COURSE CODE:	ABG 506
COURSE TITLE:	BIOCHEMICAL GENETICS
NUMBER OF UNITS:	2 Units
COURSE DURATION:	Two hours per week

COURSE DETAILS:

Course Coordinator: **Prof. Olufunmilayo, A. Adebambo**
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Office Location: Department of Animal Breeding and Genetics
Other Lecturers: **Dr. A.O. Adebambo**

COURSE CONTENT:

The Chemistry of Genetics, Genetics of disease resistance, Immunoglobulins, Immune response, Humoral-cell mediated histocompatibility system, Genetic regulation of disease, Genetic regulation of immune responsiveness, Blood groups, Disease resistance in animals, The immune system.

COURSE REQUIREMENTS:

This is a required course for all students in the Department of Animal Breeding and Genetics. In view of this, students are expected to participate in all the lectures and practical and must meet up with a minimum of 75% attendance to qualify for the final examination.

READING LIST:

Hartl. D.L. and Jones, E.W. 2005.Genetics: Analysis of genes and genomes, 6th edition, Jones and Bartlett publishers, Inc. Pg. 854.

Klung, W.S. and Cummings, M.R. 2000. Genetics, 6th edition, Prentice Hall, Inc. pg. 816.

Stansfield, W.D. 1986. Schaum's outiline of theory and problems of genetics, 2nd edition, McGraw-Hill Book Company. Pg. 392.

SECTION A: Prof Adebambo: Immunology

INTRODUCTION

Immunology

Immunology is a broad branch of biomedical science that covers the study of all aspects of the immune system in all organisms. It deals with, among other things, the physiological functioning of the immune system in states of both health and disease; malfunctions of the immune system in immunological disorders (autoimmune diseases, hypersensitivities, immune deficiency, transplant rejection); the physical, chemical and physiological characteristics of the components of the immune system in vitro, in situ, and in vivo. Immunology has applications in several disciplines of science, and as such is further divided.

The immune system

The key primary lymphoid organs of the immune system are thymus and bone marrow, and secondary lymphatic tissues such as spleen, tonsils, lymph vessels, lymph nodes, adenoids, and skin. When health conditions warrant, immune system organs including the thymus, spleen, portions of bone marrow, lymph nodes and secondary lymphatic tissues can be surgically excised for examination while patients are still alive.

Many components of the immune system are actually cellular in nature and not associated with any specific organ but rather are embedded or circulating in various tissues located throughout the body.

IMMUNITY

The earliest written mention of immunity can be traced back to the plague of Athens in 430 BCE. Thucydides noted that people who had recovered from a previous bout of the disease could nurse the sick without contracting the illness a second time. Many other ancient societies have references to this phenomenon, but it was not until the 19th and 20th centuries before the concept developed into scientific theory.

The study of the molecular and cellular components that comprise the immune system, including their function and interaction, is the central science of immunology.

THE IMMUNE SYSTEM

The immune system has been divided into a more primitive innate immune system, and acquired or adaptive immune system of vertebrates, the latter of which is further divided into humoral and cellular components.

Components of the immune system	
Innate immune system	Adaptive immune system
Response is non-specific	**Pathogen and antigen specific response**
Exposure leads to immediate maximal response	**Lag time between exposure and maximal response**
Cell-mediated and humoral components	**Cell-mediated and humoral components**
No immunological memory	**Exposure leads to immunological memory**
Found in nearly all forms of life	**Found only in jawed vertebrates**

Both innate and adaptive immunity depend on the ability of the immune system to distinguish between self and non-self molecules. In immunology, *self* molecules are those components of an organism's body that can be distinguished from foreign substances by the immune system. Conversely, *non-self* molecules are those recognized as foreign molecules. One class of non-self molecules are called antigens (short for *anti*body *gen*erators) and are defined as substances that bind to specific immune receptors and elicit an immune response.

The humoral (antibody) response is defined as the interaction between antibodies and antigens. Antibodies are specific proteins released from a certain class of immune cells (B lymphocytes). Antigens are defined as anything that elicits generation of antibodies, hence they are **Anti**body **Gen**erators. Immunology itself rests on an understanding of the properties of these two biological entities. However, equally important is the cellular response, which can not only kill infected cells in its own right, but is also crucial in controlling the antibody response. Put simply, both systems are highly interdependent.

In the 21st century, immunology has broadened its horizons with much research being performed in the more specialized niches of immunology. This includes the immunological function of cells, organs and systems not normally associated with the immune system, as well as the function of the immune system outside classical models of immunity, such as in clinical, developmental and reproductive immunology.

Clinical immunology

Clinical immunology is the study of diseases caused by disorders of the immune system (failure, aberrant action, and malignant growth of the cellular elements of the system). It also involves diseases of other systems, where immune reactions play a part in the pathology and clinical features.

The diseases caused by disorders of the immune system fall into two broad categories: immunodeficiency, in which parts of the immune system fail to provide an adequate response (examples include chronic granulomatous disease), and autoimmunity, in which the immune system attacks its own host's body (examples include systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's disease and myasthenia gravis). Other immune system disorders include different hypersensitivities, in which the system responds inappropriately to harmless compounds (asthma and other allergies) or responds too intensely.

The most well-known disease that affects the immune system itself is AIDS, caused by HIV. AIDS is an immunodeficiency characterized by the lack of CD4+ ("helper") T cells and macrophages, which are destroyed by HIV. Clinical immunologists also study ways to prevent transplant rejection, in which the immune system attempts to destroy allografts or xenografts.

Developmental Immunology

Adolescence is the age or biological time at which the human body starts to develop from an infantile form to a fully-grown adult. During this time several physical, physiological and immunological changes start to occur inside the developing human body. These changes are started and mediated by different hormones. Depending on the sex either testosterone or 17-β-oestradiol, act on male and female bodies accordingly, start acting at ages of 12 and 10 years (2). There is evidence that these steroids act directly not only on the primary and secondary sexual characteristics, but also have an effect on the development and regulation of the immune system (3). There is an increased risk in developing autoimmunity for pubescent and post pubescent females and males (4). There is also evidence of cell surface receptors on T cells and Macrophages that detect sex hormones in the system (5). The female sex hormone 17-β-oestradiol has been shown to regulate the level of immunological response (6). For example, compared to adult females, there is a greater drop in IgG levels than in those of IgA during the follicular phase of the menstrual cycle in adolescent females; also, other immune cells, like macrophages and Antigen Presenting Cells (APC), seem to respond to this fluctuation of 17-β-oestradiol, specifically in the mucosal layer on the womb of post pubescent females (7). It has been suggested that this level of control is achieved by the stimulation of peripheral blood monocytes cells (PBMC) by 17-β-oestradiol (7). Some male androgens, like testosterone, seem to suppress the stress response to infection; but other androgens like DeHydroepiandrosteron(DHEA) have the opposite effect, as it increases the immune response instead of down playing it (8). As in females, the male sex hormones seem to have more control of the immune system during puberty and the time right after than in fully developed adults. Other than hormonal changes physical changes like the involution of the Thymus during puberty will also affect the immunological response of the subject or patient (9). These differences occurring not only during development but also in sex hormones makes the development of vaccines or antitoxins extra challenging for the immunologist

because it simply presents more variables to take into account at the time of designing and testing new treatment and vaccines (8)

Neonates are said to be in a state of physiological immunodeficiency, because both their innate and adaptive immunological responses are greatly suppressed. In fact, many of the infections they acquire are caused by low virulence organisms like Staphylococcus and Pseudomonas. In neonates, opsonic activity and the ability to activate the complement cascade is very limited. For example, the mean level of C3 in a newborn is approximately 65% of that found in the adult. Phagocytic activity is also greatly impaired in newborns. This is not only due to lower opsonic activity, but mainly to a diminished up-regulation of integrin and selectin receptors, which limit the ability of neutrophils to interact with adhesion molecules in the endothelium. Their monocytes are slow and have a reduced ATP production, which also limits the newborns phagocitic activity. Although, the number of total lymphocytes is significantly higher than in adults, the cellular and humoral immunity is also impaired. Antigen presenting cells in newborns have a reduced capability to activate T cells. Also, T cells of a newborn proliferate poorly and produce very little amount, if any, of cytokines like IL-2, IL-4, IL-5, IL-12, and IFN-g which limits their capacity to activate the humoral response as well as the phagocitic activity of macrophage. B cells develop early in gestation but are not fully active. At birth most of the immunoglobulin present is maternal IgG. Because IgM, IgD, IgE and IgA don't cross the placenta, they are almost undetectable at birth. By breast feeding the mother provides the newborn with some IgA. The passively acquired antibodies can protect the newborn up to 18 months, but their response is usually short-live and of low affinity.

ABILITY TO RESPOND TO ANTIGENIC CHALLENGES

The body's capability to react to antigen depends on:

- age (of the person),
- antigen type,
- maternal factors and
- the area where the antigen is presented.

AGE

Once born, a child's immune system responds favorably to protein antigens but not as well to glycoproteins and polysaccharies. By 6-9 months after birth, a child's immune system begins to respond better (more strongly) to glycoproteins. Not until 12-24 months of age is there a marked improvement in the body's response to polysaccharides.

This can be the reason for the specific time frames found in vaccination schedules.

MATERNAL FACTOR

Maternal factors also play a role in the body's immune response. As we know a child receives antibodies from the mother through breast milk and through the placenta. These antibodies have a beneficial and a negative response. If a child is exposed to the antibody for a particular antigen before being exposed to the antigen itself then the child will have a dampened response. According to Jaspen, the passively acquired maternal antibodies suppress the antibody response to active immunization. (We see it as not giving the child a chance to experience the antigen for itself; therefore the child is not exposed to as many antigen possibilities were it to experience the real thing).

Similarly the response of T-cells to vaccination differs in children compared to adults. "Vaccines that induce Th1 responses in adults do not readily elicit neonatal Th1 responses".

AREA OF ANTIGEN PRESENTATION

Location where the antigen is found by the body is also an important factor. This is due to the ability (or lack thereof) of APC's to migrate to specific tissue. An example given is that when an antigen is presented in mucosa the local cells will have access to the antigen, and transfer that antigen to a central lymphatic node where it will be presented (simultaneously, APC's generally have a harder time reaching mucosa). Nevertheless, antigen found in the mucosa of the nasal cavity will induce a more wide spread response by activating both a mucosal and systemic response

resulting in a response in the nasal lymphoid tissue, saliva and female genital tract [12]. In terms of general vaccination, the cellular response to live vaccines generally induces a stronger immune response unless the aforementioned circumstances are present.

Evolutionary immunology

Study of the immune system in extant and extinct species is capable of giving us a key understanding of the evolution of species and the immune system.

A development of complexity of the immune system can be seen from simple phagocytotic protection of single celled organisms, to circulating antimicrobial peptides in insects to lymphoid organs in vertebrates. Of course, like much of evolutionary observation, these physical properties are often seen from the anthropocentric aspect. It should be recognized that every organism living today has an immune system absolutely capable of protecting it from most forms of harm; those organisms that did not adapt their immune systems to external threats are no longer around to be observed.

Insects and other arthropods, while not possessing true adaptive immunity, show highly evolved systems of innate immunity, and are additionally protected from external injury (and exposure to pathogens) by their chitinous shells.

Reproductive immunology

This area of the immunology is devoted to the study of immunological aspects of the reproductive process including fetus acceptance. The term has also been used by fertility clinics to address fertility problems, recurrent miscarriages, premature deliveries, and dangerous complications such as pre-clampsia.

Major discoveries in the study of humoral immunity

Substance	Activity	Discovery
Alexin(s) Complement	Soluble components in the serum that are capable of killing microorganisms	Buchner (1890), Ehrlich (1892)
Antitoxins	Substances in the serum that can neutralize the activity of toxins, enabling passive immunization	von Behring and Kitasato (1890)
Bacteriolysins	Serum substances that work with the complement proteins to induce bacterial lysis	Richard Pfeiffer (1895)
Bacterial agglutinins & precipitins	Serum substances that agglutinate bacteria and precipitate bacterial toxins	von Gruber and Durham (1896), Kraus (1897)
Hemolysins	Serum substances that work with complement to lyse red blood cells	Belfanti and Carbone (1898) Jules Bordet (1899)
Opsonins	serum substances that coat the outer membrane of foreign substances and enhance the rate of phagocytosis by macrophages	Wright and Douglas (1903)
Antibody	formation (1900), antigen-antibody binding hypothesis (1938), produced by B cells (1948), structure (1972), immunoglobulin genes (1976)	Founder: P Ehrlich

THE THYMUS

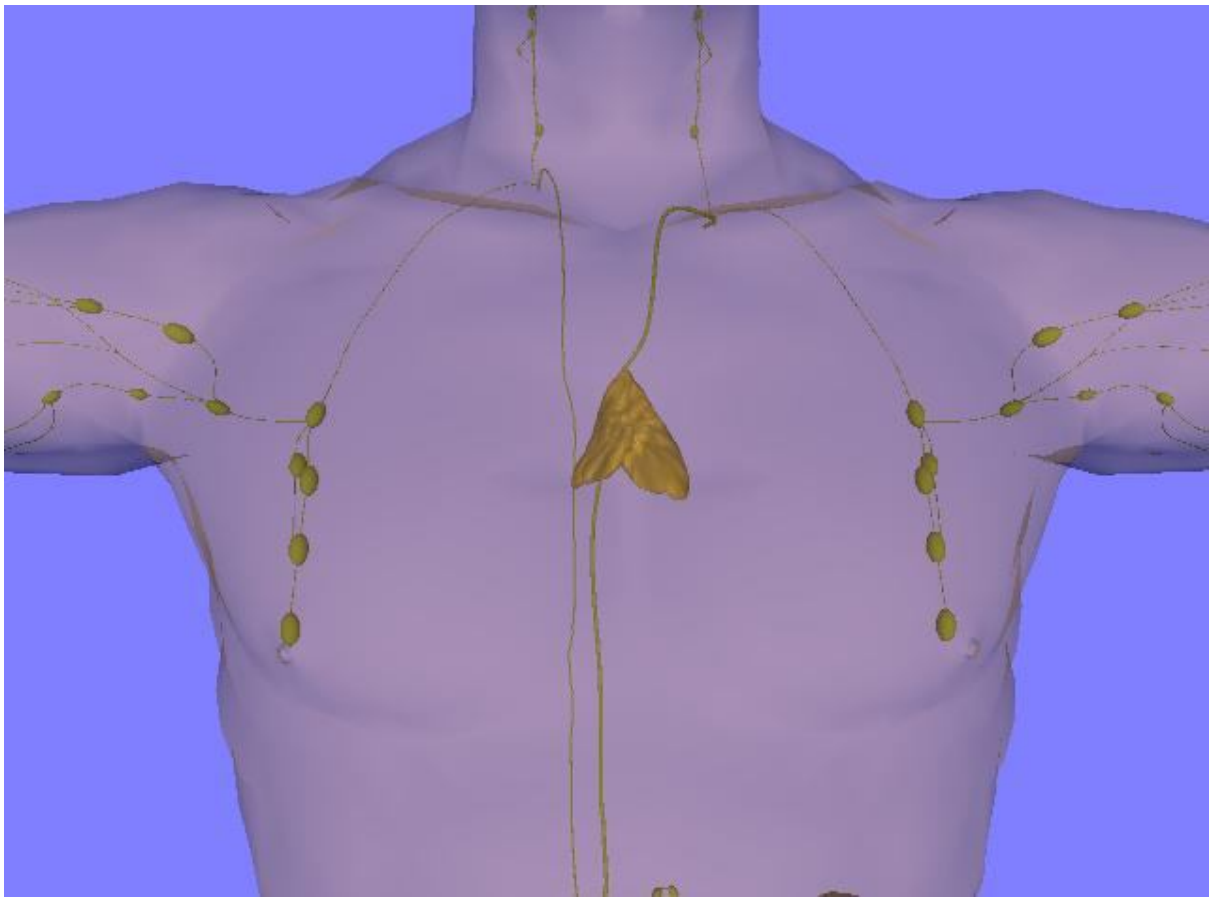


Fig 1. Anterior view of chest showing location and size of adult thymus

In human anatomy, the **thymus** is an organ located in the upper anterior portion of the chest cavity just behind the sternum. The main function of the thymus is to provide an area for maturation, and is vital in protecting against autoimmunity.

The thymus was known to the Ancient Greeks. Galen was the first to note that the size of the organ changed over the duration of a person's life.

Due to the large numbers of apoptotic lymphocytes, the thymus was originally dismissed as a "lymphocyte graveyard", without functional importance. The importance of the thymus in the immune system was discovered in 1961 by Jacques Miller, by surgically removing the thymus from three day old mice, and observing the subsequent deficiency in a lymphocyte population, subsequently named T cells after the organ of their origin. Recently, advances in immunology have allowed the function of the thymus in T cell maturation to be more fully understood.

Function

In the two thymic lobes, lymphocyte precursors from the bone-marrow become thymocytes, and subsequently mature into T cells. Once mature, T cells emigrate from the thymus and constitute the peripheral T cell repertoire responsible for directing many facets of the adaptive immune system. Loss of the thymus at an early age through genetic mutation (as in DiGeorge Syndrome) or surgical removal results in severe immunodeficiency and a high susceptibility to infection.

The stock of T-lymphocytes is built up in early life, so the function of the thymus is diminished in adults. It is largely degenerated in elderly adults and is barely identifiable, consisting mostly of fatty tissue, but it continues to function as

an endocrine gland important in stimulating the immune system. Involution of the thymus has been linked to loss of immune function in the elderly, susceptibility to infection and to cancer.

The ability of T cells to recognize foreign antigens is mediated by the T cell receptor. The T cell receptor undergoes genetic rearrangement during thymocyte maturation, resulting in each T cell bearing a unique T cell receptor, specific to a limited set of peptide:MHC combinations. The random nature of the genetic rearrangement results in a requirement of central tolerance mechanisms to remove or inactivate those T cells which bear a T cell receptor with the ability to recognise self-peptides.

Phases of thymocyte maturation

The generation of T cells expressing distinct T cell receptors occurs within the thymus, and can be conceptually divided into three phases:

1. A rare population of hematopoietic progenitor cells enter the thymus from the blood, and expands by cell division to generate a large population of immature thymocytes.
2. Immature thymocytes each make distinct T cell receptors by a process of gene rearrangement. This process is error-prone, and some thymocytes fail to make functional T cell receptors, whereas other thymocytes make T cell receptors that are autoreactive. Growth factors include thymopoietin and thymosin.
3. Immature thymocytes undergo a process of selection, based on the specificity of their T cell receptors. This involves selection of T cells that are *functional (positive selection)*, and elimination of T cells that are *autoreactive (negative selection)*.

THE BONES

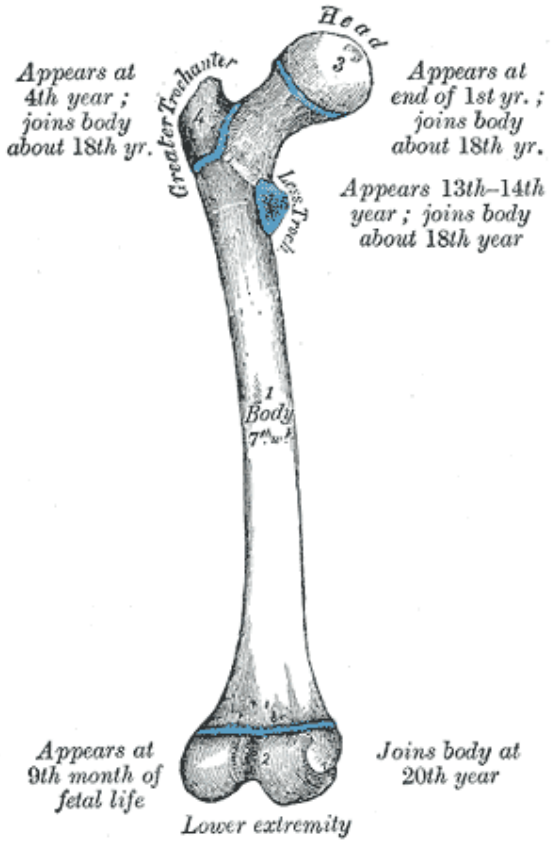


Fig 2. Illu compact spongy bone.

Bones are rigid organs that form part of the endoskeleton of vertebrates. They function to move, support, and protect the various organs of the body, produce red and white blood cells and store minerals. Bone tissue is a type of dense connective tissue. Because bones come in a variety of shapes and have a complex internal and external

structure they are lightweight, yet strong and hard, in addition to fulfilling their many other functions. One of the types of tissue that makes up bone is the mineralized osseous tissue, also called bone tissue, that gives it rigidity and a honeycomb-like three-dimensional internal structure. Other types of tissue found in bones include marrow, endosteum and periosteum, nerves, blood vessels and cartilage. There are 206 bones in the adult human body[1] and 270 in an infant.

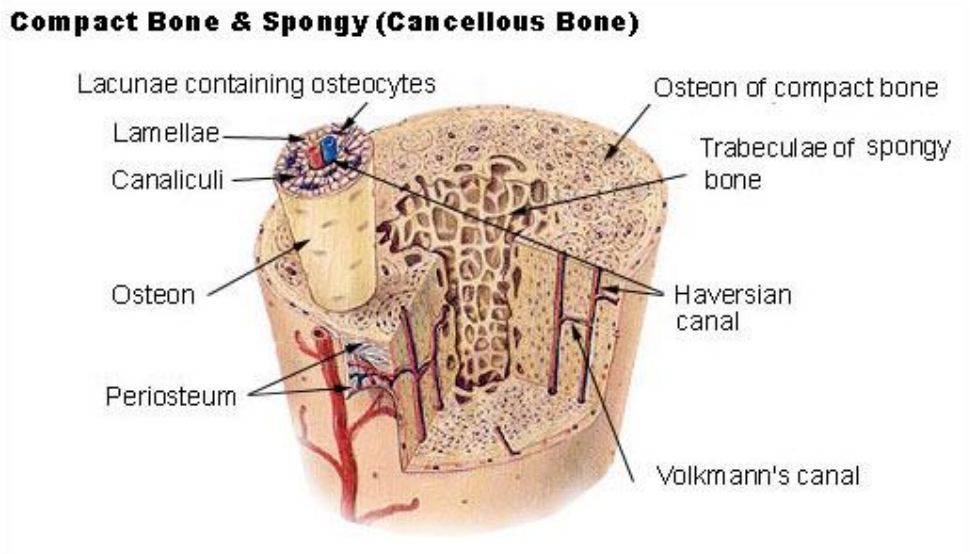


Fig 3.Caput femoris cortex medulla.

Functions of the bone

Bones have eleven main functions:

Mechanical

- Protection — Bones can serve to protect internal organs, such as the skull protecting the brain or the ribs protecting the heart and lungs.
- Shape — Bones provide a frame to keep the body supported.
- Movement — Bones, skeletal muscles, tendons, ligaments and joints function together to generate and transfer forces so that individual body parts or the whole body can be manipulated in three-dimensional space. The interaction between bone and muscle is studied in biomechanics.
- Sound transduction — Bones are important in the mechanical aspect of overshadowed hearing.

Synthetic

- Blood production — The marrow, located within the medullary cavity of long bones and interstices of cancellous bone, produces blood cells in a process called haematopoiesis.

Metabolic

- Mineral storage — Bones act as reserves of minerals important for the body, most notably calcium and phosphorus.
- Growth factor storage — Mineralized bone matrix stores important growth factors such as insulin-like growth factors, transforming growth factor, bone morphogenetic proteins and others.
- Fat Storage — The yellow bone marrow acts as a storage reserve of fatty acids
- Acid-base balance — Bone buffers the blood against excessive pH changes by absorbing or releasing alkaline salts.
- Detoxification — Bone tissues can also store heavy metals and other foreign elements, removing them from the blood and reducing their effects on other tissues. These can later be gradually released for excretion.
- Endocrine organ - Bone controls phosphate metabolism by releasing fibroblast growth factor - 23 (FGF-23), which acts on kidney to reduce phosphate reabsorption.

Characteristics

The primary tissue of bone, osseous tissue, is a relatively hard and lightweight composite material, formed mostly of calcium phosphate in the chemical arrangement termed calcium hydroxylapatite (this is the osseous tissue that gives bones their rigidity). It has relatively high compressive strength but poor tensile strength of 104-121 MPa, meaning it resists pushing forces well, but not pulling forces. While bone is essentially brittle, it does have a significant degree of elasticity, contributed chiefly by collagen. All bones consist of living and dead cells embedded in the mineralized organic *matrix* that makes up the osseous tissue.

Bone marrow

Bone marrow is the flexible tissue found in the hollow interior of bones. In adults, marrow in large bones produces new blood cells. It constitutes 4%[1] of total body weight, i.e. approximately 2.6 kg (5.7 lbs.) in adults.

Anatomy

Marrow types

There are two types of bone marrow: **red marrow** (consisting mainly of myeloid tissue) and **yellow marrow** (consisting mainly of fat cells).

Red blood cells, platelets and most white blood cells arise in red marrow. Both types of bone marrow contain numerous blood vessels and capillaries.

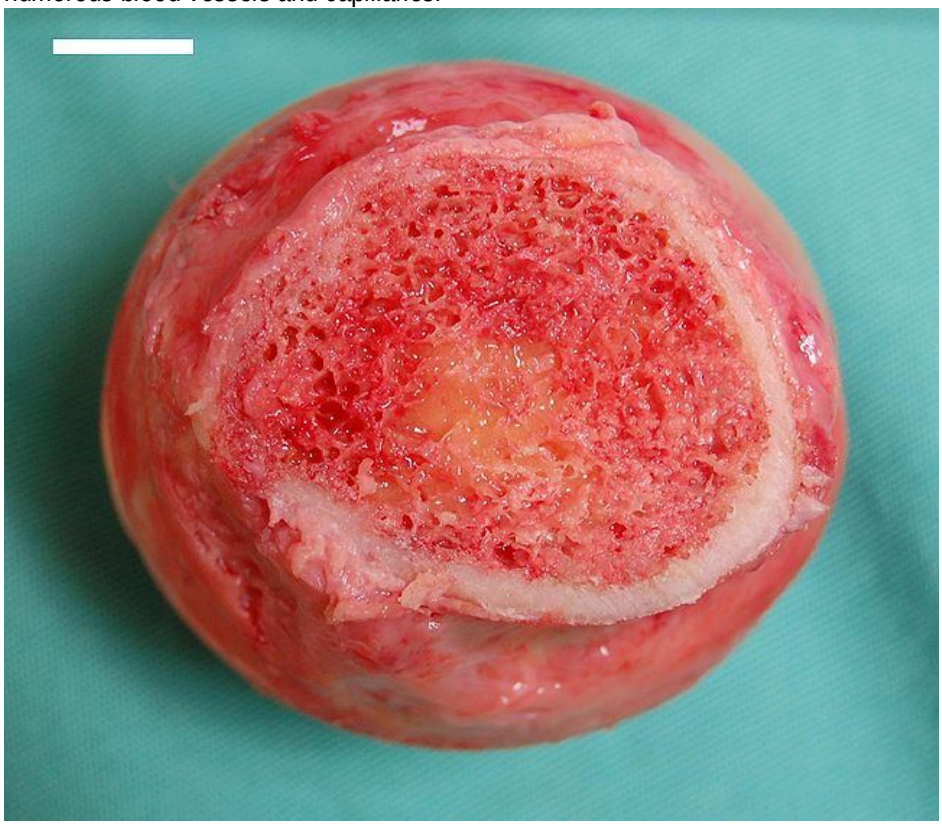


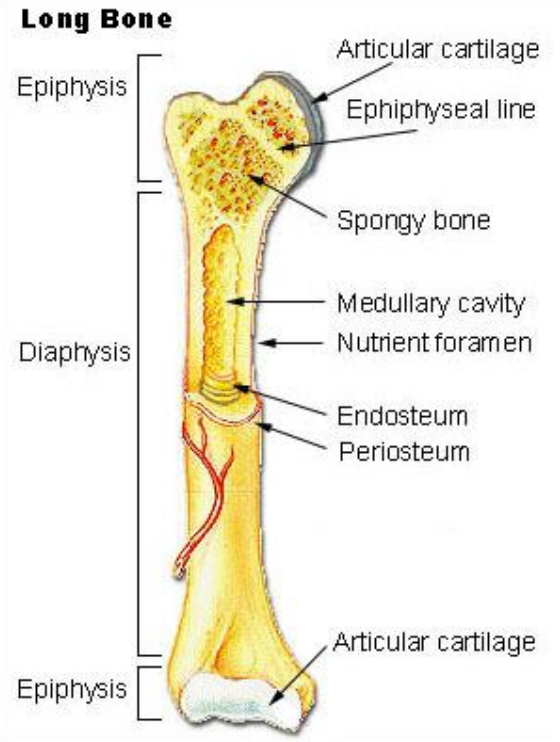
Fig 4.A femur head with a cortex of compact bone and medulla of trabecular bone.

At birth, all bone marrow is red. With age, more and more of it is converted to the yellow type. About half of adult bone marrow is red.[1] Red marrow is found mainly in the flat bones, such as the hip bone, breast bone, skull, ribs, vertebrae and shoulder blades, and in the cancellous ("spongy") material at the epiphyseal ends of the long bones such as the femur and humerus. Yellow marrow is found in the hollow interior of the middle portion of long bones.

In cases of severe blood periods, the body can convert yellow marrow back to red marrow to increase blood cell production.

Stroma

The *stroma of the bone marrow* is all tissue not directly involved in the primary function of hematopoiesis. The yellow bone marrow belongs here, and makes the majority of the bone marrow stroma, in addition to stromal cells located in the red bone marrow. Yellow bone marrow is found in the Medullary cavity.



Still, the stroma is indirectly involved in hematopoiesis, since it provides the *hematopoietic microenvironment* that facilitates hematopoiesis by the parenchymal cells. For instance, they generate colony stimulating factors, affecting hematopoiesis.

Cells that constitute the bone marrow stroma are:

- fibroblasts (reticular connective tissue)
- macrophages
- adipocytes
- osteoblasts
- osteoclasts
- endothelial cells forming the sinusoids

Macrophages contribute especially to red blood cell production. They deliver iron for hemoglobin-production.

Bone marrow barrier

The blood vessels constitute a barrier, inhibiting immature blood cells from leaving the bone marrow. Only mature blood cells contain the membrane proteins required to attach to and pass the blood vessel endothelium.

Hematopoietic stem cells may also cross the bone marrow barrier, and may thus be harvested from blood .

Stem cells

Mesenchymal stem cell

The bone marrow stroma contain *mesenchymal stem cells* (also called *marrow stromal cells*). These cells are multipotent stem cells that can differentiate into a variety of cell types. Cell types that MSCs have been shown to differentiate into in vitro or in vivo include osteoblasts, chondrocytes, myocytes, adipocytes, and, as described lately, beta-pancreatic islets cells. They can also transdifferentiate into neuronal cells.

Compartmentalization

There is biologic compartmentalization in the bone marrow, in that certain cell types tend to aggregate in specific areas. For instance, erythrocytes, macrophages and their precursors tend to gather around blood vessels, while granulocytes gather at the borders of the bone marrow.

Types of stem cells

Bone marrow contains three types of stem cells:

- Hematopoietic stem cells give rise to the three classes of blood cells that are found in the circulation: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes).
- Mesenchymal stem cells are found arrayed around the central sinus in the bone marrow. They have the capability to differentiate into osteoblasts, chondrocytes, myocytes, and many other types of cells. They also function as "gatekeeper" cells of the bone marrow.

- Endothelial stem cells

Diseases involving the bone marrow

The normal bone marrow architecture can be displaced by:

- malignancies or infections such as tuberculosis, leading to a decrease in the production of blood cells and blood platelets.
- cancers of the hematologic progenitor cells in the bone marrow, these are the leukemias.

To diagnose diseases involving the bone marrow, a bone marrow aspiration is sometimes performed. This typically involves using a hollow needle to acquire a sample of red bone marrow from the crest of the ilium under general or local anesthesia. The average number of cells in a leg bone is about 440,000,000,000 ($440x10^9$).

Exposure to radiation or chemotherapy will kill many of the rapidly dividing cells of the bone marrow and will therefore result in a depressed immune system. Many of the symptoms of radiation sickness are due to damage to the bone marrow cells.

Bone marrow examination

Bone marrow examination is the pathologic analysis of samples of bone marrow obtained by *bone marrow biopsy* and *bone marrow aspiration*. Bone marrow examination is used in the diagnosis of a number of conditions, including leukemia, multiple myeloma, anemia, and pancytopenia. The bone marrow produces the cellular elements of the blood, including platelets, red blood cells and white blood cells. While much information can be gleaned by testing the blood itself (drawn from a vein by phlebotomy), it is sometimes necessary to examine the source of the blood cells in the bone marrow to obtain more information on hematopoiesis; this is the role of bone marrow aspiration and biopsy.

Donation and transplantation of bone marrow

It is possible to take hematopoietic stem cells from one person and then infuse them into another person (Allogenic) or into the same person at a later time (Autologous). If donor and recipient are compatible, these infused cells will then travel to the bone marrow and initiate blood cell production.

Transplantation from one person to another is performed in severe cases of disease of the bone marrow. **The patient's marrow is first killed off with drugs or radiation,** and then the new stem cells are introduced.

Before radiation therapy or chemotherapy in cases of cancer, **some of the patient's hematopoietic stem cells are sometimes harvested and later infused back when the therapy is finished to restore the immune system.**

Harvesting

The stem cells are harvested directly from the red marrow in the crest of the ilium, often under general anesthesia. The procedure is minimally invasive and does not require stitches afterwards. Depending on the donor health and reaction to the procedure, the actual harvesting can be an outpatient procedure or requiring 1–2 days of recovery in the hospital.[3] Another option is to administer certain drugs that stimulate the release of stem cells from the bone marrow into circulating blood.[4] An IV is inserted into the donor's arm, and the stem cells are filtered out of the blood. The procedure is similar to donating blood or platelets.

It may also be taken from the sternum. The tibia may seem a good source, since it is very superficial. However, except in children, this bone marrow does not contain any substantial amount of red bone marrow, only yellow bone marrow.[1]

In newborns, stem cells may be retrieved from the umbilical cord.[5]

Bone marrow as a food

Many cultures utilize bone marrow as a food. The Vietnamese prize bone as the soup base for their national staple *phở*; Alaskan Natives eat the bone marrow of caribou and moose; Indians use slow-cooked marrow as the core ingredient of the Indian dish Nalli Nihari; Mexicans use beef bone marrow from leg bones, called tuetano, which is cooked and served as filling for tacos or tostadas; it is also considered to be the highlight of the Italian dish ossobuco (braised veal shanks). Though once used in various preparations, including pemmican, bone marrow has fallen out of favor as a food in the United States. In the Philippines, the soup "Bulalo" is made primarily of beef stock and marrow bones, seasoned with choice vegetables and boiled meat.

Diners in the 18th century used a marrow scoop (or marrow spoon), often of silver and with a long thin bowl, as a table implement for removing marrow from a bone.

Some anthropologists believe that early humans were scavengers rather than hunters. Marrow would then have been a major protein source for tool-using hominids, who were able to crack open the bones of carcasses left by top predators such as lions.

The Antigen-presenting cell (APC)

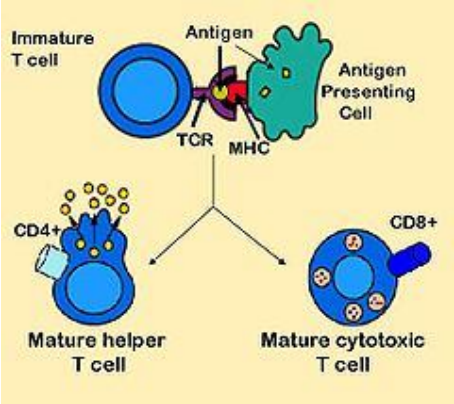


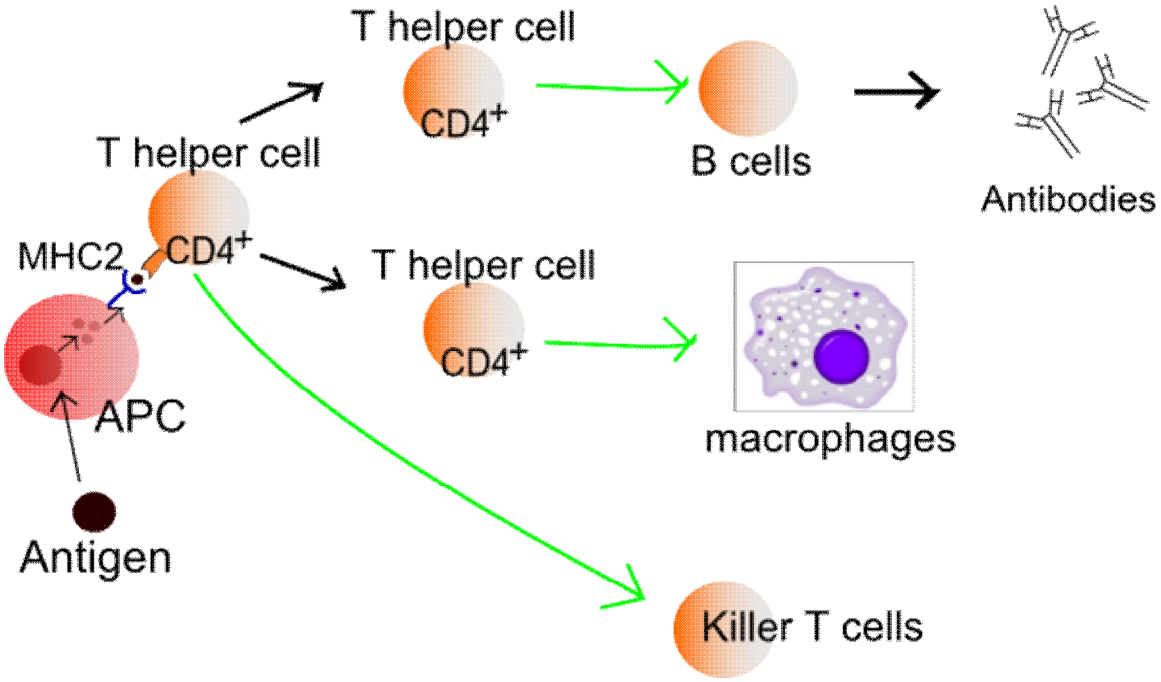
Fig 5. Antigen presentation stimulates T cells to become either "cytotoxic" CD8+ cells or "helper" CD4+ cells.

An **antigen-presenting cell** (**APC**) or **accessory cell** is a cell that displays foreign antigen complex with major histocompatibility complex (MHC) on its surface. T-cells may recognize this complex using their T-cell receptor (TCR).
Types

Most cells in the body can present antigen to $CD8^+$ T cells via MHC class I molecules and, thus, act as "APCs"; however, the term is often limited to those specialized cells that can prime T cells (i.e., activate a T cell that has not been exposed to antigen, termed a *naive T cell*). These cells, in general, express MHC class II as well as MHC class I molecules, and can stimulate $CD4^+$ ("helper") cells as well as $CD8^+$ ("cytotoxic") T cells, respectively.
To help distinguish between the two types of APCs, those that express MHC class II molecules are often called **professional antigen-presenting cells**.

APCs fall into two categories: professional or non-professional.

Lymphocyte activation



Function of T helper cells: Antigen presenting cells (APCs) present antigen on their Class II MHC molecules (MHC2). Helper T cells recognize these, with the help of their expression of CD4 co-receptor (CD4+). The activation of a resting helper T cell causes it to release cytokines and other stimulatory signals (green arrows) that stimulate the activity of macrophages, killer T cells and B cells, the latter producing antibodies. The stimulation of B cells and macrophages succeeds a proliferation of T helper cells.

Cytotoxic T cell

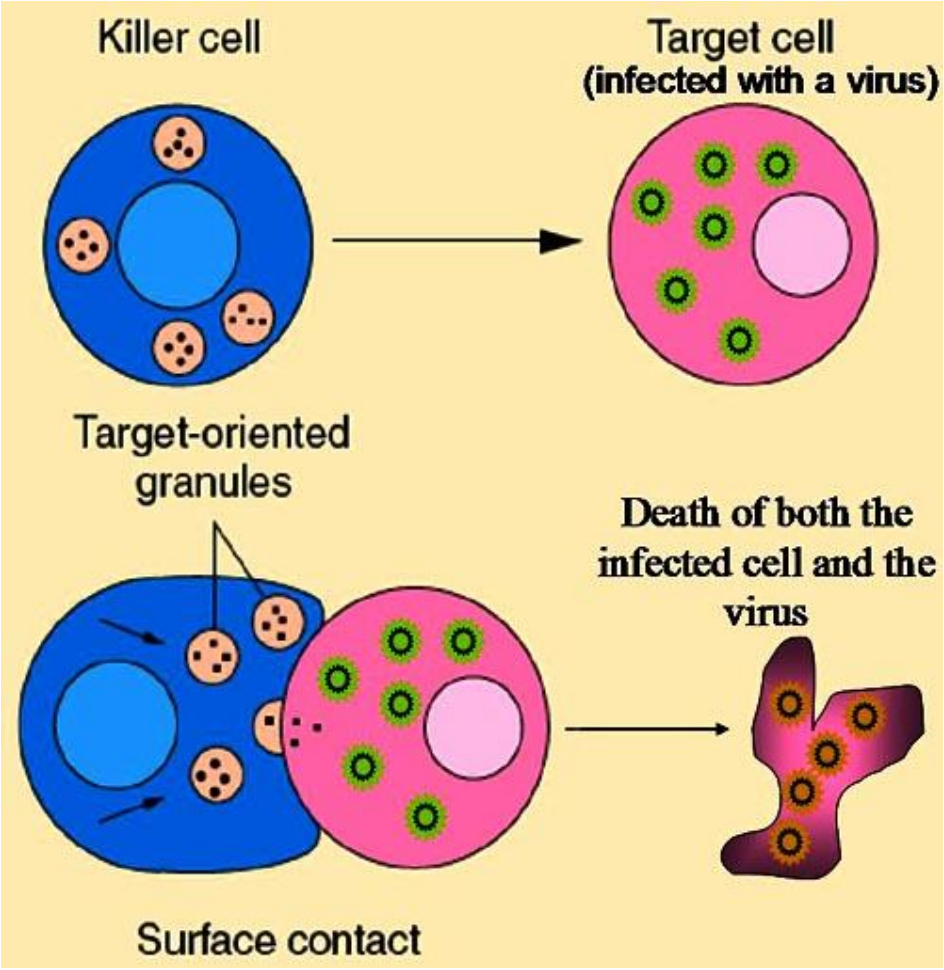


Fig 6. Killer T cells directly attack other cells carrying foreign or abnormal antigens on their surfaces.

Professional APCs

These professional APCs are very efficient at internalizing antigen, either by phagocytosis or by receptor-mediated endocytosis, and then displaying a fragment of the antigen, bound to a class II MHC molecule, on their membrane. The T cell recognizes and interacts with the antigen-class II MHC molecule complex on the membrane of the antigen-presenting cell. An additional co-stimulatory signal is then produced by the antigen-presenting cell, leading to activation of the T cell.

There are three main types of professional antigen-presenting cell:

- Dendritic cells, which have the broadest range of antigen presentation, and are probably the most important APC. Activated DCs are especially potent T_H cell activators because, as part of their composition, they express co-stimulatory molecules such as B7.
- Macrophages, which are also CD4+ and are therefore also susceptible to infection by HIV.
- B-cells, which expresses (as B cell receptor)and secretes a specific antibody, , can internalize the antigen which bind to its BCR and present it incorporated to MHC II molecule, but are inefficient APC for most other antigens.
- Certain activated epithelial cells

Non-professional

A non-professional APC does not constitutively express the Major histocompatibility complex proteins required for interaction with naive T cells; these are expressed only upon stimulation of the non-professional APC by certain cytokines such as IFN-γ. Non-professional APCs include:

- Fibroblasts (skin)

- Thymic epithelial cells
- Thyroid epithelial cells
- Glial cells (brain)
- Pancreatic beta cells
- Vascular endothelial cells

Interaction with T cells

After the APCs phagocytose pathogens, they usually migrate to the vast networks of lymph vessels and are carried via lymph flow to the draining lymph nodes (this network is collectively known as the Lymphatic system). The lymph nodes become a collection point to which APCs such as dendritic cells can interact with T cells. They do this by chemotaxis, which involves interacting with Chemokines that are expressed on the surface of cells (e.g., endothelial cells of the high endothelial venules) or have been released as chemical messengers to draw the APCs to the lymph nodes. During the migration, DCs undergo a process of maturation; in essence, they lose most of their ability to further engulf pathogens, and they develop an increased ability to communicate with T cells. Enzymes within the cell digest the swallowed pathogen into smaller pieces containing epitopes, which are then presented to T cells using MHC.

Recent research indicates that only certain epitopes of a pathogen are presented because they are immunodominant, possibly as a function of their binding affinity to the MHC. The stronger binding affinity allows the complex to remain kinetically stable long enough to be recognized by T cells.

Spleen

The **spleen** is an organ found in all vertebrate animals with important roles in regard to red blood cells and the immune system. In humans, it is located in the left upper quadrant of the abdomen. It removes old red blood cells, holds a reserve in case of hemorrhagic shock, especially in animals like horses (not in humans) and recycles iron. It synthesizes antibodies in its white pulp, removes from the circulation antibody-coated bacteria and antibody-coated blood cells. Recently it has been found to contain in reserve half the body's monocytes in its red pulp that upon moving to injured tissue such as the heart turn into dendritic cells and macrophages and aid wound healing. It is one of the centers of activity of the reticuloendothelial system, and can be considered analogous to a large lymph node. Its absence leads to a predisposition to certain infections.

Anatomy

The spleen is found in the upper left quadrant of the human abdomen. Spleens in healthy adult humans are approximately 11 centimeters in length. It usually weighs 150 grams and lies beneath the 9th to the 12th rib.

Like the thymus, the spleen possesses only efferent lymphatic vessels.

The spleen is part of the lymphatic system.

The germinal centers are supplied by arterioles called *penicilliary radicles*.

The spleen is unique with respect to its development within the gut. While most of the gut viscera are endodermally derived (with the exception of the neural-crest derived suprarenal gland), the spleen is derived from mesenchymal tissue. Specifically, the spleen forms within and from the dorsal mesentery. However, it still shares the same blood supply—the celiac trunk--as the foregut organs.

Function

Major functions of the Spleen are:

1. **The Red Pulp is responsible for the mechanical filtration of red blood cells and reservation of Monocytes**
2. **The White pulp is responsible for active immune response through Humoral and Cell Mediated pathways.**

Other functions include:

- Production of opsonins, properdin, and tuftsin.
- Creation of red blood cells. While the bone marrow is the primary site of hematopoeisis in the adult, the spleen has important hematopoietic functions up until the fifth month of gestation. After birth, erythropoietic functions cease except in some hematologic disorders. As a major lymphoid organ and a central player in the reticuloendothelial system the spleen retains the ability to produce lymphocytes and, as such, remains an hematopoietic organ.
- Storage of red blood cells and other formed elements. In horses roughly 30% of the red blood cells are stored there. The red blood cells can be released when needed. In humans, it does not act as a reservoir of blood cells. It can also store platelets in case of an emergency.
- Storage of half the body's monocytes so that upon injury they can migrate to the injured tissue and transform into dendritic cells and macrophages and so assist wound healing.

Effect of removal

Asplenia

Surgical removal causes:

- modest increases in circulating white blood cells and platelets,
- diminished responsiveness to some vaccines,
- increased susceptibility to infection by bacteria and protozoa

A 28 year follow up of 740 veterans of World War II found that those who had been splenectomised showed a significant excess mortality from pneumonia (6 from expected 1.3) and ischaemic heart-disease (41 from expected 30) but not other conditions.

Disorders

Splenic disease

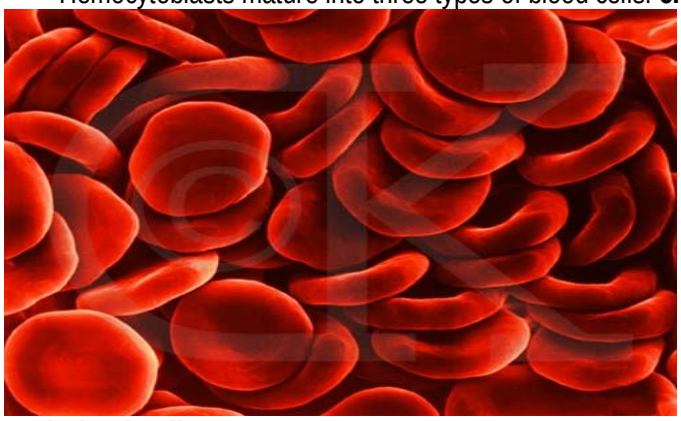
Disorders include splenomegaly, where the spleen is enlarged for various reasons, and asplenia, where the spleen is not present or functions abnormally.

Blood

Blood is composed of 52–62% liquid plasma and 38–48% cells. The plasma is mostly water (91.5%) and acts as a solvent for transporting other materials (7% protein [consisting of albumins (54%), globulins (38%), fibrinogen (7%), and assorted other stuff (1%)] and 1.5% other stuff). Blood is slightly alkaline (pH = 7.40 .05) and a tad heavier than water (density = 1.057 .009).

All blood cells are manufactured by stem cells, which live mainly in the bone marrow, via a process called **hematopoiesis**. The stem cells produce hemocytoblasts that differentiate into the precursors for all the different types of blood cells.

Hemocytoblasts mature into three types of blood cells: **erythrocytes** (red blood cells or RBCs),



Red Blood Cells

Types of white blood cells

Fig 7. Leukocytes *(white blood cells or WBCs), and* ***thrombocytes*** *(platelets).*

The leukocytes are further subdivided into **granulocytes** (containing large granules in the cytoplasm) and **agranulocytes** (without granules). The granulocytes consist of neutrophils (55–70%), eosinophils (1–3%), and basophils (0.5–1.0%). The agranulocytes are **lymphocytes** (consisting of B cells and T cells) and **monocytes**. Lymphocytes circulate in the blood and lymph systems, and make their home in the lymphoid organs.

All of the major cells in the blood system are illustrated below.

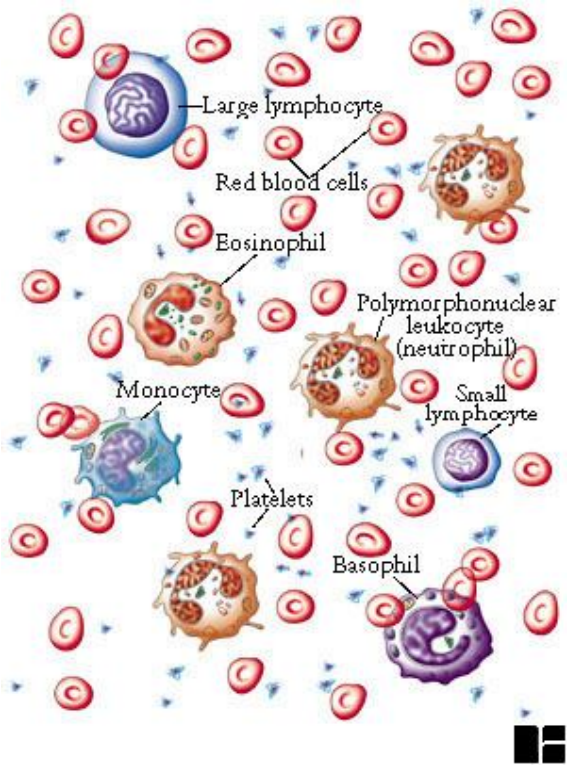


Fig. 8. Major Cells in the Blood System

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells — such as nutrients and oxygen — and transports waste products away from those same cells.

In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma, which comprises 55% of blood fluid, is mostly water (90% by volume),[1] and contains dissolved proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), platelets and blood cells themselves.

BLOOD CONSTITUENTS

The blood cells present in blood are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellularly dissolved in plasma as bicarbonate ion.

BLOOD IN OTHER ORGANISMS

Vertebrate blood is bright red when its hemoglobin is oxygenated. Some animals, such as crustaceans and mollusks, use hemocyanin to carry oxygen, instead of hemoglobin. Insects and some molluscs use a fluid called hemolymph instead of blood, the difference being that hemolymph is not contained in a closed circulatory system. In most insects, this "blood" does not contain oxygen-carrying molecules such as hemoglobin because their bodies are small enough for their tracheal system to suffice for supplying oxygen.

Jawed vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood.[2] Arthropods, using hemolymph, have hemocytes as part of their immune system.

BLOOD CIRCULATION

Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals having lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled.

Medical terms related to blood often begin with *hemo-* **or** *hemato-* **(also spelled** *haemo-* **and** *haemato-***) from the Ancient Greek word αἷμα (*haima*) for "blood". In terms of anatomy and histology, blood is considered a specialized form of connective tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen**

DEFINITIONS

A. Immunogen

A substance that induces a specific immune response.

B. Antigen (Ag)

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

C. Hapten

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

D. Epitope or Antigenic Determinant

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

E. Antibody (Ab)

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

II. FACTORS INFLUENCING IMMUNOGENICITY

A. Contribution of the Immunogen

1. Foreignness

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

2. Size

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

3. Chemical Composition

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

4. Physical form

In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.

5. Degradability

Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens, see below) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

B. Contribution of the Biological System

1. Genetic Factors

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

2. Age

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

C. Method of Administration

1. Dose

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

2. Route

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

3. Adjuvants

Substances that can enhance the immune response to an immunogen are called adjuvants. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

III. CHEMICAL NATURE OF IMMUNOGENS

A. Proteins

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

B. Polysaccharides

Pure polysaccharides and lipopolysaccharides are good immunogens.

C. Nucleic Acids

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

D. Lipids

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

IV. TYPES OF ANTIGENS

A. T-independent Antigens

T-independent antigens are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help In general, polysaccharides are T-independent antigens. The responses to these antigens differ from the responses to other antigens.

Properties of T-independent antigens

1. Polymeric structure

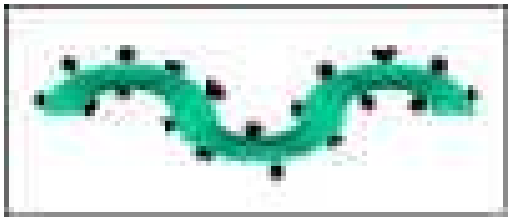
These antigens are characterized by the same antigenic determinant repeated many times as illustrated in Figure 1.

2. Polyclonal activation of B cells

Many of these antigens can activate B cell clones specific for other antigens (polyclonal activation). T-independent antigens can be subdivided into Type 1 and Type 2 based on their ability to polyclonally activate B cells. Type 1 T-independent antigens are polyclonal activators while Type 2 are not.

3. Resistance to degradation

T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.



B. T-dependent Antigens

T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are characterized by a few copies of many different antigenic determinants as illustrated in the Figure 2.

V. HAPTEN-CARRIER CONJUGATES

A. Definition

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

B. Structure

Structurally these conjugates are characterized by having native antigenic determinants of the carrier as well as new determinants created by the hapten (haptenic determinants) as illustrated in the Figure 3. The actual determinant created by the hapten consists of the hapten and a few of the adjacent residues, although the antibody produced to the determinant will also react with free hapten. In such conjugates the type of carrier determines whether the response will be T-independent or T-dependent.

VI. ANTIGENIC DETERMINANTS

A. Determinants recognized by B cells

1. Composition

Antigenic determinants recognized by B cells and the antibodies secreted by B cells are created by the primary sequence of residues in the polymer (linear or sequence determinants) and/or by the secondary, tertiary or quaternary structure of the molecule (conformational determinants).

2. Size

In general antigenic determinants are small and are limited to approximately 4-8 residues. (amino acids and or sugars). The combining site of an antibody will accommodate an antigenic determinant of approximately 4-8 residues.

3. Number

Although, in theory, each 4-8 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much lower than what would theoretically be possible. Usually the antigenic determinants are limited to those portions of the antigen that are accessible to antibodies as illustrated in the Figure 4 (antigenic determinants are indicated in black).

B. Determinants recognized by T cells

1. Composition

Antigenic determinants recognized by T cells are created by the primary sequence of amino acids in proteins. T cells do not recognize polysaccharide or nucleic acid antigens. This is why polysaccharides are generally T-independent antigens and proteins are generally T-dependent antigens. The determinants need not be located on the exposed surface of the antigen since recognition of the determinant by T cells requires that the antigen be proteolytically degraded into smaller peptides. Free peptides are not recognized by T cells, rather the peptides associate with molecules coded for by the major histocompatibility complex (MHC) and it is the complex of MHC molecules + peptide that is recognized by T cells.

2. Size

In general antigenic determinants are small and are limited to approximately 8-15 amino acids.

3. Number

Although, in theory, each 8-15 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much less than what would theoretically be possible. The antigenic determinants are limited to those portions of the antigen that can bind to MHC molecules. This is why there can by differences in the responses of different individuals.

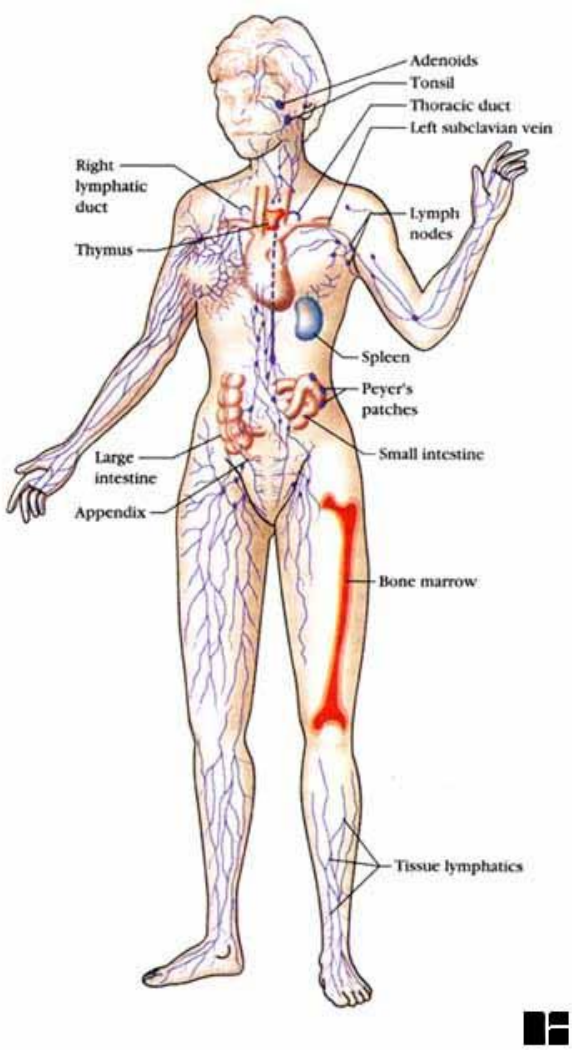


Fig 9. The Lymph System

Lymph is an alkaline (pH > 7.0) fluid that is usually clear, transparent, and colorless. It flows in the lymphatic vessels and bathes tissues and organs in its protective covering. There are no RBCs in lymph and it has a lower protein content than blood. Like blood, it is slightly heavier than water (density = 1.019 □ .003).

The lymph flows from the interstitial fluid through lymphatic vessels up to either the thoracic duct or right lymph duct, which terminate in the subclavian veins, where lymph is mixed into the blood. (The right lymph duct drains the right sides of the thorax, neck, and head, whereas the thoracic duct drains the rest of the body.) Lymph carries lipids and lipid-soluble vitamins absorbed from the gastrointestinal (GI) tract. Since there is no active pump in the lymph system, there is no back-pressure produced. The lymphatic vessels, like veins, have one-way valves that prevent backflow. Additionally, along these vessels there are small bean-shaped **lymph nodes** that serve as filters of the lymphatic fluid. It is in the lymph nodes where antigen is usually presented to the immune system.

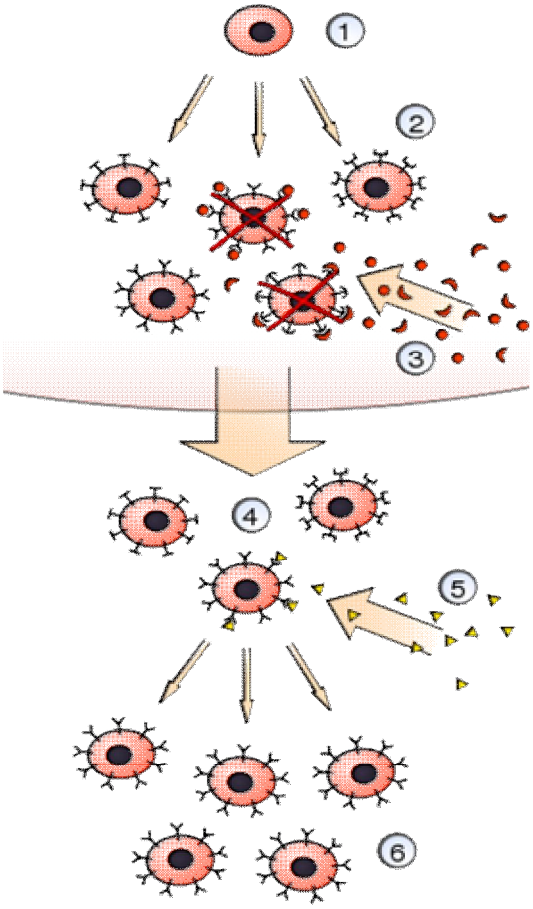
The human **lymphoid system** has the following:

* **primary organs**: bone marrow (in the hollow center of bones) and the thymus gland (located behind the breastbone above the heart), and

* **secondary organs** at or near possible portals of entry for pathogens: adenoids, tonsils, spleen (located at the upper left of the abdomen), lymph nodes (along the lymphatic vessels with concentrations in the neck, armpits, abdomen, and groin), Peyer's patches (within the intestines), and the appendix.

The clonal selection theory has become a widely accepted model for how the immune system responds to infection and how certain types of B and T lymphocytes are selected for destruction of specific antigens invading the body.

Clonal selection.



Clonal selection *of lymphocytes: 1) A hematopoietic stem cell undergoes differentiation and genetic rearrangement to produce 2) immature lymphocytes with many different antigen receptors. Those that bind to 3) antigens from the body's own tissues are destroyed, while the rest mature into 4) inactive lymphocytes. Most of these will never encounter a matching 5) foreign antigen, but those that do are activated and produce 6) many clones of themselves.*

Four Postulates of Clonal Selection Hypothesis

- Each lymphocyte bears a single type of receptor with a unique specificity.
- Receptor occupation is required for cell activation.

- The differentiated effector cells derived from an activated lymphocyte will bear receptors of identical specificity as the parental cell.
- Those lymphocytes bearing receptors for self molecules will be deleted at an early stage.

MHC expression

Overview about the correlation between the genetic origin on Chromosome 6 and the expression of the common MHC class I and II molecules in humans. Due to the codominance of the MHC alleles and the extreme polymorphism of the major histocompatibility complex genes, a great number of variant molecules is seen on the cells of one individual and throughout the whole population.

Major Histocompatibility Complex

Viruses are insidious enemies, so we must have numerous defenses against them. Antibodies are our first line of defense. Antibodies bind to viruses, mobilizing blood cells to destroy them. But what happens if viruses slip past this defense and get inside a cell? Then, antibodies have no way of finding them and the viruses are safe...but not quite.

Each cell has a second line of defense that it uses to signal to the immune system when something goes wrong inside. Cells continually break apart a few of their old, obsolete proteins and display the pieces on their surfaces. The small peptides are held in MHC, the Major Histocompatibility Complex, which grips the peptides and allow the immune system to examine them. In this way, the immune system can monitor what is going on inside the cell. If all the peptides displayed on the cell surface are normal, the immune system leaves the cell alone. But if there is a virus multiplying inside the cell, many of the MHC molecules carry unusual peptides from viral proteins, and the immune system kills the cell.

Displaying Peptides

Like many proteins used in the immune system, MHC is composed of several functional parts connected by flexible joints. The structure shown here, PDB entry 1hsa, only shows the part found on the outside of the cell. The large chain colored orange has a groove at the top, which binds to the peptide, colored red. A smaller chain, colored pink, stabilizes the structure. In the whole protein, the orange chain extends down and crosses the cell membrane at the bottom, attaching the protein to the surface of the cell. This portion of the molecule, however, is too flexible for study by x-ray crystallography and was removed for the analysis.

MHC in Action

Our itchy reaction to poison ivy is caused by the MHC system. The resins on poison ivy leaves react with proteins in the skin. These poisoned cells then break the proteins into pieces and display them using MHC molecules. The itchy rash is caused when the immune system attacks the problem. Even more serious, MHC is the cause of tissue rejection during skin grafts and organ grafts. This is how the protein got its name: the term histocompatibility refers to the difficulty of finding compatible grafts between a donor and a patient. Each person has their own collection of MHC molecules. There are hundreds of different kinds, but each person only has four types (two from each parent). If you

graft a piece of skin that has a different collection of MHC types, they will trigger the immune system to destroy the cells. So the trick is to find a compatible donor, such as a relative, who has a similar collection of MHC molecules.

The Cancer Connection

There is growing evidence that the MHC system is also important in the natural fight against cancer in your body. Cancer cells, like normal cells, display pieces of their own proteins on their surface. So, if any of these proteins carry recognizable cancer mutations, this provides a signal to the immune system that something is wrong.

MHC I vs MHC II.

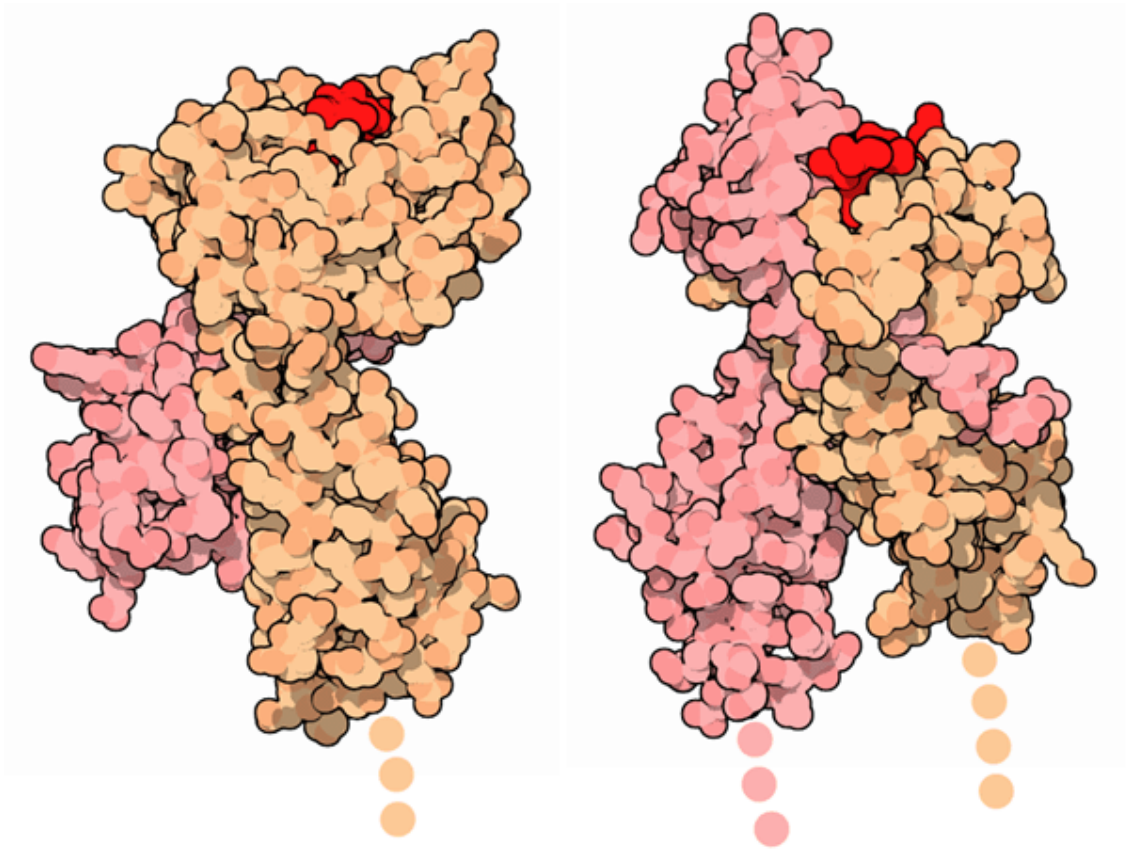
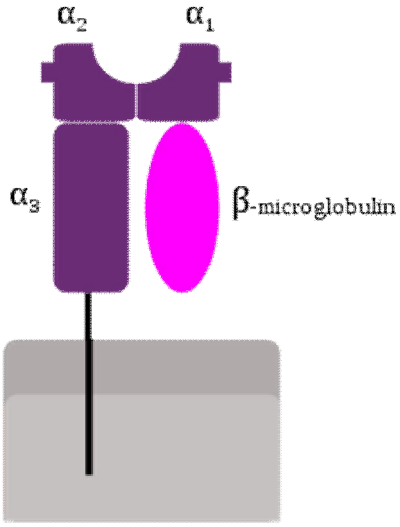


Fig 10. ***MHC_I_vs_MHC_II.***

MHC class I



Schematic representation of MHC class I

MHC class I molecules are one of two primary classes of major histocompatibility complex (MHC) molecules (the other one being simply MHC class II) and are found on every nucleated cell of the body (and thus not on red blood cells though paradoxically are found on platelets). Their function is to display fragments of proteins from within the cell to T cells; healthy cells will be ignored while cells containing foreign proteins will be attacked by the immune system. Because MHC class I molecules present peptides derived from cytosolic proteins, the pathway of MHC class I presentation is often called the *cytosolic* or *endogenous pathway*.

Structure

MHC class I molecules consist of two polypeptide chains, α and β2-microglobulin (b2m). The two chains are linked noncovalently via interaction of b2m and the α3 domain. Only the α chain is polymorphic and encoded by a HLA gene, while the b2m subunit is not polymorphic and encoded by the Beta-2 microglobulin gene. The α3 domain is plasma membrane spanning and interacts with the CD8 co-receptor of T-cells. The α1 and α2 domains fold to make up a groove for peptides to bind. MHC class I molecules bind peptides that are 8-10 amino acid in length.

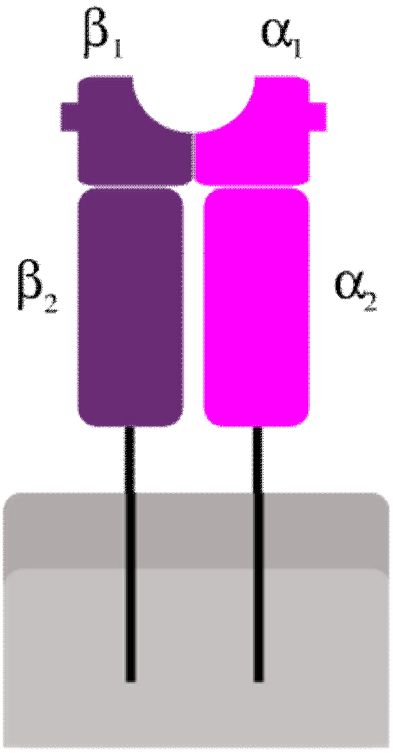
Function

MHC class I molecules bind peptides mainly generated from degradation of cytosolic proteins. The MHC-I peptide complex is then inserted into the plasma membrane of the cell. The peptide is bound to the extracellular part of the MHC-I molecule. The function of the MHC-I is thus to display to the environment, specifically cytotoxic T cells (CTLs), the proteins that are being produced within the cell.

A normal cell will display peptides from normal cellular proteins on its MHC-I, and CTLs will not be activated in response to them. When a cell expresses peptides that are not normally present in cells, such as after viral infection, these foreign peptides will be recognized by CTLs, which will become activated and kill the cell. Additionally, reduction

in the normal levels of expression of MHC-I, which can occur in some virally infected cells as well as some cancer cells, will activate Natural killer cells to destroy the cell.

MHC class II



Schematic representation of MHC class II

MHC (major histocompatibility complex) Class II molecules are found only on a few specialized cell types, including macrophages, dendritic cells and B cells, all of which are professional antigen-presenting cells (APCs).

The peptides presented by class II molecules are derived from extracellular proteins (not cytosolic as in class I); hence, the MHC class II-dependent pathway of antigen presentation is called the *endocytic* or *exogenous* pathway.

Loading of class II molecules must still occur inside the cell; extracellular proteins are endocytosed, digested in lysosomes, and bound by the class II MHC molecule prior to the molecule's migration to the plasma membrane.

Structure

Like MHC class I molecules, class II molecules are also heterodimers, but in this case consist of two homologous peptides, an α and β chain, both of which are encoded in the MHC.

Because the antigen-binding groove of MHC class II molecules is open at both ends while the corresponding groove on class I molecules is closed at each end, the antigens presented by MHC class II molecules are longer, generally between 15 and 24 amino acid residues long.

Reaction to bacteria

Because class II MHC is loaded with extracellular proteins, it is mainly concerned with presentation of extracellular pathogens (for example, bacteria that might be infecting a wound or the blood). Class II molecules interact exclusively with CD4+ ("helper") T cells (T_HC). The helper T cells then help to trigger an appropriate immune response which may

include localized inflammation and swelling due to recruitment of phagocytes or may lead to a full-force antibody immune response due to activation of B cells.

Antigen presentation

Antigen presentation and major histocompatibility complex

After the processed antigen (peptide) is complexed to the MHC molecule, they both migrate together to the cell membrane, where they are exhibited (elaborated) as a complex that can be recognized by the CD 4+ (T helper cell) – a type of white blood cell. This is known as *antigen presentation.* However, the epitopes (conformational epitopes) that are recognized by the B cell prior to their digestion may not be the same as that presented to the T helper cell. Additionally, a B cell may present different peptides complexed to different MHC-II molecules.

T helper cell stimulation

The CD 4+ cells through their T cell receptor-CD3 complex recognize the epitope-bound MHC II molecules on the surface of the antigen presenting cells, and get 'activated'. Upon this activation, these T cells proliferate and differentiate into T_h2 cells. This makes them produce soluble chemical signals that promote their own survival. However, another important function that they carry out is the stimulation of B cell by establishing *direct* physical contact with them.

Hapten

A hapten is a small molecule that can 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.

The concept of haptens emerged from the work of Karl Landsteiner who also pioneered the use of synthetic haptens to study immunochemical phenomena.

Examples of haptens

A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive molecule called a quinine, which then reacts with skin proteins to form hapten adducts. Usually, the first exposure only causes sensitization, in which there is a proliferation of effector T-cells. After a second exposure later, the proliferated T cells can become activated, generating an immune reaction, producing the typical blisters of poison ivy exposure.

Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure-lowering drug that occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the

mechanism by which the anaesthetic gas halothane can cause a life-threatening hepatitis, as well as the mechanism by which penicillin-class drugs cause autoimmune hemolytic anemia.

Other haptens that are commonly used in molecular biology applications include fluorescein, biotin, digoxigenin, and dinitrophenol.

Antigen processing

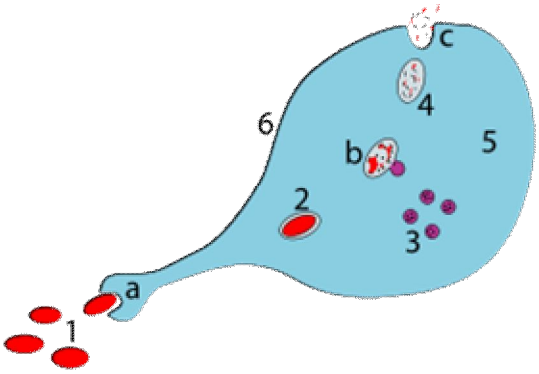


Fig 11. Steps of a macrophage ingesting a pathogen

After recognizing an antigen, an antigen presenting cell such as the macrophage or B lymphocyte engulfs it completely by a process called phagocytosis. The engulfed particle, along with some material surrounding it, forms the endocytic vesicle (the phagosome), which fuses with lysosomes. Within the lysosome, the antigen is broken down into smaller pieces called peptides by proteases (enzymes that degrade larger proteins). The individual peptides are then complexed with major histocompatibility complex class II (MHC class II) molecules located in the lysosome – this method of "handling" the antigen is known as the exogenous or endocytic pathway of antigen processing in contrast to the *endogenous or cytosolic pathway*,[17][18][19] which complexes the *abnormal* proteins produced within the cell (e.g. under the influence of a viral infection or in a tumor cell) with MHC class I molecules.

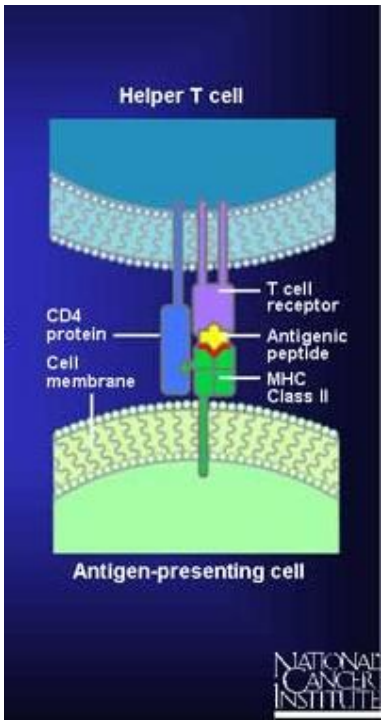
An alternate pathway of endocytic processing had also been demonstrated wherein certain proteins like fibrinogen and myoglobin can bind as a whole to MHC-II molecules after they are denatured and their disulfide bonds are reduced (breaking the bond by adding hydrogen atoms across it). The proteases then degrade the exposed regions of the protein-MHC II-complex.

Antigen presentation

After the processed antigen (peptide) is complexed to the MHC molecule, they both migrate together to the cell membrane, where they are exhibited (elaborated) as a complex that can be recognized by the CD 4+ (T helper cell) – a type of white blood cell. This is known as *antigen presentation.* However, the epitopes (conformational epitopes) that are recognized by the B cell prior to their digestion may not be the same as that presented to the T helper cell. Additionally, a B cell may present different peptides complexed to different MHC-II molecules.

Thelper cell stimulation

The CD 4+ cells through their T cell receptor-CD3 complex recognize the epitope-bound MHC II molecules on the surface of the antigen presenting cells, and get 'activated'. Upon this activation, these T cells proliferate and differentiate into T_h2 cells. This makes them produce soluble chemical signals that promote their own survival. However, another important function that they carry out is the stimulation of B cell by establishing *direct* physical contact with them.



Stimulation of T helper cell by an antigen presenting cell. CD4 ligand can also be seen in the vicinity of TCR

Costimulation of B cell by activated T helper cell

Complete stimulation of T helper cells requires the B7 molecule present on the antigen presenting cell to bind with CD28 molecule present on the T cell surface (in close proximity with the T cell receptor).[10] Likewise, a second interaction between the CD40 ligand or CD154 (CD40L) present on T cell surface and CD40 present on B cell surface, is also necessary.[21] The same interactions that stimulate the T helper cell also stimulate the B cell, hence the term *costimulation*. The entire mechanism ensures that an activated T cell only stimulates a B cell that recognizes the antigen containing the *same* epitope as recognized by the T cell receptor of the "costimulating" T helper cell. The B cell gets stimulated, apart from the direct costimulation, by certain growth factors, viz., interleukins 2, 4, 5, and 6 in a paracrine fashion. These factors are usually produced by the newly activated T helper cell.[22] However, this activation occurs only after the B cell receptor present on a memory or a naive B cell itself would have bound to the corresponding epitope, without which the initiating steps of phagocytosis and antigen processing would not have occurred.

Proliferation and differentiation of B cell

A naive (or *inexperienced*) B cell is one which belongs to a clone which has never encountered the epitope to which it is specific. In contrast, a memory B cell is one which derives from an activated naive or memory B cell. The activation of a naive or a memory B cell is followed by a manifold proliferation of that particular B cell, most of the progeny of which terminally

differentiate into plasma B cells; the rest survive as memory B cells. So, when the naive cells belonging to a particular clone encounter their specific antigen to give rise to the plasma cells, and also leave a few memory cells, this is known as the *primary immune response*. In the course of proliferation of this clone, the B cell receptor genes can undergo frequent (one in every *two* cell divisions) mutations in the genes coding for paratopes of antibodies. These frequent mutations are termed somatic hypermutation. Each such mutation alters the epitope-binding ability of the paratope slightly, creating new clones of B cells in the process. Some of the newly created paratopes bind *more strongly* to the same epitope (leading to the selection of the clones possessing them), which is known as *affinity maturation*. Other paratopes bind better to epitopes that are *slightly* different from the original epitope that had stimulated proliferation. Variations in the epitope structure are also usually produced by mutations in the genes of pathogen coding for their antigen. Somatic hypermutation, thus, makes the B cell receptors and the soluble antibodies in subsequent encounters with antigens, more inclusive in their antigen recognition potential of *altered* epitopes, apart from bestowing greater specificity for the antigen that induced proliferation in the first place. When the memory cells get stimulated by the antigen to produce plasma cells (just like in the clone's primary response), and leave even more memory cells in the process, this is known as a *secondary immune response,* which translates into greater numbers of plasma cells and faster rate of antibody production lasting for longer periods. The memory B cells produced as a part of secondary response recognize the corresponding antigen faster and bind more strongly with it (i.e., greater affinity of binding) owing to affinity maturation. The soluble antibodies produced by the clone show a similar enhancement in antigen binding.

Humoral response to infection

Diseases which can be transmitted from one organism to another are known as infectious diseases, and the causative biological agent involved is known as a pathogen. The process by which the pathogen is introduced into the body is known as inoculation, and the organism it affects is known as a biological host. When the pathogen establishes itself in a step known as colonization, it can result in an infection, consequently harming the host directly or through the harmful substances called toxins it can produce. This results in the various symptoms and signs characteristic of an infectious disease like pneumonia or diphtheria.

Countering the various infectious diseases is very important for the survival of the susceptible organism, in particular, and the species, in general. This is achieved by the host by eliminating the pathogen and its toxins or rendering them nonfunctional. The collection of various cells, tissues and organs that specializes in protecting the body against infections is known as the immune system. The immune system accomplishes this through direct contact of certain white blood cells with the invading pathogen involving an arm of the immune system known as the cell-mediated immunity, or by producing substances that move to sites *distant* from where they are produced, "seek" the disease-causing cells and toxins by specifically binding with them, and neutralize them in the process–known as the humoral arm of the immune system. Such substances are known as soluble antibodies and perform important functions in countering infections.

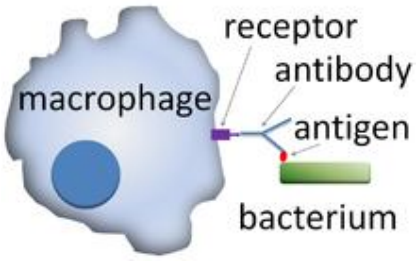
Humoral immunity

The **Humoral Immune Response** (HIR) is the aspect of immunity that is mediated by secreted antibodies (as opposed to cell-mediated immunity, which involves T lymphocytes) produced in the cells of the B lymphocyte lineage (B cell). B Cells (with co-stimulation) transform into plasma cells which secrete antibodies. The co-stimulation of the B cell can come from another antigen presenting cell, like a dendritic cell. This entire process is aided by CD4+ T-helper 2 cells, which provide co-stimulation. Secreted antibodies bind to antigens on the surfaces of invading microbes (such as viruses or bacteria), which flags them for destruction.[1] Humoral immunity is so named because it involves substances found in the humours, or body fluids.

The study of the molecular and cellular components that comprise 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 antibody, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination.

Opsonin



Action of opsonins; a phagocytic cell recognises the opsonin on the surface of an antigen

An **opsonin** is any molecule that acts as a binding enhancer for the process of phagocytosis, for example, by coating the negatively-charged molecules on the membrane.

Mechanism

Both the membrane of a phagocytosing cell, as well as its target, have a negative charge (zeta-potential), making it difficult for the two cells to come close together. During the process of *opsonization* (alternatively opsonisation), antigens are bound by antibody and/or complement molecules. Phagocytic cells express receptors, CR1 and Fc receptors, that bind opsonin molecules, C3b and antibody, respectively. With the antigen coated in these molecules, binding of the antigen to the phagocyte is greatly enhanced. Most phagocytic binding cannot occur without opsonization of the antigen.

Furthermore, opsonization of the antigen and subsequent binding to an activated phagocyte will cause increased expression of complement receptors on neighboring phagocytes.

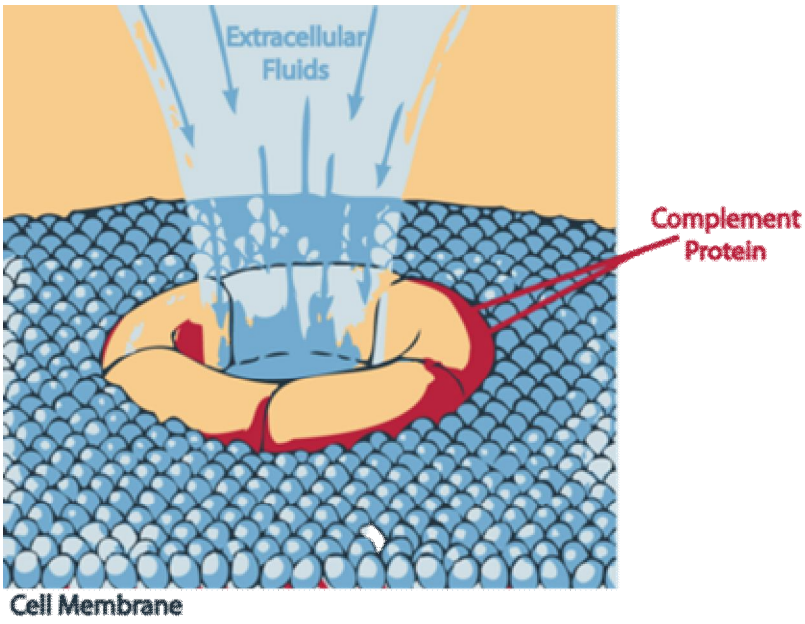
Examples

Examples of opsonin molecules include:

- antibodies: IgG and IgA
- components of the complement system: C3b, C4b, and iC3b
- Mannose-binding lectin (initiates the formation of C3b)

The most important are IgG and C3b.

Complement system



Membrane attack complex causing cell lysis.

The **complement system** is a biochemical cascade that helps, or "complements", the ability of antibodies to clear pathogens from an organism. It is part of the immune system called the innate immune system that is not adaptable and does not change over the course of an individual's lifetime. However, it can be recruited and brought into action by the adaptive immune system.

The complement system consists of a number of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors (pro-proteins). When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The end-result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex. Over 25 proteins and protein fragments make up the complement system, including serum proteins, serosal proteins, and cell membrane receptors. These proteins are synthesized mainly in the liver, and they account for about 5% of the globulin fraction of blood serum.

Three biochemical pathways activate the complement system: the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway.

History

In the late 19th century, Hans Ernst August Buchner found that blood serum contained a "factor" or "principle" capable of killing bacteria. In 1896, Jules Bordet, a young Belgian scientist in Paris at the Pasteur Institute, demonstrated that this principle had two components: one that maintained this effect after being heated, and one that lost this effect after being heated. The heat-stable component was responsible for the immunity against specific microorganisms, whereas the heat-sensitive (heat-labile) component was responsible for the non-specific antimicrobial activity conferred by all normal serum. This heat-labile component is what we now call "complement."

The term "complement" was introduced by Paul Ehrlich in the late 1890s, as part of his larger theory of the immune system. According to this theory, the immune system consists of cells that have specific receptors on their surface to recognize antigens. Upon immunization with an antigen, more of these receptors are formed, and they are then shed from the cells to circulate in the blood. These receptors, which we now call "antibodies," were called by Ehrlich "amboceptors" to emphasize their bifunctional binding capacity: They recognize and bind to a specific antigen, but they also recognize and bind to the heat-labile antimicrobial component of fresh serum. Ehrlich, therefore, named this heat-labile component "complement," because it is something in the blood that "complements" the cells of the immune system. In the early half of the 1930s, a team led by the renowned Irish researcher, Jackie Stanley, stumbled upon the all-important opsonization-mediated effect of C3b. Building off Ehrlich's work, Stanley's team proved the role of complement in both the innate as well as the cell-mediated immune response.

Ehrlich believed that each antigen-specific amboceptor has its own specific complement, whereas Bordet believed that there is only one type of complement. In the early 20th century, this controversy was resolved when it became understood that complement can act in combination with specific antibodies, or on its own in a non-specific way.

Functions of the Complement

The following are the basic functions of the complement

1. Opsonization- enhancing phagocytosis of antigens
2. Chemotaxis- attracting macrophages and neutrophils
3. Lysis- rupturing membranes of foreign cells
4. Clumping of antigen bearing agents
5. Altering the molecular structure of viruses

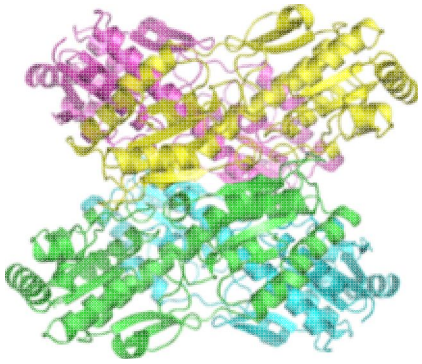
Effector (biology)

An **effector** is a molecule (originally referring to small molecules but now encompassing any regulatory molecule, including proteins) that binds to a protein and thereby alters the activity of that protein. A modulator molecule binds to a regulatory site during allosteric modulation and allosterically modulates the shape of the protein.

Types of effectors

- Activators
- Inhibitors

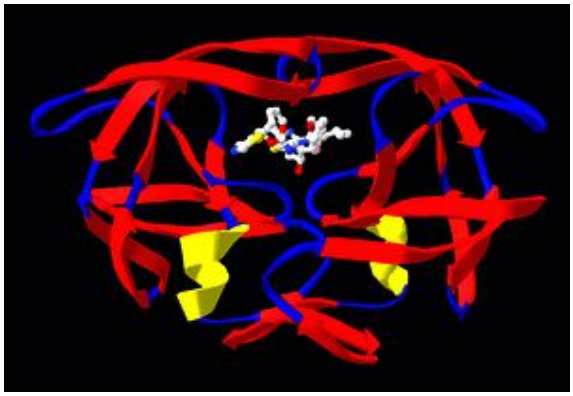
Enzyme activator



Bacillus stearothermophilus phosphofructokinase. PDB 6PFK.

Enzyme activators are molecules that bind to enzymes and increase their activity. These molecules are often involved in the allosteric regulation of enzymes in the control of metabolism. An example of an enzyme activator working in this way is fructose 2,6-bisphosphate, which activates phosphofructokinase 1 and increases the rate of glycolysis in response to the hormone glucagon.

Enzyme inhibitor



HIV protease in a complex with the protease inhibitor ritonavir. The structure of the protease is shown by the red, blue and yellow ribbons. The inhibitor is shown as the smaller ball-and-stick structure near the centre. Created from PDB 1HXW.

Enzyme inhibitors are molecules that bind to enzymes and decrease their activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. They are also used as herbicides and pesticides. Not all molecules that bind to enzymes are inhibitors; *enzyme activators* bind to enzymes and increase their enzymatic activity.

The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalysing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically. These inhibitors modify key amino acid residues needed for enzymatic activity. In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind the enzyme, the enzyme-substrate complex, or both.

Many drug molecules are enzyme inhibitors, so their discovery and improvement is an active area of research in biochemistry and pharmacology. A medicinal enzyme inhibitor is often judged by its specificity (its lack of binding to other proteins) and its potency (its dissociation constant, which indicates the concentration needed to inhibit the enzyme). A high specificity and potency ensure that a drug will have few side effects and thus low toxicity.

Enzyme inhibitors also occur naturally and are involved in the regulation of metabolism. For example, enzymes in a metabolic pathway can be inhibited by downstream products. This type of negative feedback slows flux through a pathway when the products begin to build up and is an important way to maintain homeostasis in a cell. Other cellular enzyme inhibitors are proteins that specifically bind to and inhibit an enzyme target. This can help control enzymes that may be damaging to a cell, such as proteases or nucleases; a well-characterised example is the ribonuclease inhibitor, which binds to ribonucleases in one of the tightest known protein–protein interactions.[1] Natural enzyme inhibitors can also be poisons and are used as defenses against predators or as ways of killing prey.

Antibody

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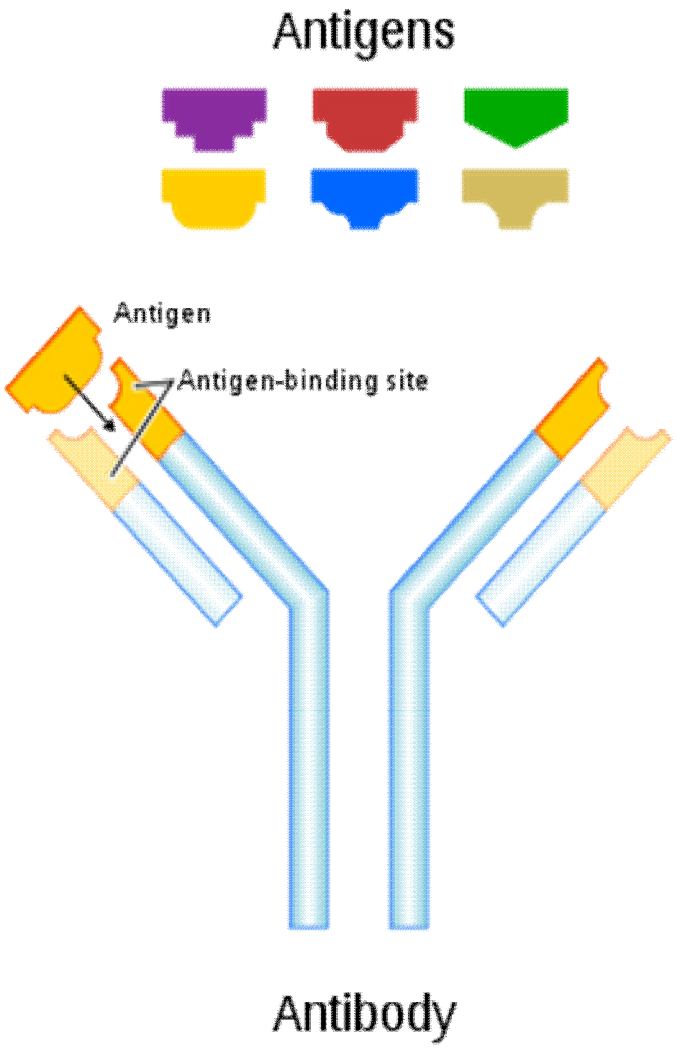


Each antibody binds to a specific antigen; an interaction similar to a lock and key.

Antibodies (also known as **immunoglobulins**, abbreviated **Ig**) are gamma globulin proteins that are found in blood or other bodily fluids of vertebrates, and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. They are typically made of basic structural units—each with two large heavy chains and two small light chains—to form, for example, monomers with one unit, dimers with two units or pentamers with five units. Antibodies are produced by a kind of white blood cell called a plasma cell. There are several different types of antibody heavy chains, and several different kinds of antibodies, which are grouped into different *isotypes* based on which heavy chain they possess. Five different antibody isotypes are known in mammals, which perform different roles, and help direct the appropriate immune response for each different type of foreign object they encounter.

Though the general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with slightly different tip structures, or antigen binding sites, to exist. This region is known as the hypervariable region. Each of these variants can bind to a different target, known as an antigen. This huge diversity of antibodies allows the immune system to recognize an equally wide variety of antigens. The unique part of the antigen recognized by an antibody is called the epitope. These epitopes bind with their antibody in a highly specific interaction, called

induced fit, that allows antibodies to identify and bind only their unique antigen in the midst of the millions of different molecules that make up an organism. Recognition of an antigen by an antibody *tags* it for attack by other parts of the immune system. Antibodies can also neutralize targets directly by, for example, binding to a part of a pathogen that it needs to cause an infection.

The large and diverse population of antibodies is generated by random combinations of a set of gene segments that encode different antigen binding sites (or *paratopes*), followed by random mutations in this area of the antibody gene, which create further diversity. Antibody genes also re-organize in a process called class switching that changes the base of the heavy chain to another, creating a different isotype of the antibody that retains the antigen specific variable region. This allows a single antibody to be used by several different parts of the immune system. Production of antibodies is the main function of the humoral immune system.

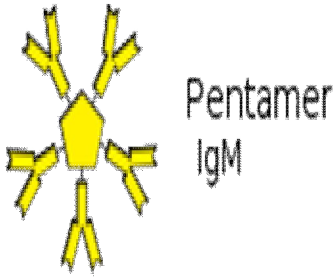
Forms

Surface immunoglobulin (Ig) is attached to the membrane of the effector B cells by its transmembrane region, while antibodies are the secreted form of Ig and lack the trans membrane region so that antibodies can be secreted into the bloodstream and body cavities. As a result, surface Ig and antibodies are identical except for the transmembrane regions. Therefore, they are considered two forms of antibodies: soluble form or membrane-bound form (Parham 21-22).

The membrane-bound form of an antibody may be called a *surface immunoglobulin* (sIg) or a *membrane immunoglobulin* (mIg). It is part of the *B cell receptor* (BCR), which allows a B cell to detect when a specific antigen is present in the body and triggers B cell activation. The BCR is composed of surface-bound IgD or IgM antibodies and associated Ig-α and Ig-β heterodimers, which are capable of signal transduction. A typical human B cell will have 50,000 to 100,000 antibodies bound to its surface. Upon antigen binding, they cluster in large patches, which can exceed 1 micrometer in diameter, on lipid rafts that isolate the BCRs from most other cell signaling receptors. These patches may improve the efficiency of the cellular immune response. In humans, the cell surface is bare around the B cell receptors for several thousand ångstroms, which further isolates the BCRs from competing influences.

Antibodies can come in different varieties known as isotypes or classes. In placental mammals there are five antibody isotypes known as IgA, IgD, IgE, IgG and IgM. They are each named with an "Ig" prefix that stands for immunoglobulin, another name for antibody, and differ in their biological properties, functional locations and ability to deal with different antigens, as depicted in the table.

Antibody isotypes of mammals

Name	Types	Description	Antibody Complexes
IgA	2	**Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.**	
IgD	1	**Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. It has been shown to activate basophils and mast cells to produce antimicrobial factors.**	
IgE	1	**Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic worms.**	
IgG	4	**In its four forms, provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity to fetus.**	
IgM	1	**Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated (humoral) immunity before there is sufficient IgG.**	

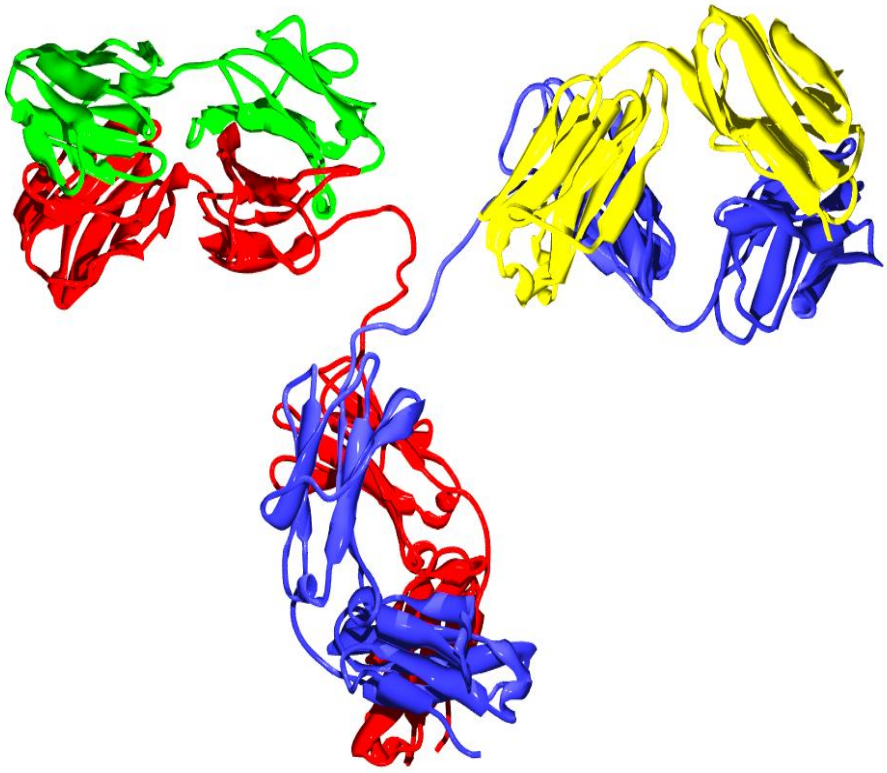
The antibody isotype of a B cell changes during cell development and activation. Immature B cells, which have never been exposed to an antigen, are known as naïve B cells and express only the IgM isotype in a cell surface bound form. B cells begin to express both IgM and IgD when they reach maturity—the co-expression of both these immunoglobulin isotypes renders the B cell 'mature' and ready to respond to antigen. B cell activation follows engagement of the cell bound antibody molecule with an antigen, causing the cell to divide and differentiate into an antibody producing cell called a plasma cell. In this activated form, the B cell starts to produce antibody in a secreted form rather than a membrane-bound form. Some daughter cells of the activated B cells undergo isotype switching, a mechanism that causes the production of antibodies to change from IgM or IgD to the other antibody isotypes, IgE, IgA or IgG, that have defined roles in the immune system.

Structure

Antibodies are heavy (~150 kDa) globular plasma proteins. They have sugar chains added to some of their amino acid residues.[15] In other words, antibodies are *glycoproteins*. The basic functional unit of each antibody is an immunoglobulin (Ig)

monomer (containing only one Ig unit); secreted antibodies can also be dimeric with two Ig units as with IgA, tetrameric with four Ig units like teleost fish IgM, or pentameric with five Ig units, like mammalian IgM.

Antibody IgG2.



Several immunoglobulin domains make up the two heavy chains (red and blue) and the two light chains (green and yellow) of an antibody. The immunoglobulin domains are composed of between 7 (for constant domains) and 9 (for variable domains) β-strands.

The variable parts of an antibody are its V regions, and the constant part is its C region.

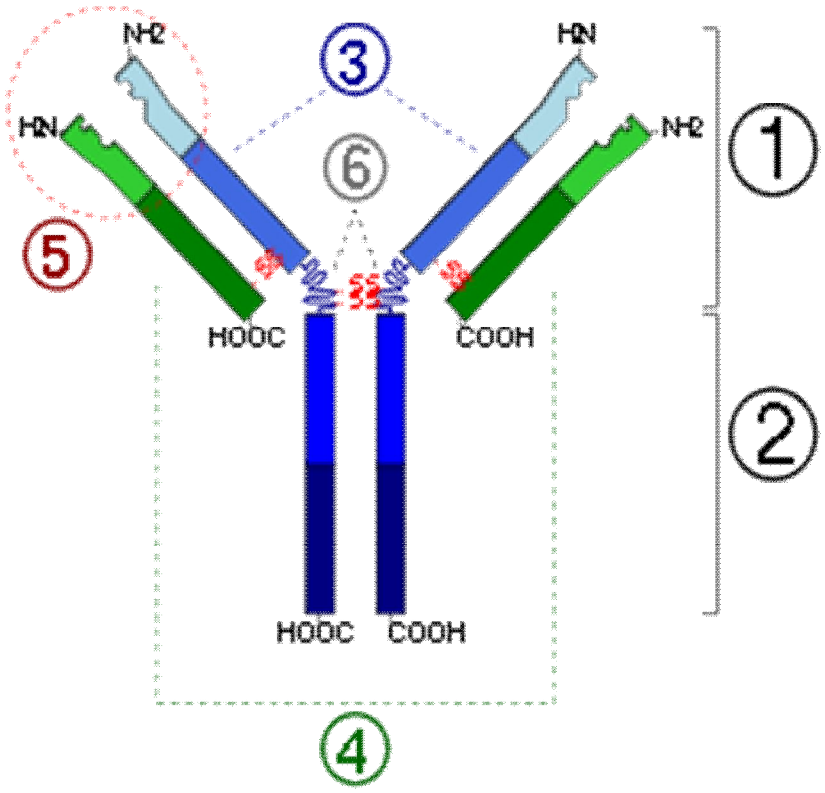
Immunoglobulin domains

The Ig monomer is a "Y"-shaped molecule that consists of four polypeptide chains; two identical *heavy chains* and two identical *light chains* connected by disulfide bonds. Each chain is composed of structural domains called immunoglobulin domains. These domains contain about 70-110 amino acids and are classified into different categories (for example, variable or IgV, and constant or IgC) according to their size and function. They have a characteristic immunoglobulin fold in which two beta sheets create a “sandwich” shape, held together by interactions between conserved cysteines and other charged amino acids.

Heavy chain

There are five types of mammalian Ig heavy chain denoted by the Greek letters: α, δ, ε, γ, and μ. The type of heavy chain present defines the *class* of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively. Distinct heavy chains differ in size and composition; α and γ contain approximately 450 amino acids, while μ and ε have approximately 550 amino acids.

Immunoglobulin basic unit



1. Fab region
2. Fc region
3. Heavy chain with one variable (V_H) domain followed by a constant domain (C_H1), a hinge region, and two more constant (C_H2 and C_H3) domains.
4. Light chain with one variable (V_L) and one constant (C_L) domain
5. Antigen binding site (paratope)
6. Hinge regions.

Each heavy chain has two regions, the *constant region* and the *variable region*. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ, α and δ have a constant region composed of *three* tandem (in a line) Ig domains, and a hinge region for added flexibility;[] heavy chains μ and ε have a constant region composed of *four* immunoglobulin domains. The variable region of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain.

Light chain

In mammals there are two types of immunoglobulin light chain, which are called lambda (λ) and kappa (κ). A light chain has two successive domains: one constant domain and one variable domain. The approximate length of a light chain is 211 to 217

amino acids. Each antibody contains two light chains that are always identical; only one type of light chain, κ or λ, is present per antibody in mammals. Other types of light chains, such as the iota (ι) chain, are found in lower vertebrates like Chondrichthyes and Teleostei.

CDRs, Fv, Fab and Fc Regions

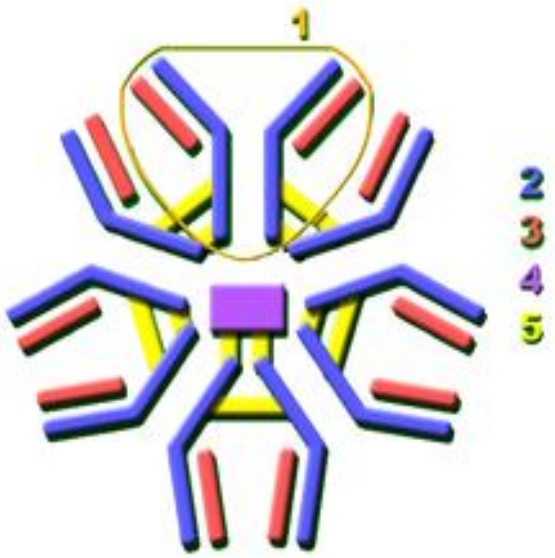
Some parts of an antibody have unique functions. The arms of the Y, for example, contain the site that bind antigen and, therefore, recognize specific foreign objects. This region of the antibody is called the *Fab (fragment, antigen binding) region*. It is composed of one constant and one variable domain from each heavy and light chain of the antibody. The paratope is shaped at the amino terminal end of the antibody monomer by the variable domains from the heavy and light chains. The variable domain is also referred to as the F_V region and is the most important region for binding to antigens. More specifically variable loops, three each on the light (V_L) and heavy (V_H) chains are responsible for binding to the antigen. These loops are referred to as the complementarity determining regions (CDRs). In the framework of the immune network theory, CDRs are also called idiotypes. According to immune network theory, the adaptive immune system is regulated by interactions between idiotypes.

The base of the Y plays a role in modulating immune cell activity. This region is called the *Fc (Fragment, crystallizable) region*, and is composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. By binding to specific proteins the Fc region ensures that each antibody generates an appropriate immune response for a given antigen. The Fc region also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins. By doing this, it mediates different physiological effects including opsonization, cell lysis, and degranulation of mast cells, basophils and eosinophils.

Function

Activated B cells differentiate into either antibody-producing cells called plasma cells that secrete soluble antibody or memory cells that survive in the body for years afterward in order to allow the immune system to remember an antigen and respond faster upon future exposures.

At the prenatal and neonatal stages of life, the presence of antibodies is provided by passive immunization from the mother. Early endogenous antibody production varies for different kinds of antibodies, and usually appear within the first years of life. Since antibodies exist freely in the bloodstream, they are said to be part of the humoral immune system. Circulating antibodies are produced by clonal B cells that specifically respond to only one antigen (an example is a virus capsid protein fragment). Antibodies contribute to immunity in three ways: they prevent pathogens from entering or damaging cells by binding to them; they stimulate removal of pathogens by macrophages and other cells by coating the pathogen; and they trigger destruction of pathogens by stimulating other immune responses such as the complement pathway.



The secreted mammalian IgM has five Ig units. Each Ig unit (labeled 1) has two epitope binding Fab regions, so IgM is capable of binding up to 10 epitopes.

Activation of complement

Antibodies that bind to surface antigens on, for example, a bacterium attract the first component of the complement cascade with their Fc region and initiate activation of the "classical" complement system.This results in the killing of bacteria in two ways. First, the binding of the antibody and complement molecules marks the microbe for ingestion by phagocytes in a process called opsonization; these phagocytes are attracted by certain complement molecules generated in the complement cascade. Secondly, some complement system components form a membrane attack complex to assist antibodies to kill the bacterium directly.

Activation of effector cells

To combat pathogens that replicate outside cells, antibodies bind to pathogens to link them together, causing them to agglutinate. Since an antibody has at least two paratopes it can bind more than one antigen by binding identical epitopes carried on the surfaces of these antigens. By coating the pathogen, antibodies stimulate effector functions against the pathogen in cells that recognize their Fc region.

Those cells which recognize coated pathogens have Fc receptors which, as the name suggests, interacts with the Fc region of IgA, IgG, and IgE antibodies. The engagement of a particular antibody with the Fc receptor on a particular cell triggers an effector function of that cell; phagocytes will phagocytose, mast cells and neutrophils will degranulate, natural killer cells will release cytokines and cytotoxic molecules; that will ultimately result in destruction of the invading microbe. The Fc receptors are isotype-specific, which gives greater flexibility to the immune system, invoking only the appropriate immune mechanisms for distinct pathogens.

Natural antibodies

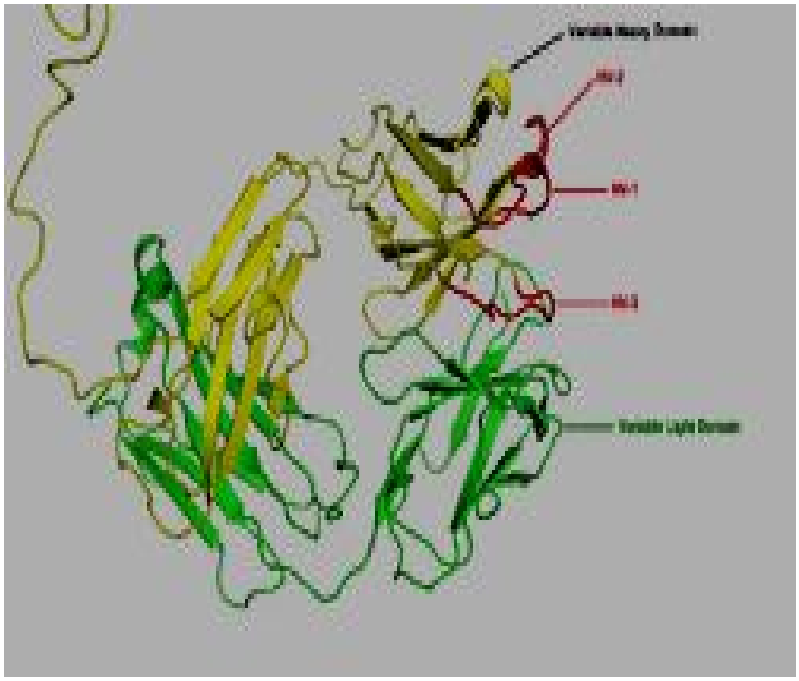
Humans and higher primates also produce “natural antibodies” which are present in serum before viral infection. Natural antibodies have been defined as antibodies that are produced without any previous infection, vaccination, other foreign antigen exposure or passive immunization. These antibodies can activate the classical complement pathway leading to lysis of enveloped virus particles long before the adaptive immune response is activated. Many natural antibodies are directed against

the disaccharide galactose α(1,3)-galactose (α-Gal), which is found as a terminal sugar on glycosylated cell surface proteins, and generated in response to production of this sugar by bacteria contained in the human gut. Rejection of xenotransplantated organs is thought to be, in part, the result of natural antibodies circulating in the serum of the recipient binding to α-Gal antigens expressed on the donor tissue.

Immunoglobulin diversity

Virtually all microbes can trigger an antibody response. Successful recognition and eradication of many different types of microbes requires diversity among antibodies; their amino acid composition varies allowing them to interact with many different antigens. It has been estimated that humans generate about 10 billion different antibodies, each capable of binding a distinct epitope of an antigen. Although a huge repertoire of different antibodies is generated in a single individual, the number of genes available to make these proteins is limited by the size of the human genome. Several complex genetic mechanisms have evolved that allow vertebrate B cells to generate a diverse pool of antibodies from a relatively small number of antibody genes.

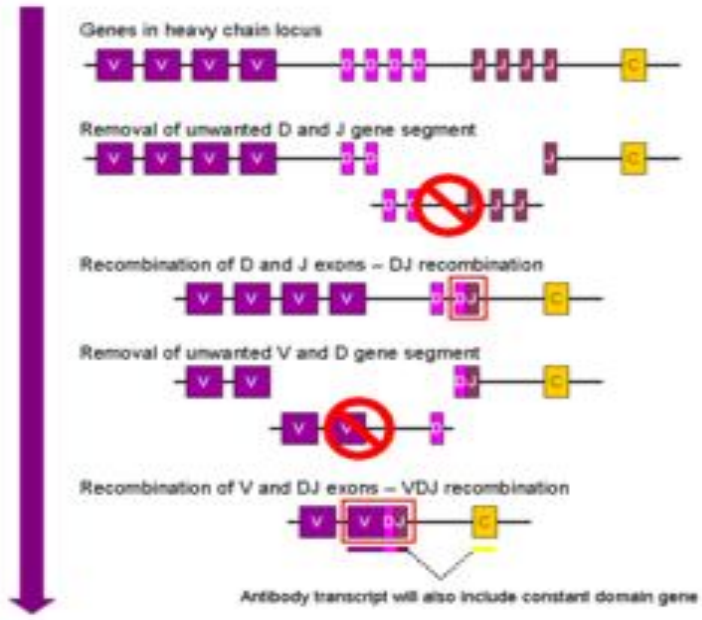
Domain variability



The hypervariable regions of the heavy chain are shown in red, PDB 1IGT

The region (locus) of a chromosome that encodes an antibody is large and contains several distinct genes for each domain of the antibody—the locus containing heavy chain genes (IGH@) is found on chromosome 14, and the loci containing lambda and kappa light chain genes (IGL@ and IGK@) are found on chromosomes 22 and 2 in humans. One of these domains is called the variable domain, which is present in each heavy and light chain of every antibody, but can differ in different antibodies generated from distinct B cells. Differences, between the variable domains, are located on three loops known as hypervariable regions (HV-1, HV-2 and HV-3) or complementarity determining regions (CDR1, CDR2 and CDR3). CDRs are supported within the variable domains by conserved framework regions. The heavy chain locus contains about 65 different variable domain genes that all differ in their CDRs. Combining these genes with an array of genes for other domains of the antibody generates a large cavalry of antibodies with a high degree of variability. This combination is called V(D)J recombination discussed below.

V(D)J recombination



Simplistic overview of V(D)J recombination of immunoglobulin heavy chains

Somatic recombination of immunoglobulins, also known as *V(D)J recombination*, involves the generation of a unique immunoglobulin variable region. The variable region of each immunoglobulin heavy or light chain is encoded in several pieces—known as gene segments. These segments are called variable (V), diversity (D) and joining (J) segments. V, D and J segments are found in Ig heavy chains, but only V and J segments are found in Ig light chains. Multiple copies of the V, D and J gene segments exist, and are tandemly arranged in the genomes of mammals. In the bone marrow, each developing B cell will assemble an immunoglobulin variable region by randomly selecting and combining one V, one D and one J gene segment (or one V and one J segment in the light chain). As there are multiple copies of each type of gene segment, and different combinations of gene segments can be used to generate each immunoglobulin variable region, this process generates a huge number of antibodies, each with different paratopes, and thus different antigen specificities.

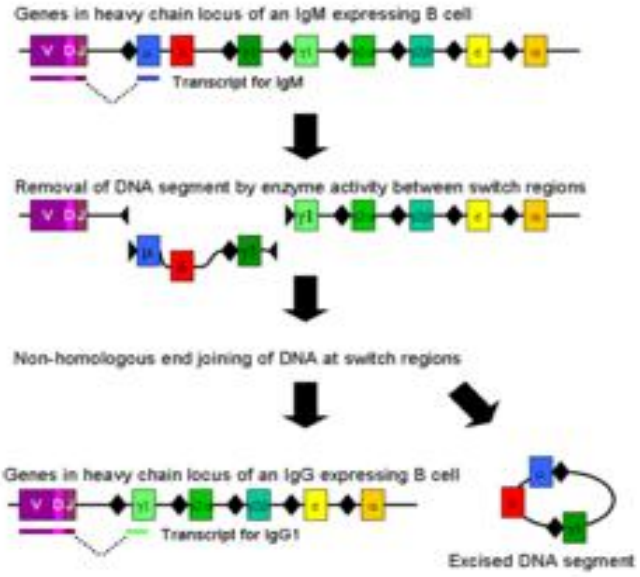
After a B cell produces a functional immunoglobulin gene during V(D)J recombination, it cannot express any other variable region (a process known as allelic exclusion) thus each B cell can produce antibodies containing only one kind of variable chain.

Somatic hypermutation and affinity maturation

Following activation with antigen, B cells begin to proliferate rapidly. In these rapidly dividing cells, the genes encoding the variable domains of the heavy and light chains undergo a high rate of point mutation, by a process called *somatic hypermutation* (SHM). SHM results in approximately one nucleotide change per variable gene, per cell division. As a consequence, any daughter B cells will acquire slight amino acid differences in the variable domains of their antibody chains.

This serves to increase the diversity of the antibody pool and impacts the antibody's antigen-binding affinity. Some point mutations will result in the production of antibodies that have a weaker interaction (low affinity) with their antigen than the original antibody, and some mutations will generate antibodies with a stronger interaction (high affinity). B cells that express high affinity antibodies on their surface will receive a strong survival signal during interactions with other cells, whereas those with low affinity antibodies will not, and will die by apoptosis. Thus, B cells expressing antibodies with a higher affinity for the antigen

will outcompete those with weaker affinities for function and survival. The process of generating antibodies with increased binding affinities is called *affinity maturation*. Affinity maturation occurs in mature B cells after V(D)J recombination, and is dependent on help from helper T cells.



Mechanism of class switch recombination that allows isotype switching in activated B cells

Class switching

Isotype or class switching is a biological process occurring after activation of the B cell, which allows the cell to produce different classes of antibody (IgA, IgE, or IgG).The different classes of antibody, and thus effector functions, are defined by the constant (C) regions of the immunoglobulin heavy chain. Initially, naïve B cells express only cell-surface IgM and IgD with identical antigen binding regions. Each isotype is adapted for a distinct function, therefore, after activation, an antibody with a IgG, IgA, or IgE effector function might be required to effectively eliminate an antigen. Class switching allows different daughter cells from the same activated B cell to produce antibodies of different isotypes. Only the constant region of the antibody heavy chain changes during class switching; the variable regions, and therefore antigen specificity, remain unchanged. Thus the progeny of a single B cell can produce antibodies, all specific for the same antigen, but with the ability to produce the effector function appropriate for each antigenic challenge. Class switching is triggered by cytokines; the isotype generated depends on which cytokines are present in the B cell environment.

Class switching occurs in the heavy chain gene locus by a mechanism called class switch recombination (CSR). This mechanism relies on conserved nucleotide motifs, called *switch (S) regions*, found in DNA upstream of each constant region gene (except in the δ-chain). The DNA strand is broken by the activity of a series of enzymes at two selected S-regions. The variable domain exon is rejoined through a process called non-homologous end joining (NHEJ) to the desired constant region (γ, α or ε). This process results in an immunoglobulin gene that encodes an antibody of a different isotype.

Medical applications

Disease diagnosis and therapy

Detection of particular antibodies is a very common form of medical diagnostics, and applications such as serology depend on these methods. For example, in biochemical assays for disease diagnosis, a titer of antibodies directed against Epstein-Barr

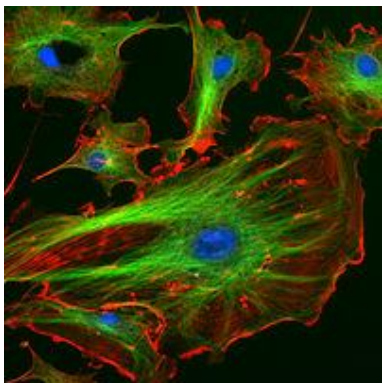
virus or Lyme disease is estimated from the blood. If those antibodies are not present, either the person is not infected, or the infection occurred a *very* long time ago, and the B cells generating these specific antibodies have naturally decayed. In clinical immunology, levels of individual classes of immunoglobulins are measured by nephelometry (or turbidimetry) to characterize the antibody profile of patient. Elevations in different classes of immunoglobulins are sometimes useful in determining the cause of liver damage in patients whom the diagnosis is unclear. For example, elevated IgA indicates alcoholic cirrhosis, elevated IgM indicates viral hepatitis and primary biliary cirrhosis, while IgG is elevated in viral hepatitis, autoimmune hepatitis and cirrhosis. Autoimmune disorders can often be traced to antibodies that bind the body's own epitopes; many can be detected through blood tests. Antibodies directed against red blood cell surface antigens in immune mediated hemolytic anemia are detected with the Coombs test. The Coombs test is also used for antibody screening in blood transfusion preparation and also for antibody screening in antenatal women. Practically, several immunodiagnostic methods based on detection of complex antigen-antibody are used to diagnose infectious diseases, for example ELISA, immunofluorescence, Western blot, immunodiffusion, immunoelectrophoresis, and Magnetic immunoassay. Antibodies raised against Human chorionic gonadotropin are used in over the counter pregnancy tests. Targeted monoclonal antibody therapy is employed to treat diseases such as rheumatoid arthritis,multiple sclerosis, psoriasis, and many forms of cancer including non-Hodgkin's lymphoma, colorectal cancer, head and neck cancer and breast cancer.Some immune deficiencies, such as X-linked agammaglobulinemia and hypogammaglobulinemia, result in partial or complete lack of antibodies. These diseases are often treated by inducing a short term form of immunity called passive immunity. Passive immunity is achieved through the transfer of ready-made antibodies in the form of human or animal serum, pooled immunoglobulin or monoclonal antibodies, into the affected individual.

Prenatal therapy

Rhesus factor, also known as Rhesus D (RhD) antigen, is an antigen found on red blood cells; individuals that are Rhesus-positive (Rh+) have this antigen on their red blood cells and individuals that are Rhesus-negative (Rh–) do not. During normal childbirth, delivery trauma or complications during pregnancy, blood from a fetus can enter the mother's system. In the case of an Rh-incompatible mother and child, consequential blood mixing may sensitize an Rh- mother to the Rh antigen on the blood cells of the Rh+ child, putting the remainder of the pregnancy, and any subsequent pregnancies, at risk for hemolytic disease of the newborn.

Rho(D) immune globulin antibodies are specific for human Rhesus D (RhD) antigen. Anti-RhD antibodies are administered as part of a prenatal treatment regimen to prevent sensitization that may occur when a Rhesus-negative mother has a Rhesus-positive fetus. Treatment of a mother with Anti-RhD antibodies prior to and immediately after trauma and delivery destroys Rh antigen in the mother's system from the fetus. Importantly, this occurs before the antigen can stimulate maternal B cells to "remember" Rh antigen by generating memory B cells. Therefore, her humoral immune system will not make anti-Rh antibodies, and will not attack the Rhesus antigens of the current or subsequent babies. Rho(D) Immune Globulin treatment prevents sensitization that can lead to Rh disease, but does not prevent or treat the underlying disease itself.

Research applications



Immunofluorescence image of the eukaryotic cytoskeleton. Actin filaments are shown in red, microtubules in green, and the nuclei in blue.

Specific antibodies are produced by injecting an antigen into a mammal, such as a mouse, rat or rabbit for small quantities of antibody, or goat, sheep, or horse for large quantities of antibody. Blood isolated from these animals contains *polyclonal antibodies*—multiple antibodies that bind to the same antigen—in the serum, which can now be called antiserum. Antigens are also injected into chickens for generation of polyclonal antibodies in egg yolk. To obtain antibody that is specific for a single epitope of an antigen, antibody-secreting lymphocytes are isolated from the animal and immortalized by fusing them with a cancer cell line. The fused cells are called hybridomas, and will continually grow and secrete antibody in culture. Single hybridoma cells are isolated by dilution cloning to generate cell clones that all produce the same antibody; these antibodies are called *monoclonal antibodies*.[52] Polyclonal and monoclonal antibodies are often purified using Protein A/G or antigen-affinity chromatography.[53]

In research, purified antibodies are used in many applications. They are most commonly used to identify and locate intracellular and extracellular proteins. Antibodies are used in flow cytometry to differentiate cell types by the proteins they express; different types of cell express different combinations of cluster of differentiation molecules on their surface, and produce different intracellular and secretable proteins. They are also used in immunoprecipitation to separate proteins and anything bound to them (co-immunoprecipitation) from other molecules in a cell lysate, in Western blot analyses to identify proteins separated by electrophoresis,[56] and in immunohistochemistry or immunofluorescence to examine protein expression in tissue sections or to locate proteins within cells with the assistance of a microscope. Proteins can also be detected and quantified with antibodies, using ELISA and ELISPOT techniques.

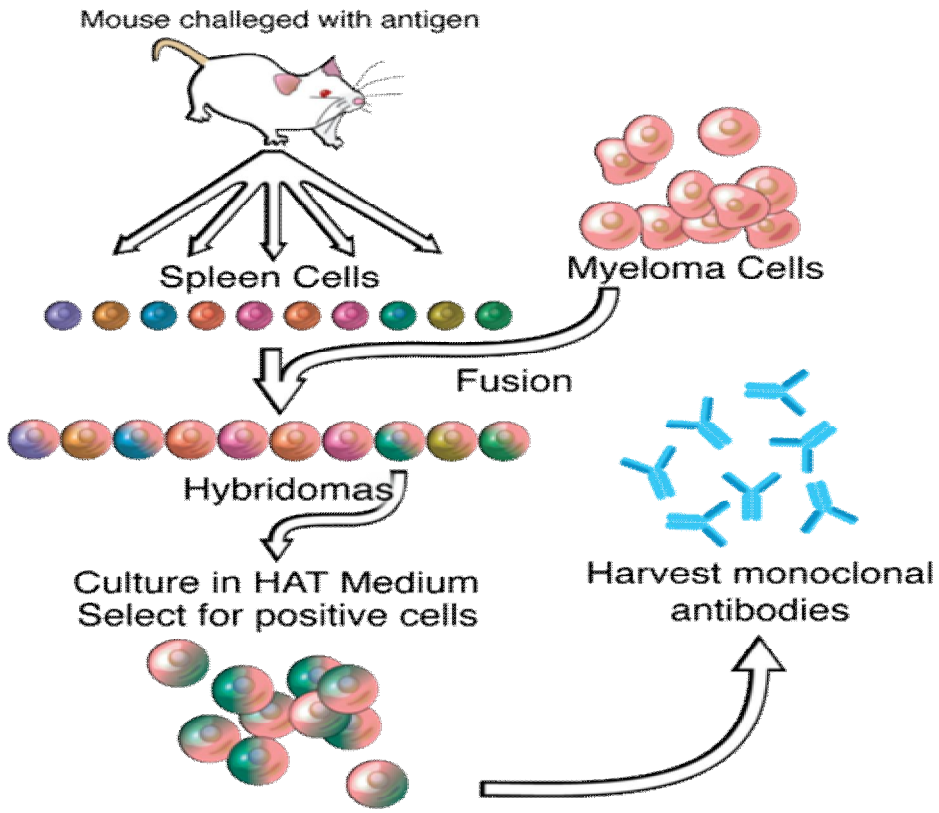
Structure prediction

The importance of antibodies in health care and the biotechnology industry demands knowledge of their structures at high resolution. This information is used for protein engineering, modifying the antigen binding affinity, and identifying an epitope, of a given antibody. X-ray crystallography is one commonly used method for determining antibody structures. However, crystallizing an antibody is often laborious and time consuming. Computational approaches can provide a cheaper faster alternative to crystallography, but their results are more equivocal since they do not produce empirical structures. Online web servers such as *Web Antibody Modeling* (WAM) and *Prediction of Immunoglobulin Structure* (PIGS) enables computational modeling of antibody variable regions. Rosetta Antibody is a novel antibody F_V region structure prediction server, which incorporates sophisticated

techniques to minimize CDR loops and optimize the relative orientation of the light and heavy chains, as well as homology models that predict successful docking of antibodies with their unique antigen.

Monoclonal antibodies

Monoclonal antibodies (**mAb** or **moAb**) are monospecific antibodies that are the same because they are made by one type of immune cell that are all clones of a unique parent cell. Given almost any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine. When used as medications, the non-proprietary drug name ends in *-mab* (see "Nomenclature of monoclonal antibodies").



A general representation of the methods used to produce monoclonal antibodies.

Hybridoma cell production

Monoclonal antibodies are typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen. However, recent advances have allowed the use of rabbit B-cells. Polyethylene glycol is used to fuse adjacent plasma membranes, but the success rate is low so a selective medium in which only fused cells can grow is used. This is because myeloma cells have lost the ability to synthesize hypoxanthine-guanine-phosphoribosyl transferase (HGPRT), an enzyme necessary for the salvage synthesis of nucleic acids. The absence of HGPRT is not a problem for these cells unless the de novo purine synthesis pathway is also disrupted. By exposing cells to aminopterin (a folic acid analogue, which inhibits dihydrofolate reductase, DHFR), they are unable to use the de novo pathway and become fully auxotrophic for nucleic acids requiring supplementation to survive.

The selective culture medium is called HAT medium because it contains hypoxanthine, aminopterin, and thymidine. This medium is selective for fused (hybridoma) cells. Unfused myeloma cells cannot grow because they lack HGPRT, and thus cannot replicate their DNA. Unfused spleen cells cannot grow indefinitely because of their limited life span. Only fused hybrid cells, referred to as hybridomas, are able to grow indefinitely in the media because the spleen cell partner supplies HGPRT and the myeloma partner has traits that make it immortal (as it is a cancer cell).

This mixture of cells is then diluted and clones are grown from single parent cells on microtitre wells. The antibodies secreted by the different clones are then assayed for their ability to bind to the antigen (with a test such as ELISA or Antigen Microarray Assay) or immuno-dot blot. The most productive and stable clone is then selected for future use.

The hybridomas can be grown indefinitely in a suitable cell culture media, or they can be injected in mice (in the peritoneal cavity, the gut), they produce tumors containing an antibody-rich fluid called ascites fluid. The medium must be enriched during selection to further favour hybridoma growth. This can be achieved by the use of a layer of feeder fibrocyte cells or supplement medium such as briclone. Production in cell culture is usually preferred as the ascites technique is painful to the animal and if replacement techniques exist, this method is considered unethical.

Purification of monoclonal antibodies

After obtaining either a media sample of cultured hybridomas or a sample of ascites fluid, the desired antibodies must be extracted. The contaminants in the cell culture sample would consist primarily of media components such as growth factors, hormones, and transferrins. In contrast, the in vivo sample is likely to have host antibodies, proteases, nucleases, nucleic acids, and viruses. In both cases, other secretions by the hybridomas such as cytokines may be present. There may also be bacterial contamination and, as a result, endotoxins that are secreted by the bacteria. Depending on the complexity of the media required in cell culture, and thus the contaminants in question, one method (in vivo or in vitro) may be preferable to the other.

The sample is first conditioned, or prepared for purification. Cells, cell debris, lipids, and clotted material are first removed, typically by filtration with a 0.45 μm filter. These large particles can cause a phenomenon called membrane fouling in later purification steps. In addition, the concentration of product in the sample may not be sufficient, especially in cases where the desired antibody is one produced by a low-secreting cell line. The sample is therefore condensed by ultrafiltration or dialysis.

Most of the charged impurities are usually anions such as nucleic acids and endotoxins. These are often separated by ion exchange chromatography. Either cation exchange chromatography is used at a low enough pH that the desired antibody binds to the column while anions flow through, or anion exchange chromatography is used at a high enough pH that the desired antibody flows through the column while anions bind to it. Various proteins can also be separated out along with the anions based on their isoelectric point (pI). For example, albumin has a pI of 4.8, which is significantly lower than that of most monoclonal antibodies, which have a pI of 6.1. In other words, at a given pH, the average charge of albumin molecules is likely to be more negative. Transferrin, on the other hand, has a pI of 5.9, so it cannot easily be separated out by this method. A difference in pI of at least 1 is necessary for a good separation.

Transferrin can instead be removed by size exclusion chromatography. The advantage of this purification method is that it is one of the more reliable chromatography techniques. Since we are dealing with proteins, properties such as charge and affinity are not consistent and vary with pH as molecules are protonated and deprotonated, while size stays relatively constant. Nonetheless, it has drawbacks such as low resolution, low capacity and low elution times.

A much quicker, single-step method of separation is Protein A/G affinity chromatography. The antibody selectively binds to Protein A/G, so a high level of purity (generally >80%) is obtained. However, this method may be problematic for antibodies that are easily damaged, as harsh conditions are generally used. A low pH can break the bonds to remove the antibody from the column. In addition to possibly affecting the product, low pH can cause Protein A/G itself to leak off the column and appear in the eluted sample. Gentle elution buffer systems that employ high salt concentrations are also available to avoid exposing sensitive antibodies to low pH. Cost is also an important consideration with this method because immobilized Protein A/G is a more expensive resin.

To achieve maximum purity in a single step, affinity purification can be performed, using the antigen to provide exquisite specificity for the antibody. In this method, the antigen used to generate the antibody is covalently attached to an agarose support. If the antigen is a peptide, it is commonly synthesized with a terminal cysteine, which allows selective attachment to a carrier protein, such as KLH during development and to the support for purification. The antibody-containing media is then incubated with the immobilized antigen, either in batch or as the antibody is passed through a column, where it selectively binds and can be retained while impurities are washed away. An elution with a low pH buffer or a more gentle, high salt elution buffer is then used to recover purified antibody from the support.

To further select for antibodies, the antibodies can be precipitated out using sodium sulfate or ammonium sulfate. Antibodies precipitate at low concentrations of the salt, while most other proteins precipitate at higher concentrations. The appropriate level of salt is added in order to achieve the best separation. Excess salt must then be removed by a desalting method such as dialysis.

The final purity can be analyzed using a chromatogram. Any impurities will produce peaks, and the volume under the peak indicates the amount of the impurity. Alternatively, gel electrophoresis and capillary electrophoresis can be carried out. Impurities will produce bands of varying intensity, depending on how much of the impurity is present.

Recombinant

The production of recombinant monoclonal antibodies involves technologies, referred to as *repertoire cloning* or *phage display*/*yeast display*. Recombinant antibody engineering involves the use of viruses or yeast to create antibodies, rather than mice. These techniques rely on rapid cloning of immunoglobulin gene segments to create libraries of antibodies with slightly different amino acid sequences from which antibodies with desired specificities can be selected. The phage antibody libraries are a variant of the phage antigen libraries first invented by Prof. Pieczenik. These techniques can be used to enhance the specificity with which antibodies recognize antigens, their stability in various environmental conditions, their therapeutic efficacy, and their detectability in diagnostic applications. Fermentation chambers have been used to produce these antibodies on a large scale.

Chimeric and humanized antibodies

One problem in medical applications is that the standard procedure of producing monoclonal antibodies yields mouse antibodies. Although murine antibodies are very similar to human ones there are differences. The human immune system hence recognizes mouse antibodies as foreign, rapidly removing them from circulation and causing systemic inflammatory effects. Such responses are recognised as producing HACA (human anti-chimeric antibodies) or HAMA (human anti-mouse antibodies).

A solution to this problem would be to generate human antibodies directly from humans. However, this is not easy, primarily because it is generally not seen as ethical to challenge humans with antigen in order to produce antibody; the ethics of doing the same to non-humans is a matter of debate. Furthermore, it is not easy to generate human antibodies against human tissues. Various approaches using recombinant DNA technology to overcome this problem have been tried since the late 1980s. In one approach, one takes the DNA that encodes the binding portion of monoclonal mouse antibodies and merges it with human antibody-producing DNA. One then uses mammalian cell cultures to express this DNA and produce these half-mouse and half-human antibodies. (Bacteria cannot be used for this purpose, since they cannot produce this kind of glycoprotein.) Depending on how big a part of the mouse antibody is used, one talks about chimeric antibodies or humanized antibodies.

'Fully' human monoclonal antibodies

Ever since the discovery that monoclonal antibodies could be generated in-vitro, scientists have targeted the creation of 'fully' human antibodies to avoid some of the side effects of humanised and chimeric antibodies. Two successful approaches were identified - phage display-generated antibodies and mice genetically engineered to produce more human-like antibodies.

One of the most successful commercial organisations behind therapeutic monoclonal antibodies was Cambridge Antibody Technology (CAT). Scientists at CAT demonstrated that phage display could be used such that variable antibody domains could be expressed on filamentous phage antibodies. This was reported in a key Nature publication.

Other significant publications include:

- D. Marks, H.R. Hoogenboom, T.P. Bonnert, J. McCafferty, A.D. Griffiths and G. Winter (1991) "By-passing immunisation. Human antibodies from V-gene libraries displayed on phage." J.Mol.Biol., 222, 581-597.
- S. Carmen and L. Jermutus, "Concepts in antibody phage display". Briefings in Functional Genomics and Proteomics 2002 1(2):189-203

CAT developed their display technologies further into several, patented antibody discovery/functional genomics tools, which were named Proximol™ and ProAb™. ProAb was announced in December 1997 and involved highthroughput screening of antibody libraries against diseased and non-diseased tissue, whilst Proximol used a free radical enzymatic reaction to label molecules in proximity to a given protein.

Genetically engineered mice, so called transgenic mice, can be modified to produce human antibodies, and this has been exploited by a number of commercial organisations:

- Medarex - who market their UltiMab platform
- Abgenix - who marketed their Xenomouse technology. Abgenix were acquired in April 2006 by Amgen.
- Regeneron's VelocImmune technology.

Monoclonal antibodies have been generated and approved to treat: cancer, cardiovascular disease, inflammatory diseases, macular degeneration, transplant rejection, multiple sclerosis, and viral infection (see monoclonal antibody therapy).

In August 2006 the Pharmaceutical Research and Manufacturers of America reported that U.S. companies had 160 different monoclonal antibodies in clinical trials or awaiting approval by the Food and Drug Administration.[18]

Applications

Diagnostic tests

Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance. The Western blot test and immuno dot blot tests detect the protein on a membrane. They are also very useful in

immunohistochemistry, which detect antigen in fixed tissue sections and immunofluorescence test, which detect the substance in a frozen tissue section or in live cells.

Polyclonal antibodies

Polyclonal antibodies (or antisera) are antibodies that are obtained from different B cell resources. They are a combination of immunoglobulin molecules secreted against a specific antigen, each identifying a different epitope.

Production

These antibodies are typically produced by immunization of a suitable mammal, such as a mouse, rabbit or goat. Larger mammals are often preferred as the amount of serum that can be collected is greater. An antigen is injected into the mammal. This induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen. This polyclonal IgG is polyclonal purified from the mammal's serum.

By contrast, monoclonal antibodies are derived from a single cell line.

Many methodologies exist for polyclonal antibody production in laboratory animals. Institutional guidelines governing animal use and procedures relating to these methodologies are generally oriented around humane considerations and appropriate conduct for adjuvant (agents which modify the effect of other agents while having few if any direct effects when given by themselves) use. This includes adjuvant selection, routes and sites of administration, injection volumes per site and number of sites per animal. Institutional policies generally include allowable volumes of blood per collection and safety precautions including appropriate restraint and sedation or anesthesia of animals for injury prevention to animals or personnel.

The primary goal of antibody production in laboratory animals is to obtain high titer, high affinity antisera for use in experimentation or diagnostic tests. Adjuvants are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site, antigen depot which allows for a slow release of antigen into draining lymph nodes.

Many adjuvants also contain or act directly as:

1. surfactants which promote concentration of protein antigens molecules over a large surface area, and
2. immunostimulatory molecules or properties. Adjuvants are generally used with soluble protein antigens to increase antibody titers and induce a prolonged response with accompanying memory.

Such antigens by themselves are generally poor immunogens. Most complex protein antigens induce multiple B-cell clones during the immune response, thus, the response is polyclonal. Immune responses to non-protein antigens are generally poorly or enhanced by adjuvants and there is no system memory.

Animal selection

Animals frequently used for polyclonal antibody production include chickens, goats, guinea pigs, hamsters, horses, mice, rats, and sheep. However, the rabbit is the most commonly used laboratory animal for this purpose. Animal selection should be based upon:

1. the amount of antibody needed,
2. the relationship between the donor of the antigen and the recipient antibody producer (generally the more distant the phylogenetic relationship, the greater the potential for high titer antibody response) and
3. the necessary characteristics [e.g., class, subclass (isotype), complement fixing nature] of the antibodies to be made. Immunization and phlebotomies are stress associated and, at least when using rabbits and rodents, specific pathogen

free (SPF) animals are preferred. Use of such animals can dramatically reduce morbidity and mortality due to pathogenic organisms, especially *Pasteurella multocida* in rabbits.

Goats or horses are generally used when large quantities of antisera are required. Many investigators favor chickens because of their phylogenetic distance from mammals. Chickens transfer high quantities of IgY (IgG) into the egg yolk and harvesting antibodies from eggs eliminates the need for the invasive bleeding procedure. One week's eggs can contain 10 times more antibodies than the volume of rabbit blood obtained from one weekly bleeding. However, there are some disadvantages when using certain chicken derived antibodies in immunoassays. Chicken IgY does not fix mammalian complement component C1 and it does not perform as a precipitating antibody using standard solutions.

Although mice are used most frequently for monoclonal antibody production, their small size usually prevents their use for sufficient quantities of polyclonal, serum antibodies. However, polyclonal antibodies in mice can be collected from ascites fluid using any one of a number of ascites producing methodologies.

When using rabbits, young adult animals (2.5–3.0 kg or 5.5-6.5lbs) should be used for primary immunization because of the vigorous antibody response. Immune function peaks at puberty and primary responses to new antigens decline with age. Female rabbits are generally preferred because they are more docile and are reported to mount a more vigorous immune response than males. At least two animals per antigen should be used when using outbred animals. This principle reduces potential total failure resulting from non-responsiveness to antigens of individual animals.

Antigen preparation

The size, extent of aggregation and relative nativity of protein antigens can all dramatically affect the quality and quantity of antibody produced. Small polypeptides (<10 ku) and non-protein antigens generally need to be conjugated or crosslinked to larger, immunogenic, carrier proteins to increase immunogenicity and provide T cell epitopes. Generally, the larger the immunogenic protein the better. Larger proteins, even in smaller amounts, usually result in better engagement of antigen presenting antigen processing cells for a satisfactory immune response. Injection of soluble, non-aggregated proteins has a higher probability of inducing tolerance rather than a satisfactory antibody response.

Keyhole limpet hemocyanin (KLH) and bovine serum albumin are two widely used carrier proteins. Poly-L-lysine has also been used successfully as a backbone for peptides. Although the use of Poly-L-lysine reduces or eliminates production of antibodies to foreign proteins, it may result in failure of peptide-induced antibody production. Recently, liposomes have also been successfully used for delivery of small peptides and this technique is an alternative to delivery with oily emulsion adjuvants.

Antigen quantity

Selection of antigen quantity for immunization varies with the properties of the antigen and the adjuvant selected. In general, microgram to milligram quantities of protein in adjuvant are necessary to elicit high titer antibodies. Antigen dosage is generally species, rather than body weight, associated. The so called "window" of immunogenicity in each species is broad but too much or too little antigen can induce tolerance, suppression or immune deviation towards cellular immunity rather than a satisfactory humoral response. Optimal and usual protein antigen levels for immunizing specific species have been reported in the following ranges:

1. rabbit, 50–1000 µg;
2. mouse, 10–200 µg;
3. guinea pig, 50–500 µg; and

4. goat, 250–5000 µg. Optimal "priming" doses are reported to be at the low end of each range.

The affinity of serum antibodies increases with time (months) after injection of antigen-adjuvant mixtures and as antigen in the system decreases. Widely used antigen dosages for "booster" or secondary immunizations are usually one half to equal the priming dosages. Antigens should be free of preparative byproducts and chemicals such as polyacrylamide gel, SDS, urea, endotoxin, particulate matter and extremes of pH.

Peptide Antibodies

When a peptide is being used to generate the antibody, it is extremely important to design the antigens properly. There are several resources that can aid in the design as well as companies that offer this service. Expasy has aggregated a set of public tools under its ProtScale page that require some degree of user knowledge to navigate. For a more simple peptide scoring tool there is a Antigen Profiler tool available that will enable you to score individual peptide sequences based upon a relation epitope mapping database of previous immunogens used to generate antibodies. Finally, as a general rule peptides should follow some basic criteria.

When examining peptides for synthesis and immunization, it is recommended that certain residues and sequences be avoided due to potential synthesis problems. This includes some of the more common characteristics:

• Extremely long repeats of the same amino acid (e.g. RRRR) • Serine (S), Threonine (T), Alanine (A), and Valine (V) doublets • Ending or starting a sequence with a proline (P) • Glutamine (Q) or Asparagine (N) at the n-terminus • Peptides over weighted with hydrophobic residues (e.g. V,A,L,I, etc...)

Reactivity

Investigators should also consider the status of nativity of protein antigens when used as immunogens and reaction with antibodies produced. Antibodies to native proteins react best with native proteins and antibodies to denatured proteins react best with denatured proteins. If elicited antibodies are to be used on membrane blots (proteins subjected to denaturing conditions) then antibodies should be made against denatured proteins. On the other hand, if antibodies are to be used to react with a native protein or block a protein active site, then antibodies should be made against the native protein. Adjuvants can often alter the nativity of the protein. Generally, absorbed protein antigens in a preformed oil-in-water emulsion adjuvant, retain greater native protein structure than those in water-in-oil emulsions.

Asepticity

Antigens should always be prepared using techniques that ensure that they are free of microbial contamination. Most protein antigen preparations can be sterilized by passage through a 0.22u filter. Septic abscesses often occur at inoculation sites of animals when contaminated preparations are used. This can result in failure of immunization against the targeted antigen.

Adjuvants

There are many commercially available immunologic adjuvants. Selection of specific adjuvants or types varies depending upon whether they are to be used for research and antibody production or in vaccine development. Adjuvants for vaccine use only need to produce protective antibodies and good systemic memory while those for antiserum production need to rapidly induce high titer, high avidity antibodies. No single adjuvant is ideal for all purposes and all have advantages and disadvantages. Adjuvant use generally is accompanied by undesirable side effects of varying severity and duration. Research on new adjuvants focuses on substances which have minimal toxicity while retaining maximum immunostimulation. Investigators should always be aware of potential pain and distress associated with adjuvant use in laboratory animals.

The most frequently used adjuvants for antibody production are Freund's, Alum, the Ribi Adjuvant System and Titermax.

Freund's adjuvants

There are two basic types of Freund's adjuvants: Freund's Complete Adjuvant (FCA) and Freund's Incomplete Adjuvant (FIA). FCA is a water-in-oil emulsion that localizes antigen for release periods up to 6 months. It is formulated with mineral oil, the surfactant mannide monoleate and heat killed *Mycobacterium tuberculosis*, *Mycobacterium butyricum* or their extracts (for aggregation of macrophages at the inoculation site). This potent adjuvant stimulates both cell mediated and humoral immunity with preferential induction of antibody against epitopes of denatured proteins. Although FCA has historically been the most widely used adjuvant, it is one of the more toxic agents due to non-metabolizable mineral oil and it induces granulomatous reactions. Its use is limited to laboratory animals and it should be used only with weak antigens. It should not be used more than once in a single animal since multiple FCA inoculations can cause severe systemic reactions and decreased immune responses. Freund's Incomplete Adjuvant has the same formulation as FCA but does not contain mycobacterium or its components. FIA usually is limited to booster doses of antigen since it normally much less effective than FCA for primary antibody induction. Freund's adjuvants are normally mixed with equal parts of antigen preparations to form stable emulsions.

Ribi Adjuvant System

Ribi adjuvants are oil-in-water emulsions where antigens are mixed with small volumes of a metabolizable oil (squalene) which are then emulsified with saline containing the surfactant Tween 80. This system also contains refined mycobacterial products (cord factor, cell wall skeleton) as immunostimulants and bacterial monophosphoryl lipid A. Three different species oriented formulations of the adjuvant system are available. These adjuvants interact with membranes of immune cells resulting in cytokine induction, which enhances antigen uptake, processing and presentation. This adjuvant system is much less toxic and less potent than FCA but generally induces satisfactory amounts of high avidity antibodies against protein antigens.

Titermax

Titermax represents a newer generation of adjuvants that are less toxic and contain no biologically derived materials. It is based upon mixtures of surfactant acting, linear, blocks or chains of nonionic copolymers polyoxypropylene (POP) and polyoxyethylene (POE). These copolymers are less toxic than many other surfactant materials and have potent adjuvant properties which favor chemotaxis, complement activation and antibody production. Titermax adjuvant forms a microparticulate water-in-oil emulsion with a copolymer and metabolizable squalene oil. The copolymer is coated with emulsion stabilizing silica particles which allows for incorporation of large amounts of a wide variety of antigenic materials. The adjuvant active copolymer forms hydrophilic surfaces, which activate complement, immune cells and increased expression of class II major histocompatibility molecules on macrophages. Titermax presents antigen in a highly concentrated form to the immune system, which often results in antibody titers comparable to or higher than FCA.

Specol: Specol is a water in oil adjuvant made of purified mineral oil. It has been reported to induce immune response comparable to Freund's adjuvant in rabbit and other research animal while producing fewer histological lesions.

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2. ^ Landsteiner, Karl (1990). *The Specificity of Serological Reactions, 2nd Edition, revised*. Courier Dover Publications. ISBN 0486662039.
3. ^ Shreder, Kevin (March 2000). "Synthetic Haptens as Probes of Antibody Response and Immunorecognition". *Methods* (Academic Press) **20** (3): 372–379. doi:10.1006/meth.1999.0929. PMID 10694458.

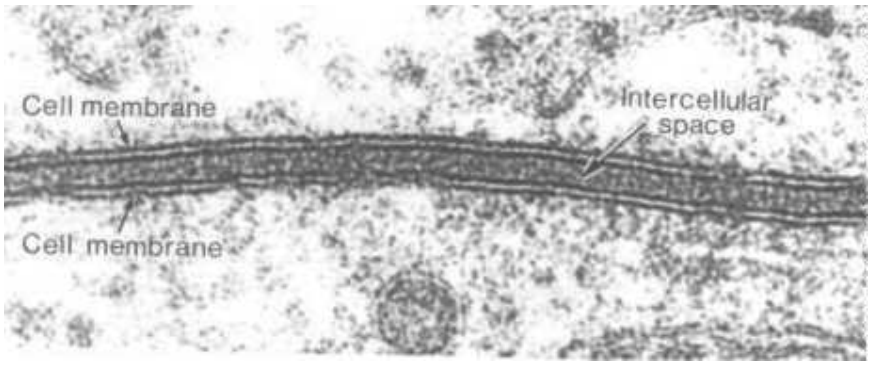
4 Kurland IJ, Pilkis SJ (06/01/1995). "Covalent control of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: insights into autoregulation of a bifunctional enzyme". *Protein Sci.* **4** (6): 1023–37. doi:10.1002/pro.5560040601 (inactive 2010-01-08). PMID 7549867. PMC 2143155. http://www.proteinscience.org/cgi/reprint/4/6/1023.

5 Okar DA, Lange AJ (1999). "Fructose-2,6-bisphosphate and control of carbohydrate metabolism in eukaryotes". *Biofactors* **10** (1): 1–14. doi:10.1002/biof.5520100101. PMID 10475585.

Section B: Dr Adebambo. Blood Groups

INTRODUCTION TO BLOOD GROUP SEROLOGY

CELL LAYER.....



Membrane Structure

The cell is highly organized with many functional units or organelles. Most of these units are limited by one or more membranes. To perform the function of the organelle, the membrane is specialized in that it contains specific proteins and lipid components that enable it to perform its unique roles for that cell or organelle. In essence membranes are essential for the integrity and function of the cell.

Membrane components may:

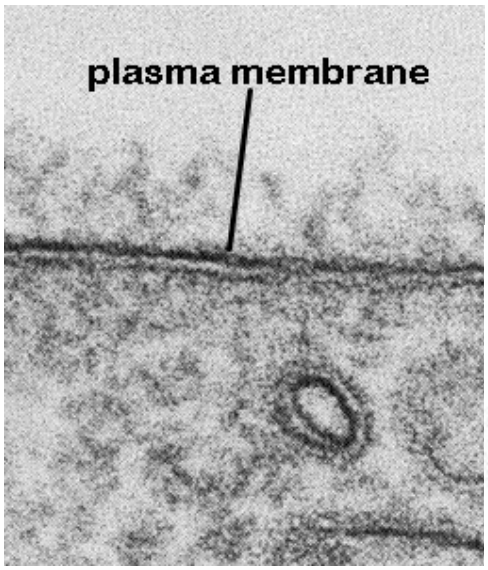
- be protective
- regulate transport in and out of cell or subcellular domain
- allow selective receptivity and signal transduction by providing transmembrane receptors that bind signaling molecules
- allow cell recognition
- provide anchoring sites for cytoskeletal filaments or components of the extracellular matrix. This allows the cell to maintain its shape and perhaps move to distant sites.
- help compartmentalize subcellular domains or microdomains
- provide a stable site for the binding and catalysis of enzymes.
- regulate the fusion of the membrane with other membranes in the cell via specialized junctions)
- provide a passageway across the membrane for certain molecules, such as in gap junctions.
- allow directed cell or organelle motility

Membrane Structure and Function

Introduction:

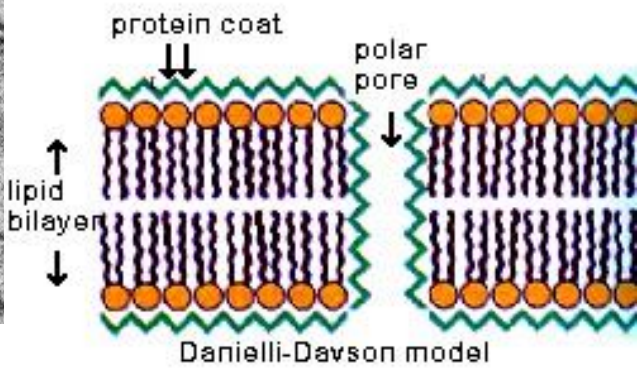
Membranes are vital because they separate the cell from the outside world. They also separate compartments inside the cell to protect important processes and events.

Cellular membranes have diverse functions in the different regions and organelles of a cell. However, at the electron microscopic level, they share a common structure following routine preparative steps. The above figure shows the typical **"Unit" membrane** which resembles a railroad track with two dense lines separated by a clear space. This figure actually shows two adjacent plasma membranes, both of which have the **"unit membrane"** structure. *(Above figure taken from Bloom and Fawcett, A Textbook of Histology, Chapman and Hall, N.Y., 12th edition, 1994, Figure 1-2.)*



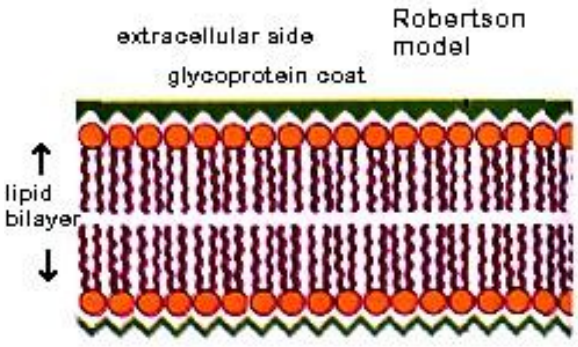
How did early cell biologists deduce membrane structure from electron microscopic images and the knowledge that membranes were lipoprotein complexes?

Note the material projecting from the plasma membrane both inside and outside of the cell. What do you think this is? Also, outside of the cell is in the upper half of photo. Can you find a vesicle with a unit membrane structure?



A historical perspective

In the early 1930's-40's, Danielli and Davson studied triglyceride lipid that they arranged bilayers over a water surface. They found themselves with the polar heads facing outward. However, they always formed droplets (oil in water) and the surface tension was much higher than that of cells. However, if you added proteins, the surface tension was reduced and the membranes flattened out.



Here is a diagram of their early model of the cell membrane.

What membrane functions might be allowed by this model?

In the 1950's Robertson noted the structure of membranes seen in the above electron micrographs. He saw no spaces for pores in the electron micrographs. He hypothesized that the railroad track appearance came from the binding of osmium tetroxide to proteins and polar groups of lipids.

What is missing in Robertson's model?

In 1966, Lenard and Singer noted that over 30% of membrane proteins were twisted into an alpha helix. This made it likely that there were many spherical proteins.

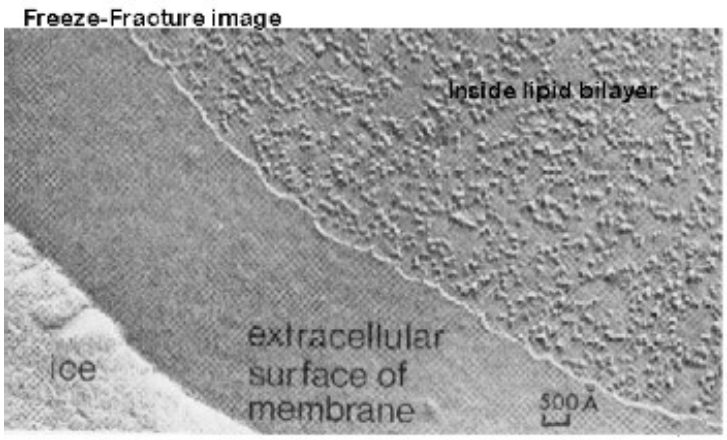
Could one build a Davson-Danielli or Robertson model with spherical proteins? What would happen to the size of the membrane?

Furthermore, what would happen if you unfolded the proteins, exposing non-polar (hydrophobic amino acid side chains) groups to the aqueous environment?

What kind of energy would the cell have to expend to keep proteins flattened in this state?

Singer studied phospholipids bilayers and found that they can form a flattened surface on water, with no requirement for a protein coat. The turning point in the modeling came with the advent of freeze fracture techniques. This figure shows the inside of a membrane and the "bumps, grooves, ridges". These were later found to be proteins.

What conclusions could you draw about membrane structure from the photo to the left?

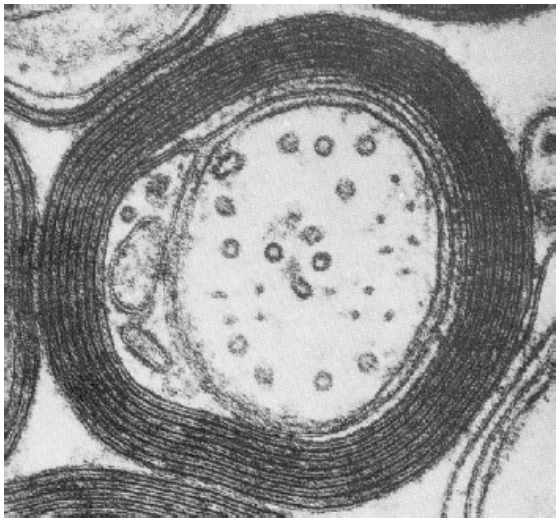


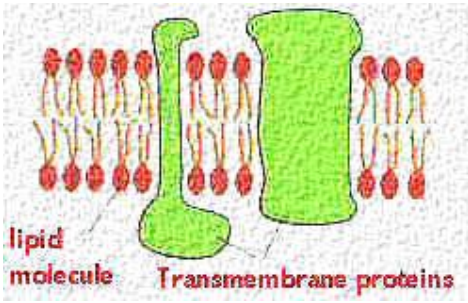
Basic Membrane Architecture

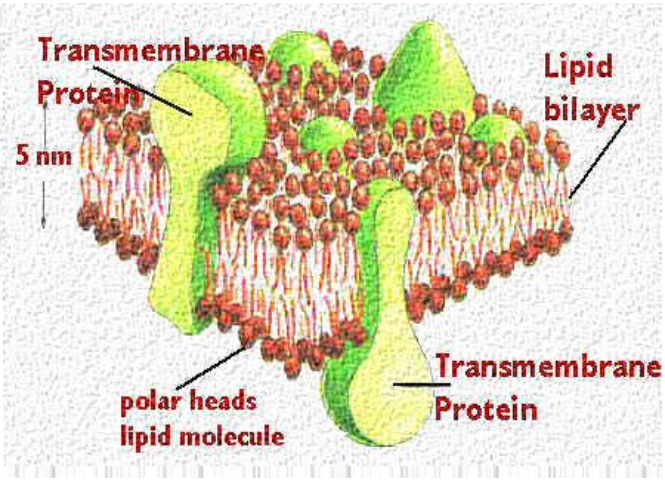
The purpose of this presentation will be to show how membranes are studied by the cell biologist. First, we will look at basic **unit membrane architecture**. All membranes contain proteins and lipid. However, the proportion of each varies depending on the membrane. For example:

- Myelin, which insulates nerve fibers, contains only 18% protein and 76% lipid. A electron micrograph of myelin is to the right.
- Mitochondrial inner membrane contain 76% protein and only 24% lipid.
- Plasma membranes of human red blood cells and mouse liver contain nearly equal amounts of proteins (44, 49% respectively) and lipids (43, 52% respectively).

Considering what you already know about these cells or organelles, what would be the significance of these different proportions of lipids and proteins?





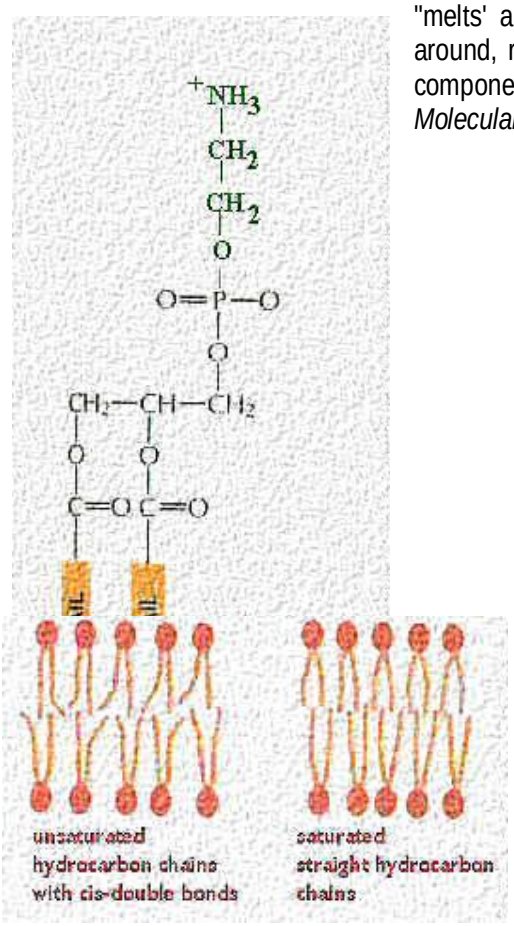


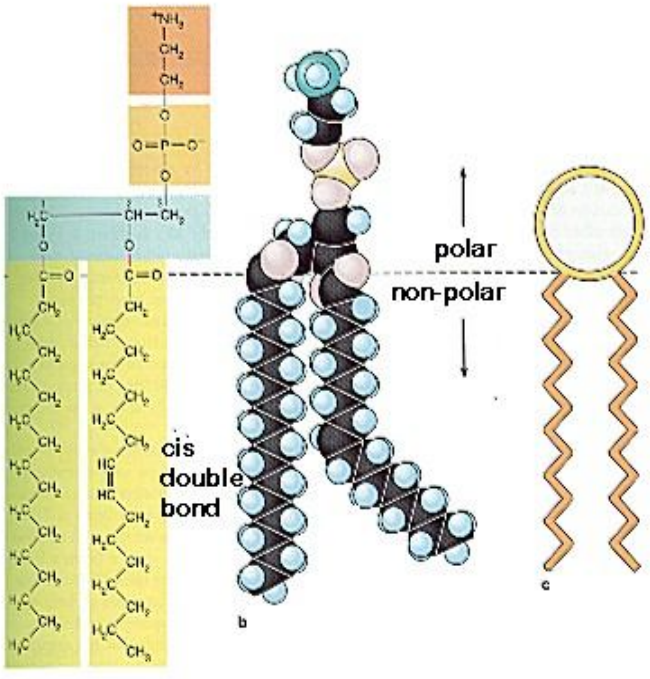
As we said above, membrane architecture is that of a **lipid bilayer**. The lipids are amphipathic in that they have **hydrophilic polar heads** pointing out and the **hydrophobic portion** forming the core. Proteins are embedded in the bilayer. They may pass through the bilayer (as **transmembrane**

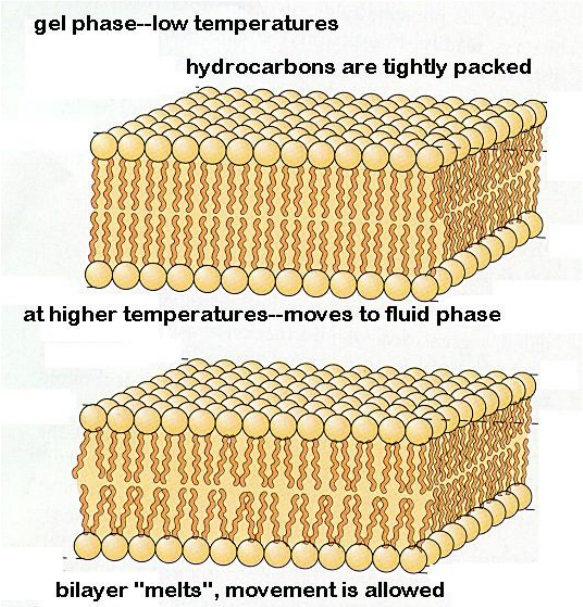
proteins), or they may be inserted at the cytoplasmic or exterior face.
A cross section of the bilayer is seen in this figure. As we will see in more detail below, the lipid molecules have a **globular (polar) head** and a straight region **(non-polar)**. Each row of lipids is a leaflet. Therefore, the plasma membrane consists of two leaflets with the non-polar regions pointing inward.

Membrane Phospholipids
Lets take apart the membrane and examine each of its components.
One of the principal types of lipid in the membrane include the **phospholipids** . These have a polar head group and two hydrocarbon tails. An example of a phospholipid is shown in this figure (right). The top region beginning with the NH3 is the polar group. It is connected by glycerol to two fatty acid tails. One of the tails is a straight chain fatty acid (saturated). The other has a kink in the tail because of a cis double bond (unsaturated). This kink influences packing and movement in the lateral plane of the membrane. Figure to the left is modified from *Alberts et al. Molecular Biology of the Cell, Garland Publishing, N.Y., 1994, Third Edition, Figure 10-10.* The figure below is from *Wolfe S.L., Molecular and Cellular Biology, Wadsworth Publishing Company, 1993, p 155.*

The figure in this paragraph shows how the phospholipids pack together in the two leaflets in the membrane. The presence of the cis double bond makes prevents tight packing and makes the bilayer difficult to freeze.
The lipid bilayer gives the membranes its fluid characteristics. The following cartoon shows the effect of temperature on the packing of the hydrocarbons. Note that at low temperatures, the bilayer is in a gel state and tightly packed. At higher (body) temperatures, the bilayer actually "melts' and the interior is fluid allowing the lipid molecules to move around, rotate, exchange places. This also allows movement of other components of the membrane. The figure below is from *Wolfe S.L., Molecular and Cellular Biology, Wadsworth Publishing Company, 1993.*

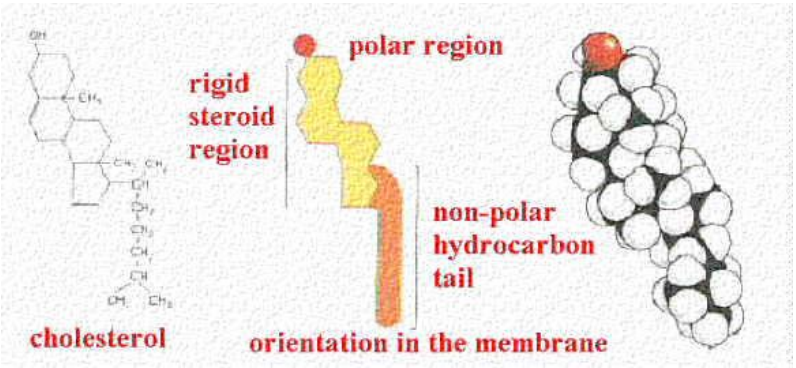




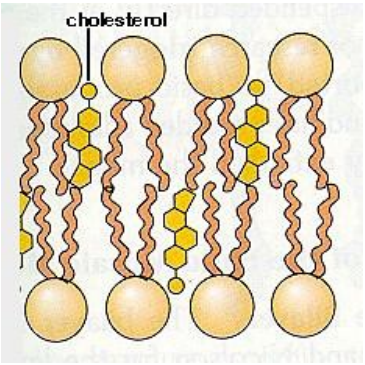


Membrane Cholesterol

Another type of lipid in the membrane is **cholesterol**. The amount of cholesterol may vary with the type of membrane. Plasma membranes have nearly one cholesterol per phospholipid molecule. Other membranes (like those around bacteria) have no cholesterol. The following figure shows the steroid structure of cholesterol. The non-polar and polar regions are also illustrated in b) (Figure modified from *Alberts et al. Molecular Biology of the Cell, Garland Publishing, N.Y., 1994, Third Edition, Figure 10-8.*

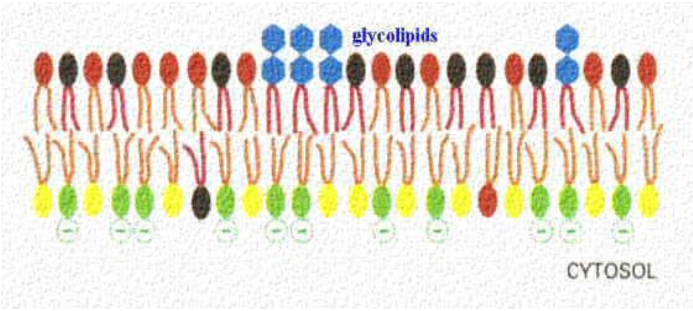


The cholesterol molecule inserts itself in the membrane with the same orientation as the phospholipid molecules. The figures show phospholipid molecules with a cholesterol molecule inbetween. Note that the polar head of the cholesterol is aligned with the polar head of the phospholipids. Figure modified from *Alberts et al. Molecular Biology of the Cell, Garland Publishing, N.Y., 1994, Third Edition, Figure 10-9; or Wolfe S.L., Molecular and Cellular Biology, Wadsworth Publishing Company, 1993 (figure below).*



Cholesterol molecules have several functions in the membrane:

- They immobilize the first few hydrocarbon groups of the phospholipid molecules. This makes the lipid bilayer less deformable and decreases its permeability to small water-soluble molecules. Without cholesterol (such as in a bacterium) a cell would need a cell wall. Cholesterol prevents crystallization of hydrocarbons and phase shifts in the membrane.



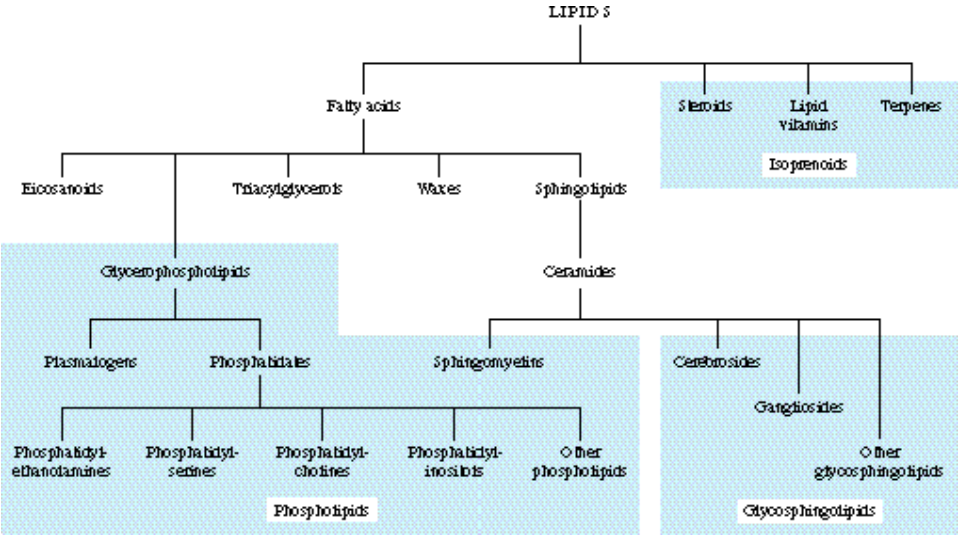
Membrane Glycolipids

Glycolipids are also a constituent of membranes. In this figure, they are shown as blue sugar groups projecting into the extracellular space. They may microaggregate in the membrane. These components of the membrane may be protective, insulators, and sites of receptor binding. Among the molecules bound by glycososphingolipids include cell poisons such as cholera and tetanus toxins. The lower figure shows the chemical structure of two examples of glycososphingolipids.

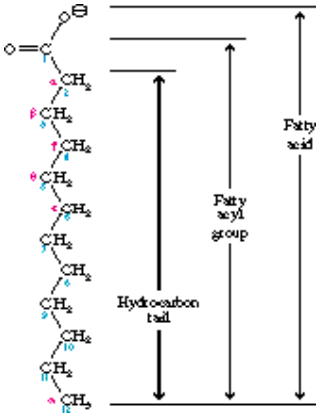
Formation of "Microdomains"

Sphingolipids and cholesterol work together to help cluster proteins in a region called a "microdomain". They function as "rafts" or platforms for the attachment of proteins as membranes are moved around the cell and also during signal transduction.

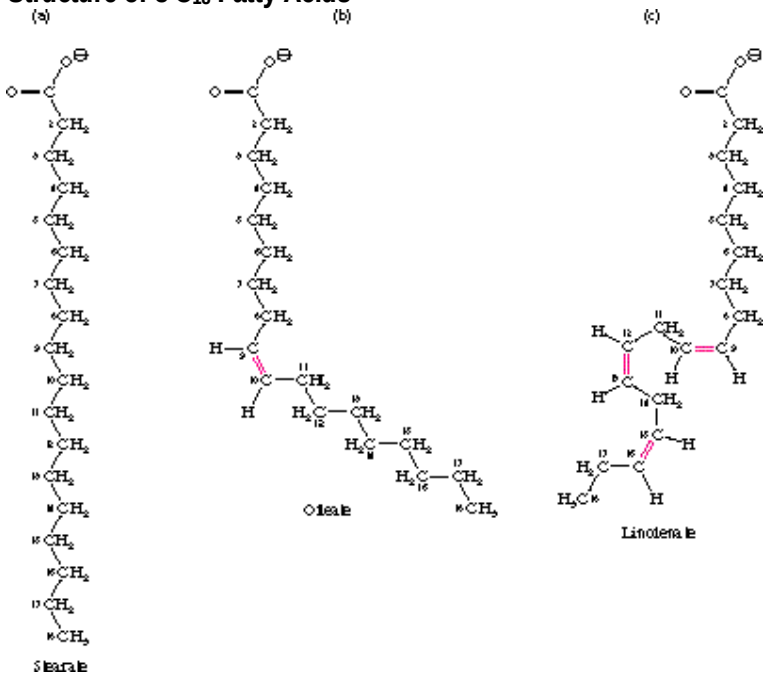
LIPID STRUCTURES



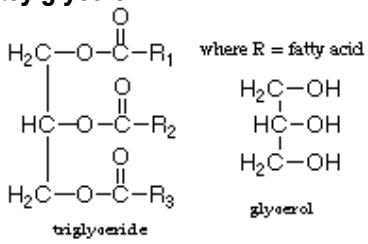
Structure and Nomenclature of Fatty Acids

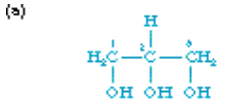


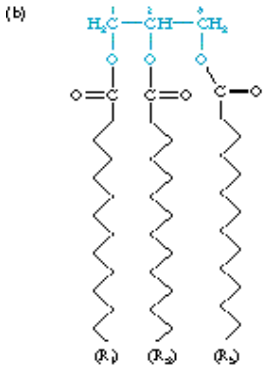
Structure of 3 C_{18} Fatty Acids



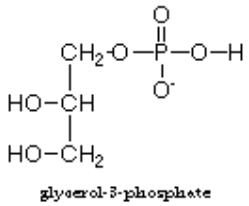
Triacylglycerol

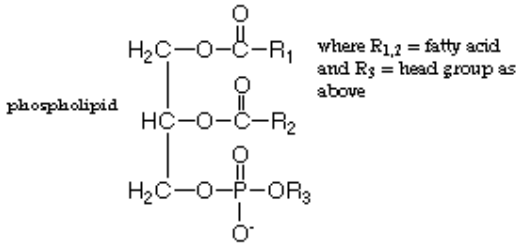






Glycerophospholipids or phoshoglycerides





a) Glycerol 3-Phosphate and Phosphatidate

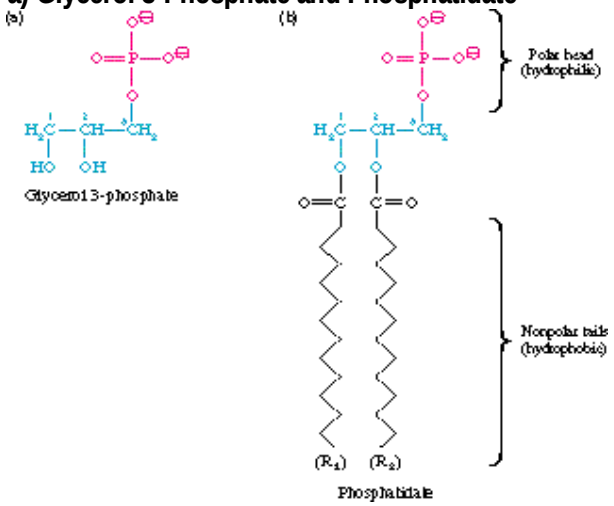
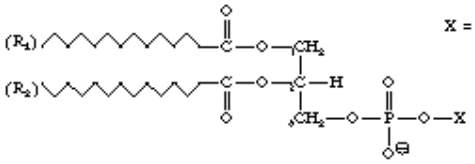
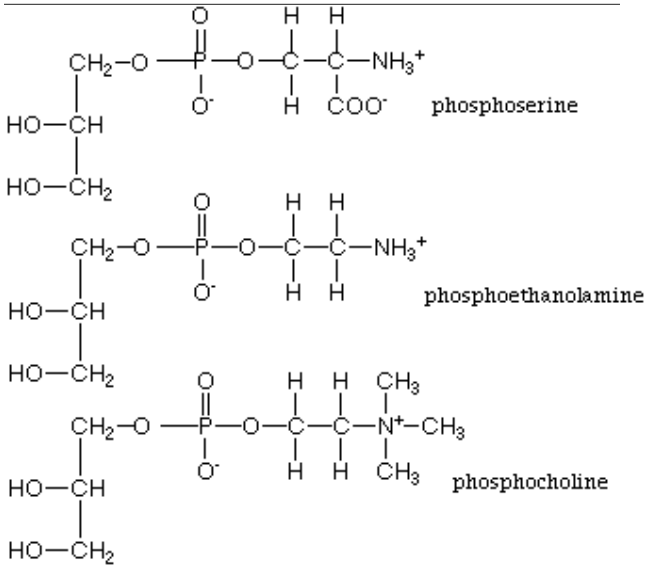


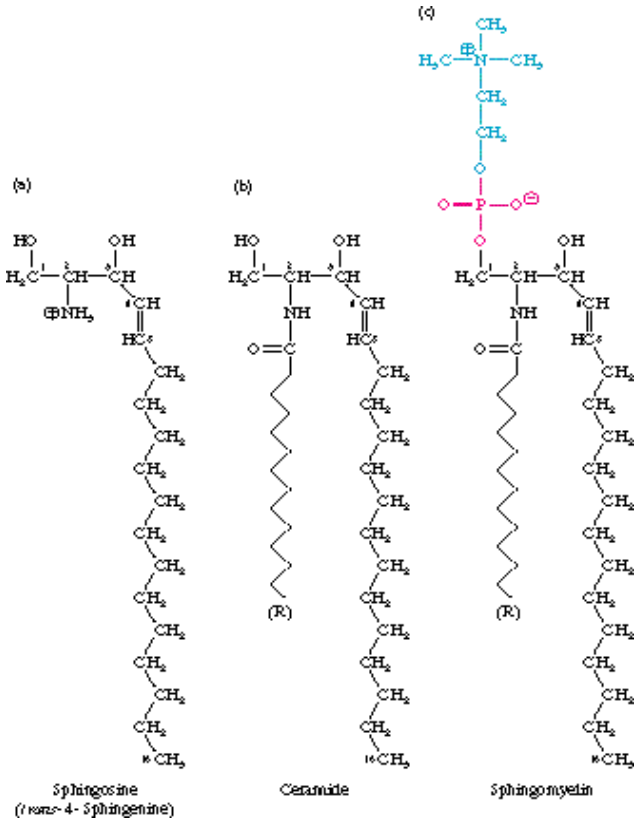
Table 10-2 Some common types of glycerophospholipids

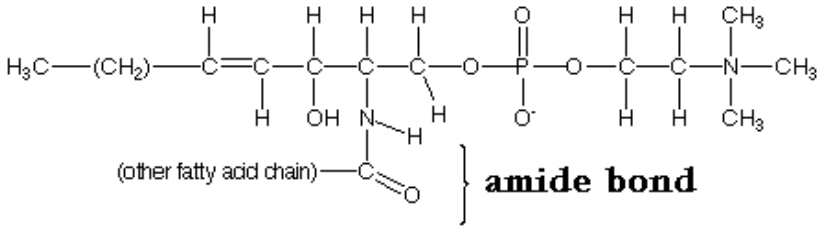


Precursor of X (HO—X)	Formula of —O—X	Name of resulting glycerophospholipid
Water	—O—H	Phosphatidate
Choline	—O—$CH_2CH_2\overset{\oplus}{N}(CH_3)_3$	Phosphatidylcholine
Ethanolamine	—O—$CH_2CH_2\overset{\oplus}{N}H_3$	Phosphatidylethanolamine
Serine	—O—CH_2—$CH(\overset{\oplus}{N}H_3)(COO^{\ominus})$	Phosphatidylserine
Glycerol	—O—$CH_2CH(OH)CH_2OH$	Phosphatidylglycerol
Phosphatidyl-glycerol	—O—$CH_2CH(OH)CH_2$—O—P(O)(O$^{\ominus}$)—O—CH_2	Diphosphatidylglycerol (Cardiolipin)
myo-Inositol	—O—	Phosphatidylinositol



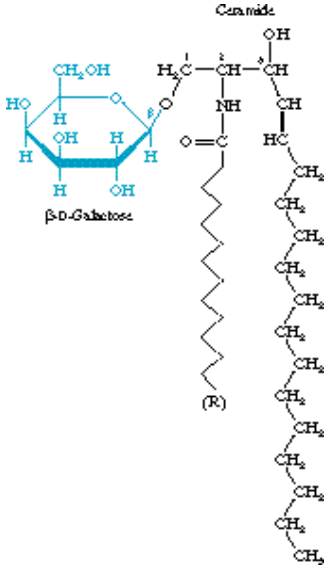
Sphingolipids
a) Sphingosine, b) ceramide, and c) Sphingomyelin



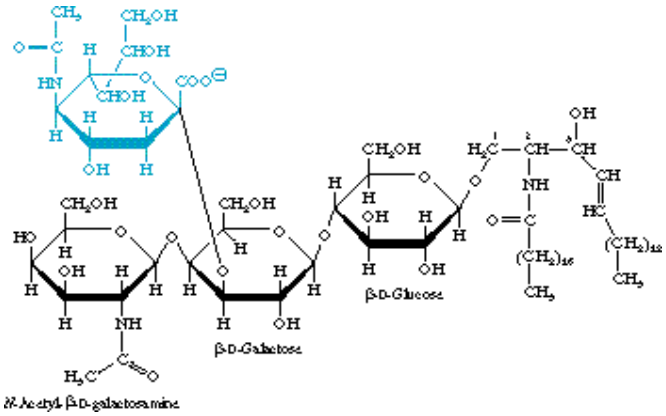


sphingomyelin

Galactocerebroside

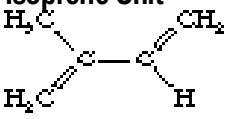


Ganglioside G_{M2}



Isoprenoids (based on 5-carbon isoprene)

Isoprene Unit



Membrane Proteins

As you study different organelles, you will learn about important membrane proteins that function for that particular organelle. **Transmembrane proteins** are amphipathic, in that they have hydrophobic and hydrophilic regions that are oriented in the same regions in the lipid bilayer. Another name for them is **"integral proteins"**. Other types of proteins may be linked only at the cytoplasmic surface (by attachment to a fatty acid chain), or at the external cell surface, attached by a oligosaccharide. Or, these non-transmembrane proteins may be bound to other membrane proteins. Collectively these are called **"peripheral membrane proteins".** Proteins inserted once through the membrane are called "single-pass transmembrane proteins." Those that pass through several times are called "multipass transmembrane proteins". They form loops outside the membrane.

Glycoproteins are the proteins covalently attached to carbohydrates such as glucose, galactose, lactose, fucose, sialic acid, N-acetylglucosamine, N-acetylgalactosamine, etc. Glycolipids are carbohydrate-attached lipids. Their role is to provide energy and also serve as markers for cellular recognition.

The antigens which determine blood types belong to glycoproteins and glycolipids. The antigens which determine blood types belong to glycoproteins and glycolipids. There are three types of blood-group antigens: O, A, and B. They differ only slightly in the composition of carbohydrates.

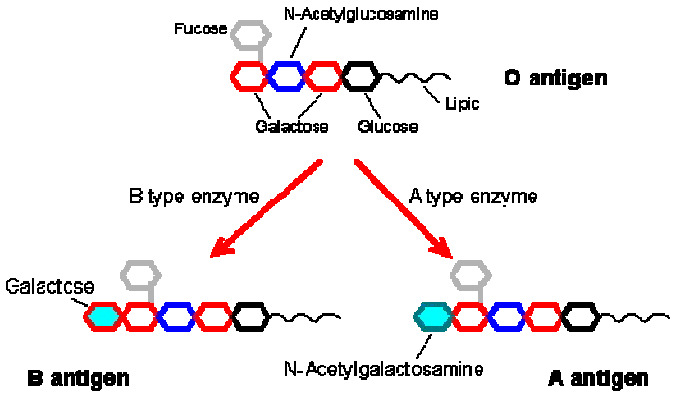


Figure. Blood-group antigens.

All humans contain enzymes which catalyze the synthesis of the O antigen. Humans with A-type blood also contain an additional enzyme (called A-type enzyme here) which adds N-Acetylgalactosamine to the O antigen. Humans with B-type blood contain another enzyme (called B-type enzyme here) which adds Galactose to the O antigen. Humans with AB-type blood contain both A-type and B-type enzymes while humans with O-type blood lack both types of enzymes.

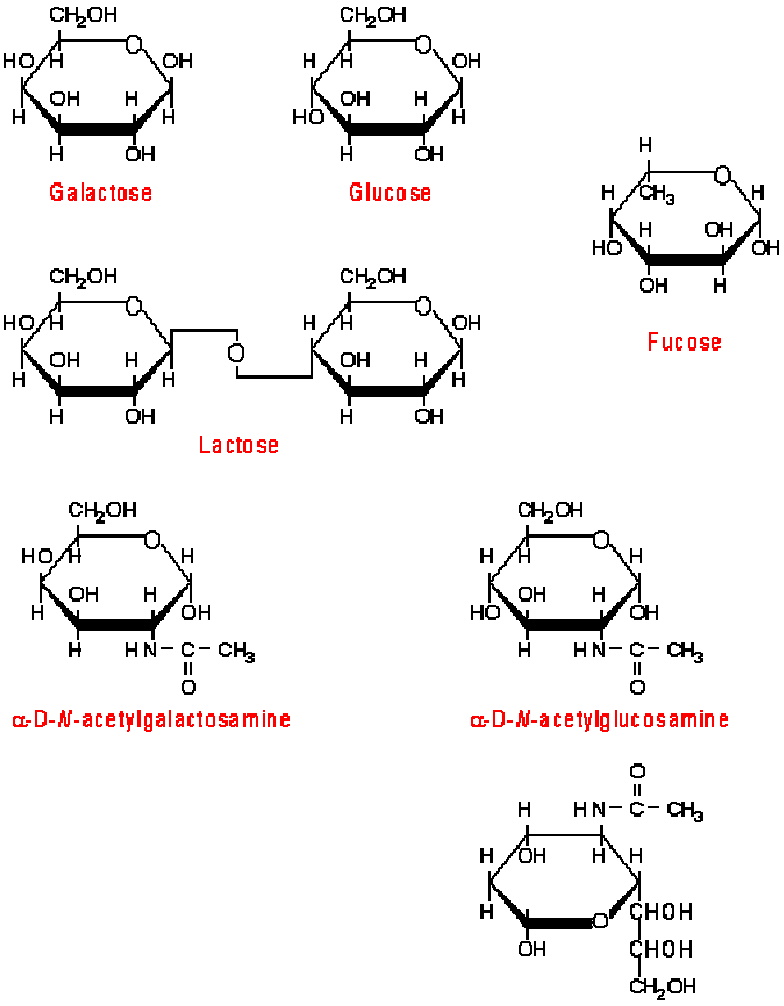
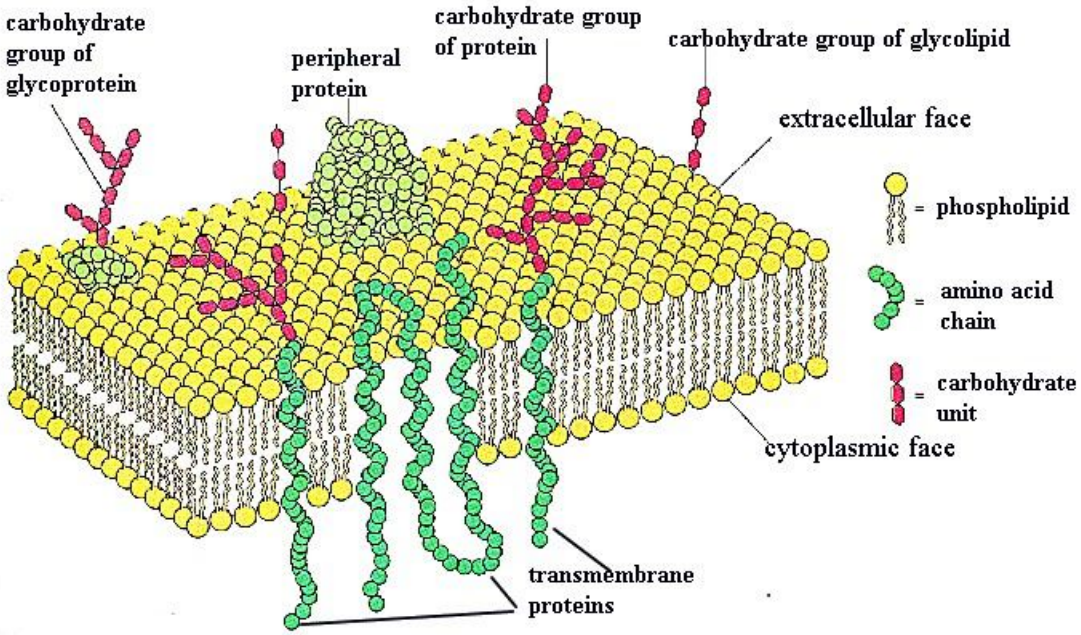
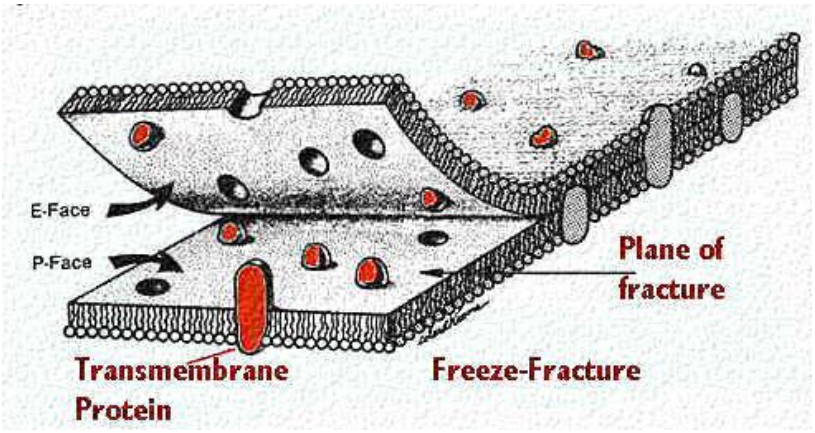


Figure 1-B-4. Structures of some sugar molecules.

The following figure shows transmembrane proteins passing through the lipid bilayer. Can you find the multipass and single-pass transmembrane proteins in this figure?



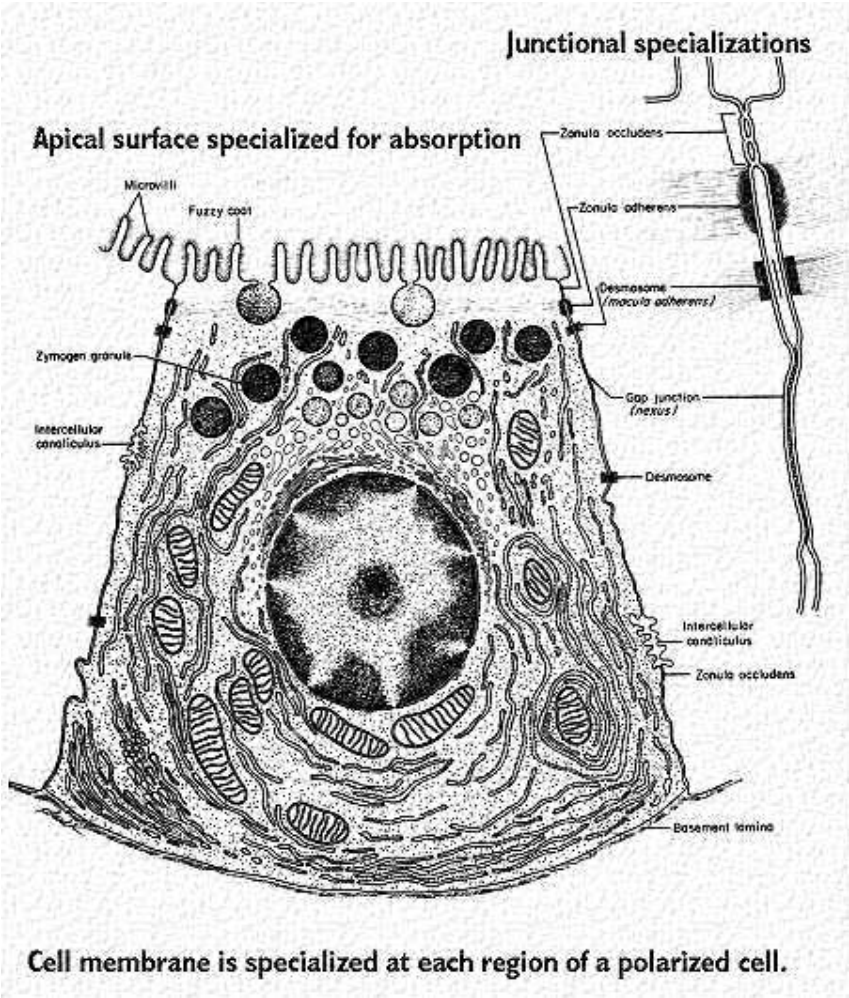


An EM view of membranes via freeze fracture/freeze etch .

You can best see protein distribution via a technique called freeze fracture/freeze etch. The freeze-fracture/freeze etch technique starts with rapid freezing of a cell. Then the frozen cells are cleaved along a fracture plane. This fracture plane is in between the leaflets of the lipid bilayer , as shown by this cartoon. The two fractured sections are then coated with heavy metal (etched) and a replica is made of their surfaces. This replica is then viewed in an electron microscope. One sees homogeneous regions where there was only the exposed lipid leaflet (Is the exposed surface made of polar or nonpolar groups?

In certain areas of the cell, one also sees protrusions or bumps. These are colored red in the cartoon. Sometimes one can see structure within the bumps themselves. These are the transmembrane proteins.

The following illustration will show you a freeze-fracture/freeze etch view. The organization or structure of the transmembrane proteins can often be visualized.

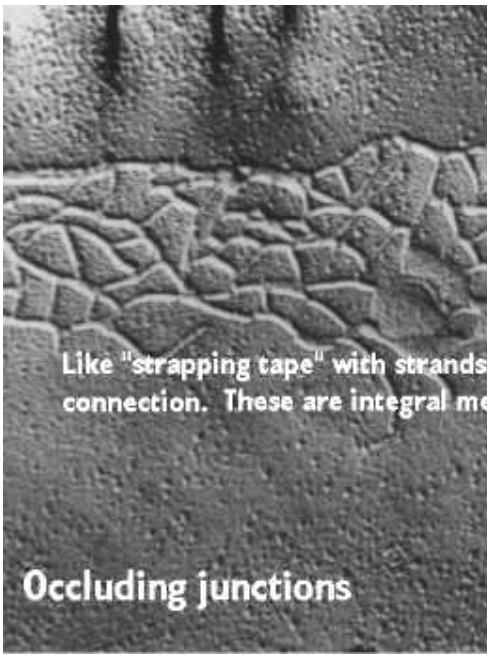


Membrane specializations (Adhesions): Junctions

The drawing is of a polarized cell. The top is specialized for absorption and the bottom for transfer of materials to the blood stream. The sides have specialized junctions that keep the nutrients from entering the space between the cells.

Tight Junctions

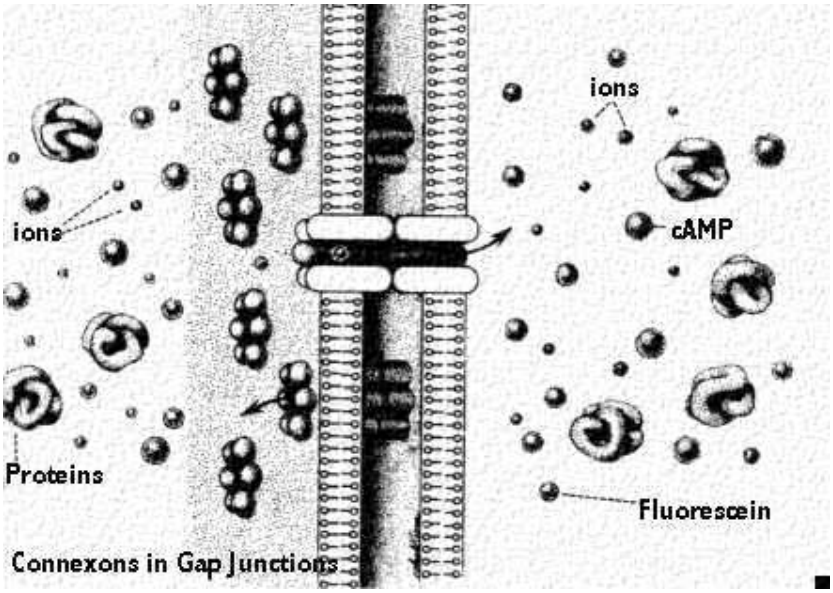
One of these is called a **tight junction or "occluding junction" (zonula occludens)**. This is shown as the top junction in the above drawing. At this site, membrane glycoproteins and associated "glue" bind the cells together like double-sided "strapping tape" The freeze-fracture/freeze etch view of this junction (shown below) illustrates the ridges in the plane of the exposed leaflet. These are the proteins that bind to the proteins from the adjacent cell.



Gap Junctions

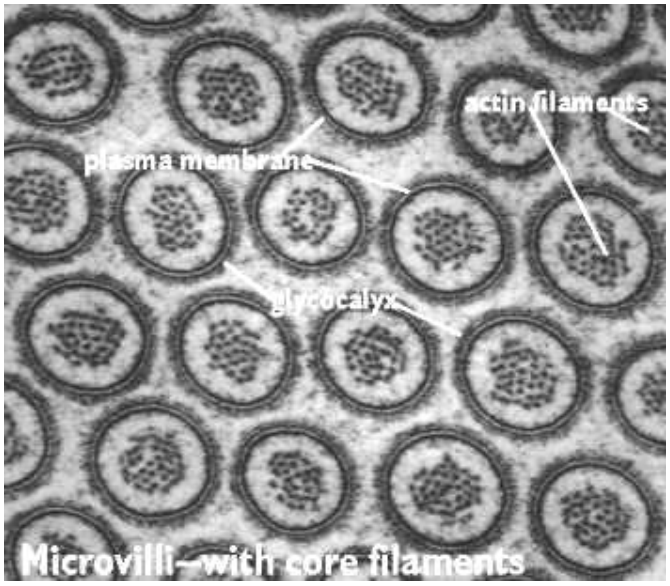
Another type of junction allows communication between cells. This type is called a **gap junction**. Small molecules or ions can pass through, as we will see by the following figures.

The above freeze-fracture /freeze etch image shows the internal view of the **gap junction** on the left. The proteins look like little donuts which reflects the fact that they are actually a channel. These proteins are "connexon" molecules. The side facing the cytoplasm (called the P face) is shown in the center panel. The region looks like aggregated lumps. Finally, the typical electron microscopic view is seen in the third panel. This shows a thin line between the two plasma



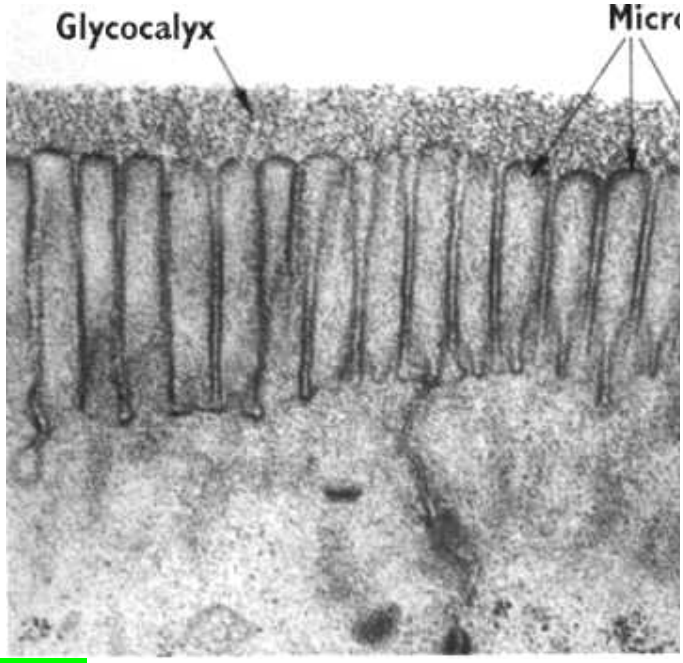
membranes indicating a "gap junction".

There are several ways to prove the cells are communicating by gap junctions. First, one can identify the connexon molecules by immunocytochemical labeling. Second, one can identify the actual junctional complex with freeze-fracture/freeze etch. To see if they are functional, however, one needs to inject one cell with a dye and watch to see if it is transferred to another cell. This cartoon diagrams a view of a gap junction showing molecules that can freely pass. Ions pass and in this way the cells can be electrically coupled together. Other small molecules that pass through include cyclic AMP (a second messenger) and the dye marker fluorescein. This last compound enables the scientist to study transport throught the gap junction.



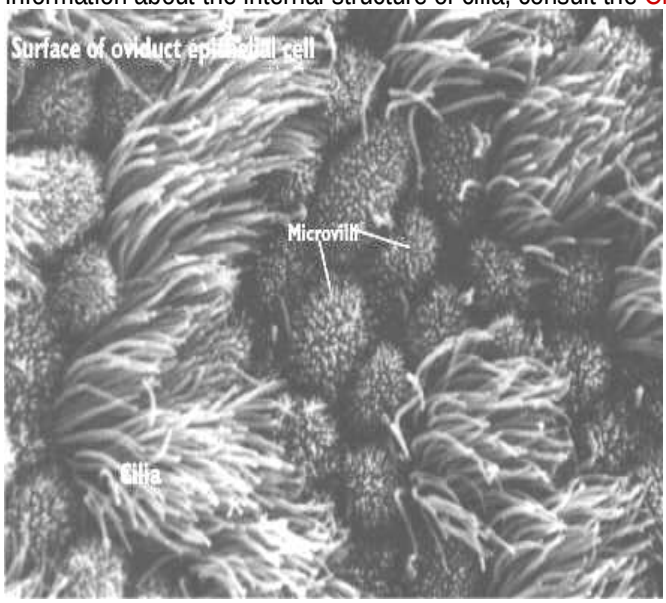
Membrane Specializations: Microvilli

The purpose of this final presentation is to introduce a surface specialization that projects from membranes called the microvillus. It is covered by a plasma membrane and encloses cytoplasm and microfilaments. Typically microvilli are found in absorptive cells, whenever there is a need for an increase in surface area.



It is also covered by a glycocalyx which are peripheral glycoproteins that attach themselves to the membrane. It might be used to trap nutrients, protect against toxic subxtances, or adhere to substances needed for uptake. Enzymes used for the cell's function are stored in this region, depending on the cell type. The figures to the right show views of microvilli cut transversely. Note that the microvilli are lined with the Unit membrane . (top figure) The core of filaments may allow them to move, although such movement is not as great as that of cilia or flagella

The lower figure shows a scanning electron micrograph of the luminal surface of the oviduct. It illustrates one difference between cilia and microvilli. The longer projections are cilia and the shorter projections are microvilli. For more information about the internal structure of cilia, consult the Cilia Web page



02 Red Cell Membrane Structure – Antigens

ANTIGEN - DEFINITION

An antigen is any substance, which in appropriate biological circumstances can stimulate an immune response; e.g. the formation of antibody or the activation of antigen specific effector cells.

WHAT ARE BLOOD GROUP ANTIGENS?

Blood group antigens are located within many red cell membrane chemical structures and are inherited characteristics. Inheritance of genetic material results in the production of different blood group system structures, i.e. ABO, Rh, etc. The presence of alternative genes (alleles) at these genetic loci result in the production of different antigen substances, e.g. the A or B antigens of the ABO blood group system. The different alleles at a particular gene locus have arisen due to genetic mutation, which occurs spontaneously. This provides the mechanism by which the diversity of red cell antigen structures are produced. The majority of these alternative structures do not however appear to affect red cell function. 'Red cell antigens' are therefore variable parts of red cell membrane extracellular protein or carbohydrate structures that are capable of eliciting an immune response.

The blood group genes, via mRNA, either code for red cell membrane proteins directly or code for enzymes that cause the production of specific red cell membrane carbohydrates (i.e. sugars).

BASIC RED CELL MEMBRANE COMPOSITION

The red cell membrane is composed of approximately equal amounts of lipid (approximately 44%) and protein (approximately 49%) together with a small amount (approximately 7%) of carbohydrate.

Lipid

The major lipid component (approximately 75%) of the red cell membrane is phospholipid, which are molecules having hydrophilic (water-soluble) polar ("head") groups and two hydrophobic (water insoluble) low viscosity chain ("tail") groups. The hydrophobic areas align to form a basic phospholipid bilayer structure, which is approximately 7nm across. This structure provides the red cell membrane with its major properties of impermeability (i.e. to ions, water and metabolites) and fluidity (i.e. flexibility and deformability within the plane of the membrane).

Protein

The presence of protein within the phospholipid bi-layer provides the capacity for selective transport across the membrane barrier, as well as providing a skeletal function. The protein may be extrinsic (projecting above the phospholipid bi-layer) or intrinsic (on the inside or across the phospholipid bilayer). Red cell protein may be free within the phospholipid bi-layer or anchored to the ankrin and spectrin protein underneath the phospholipid bilayer.

Carbohydrate

The ABO, Lewis, P_1, I and H antigens are carbohydrate structures. The carbohydrate present in the red cell membrane is associated with either protein, (i.e. as glycoprotein), or with lipid (i.e. as glycolipid):

GLYCOPROTEIN

These are mainly long chain structures (e.g. sialoglycoprotein) extending above the red cell membrane surface. These molecules contain most of the red cell membrane sialic acid (i.e. N-acetyl-neuraminic acid). Sialic acid is one of the major charged molecules of the red cell membrane. Glycoprotein comprises the major charged molecules within the red cell membrane and as such it confers the red cell with a net negative charge.

The most common types of red cell sialoglycoprotein are glycophorin A (GPA), which includes the MN antigen structures and glycophorin B (GPB), which includes the Ss antigen structures. Since these rod-like glycoprotein structures extend some

distance above the red cell membrane, they are (like the Duffy glycoprotein) sensitive to proteolytic enzyme treatment (e.g. by papain, etc.). The Rh, Kell, Kidd and Lutheran antigen structures are also glycoprotein.

GLYCOLIPID

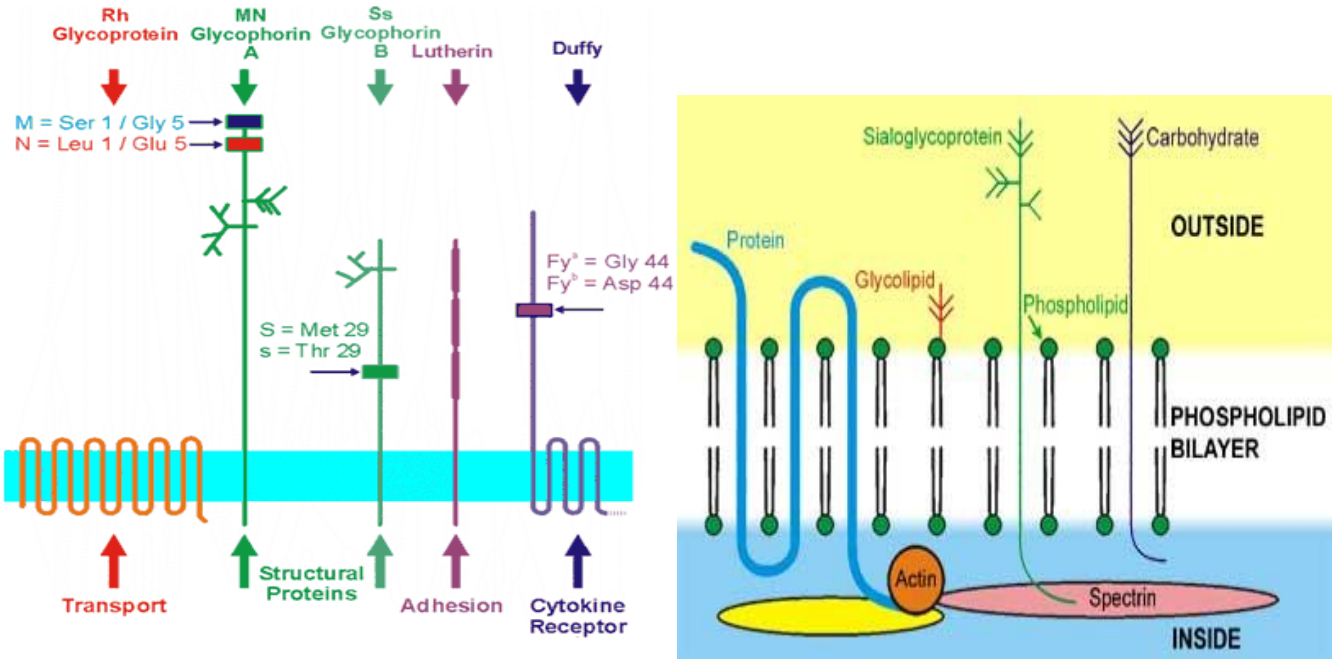
These molecules form only approximately 5% of the total lipid presence and are known as sphingosine molecules (i.e. long chain fatty acids).

INDIVIDUAL ANTIGENIC SPECIFICITIES

The difference between antithetical blood group antigens has been identified in some instances to be due to very minor differences within the 'parent' protein. The M and N antigen structure difference is produced by a change to two amino acids (at positions 1 and 5) within gylcophorin A, a protein consisting of 131 amino acids. The difference between the S and s antigens is due to a single amino acid change at position 29 within glcophorin B, a protein consisting of 72 amino acids. These differences do not appear to affect the specific function of the different 'parent' glycoprotein structures (e.g. as a structural, receptor molecule, trans-membrane transport function, etc.).

The very minor structural variations associated with some antithetical antigens, though capable of eliciting an antibody response in some patients results (to some degree) in their relatively low immunogenicity. This situation is in sharp contrast to the immunogenicity of the RhD protein, which has no antithetical antigen option (i.e. D has no allelic gene option). As such, a D-recipient sees the large extracellular areas of the RhD protein (consisting of 417 amino acids) as foreign. This accounts for the high immunogenicity of the RhD protein, compared with other (non-ABO) red cell antigens.

Diagrammatic representation of some of the major red cell membrane glycoproteins and variations in antigenic structures (not to scale)



Diagrammatic cross-sectional representation of the red cell membrane structures (not to scale)

Blood group antigens are associated with a variety of red cell membrane structures, i.e.

NAME	ASSOCIATED MEMBRANE STRUCTURE *	CHROMOSOME GENE LOCUS
ABO	Carbohydrate	9
MNS	Sialoglycoprotein (GPA / GPB)	4
P	Glycolipid	22
Rh	Proteins	1
Lutheran	Glycoprotein	19
Kell	Glycoprotein	7
Lewis	Carbohydrate	19
Duffy	Glycoprotein	1
Kidd	Glycoprotein	18

* For further information regarding the functions associated with these red cell membrane structures, refer to the individual blood group systems.

Therefore 'red cell antigens' can be identified to be associated with specific red cell membrane structures, the majority of which have been identified to have a specific function. Since antigen specificity may be defined by something as small as a single carbohydrate (e.g. A and B antigens) or alternative amino-acids within a single protein (e.g. M and N antigens), each red cell can have an enormous number of each antigen expressed within its membrane, i.e.

BLOOD GROUP	ANTIGEN	APPROXIMATE NUMBER OF ANTIGEN SITES PER RED CELL *
ABO	A	810,000 - 1,200,000
	B	600,000 - 850,000
Rh	D	10,000 - 40,000
Kell	K	3,000 - 4,000
Duffy	Fy^a	10,000 - 15,000

* As calculated by radioisotope (125I) labelled antibody binding and flow cytometry experiments (performed by different research groups).

Generally, the same antibody binding experiments have demonstrated that there are more antigen sites detectable on red cells of the homozygote rather than the heterozygote genotype, i.e.

DD homozygote: approximately 25,000 - 37,000 RhD antigen sites / red cell.

Dd heterozygote: approximately 10,000 - 15,000 RhD antigen sites / red cell.

This factor is a major reason why homozygous antigen expression is preferred for the red cells that are used for antibody detection, since they offer a potential for improved reactivity (i.e. more antigen available for the antibody to react with). The location and number of antigen sites on each red cell can affect antigen antibody reactivity in a variety of ways:

1. The type of laboratory technique used to demonstrate the antigen-antibody reaction (i.e. IgG ABO antibodies are able to work in a saline medium, whereas AHG and/or enzyme techniques must be used to detect IgG Rh antibodies).
2. The effects of the action of enzymes (e.g. papain) on the antigen-antibody reaction, i.e. as to whether antigen reactivity is enhanced (e.g. Rh) due to the removal of adjacent (interfering) proteins or destroyed (e.g. Duffy) due to the antigen structure being removed by the enzyme treatment.
3. The strength (avidity) of the antigen-antibody reaction produced, i.e. the ease with which the antibody is able to react with its antigen.

PRINCIPLES OF TRANSPLANTATION

The Immune System

The continuing health of an animal depends upon its ability to recognise and repel disease; this ability is called **immunity**.

Two types of immunity exist, innate and adaptive.

Innate immunity, a first line of defence, is furnished by barriers such as skin, tears, saliva, and mucus, and the tissue inflammation that occurs after injury or infection.

Adaptive immunity develops specific defences against an invader that can be invoked whenever this particular intruder attacks again.

Forms of adaptive immunity.

The immune system responds to surface structures of the invading organism called antigens. There are two types of adaptive immune responses: **humoral** and **cell mediated**.

In **humoral** immune responses antibodies appear in the body fluids and stick to and destroy antigens. The response is to toxic substances outside of the cell.

In the **cell-mediated** immune response cells that can destroy other cells become active (**T-cells**). They destroy disease infected cells or cells making mutant forms of normal molecules.

Cell-mediated response molecules.

When disease associated proteins occur in a cell they are broken into pieces by the cells proteolytic machinery. Cell proteins become attached to antigen fragments and transport them to the surface of the cell, where they are "presented" to the bodies defence mechanisms.

These transport molecules are called the Major Histocompatibility Complex (**MHC**) proteins. Without these, there would be no presentation of internal or external antigens to the **T cells**. The importance of MHC proteins is that they allow T cells to distinguish self from non-self. In every cell in your body, antigens are constantly broken up and presented to passing T cells. Without this presentation, other aspects of the immune response cannot occur.

Class I MHC proteins (found on all nucleated cell surfaces) present antigens to cytotoxic T lymphocytes (**CTLs**) . Most CTLs possess both T-cell receptors (**TCR**) and CD8 molecules on their surfaces. These TCRs are able to recognize peptides when they are expressed in complexes with MHC Class I molecules. For the TCR to bind a peptide-MHC complex two conditions must be met. Firstly, the TCR must have a structure which allows it to bind the peptide-MHC complex. Secondly, the accessory molecule CD8, must bind to the alpha-3 domain of the MHC Class I molecule. Due to genetic recombination events each CTL expresses a unique TCR which only binds a specific MHC-peptide complex. CTLs which recognize self-peptides (i.e. peptides produced by the normal host body as opposed to a foreign or cancerous cells) are removed in the thymus or tolerized after their release from the thymus. So, if a CTL can bind to a MHC-peptide complex on the cell surface, that cell is producing a peptide which is not native to the host.

The MHC Class II proteins (found only on B lymphocytes, macrophages, and other cells that present antigens to T cells), which primarily present peptides, which have been digested from external sources, are needed for T-cell communication with B-cells and macrophages. Class II MHC proteins presenting antigens are detected by a different group of T cells (called T-helper or TH cells) to Class I MHC proteins (which are detected by CTLs cells).

The MHC proteins, and several closely associated with them in the carrying out of their functions, are coded for by loci that are close together within the Human Genome. Major Histocompatibility Complex proteins and their associated molecules are fundamental in the process of antigen presentation.

DEFINITIONS:

- **Histocompatibility (transplantation) antigens: Antigens on tissues and cells that determine their rejection when grafted between two genetically different individuals**
- **Major histocompatibility (MHC) antigens: Histocompatibility antigens that cause a very strong immune response and are most important in rejection**
- **MHC complex: Group of genes on a single chromosome encoding the MHC antigens**
- **HLA (human leukocyte antigens): MHC antigens of man (first detected on leukocytes)**
- **H-2 antigens: MHC antigens of mouse**

Types of graft

- **Xenograft: Grafts between members of different species (also known as heterologous, xenogeneic or heterografts)**
- **Allograft: Grafts between two members of the same species (also known as allogeneic or homograft)**
- **Isograft: Grafts between members of the same species with identical genetic makeup (identical twins or inbred animals)**

Haplotype: a group of genes on a single chromosome

An immunocompetent host recognizes the foreign antigens on grafted tissues (or cells) and mounts an immune response which results in rejection. On the other hand, if an immunocompromised host is grafted with foreign immunocompetent lymphoid cells, the immunoreactive T-cells in the graft recognize the foreign antigens on the host tissue, leading to damage of the host tissue.

Host-versus-graft-reaction

The duration of graft survival follows the order, xeno- < allo- < iso- = auto- graft. The time of rejection also depends on the antigenic disparity between the donors and recipient. MHC antigens are the major contributors in rejection, but the **minor histocompatibility antigens** also play a role. Rejection due to disparity in several minor histocompatibility antigens may be as quick or quicker than rejection mediated by an MHC antigen. As in other immune responses, there is immunological memory and secondary response in graft rejection. Thus, once a graft is rejected by a recipient, a second graft from the same donor, or a donor with the same histocompatibility antigens, will be rejected in a much shorter time.

Graft-versus-host (GVH) REACTION

Histocompatible lymphoid cells, when injected into an immunocompromised host, are readily accepted. However, the immunocompetent T lymphocytes among the grafted cells recognize the alloantigens and, in response, they proliferate and progressively cause damage to the host tissues and cells. This condition is known as graft-versus-host (GVH) disease and is often fatal. Common manifestations of GVH reaction are diarrhea, erythema, weight loss, malaise, fever, joint pains, etc. and ultimately death.

THE MHC GENE COMPLEX

The MHC complex contains a number of genes that control several antigens, most of which influence allograft rejection. These antigens (and their genes) can be divided into three major classes: **class I**, **class II** and **class III**. The class I and class II

antigens are expressed on cells and tissues whereas class III antigens are represented on proteins in serum and other body fluids (*e.g.*C4, C2, factor B, TNF). Antigens of class III gene products have no role in graft rejection.

ALLOGRAFT REJECTION

The clinical significance of the MHC is realized in organ transplantation. Cells and tissues are routinely transplanted as a treatment for a number of diseases. However, reaction of the host against allo-antigens of the graft (HVG) results in its rejection and is the major obstacle in organ transplantation. The rejection time of a graft may vary with the antigenic nature of the graft and the immune status of the host and is determined by the immune mechanisms involved.

Hyper-acute rejection

This occurs in instances when the recipient has preformed high titer antibodies. A graft may show signs of rejection within minutes to hours due to immediate reaction of antibodies and complement.

Accelerated (2nd set; secondary) rejection

Transplantation of a second graft, which shares a significant number of antigenic determinants with the first one, results in a rapid (2 - 5 days) rejection. It is due to presence of T-lymphocytes sensitized during the first graft rejection. Accelerated rejection is mediated by immediate production of lymphokines, activation of monocytes and macrophages, and induction of cytotoxic lymphocytes.

Table 1. Different patterns of graft rejection

Type of rejection	Time taken	Cause
Hyper-acute Accelerated Acute Chronic	Minutes-hours Days Days - weeks Months – years	Preformed anti-donor antibodies and complement. Reactivation of sensitized T cells Primary activation of T cells Causes unclear: antibodies, immune complexes, slow cellular reactions, recurrence of disease.

Acute (1st set; primary) rejection

The normal reaction that follows the first grafting of a foreign transplant takes 1 - 3 weeks. This is known as acute rejection and is mediated by T lymphocytes sensitized to class I and class II antigens of the allograft, elicitation of lymphokines and activation of monocytes and macrophages.

Chronic rejection

Some grafts may survive for months or even years, but suddenly exhibit symptoms of rejection. This is referred to as chronic rejection, the mechanism of which is not entirely clear.

PROCEDURES TO ENHANCE GRAFT SURVIVAL

In clinical practice, the most successful transplantation programs have been with kidneys and corneas. However, other organs are being transplanted with increasing frequency. The success in these programs has been due to a better understanding of immunological mechanisms, definition of MHC antigens and development of more effective immunosuppressive agents.

Strategies for bone marrow transplantation

In bone marrow transplantation, the most crucial factor in donor selection is class II MHC compatibility. Once again an identical twin is the ideal donor. From poorly matched grafts, T lymphocytes can be removed using monoclonal antibodies. The recipient must be immunosuppressed. Malignant cells must be eliminated from the recipient blood (in case of blood-borne malignancies). Methotrexate, cyclosporin and prednisone are often used to control GVH disease.

Other grafts

Corneal grafts do not contain D region antigens and consequently survival is frequent. Small grafts are better and corticosteroids are helpful.

Skin allografts have a very poor success rate and immunosuppressive therapy is relatively ineffective. Nevertheless, they are often used to provide a temporary covering to promote healing in severe skin damage. Indeed, there will be no rejection if the host and donor are perfectly matched (identical twins) or the recipient is tolerant to the donor MHC antigens (bone marrow chimeras).

Disease	Associated Alleles	Frequency in		Relative Risk
		Patients	Control	
Ankylosing spondylitis	B27	90	9	87.4
Reiter's disease (syndrome)	B27	79	9	37.0
Acute anterior uveitis	B27	52	9	10.4
Psoriasis vulgaris	Cw6	87	33	13.3
Dermatitis herpetiformis	DR3	85	26	15.4
Celiac Disease	DR3	79	26	10.8
Insulin-dependent diabetes mellitus	DR3/4	91	57	7.9

Table 2. Examples of selected immunosuppressive agents

agent	possible mode of action	application(s)
corticosteroids, prednisone	anti-inflammatory, altering T-cell and PMN traffic	organ transplant, hypersensitivity, autoimmune diseases
cyclosporin, FK-506	inhibition of IL-2 synthesis	organ transplant
rapamycin	blocking of IL2-IL2R signal	organ transplant
azathioprine, 6-MP	purine metabolism	organ transplant, autoimmuniy
methotrexate	folate metabolism	organ transplant, autoimmuniy
cyclophosphamide, melphalan	alkylation of DNA, RNA and proteins	organ transplant, autoimmuniy

MHC association with diseases

A number of diseases have been found to occur at a higher frequency in individuals with certain MHC haplotypes. Most prominent among these are ankylosing spondylitis (B27), celiac disease (DR3) and Reiter's syndrome (B27). Other diseases associated with different specificities of the MHC are listed in Table 3. No definite reason is known for this association. However, several hypotheses have been proposed: antigenic similarity between pathogens and MHC, antigenic hypo- and hyper-responsiveness controlled by the class II genes are included among them.

You have learned about

The role of MHC in host-versus-graft (HGV) and graft-versus-host (GVH) disease.

Genetics of the two MHC molecules.

The role of polymorphism and crossover in heterogeneity of MHC antigens in a population.

Methods for detecting MHC antigens (tissue typing).

Immune mechanisms in transplant rejection.

Strategies for successful transplantation.

The ABO Blood Group System

The Early History of Transfusion before Blood Groups

It was recorded in the Bible that the "life of the flesh was in the blood". So from ancient time blood has been intimately associated with life. The most obvious proof of this to the ancients was acute blood loss due to injury or weapons.

The major ancient civilizations of Greece and Rome deduced from their many wars that acute blood loss was one of the most rapidly lethal consequences of any wound. As a consequence one of the main goals was to stop blood loss. The **ancient Greeks** were the first recorded to attempt this by the process of using a tourniquet. This involved placing a tourniquet, on a limb, above the wound. The aim was to allow the body to form a stable clot and thereby stop bleeding. The major problem was that if left on too long, the tourniquet would deprive the tissue below it of oxygen and gangrene would set in.

It was left to the **Romans** to refine medical treatments of acute blood loss to include not only tourniquets but ligature (the tying off of a blood vessel). The Romans also invented the first surgical clamp that was able to tie off a severed artery while still allowing blood to flow to the limb thereby reducing the risk of fatal gangrene. It is interesting to note that after the fall of Rome these same skills were not rediscovered till the seventeenth century.

Ancients only devised ways of stopping acute blood loss. The first suggested case of transfusion was rumoured to have been given to Pope Innocent VIII in **July 1492.** It was widely believed in the middle age that the drinking of human blood was a method where a person's health could be restored. This should be viewed in the context that medieval "medicine" also considered swallowing paper with the Lords prayer or ground bones of saints and bleeding various parts of the body according to the phases of the moon valid and beneficial therapies. In the case of Pope Innocent VIII a Jewish physician **Abraham Myere of Balmes** suggested sucking the blood of a youth for a restorative tonic for the Pope. As a result three "volunteer" youths were said to have donated a restorative tonic for the Pope and were paid one ducat each. Whether the Pope drank it or it was transfused (unlikely as the circulatory system was not discovered until 1628 by William Harvey) is dubious as it was not reported by any court ambassadors of the time. As for the three young donors, they died of blood loss but had the consolation of being considered martyrs.

The first truly verified accounts of transfusion occurred in the mid seventeenth century. The first transfusions were animal to animal. In **1665 John Wilkins** was the first person, via a syringe, to intravenously transfuse two ounces of blood from one dog to another with no harmful effect. These experiments were extended by **Richard Lower who in 1666** continued transfusion experimentation in dogs with vein to vein and artery to vein transfusions. He discovered that artery to vein transfusions worked due to the higher arterial blood pressure (4).

The first human involvement as a transfusion recipient was performed by a Frenchman **Jean Denys on the 25 of June 1667**. The "scientific" principle behind the use of animals blood was that animals possessed purer blood than humans as they were not prone to the vices of drinking and eating that humans were (4). To this end a transfusion was used to treat Antoine Mauroy, a newly wed, who was prone to run away to Paris for extended bouts of debauchery and vice. To cure this Jean Denys selected calf's blood due to the animals gentle nature. To consolidate the treatment a second transfusion was performed a week later. One small problem is the immune system is very efficient, and antibodies to the calf cells would have formed. This became immediately evident when Mauroy complained of kidney pain and a heavy sensation in the chest and passing of dark urine. This was undoubtedly the first recorded case of an acute haemolytic transfusion reaction. Later attempts to repeat the treatment were refused by the patient (1).

Fig1 Animal to human transfusion 1667

The practice of transfusion remained at this level until the appearance of **James Blundell** in the early 19th Century. James Blundell was motivated by the consequences of post partum haemorrhages who often went into shock and died from acute blood loss. Unlike earlier attempts he refused to use animal blood based on work by **Dr John Leacock** whose experiments showed the blood of one species may be harmful to another (1). In **1818 Blundell** transfused 12 - 14 ounces of blood via a brass syringe into a patient who temporarily improved but died of their initial disease(1). However the practice of transfusions from animals did not disappear with papers as late as **1874 Franz Gesellius** and **Oscar Hasse advocated the use of lambs blood**.

The major problem before the discovery of anticoagulants was that blood collected for transfusion clotted. To get around this two methods were devised. The first was by **Jean Prevost** and Jean Baptiste Dumas who found that stirring collected blood caused the creation of fibrin and preventing the remaining blood from clotting. They also found that defibrinated blood was just as good as untreated blood for resuscitation (1). The second method invented by Gesellius involved capillary transfusions via a device (fig 2) which simultaneously punctured the skin in many places. The blood was then sucked into a bowl and transfused. The end of animals being used as donors was accomplished in **May 1874** by **Ponfick** and **Landois** who studied the death of a 34 year old woman transfused by a sheep and noticed lysed red cells in her serum and haemoglobinuria. This problem of red cell lysis was noted when blood was transfused between species (1).

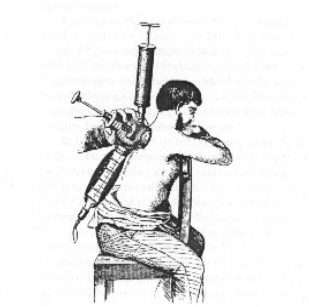


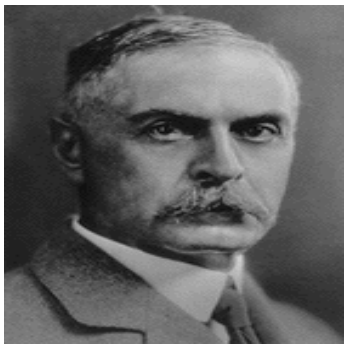
Fig 2 . Gesellius' capillary transfusion
(Transfusion 1997; 37 : pg 555)

Once the use of animals ceased there was the major problem of deaths resulting even when human blood was used. This was due to the major barrier posed by the **ABO blood group system.** By pure random chance based upon caucasian frequencies of the ABO blood groups the chance of a compatible transfusion is 64.4 % (1). The evolution of safer transfusions that we know today was heralded by the the **discovery of ABO blood groups by Karl Landsteiner** in 1901.

The Discovery of ABO Blood Groups

As has been briefly examined, transfusion success was a very hit and miss affair even when humans were used as blood donors. This was directly due to the major barrier posed by the ABO blood group system. The consequences of an ABO incompatible blood donation is acute and potentially lethal intravascular destruction of the transfused red cells.

The ABO blood group system was discovered by **Karl Landsteiner** who was born in Vienna on the 14 / 06 / 1868. He studied medicine at the University of Vienna, graduating in 1891.



In 1896 he became an assistant to Max von Gruber in the Hygiene Institute of Vienna and became interested in the mechanisms of immunity and the nature of antibodies. In 1898 he became an assistant in the university Department of Pathological Anatomy in Vienna (5). It was during this time he started to investigate whether differences existed between different peoples red cells. This was inspired by work done by **Landois** and **Ponfick** who in **1874** discovered that transfused red cells from one species to another and some humans to other humans lysed in the circulation. Death was attributed to the organ damage and hyperkalemia from the rapid release of potassium from the lysed red cells (1).

Fig 1. Karl Landsteiner

To investigate for any potential differences between human red blood cells **Landsteiner** in **1901** chose a simple experiment. He mixed the serum and red cells from different people and observed the reaction. As a result of observing the agglutination patterns he described three distinct groups whose serum possessed naturally occurring antibodies which could react with some other peoples red cells. As a result Landsteiner divided these individuals into three groups called groups A, group B and group C. The group C was later changed to group O (1, 6, 7). In **1902** the fourth group of the ABO system was defined by **Decastello** and **Sturli** who identified people whose serum did not naturally produce antibodies that agglutinated other human red cells. This group was called AB.

Summary of Early Experiments on the nature of the ABO blood Groups

- **Group A** - **Possessed A antigen** on their red cells and had naturally occurring **antibodies** (a**nti-B) that reacted with group B and AB people**. Their red cells were agglutinated by group O and B people.
- **Group B** - **Possessed B antigen** on their red cells and had naturally occurring **antibodies** (**anti-A) that reacted with group A and AB people.** Their red cells were agglutinated by group O and A people.
- **Group AB** - **Possessed** both **A and B antigens** on there red cells and **produced no** naturally occurring **antibodies**. Their serum reacted with no other ABO group individuals. Their cells agglutinated with the sera from all other groups.
- **Group O** - Had **neither A or B antigens** on their red cells and had naturally occurring **antibodies (anti- A and anti - B)** that agglutinated all red cells from group A, B and AB people. Their red cells were not agglutinated by serum from any ABO group (1, 7, 8).

These discoveries breached the potentially fatal barrier the ABO groups posed for human to human transfusions. The use of these discoveries in "typing" human donors and transfusion recipients received little attention until they were published in a book in 1909 defining the ABO blood groups. It also stated that red cells were not destroyed when people received transfusions from people of the same ABO blood group (5). The **inheritance of the ABO blood groups** was later suggested in 1910 (5). Dr Landsteiner died on 24 of June 1943 in his laboratory from a heart attack.

In experimental studies in **1911, Von Dunergern** and **Hirschfeld** discovered that **primates alsopossessed similar A and B like antigens** (10). The investigation and study of these similar antigens was subsequently undertaken in **1925** by Landsteiner **and Miller** (10). It was proposed that the sharing off the ABO system was due to **shared genetic evolution** between humans and primates.

These discoveries allowed the creation of the first transfusion service in the world founded by the British Red Cross in **1926**. The Australian Blood Bank was founded by **Dr Lucy Bryce**, with the first blood transfusion service operating at the Royal Melbourne Hospital in **1929**.

Inheritance and Genetics

The fact that the ABO blood group system was inherited was suggested in **1910** by **Epstein** and **Ottenberg**. The confirmation of the ABO system being genetically inherited was by **von Dungern** and **Hirszfeld**. who studied 72 families with 102 children. They found that the inheritance of the A and B agglutinogens obeyed **Mendels laws**.

They discovered that the **ABO gene was autosomal** (the gene was not on either sex chromosome) . Therefore each person has two copies of genes coding for their ABO blood group (one maternal and one paternal in origin) . It was observed that the **A and B blood group**s were **dominant over the O blood group**. It was also found that the **A and B group genes** were **co-dominant.** This meant that if a person **inherited one A group gene and one B group gene** their red cells would **possess both the A and B blood group antigens**. These alleles were termed A (which produced the A antigen), B (with produced the B antigen) and O (which was "non functional" and produced no A or B antigen) (1, 7, 8).

Table 1: Summary of Blood group, Red cell antigens and the possible inherited genotype.

Human Blood Group	Red Cell Antigens	Serum Antibodies	Possible Genotype
A	A antigen	Anti-B	AA or AO
B	B antigen	Anti-A	BB or BO
O	None	Anti A and Anti-B	OO only
AB	A and B antigen	None	AB only

Fig 1: A sample family pedigree of three generations demonstrating the basic Mendelian Inheritance of ABO blood groups.

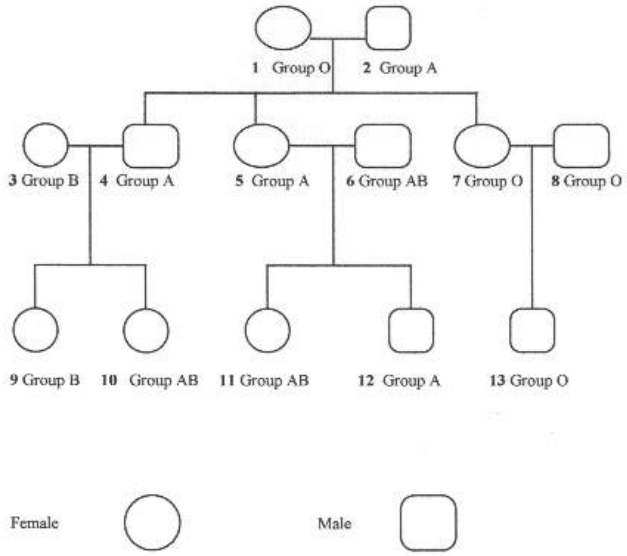


Table 2: Genotypes Of The Individuals In Figure 1

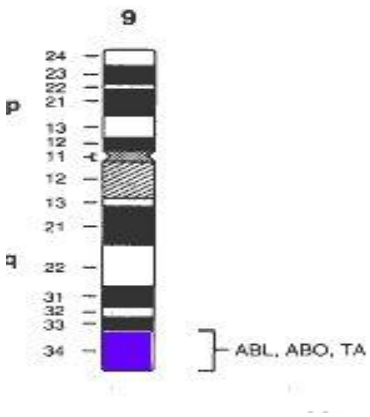
Pedigree ID number	Genotype	Reason Why
1	OO	As the O allele is recessive to both the A and B alleles to express the O blood group phenotype only the O gene can be present.
2	AO	As this male has one child who is blood group O (**#7)** he must be a heterozygote. If he was homozygote the offspring must have inherited an A gene. If this were present as it is dominant to the O gene the offspring would have been blood group A
3	BB or BO	As the B gene is dominant to the O gene this individual could possess one or two copies of the B gene. If this person and their partner # 4 had an O child then we could safely conclude that this person must have a BO genotype. Based on their offspring not enough information exists to clarify their phenotype.
4	AO	Due to Mendel's laws this person must have received one maternal and one paternal ABO allele. The mother was homozygous for the O gene so this individual must have one O allele. To express the A phenotype they require one A allele which is dominant to the O allele. which they inherited form individual **# 2**
5	AO	As for individual **# 4**
6	AB	This individual produces both the A and B antigens. They must therefore have one A gene and one B gene. These are co-dominant, hence both alleles are expressed in the phenotype.
7	OO	As for individual **# 1**
8	OO	As for individual **# 1**
9	BO	This individual could not have inherited the A gene from their father (# 4) as it would be expressed along with the maternal B gene and would have produced an AB phenotype. The must have received

		their fathers O gene which is recessive to the inherited maternal B gene. Based on Mendelian Genetics the other potential groups for this person from parents 3 and 4 could have been **A (AO), AB or O.**
10	AB	As for individual **# 6**
11	AB	As for individual **# 6**
12	AA or AO	This person could be AA if they inherited the A gene form both their mother **(#5)** and their father **(#6).** As this individuals mother is an AO heterozygote it is possible that they inherited an O gene. This would mean that the A gene was of paternal origin. As the A gene is dominant they would express the A blood group.
13	OO	As for individual **# 1**

Allelic polymorphism's of the ABO system

The ABO genes

The normal human has 46 chromosomes in their genotype. This consists of 46 chromosomes that are divided into 22 homologous paired autosomal chromosomes (one of maternal, the other paternal in origin) and two sex determining chromosomes (X and Y). These chromosomes contain all the genetic blueprints for the creation and maintenance of human life. The genetic blueprint consists of about three billion bases of Deoxyribonucleic Acid (DNA) consisting of four different bases; adenine (A) , thymine (T), gaunine (G) and cytosine (C) and protein. This DNA exists in the cell nucleus as a highly condensed strand (20, 21). The DNA in the nucleus consists of three billion bases, of this only about 10 - 15% are actually incorporated into genes. Each chromosome contains a few thousand genes. It is the gene that is responsible for the coding of a single trait (i.e. enzyme, protein, etc.) (20, 21).



The **ABO blood group phenotype is actually determined by alleles on two chromosomes**. These are **chromosome 9** which possesses the **ABO gene** allele and **chromosome 19** which possess a gene that codes for the creation of the substrate antigen (**H antigen**) which then may or may not be altered by the allele (s) present on chromosome 9. Unlike many other red cell antigens both these genes **do not code for the actual antigen.** These **genes code for enzymes** that actually create the antigen on a precursor structure on the red cell. These **enzymes determine a persons ABO blood** group by catalysing the attachment of a blood group defining sugar onto a basic carbohydrate precursor antigen. The enzyme produced by the gene on **chromosome 19 adds a sugar to** the precursor carbohydrate to **produce the H antigen**. The ABO gene is responsible for determining if a person is an A, B or O phenotype.

Fig 1: The ABO Gene

The **A and B gene** each code for **an active enzyme that recognize the H antigen** and attach a group defining sugar. The **O gene** is actually **an amorph** gene meaning that it **does not produce an active enzyme.** As a result blood group O people are not able to modify the H antigen which remains the only ABO antigen expressed on their cells.

The **ABO gene** locus is located on the **long arm of chromosome 9 (9q34)**. Except in rare ABO groups such as the **Bombay blood group** it is the alleles at this locus that defines the ABO phenotype. The ABO alleles consist of a 30 kb nucleotide which consists both exons and introns. The exon is a discontinuous nucleotide sequence that codes for the final amino acid sequence. The final coding sequence of the gene product consists of these exons being spliced together after all superfluous intervening sequences are removed (introns). The final **coding region of the gene** consists a **1062 bp nucleotide** sequence **consisting of 7 exons** . This produces an **active enzyme** that is **353 amino acid residues** in length (25, 28).

The **first 5 exons** are relatively **small** . The **sixth exon** is a 135 nucleotides and **codes** for the **C terminal catalytic domain of the enzyme.** The largest exon is the **seventh exon** which consists of 688 nucleotides. It is the seventh exon which **codes for the different transferase enzyme specificity** produced by the **A and B alleles** (25).

Differences Between the A and B Alleles

The structure of the DNA coding sequence and the enzyme produced by transcription of the ABO genes show a **high degree of similarity between the A and the B blood group gene**. The differences between the two most common forms of these two alleles results from a series of **point mutations**. The result of these mutations are **four amino acid substitutions in the enzyme at positions 176, 235, 266 and 268** (25, 26, 28, 29). These mutations result in a conformational change in the enzyme and , subsequently, the observed functional specificity (25). In particular the residue at **position 268** is **critical for A or B transferase activity**. The **A subgroups** and **B subgroups** result from different mutations in the coding DNA and result in different transferase activity in the subsequent enzyme (25, 29).

Table 1: Defining Amino Acid Differences between the enzymes Produced by the Common A and B Blood Group Alleles (25)

	Residue 176	Residue 235	Residue 266	Residue 268
A Group Allele	Arginine	Glycine	Leucine	Glycine
B Group Allele	Glycine	Serine	Methionine	Alanine

Genetics of the O Blood Group

The **O blood group** results from the gene at the **ABO locus on chromosome 9** producing a **non functional transferase enzyme** (8, 25, 26, 28, 29). This non functional transferase will not recognize the basic H antigen and is subsequently unable to add a blood group defining sugar onto the H antigen. As a result only the H antigen will be present on an O person's cells (8, 25, 26, 29).

Initially the O gene was found to be almost identical in the DNA sequence to the A allele. The only difference observed was a **point mutation at position 258** (**sixth exon**) in the coding region close to the N terminal. This **caused a frame shift** leading

to a **completely different protein** of **only 116 amino acids with no transferase activity** (25, 26, 28). This has been called the **O^1 variant**.

A **second mutation** producing the O phenotype has been identified **involving residue 268**. The A and B transferases have small non polar residues in this position. In the case of the O mutation a point mutation occurs that **substitutes arginine which is large with a basic side chain**. This **causes a radical conformational change** in the transferase enzyme rendering it inactive. This has been call the **O^2 variant (25, 28)**.

The **third mutation** identified has **two different mutations**. First a **deletion** of nucleotide at **position 1060 which alters the stop codon**. This causes a longer protein to be produced. There is also **a nucleotide insertion between position 798 and 804**. This produces a non functional transferase called the **O^3 variant** (25).

Table 2: Summary of the O allele Polymorphism's of the ABO gene (25, 30)

O Variant	Mutation observed
O^1 Variant	Point mutation at nucleotide 268 causing a truncated 116 Amino Acid protein.
O^2 Variant	Point mutation causing a Amino Acid Substitution at position 268 producing a conformational change in enzyme shape.
O^3 Variant	Two mutations: a) Deletion of nucleotide 1060 which alters the stop codon. producing a longer protein. b) Nucleotide insertion between position 798 and 804

Synthesis and Structure of ABO Blood Groups

The **ABO antigen system is a ubiquitous antigen system.** This means that the A, B or O (H antigen) is not limited only to an individuals red cells. The ABO group of an individual is expressed on diverse cells and tissues. In certain individuals who possess a specific gene, the ABO antigen is also expressed in saliva and other body fluids (secretors) (8, 25, 33).

The successful creation of the final **ABO blood group is however the final stage of a complex series of enzymatic actions** with approximately 100 glycosyl transferases being involved in the creation of the carbohydrate oligosaccharides to which the H, A or B antigen is added (8).

The **A, B or O blood group** of an individual involves the **interaction** between **enzymes** which are the product of **two genes;**

- **The H gene on chromosome 19 codes for a fucosyl transferase that synthesizes the H antigen.**
- **While the gene defining the A, B or a non functional enzyme is located at ABO gene locus on chromosome 9** (8, 25, 27, 30, 33).

The **ABO antigens expressed by an individual cell are carbohydrate antigens**. These are **constructed of oligosaccharides chains constructed in a stepwise manner** with each sugar being added to the growing chain **by a** specific enzyme (**glycosyl transferase**).

These oligosaccharides are **attached to different types of precursor chains such as polypeptides or ceramide** which act as transmembrane anchors (8, 25, 26, 33).

The synthesis of an ABO antigen is a sequential 2 step process involving:

- **H Antigen production**
- **A Antigen and / or B Antigen production**

H Antigen Precursor

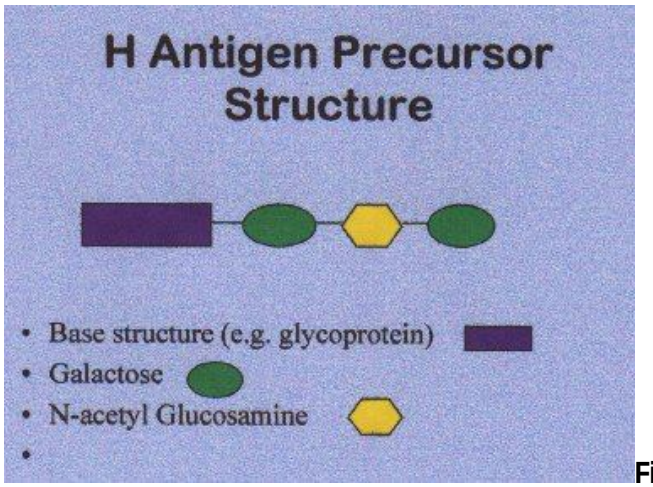


Fig 1: H Antigen Precursor.

The building block for the construction of the A,B or H antigen is a precursor oligosaccharide which can vary from a few sugar molecules in a simple linear chain to more complex structures (33). The oligosaccharide is constructed in a sequential manner by the enzymatic addition of sugars to the terminal end of the oligosaccharide chain, this is then attached to a membrane anchor which may be a glycoprotein, glycolipid, etc. No matter the molecule that the molecule is attached to the last three sugars on the chain are; galactose, N acetyl glucose and a terminal galactose (8, 25, 26, 33).

Step 1: H Antigen Synthesis

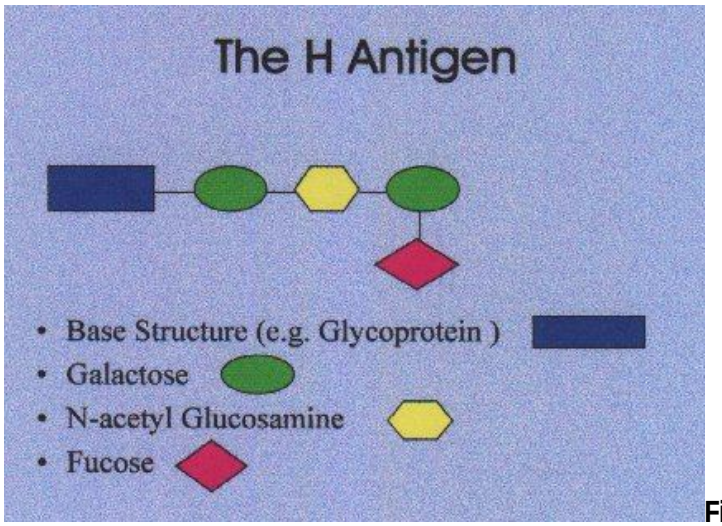


Fig 2: H antigen

The Synthesis of the H antigen is **controlled by the H gene on Chromosome 19.** This gene **codes for the synthesis of an enzyme** called **fucosyl transferase (FUT 1).** This enzyme recognizes the terminal galactose of the precursor oligosaccharide and **catalyses the addition of fucose to the terminal galactose**. This enzymatic addition creates the **H antigen** which is the antigen **expressed** on **group O individuals** and is used as **the precursor A and B group antigen**s (8, 25, 26, 33).

Step 2: Synthesis of the A and B antigens

The A Antigen:

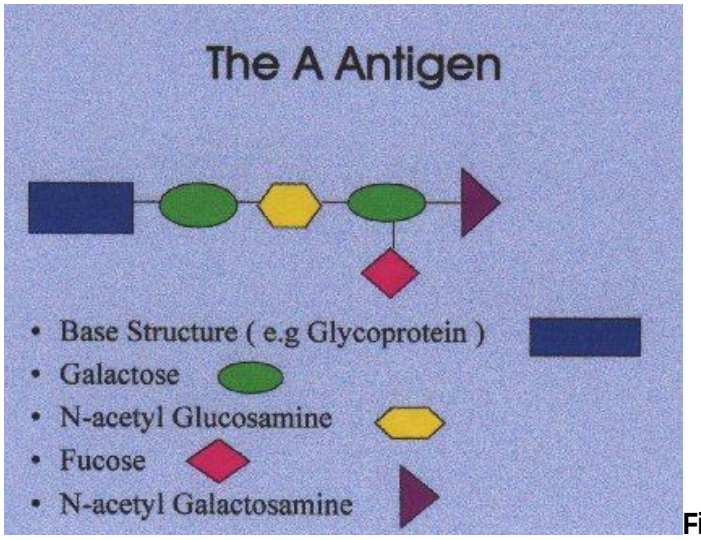


Fig 3: The A antigen.

The A antigen is produced via the action of an enzyme (a glycosyl transferase)coded on chromosome 9 at the ABO gene locus. This enzyme uses the H antigen as a substrate and **catalyses the addition of the sugar N acetyl galactosamine** to the terminal galactose t**o** produce the A antigen. The **A enzyme can only produce the A antigen if a H** a**ntigen is already presen**t (8, 25, 26, 30, 33).

The B Antigen:

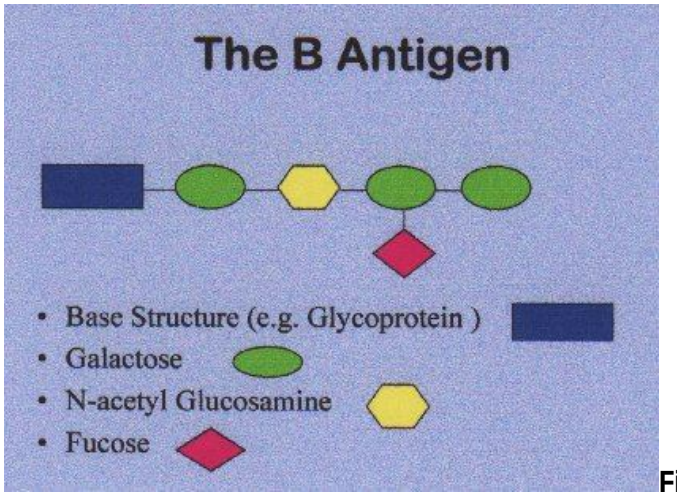


Fig 4: The B antigen

The B antigen is produced by an enzyme coded for by a gene at the ABO locus on chromosome 9. The enzyme (a glycosyl transferase) uses the H antigen as a substrate **and catalyses the** addition **of the sugar g**alactose to the terminal galactose of the H antigen**. This produces the B antigen. The B enzyme can** ONLY produce the B antigen if a H antigen is present .

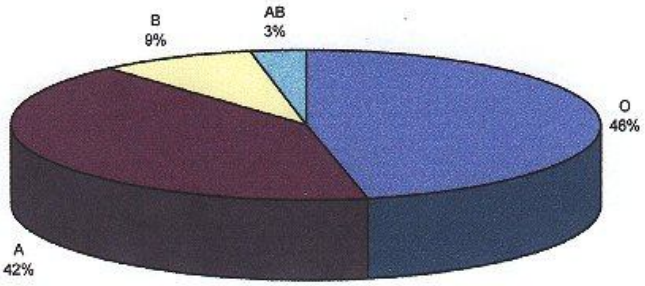
Frequencies of the ABO blood groups

Home

The frequencies of the ABO blood group system vary significantly in various populations and ethnic groups around the world. There have been several reasons given for this phenomenon such as **population migration** and **diseases** where certain blood groups were thought to provide a selective advantage or disadvantage.

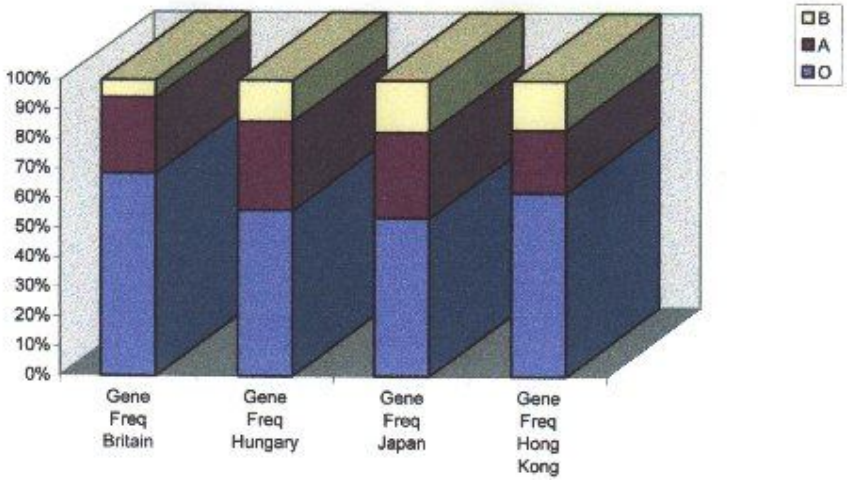
In the case of migration, **ethnic communities** that experienced **little migration** due to their location being remote maintain a **high degree of genetic similarity and relatively low ABO group polymorphism.** An example of this situation is the **Aboriginal population** of Queensland Australia which has approximately **over 75%** of the Population being **O Positive** (8). This can be compared to the frequencies of blood groups observed in the population of Britain which showed only 46% of people are of the O phenotype (Figure 1). This can be explained by the historical observation that Britain has been settled by Celts, Romans, Germanic Saxons, Scandinavian Vikings and Normans (22, 23). As the ABO blood groups are under genetic control, it would be expected that a certain gene would predominate (e.g. the O gene in Queensland Aboriginals) in a particular homogeneous tribal group.

Fig 1: The ABO Phenotype Frequency in Britain in 1946. (8)



As **tribal and ethnic groups migrated and intermarried** the **frequencies** of particular **genes** (and hence phenotypes) **would increase or decrease within the total population.** In a similar way the frequencies of the ABO genes and hence the ABO phenotype would alter if they provided selective advantages or disadvantages. As **different populations are affected by human migration to different extents** and are exposed to different pathogens the gene frequencies and sutherfore the subsequent blood group phenotypes will differ from one region / ethnic group to another. The effect of these factors on different gene frequencies is illustrated in figure 2.

Fig 2: A comparisson of the ABO Gene Frequency for Four Different Ethnic Populations. (8)(17)(18)(19)



In Australia the frequency of the various ABO phenotypes as illustrated in table 1 is similar to that observed in the study in Britain in 1946 (24). Some subtle frequency differences would reflect differences in ethnic migration frequencies when compared to Britain.

Table 1: The ABO phenotype frequency in Australia (24)	
ABO Phenotype	Frequency (%)
Group O	49
Group A	38
Group B	10
Group AB	3

The frequency of the ABO blood groups are not however as straight forward as these tables and figure indicate. This is because within the blood groups of the ABO system there exist **subgroups of the A phenotype** , **subgroups of the B phenotype** and rare groups such as the **Bombay blood** group.