UNIT	CONTENT	PAGE Nr
I	BASIC CONCEPTS OF IMMUNOLOGY	02
II	IMMUNE SYSTEM	10
III	ANTIGEN AND ANTIBODY	38
IV	ANTIGEN AND ANTIBODY REACTIONS	50
V	HYPERSENSITIVITY	59

UNIT - I
BASIC CONCEPTS OF IMMUNOLOGY

History of Immunology

Immunology begins with Edward Jenner's discovery that vaccination with cowpox protects against smallpox. An immune response was confirmed by the observations of many scientists that the same disease did not return a second time to a recovered individual. With the recognition by Friedrich Henle that germs caused disease, and the isolation of infectious bacteria by his pupil Robert Koch, the stage was set to examine how an immune response was achieved. Modern immunology begins with the research of Metchnikoff, who discovered the phenomenon of phagocytosis in starfish and extrapolated it to macrophages in humans as cells that engulf infectious agents; this was the beginning of cellular immunology. Paul Ehrlich investigated the formation of antibodies recognized as later as proteins that destroyed infectious agents. However, an explanation of how antibodies were formed and selected was puzzling. Did the body have enough genes to code for every type of antibody, and did specific cells produce antibodies, or did each cell have the ability to produce antibodies to any challenging molecule? Following the work of Karl Landsteiner, Felix Haurowitz, Niels Jerne and others, the "clonal selection theory" was proposed by MacFarlane Burnett. This theory states that each B-cell produces one type of antibody, and once activated, it expands and produces memory cells. Meanwhile, work on cellular immunity and innate immunity recognized the role of various types of T-cells, dendritic cells and cytokines in the immune response. New classes of T-cells and cytokines are constantly being found, and there is an intricate connection between these three branches of the immune system.

Immunohematology

Immunohematology, more commonly known as blood banking is a branch of hematology which studies antigen-antibody reactions and analogous phenomena as they relate to the pathogenesis and clinical manifestations of blood disorders. A person employed in this field is referred to as an immunohematologist. Their day-to-day duties include blood typing, cross-matching and antibody identification.

Immunohematology and Transfusion Medicine is a medical post graduate specialty in many countries. The specialist Immunohematology and Transfusion Physician provides expert opinion for difficult transfusions, massive transfusions, incompatibility work up, therapeutic plasmapheresis, cellular therapy, irradiated blood therapy, leukoreduced and washed blood products, stem cell procedures, platelet rich plasma therapies, HLA and cord blood banking. Other research avenues are in the field of stem cell researches, regenerative medicine and cellular therapy

Lymphocytes of the Immune System

B-Cells

B-cells (sometimes called B-lymphocytes and often named on lab reports as CD19 or CD20 cells) are specialized cells of the immune system whose major function is to produce antibodies (also called immunoglobulins or gamma-globulins). B-cells develop in the bone marrow from hematopoietic stem cells. As part of their maturation in the bone marrow, B-cells are trained or educated so that they do not produce antibodies to healthy tissues. When mature, B-cells can be found in the bone marrow, lymph nodes, spleen, some areas of the intestine, and the bloodstream.

When B-cells encounter foreign material (antigens), they respond by maturing into another cell type called plasma cells. B-cells can also mature into memory cells, which allows a rapid response if the same infection is encountered again. Plasma cells are the mature cells that actually produce the antibodies.

Antibodies, the major product of plasma cells, find their way into the bloodstream, tissues, respiratory secretions, intestinal secretions, and even tears. Antibodies are highly specialized serum protein molecules.

For every foreign antigen, there are antibody molecules specifically designed to fit that antigen, like a lock and key. For example, there are antibody molecules that physically fit the poliovirus, others that fit diphtheria, and still others that fit the measles virus. The variety of different antibody molecules is extensive so that B-cells have the ability to produce them against virtually all microbes in our environment. However, each plasma cell produces only one kind of antibody.

When antibody molecules recognize a microorganism as foreign, they physically attach to it and set off a complex chain of events involving other components of the immune system that work to eventually destroy the germ. Antibodies vary with respect to their specialized functions in the body. These variations are determined by the antibody's chemical structure, which in turn determines the class of the antibody (or immunoglobulin).

There are five major classes of antibodies (IgG, IgA, IgM, IgD and IgE). IgG has four different subclasses (IgG1, IgG2, IgG3, IgG4). IgA has two subclasses (IgA1 and IgA2).Each immunoglobulin class has distinct chemical characteristics that provide it with specific functions (Figure 3). For example, IgG antibodies are formed in large quantities, last in the circulation for a few weeks, and travel from the blood stream to the tissues easily. Only IgG crosses the placenta and passes some immunity from the mother to the newborn.

Antibodies of the IgA class are produced near mucus membranes and find their way into secretions such as tears, bile, saliva and mucus, where they protect against infection in the respiratory tract and intestines. Some of the IgA also appears in the circulation.Antibodies of the IgM class are the first antibodies formed in response to infection. They are important in protection during the early days of an infection.

Antibodies of the IgE class are responsible for allergic reactions.Antibodies protect the body against infection in a number of different ways. For example, some microorganisms, such as viruses, must attach to body cells before they can cause an infection, but antibodies bound to the surface of a virus can interfere with the virus' ability to attach to the host cell. In addition, antibodies attached to the surface of some microorganisms can cause the activation of a group of proteins called the complement system that can directly kill some bacteria or viruses.

Antibody-coated bacteria are also much easier for neutrophils to ingest and kill than bacteria that are not coated with antibodies. All of these actions of antibodies prevent microorganisms from successfully invading body tissues and causing serious infections.

The long life of plasma cells enables us to retain immunity to viruses and bacteria that infected us many years ago. For example, once people have been fully immunized with live

vaccine strains of measles virus, they will almost never catch it because they retain the plasma cells and antibodies for many years and these antibodies prevent infection.

T-Cells

T-cells (sometimes called T-lymphocytes and often named in lab reports as CD3 cells) are another type of immune cell. T-cells directly attack cells infected with viruses, and they also act as regulators of the immune system.

T-cells develop from hematopoietic stem cells in the bone marrow but complete their development in the thymus. The thymus is a specialized organ of the immune system in the chest. Within the thymus, immature lymphocytes develop into mature T-cells (the "T" stands for the thymus) and T-cells with the potential to attack normal tissues are eliminated. The thymus is essential for this process, and T-cells cannot develop if the fetus does not have a thymus. Mature T-cells leave the thymus and populate other organs of the immune system, such as the spleen, lymph nodes, bone marrow and blood.

Each T-cell reacts with a specific antigen, just as each antibody molecule reacts with a specific antigen. In fact, T-cells have molecules on their surfaces that are similar to antibodies. The variety of different T-cells is so extensive that the body has T-cells that can react against virtually any antigen.
T-cells have different abilities to recognize antigen and are varied in their function. There are "killer" or cytotoxic T-cells (often denoted in lab reports as CD8 T-cells), helper T-cells (often denoted in lab reports as CD4 T-cells), and regulatory T-cells. Each has a different role to play in the immune system.

Killer, or cytotoxic, T-cells perform the actual destruction of infected cells. Killer T-cells protect the body from certain bacteria and viruses that have the ability to survive and even reproduce within the body's own cells. Killer T-cells also respond to foreign tissues in the body, such as a transplanted kidney. The killer cell must migrate to the site of infection and directly bind to its target to ensure its destruction.

Helper T-cells assist B-cells to produce antibodies and assist killer T-cells in their attack on foreign substances.Regulatory T-cells suppress or turn off other T-lymphocytes. Without regulatory cells, the immune system would keep working even after an infection has been cured. Without regulatory T-cells, there is the potential for the body to "overreact" to the infection. Regulatory T-cells act as the thermostat of the lymphocyte system to keep it turned on just enough—not too much and not too little.

Blood Group

A blood type (also called a blood group) is a classification of blood, based on the presence and absence of antibodies and inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens can stem from one allele (or an alternative version of a gene) and collectively form a blood group system.

Blood types are inherited and represent contributions from both parents. A total of 36 human blood group systems and 346 antigens are now recognized by the International

Society of Blood Transfusion (ISBT).The two most important blood group systems are ABO and Rh; they determine someone's blood type (A, B, AB, and O, with +, − or null denoting RhD status) for suitability in blood transfusion.

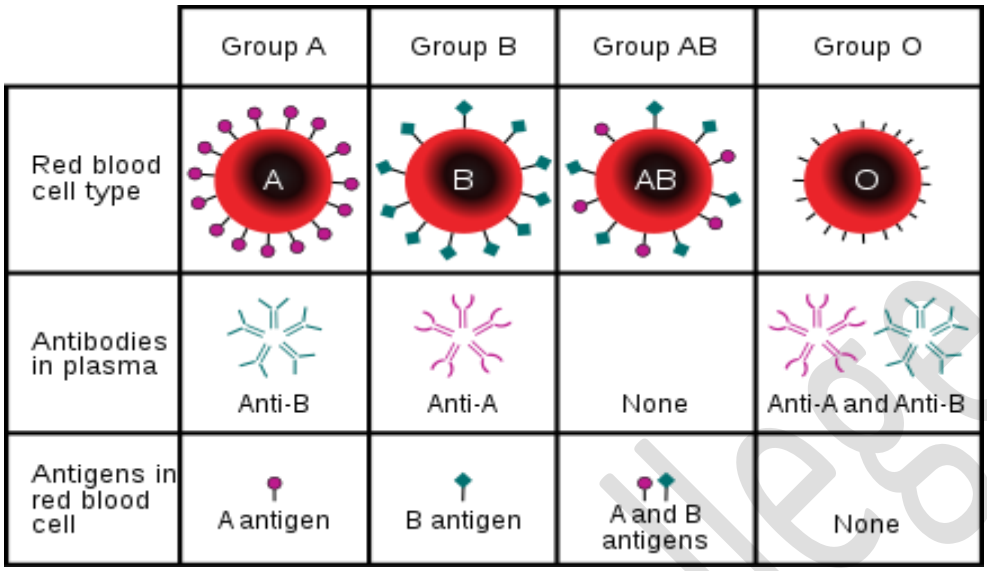

Figure1.1

Blood type (or blood group) is determined, in part, by the ABO blood group antigens present on red blood cells.

Blood transfusion

Blood transfusion is the process of transferring blood or blood products into one's circulation intravenously.Transfusions are used for various medical conditions to replace lost components of the blood. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood, such as red blood cells, white blood cells, plasma, clotting factors, and platelets.

Red blood cells (RBC) contain haemoglobin, and supply the cells of the body with oxygen. White blood cells are not commonly used during transfusion, but are part of the immune system, and fight infections. Plasma is the liquid part of the blood, which acts as a buffer, and contains proteins and important substances needed for the body's overall health. Platelets are involved in blood clotting, preventing the body from bleeding. Before these components were known, doctors believed that blood was homogenous. Because of this, many patients died because incompatible blood was transferred to them.

Rh incompatibility

Rh incompatibility is a condition that develops when a pregnant woman has Rh-negative blood and the baby in her womb has Rh-positive blood.

Causes

During pregnancy, red blood cells from the unborn baby can cross into the mother's blood through the placenta.If the mother is Rh-negative, her immune system treats Rh-positive fetal cells as if they were a foreign substance. The mother's body makes antibodies against the fetal blood cells. These antibodies may cross back through the placenta into the developing baby. They destroy the baby's circulating red blood cells.

When red blood cells are broken down, they make bilirubin. This causes an infant to become yellow (jaundiced). The level of bilirubin in the infant's blood may range from mild to dangerously high.

Firstborn infants are often not affected unless the mother had past miscarriages or abortions. This would sensitize her immune system. This is because it takes time for the mother to develop antibodies. All children she has later who are also Rh-positive may be affected.

Rh incompatibility develops only when the mother is Rh-negative and the infant is Rh-positive. This problem has become less common in places that provide good prenatal care. This is because special immune globulins called RhoGAM are routinely used.

Defense against microbes is mediated by the early reactions of innate immunity and the later responses of adaptive immunity.

Innate immunity (also called natural or native immunity) provides the early line of defense against microbes. It consists of cellular and biochemical defense mechanisms that are in place even before infection and are poised to respond rapidly to infections. The mechanisms of innate immunity are specific for structures that are common to groups of related microbes and may not distinguish fine differences between microbes.

The principal components of innate immunity are:

1. Physical and chemical barriers, such as epithelia and antimicrobial chemicals produced at epithelial surfaces;
2. Phagocytic cells (neutrophils, macrophages), dendritic cells, and natural killer (NK) cells and other innate lymphoid cells;
3. Blood proteins, including members of the complement system and other mediators of inflammation.

Adaptive immunity (also called specific or acquired immunity) system recognizes and reacts to a large number of microbial and nonmicrobial substances. The defining characteristics of adaptive immunity are the ability to distinguish different substances, called specificity, and the ability to respond more vigorously to repeated exposures to the same microbe, known as memory. The unique components of adaptive immunity are cells called lymphocytes and their secreted products, such as antibodies. Foreign substances that induce specific immune responses or are recognized by lymphocytes or antibodies are called antigens.

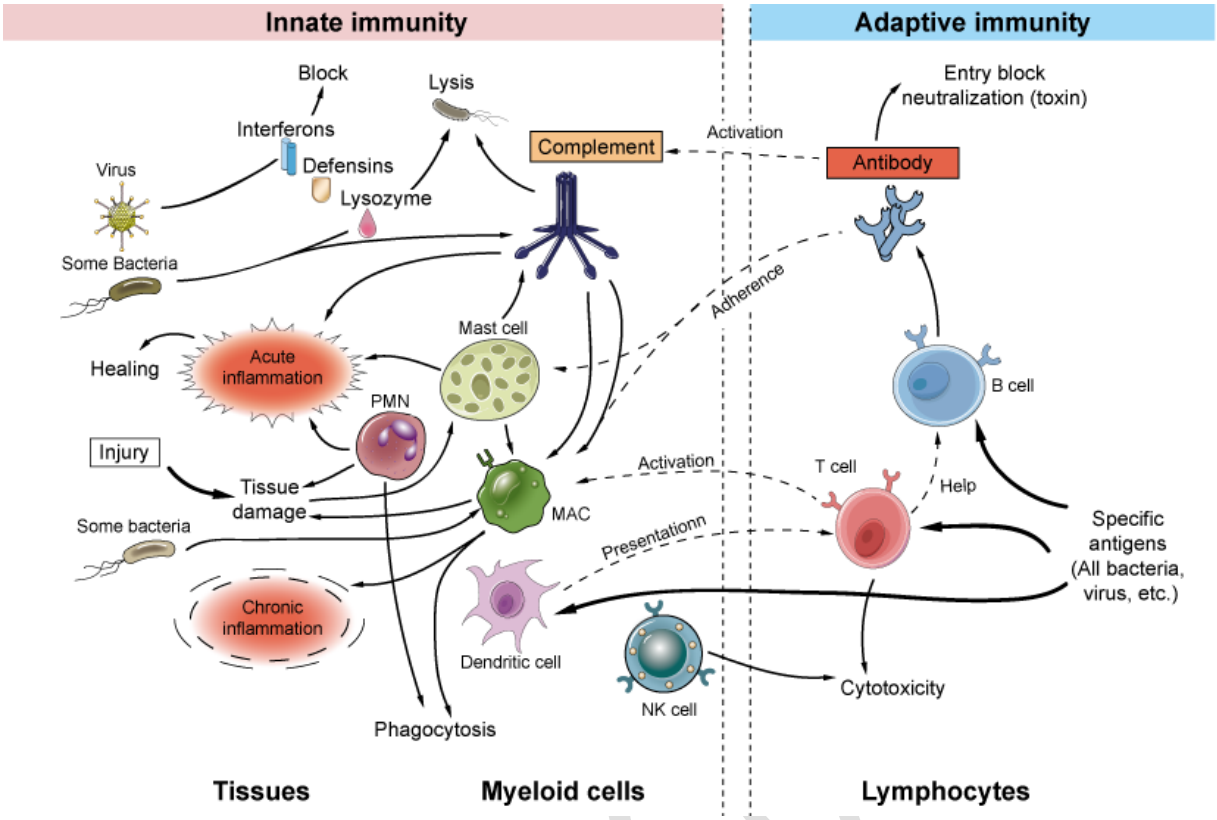

Figure 1.2. Just as resistance to disease can be innate (inborn) or acquired, the mechanisms mediating it can be correspondingly divided into innate (left) and adaptive (right), each composed of both cellular (lower half) and humoral elements (i.e. free in serum or body fluids; upper half). Adaptive mechanisms, more recently evolved, perform many of their functions by interacting with the older innate ones.

Innate immunity is activated when cells use specialized sets of receptors (Pattern recognition receptor, PRR) to recognize different types of microorganisms (bacteria, viruses, etc.) that have managed to penetrate the host. Binding to these receptors activate a limited number of basic microbial disposal mechanisms, such as phagocytosis of bacteria by macrophages and neutrophils, or the release of antiviral interferons. Many of the mechanisms involved in innate immunity are largely the same as those responsible for non-specifically reacting to tissue damage, with the production of inflammation (cover up the right-hand part of Figure 1 to appreciate this). However, as the nature of the innate immune response depends on the type of infection, the term 'nonspecific', although often used as a synonym for 'innate', is not completely accurate. Adaptive immunity is based on the special properties of lymphocytes (T and B, lower right), which can respond selectively to thousands of different non-self-materials, or 'antigens', leading to specific memory and a permanently altered pattern of response - an adaptation to the animal's own surroundings. Adaptive mechanisms can function on their own against certain antigens (cover up the left-hand part of Figure 1.2), but the majority of their effects are exerted by means of the interaction of antibody with complement and the phagocytic cells of innate immunity, and of T cells with macrophages (broken lines). Through their activation of these innate mechanisms, adaptive responses frequently provoke inflammation, either acute or chronic; when it becomes a nuisance this is called hypersensitivity.

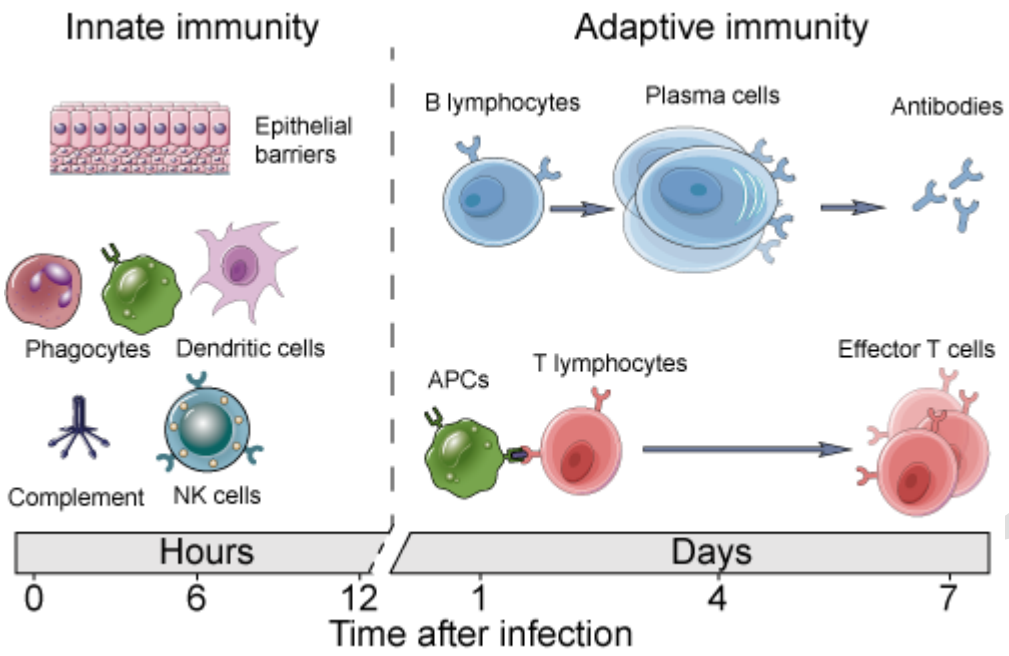

Figure 1.3. Innate and adaptive immunity time line. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and require the activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections.

Innate and adaptive immune responses are components of an integrated system of host defense in which numerous cells and molecules function cooperatively. The mechanisms of innate immunity provide effective initial defense against infections. However, many pathogenic microbes have evolved to resist innate immunity, and their elimination requires the more powerful mechanisms of adaptive immunity. There are numerous connections between the innate and adaptive immune systems. The innate immune response to microbes stimulates adaptive immune responses and influences the nature of the adaptive responses. Conversely, adaptive immune responses often work by enhancing the protective mechanisms of innate immunity, making them more capable of effectively combating pathogenic microbes.

Table 1. Features of Innate and Adaptive Immunity

Characteristics	Innate	Adaptive
Specificity	For molecules shared by groups of related microbes and molecules produced by damaged host cells	For microbial and nonmicrobial antigens
Diversity	Limited; germline encoded	Very large; receptors are produced by somatic recombination of gene segments
Memory	None	Yes
Nonreactivity to self	Yes	Yes
Components		
Cellular and chemical barriers	Skin, mucosal epithelia; antimicrobial molecules	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Blood proteins	Complement, others	Antibodies
Cells	Phagocytes (macrophages, neutrophils), natural killer cells, innate lymphoid cells	Lymphocytes

UNIT – II
IMMUNE SYSTEM

lymphoreticular system:

Also referred to as the reticuloendothelial system or mononuclear phagocytic system.It is comprised of primary lymphoid organs (bone marrow, Bursa of Fabricius, the foetal liver and the thymus) which are responsible for the production of lymphocytes, and the secondary lymphoid organs (lymph nodes, spleen and mucosal associated lymphoid tissue) which function to provide an environment where lymphocytes can react to antigen from the tissue fluid, blood and mucosal surfaces.

The main functions of the lymphoreticular system are removal of senescent cells and production of immune cells.

Introduction

The lymphatic system can be divided into two anatomical and functional subsets: lymphatic vessels that carry lymph around the body, and the lymphoreticular system which describes the lymphoid tissues. The lymphatic system has three functions - immune defence, removal of interstitial fluid from tissues and the transport of fats.

Lymphatic Vessels and Lymph

Due to their structure lymphatic capillaries are more permeable than vascular capillaries which means that not only can they remove fluid more effectively from tissues but they can also take up large molecules as well as chylomicrons. Chylomicrons transport fats and enter the lymph to eventually join the circulatory system via the thoracic duct; this enables the lipid soluble triacylglycerols (TAGs) to be transported into the bloodstream. The interstitial fluid or lymph within the lymphatic vessels passes through lymph nodes where it is surveyed by immune cells before returning to the circulation, ensuring that tissue pathogens are removed.

Lymphoreticular system

The lymphoreticular system produces immune cells and removes senescent cells. Primary (or central) lymphoid tissues can also be referred to as primary lymphoid organs. Maturation of lymphocytes and lymphopoiesis occurs in the primary lymphoid tissues, with different tissues responsible for maturing different types of lymphocyte.

The primary lymphoid tissues are:

- Bone marrow
- The Bursa of Fabricius
- The Thymus
- The Foetal Liver

Secondary (or peripheral) lymphoid tissues or secondary lymphoid organs provide a site for immune responses to occur and are populated by relatively mature T cells and B cells, macrophages and dendritic cells; each tissue seems to be preferentially populated by lymphocyte types that specialise in the antigens that are most likely to be presented at that site.

The secondary lymphoid tissues are:

- The Lymph nodes
- The Spleen

- Mucosal Associated Lymphoid Tissue or MALT
- The Tonsils
- The Appendix/caecal pouch
- The Ileal Peyer's Patch
- Regional lymphoid tissue

Functions

The lymphatic system has three functions:

Immune defence:

This is a broad topic area.Immune functions are covered broadly under the primary and secondary lymphoid tissuesof the lymphoreticular system with further information under the immunology section.

Removal of interstitial fluid from tissue: Details can be found at lymphatic vessel function.

1. Transport of fat: Chylomicrons transport digested lipids, however these are too large to enter the blood stream directly and thus enter the lymphatic system before being released into the blood stream. Further details can be found at Triacylglycerol Digestion and Absorption.

Primary (or central) lymphoid tissues can also be referred to as lymphoid organs. Maturation of lymphocytes (lymphopoiesis) occurs in the primary lymphoid tissues, with different tissues responsible for maturing different types of lymphocyte.

The primary lymphoid tissues

Bone marrow:

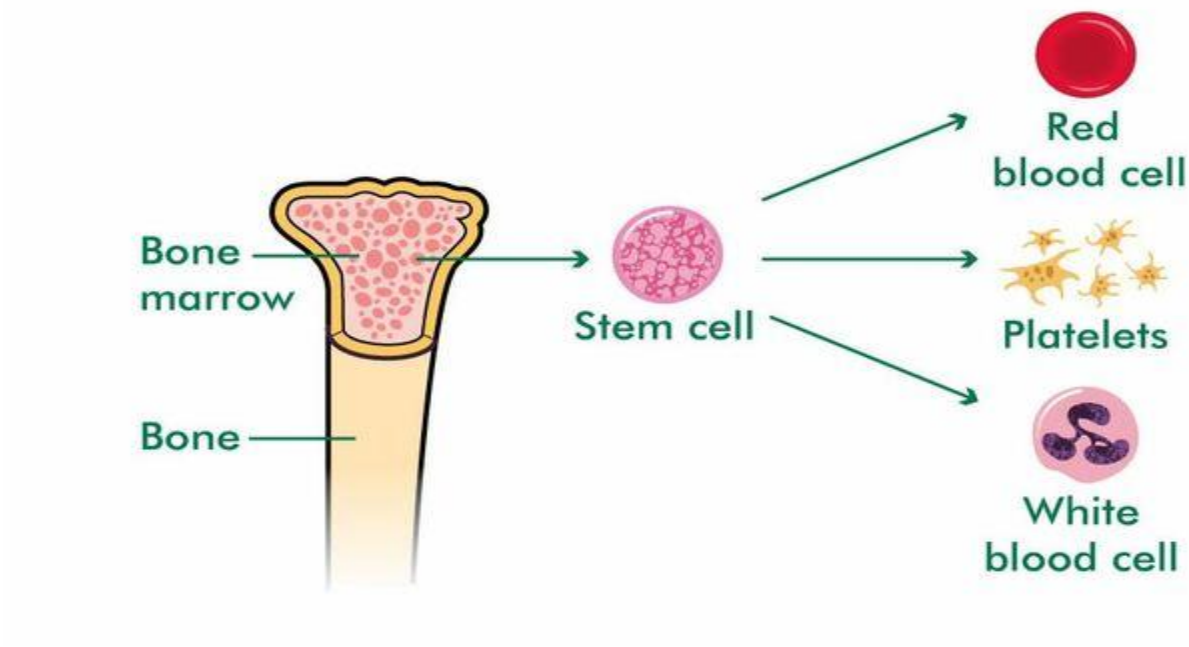

Figure 2.1

Bone marrow is referred to as red or yellow. Red bone marrow is involved with haemostasis while yellow bone marrow is adipose tissue. Bone marrow occupies the cavities in long bones and spaces in spongy bones.

Development

Pluripotential haematopoietic stem cells (PPSC) migrate into bones from the embryonic yolk sac and the foetal liver during development, a process called homing. The cells then associate closely with the connective tissues within the bone. The PPSCs continue to constantly divide in the bone marrow with one daughter cell remaining a pluripotential stem cell and the other daughter cells developing into multipotential haematopoietic stem cells. The multipotential stem cells also constantly divide with some daughter cells remaining stem cells and the other daughter cells developing into blood cells (haematopoiesis).

Red marrow

Red bone marrow consists of blood vessels, sinusoids and a network of haematopoietic cells. Sinusoids are vascular components with an endothelial layer, basal lamina and an outer adventitial cell layer. The adventitia cells are also called reticular cells and these extend into the haematopoietic cells in sheets to provide structural support. They also produce reticular fibres and cytokines to help stimulate blood cell production. Histological sections show that the haematopoietic cells lie in cords. The cells in these cords form many different blood cell types but cells producing one cell type tend to be located in groups along the cords.

Yellow marrow

In young animals the majority of marrow is red. However as the animal matures into an adult significant portions of the haematopoietic tissues is replaced by adipose tissue. In adults all of the marrow in the long bones is adipose tissue and significant portions of marrow in haematopoietically active bones is adipose tissue as well.

Functions

Functions refer to red marrow

Haematopoietic

The haematopoietic cells produce the vast majority of blood cells in the body (haematopoiesis). In young animals this occurs in most bones in the body but in mature adults this is limited to membranous bones in the body.

To enter circulation newly formed cells press against the sinusoid wall, temporally fusing to it and creating an opening. The cell then passes directly into the circulation and the membrane repairs itself. Mature erythrocytes immediately enter circulation, however the marrow stores leukocytes and consequently contains around ten times more leukocytes than found in circulation

Megakaryocytes reside alongside the sinusoid membrane but do not leave the tissue, rather they release their platelets and then withdraw from the membrane.

Lymphoid tissue

In some primates bone marrow acts as a primary lymphoid organ. Bone marrow is also a secondary lympoid tissue in other species.

It has little involvement in the primary immune response, but the migration of memory cells into the marrow from the spleen and lymph nodes means that during a subsequent exposure to an antigen it produces significant amounts of antibodies.

Others

Bone marrow is a significant source of antibodies as a large population of antibody-producing cells (plasma cells) reside there. Macrophages and dendritic cells in the marrow remove foreign substance from the blood, a process which also occurs in the lymph nodes, spleen and liver.

Bursa of Fabricius;

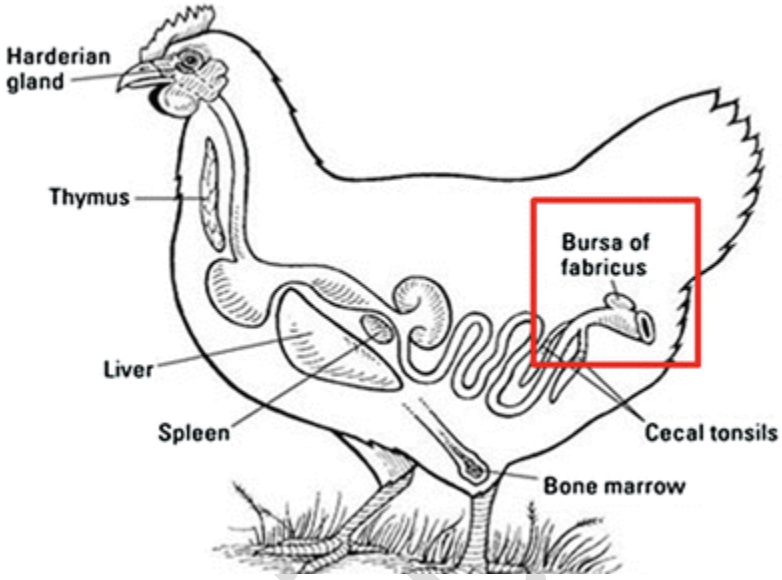

Figure 2.2

It is a primary lymphoid organ found in birds. The bursa was the first place that a certain subset of lymphocytes was observed and consequently they were named B lymphocytes (bursa of Fabricius or bursa equivalent organs). The bursa is involved in the differentiation of B lymphocytes.

Development

Lymphoid precursors migrate into the developing bursa during the first few weeks of embryo development. The bursa continues to grow through development and in chickens the bursa reaches its maximum size by six weeks. After this it slowly regresses (involutes) until only a small remnant is present in the adult.

Structure

The bursa is a round out-pouching of the cloaca. It is found on the caudodorsal surface of the cloaca (in the proctodeum) cranial to the dorsal proctodeal gland. The bursa consists of a number of lymphoid lobules and crypt-like folds surrounding a lumen, which is enclosed in a thin layer of stratified squamous epithelium. The lumen opens into the proctodeum.

Like the thymus the lobules have a cortex and medulla and the lymphocytes are supported by epithelial cells. B lymphocytes developing from the lymphoid precursors gather in the cortex, hence the cortex stains stronger than the medulla.

Histology

Functions

The bursa's primary function is maturating and causing the differentiation of B lymphocytes, it also produces the hormone bursin which activates B lymphocytes.

The bursa has some T lymphocytes present near to its opening into the proctodeum where they survey antigens.

Thymus:

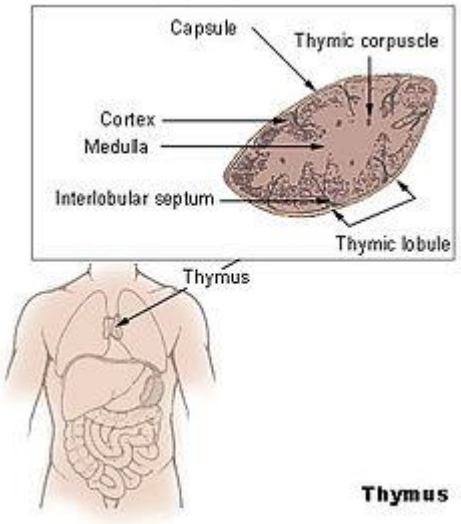

Figure 2.3

The thymus has a key role in the maturation of prothymocytes into mature T cells. In juvenile animals the thymus produces significant numbers of new T lymphocytes but as the animal matures this production decreases and T cell population is maintained by division of mature T cells.

Embryology

The thymus mainly develops from the left and right third pharyngeal pouches and extends caudally until it fuses with the pericardium. Then as the heart migrates into the thoracic cavity the thymus comes with it. At this point the thymus is Y-shaped and this shape persists in neonatal ruminants, in horses the left-right connection is lost leaving two separate lobes while in carnivores only the thoracic part remains and the cranial portion regresses.

During development some cells (CFU-L's) migrate from the bone marrow to the thymus and eventually become concentrated around the outside of the thymus, producing a dense layer of cells (the cortex) and giving rise to the inner medulla. Some of the cells in the medulla surround and form layers around enlarged endodermal cells and these give rise to the Hassall's corpuscles.

Structure

The thymus is found cranial to the heart and has a 'lobular' strucutre. Within the lobules the tissue consists of an outer cortex and an inner medulla.

General structure

The thymus is surrounded by a capsule made of thin connective tissue. Trabeculae from this extend into the thymus creating thymic 'lobules'. The connective tissues house blood vessels and efferent lymphatic vessels along with nerves.

The cortex is dense consisting of rapidly dividing thymocytes (developing T lymphocytes) which stain with H&E to give a basophilic appearance. The thymocytes are supported in a network of epithelioreticular cells ('epithelio' due to similarities with epithelium and 'reticular'

due to similarity with reticular cells in lymph tissues). Small numbers of dendritic epithelial cells are also present.

There are six types of epithelioreticular cells. Types I-III are found in the cortex and types IV-VI in the medulla. Type VI forming Hassall's (or thymic) corpuscles.

The medulla has non-dividing, more mature T cells with dendritic cells at the cortico-medullary junction. The density of thymocytes is much less than the cortex and consequently it does not stain as strongly. The cytoplasm in Hassall's corpuscles contains keratohyalin granules and intermediate fibres and may be keratinised. Function is yet to be asserted but thought to produce IL-4 and IL-7 to help with T lymphocyte development.

Blood-thymus barrier

The blood-thymus barrier protects the T lymphocytes from exposure to antigens in the blood. It is formed in the capillaries by a continuous endothelium with occluding junctions surrounded by connective tissue and then surrounded by a second layer formed from the processes of epithelioreticular cells (Type I).

Macrophages are also present around the capillaries to engulf any antigenic substances that manage to penetrate the barrier.

Changes

The thymus remains a sizeable organ until the animal reaches puberty. Once puberty is reached the lymphoid tissue is replaced with adipose tissue (involution).

Histology

Functions

The thymus has a key role in the maturation of prothymocytes into mature T cells. In juvenile animals the thymus produces significant numbers of new T lymphocytes, but as the animal matures this production decreases and the T cell population is maintained by division of mature T cells.

Endocrine

Although the Thymus shrinks after puberty, it continues to function as an endocrine gland during adulthood. It produces the hormone thymosin which stimulates the activity of the T lymphocytes.

Secondary (or peripheral) lymphoid tissues can also be referred to as lymphoid organs. They provide a site for immune responses to occur and are populated by relatively mature T cells and B cells, macrophages and dendritic cells with each tissue providing a different environment.

LYMPH NODE

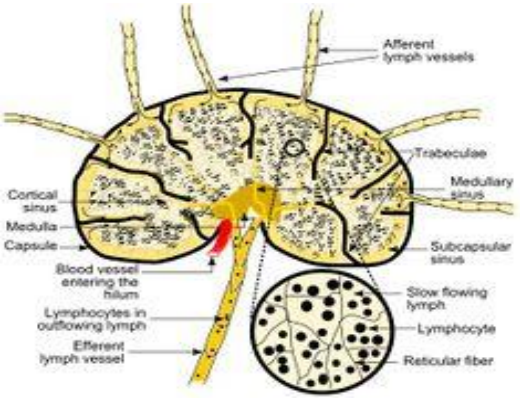

Figure 2.4

Part of the lymphatic system, the body contains hundreds of lymph nodes of varying size (1-20mm) and these are located along the routes of lymphatic vessels. They are found throughout the body but are more concentrated in the axilla, groin and mesenteries. Lymph nodes act as a filter for the lymph removing antigens and releasing immune-competent cells and immunoglobulins.

Development

Lymph nodes develop from lateral plate mesoderm in paired sacs from lymphatic vessels. These sacs undergo remodelling, and endothelial and mesenchymal outgrowths form the meshwork of channels and spaces that produces the cortex-medulla structure. Lymphocytes then populate the cortex and medulla. The subcapular sinus is a remainder of the lymphatic vessel.

Structure

General

Grossly the lymph nodes are round or bean shaped and have an outer cortex and an inner medulla. Microscopically the nodes have follicles, paracortical zones and medullary cords and sinuses. At the hilum the medulla is present on the outer part of the node. Lymph nodes are located in series with lymphatic vessels. Afferent vessels enter the node on its convex side and efferent vessels exit on its concave side.

Capsule and reticular framework

The nodes are surrounded by a fibrous capsule that extends into the node as trabeculae, which provide an overall framework. Below the capsule is the subcapsular sinus. The nodes' parenchyma contain a fine network of reticular fibres and reticular cells. Reticular cells provide "scaffolding" for other cells as well as expressing surface complexes and substance to attract T cells, B cells and dendritic cells. The cortex has aggregations of B cells (in the follicles) in its outer region and a paracortex consisting of a rim of T cells surrounding these follicles. Dendritic cells are also found in close association with the T cells. The medulla contains medullary cords of cells (B cells, plasma cells and some macrophages) and between these cords is the medullary sinus lined with endothelial cells and macrophages.

Sinuses

Three sinuses are present:

- Subcapsular/cortical
- Where afferent vessels drain

- Trabecular
- Drain lymph from subcapsular to medullary sinuses
- Medullary

Antigens and transformed cells that pass through the sinuses are filtered by macrophages and removed from the lymph.

Follicles

Lymph nodes have two types of follicles, primary and secondary. Secondary follicles contain germinal centres (sites of B-cell proliferation) and have three layers.

- The central dark zone contains a high density of dividing centroblasts, B cells without surface Ig. These centroblasts migrate to the Basal light zone.
- In the basal light zone the B cells express surface Ig and become exposed to the follicular dendritic cells.
- Here there is a high rate of apoptosis but surviving cells migrate to the apical light zone.
- Apical light zone (mantle zone) which contains cells which are destined to become B memory (lymphoblasts) or plasma cells (plasmablasts).

Follicles in the cortex of a stimulated node are larger and have a pale germinal centre. Activated B cells differentiate into plasma and memory cells. Plasma cells migrate to the medullary cords and produce immunoglobulins.

High Endothelial Venules

High endothelial venules (HEV) are composed of cuboidal/columnar epithelium and are the major route for lymphocytes to enter the lymph node. HEV contain a large number of aquaporin-1 channels allowing for a large uptake of water which in turn drives lymph flow through the cortex. This fluid is then returned directly to the bloodstream. The venules are the source of most of the node's T cells and B cells and express selectins (receptors for lymphocytes primed with antigens).

HEV express CD34 and GlyCAM-1 which bind to L-selectin on naive lymphocytes. This allows circulating lymphocytes to recognise when they have reached a secondary lymphoid organ stimulating them to leave the bloodstream and enter the lymphatic tissue.

Pig Lymph Node

As well as dolphins, hippopotamuses and rhinoceroses, the structure of the pig lymph node is inverted compared with that of most mammals.

- Most follicles are found deep in the paracortex.
- The paracortex is surrounded by loose medullary tissue
- Afferent lymphatics enter at the hilus.
- Connect with para-trabecular sinuses and exit from efferent lymphatics on the node surface.
- Blood vessels enter and leave at the hilus

Functions

The lymph nodes are secondary lymphoid tissue, and as the spleen removes antigens from the blood, lymph nodes remove antigens from tissue/lymph. Antigen presenting cells (B cells and T cells) migrate from peripheral tissue via afferent lymphatic vessels to the lymph nodes where they present their antigen to lymphocytes. B cells and T cells enter via the high

endothelial venules by diapedesis and B cells migrate to the cortex while T cells to the deep cortex.
Antibodies and immunologically competent cells leave the lymph nodes via the efferent lymphatics.

Spleen

The spleen is a major lymphoid and blood filtration organ and is located in the left cranial abdomen. It is responsible for storing and removing erythrocytes from the blood as well as antigen surveillance of the blood and antibody production.

Development

The spleen develops in association with the digestive system in the dorsal mesogastrium, and as the stomach rotates during development the spleen comes to occupy the left cranial abdomen. Haematopoietic cells in the spleen are derived from the AGM (aorta-gonad-mesonephros) and yolk sac and as the primary lymphoid organs become established it becomes populated with T and B lymphocytes.

Structure

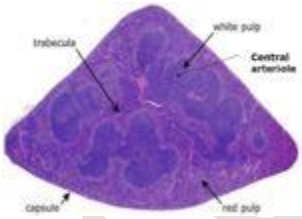

Figure 2.5

Histological section

The spleen lies vertically on the left side of the cranial abdomen. It is attached to the greater curvature of the stomach by the gastrosplenic ligament. The spleen is enclosed in a capsule of fibrous and elastic tissue that extends into the parenchyma as trabeculae.

The parenchyma is supported by a fine mesh of reticular fibres and is divided into two types of tissue, the red and the white pulp, which are separated by the marginal sinus.

Red Pulp

The red pulp makes up the majority of the spleen and is composed of a network of cell cords in series with vascular sinuses. The splenic cords contain macrophages, plasma cells, lymphocytes and other mature blood cells e.g. granulocytes and erythrocytes. While the vascular sinuses are wide vascular channels lined with endothelial cells. Blood cells and fluid can pass into the splenic cords through fenestrations in the sinus walls.

White Pulp

White pulp is organised in relation to the splenic arterioles and consists of discrete lymphoid tissue surrounding a central arteriole. There is a sheath of T cells directly around the arteriole, the periarteriolar lymphoid sheath (PALS), which is surrounded by a marginal sinus, and then a zone of B cells and macrophages (the marginal zone). B cell follicles are associated with the marginal zone and expand and develop germinal centres after antigen activation. The marginal sinuses are linked to the red pulp sinuses.White pulp stains basophilic in a H&E stain

Species Differences

The capsule and trabeculae are much more muscular in carnivores and horses than ruminants

Carnivores:
It is elongated and dumb-bell shaped (larger ventrally)
Ruminants:
It is flat and oblong shaped

Horses:
Lies under the last three ribs. Dorsally it is broad but narrows as it extends cranially and ventrally on rectal palpation it is located against the body wall and feels smooth with a sharp border.

Pigs:
Elongated and strap-like under the last few ribs

Birds (Picture here):
Lies alongside, to the right, of the proventriculus and is found caudodorsally to the liverSpherical in chickens, triangular in ducks & oval in pigeons

Vasculature
The splenic artery, a branch of the celiac artery, supplies the spleen. The artery branches into arterioles and capillaries, which may either:

- Connect with the venous sinuses, or
- Terminate with open ends in the splenic cords

Blood released into the splenic cords, either from the sinuses or capillaries, eventually filters back into the sinus network. The sinuses converge and empty into trabecular veins, which then merge into a single splenic vein which then empties into the portal vein.

Lymphocytes in the arterial blood migrate from the red pulp sinuses, through the splenic cords and through the white pulp. T cells specifically migrate through the PALS and B cells specifically migrate through the follicles. Antigen in the blood is filtered by the large numbers of macrophages in the splenic cords and white pulp.

Species Differences
The splenic artery:

- Passes through the spleen without dividing in ruminants
- Branches regularly as it passes through the spleen in horses and pigs
- Branches before it reaches the spleen in dogs and cats

Innervation
Innervation is purely sympathetic and nerve fibres travel with the artery into the spleen.

Functions
The spleen has a number of functions:

- It filters the blood removing ageing erythrocytes and antigens
- It stores erythrocytes and platelets
- Secondary lymphoid organ

Erythrocytes & Platelets

In the foetus the spleen also has a role in haematopoiesis when it becomes the main erythrocyte producing organ during the haematopoietic transitional phase.

In the developed animal the red pulp is involved in the removal of aged, damaged or abnormal erythrocytes (along with the liver and bone marrow). As erythrocytes age they become less supple and this causes them to become damaged when they pass through the very narrow capillaries of the spleen, after which they are phagocytised by splenic macrophages. If a splenectomy is performed the number of aged erythrocytes in circulation increases.

The red pulp also acts as a storage site for erythrocytes. The degree of storage is variable between species but is particularly notable in horses which, during exercise under sympathetic activity, can contract their spleen to increase the concentration of circulating erythrocytes. In some species such as cats and rodents the red pulp acts as a storage site for platelets and contains megakaryocytes.

Lymphoid

Blood flows through the marginal sinus. This means that most antigens present in the blood come into contact with the B lymphocytes and dendritic cells in the spleen. Dendritic cells in the marginal sinus and red pulp take up antigens from the blood and transport them to the primary follicles in the white pulp. If the antigen activates the B lymphocytes then a germinal centre will form in the primary follicle and this is called a splenic nodule. Antibody producing cells then migrate to the red pulp and marginal zone.

Mucosal associated lymphoid tissue (MALT)

Mucosal associated lymphoid tissue (MALT) covers lymphoid tissues associated with the mucosal surfaces of alimentary, respiratory, urinary and reproductive tracts. Due to the extent of these surfaces the mucosal lymphoid tissue contains as many lymphocytes as the rest of body. The MALT is strategically located to intercept pathogens before they enter the body.

Development

γδ T cells migrate to these tissues during foetal development from the thymus.

Structure

The MALT is found in lamina propria and is non-encapsulated lymphoid tissue. In the alimentary and respiratory tract it is more specifically called:

Gut associated lymphoid tissue (GALT)

Including the Peyer's patches, tonsils, appendix, and lymphoid follicles in large intestine and rectum

Bronchial associated lymphoid tissue (BALT)

Located at the bronchial bifurcations under non-ciliated epithelium

Lymphatic nodules are present in the mucosal surfaces and are more readily defined than other more diffuse lymphoid tissue of the MALT but are still not encapsulated. They exist with small primary nodule or generally as secondary nodules containing germinal centres. The nodules are generally found in the tonsils, Peyer's patches and appendix and become enlarged when responding to an antigen.

Functions

As with other secondary lymphoid tissue the role of the MALT is to mount an immune response to pathogenic antigens. Cells in the MALT sample antigens at the mucosal surface and then migrate to regional lymph nodes where they divide and differentiate before returning to the mucosal surface as effector cells.

In the GALT TGF-β is produced and this causes the B cells to produce IgA.
Present in rabbits, the appendix is a primary lymphoid tissue located at the ileocaecal junction and is part of the mucosal-associated lymphoid tissues (MALT) and more specifically the gut-associated lymphoid tissue (GALT).

Development

Lymphatic tissue develops during early life reaching maximum size during early adulthood. Some regression (involution) occurs as the animal ages but is not complete.

Structure

The appendix is a blind ending out pouching of the caeca at the ileocaecal junction in the intestines. The lamina propria of the appendix contains a high number of lymphocytes as well as a number of lymphatic nodules.

Function

The appendix is a bursa equivalent organ and consequently has functions associated with maturation and differentiation of B lymphocytes.

Peyer's patches

Peyer's patches are lymphoid tissues found in the wall of the small intestine. They are part of the mucosal-associated lymphoid tissues (MALT) and more specifically the gut-associated lymphoid tissue (GALT). Although nodules of lymphatic tissue are found throughout the intestines in the small intestine larger collections of nodules exist and these are referred to as Peyer's patches. In many species they act as a primary lymphoid tissue (cattle, sheep , pigs, horses, dogs and rabbits).

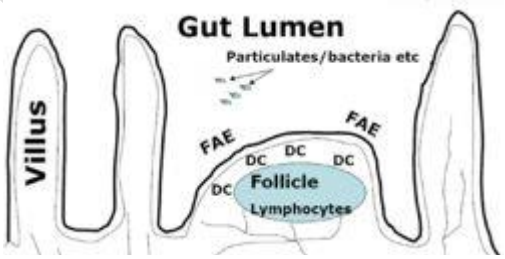

Figure 2.6

Development

In cattle, sheep , pigs, horses and dogs over eighty percent of the patches are found in the ileum where they form a continuous structure which is most developed before birth and regresses to the point that in the adult they cannot be detected. The rest of the patches are found in the jejunum and are isolated from each other, however these patches last throughout adult life.

In rabbits and rodents the patches are randomly located along both the ileum and jejunum and persist throughout life.

Structure

Peyer's patches are located in the lamina propria and submucosa of small intestine and may be distinguishable by the lack of villi covering them. The patches are regions of concentrated B lymphocyte follicles covered in a 'dome' of a specialised follicle associated epithelium (FAE) which consists of follicle associated enterocytes and M (microfold or multifold) cells.

M cells

M cells transport antigens from the intestinal lumen to the lymphocytes. Their luminal surface is folded and takes up antigens from the intestine via endocytosis and transports them to the extracellular space on their basal surface where the antigen is processed by antigen presenting cells.

Sometimes the term tonsil is used to refer to all mucosal associated lymphoid tissue (MALT). It will specifically deals with tonsils located in the nasopharynx & oropharynx.

The tonsils are part of the MALT and more specifically the GALT. They are located in the nasopharynx and oropharynx and form a ring of lymphoid tissue around the pharynx to protect the openings into the alimentary and respiratory systems.

Structure

The tonsils are non-encapsulated lymphoid tissue. They have crypts lined with stratified squamous epithelium which are infiltrated with lymphocytes.Species being covered here are dog, cat, horse, cattle and pig.Pharyngealalso called adenoids present in all the species they are located on the pharyngeal septum in the nasopharynx. In cattle it is located on the caudal end of the septum.

Soft palate

It is present in all the species but are the principal tonsils in the pig. Found on the ventral aspect of the soft palate (roof of oropharynx).

Tubal

It is absent in the cat and dog. They are located in the lateral wall of the nasopharynx and provide protection to the entrance of the auditory tubes. Compact in cattle and pigs and diffuse in horses.

Lingual

It is found in varying degrees in all species on floor of oropharynx.

Paraepiglottic

It is present in the pig and are found rostrolateral to epiglottis base.

Functions

The tonsils are a secondary lymphoid tissue and B cells in the tonsils become committed to synthesise IgA.

Cells of Immune System

Lymphocytes

Lymphocytes account for around a third of all circulating leukocytes and are formed in a variety of lymphoid tissues. They are functionally divided into T cells, B cells and Natural Killer (NK) cells. Lymphocytes vary in size (6-30µm) and are classified as small, medium or large. Large cells are either activated lymphocytes or NK cells. The vast majority of circulating lymphocytes are small and of a similar size to erythrocytes. Histologically they are round with a large densely staining nucleus and a thin, often indistinct, rim of cytoplasm. While NK cells can be distinguished by their large granules and kidney shaped nucleus, B and T cells appear the same histologically.

Lymphocytes, along with the associated supporting cells, form the immune system and recognise antigens, produce antibodies and destroy pathogens.

Development

Both T and B lymphocytes develop from a common stem cell (CFU-L's) during lymphopoiesis. Where they mature accounts for the letter the lymphocytes are given i.e. B cells in the Bone Marrow and T cells in the Thymus. Natural Killer cells develop in the Bone Marrow.

Lymphocyte Surveillance

About two thirds of lymphocytes (all immunocompetent) are circulating in the blood and lymph systems. Most of these are T cells and long lived. In the lymphatic system they survey tissues.

High endothelial venules (HEV) facilitate lymphocyte access to the lymph nodes from the bloodstream. Once inside the lymph node, the naive lymphocytes search for antigens. If there are no antigens present, the naive lymphocytes leave via the efferent lymphatic vessel and return back to the bloodstream. Each lymphocyte can search several secondary lymphoid organs each day. This process is called surveillance.If a naive lymphocyte recognises an antigen then it differentiates into its adult (mature) form. Interdigitating dendritic cells present antigen to T cells and follicular dendritic cells present antigen to B cells.B cells proliferate into plasma cells within sectors of the lymph nodes known as germinal centres, producing antibody.

T cells leave the lymph node in attack mode to locate the infectious organism. The surface molecule L-selectin (which allows the naive lymphocyte to enter the lymph node via an HEV) is replaced by the adhesion molecule VLA-4. At the site of inflammation, the VLA-4 receptor recognises VCAM-1 on endothelial cells and the T cell enters the site of disease. $CD4^+$ T cells search for infected macrophages and $CD8^+$ T cells look for virus infected cells creating an immune response. After the infection has been defeated, memory cells develop which express L-selectin (rather than VLA-4) and continue to search the body in surveillance mode in case the host is re-infected with the disease producing organism.

B cells

It is also known as B lymphocytes so named as they were initially found in the Bursa of Fabricius, B cells produce antibodies and are associated with humoral immunity (T cells are part of the cell-mediated immune response), and are an integral part of the adaptive immune system. They represent 20-30% of circulating lymphocytes.

B cells have cell surface proteins known as B cell receptors (BCRs) that are known as immunoglobulins; IgM is the membrane bound BCR that is expressed when the B cell is

immature, changing to IgD when the cell is mature. IgM has a large molecular mass and can bind up to 10 antigens simultaneously. B cells also express MHC II, CD9, CD,19, CD20 and CD24.Under antigenic stimulation B cells differentiate into plasma cells and memory cells.B-cells also act as Antigen-Presenting Cells (APCs) by presenting digested fragments to T cells on MHC II.

B cell differentiation

Mature B cells that undergo stimulation by an antigen undergo class switching, and differentiate into either plasma or memory cells.In the paracortex region of the lymph node binding to MHC II in the presence of IL-4 produced by the $CD4^+$ T cells (T_H2 type) causes the B cells to differentiate; most will become plasma cells, however a small number will become memory cells.Follicular dendritic cells present in the germinal centers of peripheral lymphoid organs can absorb intact antigen onto their surface to present to B cells to stimulate differentiation.

Plasma cells

Appearance

Plasma cells are oval, around 9µm and have a round prominent nucleus. The cytoplasm is extensive and strongly basophilic when stained. It contains large amounts of rough endoplasmic reticulum and the Golgi apparatus is large and appears as a clear crescent-shaped structure near the nucleus. Some plasma cells, known as "Mott cells", accumulate considerable quantities of, perhaps abnormal, antibody and this appears as a large eosinophilic blob filling the cytoplasm and displacing the nucleus to one side. These blobs are called "Russell Bodies".

Function

Plasma cells produce immunoglobulins/antibodies (thousands a second). The immunoglobulin binding specificity is identical to the binding specificity of the B Cell receptor (BCR) on the B cell that the cell has differentiated from. This means that when a B cell has a BCR that can effectively bind to an antigen the immunoglobulins produced by the plasma cell can bind to that antigen.Although they can live for months most plasma cells only live for a few days and do not replicate in this time.

Interaction of a B-cell with antigen results in clonal expansion, as does activation by T-cells. The majority of B-cell clones mature into plasma cells. Plasma cells are found in the splenic red pulp, lymph node medulla and bone marrow. Plasma cells are the terminal differentiation state of B-cells. They migrate to the medullary cords where their sole function is to secrete antibody.

Class switching

Initially plasma cell produce Immunoglobulin M (IgM) however this is not always the most appropriate Ig to be produced and therefore stimulation by T cells and interleukins causes the plasma cells to undergo class switching to produce different classes of Immunoglobulins.

- In mucosal B cells plasma cells CD40 interaction (with T_H2 CD40L) and Il-10 stimulates class switching to IgA
- Eosinophils produce Il-13 which promotes class switching to IgE

Plasma cells produced in the first immune response to an antigen are mainly of the class IgM whereas those produced from memory cells in the second immune response are mainly of

the IgG class.T-Cell Dependent and Independent Responsesare of two types of B cell response to antigen; one is dependant and the other independant of T cell interaction.

T-Cell Independent Response

B cells are activated in this instance by non-protinaceous antigens such aslipopolysaccharides which act as mitogens for the B cells by stimulating cell division. These antigens only stimulate primary immune responses (production of IgM) and do not produce memory cells. Initial and subsequent exposure to an antigen stimulates an identical response from the B cell.

T-Cell Dependent Response

The B cell response is to protinaceous antigens and T cells moderate the response, which occurs in the germinal centres of follicles in secondary lymphoid tissues.
B cells then act as antigen presenting cells on MHC II, with T cells interacting via cell surface receptors:

- CD40L on the T cell
- CD40 on the B cell

T cells produce cytokines such as IL-4 which induces class switching from IgM and the formation of memory cells.

Primary T Cell Dependent Response

The first exposure of an individual to a particular antigen is referred to as priming. The measurable antibody response is called the primary immune response. The delay of 5-7 days before antibody is produced is called the Lag Phase during which time the B cells undergo clonal expansion and form plasma cells. IgM antibody is produced first and will begin to appear in the blood; this stage is called the Log Phase.

The log phase will peak after about 10-14 days and the Plateau Phase will then occur. Class switching occurs replacing decreasing levels of IgM with IgG. Antibody levels then begin to decline as plasma cells undergo apoptosis.

After primary immunisation, it usually takes around 7-10 days for a measurable antibody response to become detectable. This latent period is the time necessary for the antigen to contact specific cells, for the cells involved to interact and expand, and for plasma cells to secrete antibody.During the primary response, IgG production lags about a week behind that of IgM.

Secondary T Cell Dependent Response

The production of antibody to any antigen ceases within a few weeks of immunisation as the antigen disappears from the body; However, the animal retains immunological memory of the antigen which occurs after the primary response - it is seen on the second and subsequent exposures to the same antigen. In effect, there is an expanded pool of memory B and T cells from the first exposure to the particular antigen which permits a shorter lag phase and a longer plateau phase as antibody persists for an extended time. There is a higher antibody titre overall due to cloanal expansion and Long-lived memory cells which are qualitatively distinct are produced. Antigen is retained within the immune system on the surface of follicular dendritic cells and this can stimulate the immune system for years.

The level of IgM antibody production in the secondary response is similar to the primary response; the increase in antibody production occurs with IgG.

Plasma Cell Pathology

If many plasma cells are present in blood, it is due to a plasma cell tumour that has begun to metastasise.

Memory cells

The differentiated B cells that remain in the cortex become memory cells and these proliferate and form germinal centres in the lymph node.

Memory cells are long lived and responsible for long term immunity providing the immune system with a memory of previously encountered antigens. When they experience an antigen again they proliferate and differentiate into plasma cells. This response produces up to ten times more plasma cells than the original exposure to the antigen and is why the second immune response to an antigen is both more rapid and much stronger than the first response.

T Cells

T cells are so named as they differentiate in the thymus. They are long lived and are involved in cell mediated immunity. They represent 60-80% of the circulating lymphocytes and all express the markers CD2, CD3 and CD7 as well as having T cell receptors (TCR). Each T cell has 30,000 TCRs each of which is identical and recognises antigens and major histocompatability complex (MHC) II.

Functionally they are divided into three subsets that are distinguished by the presence or absence of CD4 or CD8 markers. CD4 and CD8 cells have α/β antigen receptors while the γδ cells have the γ/δ antigens receptors.

T-cell receptors:

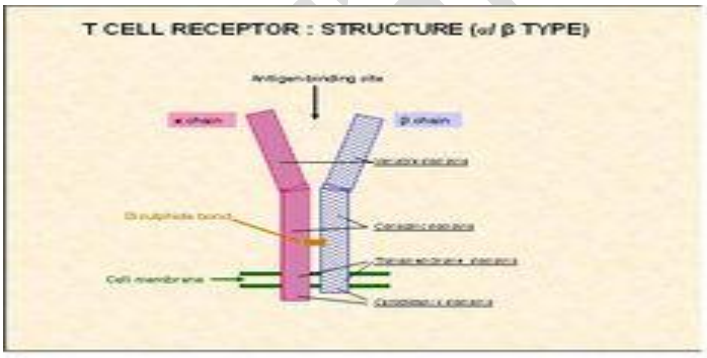

Figure 2.7

T-cell receptors are the antigen-specific receptors for T-lymphocytes, and are a combination of either α/β chains or γ/δ chains; one T-cell will express either α/β or γ/δ TCRs. The antigen-binding site of the TCR is produced by a combination of the V domains of the relevant chain. Like antibody, the specificity of the TCR is determined by the amino acid composition of the variable domains.

TCRs are linked to the cell membrane; structurally, they look like a single arm of an antibody molecule consisting of a distal domain and a membrane proximal constant domain

Helper $CD4^+$

These T cells express the marker CD4 and are categorised into three groups, T_H1 and T_H2, with a third lineage, T_H17 being recently described. These lineages are distinguished by the cytokines they produce. T helper cells recognise antigens bound to MHC II complexes.

- T_H1 cells produce IL-2, IFN-γ and TNF-α
- T_H2 cells produce IL-4, IL-5, IL-10 and IL-13
- T_H17 cells produce IL-17, IL-17F, IL-21

T_H1 cells interact with $CD8^+$, NK and dendritic cells and T_H2 cells interact with B cells. T_H1 cells are involved with the control of intracellular pathogens and T_H2 cells extracellular pathogens. IL-2 produced by T_H1 cells stimulates further proliferation of $CD4^+$ cells. T_H17 cells are produced to enhance innate immunity, with the cytokines produced increasing the extravasation of neutrophils.

T_H0 populations are $CD4^+$ cells that have yet to differentiate into T_H1, T_H2 or T_H17 cells and they secrete IL-2, IL-4, IL-5, IFN-γ. In the presence of IL-4 they develop into T_H2 cells while in the presence of IL-2 they develop into T_H1 cells. In the abscence of IL-2 T_H1 cells will change into T_H2 cells. T_H17 cells develop in the presence of TGF-β and IL-6 and can further develop into T_H1 cells depending on various conditions.

T_H1 cells have two populations; one that secretes IFN-γ and is short lived, and the other that doesn't secrete IFN-γ and is long lived. The cells that do not secrete IFN-γ are termed memory T cells
T cell differentiation is a field of continual change, with discoveries made on a regular basis.

Cytotoxic $CD8^+$

These T cells express the marker CD8 and once fully mature seek and destroy target cells (infected or neoplastic cells). When the cytotoxic T cell recognises the MHC I complex on the target cell (MHC I binds to TCR) the T cell kills that cell -for example with a viral infection, viral peptides associate with MHC I and the $CD8^+$ T cell recognises this and binds to the infected cell. Only two pathways are used by cytotoxic T cells to kill cells:

- One pathway uses the CD95 (death receptor) which triggers apoptosis in the target cell (usually other T cells)
- The other pathway uses perforins and granzymes which form pores in the target cell membrane causing cell lysisPerforins are structurally related to complement factor C9.
- Granzymes are proteolytic enzymes that target cell nucleases and cause apoptosis

In both cases direct contact is required between the T cell and target cell, and cell killing can take several minutes.Cytotoxic T cells secrete a pattern of cytokines similar to that of T_H1 cells i.e. IFN-γ but not IL-2. IFN-γ shifts the balance of the immune response in favour of TH_1 cells giving an increased level of T cell proliferation. The initiation of the immune response via cytotoxic T cells leads to the selective proliferation of cytotoxic T cells enhancing the main mechanism of killing infected cells.γδ cellsInformation on these cells is varied.

They do not express CD4 or CD8 and have γδ antigen receptors rather than α/β like other T cells. They develop in the thymus and migrate to epithelial tissues where they remain. The number present in an individual varies greatly but is generally greatest in immature ruminants and pigs.

The cells can be divided into two subsets:

- One with restricted antigen binding, that act as first line defence against invading organisms and recognises antigens bound to MHC I complex
- The other subset doesn't require the MHC complex and this subset has two further divisions
  - One producing cytokines and chemokines (T_H1 and T_H2)
  - The other having cytotoxic effects.

T Cell Differentiation

Introduction

T cells are long lived and are involved in cell mediated immunity. Functionally they are divided by the expression of $CD4^+$ or $CD8^+$ markers. $CD4^+$ T helper cells recognise antigens bound to MHC II complexes and are involved with the control of intracellular and extracellular pathogens; they can interact with $CD8^+$, NK and dendritic cells or with B cells.

Cytotoxic $CD8^+$ T cells recognise the MHC I complex and destroy infected or neoplastic cells. Within the blood and lymphoid organs the majority of T cells are antigen-naive T cells; only a small proportion are memory T cells.

Naive T cells have yet to encounter antigen and can only be activated by antigen that is presented by dendritic cells.

After initial antigenic activation, naïve T-cells develop into an intermediate stage cell called the T_H0 cell which can then be activated by any antigen-presenting cell, e.g. Dendritic cells, macrophages or B cells.

The T_H0 cells have the capacity to differentiate into T_H1, T_H2 cells and a very recently described subtype T_H17 cells. The type of cell that develops depends on the antigen presenting cell type. Macrophages cause the T_H0 cell to develop into a T_H1 cell induced by IL-12 production following macrophage-antigen interaction.

B cells cause the T_H0 cell to develop into a T_H2 cell induced by IL-10 production following B cell-antigen interaction. On antigenic stimulation the T_H1 or T_H2 cells become activated, undergo clonal expansion and secrete a range of different cytokines. The third most recently described subset, T_H17, form in the presence of IL-6 and TGF-β which are produced in the prescence of infection, and by either of the Antigen Presenting Cells (APCs). The importance of $CD4^+$ T_H cells is very clear in immunity. An example of a disease that targets $CD4^+$ T cells is the Human Immunodeficieny Viruses (HIV) and Simian Immunodeficieny Viruses (SIV) which, when the $CD4^+$ T cells are overwhelmed, causes Advanced Immunodeficiency Syndrome (AIDS).For any one cell the cytokine-secreting activation state is short-lived, lasting between 4 - 40 hours. After this time these cells either die or mature into the long-lived memory cells. The proliferation of T cells continues until the presentation of antigen ceases.

Dendritic Cells

There are two different lineages of dendritic cells:

- From myeloid precursor cells
- From plasmacytoid precursor cells

Dendritic cells stimulate a primary T cell response; they migrate through tissues, track to T cell dependent areas of the lymph nodes and cluster with the T cells. Dendritic cells have unique capabilities to take up antigen by pathways involving phagocytosis, macropinocytosis and clathrin-coated pits. The cell-surface antigen phenotype distinguishes the dendritic cell from Monocytes/macrophages and B cells. Their main function is priming T helper cells. They produce cell signaling cytokine molecules known as chemokines.

Maturation signals

Exogenous

- Bacteria or their products (LPS, LTA, lipoproteins)
- Viruses or their products (dsRNA, G-RSV)
- Protozoa or their products
- Helminths (SEA, ES 62)

Endogenous

- Inflammatory mediators (IL-1/TNFα, hsp, FcR)
- Immune cells (CD40L, CD47, FasL)

Antigen Presentation

Circulating monocytes differentiate to form immature dendritic cells called Langerhans Cells

Langerhans cells sample the tissue fluid by endocytosis:

- Foreign organisms are internalised
- Within the dendritic cells, antigen is digested to peptides
- Some of the peptides formed bind to the cell's MHC molecules

The Langerhans cells leave the epithelium and travel via the afferent lymph flow. They are now known as Veiled Cells. Veiled cells enter the paracortical region of the lymph node where they present antigen to the T cells. They are now known as Interdigitating Dendritic Cells.

T_H1 Cells

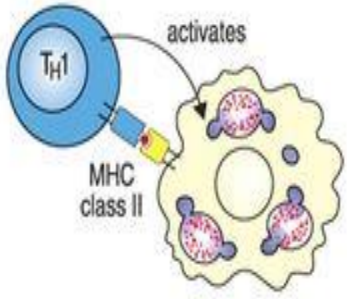

Figure 2.8

TH_1 cells help macrophages digest bacteria - the organisms are contained in cellular vesicles.

T_H1 cells secrete a range of cytokines, including:

- IL-2, which induces proliferation of both $CD4^+$ and $CD8^+$ T-cells. This stimulation of T cell proliferation is the main function of the T_H1 cell.
- Interferon gamma (IFNγ) which activates tissue macrophages and is the principal effector mechanism in the defence against intracellular bacteria and parasites such as Mycobacteria, Brucella, Rickettsia Leishmania, Coccidia, and Babesia. IFNγ activates macrophages and stimulates them to produce enzymes triggering intracellular killing mechanisms - specifically:

1. Superoxide dismutase and myeloperoxidase that produce H_2O_2 and trigger the "superoxide burst".
2. Nitric oxide synthase which produces nitric oxide.

This is another example of the immune system working through the innate immune response, and this can even act to suppress antibody synthesis.

T_H2 Cells

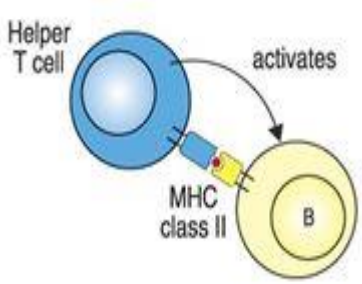

Figure 2.9

T_H2 cells help B cells produce antibody where the organism is present in tissue fluid. The T_H2 population influences B cell activation, proliferation and immunoglobulin production. T_H2 T cells also secrete a range of cytokines:

1. IL-4 which stimulates B cell growth and induces the heavy chain switch from IgM to IgG , IgA and IgE, as well as proliferation of basophils and mast cells. IL-4 can inhibit some T cell responses.
2. IL-5 which activates B cells and stimulates a high rate of proliferation. IL-5 also promotes immunoglobulin synthesis and the proliferation and differentiation of eosinophils.
3. IL-6 also activates B cells, stimulates a high rate of proliferation and promotes immunoglobulin synthesis.

Common Functions of T_H1 and T_H2 Cells

Both T_H1 and T_H2 cells produce IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF). These act to activate and induce proliferationof neutrophils and macrophages.

Neutrophils are the major phagocytic cells in the blood and the principal cells in acute inflammatory lesions whose function is chiefly the body's defence against extracellular bacteria. One of the major biological functions therefore of the activation of either T_H subset is cytokine-controlled reactive haematopoiesis.

T_H17 Cells

The T_H17 cells form when T_H0 cells are challenged with IL-6 and TGF-β to produce a number of cytokines that enhance the innate immune response.

The cytokines produced enhance the extravasation and chemotaxis of neutrophils to the site of infection, in the aim of combating extracellular bacteria. These cytokines include:

- IL-17
- IL-17F
- IL-6
- TNFα
- IL-21
- IL-22
- IL-23

They do not produce:

- IFNγ normally associated with T_H1 cells
- IL-4 normally associated with T_H2 cells

Cytotoxic T-Cells

Cytotoxic T cells kill virus infected cells where the organisms are contained in the cell cytoplasm. Viruses are obligate intracellular pathogens that use the host cell machinery for pathogen protein synthesis; viral peptides associate with MHC class I and are expressed on the cell surface. $CD8^+$ cytotoxic T lymphocytes (CTL) recognise the antigen-MHC complex. Cytotoxic

T-cells secrete a pattern of cytokines similar to that of TH_1 cells:

IFNγ but not IL-2. The IFNγ shifts the balance of the immune response in favour of TH_1 cells and there is therefore an increased level of T-cell proliferation. The initiation of the immune response via CTL leads to the selective proliferation of CTL which enhances the main mechanism of killing virally-infected cells.

Killing Mechanism

The CTl killing mechanism is initiated by direct CTL-target cell contact.

- The cells involved bind by antigen/MHC class I-TcR interaction. This allows the CTL's intracellular granules to be localised at the area of contact - the granules contain most of the molecules responsible for cytotoxicity.
- Direct cell contact stimulates the release of the granule contents into the area of contact between the two cells. The granules contain two groups of cytotoxic molecules.

1. Perforin, which is structurally related to the complement component, C9 and forms pores in the cell membrane.
2. Granzymes, which are proteolytic enzymes that target cell nucleases and cause programmed cell death.

T-Cell Activation

T cells function only after recent activation by an antigen.

- CD4 binds MHC class II - $CD4^+$ T-cells recognise antigen only in association with MHC class II.
- CD8 binds MHC class I - $CD8^+$ T-cells recognise antigen only in association with MHC class I.

Activation of T cells requires two distinct signals:

- Signal 1 is the interaction of the TcR with the antigenic peptide/MHC complex on the antigen presenting cell.
- Signal 2 is the interaction of CD28 on the T cells with its ligand, CD80, on the antigen-presenting cell (APC). APC expression of CD80 only occurs after the engagement of pattern recognition on Fc receptors or activation by the cytokines Interferon, IL-1β or TNFα.

Signal 2 only occurs after the recognition of DANGER.

Activation Scenarios

No signal 1:

T cell is not activated as there is no antigen.

Both signal 1 and signal 2

T cell is activated into clonal expansion and produces cytokines or becomes cytotoxic.

Signal 1 but no signal 2

T-cell is triggered into apoptosis and dies.

This is the basis of "clonal deletion" and is a major mechanism of the development of tolerance. It ensures that T-cells do not react with self (non-dangerous) antigens.

TCR Complex

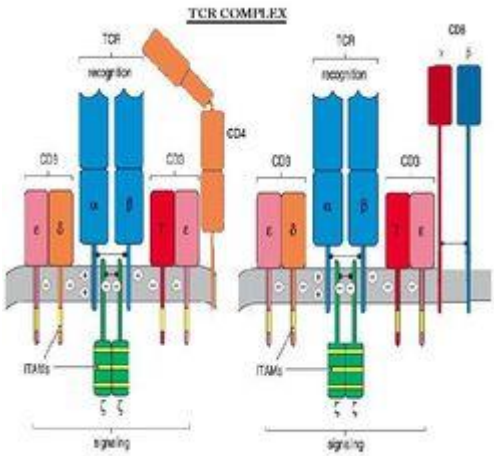

Figure 2.10

The T cell Receptor, or TCR is always associated with CD3, forming what is referred to as the TCR complex. TCR is expressed on the surface of T cells in a noncovalent association with a complex of transmembrane polypeptides.

CD3 contains 3 distinct polypeptide chains that are expressed exclusively on T cells: γ, ε, and δ. These molecules are members of the Ig superfamily - the ε chain associates with both γ and δ - and they play a 'chaperone' role in transporting newly synthesized TCR molecules to the cell surface. CD3 also contains 2 identical chains: ζ and 16 kDa, which are found on T cells, macrophages and NK cells. Mice also can have an ε (eta) form.

Response to Activation

The response of the T cells to obtaining Signals 1 and 2 is to express the receptor for the cytokine interleukin-2 (IL-2) and $CD4^+$ T-cells secrete IL-2. The final trigger for clonal expansion is the engagement of IL-2R with IL-2 from any activated $CD4^+$ T-cell. IL-2 produced by a $CD4^+$ cell may also stimulate clonal expansion of the $CD4^+$ cell.

T-Helper Cell Function

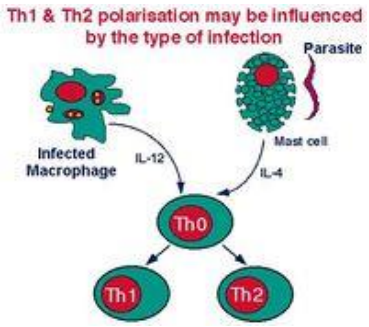

Figure 2.11

The function of T helper cells is to regulate the immune response. The cytokines they secrete exert their influence on other cell populations; most of the different effector cells of the immune system are affected by one or more of the cytokines secreted by T_H cells.

TH cells secrete cytokines for only a short period after they have been activated; the range of cytokines that T_H cells secrete after activation chiefly determines their function. Different T-helper cell subpopulations (T_H1, T_H2 and T_H17 cells) secrete different sets of cytokines.

NK Cells

NK cells can be classified as lymphocytes because they are capable of recognising antigen, however they are more often associated with the innate immune response. They target cells by monitoring MHC production, which is expressed by healthy cells to present antigen to T-cells. Low MHC levels can be used as a marker for a cell whose machinery is compromised by a replicating virus. When MHC levels drop, it acts as a danger signal to the NK cells, which then release enzymes to kill the infected cells.

NK cells do not develop in the thymus and represent 5-10% of the circulating lymphocytes. They recognise and kill transformed cells by releasing perforins and granzymes which create channels in the target cell membrane causing lysis. They express the markers CD16, CD56 and CD94.

Natural Killer cells also play a role in antibody-dependent cell-mediated cytotoxicity

NK Receptors

Some viruses are able to down-regulate MHC expression of the infected cell; this mechanism is also used as a protection against the host immune system as a lack of MHC inhibits normal T-cell activity. NK cells can counteract the down-regulation tactic and in this regard are mainly associated with activity against virus-infected cells and tumour cells, which can also have lowered MHC expression.

The receptors on NK cells do not act like antigen-specific receptors because although they trigger functional activity within the cell, they do not stimulate proliferation and there is no clonal expansion of NK cells. NK cells work through two different types of receptors:

1. Activating receptors, R1 which recognise pathogen-associated glycolipids or Fc receptors (E.g. CD16 recognises Ig that is bound to pathogen antigens)
2. Suppressing receptors, R2 which recognise target cell MHC molecules.

When an NK cell interacts with a target cell it will be activated via R1 - if the target cell expresses MHC this will be seen by R2. R2 suppresses the activities of NK cells.If the target cell does not express MHC, the suppressing receptors are not engaged; the engagement of R1 therefore causes activation of the NK cells

Activated NK cell Response

NK cells secrete a range of cytokines, including:

- Tumour necrosis factor alpha; (TNFα) - a potent stimulator of acute inflammation which can cause target cell killing directly and also via stimulated macrophages.
- Interferon gamma; (IFNγ) which stimulates macrophages to be active against the target cell and stimulates target cell expression of MHC.

Humoral immunity

Humoral immunity or humoural immunity is the aspect of immunity that is mediated by macromolecules found in extracellular fluids such as secreted antibodies, complement proteins, and certain antimicrobial peptides. Humoral immunity is so named because it involves substances found in the humors, or body fluids. It contrasts with cell-mediated immunity. Its aspects involving antibodies are often called antibody-mediated immunity.

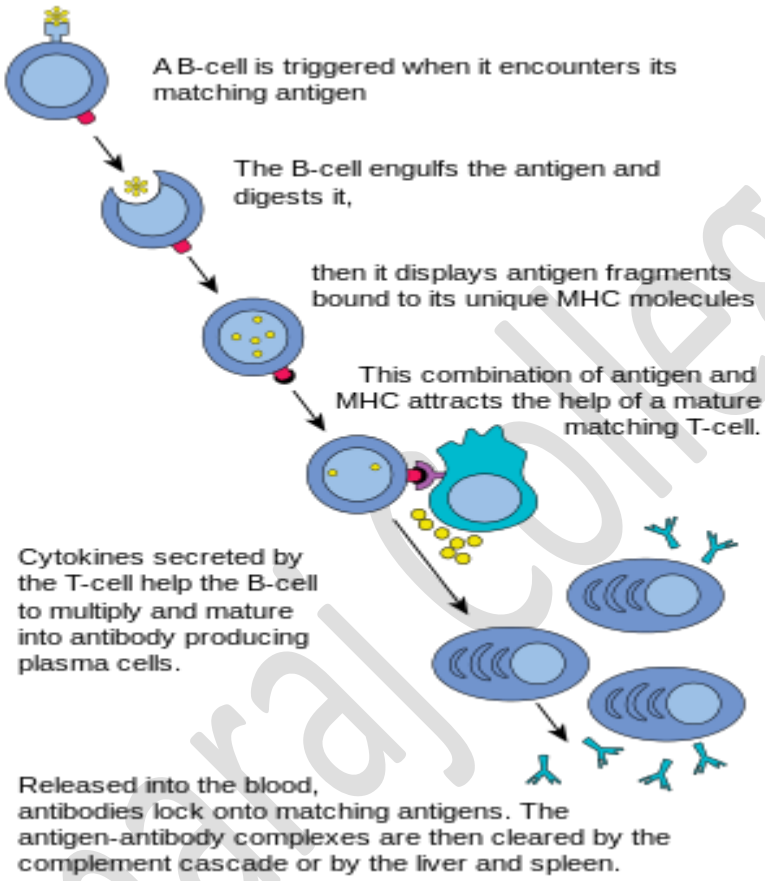

Figure 2.12

The study of the molecular and cellular components that form the immune system, including their function and interaction, is the central science of immunology. The immune system is divided into a more primitive innate immune system, and acquired or adaptive immune system of vertebrates, each of which contains humoral and cellular components.Humoral immunity refers to antibody production and the accessory processes that accompany it, including: Th2 activation and cytokine production, germinal center formation and isotype switching, affinity maturation and memory cell generation. It also refers to the effector functions of antibodies, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination.

In humoral immune response, first the B cells mature in the bone marrow and gain B-cell receptors (BCR's) which are displayed in large number on the cell surface.

These membrane-bound protein complexes have antibodies which are specific for antigen detection. Each B cell has a unique antibody that binds with an antigen. The mature B

cells migrate from the bone marrow to the lymph nodes or other lymphatic organs, where they begin to encounter pathogens.

Cell-mediated immunity

Cell-mediated immunity is an immune response that does not involve antibodies. Rather, cell-mediated immunity is the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to antigen. Historically, the immune system was separated into two branches: humoral immunity, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or serum) and cellular immunity, for which the protective function of immunization was associated with cells. CD4 cells or helper T cells provide protection against different pathogens. Naive T cells, which are mature T cells that have yet to encounter an antigen, are converted into activated effector T cells after encountering antigen-presenting cells (APCs). These APCs, such as macrophages, dendritic cells, and B cells in some circumstances, load antigenic peptides onto the MHC of the cell, in turn presenting the peptide to receptors on T cells. The most important of these APCs are highly specialized dendritic cells; conceivably operating solely to ingest and present antigens.

Activated Effector T cells can be placed into three functioning classes, detecting peptide antigens originating from various types of pathogen: The first class being

1) Cytotoxic T cells, which kill infected target cells by apoptosis without using cytokines,
2) TH1 cells, which primarily function to activate macrophages
3) TH2 cells, which primarily function to stimulate B cells into producing antibodies.

The innate immune system and the adaptive immune system each comprise both humoral and cell-mediated components.

Cellular immunity protects the body through:

T-cell mediated immunity or T-cell immunity:

Activating antigen-specific cytotoxic T cells that are able to induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;

Macrophage and natural killer cell action:

Enabling the destruction of pathogens via recognition and secretion of cytotoxic granules (for natural killer cells)and phagocytosis (for macrophages); and

Stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily at microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in removing virus-infected cells, but also participates in defending against fungi, protozoans, cancers, and intracellular bacteria. It also plays a major role in transplant rejection.

Lymphokines are a subset of cytokines that are produced by a type of immune cell known as a lymphocyte. They are protein mediators typically produced by T cells to direct the immune system response by signaling between its cells. Lymphokines have many roles,

including the attraction of other immune cells, including macrophages and other lymphocytes, to an infected site and their subsequent activation to prepare them to mount an immune response. Circulating lymphocytes can detect a very small concentration of lymphokine and then move up the concentration gradient towards where the immune response is required. Lymphokines aid B cells to produce antibodies.

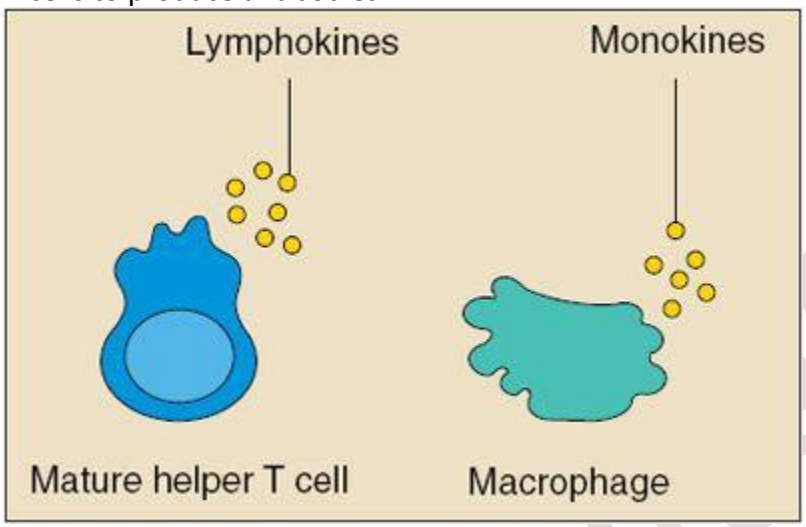

Figure 2.13

Important lymphokines secreted by the T helper cell include:

- Interleukin 2
- Interleukin 3
- Interleukin 4
- Interleukin 5
- Interleukin 6
- Granulocyte-macrophage colony-stimulating factor
- Interferon-gamma

Cytokines

Cytokines are a broad and loose category of small proteins (~5–20 kDa) important in cellsignalling. Cytokines are peptides and cannot cross the lipid bi-layer of cells to enter the cytoplasm. Cytokines have been shown to be involved in autocrine, paracrine and endocrine signalling as immunemodulating agents. Their definite distinction from hormones is still part of ongoing research.

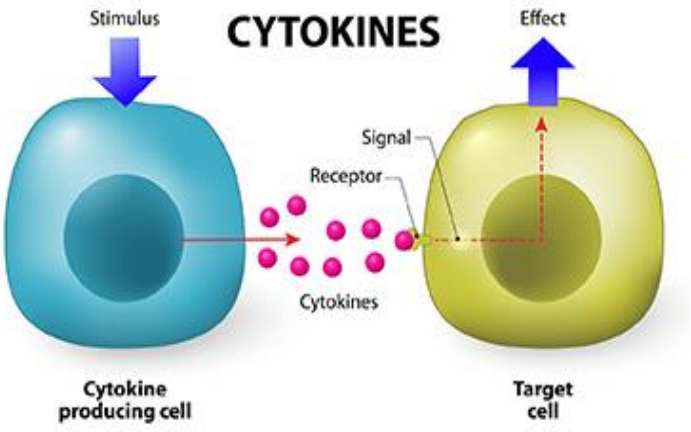

Figure 2.14

Cytokines include chemokines, interferons, interleukins, lymphokines, and tumour necrosis factors, but generally not hormones or growth factors (despite some overlap in the terminology). Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells; a given cytokine may be produced by more than one type of cell. They act through cell surface receptors and are especially important in the immune system; cytokines modulate the balance between humoral and cell-based immune responses, and they regulate the maturation, growth, and responsiveness of particular cell populations. Some cytokines enhance or inhibit the action of other cytokines in complex ways. They are different from hormones, which are also important cell signaling molecules. Hormones circulate in higher concentrations, and tend to be made by specific kinds of cells. Cytokines are important in health and disease, specifically in host immune responses to infection, inflammation, trauma, sepsis, cancer, and reproduction.

UNIT – III
ANTIGEN AND ANTIBODY

Antigen

Antigen, substance that is capable of stimulating an immune response, specifically activating lymphocytes, which are the body's infection-fighting white blood cells. In general, two main divisions of antigens are recognized: foreign antigens (or heteroantigens) and autoantigens (or self-antigens). Foreign antigens originate from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals. Autoantigens, on the other hand, originate within the body. Normally, the body is able to distinguish self from nonself, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. An antigen that induces an immune response—i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly—is called an immunogen.

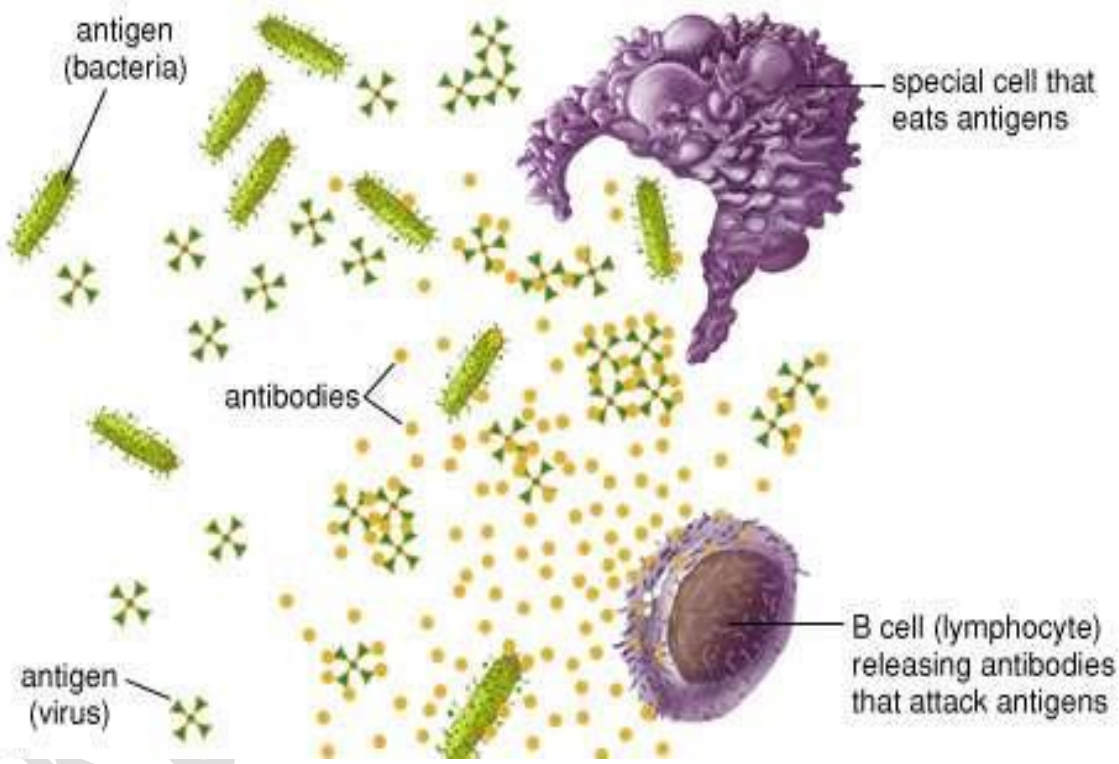

Figure 3.1

Antigen; antibody; lymphocytePhagocytic cells destroy viral and bacterial antigens by eating them, while B cells produce antibodies that bind to and inactivate antigens.

On the surface of antigens are regions, called antigenic determinants, that fit and bind to receptor molecules of complementary structure on the surface of the lymphocytes. The binding of the lymphocytes' receptors to the antigens' surface molecules stimulates the lymphocytes to multiply and to initiate an immune response—including the production of antibody, the activation of cytotoxic cells, or both—against the antigen. The amount of antibody formed in response to stimulation depends on the kind and amount of antigen involved, the route of entry to the body, and individual characteristics of the host.

Chemical Nature

The antigens are mostly the conjugated proteins like lipoproteins, glycoproteins and nucleoproteins.

Structure:

Antigenic determinants or epitopes (Gk. epi – upon, topos- place) are components of antigen. Each antigen carries many epitopes. Each Y-shaped antibody molecule has atleast two binding sites that can attach to a specific epitope on an antigen. An antibody can also bind to identical epitopes of two different cells at the same time which can cause neighbouring cells to aggregate. Antigens combine with the antibody. The combination is very much like the lock and key analogy.

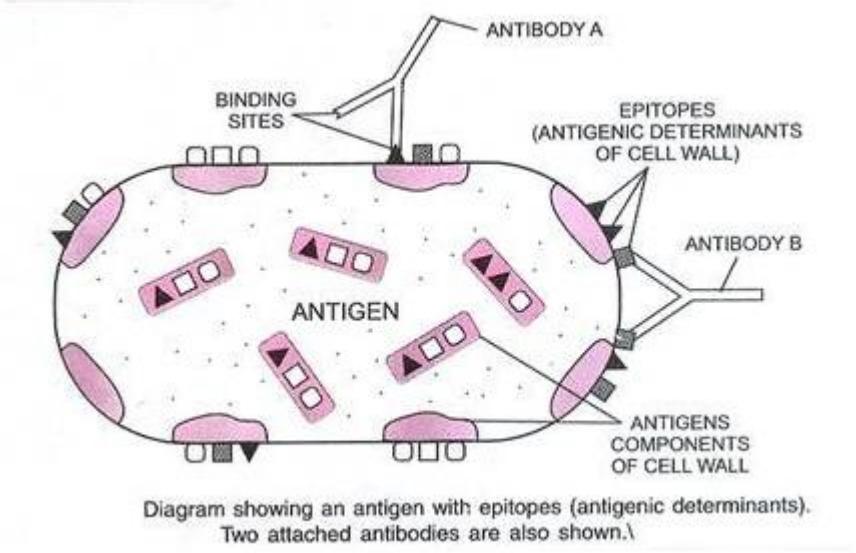

Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.\

Figure 3.2

Types:

Based upon the ability of antigens to carry out their functions, antigens are of two types: complete antigens and incomplete antigens (haptens). A complete antigen is able to induce antibody formation and produce a specific and observable reaction with the antibody so produced.

Haptens (Gr. hapten to grasp; partial antigens) are substances which are incapable of inducing antibody formation by themselves, but can be capable of inducing antibodies on combining with larger molecules (normally proteins) which serve as carriers.

Antigens which are present on the body's own cells are called the auto-antigens or self antigens. The antigens on the non-self cells are known as foreign antigens or non-self antigens.

H antigen:

Red blood corpuscles of all ABO blood groups possess a common antigen, the H antigen, which is a precursor for the formation of A and B antigens. Due to universal distribution, H antigen is not ordinarily important in grouping or blood transfusion.

However, Bhende et al (1952) from Mumbai reported a very rare example in which A and B antigens and H antigens were absent from the red blood corpuscles. This is known as Bombay or Oh blood group. Such individuals will have anti A, anti B and anti H antibodies. Therefore, they can accept the blood only from their own group.

Haptens

Haptens are relatively small molecules that elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself (in general, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body). Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody, a process called hapten inhibition.

The mechanisms of absence of immune response may vary and involve complex immunological mechanisms, but can include absent or insufficient co-stimulatory signals from antigen-presenting cells.

Haptens have been used to study allergic contact dermatitis (ACD) and the mechanisms of inflammatory bowel disease (IBD) to induce autoimmune-like responses.
The concept of haptens emerged from the work of Karl Landsteiner who also pioneered the use of synthetic haptens to study immunochemical phenomena.

Adjuvant

An adjuvant is a pharmacological or immunological agent that modifies the effect of other agents. Adjuvants may be added to a vaccine to boost the immune response to produce more antibodies and longer-lasting immunity, thus minimizing the dose of antigen needed. Adjuvants may also be used to enhance the efficacy of a vaccine by helping to modify the immune response to particular types of immune system cells: for example, by activating T cells instead of antibody-secreting B cells depending on the purpose of the vaccine. Adjuvants are also used in the production of antibodies from immunized animals. There are different classes of adjuvants that can push immune response in different directions, but the most commonly used adjuvants include aluminum hydroxide and paraffin oil.

Adjuvants are needed to improve routing and adaptive immune responses to antigens. This reaction is mediated by two main types of lymphocytes, B and T lymphocytes. Adjuvants apply their effects through different mechanisms. Some adjuvants, such as alum, function as delivery systems by generating depots that trap antigens at the injection site, providing a slow release that continues to stimulate the immune system. This is now under debate, as studies have shown that surgical removal of these depots had no impact on the magnitude of IgG1 response.

Types

- Analgesic adjuvants
- Inorganic compounds: alum, aluminum hydroxide, aluminum phosphate, calcium phosphate hydroxide
- Mineral oil: paraffin oil
- Bacterial products: killed bacteria Bordetella pertussis, Mycobacterium bovis, toxoids
- Nonbacterial organics: squalene
- Delivery systems: detergents (Quil A)
- Plant saponins from Quillaja (See Quillaia), soybean, Polygala senega
- Cytokines: IL-1, IL-2, IL-12

- Combination: Freund's complete adjuvant, Freund's incomplete adjuvant
- Food-based oil: Adjuvant 65, which is a product based on peanut oil.[8] Adjuvant 65 was tested in influenza vaccines in the 1970s, but was never released commercially.

Vaccine

A vaccine is a biological preparation that provides active acquired immunity to a particular infectious 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 a threat, destroy it, and to further recognize and destroy any of the microorganisms associated with that agent that it may encounter in the future. Vaccines can be prophylactic (to prevent or ameliorate the effects of a future infection by a natural or "wild" pathogen), or therapeutic (e.g., vaccines against cancer, which are being investigated).

The administration of vaccines is called vaccination. Vaccination is the most effective method of preventing infectious diseases; widespread immunity due to vaccination is largely responsible for the worldwide eradication of smallpox and the restriction of diseases such as polio, measles, and tetanus from much of the world. The effectiveness of vaccination has been widely studied and verified; for example, vaccines that have proven effective include the influenza vaccine, the HPV vaccine, and the chicken pox vaccine. The World Health Organization (WHO) reports that licensed vaccines are currently available for twenty-five different preventable infections.

The terms vaccine and vaccination are derived from Variolae vaccinae (smallpox of the cow), the term devised by Edward Jenner to denote cowpox. He used it in 1798 in the long title of his Inquiry into the Variolae vaccinae Known as the Cow Pox, in which he described the protective effect of cowpox against smallpox. In 1881, to honor Jenner, Louis Pasteur proposed that the terms should be extended to cover the new protective inoculations then being developed.

Types

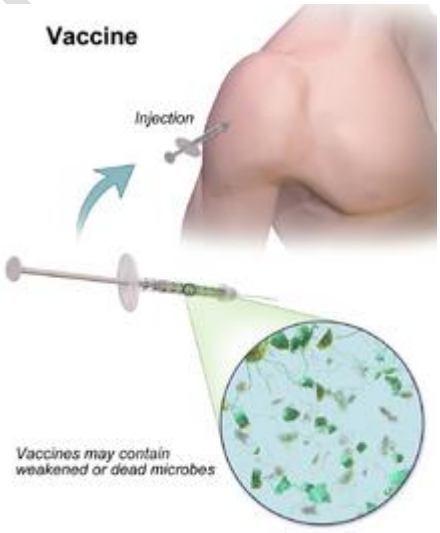

Figure 3.3

Vaccine:

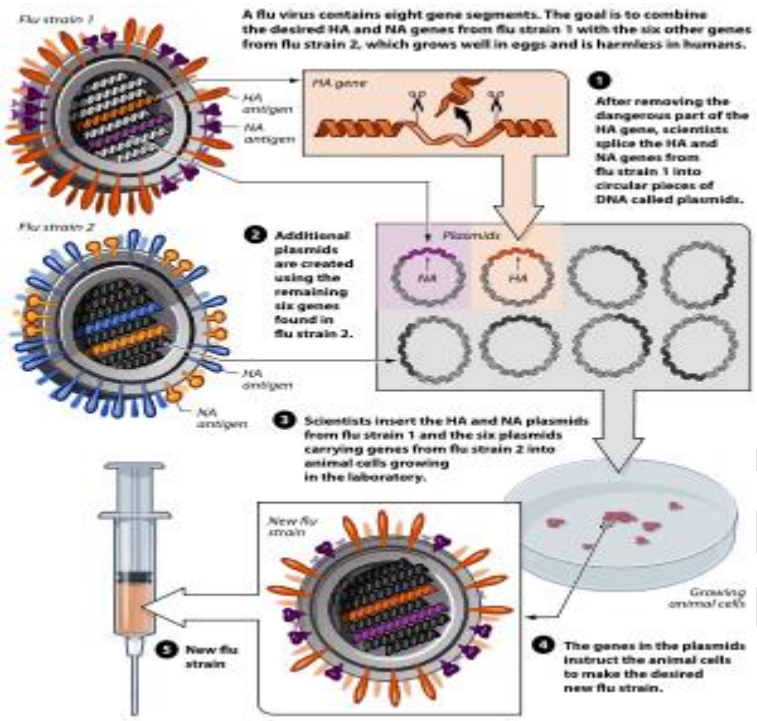

Figure 3.4

Avian flu vaccine development by reverse genetics techniques.Vaccines contain dead or inactivated organisms or purified products derived from them.There are several types of vaccines in use. These represent different strategies used to try to reduce the risk of illness while retaining the ability to induce a beneficial immune response.

Inactivated

Some vaccines contain inactivated, but previously virulent, micro-organisms that have been destroyed with chemicals, heat, or radiation. Examples include the polio vaccine, hepatitis A vaccine, rabies vaccine and some influenza vaccines.

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, mumps, and rubella, and the bacterial disease typhoid. The live Mycobacterium tuberculosis vaccine developed by Calmette and Guérin is not made of a contagious strain but contains a virulently modified strain called "BCG" used to elicit an immune response to the vaccine. The live attenuated vaccine containing strain Yersinia pestis EV is used for plague immunization. Attenuated vaccines have some advantages and disadvantages. They typically provoke more durable immunological responses and are the preferred type for healthy adults. But they may not be safe for use in immune compromised individuals, and on rare occasions mutate to a virulent form and cause disease.[36]

Toxoid

Toxoid vaccines are made from inactivated toxic compounds that cause illness rather than the micro-organism. Examples of toxoid-based vaccines include tetanus and diphtheria. Toxoid vaccines are known for their efficacy. Not all toxoids are for micro-organisms; for example, Crotalus atrox toxoid is used to vaccinate dogs against rattlesnake bites.

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) or as an edible algae vaccine, the virus-like particle (VLP) vaccine against human papillomavirus (HPV) that is composed of the viral major capsid protein, and the hemagglutinin and neuraminidase subunits of the influenza virus. Subunit vaccine is being used for plague immunization.

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.

A number of innovative vaccines are also in development and in use:

- Dendritic cell vaccines combine dendritic cells with antigens in order to present the antigens to the body's white blood cells, thus stimulating an immune reaction. These vaccines have shown some positive preliminary results for treating brain tumors and are also tested in malignant melanoma.
- DNA vaccination – an alternative, experimental approach to vaccination called DNA vaccination, created from an infectious agent's DNA, is under development. The proposed mechanism is the insertion (and expression, enhanced by the use of electroporation, triggering immune system recognition) of viral or bacterial DNA into human or animal cells. Some cells of the immune system that recognize the proteins expressed will mount an attack against these proteins and cells expressing them. Because these cells live for a very long time, if the pathogen that normally expresses these proteins is encountered at a later time, they will be attacked instantly by the immune system. One potential advantage of DNA vaccines is that they are very easy to produce and store. As of 2015, DNA vaccination is still experimental and is not approved for human use.
- Recombinant vector – by combining the physiology of one micro-organism and the DNA of another, immunity can be created against diseases that have complex infection processes. An example is the RVSV-ZEBOV vaccine licensed to Merck that is being used in 2018 to combat ebola in Congo.
- RNA vaccine is a novel type of vaccine which is composed of the nucleic acid RNA, packaged within a vector such as lipid nanoparticles. A number of RNA vaccines are under development to combat the 2019–20 coronavirus pandemic.
- T-cell receptor peptide vaccines are under development for several diseases using models of Valley Fever, stomatitis, and atopic dermatitis. These peptides have been shown to modulate cytokine production and improve cell-mediated immunity.

- Targeting of identified bacterial proteins that are involved in complement inhibition would neutralize the key bacterial virulence mechanism

While most vaccines are created using inactivated or attenuated compounds from micro-organisms, synthetic vaccines are composed mainly or wholly of synthetic peptides, carbohydrates, or antigens.

Valence

Vaccines may be monovalent (also called univalent) or multivalent (also called polyvalent). A monovalent vaccine is designed to immunize against a single antigen or single microorganism. A multivalent or polyvalent vaccine is designed to immunize against two or more strains of the same microorganism, or against two or more microorganisms. The valency of a multivalent vaccine may be denoted with a Greek or Latin prefix (e.g., tetravalent or quadrivalent). In certain cases, a monovalent vaccine may be preferable for rapidly developing a strong immune response.

Heterotypic

Also known as heterologous or "Jennerian" vaccines, these are vaccines that are pathogens of other animals that either do not cause disease or cause mild disease in the organism being treated. The classic example is Jenner's use of cowpox to protect against smallpox. A current example is the use of BCG vaccine made from Mycobacterium bovis to protect against human tuberculosis.

Toxoid

A toxoid is an inactivated toxin (usually an exotoxin) whose toxicity has been suppressed either by chemical (formalin) or heat treatment, while other properties, typically immunogenicity, are maintained. Toxins are secreted by bacteria, whereas toxoids are altered form of toxins; toxoids are not secreted by bacteria. Thus, when used during vaccination, an immune response is mounted and immunological memory is formed against the molecular markers of the toxoid without resulting in toxin-induced illness. Such a preparation is also known as an anatoxin.There are toxoids for prevention of diphtheria, tetanus and botulism.

Toxoids are used as vaccines because they induce an immune response to the original toxin or increase the response to another antigen since the toxoid markers and toxin markers are preserved. For example, the tetanus toxoid is derived from the tetanospasmin produced by Clostridium tetani. The latter causes tetanus and is vaccinated against by the DTaP vaccine. Botulin is produced by Clostridium botulinum and causes the deadly disease botulism. While patients may sometimes complain of side effects after a vaccine, these are associated with the process of mounting an immune response and clearing the toxoid, not the direct effects of the toxoid. The toxoid does not have virulence as the toxin did before inactivation.

Multiple doses of tetanus toxoid are used by many plasma centers in the United States for the development of highly immune persons for the production of human anti-tetanus immune globulin (tetanus immune globulin (TIG), HyperTet (c)), which has replaced horse serum-type tetanus antitoxin in most of the developed world.

Antitoxin

An antitoxin is an antibody with the ability to neutralize a specific toxin. Antitoxins are produced by certain animals, plants, and bacteria in response to toxin exposure. Although they

are most effective in neutralizing toxins, they can also kill bacteria and other microorganisms. Antitoxins are made within organisms, and can be injected into other organisms, including humans, to treat an infectious disease. This procedure involves injecting an animal with a safe amount of a particular toxin. The animal's body then makes the antitoxin needed to neutralize the toxin.

Later, blood is withdrawn from the animal. When the antitoxin is obtained from the blood, it is purified and injected into a human or other animal, inducing temporary passive immunity. To prevent serum sickness, it is often best to use an antitoxin obtained from the same species (e.g. use human antitoxin to treat humans).

Structure of immunoglobulins

Antibody (or immunoglobulin) molecules are glycoproteins composed of one or more units, each containing four polypeptide chains: two identical heavy chains (H) and two identical light chains (L). The amino terminal ends of the polypeptide chains show considerable variation in amino acid composition and are referred to as the variable (V) regions to distinguish them from the relatively constant (C) regions. Each L chain consists of one variable domain, VL, and one constant domain, CL. The H chains consist of a variable domain, VH, and three constant domains CH1, CH2 and CH3. Each heavy chain has about twice the number of amino acids and molecular weight (~50,000) as each light chain (~25,000), resulting in a total immunoglobulin monomer molecular weight of approximately 150,000.

Generalized structure of an immunoglobulin (IgG):

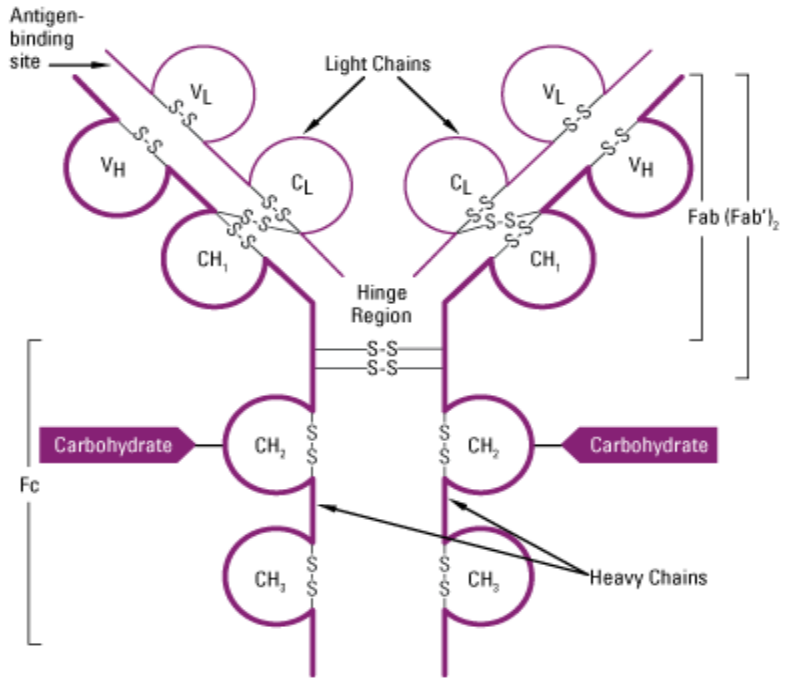

Figure 3.5

Heavy and light chains are held together by a combination of non-covalent interactions and covalent interchain disulfide bonds, forming a bilaterally symmetric structure. The V regions of H and L chains comprise the antigen-binding sites of the immunoglobulin (Ig) molecules. Each Ig monomer contains two antigen-binding sites and is said to be bivalent.The hinge region is the area of the H chains between the first and second C region domains and is

held together by disulfide bonds. This flexible hinge (found in IgG, IgA and IgD, but not IgM or IgE) region allows the distance between the two antigen-binding sites to vary.

Classes of immunoglobulins

The five primary classes of immunoglobulins are IgG, IgM, IgA, IgD and IgE. These are distinguished by the type of heavy chain found in the molecule. IgG molecules have heavy chains known as gamma-chains; IgMs have mu-chains; IgAs have alpha-chains; IgEs have epsilon-chains; and IgDs have delta-chains.

Differences in heavy chain polypeptides allow these immunoglobulins to function in different types of immune responses and at particular stages of the immune response. The polypeptide protein sequences responsible for these differences are found primarily in the Fc fragment. While there are five different types of heavy chains, there are only two main types of light chains: kappa (κ) and lambda (λ).Antibody classes differ in valency as a result of different numbers of Y-like units (monomers) that join to form the complete protein. For example, in humans, functioning IgM antibodies have five Y-shaped units (pentamer) containing a total of 10 light chains, 10 heavy chains and 10 antigen-binding.

IgG class

Figure 3.6

Properties of IgG:

- Molecular weight: 150,000
- H-chain type (MW): gamma (53,000)
- Serum concentration: 10 to 16 mg/mL
- Percent of total immunoglobulin: 75%
- Glycosylation (by weight): 3%
- Distribution: intra- and extravascular
- Function: secondary response

IgM class

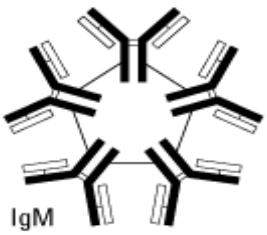

Figure 3.7

Properties of IgM:

- Molecular weight: 900,000
- H-chain type (MW): mu (65,000)
- Serum concentration: 0.5 to 2 mg/mL
- Percent of total immunoglobulin: 10%
- Glycosylation (by weight): 12%
- Distribution: mostly intravascular
- Function: primary response

IgA class

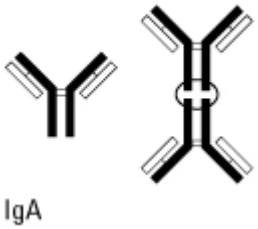

Figure 3.8

Properties of IgA:

- Molecular weight: 320,000 (secretory)
- H-chain type (MW): alpha (55,000)
- Serum concentration: 1 to 4 mg/mL
- Percent of total immunoglobulin: 15%
- Glycosylation (by weight): 10%
- Distribution: intravascular and secretions
- Function: protect mucus membranes

IgD and IgE class

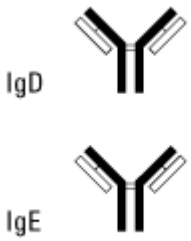

Figure 3.9

Properties of IgD:

- Molecular weight: 180,000
- H-chain type (MW): delta (70,000)
- Serum concentration: 0 to 0.4 mg/mL
- Percent of total immunoglobulin: 0.2%
- Glycosylation (by weight): 13%
- Distribution: lymphocyte surface
- Function: unknown

Properties of IgE:

- Molecular weight: 200,000

- H-chain type (MW): epsilon (73,000)
- Serum concentration: 10 to 400 ng/mL
- Percent of total immunoglobulin: 0.002%
- Glycosylation (by weight): 12%
- Distribution: basophils and mast cells in saliva and nasal secretions
- Function: protect against parasites

Subclasses of immunoglobulins

In addition to the major immunoglobulin classes, several Ig subclasses exist in all members of a particular animal species. Antibodies are classified into subclasses based on minor differences in the heavy chain type of each Ig class. In humans there are four subclasses of IgG: IgG1, IgG2, IgG3 and IgG4 (numbered in order of decreasing concentration in serum).

Variance among different subclasses is less than the variance among different classes. For example, IgG1 is more closely related to IgG2, IgG3 and IgG4 than to IgA, IgM, IgD or IgE. Consequently, antibody-binding proteins (e.g., Protein A or Protein G) and most secondary antibodies used in immunodetection methods cross-react with multiple subclasses but usually not multiple classes of Ig.

The complement system

The complement system is an enzyme cascade that is a collection of blood and cell surface proteins to help the abilities of antibodies to clear pathogens from an organism. The complement system that comprises 30 different proteins, including serum proteins, serosal proteins, and cell membrane receptors is an important part of the innate immune system. 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 and initiate an amplifying cascade of further cleavages. The end-result of this cascade is massive amplification of the response and activation of the cell-killing membrane attack complex. The complement system has four major function, including lysis of infectious organisms, activation of inflammation, opsonization and immune clearance.There are three different complement pathways, the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway. The classic complement pathway is triggered when antibody-antigen complex interact with C1-complex, which consists of C1q, two molecules of C1r, and two molecules of C1s. The C1-complex cleaves C2 and C4, which then form C3 convertase (C4b2a). C3 is then cleaved by the C3 convertase, and forms C5 convertase in association with C4b and C2a. The generation of C5 convertase is the end of the classical pathway. The lectin pathway is very similar to the classical pathway. It is stimulated when the mannose-binding lectin (MBL) binds to mannose residues on the pathogen surface. The MBL-associated serine proteases, MASP-1, and MASP-2, are activated and cleave C4 and C2, which then form the C3 convertase as in the classical pathway. The alternative complement pathway begins with the activation of C3 and requires factor B and factor D. All three pathways merge at C3, which is then converted into C3a and C3b.

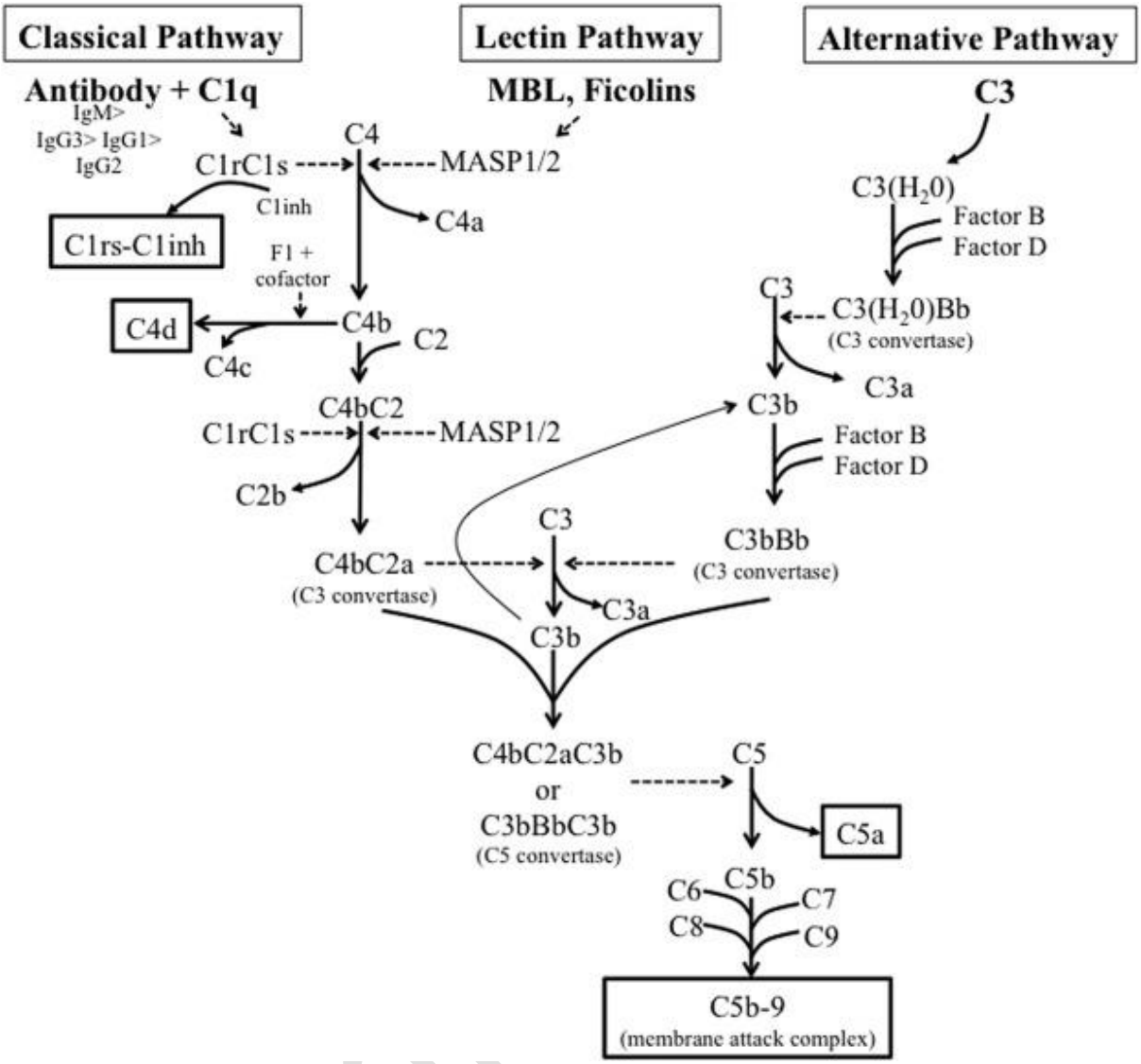
Classical Pathway
Lectin Pathway
Alternative Pathway
Antibody + C1q
IgM>
IgG3> IgG1>
IgG2
MBL, Ficolins
C3
C4
C1rC1s
MASP1/2
C1inh
C4a
C1rs-C1inh
C3(H20)
Factor B
Factor D
F1 +
cofactor
C4d
C4b
C2
C4c
C3
C3(H20)Bb
(C3 convertase)
C3a
C4bC2
C1rC1s
MASP1/2
C3b
Factor B
Factor D
C2b
C3
C3bBb
(C3 convertase)
C4bC2a
(C3 convertase)
C3a
C3b
C4bC2aC3b
or
C3bBbC3b
(C5 convertase)
C5
C5a
C5b
C6
C7
C8
C9
C5b-9
(membrane attack complex)

Figure 3.10

UNIT – IV
ANTIGEN AND ANTIBODY REACTIONS

Antigen Antibody Reaction

Antigen can react with antibodies in vivo or in vitro. The in vivo reaction can be beneficial for the organism (immunity), harmful (immunopathological reactions) or indifferent (immune system tolerates, rather than responds to the antigen). The in vitro reactions are the basis for immunochemical methods which depend on biospecific binding between binding sites of the antibody and determinant groups of the antigen resulting in formation of antibody-antigen complexes (immune complexes).

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. Antigens are soluble molecules and larger in size in precipitation reactions. 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, or non-gel support media such as cellulose acetate.

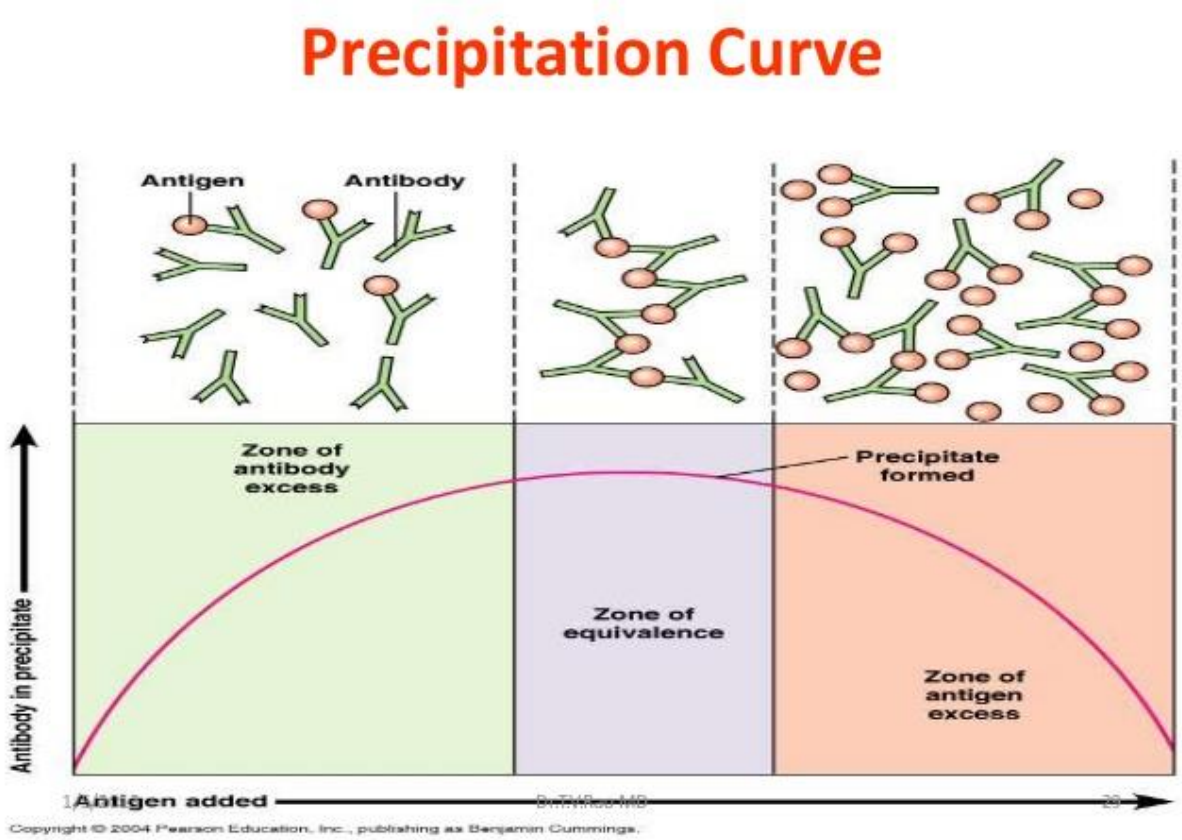

Figure 4.1

Precipitation methods include double immunodiffusion (qualitative gel technique that determines the relationship between antigen and antibody), radial immunodiffusion (semi-

quantitation of proteins by gel diffusion using antibody incorporated in agar), and electroimmunodiffusion (variation of the double immunodiffusion method reaction that uses an electric current to enhance the mobility of the reactants toward each other).

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). In the double immunodiffusion technique, three basic reaction patterns result from the relationship of antigens and antibodies. These patterns are identity, non-identity, and partial identity. 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.

Agglutination

Agglutination is the clumping of particles. The word agglutination comes from the Latin agglutinare (glueing to).

Agglutination is the process that occurs if an antigen is mixed with its corresponding antibody called isoagglutinin. This term is commonly used in blood grouping.

This occurs in biology in two main examples:

1. The clumping of cells such as bacteria or red blood cells in the presence of an antibody or complement. The antibody or other molecule binds multiple particles and joins them, creating a large complex. This increases the efficacy of microbial elimination by phagocytosis as large clumps of bacteria can be eliminated in one pass, versus the elimination of single microbial antigens.
2. When people are given blood transfusions of the wrong blood group, the antibodies react with the incorrectly transfused blood group and as a result, the erythrocytes clump up and stick together causing them to agglutinate. The coalescing of small particles that are suspended in a solution; these larger masses are then (usually) precipitated.

Hemagglutination

The 'bedside card' method of blood typing, in this case using a Serafol card. The result is blood group A positive.

Hemagglutination is the process by which red blood cells agglutinate, meaning clump or clog. The agglutin involved in hemagglutination is called hemagglutinin.

In cross-matching, donor red blood cells and recipient's serum or plasma are incubated together. If agglutination occurs, this indicates that the donor and recipient blood types are incompatible.

Leukoagglutination

Leukoagglutination occurs when the particles involved are white blood cells.
An example is the PH-L form of phytohaemagglutinin.

Agglutination is commonly used as a method of identifying specific bacterial antigens, and in turn, the identity of such bacteria. Because the clumping reaction occurs quickly and is easy to produce, agglutination is an important technique in diagnosis.

Complement fixation

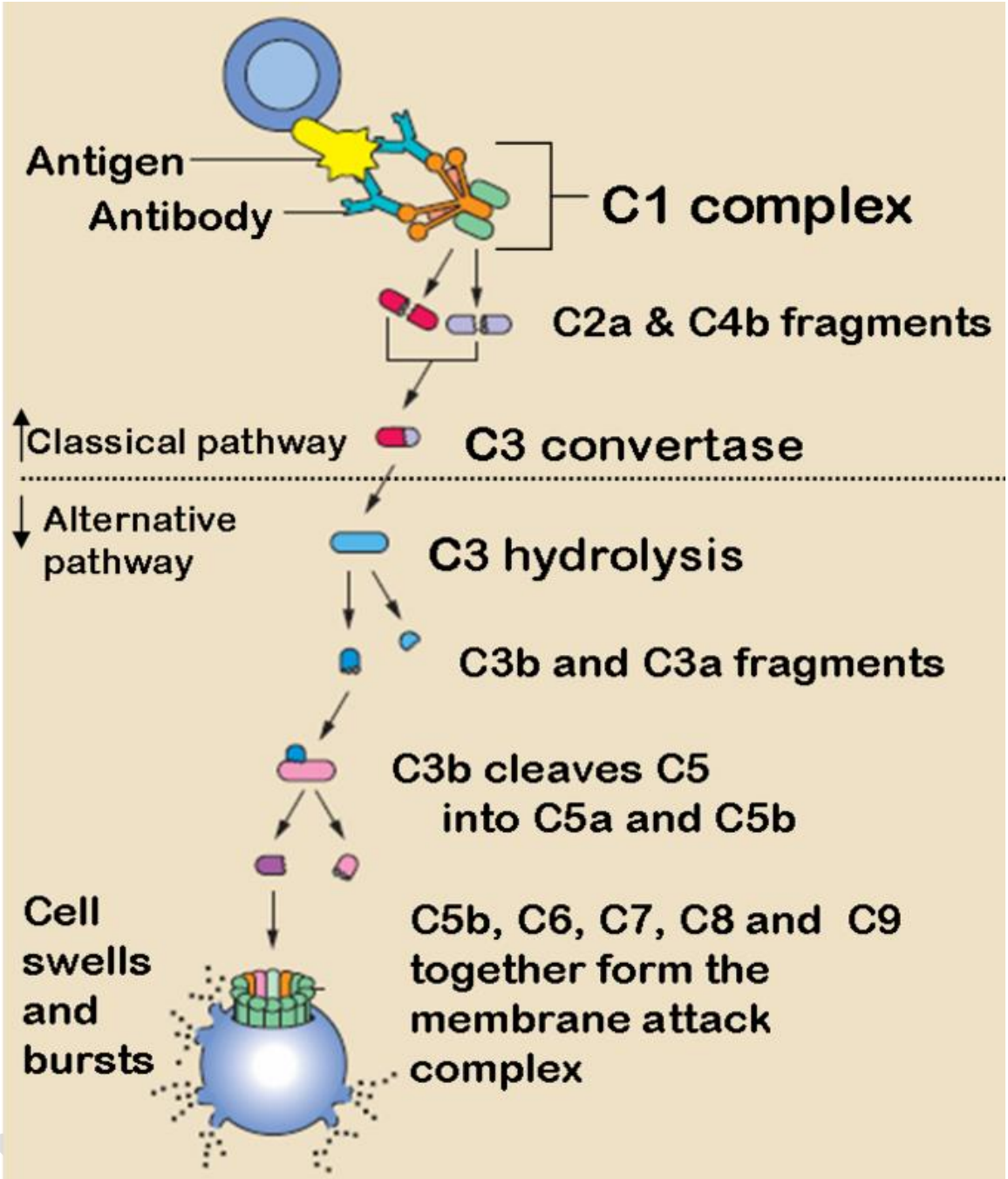

Figure 4.2

Complement fixation is a classic method for demonstrating the presence of antibody in patient serum. The complement fixation test consists of two components.

The first component is an indicator system that uses combination of sheep red blood cells, complement-fixing antibody such as immunoglobulin G produced against the sheep red blood cells and an exogenous source of complement usually guinea pig serum. When these elements are mixed in optimum conditions, the anti-sheep antibody binds on the surface of red blood cells. Complement subsequently binds to this antigen -antibody complex formed and will cause the red blood cells to lyse.

The second component is a known antigen and patient serum added to a suspension of sheep red blood cells in addition to complement. These two components of the complement fixation method are tested in sequence. Patient serum is first added to the known antigen, and complement is added to the solution. If the serum contains antibody to the antigen, the resulting antigen-antibody complexes will bind all of the complement.

Immunofluorescence

Immunofluorescence is an assay which is used primarily on biological samples and is classically defined as a procedure to detect antigens in cellular contexts using antibodies. The specificity of antibodies to their antigen is the base for immunofluorescence. The biological samples include tissue and cells. Immunofluorescence allows researchers to evaluate whether or not cells in a particular sample express the antigen in question. In cases where an immunopositive signal is found, immunofluorescence also allows researchers to determine which subcellular compartments are expressing the antigen. Immunofluorescence can be used on cultured cell lines, tissue sections, or individual cells.

Immunofluorescence may be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. Immunofluorescence has been widely used in biological research and medical research yield and becomes one of the most important and effective method

There are two different immunofluorescence assay which include indirect immunofluorescence assay and direct immunofluorescence assay.For indirect immunofluorescence assay, the protocol mainly include tissue or tell treparation, tissue or cell fixation, serum blocking, primary antibody incubation, marked second antibody incubation, staining, result judgment and imaging. For direct immunofluorescence assay, there are only marked primary antibody been incubated without second antibody and other steps are same.

Indirect Immunofluorescence:

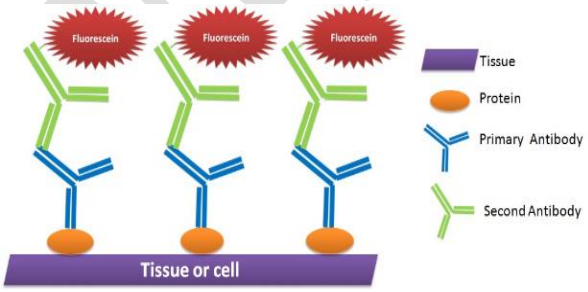

Figure 4.3

Direct Immunofluorescence:

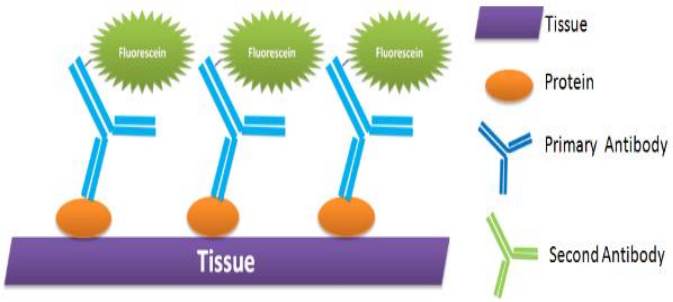

Figure 4.4

ELISA (enzyme-linked immunosorbent assay)

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. In an ELISA, an antigen must be immobilized on a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISAs can be performed with a number of modifications to the basic procedure. The key step, immobilization of the antigen of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (labeled secondary antibody). The most powerful ELISA assay format is the sandwich assay. This type of capture assay is called a “sandwich” assay because the analyte to be measured is bound between two primary antibodies – the capture antibody and the detection antibody. The sandwich format is used because it is sensitive and robust.

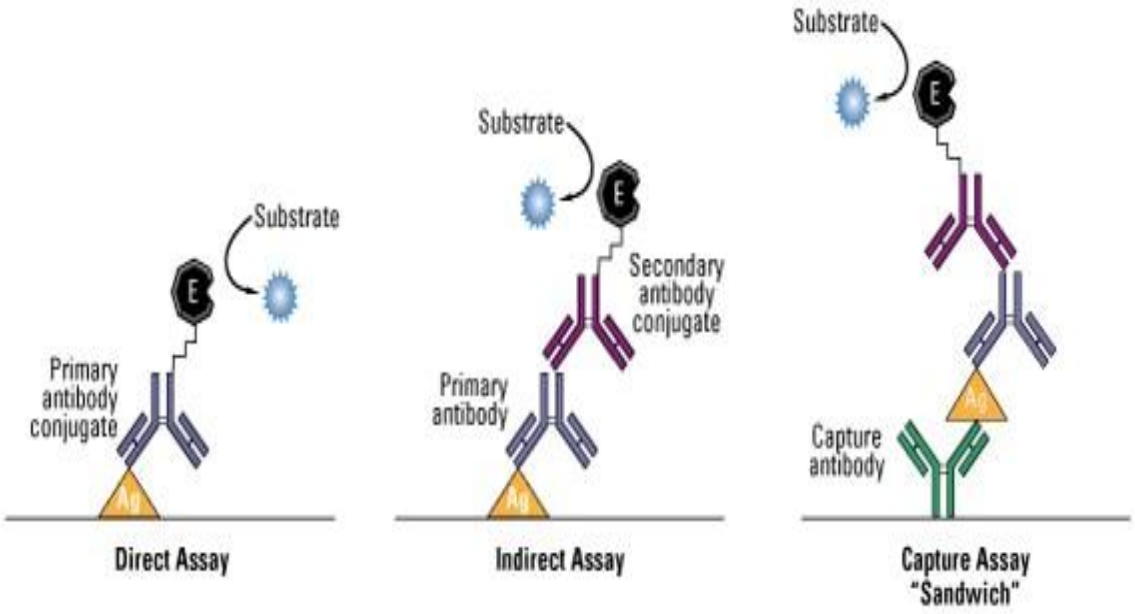

Figure 4.5

Direct vs. indirect detection ELISA strategies

Among the standard assay formats discussed and illustrated above, where differences in both capture and detection were the concern, it is important to differentiate between the particular strategies that exist specifically for the detection step. Irrespective of the method by which an antigen is captured on the plate (by direct adsorption to the surface or through a pre-coated "capture" antibody, as in a sandwich ELISA), it is the detection step (as either direct or indirect detection) that largely determines the sensitivity of an ELISA.

Comparison of direct and indirect ELISA detection methods

Direct ELISA detection	
Advantages	• Quick because only one antibody and fewer steps are used. • Cross-reactivity of secondary antibody is eliminated.
Disadvantages	• Immunoreactivity of the primary antibody might be adversely affected by labeling with enzymes or tags. • Labeling primary antibodies for each specific ELISA system is time-consuming and expensive. • No flexibility in choice of primary antibody label from one experiment to another. • Minimal signal amplification.
Indirect ELISA detection	
Advantages	• A wide variety of labeled secondary antibodies are available commercially. • Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection. • Maximum immunoreactivity of the primary antibody is retained because it is not labeled. • Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification. • Different visualization markers can be used with the same primary antibody.
Disadvantages	• Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal. • An extra incubation step is required in the procedure.

Fluorescent tags and other alternatives to enzyme-based detection can be used for plate-based assays. Despite not involving reporter-enzymes, these methods are also generally referred to as a type of ELISA. Likewise, wherever detectable probes and specific protein binding interactions can be used in a plate-based method, these assays are often called ELISAs despite not involving antibodies.

Other ELISA formats

Besides the standard direct and sandwich formats described above, several other styles of ELISA's exist:

Competitive ELISA is a strategy that is commonly used when the antigen is small and has only one epitope, or antibody binding site. One variation of this method consists of labeling purified antigen instead of the antibody. Unlabeled antigen from samples and the labeled antigen compete for binding to the capture antibody. A decrease in signal from the purified antigen indicates the presence of the antigen in samples when compared to assay wells with labeled antigen alone.

Radioimmunoassay- Principle, Uses and Limitations

When radioisotopes instead of enzymes are used as labels to be conjugated with antigens or antibodies, the technique of detection of the antigen-antibody complex is called radioimmunoassay (RIA). Radioimmunoassay (RIA) is an in vitro assay that measures the

presence of an antigen with very high sensitivity. RIA was first described in 1960 for the measurement of endogenous plasma insulin by Solomon Berson and Rosalyn Yalow of the Veterans Administration Hospital in New York.

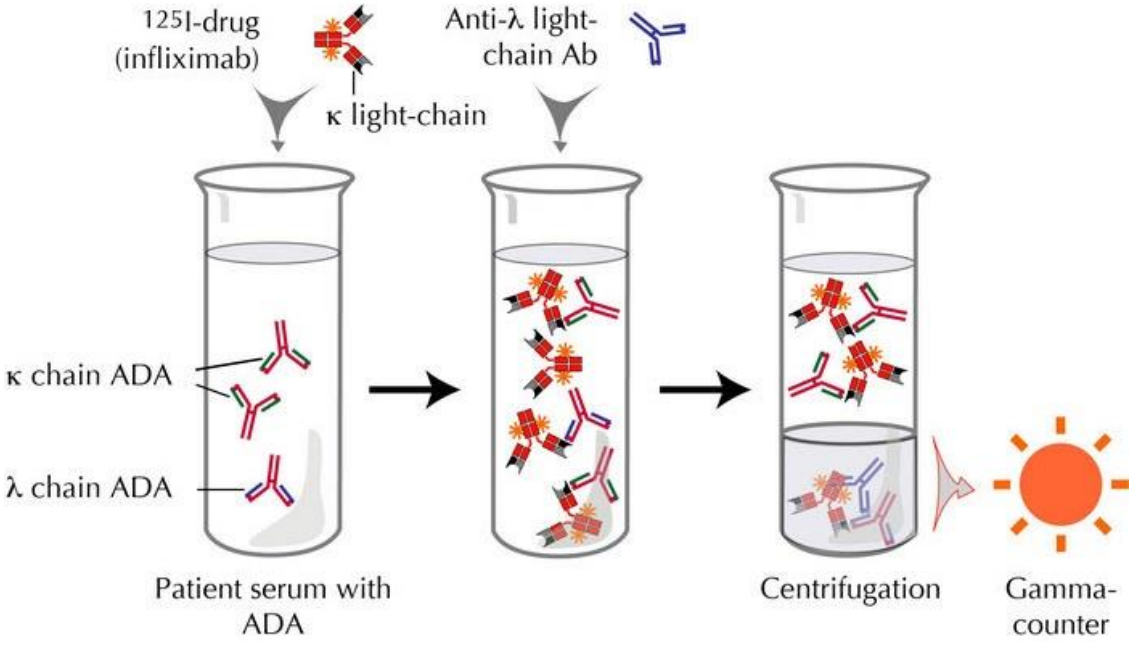

Figure 4.6

The classical RIA methods are based on the principle of competitive binding. In this method, an unlabeled antigen competes with a radiolabeled antigen for binding to an antibody with the appropriate specificity. Thus, when mixtures of radiolabeled and unlabeled antigen are incubated with the corresponding antibody, the amount of free (not bound to antibody) radiolabeled antigen is directly proportional to the quantity of unlabeled antigen in the mixture.

It involves a combination of three principles:

1. An immune reaction i.e. antigen, antibody binding.
2. A competitive binding or competitive displacement reaction. (It gives specificity)
3. Measurement of radio emission. (It gives sensitivity)

Immune Reactions

When a foreign biological substance enters into the body bloodstream through a non-oral route, the body recognizes the specific chemistry on the surface of foreign substance as antigen and produces specific antibodies against the antigen so as nullify the effects and keep the body safe. The antibodies are produced by the body's immune system so, it is an immune reaction. Here the antibodies or antigens bind move due to chemical influence. This is different from principle of electrophoresis where proteins are separated due to charge.

Competitive binding or competitive displacement reaction:

This is a phenomenon wherein when there are two antigens that can bind to the same antibody, the antigen with more concentration binds extensively with the limited antibody displacing others. So here in the experiment, a radiolabelled antigen is allowed to bind to high-affinity antibody. Then when the patient serum is added unlabeled antigens in it start binding to the antibody displacing the labeled antigen.

Measurement of radio emission:

Once the incubation is over, then washings are done to remove any unbound antigens. Then radio emission of the antigen-antibody complex is taken, and the gamma rays from radiolabeled antigen are measured.

The target antigen is labeled radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added). A sample, for e.g. blood-serum, is added in order to initiate a competitive reaction of the labeled antigens from the preparation, and the unlabeled antigens from the serum-sample, with the specific antibodies. The competition for the antibodies will release a certain amount of labeled antigen. This amount is proportional to the ratio of labeled to an unlabeled antigen. A binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived. That means as the concentration of unlabeled antigen is increased, more of it binds to the antibody, displacing the labeled variant. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigens remaining in the supernatant is measured.

Antigen-antibody complexes are precipitated either by crosslinking with a second antibody or by means of the addition of reagents that promote the precipitation of antigen-antibody complexes. Counting radioactivity in the precipitates allows the determination of the amount of radiolabeled antigen precipitated with the antibody. A standard curve is constructed by plotting the percentage of antibody-bound radiolabeled antigen against known concentrations of a standardized unlabeled antigen, and the concentrations of antigen in patient samples are extrapolated from that curve.

The extremely high sensitivity of RIA is its major advantage:
Uses of Radioimmunoassay

1. The test can be used to determine very small quantities (e.g. nanogram) of antigens and antibodies in the serum.
2. The test is used for quantitation of hormones, drugs, HBsAg, and other viral antigens.
3. Analyze nanomolar and picomolar concentrations of hormones in biological fluids.

The limitations of the RIA include:

1. The cost of equipment and reagents
2. Short shelf-life of radiolabeled compounds
3. The problems associated with the disposal of radioactive waste.

Skin Test

Allergic diseases, such as allergic rhinitis and atopic dermatitis, are caused by abnormal responses to substances such as pollens and foods. These substances, when recognized by the cells and antibodies that cause an allergic response, are called allergens. It is essential to the care of allergic patients to determine which allergens may be inciting their disease because this information is used to direct allergy prevention and treatment. This determination starts with a good history and physical examination, which provide important clues. For example, a patient who awakens every day with itchy, watery eyes and a stuffy nose but who otherwise feels well likely has an allergy to the dust mite allergens that are present in his or her bedding; however, a patient with similar symptoms that vary with the seasons likely has a pollen allergy. Another patient, for example, who experiences shortness of breath, mouth and tongue swelling, and an urticarial rash after eating shellfish very likely has a food allergy to shellfish. With the

information provided by a good history and physical, it is possible to identify specific allergies that a patient is likely to have and, subsequently, to order specific, cost-effective allergy testing.

The allergist has a variety of tests to identify the allergens that may be responsible for an individual's allergic disease. These tests include in vivo skin tests, such as prick and intradermal skin tests, and in vitro tests, such as radioallergosorbent tests (RAST). The choice of which appropriate tests to order and perform requires a fundamental understanding of the allergic response, the mechanism of action of in vivo and in vitro testing, and the relative risks and benefits of the different tests available.

Immune complex in tissue demonstration

Clinical and laboratory observations have strongly suggested that leukocytoclastic angitis is an immune complex disease. Since immune complexes can be visualized as electron-dense deposits by electron microscopy (EM), this method was used in conjunction with direct immunofluorescence (IF) to determine whether complexes could be demonstrated in spontaneous lesions, and in uninvolved skin in which the vessels were made permeable by the local injection of histamine. Histamine-induced wheals were produced in the uninvolved skin of patients with active angitis. In the resulting wheal, EM studies revealed electron-dense deposits characteristic of immune complexes in postcapillary venules and direct IF studies demonstrated complement and immunoglobulins in the vessel walls. Neutrophils in varying stages of disintegration were present thereby reproducing the histopathologic changes of spontaneous lesions. EM and IF studies of nonmanipulated uninvolved skin also revealed electron-dense deposits and immune reactants in the vessel walls. Neutrophils were not present, however. This observation indicates that immune complexes are deposited in vessels before tissue damage ensues. Study of spontaneous lesions older than 24 hr revealed only fibrin by EM and no immune reactants by direct IF. In spontaneous lesions less than 24 hr old, electron-dense deposits and fibrin were seen by EM, and complement and immunoglobulins by IF. Histamine-induced wheals should be a useful device to investigate patients with disorders that have an immune complex pathogenesis.

UNIT – V
HYPERSENSITIVITY

Hypersensitivity reactions

Hypersensitivity reactions (HR) are immune responses that are exaggerated or inappropriate against an antigen or allergen. Coombs and Gell classified hypersensitivity reactions into four forms. Type I, type II, and type III hypersensitivity reactions are known as immediate hypersensitivity reactions (IHR) because thy occur within 24 hours. Antibodies including IgE, IgM, and IgG mediate them.

Type I or Anaphylactic Response

Anaphylactic Responseis mediated by IgE antibodies that are produced by the immune system in response to environmental proteins (allergens) such as pollens, animal danders or dust mites. These antibodies (IgE) bind to mast cells and basophils, which contain histamine granules that are released in the reaction and cause inflammation. Type I hypersensitivity reactions can be seen in bronchial asthma, allergic rhinitis, allergic dermatitis, food allergy, allergic conjunctivitis, and anaphylactic shock.

Anaphylaxis

Anaphylaxis is a medical emergency because can lead to an acute, life-threatening respiratory failure. It is an IgE-mediated process. It is the most severe form of an allergic reaction, where mast cells suddenly release a large amount of histamine and later on leukotrienes. In severe cases intense bronchospasm, laryngeal edema, cyanosis, hypotension, and shock are present.

Allergic bronchial asthma

Allergic bronchial asthma is an atopic disease, characterized by bronchospasm. It may also be a chronic inflammatory disease. In its etiology, and environmental factors along with a genetic background play an important role. The diagnosis is dependent on history and examination. In allergic bronchial asthma, IgE is elevated, and sputum eosinophilia is common. Epidemiologically, a positive skin prick test or specific IgE are risk factors for asthma.

Allergic rhinitis

Allergic rhinitis is another atopic disease where histamine and leukotrienes are responsible for rhinorrhea, sneezing and nasal obstruction. Allergens are similar to those found in bronchial asthma. Nasal polyps may be seen in chronic rhinitis.

Allergic conjunctivitis

Allergic conjunctivitis presents with rhinitis and is IgE-mediated. Itching and eye problems including watering, redness, and swelling always occur.

Food allergy

One must differentiate food allergy (IgE-mediated) from food intolerance that can be cause for a variety of etiology including malabsorption and celiac disease. It is more frequent in children as seen in cow's milk allergy. Food allergy symptoms mostly affect the respiratory tract, the skin, and the gut. Skin prick tests are helpful to test for food allergens that can trigger severe reactions, e.g., peanuts, eggs, fish, and milk.

Atopic eczema

Atopic eczema is an IgE-mediated disease that affects the skin and has an immunopathogenesis very similar to that of allergic asthma and allergic rhinitis, which are present in more than half of the diseased. Radioallergosorbent (RAST) may reveal the specificity of the IgE antibody involved but has little help in management.

Drug allergy

Drugs may cause allergic reactions by any mechanism of hypersensitivity. For example, penicillin may cause anaphylaxis, which is IgE-mediated but must responses be trivial. Penicillin cross-reacts with other semisynthetic penicillins including monobactams and carbapenems and may also cross-react with other antibiotics such as cephalosporins.

Type II or Cytotoxic-Mediated Response

IgG and IgM mediate cytotoxic-mediated response against cell surface and extracellular matrix proteins. The immunoglobulins involved in this type of reaction damages cells by activating the complement system or by phagocytosis. Type II hypersensitivity reactions can be seen in immune thrombocytopenia, autoimmune hemolytic anemia, and autoimmune neutropenia.

Immune thrombocytopenia (ITP)

ITP is an autoimmune disorder that occurs at any age. Phagocytes destroy sensitized platelets in the peripheral blood. Clinically, it manifests by thrombocytopenia with shortened platelet survival and increased marrow megakaryocytes. Sudden onset of petechiae and bleeding from the gums, nose, bowel, and urinary tract occurs. Bleeding can accompany infections, drug reactions, malignancy and other autoimmune disorders such as thyroid disease and SLE.

Autoimmune hemolytic anemia (AIHA)

There are two types of immune hemolytic anemia: IgG-mediated (warm AIHA) and IgM-mediated (cold AIHA). The warm type may be idiopathic autoimmune or secondary to other diseases such as malignancy affecting the lymphoid tissues. The cold type may be idiopathic or secondary to infections such as Epstein-Barr virus. The primary clinical sign of the two is jaundice. The laboratory diagnosis is made by a positive Coombs test, which identifies immunoglobulins and C3 on red blood cells.

Autoimmune neutropenia

Autoimmune neutropenia may be present with bacterial and fungal infections, or it may occur alone or with autoimmune diseases (SLE, RA, autoimmune hepatitis), infections and lymphoma. Bone marrow examination is needed if neutropenia is severe. For associated autoimmune disorders, an autoimmune antibody panel is necessary (ANA, ENA, and dsDNA).
Hemolytic disease of the fetus and the newborn (erythroblastosis fetalis).

The maternal immune system suffers an initial sensitization to the fetal Rh+ red blood cells during birth, when the placenta tears away. The first child escapes disease but the mother, now sensitized, will be capable of causing a hemolytic reaction against a second Rh+ fetus, which develops anemia and jaundice once the maternal IgG crosses the placenta.

Myasthenia gravis is an autoimmune disorder caused by antibodies to post-synaptic acetylcholine receptors that interfere with the neuromuscular transmission. It is characterized by extreme muscular fatigue, double vision, bilateral ptosis, deconjugate eye movements, difficulty swallowing, and weakness in upper arms. Babies born to myasthenic mothers can have transient muscle weakness due to pathogenic IgG antibodies that cross the placenta.

Goodpasture syndrome

Goodpasture syndrome is a type II hypersensitivity reaction characterized by the presence of nephritis in association with lung hemorrhage. In most patients, it is caused by cross-reactive autoantigens that are present in the basement membranes of the lung and kidney. A number of patients with this problem exhibit antibodies to collagen type IV, which is an important component of basement membranes.

Pemphigus

Pemphigus causes a severe blistering disease that affects the skin and mucous membranes. The sera of patients with pemphigus have antibodies against desmoglein-1 and desmoglein-3, which are components of desmosomes, which form junctions between epidermal cells. Pemphigus is strongly linked to HLA-DR4 (DRB1*0402), which is a molecule that presents one of the autoantigens involved in the immunopathogenesis of this disease (desmoglein-3).

Type III or Immunocomplex Reactions

These are also mediated by IgM and IgG antibodies that react with soluble antigens forming antigen-antibody complexes. The complement system becomes activated and releases chemotactic agents that attract neutrophils and cause inflammation and tissue damage as seen in vasculitis and glomerulonephritis. Type III hypersensitivity reactions can classically be seen in serum sickness and Arthus reaction.

Serum sickness

Serum sickness can be induced with massive injections of foreign antigen. Circulating immune complexes infiltrate the blood vessel walls and tissues, causing an increased vascular permeability and leading to inflammatory processes such as vasculitis and arthritis. It was a complication of anti-serum prepared in animals to which some individuals produced antibodies to the foreign protein. It was also experienced in the treatment with antibiotics such as penicillin.

Arthus reaction

Arthus reaction is a local reaction seen when a small quantity of antigens is injected into the skin repeatedly until detectable levels of antibodies (IgG) are present. If the same antigen is inoculated, immune complexes develop at the mentioned local site and in the endothelium of small vessels. This reaction is characterized by the presence of marked edema and hemorrhage, depending on the administered dose of the foreign antigen.

Etiology

Multiple causes of IHR depend on the type of antigen or allergen that trigger this inappropriate immune reactivity. In type I hypersensitivity reactions, the allergens are proteins with a molecular weight ranging from 10 to 40 kDa. These include cats, dust mite, German cockroaches, grass, rats, fungi, plants, and drugs. They stimulate the IgE production.

Bee and wasp venoms, tree nuts (e.g., almond, hazelnut, walnut, and cashew), eggs, milk, latex, antibiotics (e.g., cephalosporins), heterologous antisera, hormones (e.g., insulin) and others including shellfish and anesthetics can trigger anaphylaxis.

In type II hypersensitivity reactions, the antigens can be found in the membrane of erythrocytes (e.g., A, B, O, C, c, D, d, E, e, K, k, Fy, M, and N). In transfusion reactions, all blood groups are not equally antigenic, e.g., A or B evoke stronger hypersensitivity reactions in an incompatible recipient than other antigens such as Fy.In type III hypersensitivity reactions, the persistence of antigen from chronic infection or autoimmune diseases can develop complex immune diseases, including vasculitis and glomerulonephritis. Penicillin as an antigen can produce any hypersensitivity reactions, e.g., anaphylactic shock, hemolytic anemia, and serum sickness.

Epidemiology

Hypersensitivity reactions are very common. Fifteen percent of the world population will be affected by any type of allergic reaction during their lives. In the second half of this century, allergic diseases have increased. The cause of the increase is unknown, but it may reflect lifestyle changes, decreased breastfeeding, and air pollution. The hygiene hypothesis proposes that since IgE is no longer needed to protect against parasites in the Western world, the IgE-mast cell axis has evolved in type I hypersensitivity reaction.

European data estimate that 0.3% of the population will be troubled by anaphylaxis at some point in their lives. In addition, 1 out of 3000 inpatients in the United States experiences a severe allergic reaction every year. However, the prevalence of bronchial asthma was 1.5% in Korea. Fernández-Soto et al., 2018 reported that fungal infections could be as high as 50% in inner cities and constitute a risk factor predisposed to the development of allergic bronchial asthma.Worldwide epidemiological data of anaphylaxis are scanty and remain unavailable in many countries.

Pathophysiology

In type I hypersensitivity reactions after a previous sensitization, the immunoglobulin (Ig) E is produced and binds to Fc receptors on mast cells and basophils. On encountering the allergen, it triggered cross-linking of mast-cell cytophilic IgE, causing the activation of mast cells and their degranulation of mediators that cause an allergic reaction. The mediators that participate in this type of hypersensitivity reaction include histamine and lipid mediators such as PAF, LTC4, and PGD2 that cause a vascular leak, bronchoconstriction, inflammation, and intestinal hypermotility. Enzymes (e.g., tryptase causes tissue damage) and TNF causes inflammation. Eosinophils release cationic granule proteins, e.g., major basic protein (causes killing of host cells and parasites) and enzymes (e.g., eosinophil peroxidase, which participates in tissue remodeling).

In type II hypersensitivity reaction antibodies against basement membranes produce nephritis in Goodpasture's syndrome. Myasthenia gravis and Lambert-Eaton syndrome are caused by antibodies that reduce the amount of acetylcholine at motor endplates, and autoantibodies to an intercellular adhesion molecule cause pemphigus.In type III hypersensitivity reactions immune-complex deposition (ICD) causes autoimmune diseases, which is often a complication. As the disease progresses a more accumulation of immune-complexes occur, and when the body becomes overloaded the complexes are deposited in the

tissues and cause inflammation as the mononuclear phagocytes, erythrocytes, and complement system fail to remove immune complexes from the blood.

Histopathology

Human basophils contains multi-lobed nuclei and distinctive granules. They can be found in local tissues including the nose, lungs, skin or gut in response to allergic and immune responses. The two populations of mast cells are mucosal and connective tissue. They have morphological and pharmacological differences. The mucosal mast cells can associate with a parasitic infestation, and connective tissue mast cells are smaller and live shorter. Both contain histamine and serotonin in their granules. Skin biopsy of patients with allergic dermatitis shows inflammatory infiltrate with few eosinophils, but their degranulation in the skin demonstrated in the biopsy stained with antibodies against eosinophil major basic protein (MBP). In the nasal smear of a patient with acute bronchial asthma, an infiltrate consistent of eosinophils, and polymorphonuclear cells with a normal cytoplasm stained with hematoxylin and eosin were shown.

In type II hypersensitivity reactions, autoantibodies bind to desmosome involved in cell adhesion, and autoantibodies in diabetes mellitus bind to islet cells. They can be demonstrated in tissues by immunofluorescence. The method that uses fluorescent antibodies has also been used in type III hypersensitivity reactions to demonstrate the presence of immune complexes in the intima and media of the arterial wall, as well as IgG and C3 deposits in kidney, joints, arteries, and skin. In Goodpasture syndrome, the antibodies involved are IgG and have the capacity to fix complement. Necrosis of the glomerulus, with fibrin deposition, is a major feature of this syndrome.

History and Physical

In type I hypersensitivity reactions there is a history of atopy or a patient suffering from an allergic condition (e.g., bronchial asthma, allergic rhinitis, or food allergy). It may associate with recurrent infections caused by viruses and bacteria. For instance, bronchial asthma may link to recurrent bacterial pneumonia. Clinically allergic disorders may accompany by airways inflammation, wheezing attack, bronchial hyper-responsiveness, tachycardia, tachypnea, intense itching of the eyes and nose, sneezing, rhinorrhea, dermatitis, and gastrointestinal symptoms. Anaphylaxis, the most severe type of allergy, is clinically characterized by bronchospasm, angioedema, hypotension, loss of consciousness, generalized skin rash, nausea, vomiting, and abdominal cramps among other symptoms.

In type II hypersensitivity reactions, a patient may report multiple blood transfusions, rhesus incompatibility, and drug history. Clinically, it may manifest as autoimmunity, e.g., autoimmune hemolytic anemia (characterized by jaundice), immune thrombocytopenia (characterized by bleeding disorders), and other blood dyscrasia (autoimmune neutropenia). In this type of hypersensitivity, drugs may attach to red blood cells and stimulate the production of anti-red blood cell antibodies or anti-dsDNA antibody that causes drug-induced systemic lupus erythematosus (SLE).

Type III hypersensitivity reactions may manifest as immune complex-mediated diseases including glomerulonephritis, vasculitis, serositis, arthritis, and skin manifestations of autoimmunity such as malar rash, which is due to photosensitivity. The prevalence of serum sickness has decreased dramatically because animal anti-serum is rarely used to treat or

prevent infectious diseases. General manifestations of disease including anorexia, loss of weight, and asthenia may report in IHR.

Evaluation

The evaluation of immediate hypersensitivity includes complete blood cell count, assessment of immunoglobulins, skin prick test, and detection of autoantibodies.

Quantitative Serum Immunoglobulins

- IgG (involved in Type II and III HR)
- IgM (involved in Type II and III HR)
- IgE (elevated in allergic diseases)

Total Leukocyte Count and Differential:

- Hb (decreased in autoimmune hemolytic anemia)
- Neutrophils (decreased in autoimmune neutropenia)
- Lymphocytes (decreased in autoimmune lymphopenia)
- Platelets (decreased in immune thrombocytopenia)

Autoimmunity Studies

- Anti-nuclear antibodies (ANA, present in systemic autoimmune disorders, such as SLE and RA)
- Detection of specific auto-immune antibodies for systemic disorders, e.g., anti-ds DNA, rheumatoid factor, anti-histones, anti-Smith, anti-(SS-A) and anti-(SS-B)
- Detection of anti-RBC, antiplatelet, and anti-neutrophil antibodies
- Testing for organ-specific auto-immune antibodies, e.g., the anti-Islet cell autoantibody that is present in diabetes mellitus
- Coombs test (positive in autoimmune hemolytic anemia)

Allergic test

- Skin prick tests using various allergens from animal, plants, food, pathogens and environmental pollutants
- Radioallergosorbent test (RAST): Use to determine specific IgE antibodies

Treatment / Management

The treatment of immediate hypersensitivity reactions includes the management of anaphylaxis with intramuscular adrenaline (epinephrine), oxygen, intravenous (IV) antihistamine, support blood pressure with IV fluids, avoid latex gloves and equipment in patients who are allergic, and surgical procedures such as tracheotomy if there is severe laryngeal edema. Allergic bronchial asthma can be treated with any of the following: inhaled short- and long-acting bronchodilators (anticholinergics) along with inhaled corticosteroids, leukotriene antagonists, use of disodium cromoglycate, and environmental control. Experimentally, a low dose of methotrexate or cyclosporin and omalizumab (a monoclonal anti-IgE antibody) has been used. Treatment of autoimmune disorders (e.g., SLE) include one or a combination of NSAIDs and hydroxychloroquine, azathioprine, methotrexate, mycophenolate, cyclophosphamide, low dose IL-2, intravenous immunoglobulins, and belimumab. Omalizumab is a monoclonal antibody that interacts with the binding site of the high-affinity IgE receptor on mast cells. It is an engineered, humanized recombinant immunoglobulin. Moderate to severe allergic bronchial asthma can improve with omalizumab.

Differential Diagnosis

Allergic bronchial asthma must be ruled out from other classes of asthma based on the family history of atopy and a positive skin prick test. Chronic allergic bronchial asthma loses reversibility and is indistinguishable from chronic obstructive pulmonary disease (COPD).

Allergic rhinitis must rule out other causes of rhinitis including vasomotor, non-allergic rhinitis with eosinophilia, drug-induced (cocaine abuse), mechanical (tumors, foreign body, sarcoidosis) and infectious including viral, bacterial and leprosy. In allergic rhinitis, IgE is elevated, and prick test is positive for similar allergens as those in allergy bronchial asthma. Also, family predisposition to allergies may be present.

Autoimmune hemolytic anemia (AIHA) can rule out from other anemias based on the presence of a positive direct Coombs' test. Sometimes the AIHA is secondary to lymphoma or autoimmune disease, especially SLE, where other blood dyscrasias including immune thrombocytopenia and autoimmune neutropenia may be present besides with the presence of anti-dsDNA antibodies, and clinical signs including malar rash, nephropathy, vasculitis, serositis, neuropathy, and among other problems.

Prognosis

The prognosis of IHR depends on the severity of the disorders, the extension of the inflammation and tissue damage, and the available treatment and their effectiveness to control the disease. Relapsing or slow progression characterizes myasthenia gravis. If present with thymoma, 68% of the affected have a 5-year survival. In SLE, approximately 80% survive for 15 years if treated. Atopic eczema (dermatitis) is usually most severe in infancy and improves with age in 80% of the cases. Allergic bronchial asthma that does not respond to steroids has a reserved prognosis.

The prognosis of other allergic disorders, including food allergy, drug allergy, latex allergy, allergic conjunctivitis, and allergic rhinitis is good once the triggers identified using skin prick test or RAST and treatment with anti-histamine occurs. The use of monoclonal antibodies directed to IgE (e.g., omalizumab) has improved the prognosis of patients that do not respond well to conventional therapy, although the acquisition of these biologicals is expensive. The use of vaccines, some classic and recently experimental, is another avenue of treatment of allergic disorders that improve the life expectancy and quality of individuals with allergies.

Complications

Some of the complications of immediate hypersensitivity reactions are:

Status Asthmaticus

This is a type I hypersensitivity reaction, an acute exacerbation of bronchial asthma that does not respond to the standard therapy with bronchodilators. It is a medical emergency and must require aggressive treatment.

Anaphylactic Shock

This is an allergic reaction, often life-threatening, triggered by an allergen to which the immune system over-reacts.

Post-Transfusion Reaction

This is a hypersensitivity reaction that occurs within 24 hours of a blood transfusion. Hemoglobinuria that appears during or after the procedure becomes an alarming sign. Other manifestations include back pain, fever, chills, dizziness, and dyspnea.

Serum Sickness

This is a type III hypersensitivity reaction that commences after the administration of a drug (e.g., penicillin) or heterologous anti-serum or plasma. Clinically, it is characterized by skin rash, fever, arthralgias, or arthritis. Immune-complexes mediate this complication, and it may affect many organs.

MHC and Transplantation

The transplant of organs is one of the greatest therapeutic achievements of the twentieth century. In organ transplantation, the adaptive immunity is considered the main response exerted to the transplanted tissue, since the principal target of the immune response is the MHC (major histocompatibility complex) molecules expressed on the surface of donor cells. However, we should not forget that the innate and adaptive immunities are closely interrelated and should be viewed as complementary and cooperating. When a human transplant is performed, HLA (human leukocyte antigens) molecules from a donor are recognized by the recipient's immune system triggering an alloimmune response Matching of donor and recipient for MHC antigens has been shown to have a significant positive effect on graft acceptance.

The primary function of the immune system is to protect the host from infectious microbes in its environment. This system has evolved over millions of years, in response of coexistence with microorganisms. Basically, the system can be divided in two components, the innate and adaptive immunities.

The innate also called natural immunity refers to a nonspecific response that involves the recruitment of diverse components of the immune system such as macrophages, neutrophils, natural killer cells (NK cells), cytokines, several cellular receptors, complement components, cytokines, Toll-like receptors (TLRs), and antimicrobial peptides (AMPs). This response is phylogenetically older in comparison to the adaptive immunity, which involves recognition of specific antigen, conferring both specificity and a memory effect. The main effectors of the adaptive immunity are the T and B cells. T cells recognize antigen in the form of peptide bound to major histocompatibility complex (MHC) molecules. B cells have immunoglobulin receptors that recognize the antigenic portions of determined molecules.

In organ transplantation, the adaptive immunity is considered the main response exerted to the transplanted tissue, since the principal target of the immune response is the MHC molecules expressed on the surface of donor cells. However, we should not forget that the innate and adaptive immunities are divided only by educational purposes, since both are codependent. For example, T-cell activation leads to the production of cytokines and chemokines which in turn may recruit components of the innate immunity like NK cells or macrophages. Furthermore, local tissue production of complement components seems to be essential for full T-cell activation, and some AMPs like defensins and cathelicidin have chemoattractant properties on T lymphocytes.

Because the immune system uses many different effector mechanisms to destroy the broad range of microbial cells and particles that it encounters, it is critical for the immune

response to avoid unleashing these destructive mechanisms against its own tissues. This avoidance of destruction of self-tissues is referred to as self-tolerance. Mechanisms to avoid reaction against self-antigens are expressed in many parts of both the innate and the adaptive immune responses. Failure of self-tolerance underlies the broad class of autoimmune diseases. Unfortunately, transplanted tissues from individuals of the same species (allogenic) or different species (xenogeneic) are recognized as nonself, causing graft rejection. The process by which the immune system recognizes pathogens, tumors, and transplantation antigens involves the same antigen recognition molecules.

Transplantation Antigens

The rejection response to grafted tissue is caused by cell surface molecules that induce an antigenic stimulus. A wide variety of transplantation antigens have been described, including the MHC molecules, minor histocompatibility antigens, ABO blood group antigens, and monocytes/endothelial cell antigens. The minor histocompatibility antigens are processed peptides derived from cellular antigens that are presented by MHC molecules but are not derived from the MHC. ABO compatibility is of much less importance than MHC compatibility in graft survival. However, ABO incompatibility can result in hyperacute rejection of primarily vascularized grafts, such as kidney and heart. The principal target of the transplantation immune response is the MHC molecules expressed on the surface of donor cells.

The Major Histocompatibility Complex

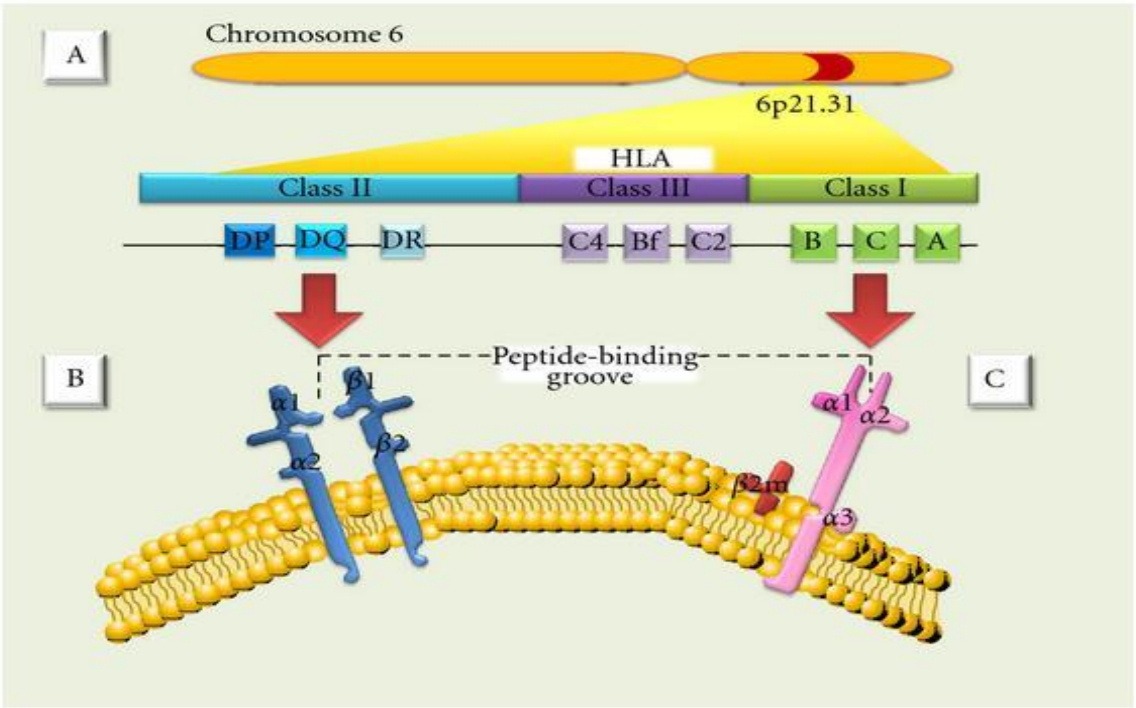

Figure 5.1

According to their relative potencies in eliciting rejection, the major antigens in mammalian species are encoded by a closely linked series of genes called MHC. In humans, these genes reside in the short arm of chromosome 6. Organs transplanted between MHC identical individuals are readily accepted, whereas organs transplanted between MHC antigen-mismatched individuals are rejected in the absence of immunosuppressive therapy . Since the

MHC was first defined in mice by Gorer and Snell, the World Health Organization Nomenclature Committee has named HLA (human leukocyte antigen) to the human MHC.

MHC (major histocompatibility complex). (B) Class II antigens are expressed only on B lymphocytes, activated T lymphocytes, monocytes, macrophages, Langerhans cells, dendritic cells, endothelium, and epithelial cells. They are heterodimers composed of noncovalently associated α and β polypeptide chains chains encoded by genes of the HLA-D region.Class I MHC antigens are present on all nucleated cells and are composed of a 45-kd transmembrane α heavy chain encoded by genes of the HLA-A, HLA-B, or HLA-C loci on chromosome 6.

The HLA complex genes and their protein products have been divided into three classes (I, II, and III) on the basis of their tissue distribution, structure, and function. MHC class I and II genes encode codominantly expressed HLA cell surface antigens, and class III genes encode several components of the complement system; all share important roles in immune function. Class I MHC antigens are present on all nucleated cells and are composed of a 45-kd transmembrane α heavy chain encoded by genes of the HLA-A, HLA-B, or HLA-C loci on chromosome 6; the α heavy chains are associated noncovalently with a 12-kd protein, β2-microglobulin, encoded by a gene on chromosome 15. Additional (nonclassical) class I molecules, like those encoded by the HLA-E, -F, -G, -H loci, have been described and show limited variability and tissue distribution. The precise functions of these molecules are not yet clear, although they have been implied in presenting carbohydrate and peptide fragments to γδ T cells and mother's immunological tolerance of the fetus . MHC class II antigens are expressed only on B lymphocytes, activated T lymphocytes, monocytes, macrophages, Langerhans cells, dendritic cells, endothelium, and epithelial cells. Class II molecules are heterodimers composed of noncovalently associated α and β polypeptide chains chains encoded by genes of the HLA-D region. There are 3 major class II proteins designated, HLA-DP, HLA-DQ, and HLA-DR. Class III genes are located between the HLA-B and HLA-D loci and determine the structure of three components of the complement system: C2, C4, and factor B. Class I MHC molecules present cytoplasm-derived peptides, or intracellular parasites, principally viruses; whereas MHC class II molecules bind peptides derived from extracellular proteins. HLA class I and II molecules are recognized by CD8 and CD4 positive T cells, respectively. Also, NK cells may recognize HLA classical and nonclassical type I.

HLA antigens are inherited in a Mendelian dominant manner. HLA genes are almost always inherited together, thus the antigens of the entire HLA region inherited from one parent collectively are called haplotype. Because chromosome 6 is an autosome (a chromosome with two pairs), all individuals have two HLA haplotypes (one for each chromosome). According to this, any sibling pair has a 25% chance of inheriting the same two parental haplotypes, a 50% chance of sharing one haplotype, and a 25% chance of having two completely different haplotypes. All children are haploidentical with each parent .

Since the biologic function of the HLA molecules is presenting endogenous and exogenous antigens, they manifest high structural polymorphism. Until 2010, 2558 HLA class I and II alleles have been recognized. Mutations in microbial antigens might permit the microbe to avoid binding (and, consequently, recognition) by a few HLA alleles, but no mutations will permit the microbe to avoid recognition broadly throughout the population; assuring then, the continuity of species in the presence of pandemic infection.