

Directions: Choose a partner and read the following explanation of immune cells and immune reactions in the blood. Practice explaining what you have read to each other. Then follow the directions in the protocol.

The Immune System of Mammals

The large size and relatively constant internal conditions of mammals make them function effectively as a source of food and protection for viruses and many types of small organisms, e.g., bacteria, protozoans, and various types of worms. There has been a continual evolutionary battle over millions of years between such invading pathogens and their hosts, with strong selection acting on the pathogens to evade each new defense mechanism the host has evolved. In many cases, the result has been a stalemate with neither side a complete victor. The battle continues today between humans and their pathogens, even as human evolution is largely cultural. Many pathogens can now tolerate the most potent of defenses our medical technology has to offer. With widespread use of antibiotics in people and livestock there has been continued selection for drug-resistant strains of bacteria. Strains susceptible to the drugs have been killed allowing resistant strains to spread more rapidly with less competition. For example, although penicillin is still widely effective against many bacterial strains, there are other strains now resistant to this antibiotic.

The skin of mammals represents a first line of defense against potential pathogens which must penetrate it in some way. Most pathogens cannot do so directly. However, no mammal can be completely separated from the external environment by a thick tough skin -- all organisms need to take in food and oxygen and release wastes. For example, the thin surface of the lungs allows oxygen to diffuse readily through the intervening cell layers to reach the blood and allows carbon dioxide to escape. Lungs, the digestive tract, the urinary tract and the reproductive tracts are all surfaces where the body interfaces with the environment to some degree. All these surfaces are vulnerable to penetration by pathogens such as viruses, bacteria and fungi. Cuts, tears, and other injuries also break down the tough skin barrier and allow entry of pathogens.

Once a pathogen enters the circulatory system it will be rapidly disseminated throughout the body. Therefore, the most likely location for a second major defense system against pathogens is the blood itself. Many of the components of blood are involved in the defense against invading pathogens and in the surveillance of cells that may have undergone mutation and become cancerous. This defense system, known as the immune system, is most complexly developed in mammals. Not all cells of the immune system are confined to the blood; some are found in the lymphatic system and other spaces among the varied cells of the body.

Immunity

The immune system can recognize and react specifically against many millions of potential invaders. There are two distinct components to the immune system. Both components involve the activities of lymphocytes, a subset of white blood cells. B-lymphocytes produce the first component, soluble proteins called antibodies. T-lymphocytes are responsible for the second component, termed cellular immunity (described later in this section). Both T-lymphocytes and B-lymphocytes are present in the blood stream. They look alike under the microscope after ordinary staining, but it is possible to identify each subtype by applying special immunological procedures.

B-lymphocytes recognize polysaccharide or protein on the surface of an invading pathogen and respond by producing antibodies. The molecules or portions of molecules on the surface of the pathogen that elicit antibody production are called antigens. The surface configuration of antigens and their specific antibodies are complementary, and antigens and their specific antibodies form tight complexes with one another. All of the cells of the body carry specific proteins and polysaccharides that are unique for each individual. These surface proteins and polysaccharides might be expected to have the potential to serve

as antigens and elicit the production of antibodies. The immune system "learns" to recognize and tolerate its own cells during early embryonic development, and does not produce antibodies against its normal cell surface constituents. Thereafter, any cell that changes its surface molecules sufficiently is susceptible to being regarded as "foreign". So-called "auto-immune diseases" are thought to be the consequence of the inappropriate production of antibodies against protein or polysaccharide constituents of one's own cells.

Cells which have become cancerous may have altered surface molecules that may be recognized as foreign, and the immune system may respond by attacking and attempting to destroy these altered cells. Thus, a system which appears to have evolved initially in order to recognize and destroy foreign pathogens may provide some defense against cancers as well.

Red blood cells carry different kinds of polysaccharides on their surfaces. These polysaccharides are not foreign to their host and therefore are not antigenic to the host. These surface molecules will, however, serve as antigens if this blood is injected into a person whose own red cells carry cell surface molecules other than these. The recipient of such foreign blood recognizes these polysaccharides as antigens and will respond by producing antibodies directed against them. Such antibodies will produce agglutination of those red cells having foreign polysaccharide antigens recognized by the antibodies. There is genetic variation in the varieties of polysaccharides on red cells which are capable of eliciting antibody production in persons who do not have these same antigens on their red cell surfaces. Therefore blood from one person may not be compatible with that of another. Transfusion of incompatible blood can lead to massive agglutination of cells, blockage of vessels, and death.

The other type of lymphocyte, the T-cell, participates mainly by cell-to-cell interaction. T-lymphocytes are involved in rejection of grafted foreign tissue, and in attacking cancer cells. T-cells also release substances which affect the activities of other cells in a variety of non-specific ways. Phagocytes, cells that migrate throughout the body and engulf pathogens and dead or worn cells, are one type of cell influenced by T-cells. T-cell substances may inhibit the migration of phagocytes away from the area of foreign cell invasion. Factors produced by other T-cells may stimulate phagocytic cells to move into the area of invasion and ingest and kill foreign cells. Some varieties of T-cells produce interferon, an antiviral agent whose mechanism of action is currently under intensive investigation. Still other T-cells produce cytotoxic factors which are able to destroy foreign cells (or cells of the individual's body that carry abnormal proteins on their surface). Finally, a subset of T-cells, known as helper cells, assists other T cells and modulates the effectiveness with which B-cells are able to produce antibodies.

White Blood Cells

In addition to lymphocytes, there are several other types of white blood cells that participate in the defense of the body against foreign substances.

Neutrophils are the most numerous of the white blood cells and act first against invading pathogens, particularly bacteria. When alerted to the presence of foreign particles or cells by antibodies, the neutrophils leave the blood to enter the infected tissue. Here they engulf and destroy the pathogens; neutrophils frequently die while acting in defense of the body. The accumulation of dead neutrophils produce a substance known as pus. Ordinarily, a neutrophil does not exist in circulation more than twelve hours and cannot reenter the blood stream once it has left the blood. Large numbers of neutrophils are stored in the bone marrow, and these can be called upon if needed.

Eosinophils defend the body against multi-cellular invertebrate parasites such as liver flukes and are important in the development of the inflammation of airways that is characteristic of asthma. Both functions involve degranulation of the large, eosinophilic granules in the cytoplasm of the cells in response to chemical signals received by the surface of the cells. The role of eosinophils in asthma is the subject of intensive basic research at the University of Chicago Medical Center.

Basophils are normally the rarest of white blood cells. Because less than 1% of white blood cells are basophils, you may not find one in your own smear. If you do, you will recognize it by its large, very basic granules. Basophils are indistinguishable in form from mast cells in the tissues. Mast cells have attached antibodies which cause the mast cells to release histamines and other substances when these antibodies recognize a foreign substance (allergen). The release of mast cell granules results in sneezing, tearing, bronchial asthma, and other allergic reactions. Basophils are thought to be involved in the same sorts of allergic mechanisms in the circulating blood. Basophils also contain histamine and "degranulate" when they encounter an allergen.

Monocytes are the precursors of tissue macrophages. Like macrophages, monocytes are phagocytic and can engulf foreign particles. Unlike neutrophils, they do so in conjunction with certain types of lymphocytes as discussed below. Monocytes will also leave the blood to enter tissues and engulf and destroy pathogens. They differ in distribution from neutrophils and in killing mechanism as well.

Blood Typing and the ABO and Rh Blood Group Systems

Oxygen is transported by a special molecule, hemoglobin, which in mammals and other vertebrates is contained within a special type of cell, the red blood cell (RBC) or erythrocyte. Red blood cells are by far the most numerous cells in the blood and give it its red color.

Red blood cells have numerous types of molecules on their surfaces, some of which are recognized as antigens by certain antibodies produced by lymphocytes. Approximately 300 different antigen systems have been described but the best known and most important are the ABO and Rh blood group systems. You will be typing your own blood to determine your blood type with respect to A antigens, B antigens, and the Rh factor.

ABO Blood Group

The ABO blood group system is due to a set (one locus) of three basic types of alleles (I^A , I^B , I^O) that determine the type of antigens (A or B) or lack of antigen (O) found on the red blood cells. The I gene is a typical Mendelian gene. Since individuals are diploid and carry two alleles for each gene, the possible genotypes are $I^A I^A$, $I^A I^B$, $I^A I^O$, $I^B I^B$, $I^B I^O$, and $I^O I^O$. Given that I^O is recessive, what are the phenotypes? Four blood types are possible: A (one or both alleles for A, none for B); B (one or both alleles for B, none for A); AB (one allele for A, one for B); O (alleles for neither A nor B). Each parent will contribute one of his/her alleles to each offspring. Blood typing using only the ABO system can be used to show that people with particular phenotypes could not be the biological parents of a child, but ABO blood typing alone certainly cannot prove that an individual is the parent. (DNA methods can give such evidence with very high probability.)

The biochemistry of this set of alleles is well known. The A and B alleles code for the production of slightly different enzymes involved in attaching the last sugar to a glycolipid on the surface of the red blood cell. The enzyme produced by allele A attaches a different sugar than does that produced by allele B. The allele O is associated with a failure to produce either enzyme, so the last sugar is not attached to the glycolipid. The antibodies, anti-A and anti-B, specifically recognize the last sugar molecule of this glycolipid. They will not be produced by individuals whose red cells bear the antigens. During development, the production of antibody specific for the antigen present on the red blood cells is suppressed. Thus, individuals with Type A blood lack the antibody, anti-A, whereas those with type O blood usually possess both anti-A and anti-B antibodies, as neither was suppressed during development. Note an unusual feature of the anti-A and anti-B antibodies is that they are usually produced even by individuals whose immune systems have never been exposed to these antigens on the surface of red

blood cells. When bloods of different types are mixed, they may prove to be incompatible because of the mixing of antigen with its associated antibody.

The following types of blood are compatible:

A type AB recipient can receive any blood because the AB recipient lacks both antibodies, whereas a type O recipient can receive only O because the type O recipient has both antibodies. Incompatibility between blood types occurs when a foreign antigen is introduced and elicits the production of antibodies. This is why type O blood can be transfused to a type AB donor despite the presence of both anti-A and anti-B antibodies in the donor's blood. In actual practice, every reasonable effort is made to transfuse only red cells of identical type.

For any genetic system within a species, there is usually some geographic variation in the frequency of the different alleles found within a population. Reproductive barriers between populations maintain these differences that arise because of chance or because of natural selection favoring phenotypes with different alleles in different environments. The ABO blood groups in humans show significant geographic variation, part of which is related to different frequencies in the different races.

Rh Antigen

The Rh antigen (so named because it was first studied in rhesus monkeys) is one of several other red blood cell surface antigens. It is present on RBCs of the majority of humans, in 93% of black individuals and 85% of whites. These people are called Rh⁺. Individuals who lack the antigen on their cells (Rh⁻) produce antibodies to this antigen only when they are exposed to it, thus making this system quite different from the ABO system. This situation may lead to complications for pregnancies in which the mother is Rh⁻ and the fetus is Rh⁺. Primarily at the time of delivery, fetal blood will cross the placenta and enter the mother's circulation. Exposure to the Rh antigens (or D antigens as they are called in humans) on the red blood cells of the Rh⁺ fetus will sensitize the Rh⁻ mother and cause her to produce anti-D antibodies. Subsequent pregnancies with Rh⁺ fetuses are potentially fatal to the fetus, because the anti-D antibodies now present in the mother's blood may cross the placenta and attack the D antigen-bearing red blood cells of the fetus. This condition is known as erythroblastosis fetalis, and in the past accounted for a significant number of fetal deaths.

Today, however, this very serious problem of incompatibility can generally be eliminated by treating the Rh⁻ mother with anti-D antibodies beginning at the sixth month of any pregnancy involving an Rh⁺ fetus. When the mother is treated with anti-D antibodies, the antibodies will coat any fetal Rh⁺ erythrocytes that are in her bloodstream and lead to their destruction before they can sensitize (immunize) the mother. Providing that this treatment is repeated with each pregnancy in which the fetus is Rh⁺, subsequent pregnancies should be at no greater risk than the first.

Super
copy

Today's lab will include 2 basic immunology-related lab procedures:

1. Typing your red blood cells---a quick visual method of detecting the presence of specific *antigens* on rbc's. Our first exposure to an antibody -antigen interaction. Good review of concepts of Mendelian genetics. And a chance to understand the basis of blood donation.
2. Examination of your peripheral blood---blood smears-- Practice the procedures as well as become familiar with the relative sizes, shapes, frequency and staining patterns of WBCs. Observe blood smears from individuals with leukemia.

Notes on Blood Smears

Most of the cells you see are red blood cells which are stained pink. The white blood cells are somewhat larger and their nuclei stain a conspicuous blue color. Use the descriptions of the different types of blood cells below to identify each type you encounter based on size, shape of the nucleus, and color. You may or may not see all the different types of WBCs - some are quite rare. Consult figure 1.6 in your text for help in distinguishing cell types.

Red blood cells are the most numerous cell type in your smear. They stain pink, lack a nucleus, and may have a donut-like appearance.

Platelets are very small cell fragments which are involved in the clotting reaction. They also lack a nucleus and are much smaller than any whole cell.

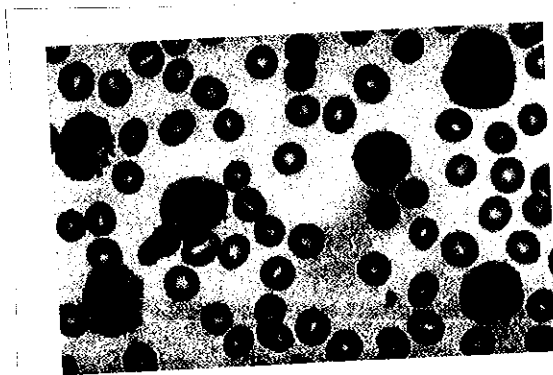
Neutrophils (frequency = 50-70% of WBCs): These are cells with dark, irregularly shaped nuclei with 2 to 5 lobes and pale cytoplasm.

Eosinophils (frequency = 1-4% of WBCs): These cells also have a dark lobed nucleus (2-3 lobes) but have large red-orange granules in their cytoplasm.

Basophils (frequency = 0.1-0.8% of WBCs): These quite rare cells are similar in appearance to eosinophils except that the granules in the cytoplasm are a dark blue color.

Monocytes (frequency = 2-8% of WBCs): These cells are distinguished by their large size, up to twice as large as other WBCs. They have few or no granules in their cytoplasm; their nucleus is dark and round, bean-shaped or lobulated.

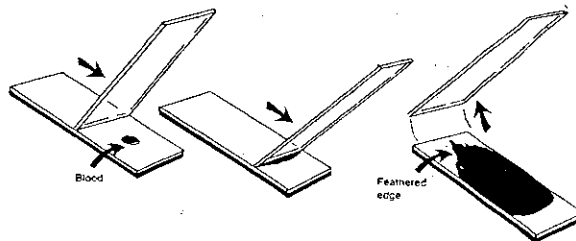
Lymphocytes (frequency = 20-40% of WBCs): These are similar in appearance to monocytes but tend to be smaller; many are about the size of red blood cells. The dark nucleus of a lymphocyte takes up nearly the whole cell in small lymphocytes and is generally round or oval.



I. Protocol---Blood Smear Preparation/Staining

Thin blood smear

1. Obtain 2 clean microscope slides, alcohol wipes, and lancet
2. Clean a finger with an alcohol and puncture with lancet
3. Place a small drop of blood at the end of one slide.
4. Use the second slide to make a thin blood film as directed below:
 - a. Place the second slide at a 30 degree angle and touch the slide with the blood drop
 - b. Move the spreader slide to touch the blood drop allowing the drop to spread by capillary action along the edge of the slide
 - c. Immediately pull/push the slide away from the blood drop, making a thin smear that should dry quickly as you move away from the drop.
 - d. A perfect smear will have a "feathered" edge and separated RBCs when you view it with the microscope. (see below).



Staining the blood smear(Horizontal staining procedure)

1. Place thoroughly dried smear on horizontal staining rack
2. Flood smear with Fixative (light blue) for 10 seconds, (fixes cells to slide/prepares cells for dyes) drain
3. Flood smear with Dye 1 (red) for 10 seconds, drain
4. Flood smear with Dye 2 (dark blue) for 10 seconds, drain
5. Rinse the smear with distilled water for 1 minute
6. Air dry and examine under the microscope, using low power first, then high power.
7. Observe as many different types of blood cells as possible. Pay close attention to size, frequency, and nuclear features.
8. Compare your slide with some of the prepared slides, including some of the hematologic abnormalities, such as leukemias.

*****Make observations that allow you to do the following:**

1. Give a written description of the WBCs you observed, including approximate frequencies of each (was one more common?; did you rarely see one type?). Comment on any difficulties you have in discerning different WBCs. Propose a method for clearing up these difficulties.
2. Compare your blood smear with one of the slides from leukemia patients. How are these slides different from your slide? How are they beneficial to a student? How would you suppose blood smears are used in leukemia diagnosis and treatment?

II. Protocol---Blood Typing

1. Obtain a blood typing card and orient yourself to its use.
2. Obtain a set of antisera from the ice bucket.
3. Place a drop of each type of antiserum in the appropriate spots.
4. Clean a finger with an alcohol and puncture with lancet. (or Dr. Super will puncture---do not allow other students to do this.)
5. Once a significant drop has formed on your finger, lightly touch the card in the appropriate sections.
6. Mix the blood and each antiserum drop separately with a fresh toothpick for each.
7. Rock the card for at least 60 seconds to thoroughly mix the blood and antisera drops (do not let them cross contaminate)
8. Evaluate the changes in the blood.
 - a. Positive reactions show subtle or drastic changes in the texture of the blood drop (grainy to clotted)
 - b. Negative reaction---no change in appearance of the blood/serum mixture.
 - c.

Use the results to do the following with your partner or group:

1. Indicate your blood types and summarize the evidence for the results for each person in your group.
2. Explain whether you and your partner could donate blood to each other in the event of an emergency.
3. Predict the possible genotypes of your children if you and your partner were somehow capable of having children. Explain whether you would need to worry about complications of a pregnancy due to Rh type.

Write-up

Each student should write a 2-3 page report. Include the basic purpose of today's lab as well as the procedures you used (you may cut and past/copy the procedure from this handout). Include sketches with labels of all the WBCs you could identify, showing their size and appearance relative to the RBCs (show a representative field). All writing should be in complete sentences. Use the suggested questions/activities to formulate some conclusions from your lab. Be sure to always support general statements and conclusions with evidence or details.