Lab Exercise#6 - INSTRUCTIONS
Immune Response: Leukocytes, Antibodies & Blood Typing

I. OBJECTIVES:

- ✓ Observe the results of antigen -antibody interactions.
- ✓ Observe and identify and cells of the peripheral circulation (especially those involved in the immune system).
- ✓ Describe the conditions under which antibodies are formed
- ✓ Distinguish the structure and function of the 5 classes of immunoglobulins.
- ✓ Predict ABO and Rh blood type on the basis of agglutination of antisera.
- ✓ Predict the ABO and/or Rh incompatibility and the consequences of the incompatibility.

II. TERMINOLOGY

Students should define and use the following terms:

agglutination	eosinophil	red blood cell
agranulocyte	erythroblastosis fetalis	Rh negative
antibody	humoral immune system	Rh positive
antigen	lymphocyte	Type A
Antigenic determinant	monocyte	Type AB
antisera	neutrophil	Type B
basophil	opsonin	Type O
blood typing	Peripheral circulation	white blood cell
cell mediated immune system	plasma	Wright's stain

III: INTRODUCTION

Immunology: Antibody – Antigen Interaction

Antibodies are produced by a special type of white blood cell known as a B lymphocyte or B cell that has come in contact with the foreign invader (aka antigen). The B lymphocyte along with its morphologically indistinguishable cousin, the T lymphocyte (T cell), is found in peripheral circulation. The antigen processing macrophage displays the antigenic determinant of the foreign invader on its membrane. The Helper T cell recognizes the antigenic determinant by means of its T cell receptors and sends a message to the B cell with the same receptor. The B cells are stimulated to differentiate into a plasma cell. The plasma cell makes and secretes the antibody molecules. Each antibody molecule has at least two and as many as 20 receptor sites (dependent upon the class of antibody molecule) each specific for the antigenic determinant of the foreign invader.

When the antibody molecule comes in contact with the antigenic determinant aspect of the antigen for which it was designed, the lock and key fit of the antibody and antigen molecules allows for antigen removal from the host system. Antigen-Antibody interaction results in neutralization, opsonization (promotion of phagocytosis) and agglutination. The agglutinated antigen is easily removed by the lymphoid tissue. In this simulated lab exercise of blood typing, agglutination of the antigen by the antibody is observable.

IV. EXERCISES

1. Blood Typing

The ABO and Rh blood group systems that designate 'blood type' are based on a set of inheritable proteins embedded in red blood cell (RBC) membranes are often used to refer to 'blood type'. Some of these proteins are designated type 'A' proteins. If the 'A' proteins are

Phenotypes	Genotypes	Incidence in US
A	I^AI^A or I^AI^O	39%
B	I^BI^B or I^BI^O	2%
AB	I^AI^B	4%
O	I^OI^O	42%

present the blood is identified as Type A blood. Likewise, if a protein known as type 'B' is present the blood is known as Type B. When both A and B type proteins are embedded in the RBC membrane the blood type is known as Type AB. When RBC membrane lacks both the 'A' and 'B' type proteins the blood type is identified as Type O. This is known as the ABO blood group system. This blood typing system is one of many different blood group systems. Human blood types (A, B, AB and O) are inherited by multiple alleles (3 or more genes that occupy a single locus on a chromosome). Gene I^A codes for the synthesis of antigen A (or protein A), Gene 1^B codes for the production of antigen B and Gene i (i^O) does not produce any antigens/proteins. There are two chromosomes each with alleles for a given trait (one came from mom and the other from dad). Summarized above are the possible alleles on your chromosomes, your genetic make up or genotype, and how these genes would be expressed as your phenotype which in this case is your blood type.

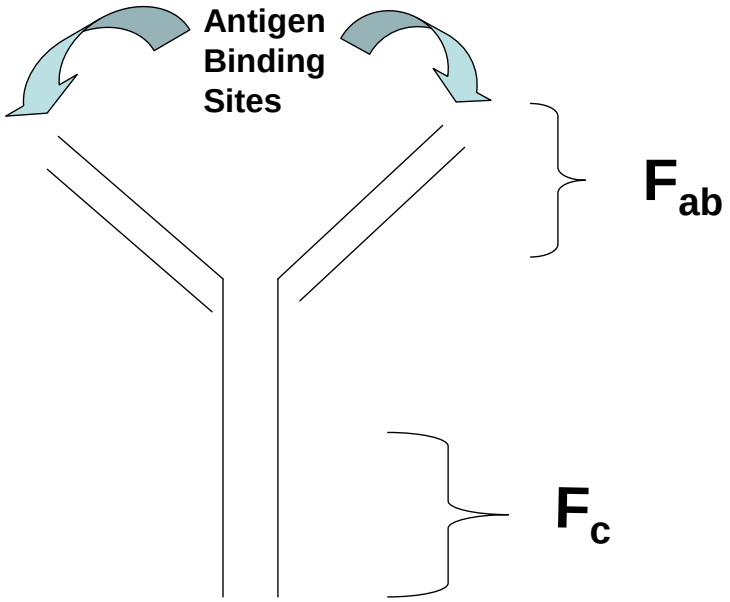
There are many other antigens on RBC's. In 1940, it was reported that rabbit sera containing antibodies for the RBC's of the Rhesus monkey would agglutinate the RBC's of 5% humans. These antigens, six in all, were designated as the Rh (Rhesus) factor; they were given the letters C,c,D,d,E and e. Of the six antigens, the D factor is found in 85% of Caucasians, 94% of African Americans, and 99% of Asians. Rh positive blood contains RBC proteins designated type 'D'. Rh negative individuals lack this membrane protein.

Blood typing is the procedure used to determine the type of proteins on the red blood cell surface. The blood typing procedure employs the use of antisera, a solution containing antibodies. There are two types of antisera: Antisera A is a solution of antibodies with receptor sites for A proteins and antisera B is a solution of antibodies with receptor sites for B proteins. The antibodies in the antisera are known as Immunoglobulin class M (IgM) antibodies. A antibodies contain 20 receptor sites specific for the A antigen on each antibody. B antibodies contain 20 receptor sites specific for the A antigen on each antibody. When the antibody receptor site contacts the membrane protein for which it is specific, the antibody binds this protein antigen. As 20 or so red blood cells attach to the same antibody a clumping or agglutination occurs. Agglutination only occurs when the antisera contacts the antigen, which in this case is the A or B protein in the red blood cell membrane. Thus, if antisera A agglutinates red blood cells the A protein is present on the red cell membrane; B antisera agglutinates only cells with B proteins.

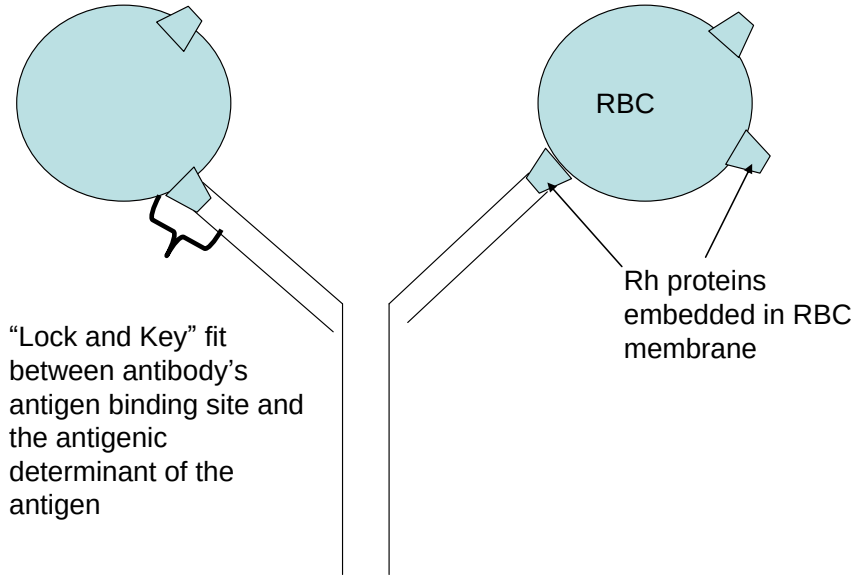
The Rh blood type is determined by mixing red blood cells with anti Rh (anti D) antisera. The anti-D antisera contains antibodies of the Immunoglobulin class G type (IgG). This is a much

smaller antibody than the IgM class. It contains only two binding sites per molecule. Anti-D antisera contacts Rh positive blood and the red blood cells agglutinate. Blood that lacks the Rh protein will not agglutinate in the presence of the anti-D antisera. The anti-Rh antibodies of the system are not normally present in the plasma, but anti-Rh antibodies can be produced upon exposure and sensitization to Rh antigens. There are several ways sensitization can occur. Two of the most common means of sensitization are an Rh positive blood transfusion into an Rh negative recipient, and the presence of an Rh positive fetus in an Rh negative mother. The latter scenario may lead to the subsequent spontaneous abortion of the fetus as it is attacked by anti-Rh antibodies manufactured by material plasma cells. This condition is known as erythroblastosis fetalis.

There are 5 classes of antibody molecules as previously discussed. The antigen binding occurs at a similar site in the various classes of antibodies. The IgG antibody is depicted as such:

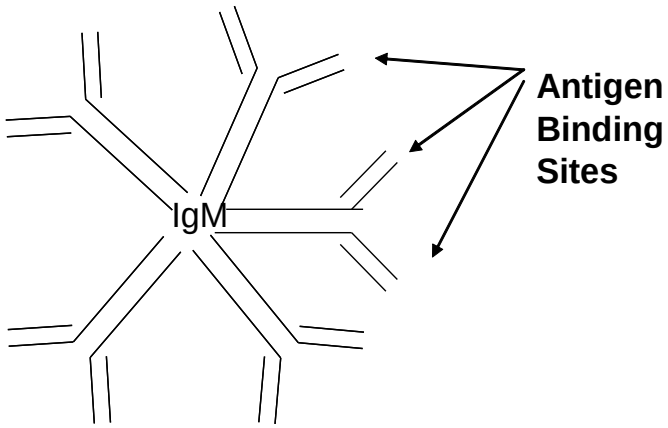


Variation in the sequence of amino acids in the antigen binding site in the Fab areas accounts for the 'lock and key' fit between antigen and antibody. The antibody molecule's antigen binding sites on a particular antibody are identical and specific. For instance, if the antigen binding site on the antibody above were specific for the Rh antigen only the Rh protein would bind to this site. These proteins are embedded in RBC membranes.



Imagine the agglutination you will see as two RBC containing the antigenic determinant of the Rh antigen attach to the antigen binding site of the antibody for each antibody molecule in the antisera.

The IgM molecule is much larger than the IgG molecule. There are 5 sets of antigen binding sites or a total of 10 sites each specific for the same antigen for which the antibody was directed.



The agglutination appears much larger with the IgM antibody as the ten RBC containing the antigenic determinant of the A or B antigen/protein of the ABO blood group system attach to the antigen binding sites of the antibody for each antibody molecule in the antisera.

The blue antisera contains millions of IgM antibodies specific for the A antigen. Likewise the yellow antisera contains IgM antibodies specific for the B antigen.

Blood Typing Procedure

A. Access the website:
http://nobelprize.org/educationalgames/medicine/landsteiner/index.html

B. Play the blood typing game after you are certain you understand the principles behind the test.

1. View the see how to play directions for more information before you play the game

2. You will need to use the cursor to move patients into the emergency room. Use the cursor again to access the needle to draw blood from the patient's arm.
3. Move the cursor to the side of the field where the tubes of antisera are found and dispense blood into each of the antisera tubes.
4. Read the results of the blood typing and record those results. Give the patient blood as directed.

C. Record the results of blood typing in the following chart:

	Patient #1	Patient #2	Patient #3
Blood type			
First unit:			
Second unit			
Third unit			
Fourth unit			
Record any errors			

D. Record the number of drops of blood you receive at the party _____ and the hiring recommendation that is made:_____.

2. White Blood Cells

Leukocytes, white blood cells, play an integral role in the immune response. They are found in peripheral circulation (i.e. in your blood vessels) along with red blood cells and platelets. There are five types of white blood cells divided into two categories: granulocytes and agranulocytes. We will observe the blood cells after they have been prepared on a microscope slide and stained with the differential Wright's stain.

Neutrophils, eosinophils and basophils are granulocytes, named because of the presence of distinctive granules in the cytoplasm. Neutrophils, the most numerous granulocyte, normally makes up 50-70% of white blood cells. It is a phagocytic cell and functions in the second line of defense. Neutrophils have large purple nuclei that take on many different shapes. For this reason they are often referred to as polymorphs (short for polymorphonuclear cell) or segs (short for segmented nucleus). They have pink cytoplasm with granules. Only 1-3% of white blood cells are eosinophils. These cells have large red/pink granules that at times obscure the nucleus. Basophils make up 0-1% of white blood cells and in contrast to eosinophils have large blue granules in Wright's stain.

The agranulocytes are monocytes and lymphocytes. The monocytes are larger with a less densely packed nucleus and more blue staining cytoplasm compared to the lymphocyte. Monocytes are phagocytic cells that can migrate from peripheral circulation to tissues where they enlarge and become known as tissue macrophages. These cells play a pivotal role in the second and third lines of defense. Approximately 2-8% of leukocytes are monocytes. The lymphocyte, with its densely packed nucleus and rim of blue cytoplasm make up 20-40% of white blood cells. They function in the cell mediated and humoral immune response.

White Blood Cells Procedure

1. Obtain a Wrights stain of blood cells. Observe the cells under oil immersion and draw red blood cells, neutrophils, lymphocytes and platelets.

2. Use the digital camera to take pictures of a neutrophil and lymphocyte