ANTIGEN-ANTIBODY REACTIONS and their IMPORTANCE in BLOOD BANKING
The separation of antigen-antibody reactions from bloodbanking is literally impossible. Virtually every reaction in Blood Bank relies on antigen-antibody interactions, and the resultant agglutination or hemolysis. A general overview of the mechanisms of an antigen-antibody reaction will first be explained, followed by a brief discussion of the importance of those reactions in the major areas of the Blood Bank. These will include blood group typing, crossmatching, transfusion reactions and hemolytic diseases of the newborn, along with short discussions of some associated problems.

The basis of bloodbanking work relies on the antigens and antibodies of red cells. An antigen is any substance capable of causing the generation of an antibody. An antibody is a substance generated in response to a foreign substance; that is, an antigen which does not occur naturally in the host tissue. Antibodies are a very necessary part of the body's immune system, especially in the overcoming of viral, bacterial, and fungal infections.

In order for an antigen-antibody reaction to take place, the antigen and antibody must have very complementary structures, that is, they must fit to each other much like a lock and key. When the antigen and antibody combine, the antigen is rendered inactive.

The two main reactions that may occur when red cell antigens and antibodies are allowed to interact are agglutination and/or hemolysis. When red cells with one antigen are placed with the corresponding antibody, cellular agglutination may occur. The antibody will
bind to one of the receptor sites on several of the antigenic red cells, forming a kind of lattice work between the red cells, allowing the cells to stick together, or agglutinate. Antibodies vary in size, and in order for the smaller antibodies to bridge the distance between red cells, it is sometimes necessary to add a substance to bring the red cells closer together, thereby facilitating agglutination. The second type of reaction is very important to bloodbanking. This is the hemolytic reaction, involving the lysis of red cells, and is accomplished by the activation of the complement system.

Antibodies play a very important role in this hemolytic reaction. An antibody is made up of a Y-shaped structure, which contains two Fab portions and one Fc part. Some larger antibodies are made up of as many as five Y-shaped pieces, thus having ten Fab pieces and five Fc portions. In order for the complement system to begin to be activated, there must be two Fc portions within close distance of each other, but still attached to the red cell membrane. These two Fc portions combine with a gamma globulin Clq, and in the presence of calcium ions this Fc-Clq complex combines withClr andCls, both of which are proenzymes in their native state. Upon binding to the Fc-Clq complex,Clr develops protease activity andCls develops esterase activity yielding an activated Fc-CIqrs complex. This complex then comes in contact with the next component, C4, and splits C4 into two particles, C4a andC4b, the latter of which binds to the red cell membrane. The Clqrs complex also acts on the next component, C2, yielding both active C2a and nonessential C2b fragments. The C2a must complex with the C4b molecules that have attached to
the red blood cell membrane surface. Many C2a and C4b molecules never become effective activators on the red cell surface. The next step in the sequence of activation involves the C4b-C2a complex, for convenience shortened to C42. This substance is also called C3 convertase and is dependent on the availability of magnesium ions. This C3 convertase converts C3 to C3a and C3b; C3b being a complex of C3c and C3d. Both C3c and C3d are capable of binding to the red cell membrane. The binding of either of these to the red cell membrane initiates the binding of C5b7, which in turn activates C59 to result in the lysis of the red cell. The lesions of the red cell membrane formed by this complement system allow the passage of ions into and out of the red cell, thereby causing a loss of the regulatory abilities of the cell, with respect to its internal environment, and rapid lysis of the cell.²

In the typing of blood cells, the cellular agglutination reaction is of the most importance. In the testing of red cells, there are four antigenic types. These are designated as types A, B, AB, and 0. Each individual has one of these blood types and naturally occurring antibodies against the other antigenic types. For example, a person with A antigens on his red cells will have a natural antibody against B antigens; a person with 0 antigen, which is really thought to be a lack of both A and B antigens, will have antibodies to both A and B antigens; while a person with both A and B antigens will have no antibodies to either A or B.

There can be several parts to typing red cells. The main section involves the use of typing sera that contains the antibody substance
specific for either A or B antigens. When red cells with the proper antigenic sites are added to the antisera, agglutination is the result.

A second part of the typing procedure is testing for the patient's Rh-D factor. This is done using a D antibody. If the patient has D antigen sites on his red cells, agglutination will result. There is a variant of D known as Dv, which takes special techniques to detect. Due to a missing part of the D molecule, no agglutination will occur, and the test result will be negative when in reality the cells are D positive. In order to determine whether a D-negative is really negative or a Dv, all D negative reactions should be additionally tested. This usually involves lengthened incubation time at 37 Centigrade, and the addition of a substance to bring the red cells closer together, thereby facilitating agglutination.3

The third part of cell typing, the back typing, is helpful in that it may detect an antibody not expected by the results of the front type. Reagent red cells, commercially prepared, with only specific antigen sites are used. For example, A1 cells have only A antigen sites; B cells have only B antigen sites. A1 cells are used because A1 antigens are stronger than other A antigens and will be more likely to pick up weak antibodies. A1 cells will also detect anti-A1 in the serum of a person belonging to a weak subgroup of A. The test uses the patient's serum and tests for antibodies in that serum. There should be no agglutination in the B cells if the sample has front typed as a B, because a person will not normally produce antibodies to his own antigens. While the back type is only a check of the front type, it can be helpful in certain subgroups of antigens.
The most common cause of back and front typing discrepancies involves weak subgroups of the A antigen, designated as $A_2$, $A_3$, and so on. For instance, a person with $A_2$ antigen sites on his cells will not agglutinate A antisera, however, on the back type he would be expected to then agglutinate the $A_1$ cells, and he will not. Thus the person may front type as an O person, but he will still back type correctly as an A. In cases such as this, there are special antisera available to test for weak subgroup types.

Another problem that arises mainly in patients with leukemia and with elderly patients involves weakened or missing antigens. The antigenic strength of blood group antigens decreases with increasing age and in certain disease states. The antigens may even disappear altogether. In these cases, the back type may show up the discrepancy, or knowledge of the patient's former blood type can help clarify the situation. However, sometimes the back type may not show up any discrepancies, and unless there is a record of the patient's proper blood type, there is no way of knowing whether your typing results are valid. Fortunately, this type of problem does not arise often.

Occasionally, a problem may arise with an acquired "B like" antigen. This usually is in conjunction with cancer of the colon or rectum, peritonitis, or appendicitis. It is thought that the acquisition of this B-like substance involves a bacterial enzyme modification of the red cell membrane. The patient's cells will react with group B antiserum however the condition is temporary and reversible.

Very rarely, bacterial contamination of the testing sera and cells will result in agglutination of both the patient's cells
and serum. Retyping the patient with fresh typing sera and cells will remedy the situation.

It is very important that the typing of a patient be correct, especially in instances where a patient needs a transfusion immediately and the doctor does not want to wait for the completion of a crossmatch. Giving the wrong group of blood, due to an error in the typing of the patient, may result in very serious complications for an already debilitated patient.

Another major area of antigen-antibody importance in Blood Bank is in the crossmatching of units of blood for transfusion purposes. This procedure consists of a major and a minor side. The major side involves the mixing of the patient's serum with the donor unit red cells, and will show up antibodies the patient may have that would react with the donor cells. The minor side involves adding the patient's cells to the donor serum, and is a check for antibodies that may be present in the donor unit. These mixtures are then checked for agglutination or hemolysis. This is followed by a half hour incubation time, followed by the addition of a substance to decrease the space between red cells and facilitate the agglutination of certain smaller antibodies.

In crossmatches, any agglutination or hemolysis indicates that the donor unit and patient are not compatible. When agglutination or hemolysis does occur, the patient's serum and cells should be examined for antibodies or substances attached to the red blood cells.

If the patient's red cells are found to be coated with a substance that is causing the cells to agglutinate upon the addition
of the Coombs sera (a substance used to bring red cells closer together to facilitate the agglutination of small antibodies), they are said to be Direct Coombs positive. The interfering substance is a specific Coombs reactive antibody that has been adsorbed onto the patient's red cells. An elution is necessary to try to remove the interfering antibody from the red cells, and then the crossmatch is repeated. In a case where the elution does not show an identifiable antibody, the most compatible unit must be chosen for the transfusion.

Agglutination or hemolysis in the major side of the crossmatch is probably due to an antibody or antibodies in the patient's serum. These antibodies must be identified and blood must be selected that lacks the antigen(s) for use in the transfusion. Agglutination in the minor side of the crossmatch usually indicates an antibody in the donor's serum. Occasionally, the donor may have a positive direct coombs, which will cause the crossmatch to agglutinate only after the addition of the Coombs serum. This unit of blood can never be successfully crossmatched and should not be used for any further crossmatch procedures.

When a unit of blood is incompatible with the patient, but is given, the antigen-antibody complexes that form will result in mild to severe transfusion reactions. The most important of the transfusion reactions is the intravascular hemolytic type. This usually occurs as a result of A, B, or O group incompatibilities and is a most severe problem. The patient will destroy all incompatible red cells via the complement system, releasing large amounts of free hemoglobin and cellular elements into the blood stream. This causes severe shock,
lung congestion, kidney failure, coma and many times death or permanent kidney damage. Even if the foreign red cells are not able to activate the complement system sufficiently to cause their immediate lysis, they may still have some portion of the complement system bound to their membranes, or may be coated with the appropriate antibody from the patient. In this case the cells will be removed extravascularly by the reticuloendothelial system, mostly by tissue bound macrophages. This type of reaction involves mostly the Rh, Kidd, Kell, and Duffy systems of antigens. This is still classified as a hemolytic reaction because of the destruction of red cells, but is not usually as severe a reaction as the intravascular hemolysis type.

Occasionally, a delayed hemolytic reaction will occur several days after a unit of apparently compatible blood is given. An antibody screen done at the time the unit was crossmatched will have shown no antibodies present and the unit will have appeared to be compatible. However, a new antibody screen and identification done after the adverse reaction will show up an antibody. This antibody may stay at significant levels for several weeks to several months, but will then disappear until another incompatible unit is given to the patient, at which time it will reappear for a short time.

One of the more common reactions during the administration of a unit of blood is a febrile reaction. The patient usually exhibits chills and fever, but rarely any more serious effects. This reaction may be due to waste products from blood glycolysis in the unit or anticoagulants used in the unit, but the most common cause is white cell antibodies in the serum of the patient. The easiest way to combat
this problem is to give either packed red cells or white cell poor
blood to patients known to exhibit febrile reactions. It would be
too time consuming and expensive to type every patient's white cells
as well as his red cells for every unit of blood; however, in the case
of leukemia patients who may need to have repeated infusions of white
cells, and in transplant candidates, it is advantageous to give
leukocyte poor or leukocyte typed blood to avoid an increased pro-
duction of white cell antibodies.

A third important involvement of the antigen-antibody system in
Blood Bank is in the identification and treatment of Hemolytic Disease
of the Newborn. Hemolytic Disease of the Newborn is an antibody-
induced hemolytic anemia that occurs because of passive transfer of
a blood group antibody from a mother to her unborn child. Red cell
destruction takes place via the complement system in the fetus. In
order for this reaction to take place, the baby must have one or more
blood group antigens that the mother lacks, therefore, she can form
antibodies to her baby's antigens. Hemolytic Disease of the Newborn
can only be caused by IgG antibodies, because only the Fc pieces of
the IgG antibodies are able to cross the placenta. In some cases,
antibodies can form during the first pregnancy, as in A, B, C in-
compatibilities. Anti-A and Anti-B are I-M antibodies too large
to cross the placenta, however type O mothers have naturally occurring
IgG Anti-A, B in their serum and this can cause Hemolytic Disease of
the Newborn. In Rh incompatibilities (Anti-D), there must first be
a stimulus of the antigen in the mother before she will produce anti-
bodies. This could happen when a D negative mother is given D positive
blood, or from fetal bleed back during a previous delivery of a D positive child. The first D positive child delivered by the mother will suffer no ill consequences as a result of it's mother's D negative state, unless she has been sensitized by transfusion. When she becomes pregnant a second time, if the baby is again a D positive, she will have circulating antibodies to its D positive cells, which may cause serious disturbances in the fetus. This Rh-D type of Hemolytic Disease of the Newborn is a most serious form of HDNB, and can lead to marked anemia, edema, and in some cases intrauterine death. If the baby survives the intrauterine problems, there are additional problems once it is born. The red cells destroyed in utero are removed from the baby's system by its mother, but after the baby is born it must take over the job of filtering out the lysed hemoglobin and cellular elements in its blood stream. Often the baby's liver is not able to take on this large task and brain damaging bilirubin builds up in its blood stream. It is sometimes necessary to do exchange transfusions on infants, and in these cases blood must be given that lacks the antigen for which the mother has antibodies. That means that even though the baby may be an O positive, it would be transfused with O negative cells.

This paper is by no means an extensive look into all the antigens and antibodies associated with Blood Bank. It is meant as an introductory essay for those students desiring basic knowledge on the mechanism and importance of antibodies and antigens and their uses in Blood Bank. Bloodbanking is a rapidly expanding field, with new antigens and antibodies being discovered every day. One thing that
must be stressed is the importance of accuracy in all aspects of Blood Bank work. A mistyped antigen, or a carelessly overlooked antibody may result in very serious and even fatal consequences.
FOOTNOTES


2. Et. al., p. 46-51.

3. Et. al., p. 120.

4. Et. al., p. 79-82.

5. Et. al., p. 84.


7. Et. al., p. 284.

8. Et. al., p. 325.
REFERENCES

