either unable to attach to receptor sites on host target cells, unable to enter the cell, or it is ingested by macrophages. For example, diphtheria toxin inhibits protein synthesis after binding to the cell surface by the B fragment and subsequent passage of the active A fragment into the cytoplasm of the target cell. Thus the antibody blocks the toxic effect by inhibiting the entry of the A fragment or the binding of the B fragment. An antibody capable of neutralizing a toxin or antiserum containing neutralizing antibody against a toxin is called **antitoxin.**

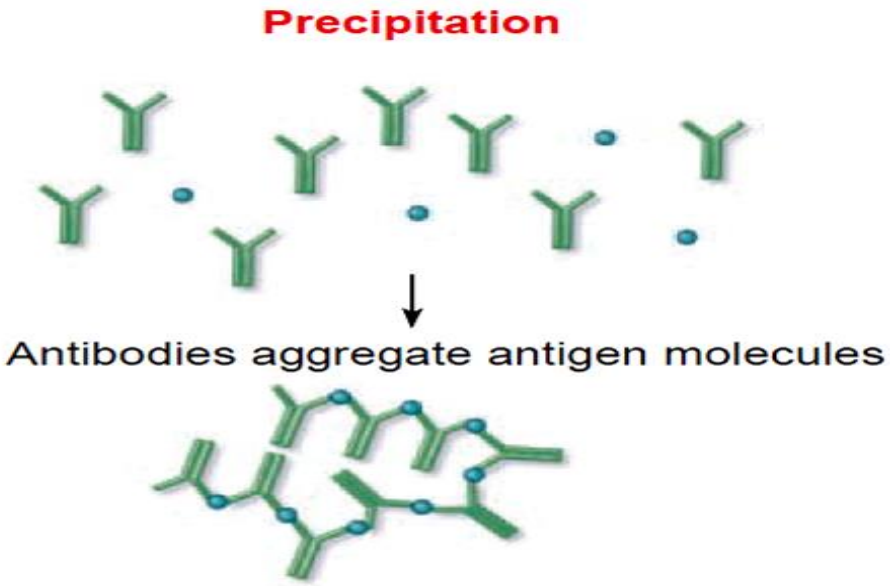


(**b**) Neutralization of the toxin by antitoxin.

Precipitin

A **precipitin** is an antibody which can precipitate out of a solution upon antigen binding. The precipitin reaction provided the first quantitative assay for antibody, which has since been rendered obsolete (out dated) by current diagnostic techniques such as ELISA (enzyme linked immunosorbant assay) and RIA (Radioimmunoassay). The precipitin reaction is based upon the interaction of antigen with antibody leading to the production of antigen:antibody complexes.

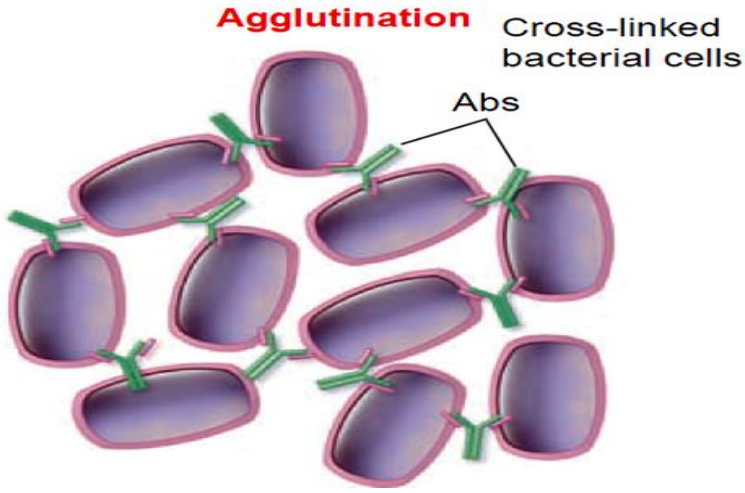
In the precipitin reaction, varying amounts of soluble antigens are added to a fixed amount of serum containing antibody.



Agglutinins

Agglutinins are antibodies that cause antigens to aggregate by binding to the antigen-binding sites of antibodies

Agglutinins work by clumping on particles causing the particles to change from fluid-like state to thickened-mass state. When an agglutinin is added to a uniform suspension of particles such as bacteria or blood, agglutinin binds to the agglutinin-specific structure on the particle causing the particles to aggregate and fall to the bottom leaving a clear suspension. This phenomenon known as agglutination is of great importance to the medical world as it serves as a diagnostic tool.



Bacteriolysin

An antibody that, together with other substances, destroys a bacterium. Specific antibody that combines with bacterial cells (i.e., antigen) and, in the presence of complement, causes lysis or dissolution of the cells.

Bacteriocidin

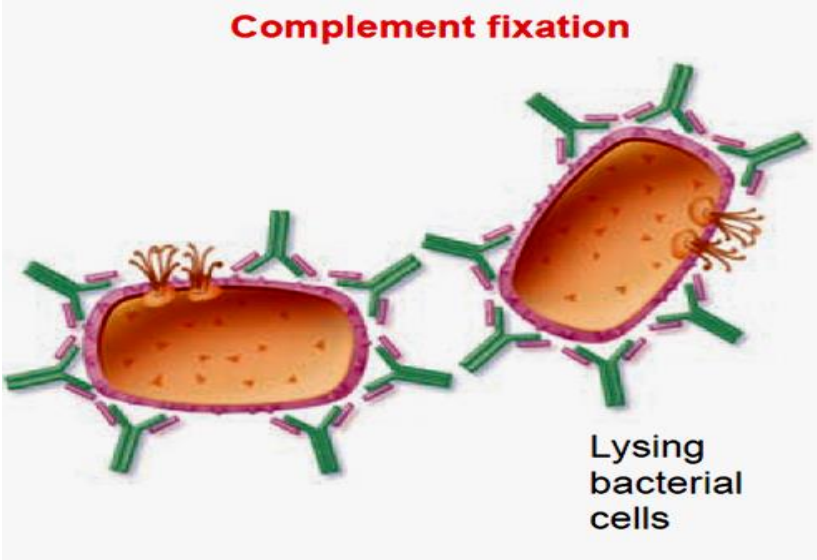
An antibody capable of destroying bacteria.

Bacteriotropin

An antibody that is increased in amount during specific immunization and that renders the corresponding bacterium more susceptible to phagocytosis.

Complement fixing

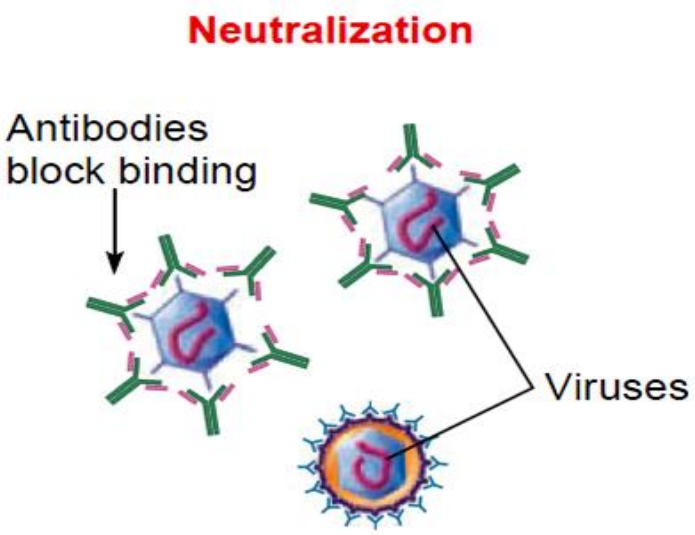
Complement-fixing antibody is one that activates complement when reacted with antigen: IgM and IgG fix complement by the classical pathway; IgA, by the alternative pathway. An antibody that combines with and sensitizes an antigen, leading to activation of complement and sometimes lysis. Fixation of the classical pathway complement component C3b to the virus aids the neutralization process. Viral neutralization prevents a viral infection due to the inability of the virus to bind to its target cell.



Neutralizing

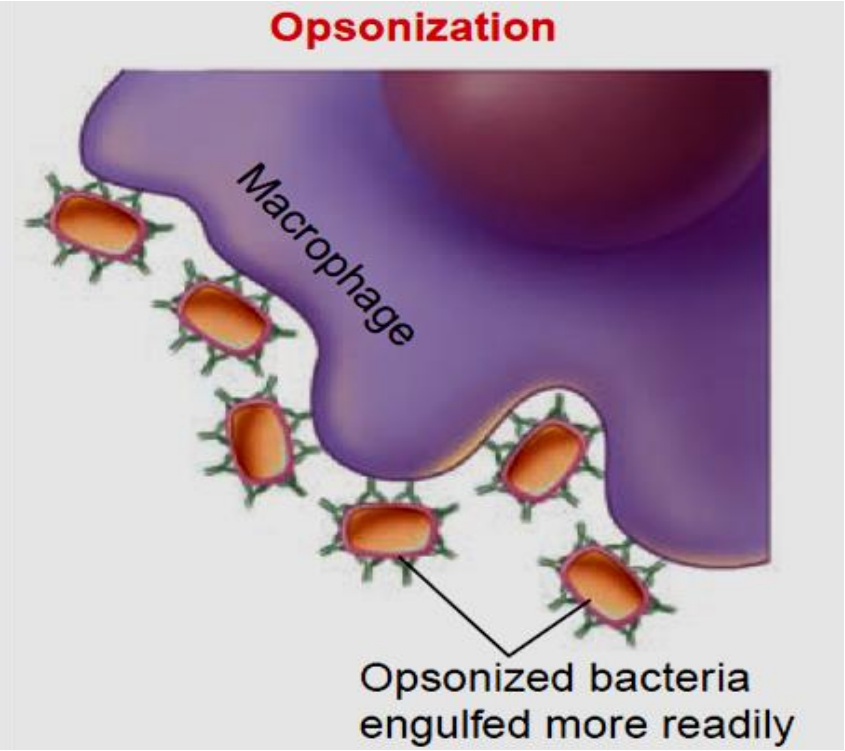
A **Neutralizing antibody**, or **NAb** is an antibody which defends a cell from an antigen or infectious body by inhibiting or neutralizing any effect it has biologically. An example of a neutralizing antibody is diphtheria antitoxin, which can neutralize the biological effects of diphtheria toxin. Neutralizing antibodies neutralize the biological effects of the antigen.

IgG, IgM, and IgA antibodies can bind to some viruses during their extracellular phase and inactivate them. This antibody mediated viral inactivation is called **viral neutralization.**



Opsonizing antibodies

Phagocytes have an intrinsic ability to bind directly to microorganisms by nonspecific cell surface receptors, engulf the microorganisms, form phagosomes, and digest the microorganisms. This phagocytic process can be greatly enhanced by opsonization. Opsonization is the process in which microorganisms or other particles are coated by antibody and/or complement, and thus prepared for "recognition" and ingestion by phagocytic cells. Opsonizing antibodies, especially IgG1 and IgG3, bind to Fc receptors on the surface of macrophages and neutrophils. This binding forms a bridge between the phagocyte and the antigen.


Opsonization
Macrophage
Opsonized bacteria
engulfed more readily

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