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- IMMUNOLOGY by Kuby, J., Goldsby, R., Kindt, T.J. and Osbourne, B.A., W.H. Freeman, Oxford, UK.
- MEDICAL IMMUNOLOGY FOR STUDENTS by Playfair, J.H.L. and Lydyard, P.M. Churchill Livingstone, Edinburgh, UK.
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IMMUNITY

The Latin term *immunis*, meaning "exempt," gave rise to the English word immunity, which refers to all the mechanisms used by the body as protection against environmental agents that are foreign to the body. These agents may be microorganisms or their products, foods, chemicals, drugs, pollen, or animal hair and dander."

Every living organism is confronted by continual intrusions from its environment. Our immune systems are equipped with a network of mechanisms to safeguard us from infectious microorganisms that would otherwise take advantage of our bodies for their own survival. In short, the immune system has evolved as a surveillance system poised to initiate and maintain protective responses against virtually any harmful foreign elements we might encounter. These defenses range from physical barriers, such as a cell wall, to highly sophisticated systems, such as the acquired immune response. This chapter describes the defense systems: the elements that constitute the defense, the participating cells and organs, and the action of the participants in the immune response to foreign substances that invade the body.

In vertebrates, immunity against microorganisms and their products, or against other foreign substances that may invade the body, is divided into two major categories: innate or nonspecific immunity (sometimes referred to as natural immunity) and acquired immunity.

INNATE (NONSPECIFIC) IMMUNITY

Innate immunity is present from birth and consists of many factors that are relatively nonspecific; that is, they operate against almost any substance that threatens the body.

It resists infection by blocking the entry of pathogens into the body or by destroying the microbes through means other than antibodies. One form of nonspecific resistance is called **species immunity**, which implies that disease affecting one species will not affect another. For instance, humans do not contract hog cholera, while hogs do not contract polio. Cattle plague is unknown in humans, while gonorrhea does not occur in cattle. Such immunities are believed to be based on physiological and anatomical differences. In chickens, a physiological difference lends resistance to anthrax. Normal temperature of chickens is 45°C, at which anthrax bacilli do not grow well. At 37°C, susceptibility increases. This phenomenon was first demonstrated by Pasteur in 1878.

Racial immunities are those that exist among various races and people of the world. Most racial communities are due to nonspecific factors related to a people's way of life. Such immunities may reflect the evolution of resistant humans. For instance, black Africans affected by the genetic disease sickle cell anemia do not suffer from malaria, presumably because the parasite cannot penetrate distorted red blood cells. According to some such immunities exist because parasites have adapted to the body's environment. Americans, for example view measles as a mild disorder.

The nonspecific defense mechanism is further of two types: external defence or first line of defence and internal or cellular defence or second line of defence.

1. EXTERNAL DEFENCE (FIRST LINE OF DEFENCE).

This defence comprises physical and chemical barriers to the entry of pathogens into the body.

(i) Physical Barriers. These, in turn, are of two kinds: skin and mucous membranes.

A. Skin.; The skin provides a nice protective covering to the body. Its outer tough layer, called horny layer, or stratum corneum, consists of dead, fully keratinized cells. These cells contain a hard, insoluble, fibrous protein, called keratin or horn, instead of soft protoplasm. The horny layer is waterproof and germproof. It successfully prevents the entry of viruses and bacteria.

B. Mucous Membranes. The digestive, respiratory and urinogenital tracts open out at one or both the ends, and do not have a direct communication with other parts of the body. The parasites present in these tracts are not in the physiological interior of the body. The mucous membranes lining these tracts arc, therefore, treated as a part of the external defence like the skin. The mucous membranes resist the penetration of parasites into the tissues. Mucus secreted by mucous membrane traps the microorganisms and immobilises them.

(a) Buccopharyngeal Cavity. Dust particles and microbes entering the buccopharyngeal cavity via mouth are caught in the mucus and eliminated with sputum. A coating of mucus over the intestinal lining also traps the microbes for removal in the faeces.

(b) Respiratory Tract. Microorganisms and dust particles often enter the respiratory tract with air during breathing. Many of these are caught in the mesh of hair present in the nostrils. Those which pass through this filtering device, are trapped in the mucus that covers the respiratory tract. The cilia sweep the mucus loaded with pathogens and dust particles into the pharynx. From here, it is thrown out or swallowed for elimination with the faeces.

(c) Eyes. Secretion of tears and movements of eyelids flush out the microorganisms settling on the eye balls from the air.

(d) Internal Tracts. The various tracts in the body are flushed with fluids, such as saliva, digestive juices, bile, and urine, so that the microbes tend to be swept away.

(ii) Chemical Barriers. The skin and mucous membranes secrete certain chemicals which dispose of the pathogens. Specific cases of this defence are cited below —

(a) Skin Secretions and Bacteria. The oil and sweat secreted by sebaceous and sudoriferous glands contain fatty acids and lactic acid, which make the skin surface acidic (pH 3 to 5). This does not allow the microorganisms to establish on the skin. The skin harbours some friendly bacteria which release acids and other metabolic wastes that check the growth of microbes. Lysozyme the enzyme present in the sweat, kill many bacteria by destroying their cell walls. All this shows that the skin is a self-disinfecting organ.

(b) Saliva. Saliva also contains lysozyme which kills the microorganisms that are not the normal inhabitants of the buccal cavity and come with food and drinks. The dead microbes are then passively flushed by saliva to the throat where they are swallowed.

(c) Gut Secretions and Bacteria. The microorganisms may escape the action of saliva and reach the stomach with food and drinks. Bacteria trapped in mucus in the respiratory tract also reach the stomach when swallowed. Here, they are killed by hydrochloric acid and proteolytic enzymes of the gastric juice. The proteolytic enzymes in the small intestine also help in killing, the microorganisms. If the bacteria survive and reach the large intestine, they arc attacked by gut microorganisms, which secrete antibiotics that kill many pathogenic bacteria.

(d) Bile. Bile, a bitter alkaline (ph 8) secretion of the liver, checks the growth of foreign bacteria on semidigested food, the chyme, in the intestine.

(e) Tears. Tears, a slightly saline fluid, secreted by the lachrymal glands over the eyes also contain lysozyme, which prevents eye infections. Frequent washing of eyes reduces the disinfecting power of the lachrymal secretion. Tears also wash off the chemical irritants of polluted air from the eyes.

(f) Nasal Secretions. Nasal secretions also destroy the harmful, foreign germs with their lysozyme.

(g) Cerumen. Cerumen (ear wax), a bitter, brownish secretion from the ceruminous glands into the auditory canal, traps dust and bacteria. It contains an effective antibacterial component. It also prevents entry of insects.

(h) Vaginal Bacteria. Certain bacteria (*Lactobacilli*) normally live in the vagina. They produce lactic acid from glycogen of the cells that periodically break off from the mucous membrane. Lactic acid kills the foreign bacteria. The lactic acid bacteria of the vagina form female's best natural defence against infection. Hence, frequent vaginal douches particularly with disinfectants, should be avoided. Douches remove the friendly bacteria.

Penetration of the First Line of Defence. Skin and mucous membrane may fail to keep out the invaders. Some parasites make, way through the skin, e.g., hookworm. Others enter through wounds and openings of sweat glands and hair follicles. Still others are injected by blood-sucking arthropods. Microorganisms may injure and pass through the thin, moist, relatively vulnerable mucous membranes of digestive, respiratory and urinogenital tracts, and get into the tissues or blood, it is, therefore, very essential for survival to have a second line of defence for controlling the parasites that have entered the body by breaking through the first line.

2. INTERNAL OR CELLULAR DEFENSES (SECOND LINE OF DEFENCE)

Once an invading microorganism has penetrated the various physiologic and chemical barriers, the next line of defense consists of various specialized cells whose purpose is to destroy the invader. There are several cell types that fulfill this function. The developmental pathways of hematopoietic cells and interrelationship between the various cell types are shown diagrammatically in Figure

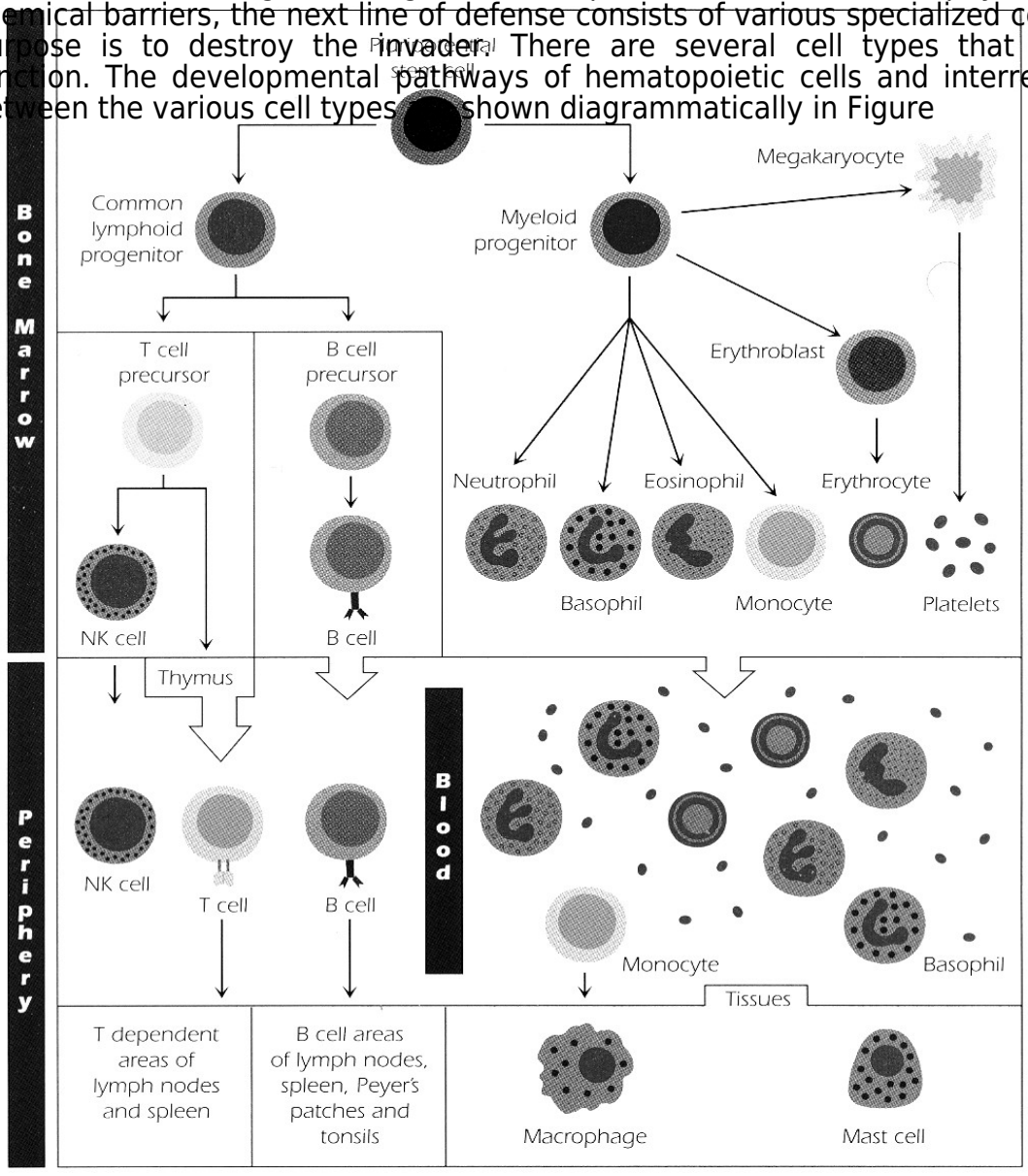


Figure The developmental pathway of various cell types from pluripotential bone marrow stem cells.

Phagocytosis or Cellular defense

Shortly after the verification of germ theory of disease of Koch, a Russian,Elie Metchnikoff (a native of Ukraine) made a chance discovery that clarified how living cells could protect themselves against microorganisms. Metchnikoff observed that motile cells in the larva of starfish gathered around a wooden splinter placed within the cell mass. He suggested that the motile cells actively sought out and engulfed foreign particles in the environment to provide resistance. His theory of phagocytosis, published in 1884 was received with skepticism since it appeared to conflict with the antitoxin theory. However, in succeeding years most persons appreciated phagocytosis. Metchnikoff later became an associate of Pasteur and was co-recipient of the 1908 Nobel Prize in Physiology or Medicine.

Cells of phagocytosis

Phagocytosis is considered a major form of nonspecific defense in the body. The cells involved are called phagocytes. They are the polymorphonuclear cells (PMNC) (also called polymorphs or neutrophils). Neutrophils are the most abundant phagocytic cells in blood. Monocytes of the circulatory system, as well as cells of the reticuloendothelial system, also called the mononuclear phagocyte system, is a collection of monocytes-derived cells that leave the circulation and undergo modification in the tissues. They include Kupffer cells of the liver and macrophages of the spleen, bone marrow, lymph nodes and connective tissues.

Steps in Phagocytosis

Mononuclear phagocytes exhibit non immune and immune phagocytosis. This process consists of several steps: (1) recognition of and attachment to the particle to be phagocytosed, (2) actual engulfment, (3) killing and digestion, and (4) post-digestion disposal of remnants.

Recognition and attachment in immune phagocytosis is mediated by the Fc’yR 111-2, CR5 (which recognizes C3d), and CD35 (CR1, which recognizes C3b) on the phagocytes. These receptors become attached to immune complexes and complement bound on opsonized target particles. During phagocytosis, foreign particles are bound to either specific or nonspecific receptors and then surrounded by the cell membrane to form a phagocytic vesicle. Alternatively, soluble macromolecules might be pinocytosed in a similar fashion. FcRs and CRs enable Ms

to recognize particles coated, or opsonized, with Ab and complement molecules. However, some Abs are cytophilic. These Abs bind to the Ms first, then bind to the Ag on the particle to be phagocytosed. In this manner, immunological specificity for target particles is acquired. Bound soluble and insoluble materials are then ingested. Following phagocytosis, formerly interiorized receptors are re expressed on Ms membranes.

Engulfment is accomplished by physical wrapping of the membrane around the particle. This creates a phagocytic vesicle, or phagosome, which is taken into the cell. Once formed, the phagosomes are moved within the directed by microtubules. They then fuse with lysosomes forming phagolysosomes. Next the contents become acidified and are digested by a veritable host of hydrolytic enzymes (table). Some intracellular parasites escape this fate by penetrating the phagosomal membrane, thereby gaining access to the more compatible cytoplasm of the Ms. This strategy is used by the protozoan, Leishmania, which preferentially infects Ms in the host. Other parasites are even capable of preventing the fusion of the phagocytic vesicle with a primary lysosome.

Enzymes	Neutrophils	Macrophages
Glycosidases		
α-L-fucosidase	Yes	
α-1,4-glucosidase	Yes	
α-mannosidase	Yes	
α-N-acetylglucosaminidase	Yes	
β-glucuronidase	Yes	Slight
β-galactosidase	Yes	
β-N-acetylglucosaminidase	Yes	
Hyaluronidase	Yes	Yes
Lysozyme	Yes	Yes
Lipases		
Acid lipase	Yes	Yes (?)
Phospholipase	Yes	Yes (?)
Nucleases		
Ribonuclease	Yes	
Deoxyribonuclease	Yes	
Phosphatases		
Acid phosphatase	Yes	Abundant
Alkaline phosphatase	Yes	
Phosphatidic acid phosphatase	Yes	
Phosphoprotein phosphatase	Yes	
Proteases		
Cathepsins B, C, D, E	Yes	Cathepsin C (?) Yes
Collagenase	Yes	Yes
Elastase	Yes	
Kininase	Yes	
Kininogenase	Yes	
Miscellaneous Substrates		
Aryl sulfatases A and B	Yes	
Esterases	Yes	Abundant
Peroxidase	Yes	Modest amounts

Two major mechanisms of killing and digestion are used by phagocytes. One depends upon oxygen, while the other is not dependent upon oxygen. The following sequence is believed to represent the steps of **oxidative (oxygen-dependent) killin**g of phagocytized microorganisms that is mediated by the enzyme myeloperoxidase:

1. Glycolysis supplies energy for engulfment (oxygen consumption is increased two- to three-fold).
2. NADPH oxidase becomes activated, leading to the generation of peroxide (H2 02).

3. The NADP made available stimulates the hexose monophosphate shunt substantially (from 1% to 10% of glucose utilization), providing increased substrate for NADPH oxidase.
4. The H202 generated interacts with myeloperoxidase, and possibly intracellular halide (Cl-)to cause bacterial killing. In this final step, toxic oxidants (for example, hypochlorite [bleach]) can be produced, which can chemically disrupt the microbial surface wall, leading to its eventual death.

A second oxidative mechanism exists that is not dependent on myeloperoxidase. Through this mechanism, microbes are destroyed by the direct effects of H202, superoxide ions (02), reactive singlet oxygen radicals (0.), and hydroxyl ions (OH-). Since Ms lack myeloperoxidase, this is their principal means of microbial killing.

Alternatively, killing can be accomplished through a **non-oxygen-dependent mechanism**. The lysosomal cationic proteins and lactoferrin are implicated in this microbicidal mechanism. Peroxide generation is not a part of this process. The cationic proteins are most active in an alkaline environment, as exists within the phagosome shortly after its creation. If the microorganisms engulfed are gram-negative bacteria, these proteins have an opportunity to cause serious damage to the cell wall before the pH shifts downward into an acidic range. Since lactoferrin can bind iron, it might bring about microbial death by depriving the bacteria of iron, which is a necessary element for their survival. This binding of iron by lactoferrin can occur at either high (alkaline) or low (acidic) pH.

As the particle is reduced by the hydrolytic enzymes to its component building blocks (amino acids, simple sugars, fatty acids, etc.), they simply diffuse, or are transported, across the vesicle membrane into the cytoplasm. While the events of microbial killing can sometimes be oxygen dependent, the actual digestive phase of phagocytosis is oxygen independent. After digestion is complete, a shriveled depleted
vesicle, called a residual body, is all that remains. The non digestible residue that remains here can then be discharged through exocytosis (a “reverse engulfment”).

Inflammation

Inflammation (Latin, inflammatio, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, inflammation which runs unchecked can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. It is for this reason that inflammation is normally tightly regulated by the body.

Causes

There are many agents that can provoke an inflammatory response.

- Burns
- Chemical irritants
- Frostbite

- Toxins
- Infection by pathogens
- Necrosis
- Physical injury, blunt or penetrating
- Immune reactions due to hypersensitivity
- Ionizing radiation
- Foreign bodies, including splinters and dirt

Types of Inflammation

There are two fundamental types of inflammation: acute and chronic. A rapid onset, short duration, and profound signs and symptoms characterize acute inflammation. On the other hand, a slow onset, long duration, and less obvious signs and symptoms characterize chronic inflammation. In addition to the two basic forms (acute and chronic), there are two others that appear less commonly: subacute and granulomatous chronic inflammation. Subacute inflammation is an ill-defined form that has some clinical features of acute and some of chronic inflammation. Granulomatous chronic inflammation, as its name signifies, is a special form of chronic inflammation. This type is associated with tuberculosis as well as some other less common diseases.

ACUTE INFLAMMATION

Acute inflammation immediately follows injury by physical, chemical, or biologic agents. The events following injury cause blood vessel changes allowing entrance of certain blood cells into the injured area. As these cells grapple with the agent that provoked their appearance, normal surrounding tissue may be damaged or even killed. The sequence of these vascular, cellular, and tissue events have been known for decades and are straightforward. More recently, however, the unfolding molecular basis for them has resulted in a maze of interacting compounds that has complicated the picture considerably. In the following discussion, microscopic and physiologic events will be emphasized more than chemical and molecular ones.

Sequence of Events

Acute inflammation unfolds as a predictable series of events. After entrance of a foreign antigenic agent into the body's connective tissue spaces, a predictable sequence of events invariably ensues. These events occur within minutes. They explain the characteristic redness, warmth, swelling, and pain accompanying acute inflammation. An initial brief contraction of blood vessels is observed experimentally. In the laboratory, the first event following tissue injury is a sudden, but short-lived, contraction of small blood vessels in the immediate area. This transient vasoconstriction may be caused by stimulation of nerves in the area. Whatever its cause, it last but a few seconds and has no apparent clinical significance.

1. Blood Vessel Dilation

Dilation of small blood vessels is the first event observed in patients. In the first minutes, small blood vessels (capillaries and venules) increase their diameter (dilate) allowing more blood to flow into the area. This increased blood flow is fed by dilation of supplying arterioles, a process known as "active hyperemia" (hyper- = increased; -emia = blood). With increased blood flow, increased numbers of blood cells enter the area too. Dilation of blood vessels makes the injured area appear red and feel warm. As more blood enters the injured area, it will be redder and warmer than surrounding unaffected areas. To the dental practitioner, then, areas of redness and warmth signify the presence of acute inflammation. Celsus used the terms "rubor" (red) and "calor" (heat) in his descriptions.

2. Increased Blood Vessel Permeability

Fluids (plasma) leak out of the dilated blood vessels into the injured area. Soon after blood vessel dilation, the blood vessels become leaky allowing the fluid portion of blood (plasma) to escape into surrounding tissues. At first, this leakage is the result of increased local blood pressure forcing a filtrate of plasma out leaving large protein molecules behind, a process known as "transudation." A short time later, changes in blood vessel endothelial cells allow plasma along with its important clotting and immunologic proteins to escape. The increased volume of proteins accumulating in the area of tissue injury further increases the rate of plasma escape by increasing osmotic tension. This rapid exodus of protein-rich plasma is known as "exudation." Transudation, then, is an early short-lived event during which protein-deficient plasma exits blood vessels; in exudation, a later and longer lasting event, protein-rich plasma leaves to accumulate in the area of tissue injury. Leakage of plasma causes swelling, pain, and loss of function.

As might be expected, increased fluid accumulation in within the area of tissue injury produces visible swelling, or "tumor" as Celsus called it. Increased pressure within the damaged tissue and increased production of acid by-products of the inflammatory reaction causes pain ("dolor") and loss of function ("functio laesa") of the inflamed part.

3. Blood Flow Stagnation

Plasma leakage causes blood flow to become stagnant. Plasma leakage causes blood cells to become more closely packed (hemoconcentration) causing sluggish flow. In fact, blood flow in the affected area may even stop. When blood flow is normal, "formed elements" normally are found in a cell-rich "axial core" separated from the endothelial lining by a thin cell-free "plasmic zone." The maintenance of the axial core and clear plasmic zone depends on a strong rapid current of blood flow. As blood flow slows during inflammation, the axial core can no longer be maintained allowing blood cells to touch the endothelial lining cells.

4. Margination

As blood flow slows, some blood cells stick to the blood vessel lining. As blood flow slows and the axial core collapses, blood cells have the opportunity to contact the surface of endothelial cells lining the vessel wall. Some blood cells ricochet off while others stick to it. In acute inflammation, neutrophils are sticky cells while later in chronic inflammation lymphocytes are the sticky ones. When blood vessels are examined with the LM at this stage of acute inflammation, neutrophils are seen to line up along the interior lining surface, a feature known as "margination" or "pavementing."

5. Emigration

Some sticky cells squeeze out of blood vessels entering the injured area. Once adherent, WBCs crawl along the lining surface until they find an open junction between endothelial cells. Finding a gap, they squeeze through it only to become trapped between the outer endothelial surface and the underlying basement membrane. The temporarily trapped WBCs crawl along its basement membrane until they find a seam to squeeze through. By such considerable effort, WBCs leave blood vessels to enter the area of tissue injury. This active process is known as "emigration" or, less commonly, "diapedesis."Neutrophils and monocytes are the first to enter the injured area.

Two leukocyte types, neutrophils and monocytes, are the first blood cells to emigrate. Neutrophils are the most common leukocyte; they compose about 65% of the circulating WBCs. These common cells soon dominate the injured area. Neutrophils die out in 48 hours or so as a consequence of self-destruction and increasing acidity of the environment. They are relatively fragile cells with a short life

span.Monocytes also emigrate early in the inflammatory reaction; however, since they comprise only 5% of the circulating WBCs, their presence in the area of inflammation is obscured for a time by overwhelming numbers of neutrophils. Once monocytes enter the injured tissues they are given a name more indicative of their function -- macrophages. Unlike neutrophils, macrophages have a long life span and have great tolerance to acidic environments. Monocytes outlive neutrophils to become more apparent later.

6. Exudation

The materials accumulating in the injured area destroy the causative agent.At this point blood plasma, neutrophils, and monocytes/macrophages have accumulated in the area of tissue injury. The term "exudate" refers to these accumulated products. From what you have learned already, the acute inflammatory exudate is composed of protein-rich plasma, of neutrophils, and of monocytes/macrophages.

Antibodies from blood plasma may destroy the causative agent. Plasma proteins leave blood vessels early in the inflammatory response. Of these, two play a particularly important role. Immunoglobulins, a group of antibodies that have the ability to react with certain antigens by destroying them or by making them vulnerable to action by neutrophils and macrophages.

Formation of a blood clot may wall off the injured area.The second are blood-clotting proteins. A blood clot is composed of a meshwork of "fibrin" a protein end product of a complex interaction of plasma, tissue, and cell factors. If fibrin is produced in the area of tissue injury, it may prevent spread of the injurious agent.

Pathogenesis of Acute Inflammation

Role of the Autonomic Nervous System; Blood vessel dilation can be caused by nerve impulses. Arterioles are "hard-wired" to the autonomic nervous system. This means that certain nerve impulses cause contraction of smooth muscle in arteriolar walls while others cause smooth muscle relaxation. Autonomic impulses play a role in relaxation of arteriole smooth muscle so that these vessels can dilate.

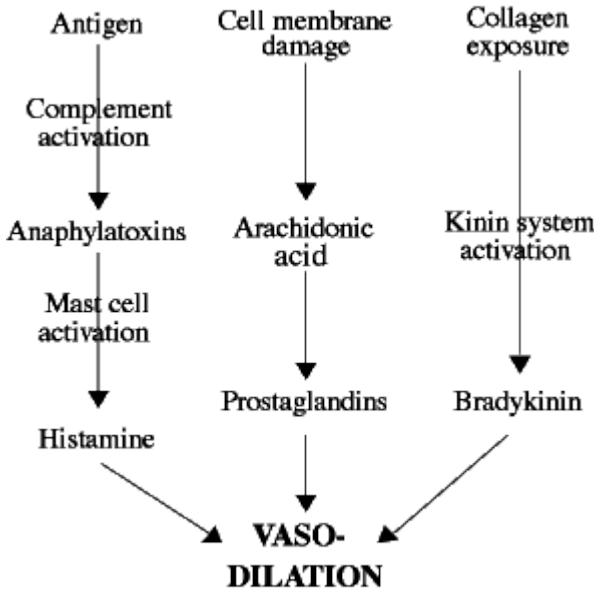
Role of Chemical Mediators; Many chemical compounds can cause blood vessel dilation. A host of chemicals have been identified that mediate or otherwise influence a number of inflammatory responses. While some of these "chemical mediators" have been known for years, others were discovered more recently. There are four classes of chemical mediators. Cell secretions can cause blood vessel dilation and leakage -- histamine & serotonin.

The first are compounds known as "vasoactive amines," histamine and serotonin, both of which are powerful vasodilators. Histamine is found in mast cells while serotonin is found in blood platelets. Beyond its known vasodilator functions, serotonin's role in inflammation is not clear. Much more is known about histamine. It is well known that mast cell granules are histamine-filled secretory vesicles which produce dilation of blood vessels. If a lot of histamine is released all at once, a life-threatening anaphylactic reaction may ensue. In run-of-the-mill inflammatory responses histamine is released in small amounts in the immediate area of tissue injury. It is in these settings that histamine acts to dilate blood vessels. Blood vessel injury stimulates production of vasodilator chemicals-bradykinin.

The second are a group of proteins constituting the "kinin system." It is the activation of this system that produces another powerful vasodilator known as "bradykinin." Initial activation results from exposure of collagen to blood plasma; such exposure is caused by injury to the endothelial blood vessel linings allowing plasma to contact collagen in underlying basement membranes. Following collagen

exposure, a series of reactions starting with activation of factor XII leads, ultimately to formation of bradykinin. It is interesting that blood vessel injury can also lead to blood clot formation by activation of a related system -- the blood-clotting cascade. In sum, following blood vessel injury, bradykinin causes dilation of small blood vessels in the injured area.Chemicals found in plasma can cause blood vessel dilation-complement.

Third, a series of plasma proteins (C_1-C_9) are activated by the presence of antigenic agents. These plasma proteins constitute the "complement system" or the "complement cascade." An activated form of at least one of these proteins (C_{5a}) binds on sensitized mast cells causing them to release histamine. Because of the anaphylactic response the release of large amounts of histamine produce, $C_{5a}$and other related complement proteins are sometimes known as "anaphylatoxins."Damaged tissue cells stimulate production of vasodilator chemicals-prostaglandins .Finally, "prostaglandins" produce vasodilatation in the areas of tissue injury. These are substances produced by a series of reactions from the damaged cell membranes and the subsequent release of "Arachidonic acid." Arachidonic acid-derivatives become vasodilator prostaglandins.



. Important Chemical Mediators

How "Increased Permeability" Occurs

Leakage of plasma is caused by contraction of blood vessel lining cells. There are two mechanisms that explain escape of plasma into the surrounding tissues in the early phase of acute inflammation: endothelial cell contraction and endothelial cell injury. The continued presence of histamine, bradykinin, and other chemical mediators cause endothelial cell contraction, an event that opens intercellular junctions allowing early transudation of protein-deficient plasma. If the inflammatory reaction is severe and long-lasting enough, endothelial cell damage (or even death) allows rapid escape of protein-rich plasma. Such injury is caused by chemicals that accumulate in the area of tissue injury and by the activation of certain white blood cells that, in turn, secrete enzymes that in the process of destroying the inciting agent kill endothelial cells.

How "Margination" Occurs

Surface receptors on WBCs and endothelial cells cause their "stickiness." There are two explanations for adherence of leukocytes (WBCs) to blood vessel walls: 1) changes in WBCs and 2) changes in the endothelial lining cells. In both cases

chemical mediators increase the numbers of surface receptors allowing increased adherence. C_{5a}increases surface receptors on neutrophils. Secretions of lymphocytes and monocytes called "cytokines" increase the surface receptor on endothelial cell surfaces.

Chemotaxis

Attraction of WBCs to injured areas is caused by chemical mediators-chemotaxis. Apparently neutrophils do not just appear in the injured area; they are enticed by chemical agents. The attraction of WBCs by chemicals has been known for decades; the term "chemotaxis" has been used to identify it. As chemotaxic chemicals appear in the area of tissue injury, neutrophils and monocytes migrate along the path of its increasing concentration. A number of chemotaxic agents have been identified: $C_{5a}$and certain leukotrienes (a product of arachidonic acid) are but two examples. These agents bind with neutrophil and monocyte/macrophage surface receptors stimulating 1) cell movement and 2) cell activation, secretion, and degranulation.

Clinical Features of Acute Inflammation

So far we have considered the minute-by-minute developments in the early phases of inflammation. Now it is time to turn to more practical considerations.

Recognizing "cardinal signs" will lead to a diagnosis of acute inflammation. Acute inflammation is easily recognized by its signs and symptoms. The inflamed area is red, warm, swollen, and painful. The part is so sore that the patient protects losing its function. These features are known as the "cardinal signs" of acute inflammation. Students seem to remember these signs (and symptoms) easier by learning the terms Celsus used two millennia ago: rubor (redness), calor (warmth), tumor (swelling), dolor (pain), and functio laesa (loss of function). Any time a patient presents with a warm, red, painful swelling it is likely that their body is fighting off some bacterial infection.

Systemic Features of Acute Inflammation

Systemic features of inflammation may cause a patient to appear sick. Severe acute inflammatory reactions produce effects far away from the area of tissue injury. Patients with serious bacterial infections are sick. The most important of systemic changes that occur with such infections are fever and elevated white cell counts.

Cardinal Signs

English	Greek/Latin	Caused By
Redness	Rubor	Hyperemia
Warmth	Calor	Hyperemia
Swelling	Tumor	Increased permeability
Pain	Dolor	Low pH
Loss of function	Functio laesa	Pain, swelling

Elevation of body temperature is a sign of acute inflammation-fever. Normal body temperature (as measured orally) is 98.6°F.; in a serious infection, temperature may rise to 103-104°. If an infection is suspected in a dental patient, her/his temperature should be measured and noted in the dental record.Secretions of inflammatory cells (cytokines) can cause fever.

Fever is caused by secretion of cytokines by cells that appear in the inflammatory reaction (e.g. macrophages). Two common cytokines are interleukin-1 (Il-1) and tumor necrosis factor (TNF). Given that these factors cause fever and are produced by inflammatory cells, it follows that a large number of cells produce large amounts of cytokines resulting in higher fever. There is, then, a direct relationship between the severity of the inflammatory response and fever.Numbers of circulating WBCs

increase in acute inflammation-leukocytosis.In severe acute inflammatory responses, greater than normal numbers of white cells appear in circulation, a condition known as "leukocytosis" (leuko- = white; -cyt- = cell; -osis = condition of). Normally, white blood cells number about 4,000 to 10,000 in each cubic millimeter of blood. In severe infections, white cell counts may reach 30,000/mm^3. The additional cells are produced in bone marrow under the influence of the same cytokines that produce fever.

The presence of immature WBCs suggests a severe infection is present. Neutrophils are the most prominent cells in acute inflammation. If extraordinary numbers of these cells are needed to fight a severe infection, bone marrow is called upon to release developing neutrophils as soon as possible. As a consequence of this demand, immature neutrophils appear in the blood stream. When performing a blood count in such patients, it is possible to differentiate immature neutrophils from mature ones because the immature nuclei are not segmented, and are horse shoe-shaped. These characteristic nuclear changes have earned immature neutrophils the names "band cells" or "non-segmented neutrophils." The appearance of many immature neutrophils is sometimes designated as a "shift to the left," a reference to a form once used for reporting blood counts. By the way, neutrophils are often known as "polys" or "PMNs."A differential blood count is a simple way to find changes in blood cells.

It is common practice to count various blood cell types and report the percentages of each when performing a blood count. This procedure is known as a "differential blood count." In a normal individual, neutrophils account for about 65% of the WBCs, lymphocytes 30%, monocytes 5%, eosinophils 1%, and basophils 0.5%. In severe acute inflammatory responses, the percentage of neutrophils (mature and immature forms) may greatly exceed the 65% rate normal for these cells.

CHRONIC INFLAMMATION

An immune reaction to some "mild" but persistent antigen producing a proliferation of lymphocytes and/or plasma cells (B cells). There is usually no pain, redness, swelling, or warmth. Scarring and persistence of etiologic agent is common.

General Features of Chronic Inflammation

Chronic inflammation is longer lasting and less dramatic.If inflammation is subdued, has a quiet onset, and lasts for days to weeks, the term "chronic inflammation" is used. This type of inflammation is, then, characterized by an insidious onset and long duration. The signs and symptoms of chronic inflammation are not as dramatic as those associated with acute inflammation.Chronic inflammation may follow acute or start anew.Sometimes chronic inflammation follows acute inflammation; other times it starts anew (de novo) without going through an acute phase first.

Etiology and Pathogenesis of Chronic Inflammation

Persistent acute inflammation will become chronic.If an acute inflammatory reaction persists, it will enter a chronic phase. There are two general causes of such persistence: the inability to eliminate or continual reacquisition of the offending agent. These situations are common in dentistry where, for example, an open pulp chamber keeps reintroducing microorganisms into the tissues around the root (periapical tissues). It also may occur when there is continual exposure to some inanimate materials like pollens and dusts.

Low-grade irritants may initiate chronic inflammation.More often than not, chronic inflammation arises without going through an acute phase first (de novo chronic inflammation). Two examples of this come to mind: persistent infections and autoimmune diseases.Microorganisms with low virulence may initiate chronic inflammation.Infection with a microorganism of low virulence that cannot be

eliminated easily may result in chronic rather than acute inflammation. Tuberculosis and some dental conditions (to be discussed later) are examples of such infections.Constant stimulation of the immune system may initiate chronic inflammation.Sometimes a patient may be "allergic" to her/his own cells. This condition is known as autoimmunity. In these cases, the affected patient's cells serve as a source of constant stimulation of the chronic inflammatory process. Systemic lupus erythematosus and rheumatoid arthritis are autoimmune diseases characterized by chronic inflammation.

The Cells of Chronic Inflammation

Mononuclear cells are characteristic of chronic inflammation.In chronic inflammation, macrophages and lymphocytes are the predominant cells; there are few, if any, neutrophils. These, along with most other cells associated with chronic inflammation, have single nuclei. Because of this feature, they are commonly known as "mononuclear cells" or "round cells."

Macrophages

Macrophages are prominent in chronic inflammatory exudates.Macrophages are monocytes that have entered an area of tissue injury. They can live for months and can thrive in acid environments. For macrophages to carry out their functions they must be stimulated (activated) by chemical mediators. Among the chemical mediators are lymphokines (cytokines secreted by lymphocytes), fibronectin-coated surfaces, and mediators that initiate acute inflammation.

Phagocytosis is the main function of macrophages. Macrophages are excellent phagocytes. They engulf and process antigens allowing them to be neutralized by other cells (lymphocytes). Activated macrophages can also engulf and kill certain microorganisms. Macrophages also secrete a number of substances that assist in the recruitment of other cells (monokines) and cause tissue destruction (collagenases, elastases, reactive oxygen).

T-Lymphocytes

T-Lymphocytes are the most characteristic cell of chronic inflammation.Lymphocytes emigrate from blood vessels late in an inflammatory reaction. Because lymphocytes account for about one-third (33%) of the circulating WBCs, they become the predominant cell in chronic inflammation. There are two types of lymphocytes: T and B. T-lymphocytes are produced in the thymus gland and are responsible for cell-based immunity. B-lymphocytes, on the other hand, arise from bone marrow and are responsible for humoral immunity.T-Lymphocytes must be activated; they also can activate macrophages.

T lymphocytes must be activated before they carry out their functions. Such activation is effected by monokines and, in some cases, directly by antigens. Once activated, lymphocytes can react with certain antigens destroying them or rendering them harmless. They also secrete lymphokines that stimulate macrophages. Thus, macrophages and lymphocytes are interdependent -- the activation of one stimulates the activation of the other.

B-Lymphocytes (Plasma Cells)

Plasma cells are activated B-lymphocytes.Plasma cells are derived from activation of a class of lymphocytes known as "B cells." They do not circulate in the blood stream but are transformed in lymphoid organs or at the site of chronic inflammation. They possess off-center nuclei, abundant basophilic cytoplasms, pale spots near the nuclei (negative Golgi images), and clock-face distribution of nuclear chromatin.Plasma cells secrete antibodies. Plasma cells manufacture and secrete antibodies against specific antigens. The antibodies circulating in blood plasma are derived from plasma cells; these circulating antibodies are called "humoral antibodies." A plasma cell produces

antibodies against a single antigen. Once a B lymphocyte is activated, it creates a clone of cells capable of producing antibodies against the antigen that stimulated it.

Eosinophils

Eosinophils can destroy parasites and certain cells.Eosinophils are related to neutrophils; both display a segmented nucleus; both are polymorphonuclear leukocytes. Eosinophils comprise about 3% of the circulating WBCs and are recognized by the bright red granules within their cytoplasm. These granules are filled with a substance called "major basic protein" that can destroy some parasites and some cells.

Eosinophils accumulate in certain diseases.These cells are not seen in all chronic inflammatory reactions. Rather, they appear in parasitic infestations, hypersensitivity reactions, and some autoimmune conditions.

Clinical Features of Chronic Inflammation

The clinical features of chronic inflammation are subdued.Acute inflammation has dramatic and easily recognized clinical features (e.g. redness, warmth, swelling, pain, loss of function, fever). These signs are absent or greatly suppressed in chronic inflammation.

Complications of Chronic Inflammation

Unlike acute inflammation where the reaction itself may be life threatening (e.g. cellulitis), the adverse effects of chronic inflammation are not so dramatic. Two complications are rather common: fibrosis and persistence.

Too much collagen production may cause disfiguring scars.

Scarring -- Much tissue can be destroyed during a long-standing chronic inflammatory reaction. This missing tissue is usually replaced by continual production of collagen by fibroblasts. If the inflammatory reaction persists for a long time, collagen build up can be significant. If this occurs, scars may form causing permanent distortion of the tissue and interfere with its function. Also, the presence of scar tissue may hinder regeneration of parenchymal cells.Chronic inflammation may persist for a long time.

Persistence -- Substances with low antigenic properties may not be eliminated quickly. If these persist, the chronic inflammatory reaction may be continually stimulated. Similarly, reactions to one's own cells (autoimmunity) may also produce long-standing chronic inflammation due to continual cellular destruction and, therefore, the unending supply of antigen.

Granulomatous Chronic Inflammation

Granulomatous chronic inflammation appears in granulomatous diseases.Under certain circumstances a chronic inflammatory reaction will acquire features so special that they will narrow a diagnosis to a group of conditions called "granulomatous diseases." These conditions include tuberculosis, syphilis, leprosy, and most fungal (mycotic) infections. The microorganisms producing these granulomatous diseases are low-virulence ones causing persistence of chronic inflammatory reactions.The granuloma consists of granulation tissue and chronic inflammation.The lesion of granulomatous chronic inflammation is the "granuloma." It is a little mass of chronic inflammation with a background of new capillaries, new fibroblasts, and new collagen. This reparative tissue is called "granulation tissue." It is the presence of granulation tissue that gives the granuloma its name.The epithelioid cell is the hallmark of granulomatous chronic inflammation.When macrophages become activated they acquire special morphologic features. These cells acquire large, round nuclei that remind pathologists of epithelial cell nuclei. It is

this feature that gives rise to their designation as "epithelioid cells." Epithelioid cells are diagnostic of granulomatous chronic inflammation.

Subacute Inflammation

Subacute inflammation is ill defined; clinicians use it more than pathologists.Pathologists do not speak of subacute inflammation often because it is so ill defined that its microscopic appearance cannot be described. However, clinicians sometimes use the term to refer to a clinical situation in which the signs and symptoms displayed by the patient are neither "acute" nor "chronic" they seem to be somewhere in between. In these cases, the reaction is neither "clinically acute" nor "clinically chronic" and, therefore, is "subacute."

ACQUIRED IMMUNITY (Specific Resistance)

In contrast to innate immunity, which is an attribute of every living organism, acquired immunity is a more specialized form of immunity. It developed late in evolution and is found only in vertebrates. The various elements that participate in innate immunity do not exhibit specificity against the foreign agents they encounter: by contrast, acquired immunity always exhibits such specificity. As its name implies, acquired immunity is a consequence of an encounter with a foreign substance. The first encounter with a foreign substance that has penetrated the body triggers a chain of events that induces an immune response with specificity against that foreign substance.

Basic concepts of immunology

Three concepts critical to the study of immunology are

1**. Specificity**. It refers to the host's response to an individual agent.

2**. Memory**, It implies that once the body has responded to an agent, it will react vigorously during a subsequent exposure. This is the reason why an episode of measles makes the person immune to future episodes.

3. **Recognition of nonself**. This means that the host will develop resistance to agents that are foreign to itself. However, this recognition sometimes fails to operate, such as in áutoimmune diseases.

The above basic concepts revealed that there are two different forms of the immune response (i) antibody-mediated immunity (also called humoral immunity), in which specific

proteins, the antibodies are made when foreign antigens are detected. An antigen is any macromolecule that elicits the formation of an antibody and that can subsequently react with an antibody. In this kind of response, plasma cells derived from certain white blood cells (B-lymphocytes) synthesize antibodies in response to the detection of a foreign macromolecule with antigenic properties. Antibodies, also called immtinoglobulins are made in response to specific antigens and react with those antigens. (ii) cell-mediated immunity (also called cellular immunity) in which certain cells of the, body acquire the ability to destroy other cells that are recognised as foreign or abnormal. This type of response depends on another class of lymphocyte cells — T- lymphocytes. These interact with "foreign" cells to destruct them. There are also interactions between B- and T-lymphocytes and between these and other blood cells that establish an integrated defense network.

The Immune System

The immune system is a general term for complex series of cells, factors and processes that provide a specific response to antigens.

The immune system appears to originate in the fetus, about two months after conception .At that time, primordial cells, called stem cells, arise in the marrow and differentiate by a complex process into either of two types of

(i) **erythropoietic cells** which will become erythrocytes. and (ii) IymIphopoietic cells which will become the lymphocytes of the immune system.

Lymphopoietic cells follow either of two courses. (i) Certain ones pass through a specialised organ of the thoracic cavity called thymus. In humans this flat, bibbed organ lies just below the thyroid gland in the upper thorax, above the heart. It increases in size untilthe age of puberty and then disintegrates. Within the thymus, lymphopoietic cells are modified to form thymus-dependent lymphocytes, or T-lymphocytès. They are also called T-cells. After they emerge, the T-lymphocytes (or T-cells) move through the circulation and colonise the lymph nodes, spleen, tonsils, and other lymphoid tissues. (ii) other lymphopoietic cells pass through an organ that has been identified in the chick but not with certainity in humans. In the chick, the organ is a gland in the lower gastrointestinal tract called the bursa of Fabricius.

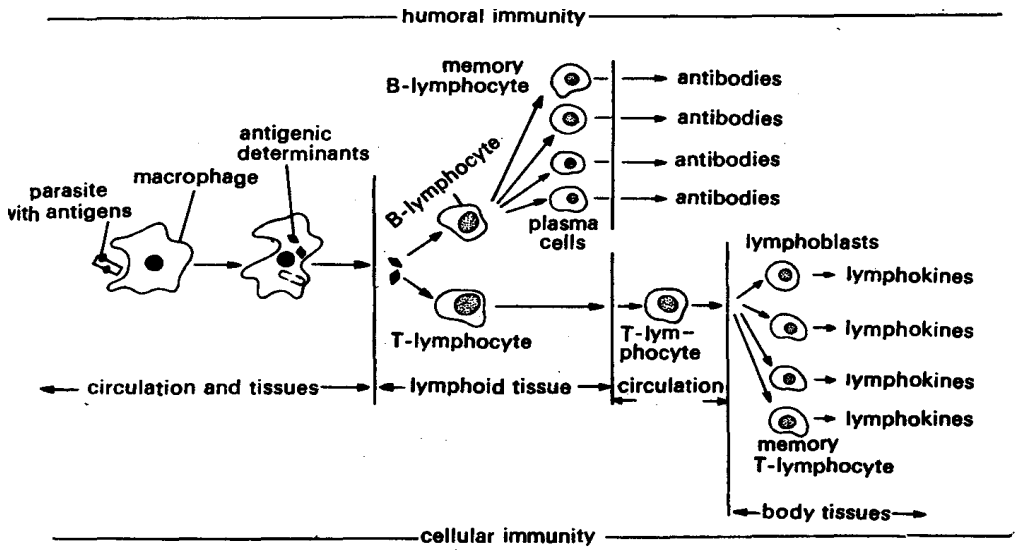
Lymphopoietic cells that pass through this gland are modified to form B-lymphocytes (B for bursa). They are also called B-cells. In humans the analogous organ is thought to be the fetal liver or bone marrow. B-lymphocytes have chemical substances on their surfaces that distinguish them from T-lymphocytes. Like T-lymphocytes, the B-lymphocytes move through the circulation to colonise the lymph nodes and other lymphoid tissues.

The T-lymphocytes and B-lymphocytes occupy a central role in the immune system. Since they accumulate in lymph nodes lying along the lymphatic vessels they encounter all antigens except those entering the cardiovascular system directly) The lymphocytes are also prominent in the tonsil and spleen, both of which are important in youth but have a diminished role in adults.-lymphocytes and T-lymphocytes are segregated into discrete areas in the lymphoid tissues. Usually, the T-lymphocyte is the smaller of the two cells. B-lymphocytes have a life span of about five to seven days, but T-lymphocytes may live for many months or years. About 65 to 80 per cent of the circulating lymphocytes are T-lymphocytes, and most of the remainder are B-lymphocytes, with a small percentage of immature cells of either type.

The production of surface components of B-lymphocytes is under the control of genes called the immune response (Ir) genes. These genes also direct the synthesis of individual markers on different B-lymphocytes and the manufacture of antibodies in immune responses. In humans Ir genes appear to occur on various chromosomes.)

Operation of the Immune System

The immune process begins with the entry of antigens to the lymphatic or cardiovascular system. The antigens are phagocytized by macrophgages, tnonocytes or polymorphonuclear cells, and the major portion of the antigenic material is digested. However, the phagocytes preserve the antigenic determinants and transport them to the immune system in the lymphoid tissue. There is evidence that unprocessed antigens (unphagocytised antigens) are poor stimulators of the immune system.

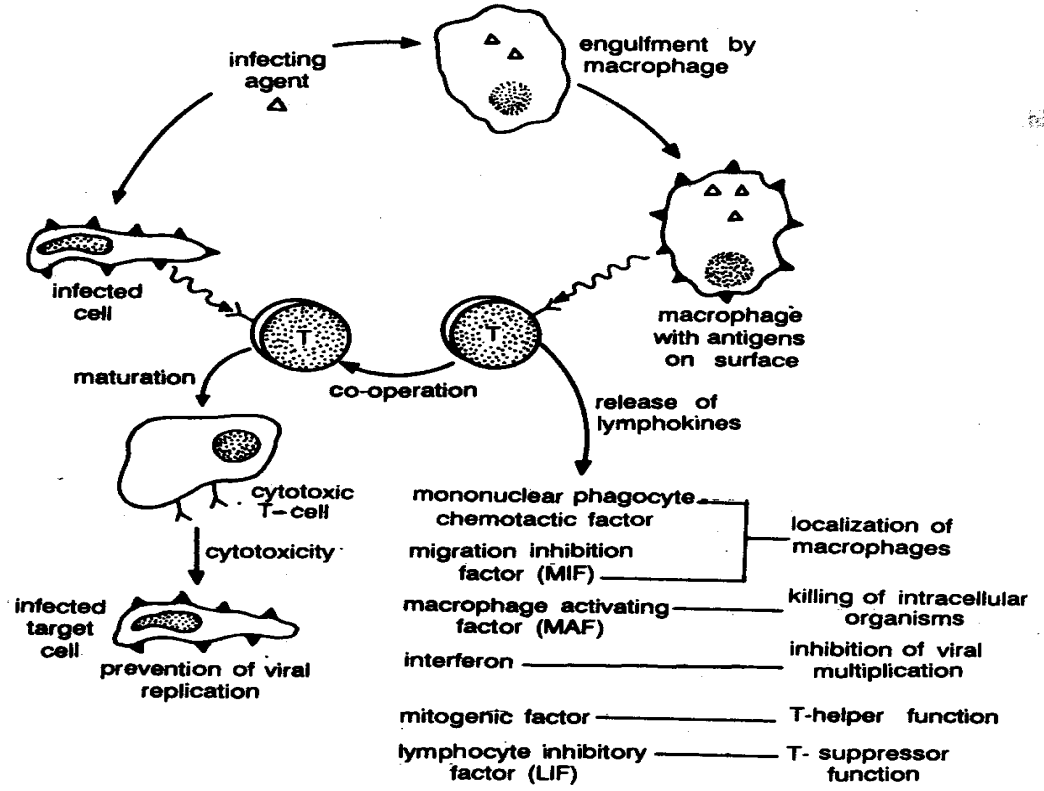


. Operation of the immune system. Parasites are engulfed by pliagocytes and the antigenic determinants are p? eserved and delivered to the lymphoid tissue. If the T-Iymphocytes are stimulated, they leave the lymphoid tissue and travel to the antigenic site in the tissue where they become lymphoblasts. The latter produce lymphokines that attract phagocytes to engulf the antigens. If the B- lymphocytes are stimulated, they remain in the lymphoid tissue and convert to plasma cells that produce antibodies. The latter enter the circulation where, by several processes, they interact with antigens and encourage phagocytosis o take place.

At the lymphoid tissue, the macrophages present the antigenic determinants to T-lymphocytes and B-lymphocytes. The lymphocytes gather about the macrophages and the cells cling to one another. An interaction then takes place between the antigenic determinants and specific receptor sites. on lymphocytes) According to some immunologists the antigenic determinants are released on disintegration of the macrophages, while others present evidence of cytoplasmic channels between the receptor cells. A third centention is that the antigen is released in microscopic vacuoles. At this point, the immunity process diverges depending upon whether T-lymphocytes or B-lymphocytes are stimulated. Two forms of immunity,. cellular immunity and humoral immunity, are possible.

CELLULAR IMMUNITY

The form of immunity arising from T-lymphocytes is termed cellular immunity because it happens on or near the body cells, and is under the direct influence of lymphocytes. It is also referred to as cell-mediated immunity (influenced by cells), or tissue immunity, because the immunity to antigens takes place within the tissues. The immunity develops as follows:



The cell-mediated immune response, the response of T cells to antigen, showing lymphokines and cytotoxic cell formation.

The antigens of many fungi and protozoa and selected viruses and bacteria stimulate the T-lymphocytes and sensitize them. Sensitized T-lymphocytes then enter the circulation and migrate to the site where the antigen was detected. The pool of lymphocytes increases as other T-lymphocytes, some from the circulation, are sensitized and drawn to the site by transfer factors from the original lymphocytes.
When stimulated by an appropriate antigen, T-lymphocytes respond by dividing and differentiating into **cytotoxic T cells** (also called Killer T cells). Various other T cells release biologically active soluble factors that mediate the response of other cells involved in the immune response. The soluble factors collectively known as **lymphokines** are effective in mediating the responses of monocytes and macrophages. Like B-lymphocytes, the T-lymphocytes have surface receptors that can react with antigen; triggering the cell-mediated response. These surface factors, however are not immunoglobulins, as they are in B-lymphocytes (humoral immunity).

Although manufactured in small concentrations, the lymphokines are extremely active. One lymphokine, called the chemotactiç factor (CF), draws phagocytes to the antigen site. Another, the migration inhibition factor (MIF) prevents macrophages from moving away. The third the macrophage aggregation factor (MAF), causes phagocytes to clump together at the site. A fourth, the macrophage activating factor (also MAF), appears to increase the mobility of phagocytes and the amount of lysosomal enzymes in each. The overall effect is to increase the efficiency of phagocytosis of antigens and bring about a specific response to disease.

Four types of lymphokines represent over 50 lymphokines that have been described so far. In 1979, the term interleukin was coined for substances produced by white blood cells (-leuko) that have effect on other white blood cells (inter.). One lymphokine, called interleukin 1, is a T-lymphocyte protein that is believed to stimulate the maturation of T-lymphocytes. Another lymphokine, interleukin 2, is also a T-lymphocyte protein, but its function is to activate T-lymphocytes to rapidly grow and divide. Interleukin 2 has found practical use in treatment of tumors_,

Lymphokines disappear rapidly once the antigen has been eliminated. However, a person will remain immune to future effects of the antigen because a colony (or clone) of identical T-lymphocytes remains in the tissues. These cells are called **memory T-lymphocytes**.

If the antigens reappear in the tissues, the memory cells will rapidly revert to lymphoblasts that secrete lymphokines to eliminate the antigens. This is one reason for long-term immunity to disease.

Cellular immunity is a chief means of resistance to bacterial diseases like leprosy and tuberculosis, fungal diseases like candidiasis and cryptococcosis, and to many pathogenic protozoa and helminthic parasites. The process is also active in many viral and rickettsial diseases because these organisms multiply within cells where antibodies are ineffective. Scientists believe that certain viruses, rickettsiae, and fungi induce antigens to form on the surface of infected cells, and that the antigens stimulate a type of T-lymphocyte called a killer T-Iymphocyte (or killer T-cell) to lyse the infected cell after contacting them. Killer T-lymphocytes also appear to be a factor in the destruction of cancer cells.

There are also two other members of the so-called T-cell family. One, **helper T-lymphocytes**, present in some immune responses, bind to antigens and assist the response by B-lymphocytes to the antigens. The collaboration is therefore essential to the immune response controlled by B-lymphocytes. Another **suppressor T-lymphocytes** apparently interfere with the function of B-lymphocytes and prevent and exaggregate immune response. They also are thought to help prevent an immunological response to oneself. In victims of AIDS, an abnormally low number of helper T-lymphocytes exists in the immune system together with an unusually high number of suppressor T-lymphocytes. These factors lead to suppression of immune system that characterises the disease. Suppressor and helper T-lymphocytes are often called regulator cells.

HUMORAL IMMUNITY

The **Humoral Immune Response** (HIR) is the aspect of immunity that is mediated by secreted antibodies, produced in the cells of the B lymphocyte lineage (B cell). Secreted antibodies bind to antigens on the surfaces of invading microbes (such as viruses or bacteria), which flags them for destruction. Humoral immunity is called as such, because it involves substances found in the humours, or body fluids.

History

The concept of humoral immunity developed based on analysis of antibacterial activity of the components of serum. Hans Buchner is credited with the development of the humoral theory. In 1890 he described alexins, or "protective substances", which exist in the serum and other bodily fluids and are capable of killing microorganisms. Alexins, later redefined "complement" by Paul Ehrlich, were shown to be the soluble components of the innate response that lead to a combination of cellular and humoral immunity, and bridged the features of innate and acquired immunity.

Following the 1888 discovery of diphtheria and tetanus, Emil von Behring and Shibasaburo Kitasato showed that disease need not be caused by microorganisms themselves. They discovered that cell-free filtrates were sufficient to cause disease. In 1890, filtrates of diphtheria (later named diphtheria toxins) were used immunize animals in an attempt to demonstrate that immunized serum contained an antitoxin that could neutralize the activity of the toxin and could transfer immunity to non immune animals. In 1897, Paul Ehrlich showed that antibodies form against the plant toxins ricin and abrin, and proposed that these antibodies are responsible for immunity. Ehrlich, with his friend Emil von Behring, went on to develop the diphtheria antitoxin, which became the first major success of modern immunotherapy. The presence and specificity of antibodies became the major tool for standardizing the state of immunity and identifying the presence of previous infections.

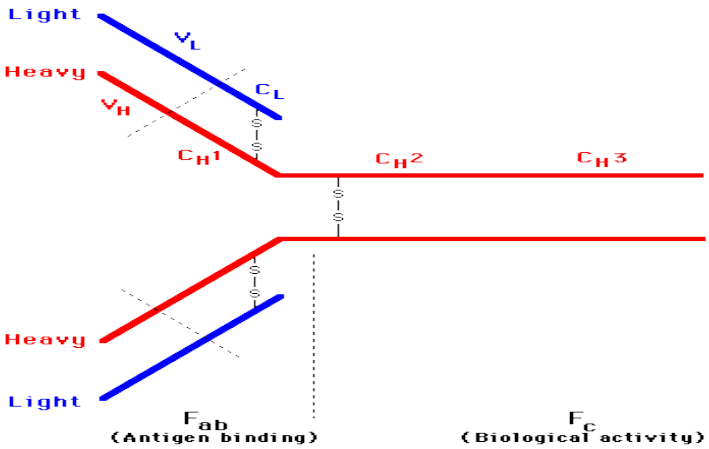
Major discoveries in the study of humoral immunity[4]

Substance	Activity	Discovery
Alexin(s) Complement	Soluble components in the serum that are capable of killing microorganisms	Buchner (1890), Ehrlich (1892)[3]
Antitoxins	Substances in the serum that can neutralize the activity of toxins, enabling passive immunization	von Bhering and Kitasato (1890)[5]
Bacteriolysins	Serum substances that work with the complement proteins to induce bacterial lysis	Richard Pfeiffer (1895)[6]
Bacterial agglutinins & precipitins	Serum substances that agglutinate bacteria and precipitate bacterial toxins	von Gruber and Durham (1896)[7], Kraus (1897)[8]
Hemolysins	Serum subtances that work with complement to lyse red blood cells	Belfanti and Carbone (1898)[9] Jules Bordet (1899)[10]
Opsonins	serum substances that coat the outer membrane of foreign substances and enhance the rate of phagocytosis by macrophages	Wright and Douglas (1903)[11]
Antibody	formation (1900), antigen-antibody binding hypothesis (1938), produced by B cells (1948), structure (1972), immunoglobulin genes (1976)	Founder: P Ehrlich[3]

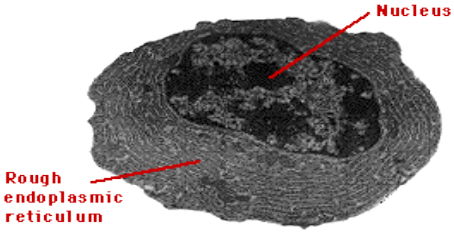
Antibodies - What Are They?

Basic Structure

Antibodies (Immunoglobulins, abbreviated Ig) are proteins of molecular weight 150,000 - 900,000 kd. They are unique molecules, derived from the 'immunoglobulin supergene'. One end of the Ig binds to antigens (the Fab portion, so called because it is the fragment of the molecule which is antigen binding), and the other end which is crystallizable, and therefore called Fc, is responsible for effector functions:



There are 5 classes ('isotypes') of Ig; IgM, IgG, IgA, IgD and IgE, plus 4 subtypes of IgG (IgG1-4), and 2 of IgA (IgA1, IgA2). Light chains exist in two classes, lambda and kappa. Each antibody molecule has either lambda or kappa light chains, not both. Igs are found in serum and in secretions from mucosal surfaces. They are produced and secreted by plasma cells which are found mainly within lymph nodes, and which do not circulate. Plasma cells are derived from B lymphocytes:



As seen in the diagram, the immunoglobulin molecule consists of two light chains, each of approximate molecular weight 25,000, and two heavy chains, each of approximate molecular weight 50,000. IgA exists in monomeric and dimeric form, IgM in pentameric form, MW 900,000 kd. The links between monomers are made by a J chain. Additionally, IgA molecules receive a secretory component from the epithelial cells into which they pass. This is used to transport them through the cell and remains attached to the IgA molecule within secretions at the mucosal surface. The heavy and light chains consist of amino acid sequences. In the regions concerned with antigen binding, these regions are extremely variable, whereas in other regions of the molecule, they are relatively constant. Thus each heavy and each light chain possesses a variable and a constant region. The isotype of an Ig is determined by the constant region.

L chains are separated from H chains by disulphide (S-S) links. Intrachain S-S links divide H and L chains into domains which are separately folded. Thus, an IgG molecule contains 3 H chain domains, written CH1, CH2 and CH3. Between CH1 and CH2, there are many cysteine and proline residues. This is known as the hinge region and confers flexibility to the Fab arms of the Ig molecule. This is used when antibody interacts with antige While antibody V_H and V_L bind antigen, antibody constant regions determine its biological functions. C_H2 domains bind complement and control the rate of Ig catabolism (breakdown). C_H2 and C_H3 domains bind phagocyte FcR (Fc Receptor) to stimulate antigen uptake. The biological functions of the C domains are independent of the antigen specificity of the molecule.

Antibody is synthesized on membrane-bound polyribosomes (**rough endoplasmic reticulum, RER**) in the cytoplasm of the B cell or plasma cell. A signal recognition protein attached to the H and L chain leader sequences sends the chains into the endoplasmic reticulum (ER). H and L chains assemble into H_2L_2 monomers with formation of the interchain disulfide bonds; carbohydrate is added to the C_H regions. The vesicle containing antibody moves via the Golgi apparatus to the plasma membrane and exocytosis releases secreted antibody from the plasma cell. Membrane-bound antibody has an additional transmembrane sequence on its carboxyl terminal C_H region which anchors the molecule to the lipid bilayer.

Types

IgM is the first antigen receptor (BCR) made during B cell development and the first antibody secreted during an immune response. Membrane IgM is a four-chain "monomer" of two μ chains and two light chains (either both κ or both λ). Serum IgM is a "pentamer" containing five four-chain monomers held together by interchain disulfide bridges in the C_H3 and C_H4 regions plus an extra polypeptide chain called J chain. Pentameric IgM is the most efficient antibody for activating complement because the five adjacent C regions easily bind two complement (C1) molecules. IgM is too large to efficiently leave the circulation, reducing its effectiveness in the tissues. Low levels of IgM are present in mucosal secretions.

IgG is the predominant serum antibody with the longest half-life. Four subisotypes of IgG in humans have somewhat varied biological functions. IgG is made later in a primary response than IgM, but it is produced more rapidly in a memory response. IgG crosses the placenta to transfer maternal immunity to the fetus and leaves the circulation to neutralize virus and toxin binding to host cells. Two molecules of IgG are required to activate complement. IgG-antigen complexes bound to FcR stimulate phagocytosis (opsonization).

IgA is present in serum and predominates in mucosal secretions: breast milk, saliva, tears, and respiratory, digestive, and genital tract mucus. Secretory IgA provides a first-line defense where pathogens enter the body. More IgA is made than any other isotype. Serum IgA is usually monomeric, although dimers, trimers and tetramers are present. Secretory IgA is dimeric or tetrameric and contains one J chain and one additional chain called secretory component (SC), which protects it from proteolytic degradation. Plasma cells make IgA and J chain and assemble and secrete polymeric IgA. IgA then travels through the circulation to the mucosal epithelial cells, which have binding molecules called poly-Ig receptor on their apical membranes. Poly-Ig receptor binds to J chain and allows IgA (and some IgM) to enter the epithelial cell, cross the cytoplasm, and exit on the luminal side with part of the poly Ig receptor still attached as secretory component.

IgE is produced in response to helminth parasites and to allergens. Epsilon chain binds very efficiently to mast cell FcεR. Antigen cross-linking of IgE on FcεR signals the mast cell to release histamine, which increases fluid entry into the tissues and mucus production. IgE also helps eosinophils destroy helminth (worm) parasites.

IgD, with IgM, is the BCR for antigen. Its presence on the B cell membrane signals that the B cell is mature and ready to leave the marrow and respond to antigen in the secondary lymphoid organs. IgD is present in serum in low amounts; no effector functions have been identified for serum IgD.

Antibodies - Where Are They Made?

Antibodies are synthesised by lymphocytes. Lymphocytes may be T (= Thymus processed), or B (= bone marrow processed). Antibodies are made by B lymphocytes and exist in 2 forms - either membrane bound or secreted. B lymphocytes use membrane bound antibody to interact with antigens. A B cell makes antibodies all of the same specificity i.e. able to react with the same antigenic determinants, and its progeny (as a consequence of mitotic division) are referred to as a clone. The clone will continue making antibody of the same specificity. Simultaneously, there will be lots of other clones of different specificity. This is known as a polyclonal response. Antigens have determinants called epitopes. Epitopes are molecular shapes recognized by antibodies, which recognise 1 epitope rather than whole antigen. Antigens may be proteins, lipids or carbohydrates, and an antigen may consist of many different epitopes, and/or may have many repeated epitopes - see figure 2.

B lymphocytes evolve into plasma cells under the influence of T cell released cytokines. Plasma cells secrete antibodies in greater amounts, but do not divide. They exist in lymphoid tissues, not blood. Other B cells circulate as memory cells.

The Life of the B cell

B lymphocytes are formed within the bone marrow and undergo their development there. They have the following functions:

- To interact with antigenic epitopes, using their immunoglobulin receptors.
- To subsequently develop into plasma cells, secreting large amounts of specific antibody, or
- To circulate as memory cells.

- To present antigenic peptides to T cells, consequent upon interiorization and processing of the original antigen. (This will be explained later in the course).

Antibodies - What Are Their Functions?

Antibodies exist free in body fluids, e.g. serum, and membrane bound to B lymphocytes. Their function when membrane bound is to capture antigen for which they have specificity, after which the B lymphocytes will take the antigen into its cytoplasm for further processing. Free antibodies have the following functions:

Agglutination of particulate matter, including bacteria and viruses. IgM is particularly suitable for this, as it is able to change its shape from a star form to a form resembling a crab.

Opsonization i.e. coating of bacteria for which the antibody's Fab region has specificity (especially IgG). This facilitates subsequent phagocytosis by cells possessing an Fc receptor, e.g. neutrophil polymorphonuclear leucocytes ("polymorphs").

Thus it can be seen that in opsonization and phagocytosis both the Fab and the Fc portions of the immunoglobulin molecule are involved.

Neutralization of toxins released by bacteria e.g. tetanus toxin is neutralized when specific IgG antibody binds, thus preventing the toxin binding to motor end plates and causing persistent stimulation, manifest as sustained muscular contraction which is the hallmark of tetanic spasms. This applies particularly to IgG. In the case of viruses, antibodies can hinder their ability to attach to receptors on host cells. Here, only Fab is involved.

Immobilization of bacteria. Antibodies against bacterial ciliae or flagellae will hinder their movement and ability to escape the attentions of phagocytic cells. Again, only Fab is involved.

Complement activation

The complement system is a biochemical cascade of the immune system that helps clear pathogens from an organism. It is derived from many small plasma proteins that work together to disrupting the target cell's plasma membrane leading to cytolysis of the cell. The complement system consists of more than 35 soluble and cell-bound proteins, 12 of which are directly involved in the complement pathways. The complement system is involved in the activities of both innate immunity and acquired immunity. Activation of this system leads to cytolysis, chemotaxis, opsonization, immune clearance, and inflammation, as well as the marking of pathogens for phagocytosis. The proteins account for 5% of the serum globulin fraction. Most of these proteins circulate as zymogens, which are inactive until proteolytic cleavage.

Three biochemical pathways activate the complement system: the classical complement pathway, the alternate complement pathway, and the mannose-binding lectin pathway. The classical complement pathway typically requires antibodies for activation and is a specific immune response, while the alternate pathway can be activated without the presence of antibodies and is considered a non-specific immune response. Antibodies, in particular the IgG1 and IgM class, can also "fix" complement.(classical pathway) especially by the Fc region of, leads eventually to death of bacteria by the terminal complement components which punch holes in the cell wall, leading to an osmotic death. Complement components also facilitate phagocytosis by cells possessing a receptor for C3b, e.g. polymorphs.

Mucosal protection. This is provided mainly by IgA, and to a lesser degree, IgG. IgA acts chiefly by inhibiting pathogens from gaining attachment to mucosal surfaces. This is a Fab function.

Expulsion as a consequence of Mast cell degranulation. As a consequence of antigen e.g. parasitic worms, binding to specific IgE attached to mast cells by their receptor for IgE Fc, there is release of mediators from the mast cell. This leads to contraction of smooth muscle, which can result in diarrhoea, and expulsion of parasites. Here we see involvement of both Fab v. Parasite antigen, and Fc anchoring the reacting participants.

Precipitation of soluble antigens by immune complex formation. These consist of antigen linked to antibody. Depending on ratio of antigen to antibody, these can be of varying size. When fixed at one site, they can be removed by phagocytic cells. They may also circulate prior to localization and removal, and can fix complement. Here Fab and Fc are involved.

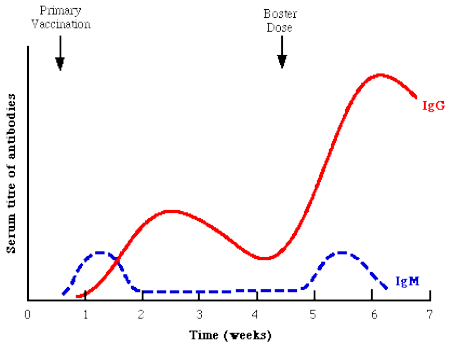
Antibody dependent cell mediated cytotoxicity (ADCC). Antibodies bind to organisms via their Fab region. Large granular lymphocytes (Natural Killer cells - abbreviated NK cells), attach via Fc

receptors, and kill these organisms not by phagocytosis but by release of toxic substances called perforins.

Conferring immunity to the foetus by the transplantal passage of IgG. IgG is the only class (isotope) of immunoglobulin that can cross the placenta and enter the foetal circulation, where it confers immune protection. This is of great importance to the foetus in the first 3 months. The precise function of IgD is not known. It may serve as a maturation marker of B lymphocytes.

Primary and Secondary Response

When we are exposed to an antigen for the first time, there is a lag of several days before specific antibody becomes detectable. This antibody is IgM. After a short time, the antibody level declines. These are the main characteristics of the primary response. If at a later date we are re-exposed to the same antigen, there is a far more rapid appearance of antibody, and in greater amount. It is of IgG class and remains detectable for months or years. These are the features of the secondary response. If at the same time that we are re-exposed to an antigen, we are exposed to a different antigen for the first time, the properties of the specific response to this antigen are those of the primary response:



Primary Response:

- Slow in Onset
- Low in Magnitude
- Short Lived
- IgM

Secondary Response:

- Rapid in Onset
- High in Magnitude
- Long Lived
- IgG (Or IgA, or IgE)

Thus we can see that the secondary response requires the phenomen known as class switching. This requires co-operation with T cells of various types, which release cocktails of substances called cytokines. These cytokines induce gene rearrangements culminating in class switching. Details of this will be given at other points in the course.

This phenomenon is possible because the immune system possesses specific memory for antigens. It occurs because during the primary response, some B lymphocytes, in addition to those differentiating into antibody secreting plasma cells, become memory cells which are long lived.