Immunology and serology L1 Dr. Muna Al-Rubiae

INTRODUCTION

Immunity refers to protection against infection. The **immune system** is the collection of cells, tissues and molecules that functions to defend us against infectious microbes. The coordinated reaction of the immune system against infections (and other foreign substances) is known as the **immune response**.

Immunology is the study of the immune system and is a very important branch of the medical and biological sciences. The immune system protects us from infection through various lines of defense.

Abnormalities of the immune system that result in defective immune response make individuals susceptible to infections by viruses, bacteria, fungi and parasites. This anti-microbial defense function of the immune system is essential for our ability to survive in an environment that is teeming with potentially deadly microbes. However, immune responses are also capable of causing damage. Many common diseases are caused by uncontrolled or excessive immune responses (examples include rheumatic fever, asthma and glomerulonephritis.

The immune system is a remarkable defense mechanism. It provides the means to make rapid, specific, and protective responses against the myriad potentially pathogenic microorganisms that inhabit the world in which we live. The tragic example of severe immunodeficiencies, as seen in both genetically determined diseases and in acquired immunodeficiency syndrome (AIDS), graphically illustrates the central role the immune

response plays in protection against microbial infection. The immune system also has a role in the rejection of tumors and may exert important effects in regulating other bodily systems.

History

Edward Jenner (1749 - 1823)

• He was a doctor in Berkeley, in England. In 1796 he carried out his now famous experiment on eight-year-old child. Jenner inserted pus taken from a cowpox pustule on the hand of milkmaid girl and inserted it into an incision on the boy's arm. He was testing his theory, drawn from the folklore of the countryside, that milkmaids who suffered the mild disease of cowpox never contracted smallpox.

• Jenner subsequently proved that having been inoculated with cowpox boy was now immune to smallpox. He submitted a paper to the Royal Society in 1797 describing his experiment but was told that his ideas were too revolutionary and that he needed more proof. Jenner experimented on several other children, including his own 11-month-old son. In 1798 the results were finally published and Jenner coined the word vaccine from the Latin vacca for cow, and called the process vaccination.

The term immunology was coined by Russian biologist Mechnikov, who advanced studies on immunology and received the Nobel Prize for his work in 1908. He pinned small thorns into starfish larvae and noticed unusual cells surrounding the thorns. This was the active response of the body trying to maintain its integrity. Mechnikov he is first observed the phenomenon of phagocytosis, in which the body defends itself against a foreign body.

The fundamental observation that led to the development of immunology as a scientific discipline was that an individual can become resistant for life to a certain disease after having contracted it only once. The founding of immunology as a discipline was closely tied to the development of microbiology. The work of Pasteur, Koch, and many other pioneers of the golden age of microbiology resulted in the rapid identification of new infectious agents, closely followed by the discovery that infectious diseases could be prevented by exposure to killed or attenuated organisms, or to compounds extracted from the infectious agents. The impact of immunization against infectious diseases such as tetanus, pertussis, diphtheria, and smallpox, which were significant causes of mortality and morbidity, are now either extinct or very rarely seen.

From Edward Jenner's pioneering work in the 18th Century that would ultimately lead to vaccination in its modern form (an innovation that has likely saved more lives than any other medical advance), the immunity lead to, safe organ transplantation, the identification of blood groups, and the now ubiquitous use of monoclonal antibodies throughout science and healthcare, immunology has changed the face of modern medicine. And vaccines for emerging pathogens, such as Ebola. Advancing our understanding of basic immunology is essential for clinical and commercial application and has facilitated the discovery of new diagnostics and treatments to manage a wide range of diseases.

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Immunity = resistance: defense against foreign material

Antigen = An antigen is anything that **elicits** the formation of a specific immune response.

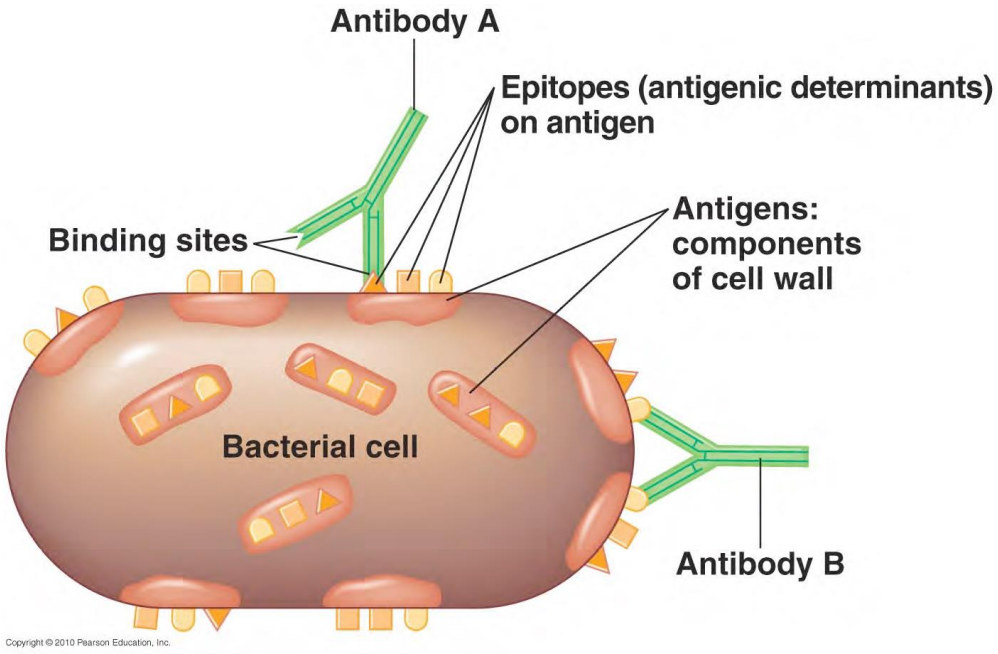
Epitopes = These are the **unique regions (or chemical groups)** on a molecule that are antigenic; i.e., that elicit a specific immune response.

Antibody = Y shape **soluble proteins** that are produced in response to foreign antigens.

Cellular immunity: or cell-mediated immunity is a protective **immune** process that involves the activation of cells such as phagocytes, T-lymphocytes, polymorphonuclear.

Humoral immunity: Immune responses mediated by circulating secreted molecule.

Immune cells or **lymphocytes**= These are the **various cells** of the specific immunity system that respond to **specific** foreign or nonself antigens.



Types of immunity

We are constantly being exposed to infectious agents and yet, in most cases, we are able to resist these infections. It is our immune system that enables us to resist infections. The immune system is composed of two major subdivisions, the innate or non-specific immune system and the adaptive or specific immune system. The innate immune system is our first line of defense against invading organisms while the adaptive immune system acts as a second line of defense and also provide protection against re-exposure to the same pathogen. Each of the major subdivisions of the immune system has both cellular and humoral components by which they carry out their protective function. In addition, the innate immune system also has anatomical features that function as barriers to infection. Although these two arms of the immune system have distinct functions, there is

interplay between these systems (i.e., components of the innate immune system influence the adaptive immune system and vice versa).

Innate (Non-Specific) Immunity

Although the innate and adaptive immune systems both function to protect against invading organisms, they differ in a number of ways. The adaptive immune system requires some time to react to an invading organism, whereas the innate immune system includes defenses that, for the most part, are present and ready to be mobilized upon infection. Second, the adaptive immune system is antigen specific and reacts only with the organism that induced the response. In contrast, the innate system is not antigen specific and reacts equally well to a variety of organisms. Finally, the adaptive immune system demonstrates immunological memory. It "remembers" that it has encountered an invading organism and reacts more rapidly on subsequent exposure to the same organism. In contrast, the innate immune system does not demonstrate immunological memory.

Table 1

Non-specific Immunity	Specific Immunity
Response is **antigen-independent**	Response is **antigen-dependent**
There is **immediate** maximal response	There is a **lag time** between exposure and maximal response
Not antigen-specific	**Antigen-specific**
Exposure results in **no immunologic memory**	Exposure results in **immunologic memory**

The elements of the innate (non-specific) immune system (Table 2) include anatomical barriers, secretory molecules and cellular components. Among the mechanical anatomical barriers are the skin and internal epithelial layers, the movement of the intestines and the movement of broncho-pulmonary cilia. Associated with these protective surfaces are chemical and biological agents.

A. Anatomical barriers to infections

1. Mechanical factors

The epithelial surfaces form a physical barrier that is very impermeable to most infectious agents. Thus, the skin acts as our first line of defense against invading organisms. The desquamation of skin epithelium also helps remove bacteria and other infectious agents that have adhered to the epithelial surfaces. Movement due to cilia helps to keep air passages and the gastrointestinal tract free from microorganisms. The trapping affect of mucus that lines the respiratory and gastrointestinal tract helps protect the lungs and digestive systems from infection.

2. Chemical factors

Fatty acids in sweat inhibit the growth of bacteria. Lysozyme and phospholipase found in tears, saliva and nasal secretions can breakdown the cell wall of bacteria and destabilize bacterial membranes. The low pH of sweat and gastric secretions prevents growth of bacteria. Defensins (low

molecular weight proteins) found in the lung and gastrointestinal tract have antimicrobial activity.

3. Biological factors
The normal flora of the skin and in the gastrointestinal tract can prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria for nutrients or attachment to cell surfaces.

B. Humoral barriers to infection

The anatomical barriers are very effective in preventing colonization of tissues by microorganisms. However, when there is damage to tissues the anatomical barriers are breached and infection may occur. Once infectious agents have penetrated tissues, another innate defense mechanism comes into play, namely acute inflammation. Humoral factors play an important role in inflammation, which is characterized by edema and the recruitment of phagocytic cells. These humoral factors are found in serum or they are formed at the site of infection.

These humoral factors include

1. Complement system – The complement system is the major humoral non-specific defense mechanism. Once activated complement can lead to increased vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.

2. Coagulation system – Depending on the severity of the tissue injury, the coagulation system may or may not be activated. Some products of the coagulation system can contribute to the non-specific defenses because of their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells. In addition, some of the products of the coagulation system are directly antimicrobial. For example, beta-lysin, a protein produced by platelets during coagulation can lyse many Gram positive bacteria by acting as a cationic detergent.

3. Lactoferrin and transferrin – By binding iron, an essential nutrient for bacteria, these proteins limit bacterial growth.

4. Interferons – Interferons are proteins that can limit virus replication in cells.

5. Lysozyme – Lysozyme breaks down the cell wall of bacteria.

6. Interleukin-1 - induces fever and the production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

Table 2

System/Organ	Active component	Effector Mechanism
Skin	Squamous cells; Sweat	Desquamation; organic acids
GI tract	Columnar cells	Peristalsis, low pH, bile acid,

Lung	Tracheal cilia	Mucocialiary elevator, surfactant
Nasopharynx and eye	Mucus, saliva, tears	Lysozyme
Circulation and lymphoid organs	Phagocytic cells	Phagocytosis and intracellular killing
	NK cells	Direct and antibody dependent cytolysis
Serum	Lactoferrin and Transferrin	Iron binding
	Interferons	Antiviral proteins
	Lysozyme	Peptidoglycan hydrolysis
	Complement	Opsonization, enhanced phagocytosis, inflammation

C. Cellular barriers to infection

Part of the inflammatory response is the recruitment of polymorphonuclear eosinophiles and macrophages to sites of infection. These cells are the main line of defense in the non-specific immune system.

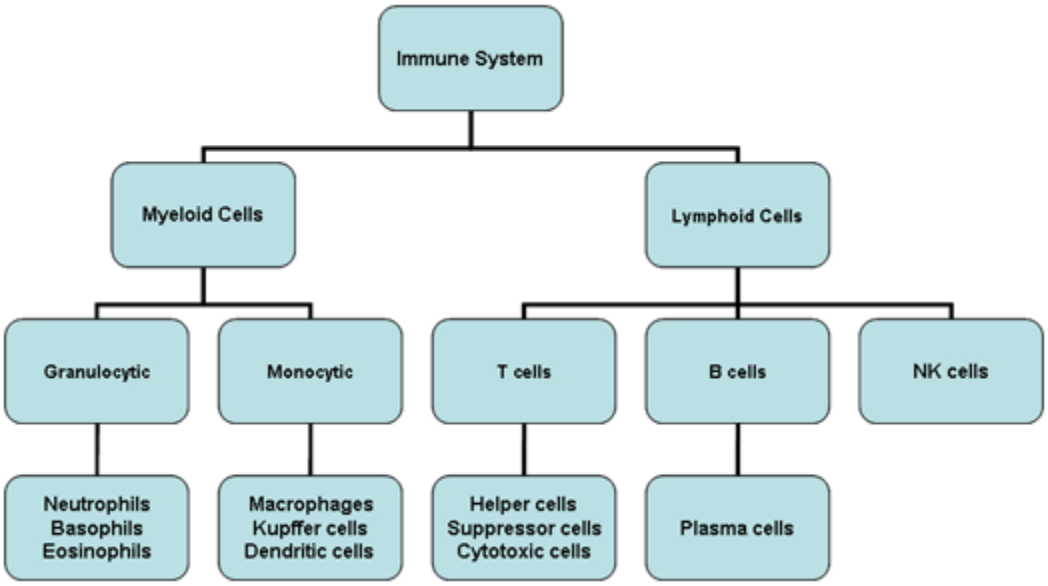
1. Neutrophils – Polymorphonuclear cells (PMNs) are recruited to the site of infection where they phagocytose invading organisms and kill them intracellularly.
2. Macrophages – Tissue macrophages and newly recruited monocytes, which differentiate into macrophages, also function in phagocytosis and intracellular killing of microorganisms. In addition, macrophages are

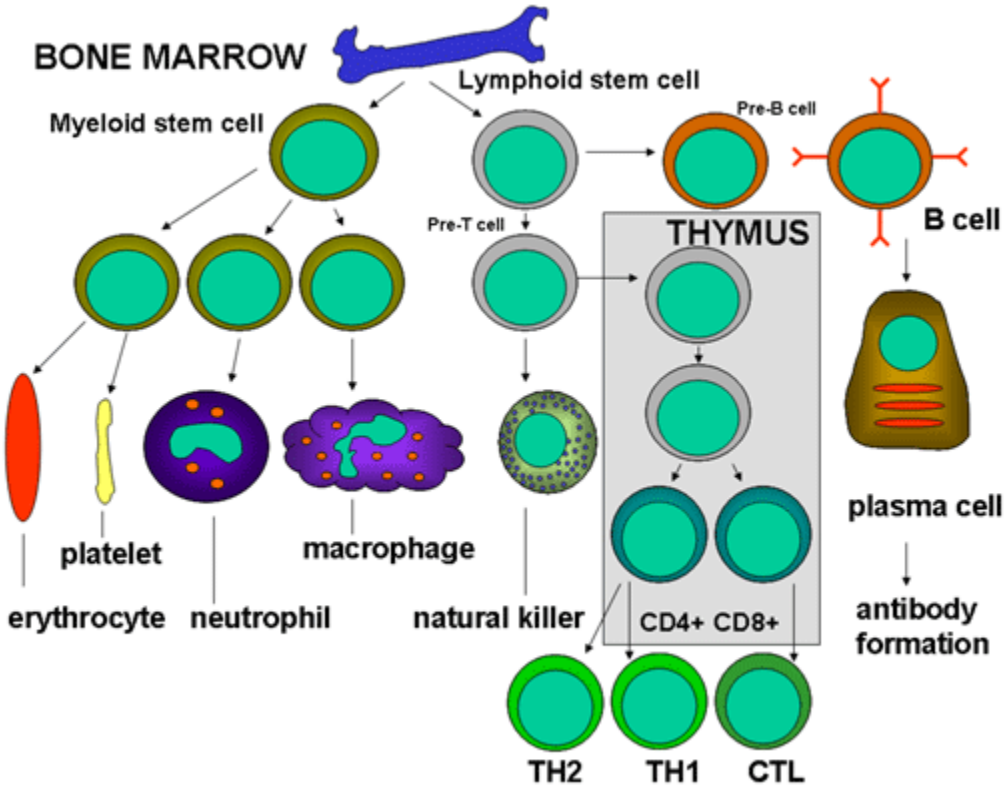
capable of extracellular killing of infected or altered self target cells. Furthermore, macrophages contribute to tissue repair and act as antigen-presenting cells, which are required for the induction of specific immune responses.

3. Natural killer (NK) can nonspecifically kill virus infected and tumor cells. These cells are not part of the inflammatory response but they are important in nonspecific immunity to viral infections and tumor surveillance.

4. Eosinophils – (acidophils) have proteins in granules that are effective in killing certain parasites.

All cells of the immune system have their origin in the bone marrow and they include myeloid (neutrophils, basophils, eosinpophils, macrophages and dendritic cells) and lymphoid (B lymphocyte, T lymphocyte and Natural Killer) cells (Figure 1), which differentiate along distinct pathways (Figure 2). The myeloid stem cell in the bone marrow gives rise to erythrocytes, platelets, neutrophils, monocytes/macrophages and dendritic cells whereas the lymphoid stem cell gives rise to the NK, T cells and B cells.


Immune System
Myeloid Cells
Lymphoid Cells
Granulocytic
Monocytic
T cells
B cells
NK cells
Neutrophils
Basophils
Eosinophils
Macrophages
Kupffer cells
Dendritic cells
Helper cells
Suppressor cells
Cytotoxic cells
Plasma cells


BONE MARROW
Lymphoid stem cell
Myeloid stem cell
Pre-B cell
B cell
Pre-T cell
THYMUS
plasma cell
platelet
macrophage
erythrocyte
neutrophil
natural killer
CD4+ CD8+
antibody formation
TH2
TH1
CTL

Adaptive or Specific Immunity

The **adaptive immune system**, also known as the **acquired immunity**, One of the two main immunity strategies found in vertebrates (the other being the innate immune system), acquired immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.

The adaptive immune or specific immune response consists of antibody responses and cell-mediated responses, which are carried out by different lymphocyte cells, B cells and T cells, respectively. B Cells are the major cells involved in the creation of antibodies that circulate in blood plasma and lymph, where they bind specifically to the foreign antigens.

Types of Adaptive or Specific Immunity

Immunity is the ability to resist infection by an invading pathogen. The body quickly launches an immune response and prevents the symptoms of disease occurring. This can happen in two ways – naturally or artificially. Natural immunity occurs without human intervention and artificial immunity occurs when antigens or antibodies are given to a person by artificial means, eg by injection. Passive immunity is when a person is given antibodies produced by someone else. This could happen naturally when a mother passes her own antibodies to her baby either

through her placenta or her breast milk. Another method of gaining passive immunity is artificially, for example when a person is given an injection of antibodies if they suspect that they have been exposed to a disease such as tetanus or diphtheria. In this situation, immunity is established immediately. This form of immunity is short lived as no memory cells are produced.

Longer-term immunity is gained via active immunity. Examples of this involve the body being stimulated to produce antibodies via a specific immune response. This occurs either by a person contracting a disease which is referred to as natural active immunity or via an injection of weakened (attenuated) or dead antigens. In this case an immune response is activated resulting in the production of antibodies and memory cells. This latter form of immunity is called artificial active immunity. The principle of immunisation is based on this.

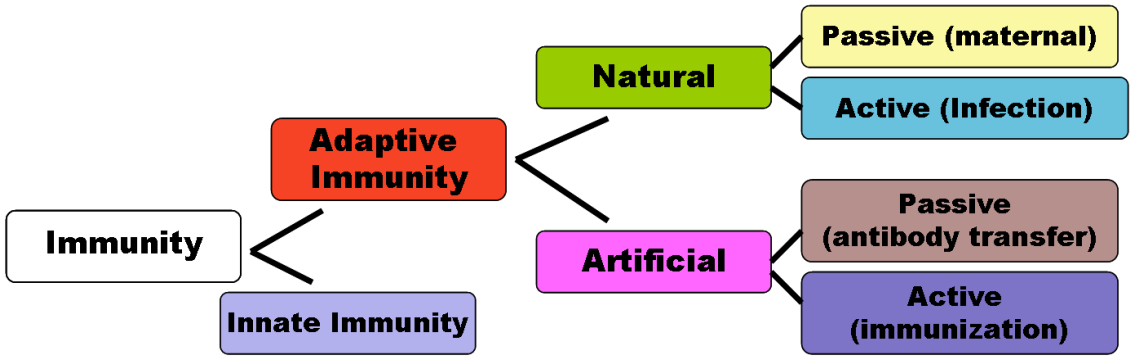
Types of natural and artificial immunity

 natural, active immunity – after having been infected by the pathogen and your immune system responds; a person is infected by the disease once and then the production of antibodies grants future immunity

 artificial, active immunity – e.g. after having been given a vaccination of a small, harmless version of a disease so that the immune system is triggered to develop appropriate antibodies, giving immunity

 natural, passive immunity – receiving antibodies not from your own immune system, but not artificially, so for example from breast milk or across the placenta during pregnancy

 artificial, passive immunity – receiving the antibodies again not from your own immune system, e.g. from an injection of the direct antibodies, rather than the disease, so the immune system does not need to respond.



A key feature of the adaptive immune system is memory, the development of immunological memory, in which each pathogen is "remembered" by a signature antibody. Part of activated B cells and T cells can develop to memory cells. Memory cells remain ready to respond rapidly and efficiently to a subsequent encounter with a pathogen. This so-called secondary response is often stronger than the primary response to infection.

The function of adaptive immune responses is to destroy invading pathogens and any toxic molecules they produce. Because these responses are destructive, it is crucial that they be made only in response to molecules that are foreign to the host and not to the molecules of the host itself. The ability to distinguish what is **foreign** from what is **self** in this way is a fundamental feature of the adaptive immune system. Occasionally, the system fails to make this distinction and reacts destructively against the host's own molecules. Such **autoimmune diseases** can be fatal.

Adaptive immune responses are carried out by white blood cells called lymphocytes. There are two broad classes of such responses—antibody responses and cell-mediated immune responses, and they are carried out by different classes of lymphocytes, called B cells and T cells, respectively. In **antibody responses**, B cells are activated to secrete antibodies, which are proteins called **immunoglobulins**. The antibodies circulate in the bloodstream and permeate the other body fluids, where they bind specifically to the foreign antigen that stimulated their production. Binding of antibody inactivates viruses and microbial toxins (such as tetanus toxin or diphtheria toxin) by blocking their ability to bind to receptors on host cells. Antibody binding also marks invading pathogens for destruction, mainly by making it easier for phagocytic cells of the innate immune system to ingest them.

Vaccine

A **vaccine** is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and keep a record of it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.

Vaccines can be prophylactic (example: to prevent the effects of a future infection by any natural or "wild" pathogen), or therapeutic (e.g., vaccines against cancer are also being investigated).

Types of vaccines

1-Inactivated

Some vaccines contain inactivated, but previously virulent, micro-organisms that have been destroyed with chemicals, heat, radioactivity, or antibiotics. Examples are influenza, cholera, polio, hepatitis A.

2-Attenuated

Some vaccines contain live, attenuated microorganisms. Many of these are active viruses that have been cultivated under conditions that disable their virulent properties, or that use closely related but less dangerous organisms

to produce a broad immune response. Although most attenuated vaccines are viral, some are bacterial in nature. Examples include the viral diseases yellow fever, measles, rubella, and mumps, and the bacterial disease typhoid. Attenuated vaccines have some advantages and disadvantages. They typically give good immunological responses and are the preferred type for healthy adults. But they may not be safe for use in immunocompromised individuals, and may rarely mutate to a virulent form and cause disease.

3-Toxoid

Toxoid vaccines are made from inactivated toxic compounds that cause illness. Examples of toxoid-based vaccines include tetanus and diphtheria. Toxoid vaccines are known for their efficacy.

4-Subunit

Protein subunit – rather than introducing an inactivated or attenuated micro-organism to an immune system (which would constitute a "whole-agent" vaccine), a fragment of it can create an immune response. Examples include the subunit vaccine against Hepatitis B virus that is composed of only the surface proteins of the virus (previously extracted from the blood serum of chronically infected patients, but now produced by recombination of the viral genes into yeast), the virus-like particle (VLP) vaccine against human papillomavirus (HPV) that is composed of the viral major capsid protein.

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5-Conjugate

Conjugate – certain bacteria have polysaccharide outer coats that are poorly immunogenic. By linking these outer coats to proteins (e.g., toxins),

the immune system can be led to recognize the polysaccharide as if it were a protein antigen. This approach is used in the *Haemophilus influenzae* type B vaccine.

6- mRNA vaccines

mRNA vaccines have strands of genetic material called mRNA inside a special coating. That coating protects the mRNA from enzymes in the body that would otherwise break it down. It also helps the mRNA enter the dendritic cells and macrophages in the lymph node near the vaccination site. Example of this vaccine Pfizer covid-19 vaccine.

mRNA can most easily be described as instructions for the cell on how to make a piece of antigens protein. Since only part of the protein is made, it does not do any harm to the person vaccinated but it is antigenic.

After the piece of the antigenic protein is made, the cell breaks down the mRNA strand and disposes of them using enzymes in the cell. It is important to note that the mRNA strand never enters the cell's nucleus or affects genetic material. This information helps counter misinformation about how mRNA vaccines alter or modify someone's genetic makeup.

Once displayed on the cell surface, the protein or antigen causes the immune system to begin producing antibodies and activating T-cells to fight off what it thinks is an infection.

Immune response to vaccines

The immune system recognizes vaccine agents as foreign, destroys them, and "remembers" them. When the virulent version of an agent is encountered, the body recognizes the protein coat on the virus, and thus is prepared to respond, by (1) neutralizing the target agent before it can enter cells, and (2) recognizing and destroying infected cells before that agent can multiply to vast numbers.

Vaccines have contributed to the eradication of smallpox, one of the most contagious and deadly diseases known to man. Other diseases such as rubella, polio, measles, mumps, chickenpox, and typhoid are nowhere near as common as they were a hundred years ago. As long as the most of people are vaccinated, it is much more difficult for an outbreak of disease to occur.. Polio, which is transmitted only between humans, is targeted by an extensive eradication campaign that has seen endemic polio restricted to only parts of four countries (Afghanistan, India, Nigeria, and Pakistan). The difficulty of reaching all children as well as cultural misunderstandings.

Examples of Vaccines

a. Smallpox – the last natural case of smallpox occurred in 1977; in 1980 the World Health Organization declared the global eradication of smallpox and recommended that all countries stop vaccination.

b. Pertussis (Whooping Cough) – causes coughing spells so bad that it is hard for infants to eat, drink, or breathe; it can lead to pneumonia, brain damage, and death

c. Diphtheria – causes a thick covering on the back of the throat; can lead to breathing problems, paralysis, heart failure, and death breathing problems, paralysis, heart failure, and death

d. Tetanus (Lockjaw) – causes painful tightening of the muscles, usually all over the body, leading to “locking” of the jaw so the victim cannot open his or her mouth or swallow. Tetanus leads to death in about 1 out of 10 cases.

e. Meningococcal disease – a serious illness caused by bacteria. It is the leading cause of bacterial meningitis in children 2–18 years old in the United States. Meningitis is an infection of the brain and spinal cord coverings.

f. Haemophilus influenzae type b (Hib) – a serious disease caused by bacteria. Hib is spread from person to person. If the bacteria remain in the nose and throat, the individual will not get sick. If it spreads to the lungs or blood stream, Hib can be serious. Before the Hib vaccine, Hib was the leading cause of bacterial meningitis among children under 5 years old in the United States. Meningitis is an infection of the brain and spinal cord coverings which can lead to lasting brain damage. Hib disease can also cause

i. Pneumonia

ii. Severe swelling in the throat, making it hard to breathe

iii. Infections of the blood, joints, bones, and covering of the heart

iv. Death

g. Influenza – caused by a virus that spreads from infected persons to the noses or throats of others. Influenza can cause fever, cough, chills, sore throat, headache, and muscle aches. People of any age can get influenza. Most people are ill with influenza for only a few days, but some get much sicker and need to be hospitalized. Influenza causes thousands of deaths each year, mostly among the elderly. Influenza viruses change often. Therefore, the influenza vaccine is updated each year to make sure it is as effective as possible.

h. Polio – caused by a virus. It enters the body through the mouth. It can cause paralysis and death. The disease is still common in some parts of the world

i. Measles – causes rash, cough, runny nose, eye irritation, and fever. It can lead to ear infection, pneumonia, brain damage, and death.

j. Mumps –causes fever, headache, and swollen glands. It can lead to deafness, meningitis, painful swelling of the testicles or ovaries, and (rarely) death.

k. Rubella (German measles) – causes rash, mild fever, and arthritis. If a woman gets rubella while she is pregnant, she could have abortion or her baby could be born with serious birth defects.

l. Anthrax – a serious disease that can affect both animals and humans

i. Caused by the bacteria *Bacillus anthracis*

ii. Contracted from infected animals, wool, meat, or hides

iii. Most commonly causing skin disease, ulcers, fever.

iv. 20 % of the cases are fatal

v. Inhaled anthrax is more serious

m. Hepatitis B (HBV) – a serious disease. The hepatitis B virus can cause a short term (acute) illness that leads to

i. Pain in the muscles, joints, and stomach

ii. Diarrhea and vomiting

iii. Jaundice (yellow tint to the skin and/or eyes)

n- Bacille Calmette-Guẻrin (BCG) vaccine

The live attenuated strain of Mycobacterium bovis known as bacillus Calmette-Guérin (BCG) uses shared antigens to stimulate the development of cross-immunity to Mycobacterium tuberculosis. It lost its virulence in humans by being specially cultured in an artificial medium for years. BCG is given as a single intradermal injection at the insertion of the left upper arm. A small bleb is raised and a successful vaccination leads to the development of a small local swelling within 2 weeks.

o- Rotavirus vaccine

A rotavirus vaccine protects children from rotaviruses, which are the leading cause of severe diarrhea among infants and young children. Each year an estimated 453,000 children die from diarrhoeal disease caused by rotavirus, most of whom live in developing countries, and another two million are hospitalised. Rotavirus is highly contagious and resistant, nearly every child in the world is at risk of infection.

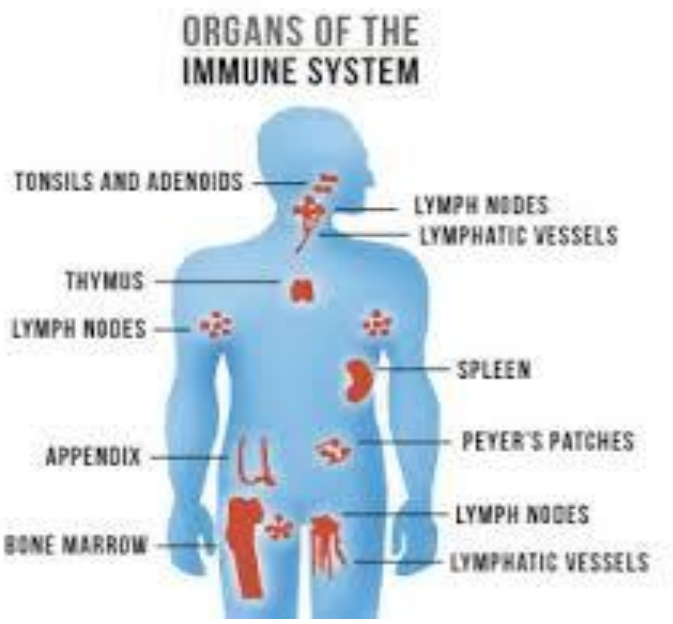
ORGANS OF IMMUNE SYSTEM

Immune (Lymphatic) System: The immune or lymphatic system consists of a complex network of specialized cells and organs designed to protect and defend the body against attacks by "foreign" invaders such as bacteria and viruses.

While the terms immune system and lymphatic system are often used interchangeably, technically the lymphatic system consists of a series of vascular-like channels (called “Lymphatics”) that drain off excess tissue fluid, returning it to the cardiovascular system via the thoracic duct). The “lymph fluid” within these channels drains through regional “lymph nodes” that serve to filter the lymph fluid on its way to the vascular system. The immune system not only protects against extrinsic pathogens but also against intrinsic pathological changes in cells and tissues that result in alterations of cell surface molecules (cancer).

There are two groups of immune system organs.

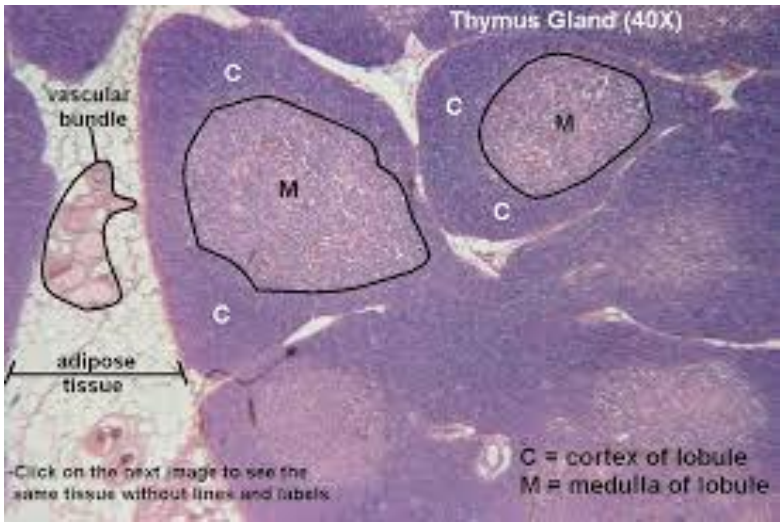
- **Primary (central)--organs where *immature* lymphocytes develop**
  - Thymus
  - Bone marrow
- **Secondary (peripheral)--tissues where antigen is localized so that it can be effectively exposed to *mature* lymphocytes**
  - Lymph nodes
  - Tonsils
  - Spleen
  - Mucosa Associated Lymphoid Tissue (MALT)

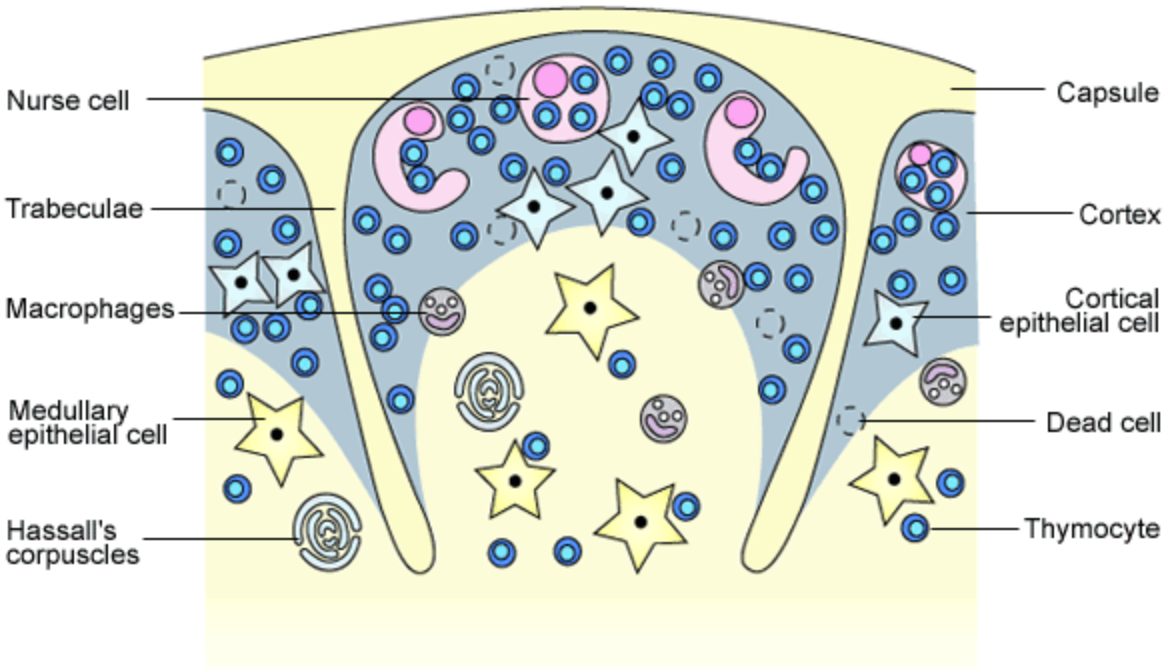


Thymus

The thymus, also called the thymus gland, is only fully developed in children. From adolescence onwards, it is slowly turned into fat tissue. The thymus organ is situated behind the breast bone above the heart. Certain defense cells are differentiated in the thymus: the so-called T lymphocytes, or T cells for short, among other things, are responsible for coordinating the innate and the adaptive immune system. The T in T lymphocytes stands for thymus, the place where they mature. T cells move through the body and constantly watch the surfaces of all cells for changes. To be able to do this job, they learn in the thymus which structures on cell surfaces are self and which are non-self. The organ itself contains two lobes, and each lobe contains numerous lobules, separated from each other by connective tissue septa known as trabeculae. Each lobule is separated into an inner medulla (with few immature thymocytes) and an outer cortex (with large numbers of

immature thymocytes). Dendritic cells, macrophages, and epithelial cells are interspersed throughout both the medulla and cortex.





Bone Marrow

Bone marrow is a sponge-like tissue situated inside of the bones. Most defense cells are produced and then also multiply here. They then migrate from the bone marrow into the bloodstream and reach other organs and tissues, where the defense cells mature and specialize. At birth, many bones contain red bone marrow, which actively builds defense cells. During the course of life, more and more red bone marrow turns into fat tissue. Adults only have red bone marrow in a few bones, for example in the ribs, in the breast bone and in the pelvic bone.

Bone marrow is the site of B cell maturation in humans. Bone marrow is also the site of hematopoiesis, the development of the myriad blood cells from progenitor cells. The tissue of bone marrow where leukocytes, red

blood cells, and platelets develop (i.e., the site of hematopoiesis) is known as myeloid tissue.

Leukemia is a cancer of the bone marrow that causes abnormal production of leukocytes (WBCs).

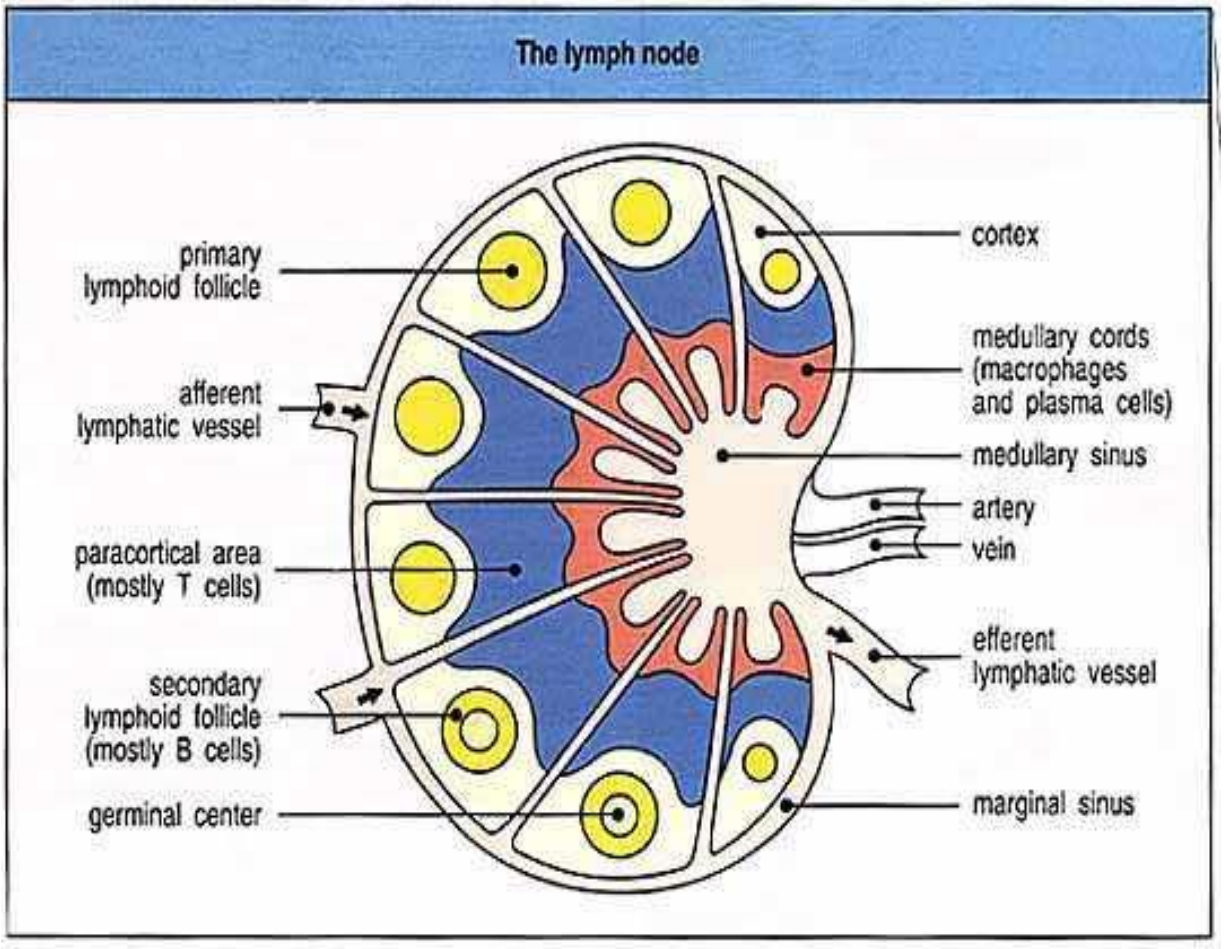
Lymph Nodes

Clusters of lymph nodes are strategically placed in the neck, axillae, groin, mediastinum and abdominal cavity, where they filter antigens from the tissue fluid and the lymph during its passage from the periphery to the thoracic duct. Each lymph node is divided into an outer cortex, inner medulla and intervening paracortical region. The cortex is also referred as B cell area, which mainly consists of B cells. The cortex is a high traffic zone where recirculating T and B lymphocytes enter from the blood.

The medulla contains a mixture of B cells, T cells, plasma cells and macrophages. The medulla consists of medullary cords that lead to the medullary sinus. The cords are populated by plasma cells and macrophages.

Extracellular fluid flows from capillary into tissue; from this tissue it enters lymphatic capillaries that are "pumped" along with the movement of skeletal muscle towards lymph nodes. APCs and antigen are sent from the tissue into the lymphatics, eventually reaching the lymph nodes where they can be exposed to the T and B cell populations. This allows a faster response, as the many combinations of T and B cell specificities are able to reside in several locations throughout the body (the lymph nodes) rather than

depending on random meetings of antigen and lymphocytes throughout the tissues themselves.



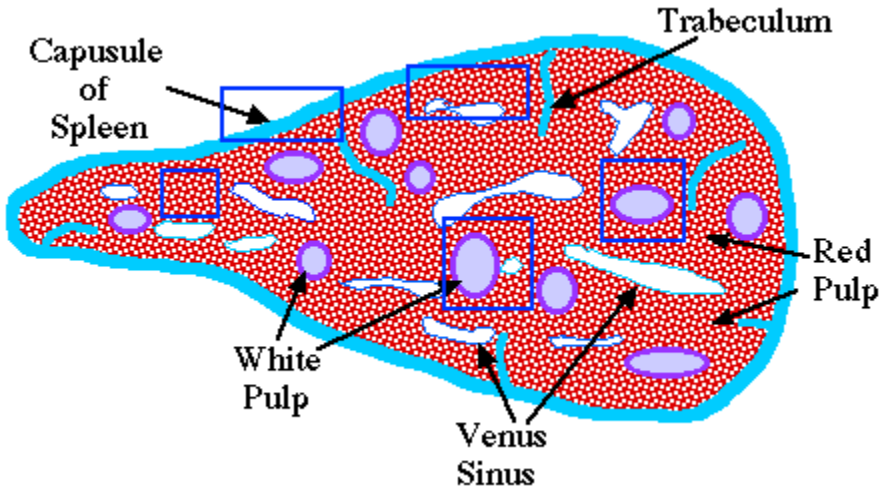
Spleen

The spleen is situated in the left upper abdomen. The spleen acts as a site of hematopoiesis during the second and third trimesters of development, before the long bones have fully developed. In the adult, the spleen acts as a site for breakdown of dying red blood cells (lifespan 120 days). For this reason, enlargement of the spleen (splenomegaly) can occur in sickle cell anemia or in certain infections. White pulp, near the arteriolar entry points

into the spleen, is where lymphocytes reside and are degraded. The central red pulp is the site of RBCs breakdown. As part of the immune defense, the functions of the spleen include the following:

- It stores different defense cells that are released into the blood to get to the organs,.
- It is responsible for removing red blood cells (erythrocytes).
- Blood platelets (thrombocytes), which are responsible for blood clotting together, are stored and removed in the spleen.

So there is always a lot of blood flowing through the spleen tissue. At the same time this tissue is very soft. In heavy injuries, in an accident, for example, the spleen can therefore rupture easily. The spleen then needs to be operated on, because otherwise there is a danger of bleeding to death. If the bleeding cannot be stopped, and the spleen has to be removed, other defense organs take on most of its tasks.



Tonsils

Tonsils also belong to the defense system; Tonsils are collections of lymphoid tissue facing into the aerodigestive tract. Tonsils are masses of lymphatic material situated at either side at the back of the human throat. The tonsils are lymphoepithelial tissues located near the nasopharynx (parts of the throat).

These immunocompetent tissues are the immune system's first line of defense against ingested or inhaled foreign pathogens. Due to their special position at the throat and palate, their defense cells come into contact with pathogens especially soon, and can activate the immune system immediately. Their tissue contains mainly lymphocytes.

Mucosa Associated Lymphoid Tissue (MALT):

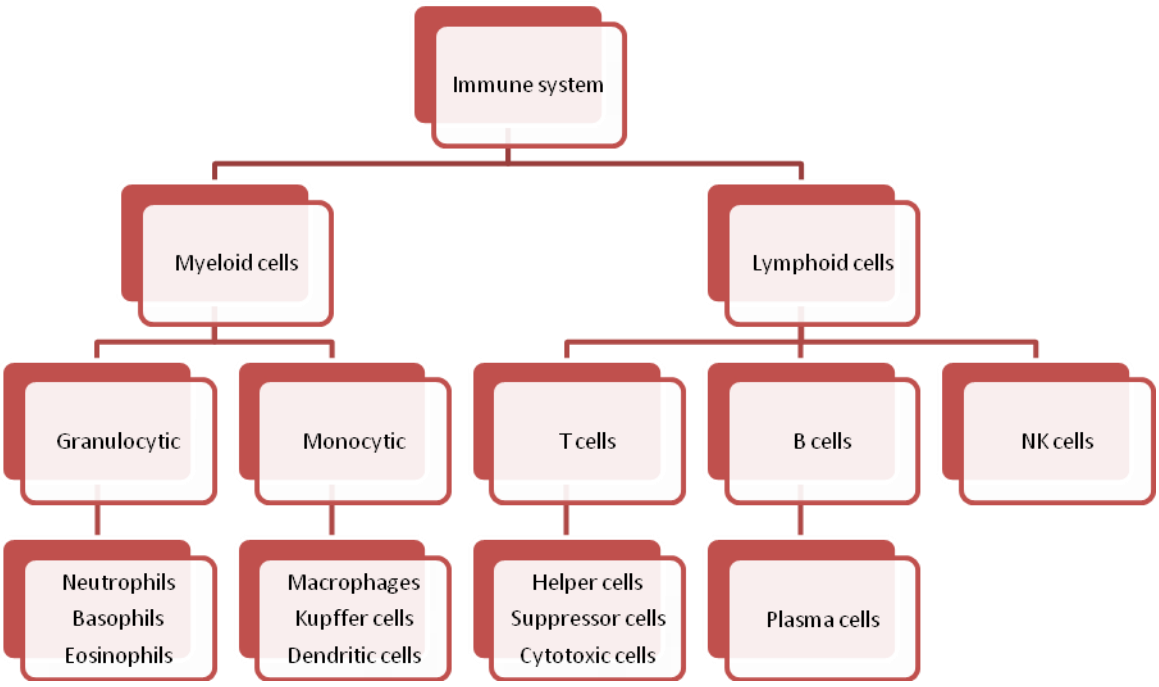
Approximately >50% of lymphoid tissue in the body is found associated with the mucosal system. MALT is composed of gut-associated lymphoid tissues (GALT) lining the intestinal tract, bronchus-associated lymphoid tissue (BALT) lining the respiratory tract, and lymphoid tissue lining the genitourinary tract. The respiratory, alimentary and genitourinary tracts are guarded by subepithelial accumulations of lymphoid tissue that are not covered by connective tissue capsule. They may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and lamina propria of intestine or as clearly organised tissue with well-formed lymphoid follicles. The well-formed follicles include the tonsils, Peyer's patches in the intestine and appendix. The major function of these organs is to provide local immunity. Diffuse accumulations of lymphoid tissue are seen in the lamina propria of the intestinal wall. The intestinal epithelium overlying the

Peyer's patches is specialized to allow the transport of antigens into the lymphoid tissue. This function is carried out by cuboidal absorptive epithelial cells termed "M" cells, so called because they have numerous microfolds on their luminal surface. M cells endocytose, transport and present antigens to subepithelial lymphoid cells.

The bowel plays a central role in defending the body against pathogens:. These cells recognize pathogens and other non-self substances, and mark and destroy them. They also store information on these non-self substances to be able to react faster the next time. The large bowel also always contains bacteria that belong to the body, the so-called gut flora. These bacteria in the large bowel make it difficult for other pathogens to settle and to enter the body. The immune system of the bowel tolerates the bacteria of the gut flora.

Cells of the Immune System

The response to pathogens is started by the complex interactions and activities of the large number of diverse cell types involved in the immune response. The innate immune response is the first line of defense and occurs soon after pathogen exposure. It is carried out by phagocytic cells such as neutrophils and macrophages, cytotoxic natural killer (NK) cells, and granulocytes. The subsequent adaptive immune response includes antigen-specific defense mechanisms and may take days to develop. Cell types with critical roles in adaptive immunity are antigen-presenting cells including macrophages and dendritic cells. Antigen-dependent stimulation of various cell types including T cell subsets, B cells, and macrophages all play critical roles in host defense.



1- Granulocytes

Granulocytes, or polymorphonuclear leukocytes, are a subgroup of white blood cells characterized by the presence of cytoplasmic granules. Granulocytes are formed in the bone marrow and can be classified as basophils, esinophils, and neutrophils. The cell types are named by their distinct staining characteristics using hematoxylin and eosin (H&E) histological preparations. Granules in basophils stain dark blue, eosinophilic components stain bright red, and neutrophilic components stain a neutral pink.

A-Neutrophils

Neutrophils are the highest numbers of leukocyte, comprising 50-70% of all white blood cells and are a critical component of the immune system. Neutrophils have a segmented nucleus. Normally, located in the circulating blood system. The main function of neutrophils is to destroy micro-organisms and foreign particles by phagocytosis. The number of circulating neutrophils is estimated using the absolute neutrophil count (ANC). An ANC may be high due to kidney failure. In contrast, leukemia and bone marrow damage result in a lower number of neutrophils (neutropenia) and an increased risk of infection.

B-Eosinophils

Eosinophils produced in the bone marrow. Eosinophils are polymorphonuclear leukocytes, characterized by eosinophilic granules in the cytoplasm. Recruited eosinophils release the toxic substances contained in their granules to destroy invasive microorganisms.

C-Basophils

Basophils are a type of granular leukocyte (white blood cell) involved in inflammatory and allergic responses. Basophils contain toxic granules which are used to destroy micro-organisms / pathogens during the process of phagocytosis.

A **mast cell** (also known as a **mastocyte**) is derived from the myeloid stem cell and a part of the immune system that contains many granules rich in histamine and heparin. Although best known for their role inallergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing and defense against pathogens.

The mast cell is very similar in both appearance and function to the basophil, another type of white blood cell. They differ in that mast cells are tissue resident, *e.g.*, in mucosal tissues, while basophils are found in the blood.

2- Natural Killer (NK) Cells

Natural killer (NK) cells are lymphocytes of the innate immune system that function as both cytolytic effectors and regulators of immune responses. NK cells express a large number of receptors that deliver either activating or inhibitory signals, and the relative balance of these signals controls NK cell activity. NK cells are activated upon detection of abnormalities in target cells such as the loss of MHC class I expression or up-regulation of stress-induced ligands in response to infection or neoplastic transformation. Many viruses have evolved strategies to evade detection by NK cells or to

modulate their activity. A variety of receptors trigger the NK cytolytic activity directed toward certain tumor targets, virally infected cells. NK cells are also important regulators of the adaptive immune system via their ability to secrete a number of cytokines in response to immune activation.

3- Dendritic Cells

Dendritic cells are best known for their role as professional antigen-presenting cells. Upon exposure and uptake of pathogens, maturing cells travel to secondary lymphoid organs where they become potent T cell activators. The upregulation of cytokines, chemokines, co-stimulatory molecules, and adhesion molecules by the active dendritic cell is a critical part of the adaptive immune response.

4- Monocytes/Macrophages

Monocytes are agranular leukocytes which originate in the bone marrow. The primary function of macrophages is believed to involve their role as critical immune effector cells, responding to signals from both innate and antigen-specific immune cells. In addition to host defense, macrophages have also been proposed to contribute to the processes of wound healing and immune regulation.

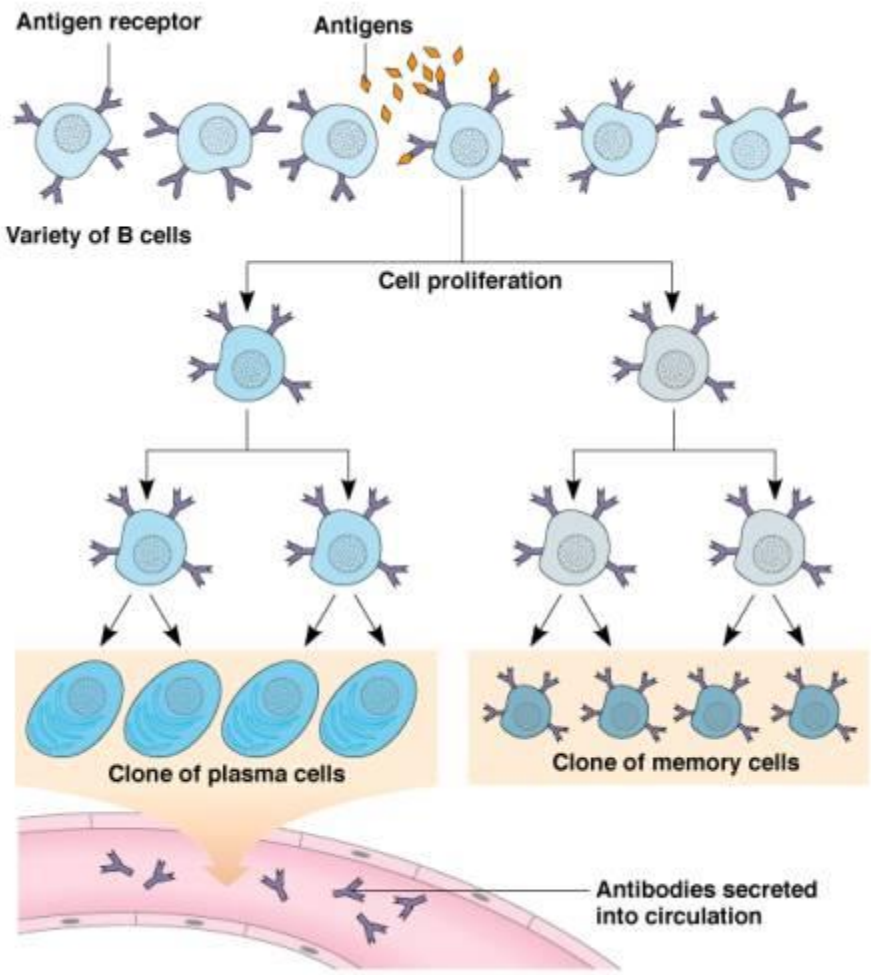
5- B Cells

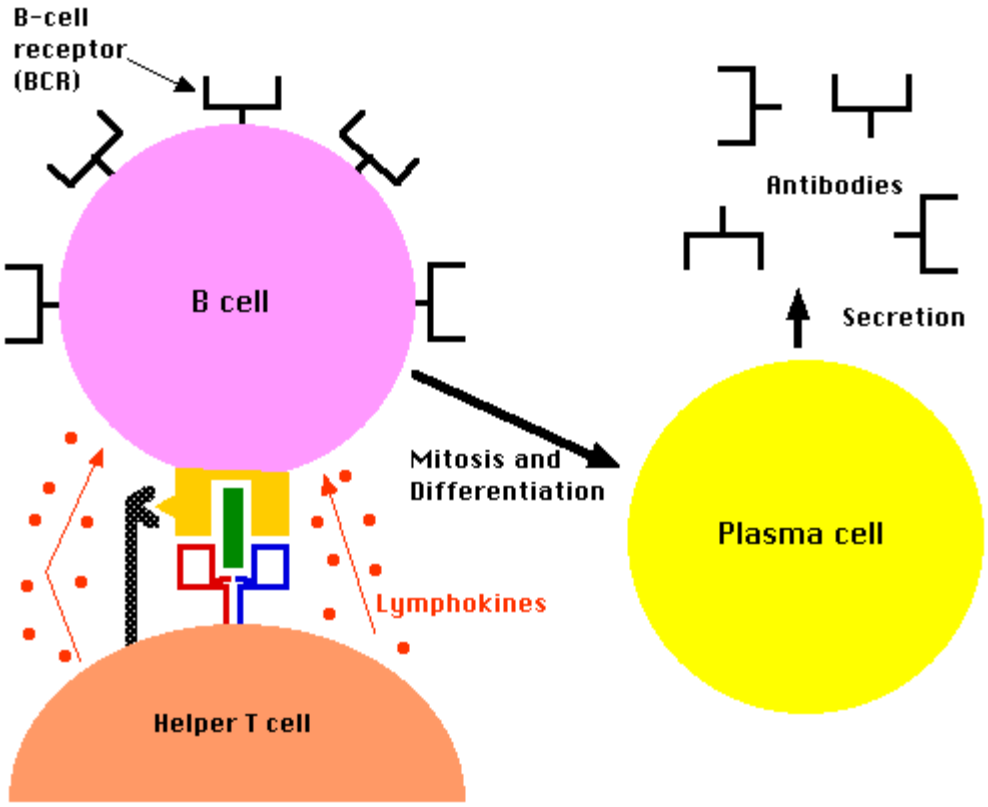
B lymphocytes (B cells) are an essential component of the humoral immune response. Produced in the bone marrow, B cells migrate to the spleen and other secondary lymphoid tissues where they mature and differentiate into

immunocompetent B cells. Part of the adaptive immune system, B cells are responsible for generating antibodies to specific antigens, which they bind via B cell receptors (BCR).

Activation of B cells occurs via antigen recognition by BCRs and a required co-stimulatory, secondary activation signal provided by either helper T cells or the antigen itself. This results in stimulation of B cell proliferation and the formation of germinal centers where B cells differentiate into plasma cells or memory B cells. Importantly, all B cells derived from a specific progenitor B cell are clones that recognize the same antigen epitope.

Plasma cells are found in the spleen and lymph nodes and are responsible for secreting different classes of clonally unique antibodies that are found in the blood. Following the primary response, a small number of B cells develop into memory B cells, which express high-affinity surface immunoglobulins (mainly IgG), survive for a longer period of time, and enable a rapid secondary response.

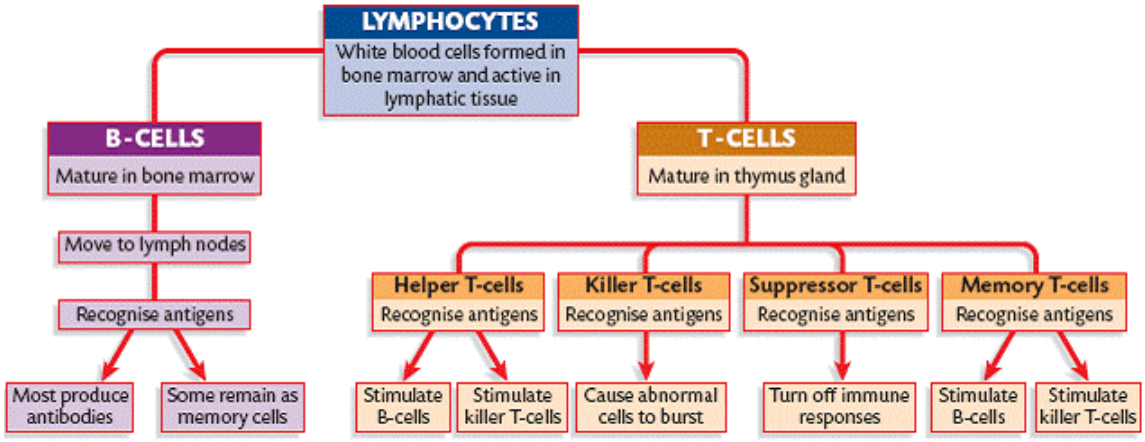




6- T Cells

T cells are distinguished from other lymphocytes by the T cell receptor (TCR). Developed from hematopoietic stem cells in the bone marrow, T cells mature in the thymus. There are several types of T cells based on their specific function: helper/effector, cytotoxic, memory, regulatory.

T cells or T lymphocytes are a type of lymphocyte that plays a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface. The several subsets of T cells each have a distinct function.



Most of the T cells in the body belong to one of two subsets. These are distinguished by the presence on their surface of **one or the other** of two glycoproteins designated (cluster of differentiation):

- **CD4**
- **CD8**

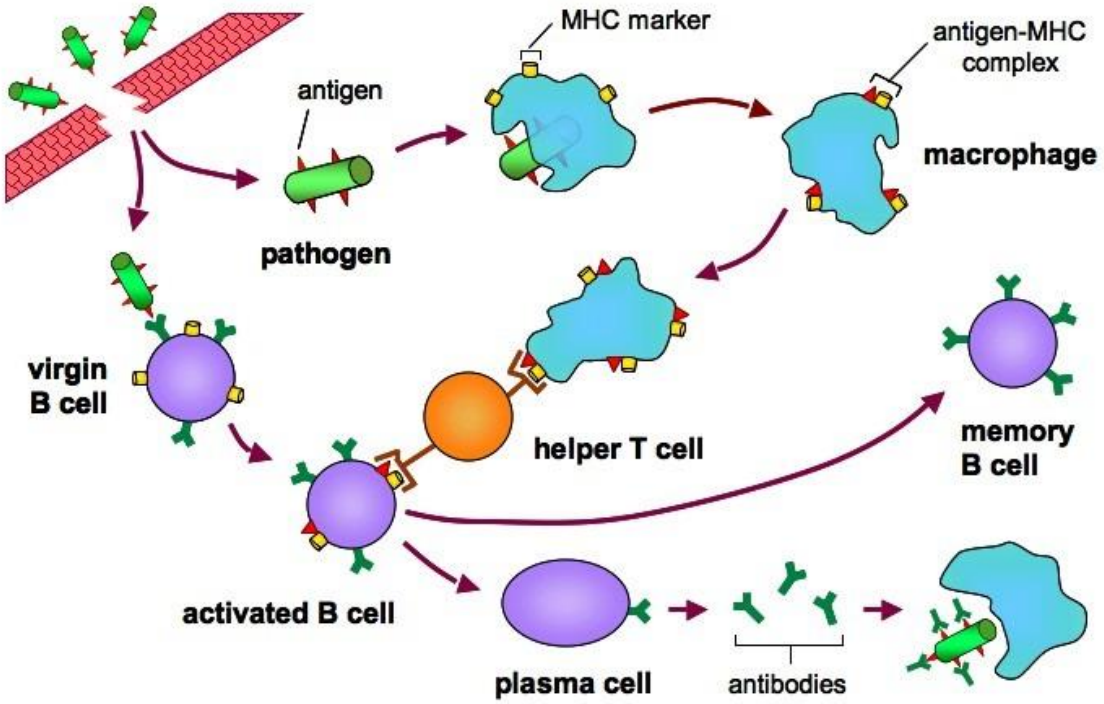
Which of these molecules is present determines what types of cells the T cell can bind to.

Types of T cell

A- Helper

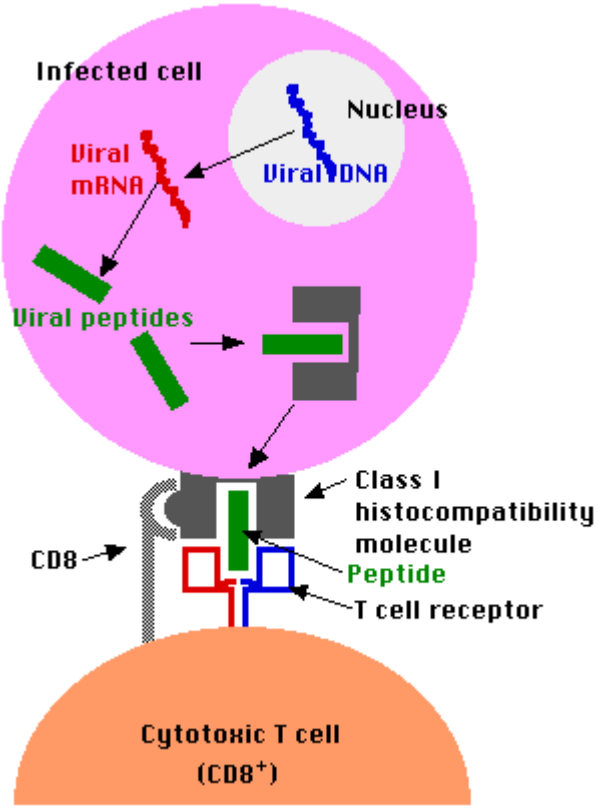
T helper cells (TH cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. These cells are also known as CD4+, (cluster of differentiation 4). T cells because they express the CD4 glycoprotein on their surfaces. Helper T cells become

activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen-presenting cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response.



B- Cytotoxic

Cytotoxic T cells (TC cells, or CTLs) destroy virus-infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as CD8+ T cells since they express the CD8 glycoprotein at their surfaces. These cells recognize their targets by binding to antigen associated with MHC class I molecules, which are present on the surface of all nucleated cells, the CD8+ cells can be inactivated to an anergic state, which prevents autoimmune diseases.



C- Memory

Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections.

D- Regulatory

Regulatory T cells (Treg cells), formerly known as suppressor T cells, are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress autoreactive T cells that escaped the process of negative selection in the thymus.

AIDS patients lose their $CD4^+$ T cells

AIDS provides a vivid illustration of the importance of $CD4^+$ T cells in immunity. The human immunodeficiency virus (**HIV**) binds to CD4 molecules and thus is able to invade and infect $CD4^+$ T cells. As the disease progresses, the number of $CD4^+$ T cells declines below its normal level of about 1000 per microliter (µl). (A partial explanation for this may be the unceasing efforts of the patient's $CD8^+$ T cells to destroy the infected $CD4^+$ cells. However, it turns out that only a small fraction of the patients $CD4^+$ T cells are infected at any given time).

When the number of $CD4^+$ T cells drops below 400 per microliter, the ability of the patient to mount an immune response declines dangerously.

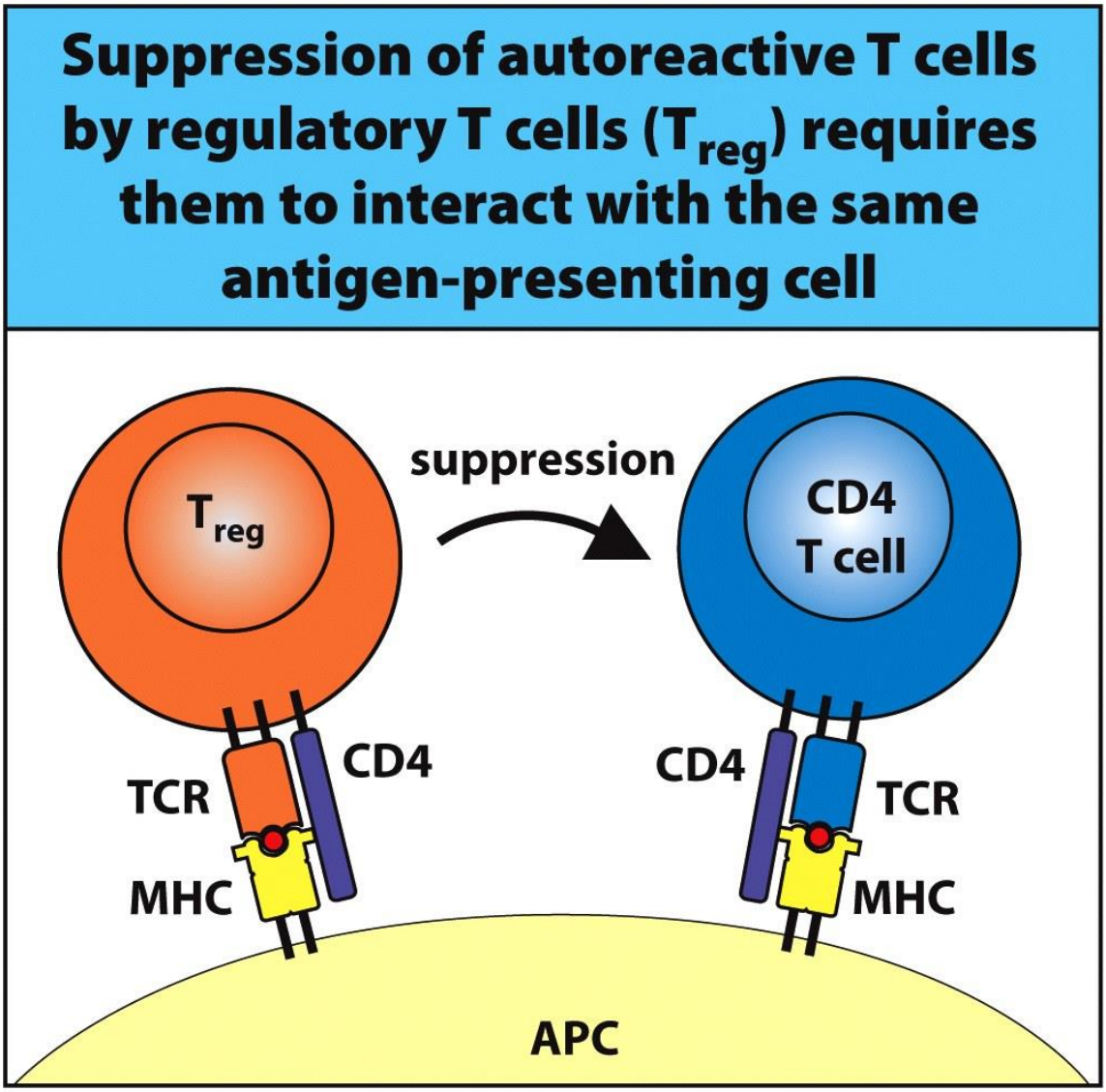


Figure 7.19 The Immune System, 3ed. (© Garland Science 2009)

Immunology and serology L11,12 Dr. Muna Al-Rubiae

Immunoglobulins - Structure and Function

Immunoglobulin (Ig)

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies.

General Functions of Immunoglobulins

A. Antigen binding

Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

B. Effector Functions

The significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

1. Fixation of complement - This results in lysis of cells and release of biologically active molecules.

2. Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placenta, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn.

Basic Structure of Immunoglobulins

Although different immunoglobulins can differ structurally, they all are built from the same basic units.

A. Heavy and Light Chains

All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD)

B. Disulfide bonds

1. Inter-chain disulfide bonds - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions. The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

2. Intra-chain disulfide binds - Within each of the polypeptide chains there are also intra-chain disulfide bonds.

C. Variable (V) and Constant (C) Regions

When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain

could be divided into two regions based on variability in the amino acid sequences.

D. Hinge Region

This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

Immunoglobulin Fragments: Structure/Function Relationships

A. Fab

Antigen binding - These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody.

B. Fc

It contains the remainder of the two heavy chains. This fragment was called Fc because it was easily crystallized.

Effector functions - The effector functions of immunoglobulins are mediated by this part of the molecule. Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen; however, there are exceptions to this rule.

Human Immunoglobulin Classes

The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class will have very similar heavy chain constant regions. These differences can be detected by sequence studies.

1. IgG - Gamma heavy chains

2. IgM - Mu heavy chains

3. IgA - Alpha heavy chains

4. IgD - Delta heavy chains

5. IgE - Epsilon heavy chains

Structure and Some Properties of Ig Classes

A. IgG

1. Structure: IgG antibodies are large molecules of about 150 kD
a composed of four peptide chains.
2. Properties
IgG is the most important immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.

a) IgG is the major Ig in serum - 75% of serum Ig is IgG

b) IgG is the major Ig in extra vascular spaces

c) Placental transfer - IgG is the only class of Ig that crosses the placenta. Transfer is mediated by a receptor on placental cells for the Fc region of IgG.

d) Fixes complement.

e) Binding to cells - Macrophages, monocytes, PMNs and some lymphocytes have Fc receptors for the Fc region of IgG. The antibody has

prepared the antigen for eating by the phagocytic cells. The term **opsonin** is used to describe substances that enhance phagocytosis. IgG is a good opsonin. Binding of IgG to Fc receptors on other types of cells results in the activation of other functions.

B. IgM

1. Structure

IgM normally exists as a pentamer. In the pentameric form all heavy chains are identical and all light chains are identical. Thus, the valence is theoretically 10.

2. Properties

a) IgM is the third most common serum Ig.

b) IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.

c) As a consequence of its pentameric structure, IgM is a good complement fixing Ig. Thus, IgM antibodies are very efficient in leading to the lysis of microorganisms.

d) As a consequence of its structure, IgM is also a good agglutinating Ig . Thus, IgM antibodies are very good in clumping microorganisms.

e) IgM binds to some cells via Fc receptors.

C. IgA

1. Structure

Serum IgA is a monomer but IgA found in secretions is a dimer. When IgA exits as a dimer, a J chain is associated with it.

2. Properties

a) IgA is the 2nd most common serum Ig.

b) IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.

c) Normally IgA does not fix complement, unless aggregated.

d) IgA can binding to some cells - PMN's and some lymphocytes.

D. IgD

1. Structure

IgD exists only as a monomer.

2. Properties

a) IgD is found in low levels in serum; its role in serum uncertain.

b) IgD is primarily found on B cell surfaces where it functions as a receptor for antigen.

c) IgD does not bind complement.

E. IgE

1. Structure

IgE exists as a monomer

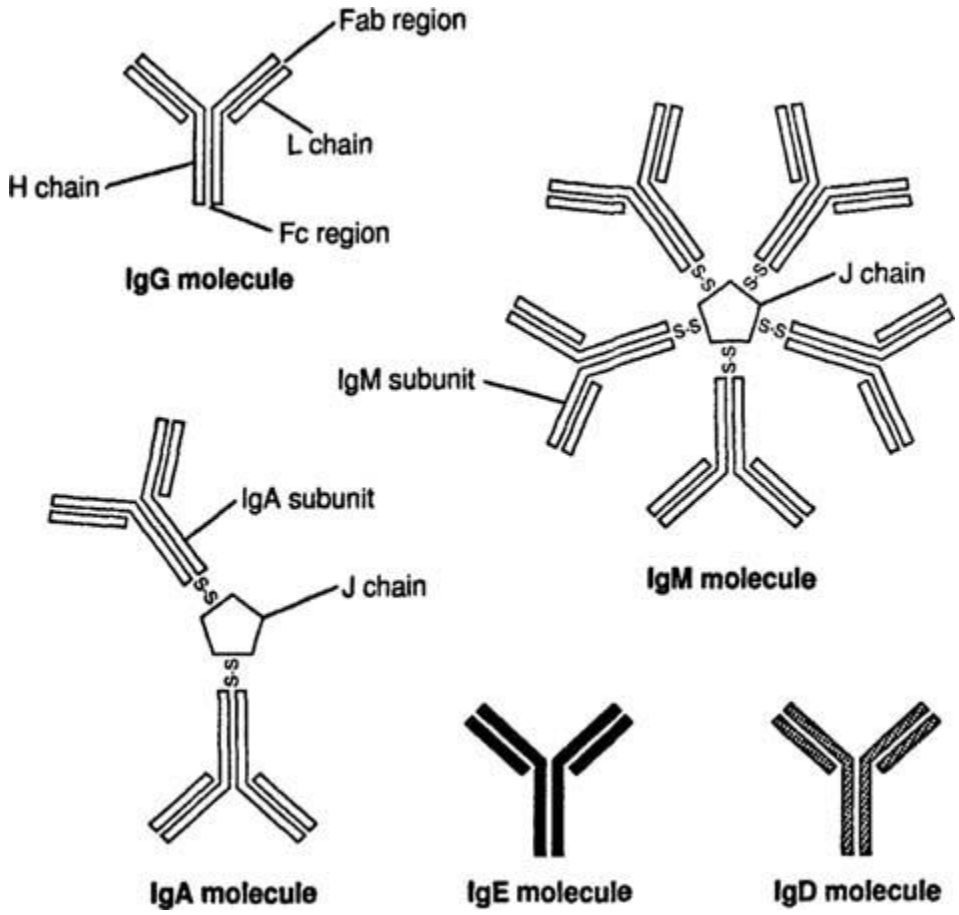
2. Properties

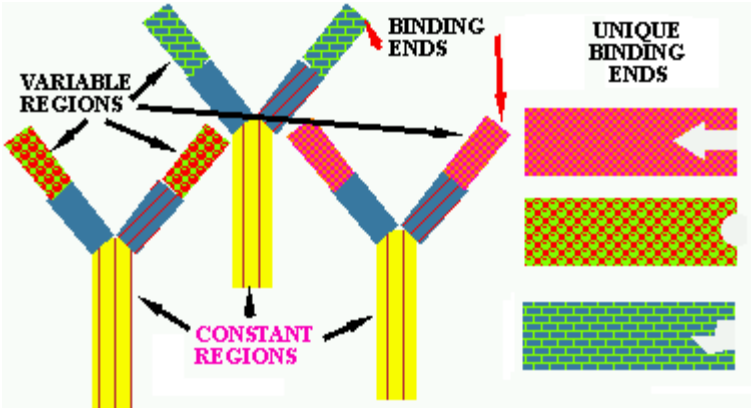
a) IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.

b) Involved in allergic reactions - As a consequence of its binding to basophils an mast cells, IgE is involved in allergic reactions. Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.

c) IgE also plays a role in parasitic helminth diseases. Since serum IgE levels rise in parasitic diseases, measuring IgE levels is helpful in diagnosing parasitic infections. Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE-coated helminths results in killing of the parasite.

d) IgE does not fix complement.


Fab region
L chain
H chain
Fc region
IgG molecule
J chain
IgM subunit
IgM molecule
IgA subunit
J chain
IgA molecule
IgE molecule
IgD molecule


BINDING ENDS
UNIQUE BINDING ENDS
VARIABLE REGIONS
CONSTANT REGIONS

Antigen

In immunology, an **antigen** (Ag), abbreviation of **anti**body **gen**erator, is any structural substance which serves as a target for the receptors of an adaptive immune response. An immunogen is an analogy to the antigen a substance (or a mixture of substances) that is able to induce an immune response if injected to the body.

Immunogenicity is the ability to induce a humoral and/or cell-mediated immune response. Antigenicity is the ability to combine specifically with the final products of the immune response (i.e. secreted antibodies and/or surface receptors on T-cells). Although all immunogenic molecules are also antigenic, the reverse is not true.

At the molecular level, an antigen can be characterized by its ability to be bound by the variable Fab region of an antibody. Note also that different antibodies have the potential to distinguish between specific epitopes present on the surface of the antigen. Hapten is a small molecule, in order to induce an immune response, it has to be attached to a large carrier molecule such as protein. Antigens are usually proteins and polysaccharides, less frequently also lipids. This includes parts (coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, and
other microorganisms. Lipids and nucleic acids are antigenic only when combined with proteins and polysaccharides. Vaccines are examples of antigens in an immunogenic form, which are to be intentionally administered to induce the memory function of adaptive immune system toward the antigens of the pathogen invading the recipient.

Binding of antibody to antigen is dependent on hydrogen bond, electrostatic attractions and Van der Waals attractions. These bonds are weak compared to covalent bonds but the large number of weak bonds results in a stable complex. Binding of antibody to antigen is reversible.

The specificity of the immune system is due to the fact that both lymphocytes and antibodies only recognize one epitope or antigenic determinant. The immune system can recognize thousands of millions of different antigens, but for each determinant, a specific lymphocyte will be induced. But, antigen recognition by an antibody is not absolute, and cross reactivity is due to antibody reacting with a partially related antigen.

Immunogen

A substance that induces a specific immune response.

Antigen (Ag)

A substance that reacts with the products of a specific immune response.

Hapten

A substance that is non-immunogenic but which can react with the products of a specific immune response. Haptens are small molecules which could never induce an immune response when administered by themselves but which can when coupled to a carrier molecule. Free haptens, however, can react with products of the immune response after such products have been elicited. Haptens have the property of antigenicity but not immunogenicity.

Epitope or Antigenic Determinant

That portion of an antigen that combines with the products of a specific immune response.

Antibody (Ab)

A specific protein which is produced in response to an immunogen and which reacts with an antigen.

Factors influencing immunogenicity

A. Contribution of the Immunogen

1. Foreignness

The immune system normally distinguishes between self and non-self such that only foreign molecules are immunogenic.

2. Size

There is not absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.

3. Chemical Composition

In general, the more complex the substance is chemically the more immunogenic it will be. The antigenic determinants are created by the primary sequence of residues in the polymer and/or by the secondary, tertiary or quaternary structure of the molecule.

4. Physical form

In general particulate antigens are more immunogenic than soluble ones.

5. Degradability

Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens) the development of

an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

B. Contribution of the Biological System

1. Genetic Factors

Some substances are immunogenic in one species but not in another. Similarly, some substances are immunogenic in one individual but not in others (*i.e.* responders and non-responders). The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells.

2. Age

Age can also influence immunogenicity. Usually the very young and the very old have a diminished ability to immune response in response to an immunogen.

C. Method of Administration

1. Dose

The dose of administration of an immunogen can influence its immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal.

2. Route

Generally the subcutaneous route is better than the intravenous or intragastric routes. The route of antigen administration can also alter the nature of the response.

3. Adjuvants

Adjuvant: a substance that, when mixed with an antigen and injected with it, serves to enhance the immune response to the antigen The use of adjuvants, however, is often associated by undesirable side effects such as fever and inflammation.

Possible mechanisms of action of adjuvants:

1- Prolong the persistence of the antigen, thus giving the immune system more time to respond.

2- Increase the “size” of the antigen by causing aggregation.

3 - Stimulate lymphocyte proliferation and/or activation

4- Stimulate a local inflammatory response, thus recruiting cells to the site of the antigen.

Chemical nature of immunogens

A. Proteins

The majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens.

B. Polysaccharides

Pure polysaccharides and lipopolysaccharides are good immunogens.

C. Nucleic Acids

Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded or when complexed with proteins.

D. Lipids

In general lipids are non-immunogenic, although they may be haptens.

Types of antigens

A. T-independent Antigens

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.

Properties of T-independent antigens

1. Polymeric structure

These antigens are characterized by the same antigenic determinant repeated many times.

2- 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.

B. T-dependent Antigens

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.

Hapten-carrier conjugates

A. Definition

Hapten-carrier conjugates are immunogenic molecules to which haptens have been covalently attached. The immunogenic molecule is called the carrier.

B. Structure

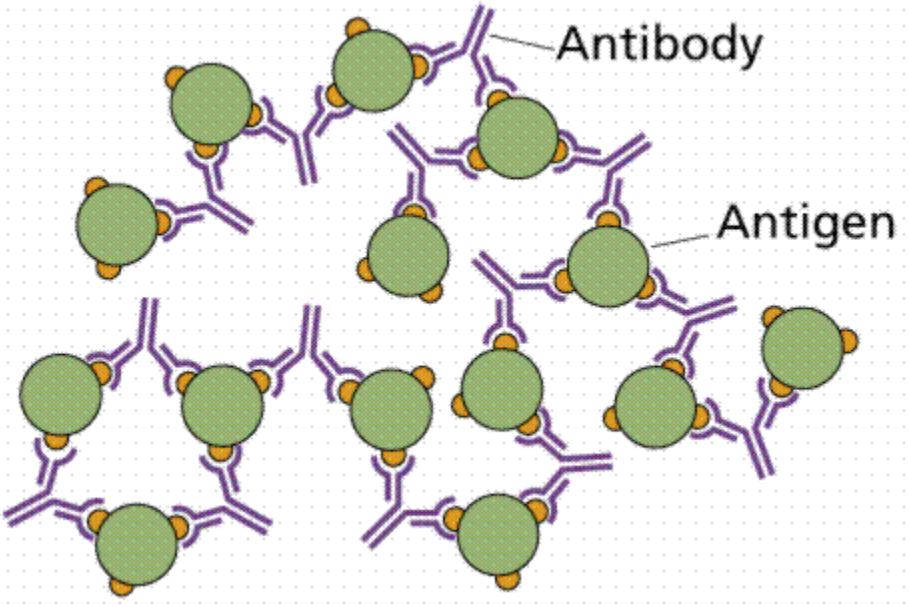
Structurally these conjugates are characterized by having native antigenic determinants of the carrier as well as new determinants created by the hapten (haptenic determinants). The actual determinant created by the hapten consists of the hapten and a few of the adjacent residues, although the antibody produced to the determinant will also react with free hapten. In such conjugates the type of carrier determines whether the response will be T-independent or T-dependent.

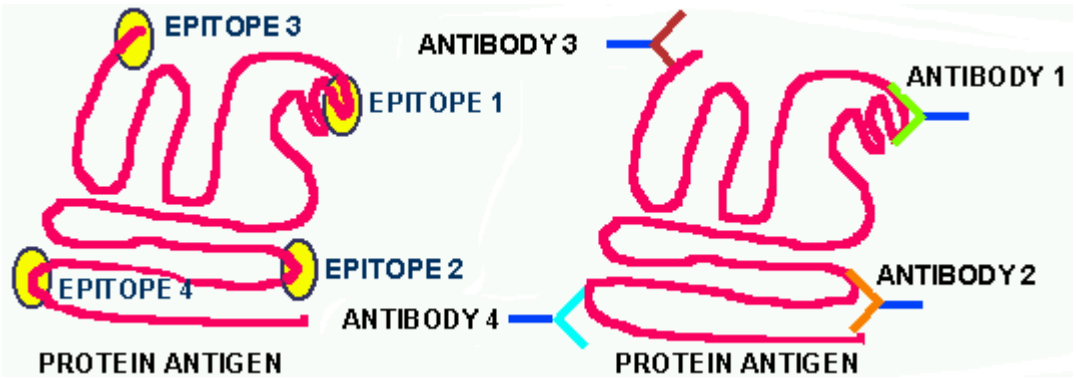
Superantigens

When the immune system encounters a T-dependent antigen, only a small fraction (1 in 10^4 -10^5) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response). However, there are some antigens which polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called **superantigens**.

Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome), Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic exotoxins (shock). Although the bacterial superantigens are the best studied there are superantigens associated with viruses and other microorganisms as well.

The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.




EPITOPE 3
EPITOPE 1
EPITOPE 2
EPITOPE 4
PROTEIN ANTIGEN
ANTIBODY 3
ANTIBODY 1
ANTIBODY 2
ANTIBODY 4
PROTEIN ANTIGEN

COMPLEMENT

Historically, the term complement (C) was used to refer to a heat-labile serum component that was able to lyses bacteria (activity is destroyed (inactivated) by heating serum at 56 degrees C for 30 minutes). However, complement is now known to contribute to host defenses in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; it can recruit and activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes. Complement can also have detrimental effects for the host; it contributes to inflammation and tissue damage and it can trigger anaphylaxis.

Complement comprises over 20 different serum proteins that are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria.

I. PATHWAYS OF COMPLEMENT ACTIVATION

Complement activation can be divided into four pathways: the classical pathway, the lectin pathway, the alternative pathway and the membrane attack (or lytic) pathway. Both classical and alternative pathways lead to the activation of C5 convertase and result in the production of C5b which is

essential for the activation of the membrane attack pathway.

1- Classical pathway

C1 activation

C1, a multi-subunit protein containing three different proteins (C1q, C1r and C1s), binds to the Fc region of IgG and IgM antibody molecules that have interacted with antigen. C1 binding does not occur to antibodies that have not complexed with antigen and binding requires calcium and magnesium ions. The binding of C1 to antibody is via C1q and C1q must cross link at least two antibody molecules before it is firmly fixed. The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated "C1qrs", which is an enzyme that cleaves C4 into two fragments C4a and C4b.

C4 and C2 activation (generation of C3 convertase)

The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated "C1qrs" also cleaves C2 into C2a and C2b. C2a binds to the membrane in association with C4b, and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b.

C3 activation (generation of C5 convertase)

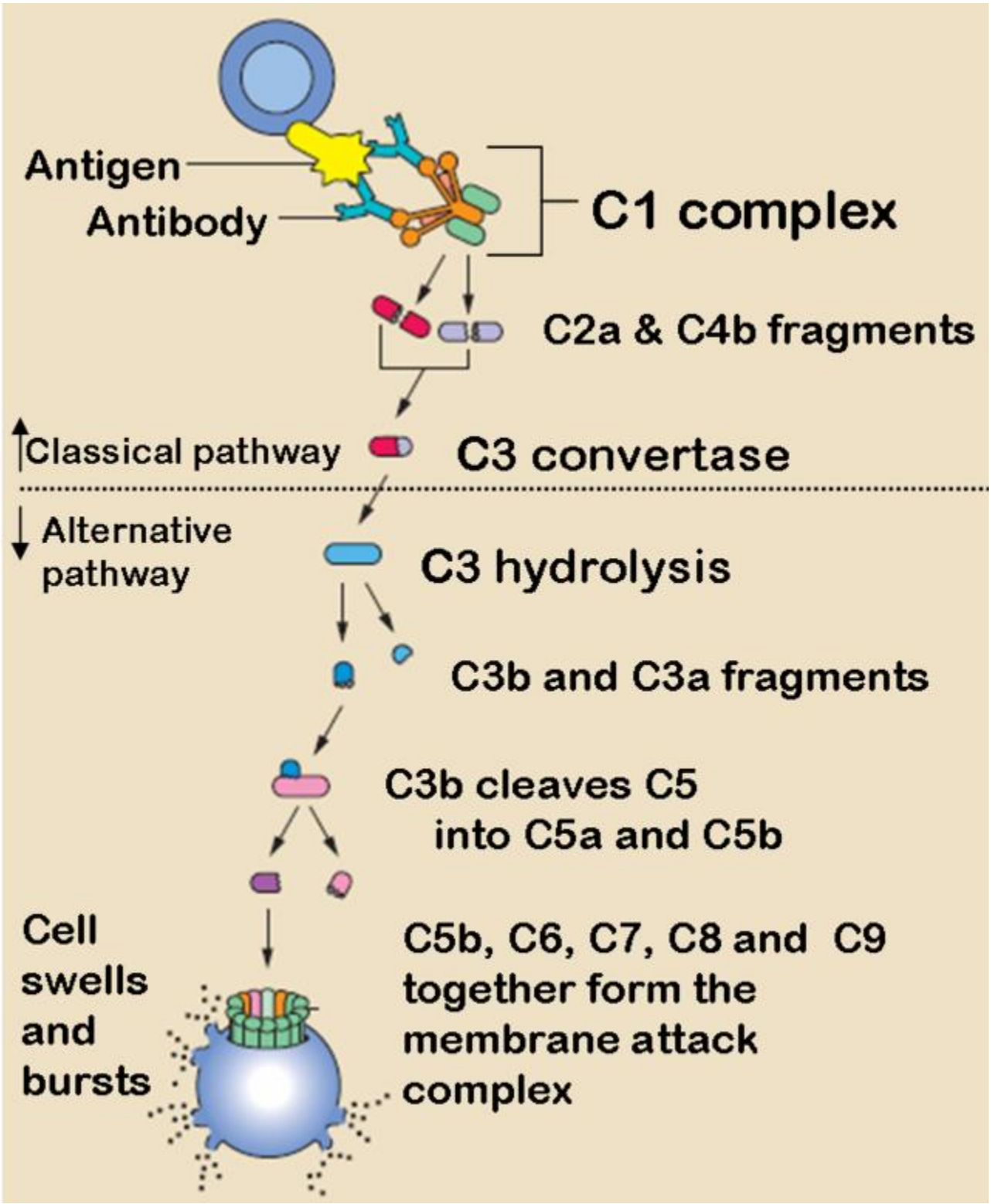
C3b binds to the membrane in association with C4b and C2a, and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the classical

pathway.

Several of the products of the classical pathway have potent biological activities that contribute to host defenses. Table 1 summarizes the biological activities of classical pathway components.

2- Lectin pathway

The lectin pathway is very similar to the classical pathway. It is initiated by the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (mannans). Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases). MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q. Formation of the MBL/MASP-1/MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated MASPs also cleave C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the lectin pathway.


Antigen
Antibody
C1 complex
C2a & C4b fragments
Classical pathway
C3 convertase
Alternative pathway
C3 hydrolysis
C3b and C3a fragments
C3b cleaves C5 into C5a and C5b
Cell swells and bursts
C5b, C6, C7, C8 and C9 together form the membrane attack complex

3- Alternative pathway

The **alternative pathway** of the complement system is an innate component of the immune system's natural defense against infections.

The alternative pathway is one of three complement pathways that opsonize and kill pathogens. The pathway is triggered when the C3b protein directly binds the microbe.

It is initiated by the spontaneous hydrolysis of C3, which is abundant in the blood plasma. Through the spontaneous cleavage of the thioester bond in C3 to form C3(H_2O).

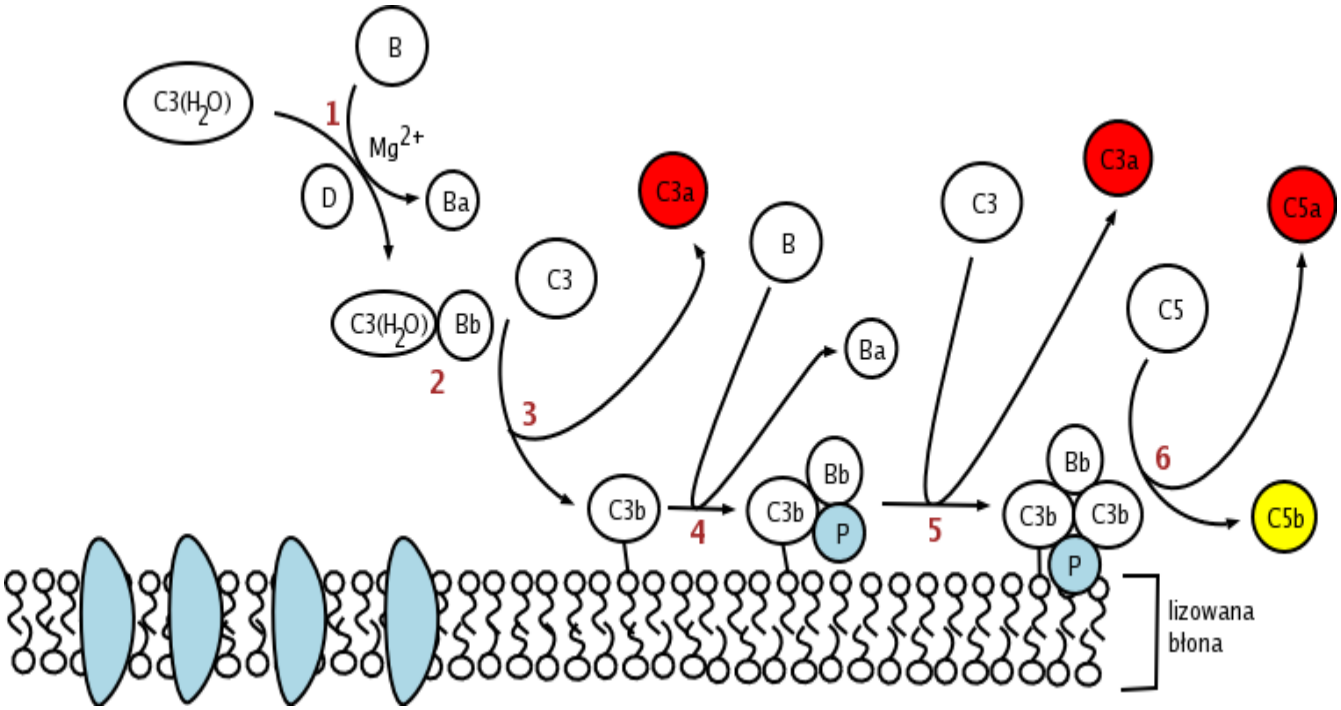
This change in shape allows the binding of plasma protein Factor B, which allows Factor D to cleave Factor B into Ba and Bb.

Bb remains part of the C3(H_2O) to form C3(H_2O)Bb. This complex is also known as a fluid-phase C3-convertase. This convertase, although only produced in small amounts, can cleave multiple C3 proteins into C3a and C3b.

The alternative pathway C3-convertase consists of the activated B and C3b factors, forming an unstable compound that can become stable after binding properdin, a serum protein.

After the creation of C3 convertase, the complement system follows the same path regardless of the means of activation (alternative, classical, or lectin). Binding of another C3b-fragment to the C3-convertase of the alternative pathway creates a C5-convertase analoguous to the lectin or classical pathway.

The C5-convertase of the alternative pathway consists of C3bBbC3b also referred to as $C3b_2Bb$ (instead of C4b2a3b in the other pathways).



Activators of the alternate pathway are components on the surface of pathogens and include: LPS of Gram-negative bacteria and the cell walls of some bacteria and yeasts. Thus, when C3b binds to an activator surface, the C3 convertase formed will be stable and continue to generate additional C3a and C3b by cleavage of C3.

The generation of C5 convertase is the end of the alternative pathway. The alternative pathway can be activated by many Gram-negative (most significantly, *Neisseria meningitidis* and *N. gonorrhoea*), some Gram-positive bacteria and certain viruses and parasites, and results in the lysis of these organisms. Thus, the alternative pathway of C activation provides another means of protection against certain pathogens before an

antibody response is mounted. A deficiency of C3 results in an increased susceptibility to these organisms. The alternate pathway may be the more primitive pathway and the classical and lectin pathways probably developed from it.

Remember that the alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents.

4- Membrane attack (lytic) pathway

C5 convertase from the classical (C4b2a3b), lectin (C4b2a3b) or alternative (C3bBb3b) pathway cleaves C5 into C5a and C5b. C5a remains in the fluid phase and the C5b rapidly associates with C6 and C7 and inserts into the membrane. Subsequently C8 binds, followed by several molecules of C9. The C9 molecules form a pore in the membrane through which the cellular contents leak and lysis occurs. Lysis is not an enzymatic process; it is thought to be due to physical damage to the membrane. The complex consisting of C5bC6C7C8C9 is referred to as the membrane attack complex (MAC).

C5a generated in the lytic pathway has several potent biological activities. It is the most potent anaphylotoxin. In addition, it is a chemotactic factor for neutrophils and stimulates the respiratory burst in them and it stimulates inflammatory cytokine production by macrophages.

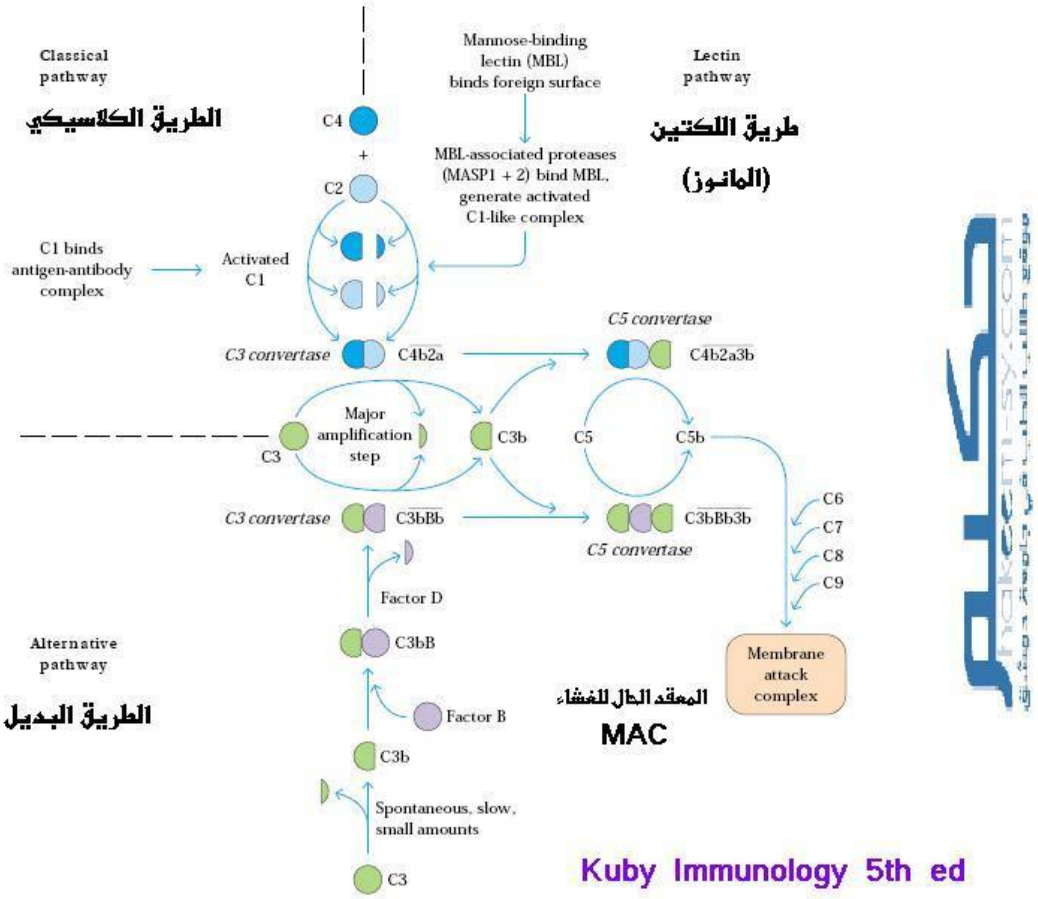
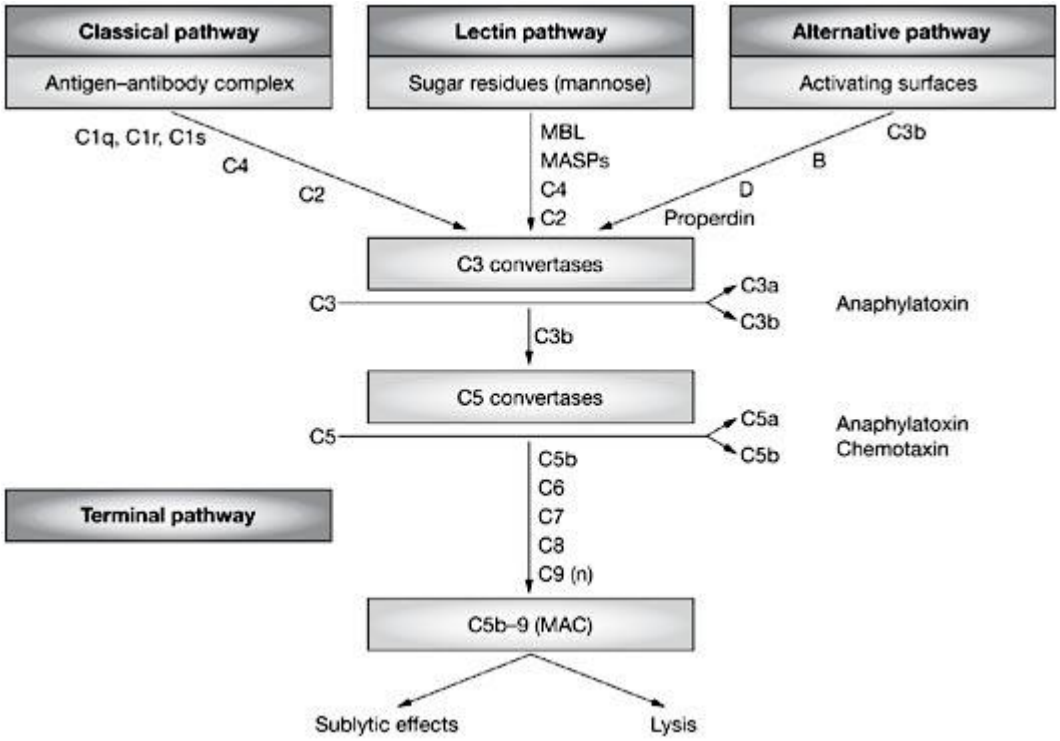


Table 1. Biological Activity of classical pathway products	
Component	**Biological Activity**
C2b	**Prokinin**; cleaved by plasmin to yield kinin, which results in edema
C3a	**Anaphylotoxin**; can activate basophils and mast cells to degranulate resulting in increased vascular permeability and

	contraction of smooth muscle cells, which may lead to**anaphylaxis**
C3b	**Opsonin**; promotes phagocytosis by binding to complement receptors Activation of phagocytic cells
C4a	**Anaphylotoxin** (weaker than C3a)
C4b	**Opsonin**; promotes phagocytosis by binding to complement receptors



Immunology and serology L17 Dr. Muna Al-Rubiae

HYPERSENSITIVITY REACTIONS

Hypersensitivity refers to excessive, undesirable (damaging, discomfort-producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. Hypersensitivity reactions can be divided into four types: type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction. Frequently, a particular clinical condition (disease) may involve more than one type of reaction.

TYPE I HYPERSENSITIVITY

Type I hypersensitivity is also known as immediate or anaphylactic hypersensitivity. The reaction may involve skin (eczema), eyes (conjunctivitis), nasopharynx , bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis). The reaction may cause a range of symptoms from minor inconvenience to death. The reaction usually takes 15 - 30 minutes from the time of exposure to the antigen, although sometimes it may have a delayed onset (10 - 12 hours).

Immediate hypersensitivity is mediated by IgE. The primary cellular component in this hypersensitivity is the mast cell or basophil. The reaction is amplified and/or modified by platelets, neutrophils and eosinophils. A biopsy of the reaction site demonstrates mainly mast cells and eosinophils.

The mechanism of reaction involves preferential production of IgE, in response to certain antigens (often called allergens).

A subsequent exposure to the same allergen cross links the cell-bound IgE and triggers the release of various pharmacologically active substances. Cross-linking of IgE Fc-receptor is important in mast cell triggering. Mast cell degranulation is preceded by increased Ca++ influx, which is a crucial process.

The agents released from mast cells and their effects are listed in Table 1. Mast cells may be triggered by other stimuli such as exercise, emotional stress, chemicals (*e.g.*, photographic developing medium), anaphylotoxins (*e.g.*, C4a, C3a, C5a, *etc.*). These reactions, mediated by agents without IgE-allergen interaction, are not hypersensitivity reactions, although they produce the same symptoms.

Table 1. Pharmacologic Mediators of Immediate Hypersensitivity

MEDIATOR	
Preformed mediators in granules	
Histamine	bronchoconstriction, mucus secretion, vasodilatation, vascular permeability
Tryptase	Proteolysis
kininogenase	kinins and vasodilatation, vascular permeability, edema

ECF-A (tetrapeptides)	attract eosinophil and neutrophils

The reaction is amplified by PAF (platelet activation factor) which causes platelet aggregation and release of histamine, heparin.

Diagnostic tests for immediate hypersensitivity include skin (prick and intradermal) tests, measurement of total IgE and specific IgE antibodies against the suspected allergens. There appears to be a genetic predisposition for atopic diseases and there is evidence for HLA association.

Symptomatic treatment is achieved with anti-histamines which block histamine receptors. Chromolyn sodium inhibits mast cell degranulation, probably, by inhibiting Ca^{++} influx. Symptomatic, although short term, relief from bronchoconstriction is provided by bronchodilators such as isoproterenol derivatives (Terbutaline, Albuterol).

The use of IgG antibodies against the Fc portions of IgE that binds to mast cells has been approved for treatment of certain allergies, as it can block mast cell sensitization.

Hyposensitization (immunotherapy or desensitization) is another treatment modality which is successful in a number of allergies, particularly to insect venoms and, to some extent, pollens. The mechanism is not clear, but there is a correlation between appearance of IgG (blocking) antibodies and relief from symptoms. Suppressor T cells that specifically inhibit IgE

antibodies may play a role.

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TYPE II HYPERSENSITIVITY

Type II hypersensitivity is also known as cytotoxic hypersensitivity and may affect a variety of organs and tissues. The antigens are normally endogenous, although exogenous chemicals (haptens) which can attach to cell membranes can also lead to type II hypersensitivity. Drug-induced hemolytic anemia, granulocytopenia and thrombocytopenia are such examples. The reaction time is minutes to hours. Type II hypersensitivity is primarily mediated by antibodies of the IgM or IgG classes and complement.

The lesion contains antibody, complement and neutrophils. Diagnostic tests include detection of circulating antibody against the tissues involved and the presence of antibody and complement in the lesion (biopsy) by immunofluorescence.

Treatment involves anti-inflammatory and immunosuppressive agents.

TYPE III HYPERSENSITIVITY

In type 3 hypersensitivity reactions, insoluble immune complexes (aggregations of antigens and IgG and IgM antibodies) form in the blood and are deposited in various tissues. This deposition of the antibodies may trigger an immune response according to the classical pathway of complement activation. There are two stages relating to the development of the complexes, firstly the complex forms when IgG and IgM antibodies are bound to an antigen, after this, the complexes can form larger ones which

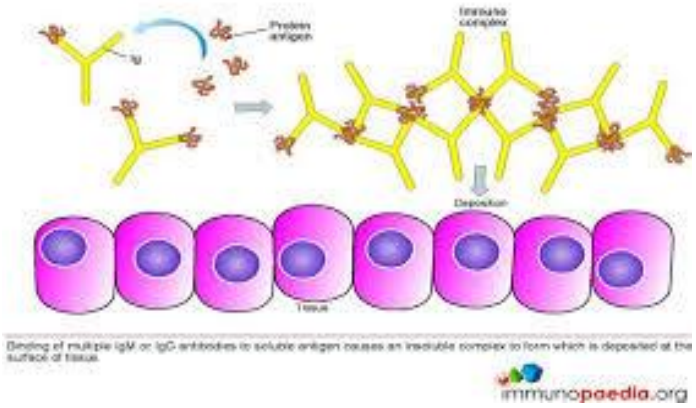
can be cleared by the body. The antigen-antibody complex will spread and deposit as stated above. The reaction takes hours to days to develop

Type III hypersensitivity is also known as immune complex hypersensitivity. The reaction may be general (*e.g.*, serum sickness) or may involve individual organs including skin (*e.g.*, systemic lupus erythematosus,), kidneys (*e.g.*, lupus nephritis), lungs (*e.g.*, aspergillosis), joints (*e.g.*, rheumatoid arthritis) or other organs. This reaction may be the pathogenic mechanism of diseases caused by many microorganisms.

The affinity of antibody and size of immune complexes are important in production of disease and determining the tissue involved. Diagnosis involves examination of tissue biopsies for deposits of immunoglobulin and complement by immunofluorescence microscopy.

Type 3 - immune complex hypersensitivity

Figure 3a



TYPE IV HYPERSENSITIVITY

Type IV hypersensitivity is also known as cell mediated or delayed type hypersensitivity. The classical example of this hypersensitivity is tuberculin (Montoux) reaction which peaks 48 hours after the injection of antigen.

The reaction to intracutaneously injected tuberculin is the classic example of a delayed (cellular) hypersensitivity reaction. T-cells sensitized by prior infection are recruited to the skin site where they release lymphokines. These lymphokines induce induration through local vasodilatation, edema, fibrin deposition, and recruitment of other inflammatory cells to the area. Features of the reaction include (*1*) its delayed course, reaching a peak more than 24 h after injection of the antigen; (*2*) its indurated character; and (*3*) its occasional vesiculation and necrosis.

Type IV hypersensitivity is involved in the pathogenesis of many autoimmune and infectious diseases (tuberculosis, leprosy, histoplasmosis, toxoplasmosis, leishmaniasis, *etc.*) due to infections and foreign antigens. Another form of delayed hypersensitivity is contact dermatitis chemicals, heavy metals, *etc.*).

Mechanisms of damage in delayed hypersensitivity include T lymphocytes and monocytes and/or macrophages. Cytotoxic T cells (Tc) cause direct damage whereas helper T (TH1) cells secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages, which cause the bulk of the damage. The delayed hypersensitivity lesions mainly contain monocytes and a few T cells.

Corticosteroids and other immunosuppressive agents are used in treatment.

Table 1 - Comparison of Different Types of hypersensitivity

Characteristic	type-I (anaphylactic)	type-II (cytotoxic)	type-III (immune complex)	type-IV (delayed type)
Antibody	IgE	IgG, IgM	IgG, IgM	None
Antigen	Exogenous	cell surface	Soluble	tissues & organs
response time	15-30 minutes	minutes-hours	3-8 hours	48-72 hours
Appearance	flare	lysis and necrosis	erythema and edema, necrosis	erythema and induration
Histology	basophils and eosinophil	antibody and complement	complement and neutrophils	monocytes and lymphocytes
transferred with	Antibody	Antibody	Antibody	T-cells
Examples	allergic asthma,	erythroblastosis fetalis,	SLE,	tuberculin test,

Immunology and serology L19-20 Dr. Muna Al-Rubiae

Tests For Antigen-Antibody Reactions

Factors affecting measurement of Ag/Ab reactions

The only way that one knows that an antigen-antibody reaction has occurred is to have some means of directly or indirectly detecting the complexes formed between the antigen and antibody. The ease with which on can detect antigen-antibody reactions will depend on a number of factors.

1. **Affinity** - The higher the affinity of the antibody for the antigen, the more stable will be the interaction. Thus, the ease with which one can detect the interaction is enhanced.

2. **Ag:Ab ratio** - The ratio between the antigen and antibody influences the detection of Ag/Ab complexes because the sizes of the complexes formed is related to the concentration of the antigen and antibody.

3. **Physical form of the antigen** - The physical form of the antigen influences how one detects its reaction with an antibody. If the antigen is a particulate, one generally looks for agglutination of the antigen by the antibody. If the antigen is soluble one generally looks for the precipitation of the antigen after the production of large insoluble Ag/Ab complexes.

Type of Antigen-Antibody Reactions Tests

1. **Agglutination Tests**

Agglutination/Hemagglutination - When the antigen is particulate the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. When the antigen is an erythrocyte the term

hemagglutination is used. All antibodies can theoretically agglutinate particulate antigens but IgM due to its high valence is particularly good agglutinin.

a) **Qualitative agglutination test** - Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen.

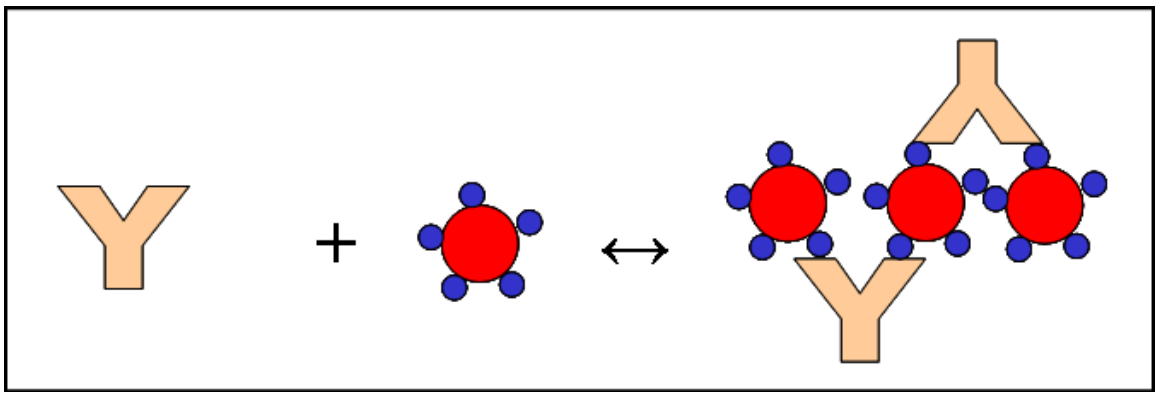
e.g. A patients red blood cells mixed with antibody to a blood group antigen to determine a person's blood type.

b) **Quantitative agglutination test** - Agglutination tests can also be used to quantitate the level of antibodies to particulate antigens. In this test one makes serial dilutions of a sample to be tested for antibody and then adds a fixed number of red blood cells or bacteria or other such particulate antigen and determines the maximum dilution which gives agglutination. The maximum dilution that gives visible agglutination is called the **titer**.

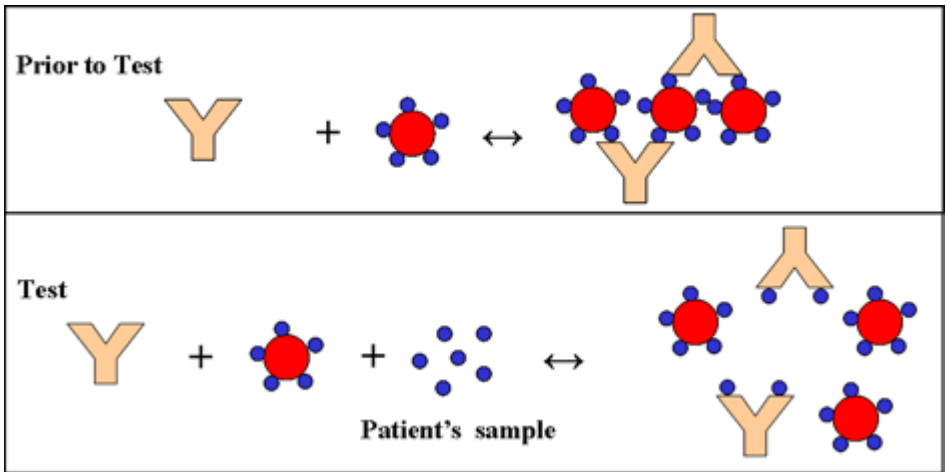
Prozone effect - when the concentration of antibody is high (i.e. lower dilutions) there is no agglutination and then as the sample is diluted agglutination occurs. The lack of agglutination at high concentrations of antibodies is called the prozone effect. Lack of agglutination in the prozone is due to antibody excess resulting in very small complexes which do not clump to form visible agglutination.

C. **Passive hemagglutination** - The agglutination test only works with particulate antigens. However, it is possible to coat erythrocytes with a soluble antigen (e.g. viral antigen, a polysaccharide or a hapten) and used the

coated red blood cells in an agglutination test for antibody to the soluble antigen. This is called passive hemagglutination. The test is performed just like the agglutination test. Applications include detection of antibodies to soluble antigens and detection of antibodies to viral antigens.



D. Hemagglutination Inhibition - The agglutination test can be modified to be used for the measurement of soluble antigens. This test is called hemagglutination inhibition. It is called hemagglutination inhibition because one measures the ability of soluble antigen to inhibit the agglutination of antigen-coated red blood cells by antibodies. In this test a fixed amount of antibodies to the antigen in question is mixed with a fixed amount of red blood cells coated with the antigen. Also included in the mixture are different amounts of the sample to be analyzed for the presence of the antigen. If the sample contains the antigen, the soluble antigen will compete with the antigen coated on the RBC for binding to the antibodies, thereby inhibiting the agglutination of the RBC



2. **Precipitation tests**

Precipitation reactions are based on the interaction of antibodies and antigens. They are based on two soluble reactants that come together to make one insoluble product, the precipitate. These reactions depend on the formation of lattices (cross-links) when antigen and antibody exist in optimal proportions. Excess of either component reduces lattice formation and subsequent precipitation. Precipitation reactions differ from agglutination reactions in the size and solubility of the antigen and sensitivity. There are several precipitation methods applied in clinical laboratory for the diagnosis of disease. These can be performed in semisolid media such as agar or agarose,

Precipitation methods include double immunodiffusion, radial immunodiffusion (semi-quantitation of proteins by gel diffusion using antibody incorporated in agar). Precipitation reactions are less sensitive than agglutination reactions but remain gold standard serological techniques. The

most commonly used serologic precipitation reactions are the Ouchterlony test (based on double immunodiffusion and named after the Swedish physician who invented it), and the Mancini method (based on single radial immunodiffusion). The Mancini method results in precipitin ring formation on a thin agarose layer. The diameter of the ring correlates with the concentration of proteins in the precipitin.

Radial Immunodiffusion (Mancini) - In radial immunodiffusion antibody is incorporated into the agar gel as it is poured and different dilutions of the antigen are placed in holes punched into the agar. As the antigen diffuses into the gel it reacts with the antibody and when the equivalence point is reached a ring of precipitation is formed. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

3.**Enzyme Linked Immunosorbent Assay (ELISA)\Radioimmunoassay (RIA)**

When enzymes (such as horseradish peroxidase) react with appropriate substrates (such as ABTS or TMB), a change in color occurs, which is used as a signal. However, the signal has to be associated with the presence of antibody or antigen, which is why the enzyme has to be linked to an appropriate antibody. Since it is necessary to remove any unbound antibody or antigen by washing, the antibody or antigen has to be fixed to the surface of the container; i.e., the immunosorbent must be prepared.

The antibody can be immobilized onto the surface of a plastic bead or coated onto the surface of a plastic plate and thus the immune complexes can easily be separated from the other components by simply washing the plate

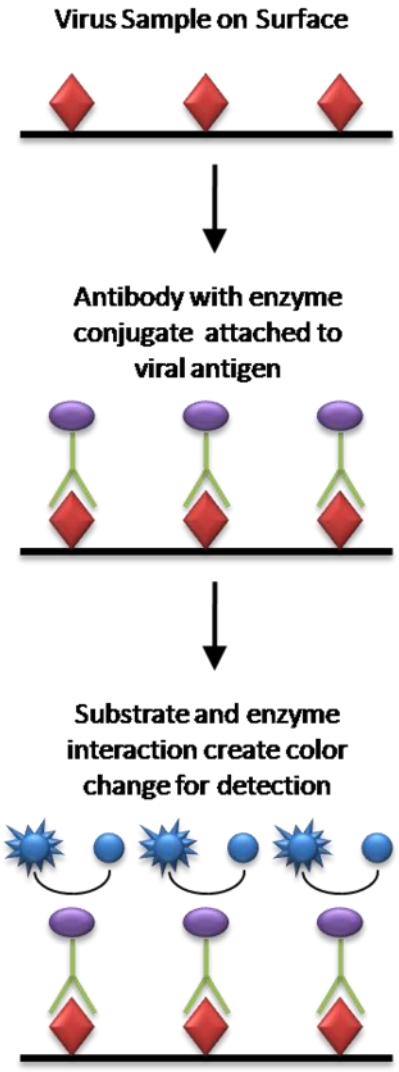
The steps of direct ELISA follow the mechanism below:-

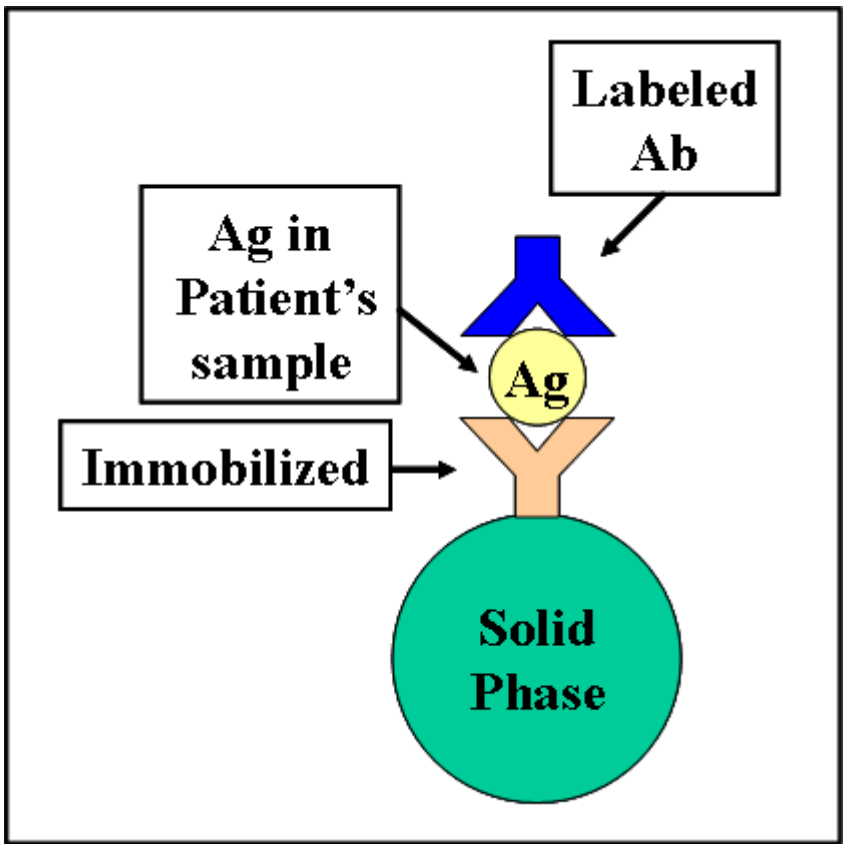
- A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
- A solution of nonreacting protein, such as bovine serum albumin or casein, is added to well in order to cover any plastic surface in the well which remains uncoated by the antigen.
- The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme.
- The stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.

A major disadvantage of the direct ELISA is the method of antigen immobilization is not specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich or indirect ELISA provides a solution to this problem, by using a "capture" antibody specific for the test antigen to pull it out of the serum's molecular mixture.

The use and meaning of the names "direct ELISA" and "indirect ELISA" differs in the literature and on web sites depending on the type of the experiment. When the presence of an antigen is analyzed, the name "direct ELISA" refers to an ELISA in which only a labelled primary antibody is

used, and the term "indirect ELISA" refers to an ELISA in which the antigen is bound by the primary antibody which then is detected by a labelled secondary antibody.





Radioimmunoassays (RIA) are assays which are based on the measurement of radioactivity associated with immune complexes. In any particular test, the label may be on either the antigen or the antibody.

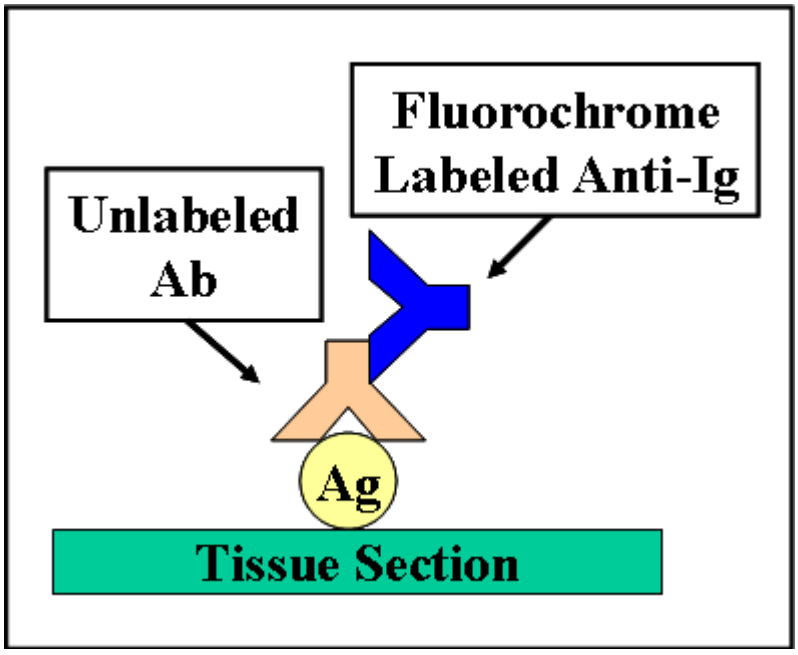
4- Tests for Cell Associated Antigens

1. Immunofluorescence - Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein or rhodamine) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.

In these assays, antibodies or other reagent proteins are tagged or labeled with fluorescent dyes. These fluorescent reagents can then be used to stain samples mounted onto microscope slides and the slides can be examined using a fluorescent microscope.

a) **Direct Immunofluorescence** - In direct immunofluorescence the antibody specific to the antigen is directly tagged with the fluorochrome.

b) **Indirect Immunofluorescence** - In indirect immunofluorescence the antibody specific for the antigen is unlabeled and a second anti-immunoglobulin antibody directed toward the first antibody is tagged with the fluorochrome. Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.

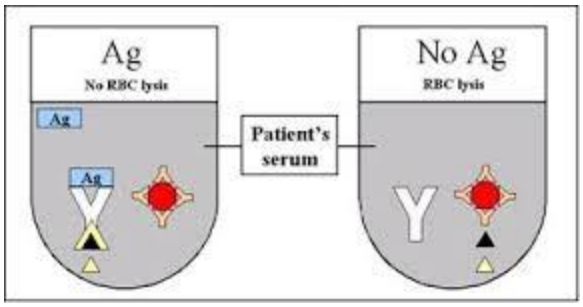


5. **Complement Fixation**

Antigen/Antibody complexes can also be measured by their ability to fix complement because an Ag/Ab complex will "consume" complement if it is present whereas free Ag's or Ab's do not. Tests for Ag/Ab complexes that depend on the consumption of complement are termed complement fixation tests and are used to quantitate Ag/Ab reactions. This test will only work with complement fixing antibodies (IgG, IgM).

Antigen is mixed with the test serum to be assayed for antibody and Ag/Ab complexes are allowed to form. A control tube in which no Ag is added is also prepared. If no Ag/Ab complexes are present in the tube, none of the complement will be fixed. However, if Ag/Ab complexes are present, they will fix complement and reduce the amount of complement in the tube. After allowing for complement fixation by any Ag/Ab complexes, a standard amount of red blood cells, which have been pre-coated with anti-erythrocyte antibodies is added.

The amount of antibody-coated RBC is predetermine to be just enough to completely use up all the complement initially added if it were still there. If all the complement was still present (i.e. no Ag/Ab complexes formed between the Ag and Ab in question), all the RBC will be lysed. If Ag/Ab complexes are formed between that Ag and Ab in question, some of the complement will be consumed and thus when the antibody-coated RBC's are added not all of them will lyse. By simply measuring the amount of RBC lysis by measuring the release of hemoglobin into the medium, one can indirectly quantitate Ag/Ab complexes in the tube. Complement fixation tests are most commonly used to assay for antibody in a test sample but they can be modified to measure antigen.


Ag
No RBC lysis
Ag
Ag
Patient's serum
No Ag
RBC lysis

Immunology and serology L21-22 Dr. Muna Al-Rubiae

The immune response to infection

Specific Immune Responses

The immune system has evolved to deal with infectious pathogens. Although all pathogens are different from each other, they can be subgrouped by the pattern of the immune response that they evolve.

In general pathogens may be divided depending of the types of pathogen

(1) Bacteria

(2) Encapsulated bacteria

(3) Viruses

(4) Parasites

(5) Fungi

1. Immune responses to bacteria

Immunity to bacteria is dependent on different effector immune cells. The following example illustrates in a simplified outline the sequence of events leading to an immune response against bacteria.

Initial phase and the activation of the innate immune system

Rapidly dividing streptococci in a surgical wound locally activate complement in the tissue. Proinflammatory complement fragments like C3a and C5a recruit neutrophils and activate local mast cells which increase blood flow and release a further cascade of proinflammatory mediators.

Uptake of antigens for presentation

Recruited neutrophils phagocytose complement coated bacteria and kill them. Dendritic cells process protein antigens derived from the bacteria and

present them. Dendritic cells migrate to the T zones of lymph nodes via the lymphatics where they present antigen to T cells.

T cell

T cells migrating form the blood through venules in lymph nodes "look" for antigen expressed by dendritic cells. T cells become primed by a combination of antigen, costimulatory molecules and cytokines expressed by activated dendritic cells.

T helper (Th cell) promote inflammation at the site of infection

The effector T cells has been described: Th cells are inflammatory T cells which secrete interferon-(□□IFN) □□interleukin-2 (IL2) and other cytokines which recruit inflammatory cells to the tissue. There they activate macrophages via IFN and by release of proinflammatory cytokines ensure the blood supply is maintained.

The acute phase response

Release of interleukin-1 and 6 by macrophages into the bloodstream stimulates the liver to make an acute phase response which includes increased synthesis of many serum proteins including complement components which are being consumed at the inflamed site, thus ensuring a supply line of immune components. One protein, C-reactive protein (CRP) functions like a primitive antibody by binding to and opsonising bacteria. Because CRP is potently upregulated, it is a useful indicator of inflammation, especially bacterial infection.

Th cells promote B cell activation and differentiation

Th T cells promote antibody formation and secrete interleukins-4, 5 and 10 . These T cells help B cells to undergo clonal expansion and affinity maturation within structures called germinal centres. As a consequence of this, high titre, high affinity antibody is generated which is effective at neutralising bacterial toxins.

B and T cell memory

In addition to generating effector B and T cells, memory B and T lymphocytes are produced which make rapid and more efficient responses upon reexposure to the infection.

2. Immunity to encapsulated bacteria

To evade the immune response, certain strains of bacteria have become encapsulated with a polysaccharide coat. Encapsulated bacteria grow less well than their nonencapsulated counterparts but can evade the immune system as they activate complement poorly, and immunity is dependent on generating antibody to the polysaccharide capsule. Three types of bacteria are clinically important in humans:

Neisseriae meningitidis

Pneumococci

Haemophilus Influenzae

All these strains of bacteria can cause sepsis and meningitis, and the latter two are common causes of pneumonia and secondary bacterial lung infections. The polysaccharide capsule of these bacteria is poorly degraded by human cells and cannot elicit T cell help.

Although the mechanism of antibody formation is poorly understood, polysaccharides probably activate B cells directly.

May be they probably receive accessory signals from macrophage cells, but they probably do not require T cell help. In normal individuals antibody is sufficient to opsonise and remove these bacteria.

For reasons that are not understood, children less than 2 years of age in particular, make poor responses to polysaccharide antigens derived from the above bacteria. In the first few months of life, infants are protected by maternal immunoglobulin, but as this decrease the incidence of infection rises.

This problem has been addressed by conjugating sugar epitopes derived from the polysaccharides with protein antigens. These conjugate vaccines induce efficient antibody responses in infants and have substantially reduced the mortality and morbidity from *H. Influenzae.*

3. Immunity to viruses

Neutralising antibody plays a crucial role in eliminating intact viruses by preventing the infection of other cells. Essentially the same mechanism which elicits antibody responses to other protein antigens (described above) operates for viruses.

To kill the intracellular phase of viral replication, the immune system has developed a number of strategies. Most cells are capable of secreting interferon □which inhibit viral replication□ Double stranded RNA (i.e. viral RNA) is particularly efficient at inducing interferons, which have been used therapeutically to help eliminate persistent viral infections in humans.

The second important mechanism is the generation of CD8 cytotoxic T cells.

Once primed, cytotoxic CD8 T cells migrate out into tissue and can kill virally infected targets. CD8 T cells play a crucial role in regulating viral infections.

4. Immunity to parasites

The next subclass of infection is parasites which are generally complex organisms which invade through mucosal surfaces or the skin. Parasites have sophisticated mechanisms for evading the immune response and the most effective strategy is to prevent infection in the first place.

Anaphylactic IgE mediated responses against parasites evolved to prevent parasites gaining access to the host by expelling the parasite as consequence of the degranulation of mast cells and release of vasoactive substances, particularly histamine.

The generation of IgE is dependent on the Th cytokine IL4. IL5 also secreted by Th cells recruits eosinophils which can kill parasites by exocytosing a cytotoxic protein, cationic basic protein.

5- Immunity to Fungal Infection

Neutrophils, macrophages, and DCs are all critical to the antifungal response. Upon infection, these innate immune cells are rapidly recruited to sites of infection by dependent on of their production of inflammatory cytokines, chemokines, and/or complement units.

One of its more recently discovered antifungal mechanisms is the neutrophil extracellular trap, which, together with the release of antimicrobials, constitutes a potent antifungal response.

The role of adaptive immunity in the antifungal immune response is also well appreciated. Immune-regulatory CD4+ T helper cells are of key importance,

Th cells can be activated by DCs , activated in response to the recognition of immutable fungal molecules. Th cells can then help to optimize the activation of phagocytes at sites of infection. Th cells can also secrete signature pro-inflammatory cytokines such as IFN. Thus, modulating Th cells can enhanced the therapeutic efficacy of antifungal agents.