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Immunohematology

Introduction

Immunohematology is the study of blood group antigens and antibodies, and their interactions in health and disease. Ehrlich and Morgenroth first described blood groups in goats based on antigens of their red cells in an article published in the *Berliner klinische Wochenschrift* in 1900. Subsequently, Karl Landsteiner, a Viennese pathologist, successfully identified the human ABO blood groups for which he was awarded the Nobel Prize 30 years later. After this initial discovery, blood grouping was developed as a science, and many different systems of grouping were designed on the basis of many isoantigens on the surface of erythrocytes. The ABO and Rh systems are among the well-known human blood groups described in the literature.

ABO Blood Group System

ABO blood group system was the first human red-cell antigen system to be characterized. The ABO blood group substances are glycopeptides with oligosaccharide side chains (Fig. 22-1). The ABO blood group specificity is determined by the presence of terminal sugar in an oligosaccharide structure. The terminal sugars of the oligosaccharides are specific for blood groups A and B. They are also immunogenic. The red cells express either A, B, both A and B, or neither, and antibodies are found in serum to antigens not expressed by the red cells.

ABO Blood Group Antigens

The blood group of an individual is determined by presence or absence of two antigens, A and B, on the surface of the red cell membrane. Red cells of blood group A carry antigen A, cells of blood group B carry antigen B, and cells of blood group AB have both A and B antigens. On the other hand, blood group O cells have neither A nor B antigens.

The blood groups are also differentiated by the presence or absence of two distinct isoantibodies in the serum. Serum of blood group A individuals have anti-B antibodies, blood group B have anti-A antibodies, and blood group O have both anti-A and anti-B antibodies. The blood group AB does not contain any anti-A and anti-B antibodies in the serum.

Soluble ABO blood group substances may be found in mucous secretions of humans, such as saliva, gastric juice, ovarian cyst fluid, etc. Such persons are termed secretors, while those without the blood group substances in their secretions are nonsecretors.

The ABO group of a given individual is determined by testing both cells and serum. In this method, the subject's red cells are mixed with serum containing known antibody and the subject's serum is tested against cells possessing known antigen. For example, the cells of a group A individual are agglutinated by anti-A serum but not by anti-B serum, and his or her serum agglutinates type B cells but not type A cells. The typing of cells as group O is done by exclusion (a cell not reacting with anti-A or anti-B is considered to be of blood group O). It is

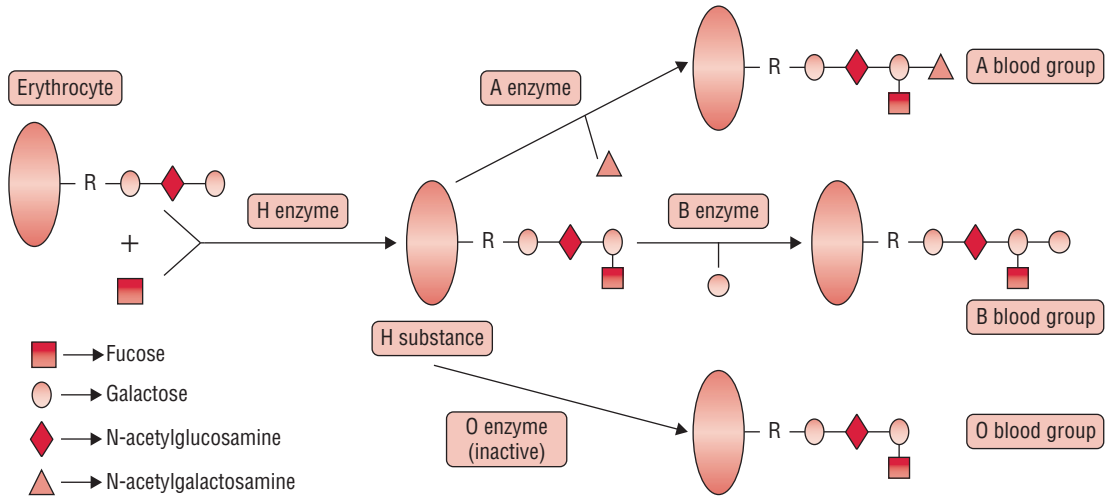

FIG. 22-1. ABO blood group system.

noteworthy that these antibodies to the isoantigens are found in all individuals including those that have had no transfusions.

It is believed that the anti-A and anti-B isoagglutinins are synthesized as a consequence of cross-immunization with bacteria of the family Enterobacteriaceae that colonize the infants' gut. These bacteria have outer membrane oligosaccharides strikingly similar to those that define the A and B antigens in the human body. For example, a newborn with group A will not have anti-B in his or her serum, since there has been no opportunity to undergo cross-immunization. When the intestine is eventually colonized by the normal microbial flora, the infant will start to develop anti-B, but will not produce anti-A because of tolerance to his or her own blood group antigens. The inheritance of the ABO groups follows simple Mendelian rules, with three common allelic genes: A, B, and O (A can be subdivided into A1 and A2), and any individual will carry two alleles, one inherited from the mother and one from the father.

H antigen

Red cells of all ABO groups possess a common antigen, the H antigen, or H substance. H antigen is a glycoprotein and structurally is an L-fucose. It is a precursor for the production of A and B antigens. A and B antigens are formed by addition of *N*-acetylgalactosamine and galactose, respectively, to L-fucose of H antigen.

The H antigen, due to its universal distribution, is not that important in blood grouping or transfusion. However, in rare instances, such as in "Bombay," or OH blood, both A and B antigens as well as H antigens are absent in the blood. Individuals with "Bombay" blood group have anti-A, anti-B, and anti-H antibodies, hence are not compatible with most of the red cells.

Rh Blood Group System

Philip Levine, in 1939, discovered that the sera of most women who gave birth to infants with hemolytic disease contained an antibody that reacted with the red cells of the infant and with the red cells of 85% of Caucasians. In 1940, Landsteiner and Wiener injected blood from the monkey *Macacus rhesus* into rabbits and guinea pigs, and discovered the resulting antibody agglutinated rhesus (Rh) red cells, which appeared to have the same specificity as the neonatal antibody. The donors whose cells were agglutinated by the antibody to Rh red cells were termed ***Rh positive***; those whose cells were not agglutinated were termed ***Rh negative***. It is now known that the antibody obtained by Landsteiner and Wiener reacts with an antigen (LW) is different but is closely related to the one that is recognized in human hemolytic disease, but nevertheless the Rh nomenclature is still retained.

Rh Blood Group Antigens

The term Rh blood group system refers to the five main Rh antigens (C, c, D, E, and e) as well as many other less frequent Rh antigens. The terms Rh factor and Rh antigen are similar, and both refer to the RhD antigen only. Of all the Rh antigens, antigen D (RhD) is most important.

D antigen

Individuals either have or do not have the RhD antigen on the surface of their red blood cells. This is usually indicated by "RhD positive" (does have the RhD antigen) or "RhD negative" (does not have the antigen) suffix to the ABO blood type. This suffix is often shortened to "D pos"/"D neg," "RhD pos"/"RhD neg," or +/−. The latter symbol is generally not preferred in research or medical situations, because it can be altered or obscured accidentally. There are several alloantigenic determinants within the Rh system.

- Clinically, the D antigen has a lot of medical importance. This is because RhD negative individuals who receive RhD positive erythrocytes by transfusion can develop alloantibodies that may lead to severe reactions with further transfusions of RhD-positive blood.
- The D antigen also poses a problem in RhD-negative mothers who bear a child with RhD-positive red cells inherited from the father. The entry of fetal erythrocytes into the maternal circulation at parturition or trauma during the pregnancy (such as in amniocentesis) can lead to alloimmunization against the RhD antigen. This may cause hemolytic disease of the newborn in subsequent pregnancies. This can now be prevented by the administration of Rh (D) immunoglobulin to these women within 72 hours of parturition.

Unlike ABO system, there are no natural antibodies against Rh antigens. Antibodies against Rh antigens develop only in certain situations, such as in Rh incompatible pregnancy or transfusion. Most of these antibodies are IgG antibodies, and few IgM antibodies. These are incomplete antibodies and can be detected in newborn blood by direct Coombs' test and in mother blood by indirect Coombs' test.

Blood Transfusion

Blood transfusion is the process of transferring blood or blood-based products from one person into the circulatory system of another. Blood transfusions have many indications as mentioned below:

- Blood transfusions can be life-saving in some situations, such as massive blood loss due to trauma, or can be used to replace blood lost during surgery.
- Blood transfusions may also be used to treat a severe anemia or thrombocytopenia caused by a blood disease.
- People suffering from hemophilia or sickle-cell disease may require frequent blood transfusions.

Before a blood transfusion, a series of procedures need to be done to establish the proper selection of blood for the patient. Basically, those procedures try:

- to establish ABO and Rh compatibility between donor and recipient and
- to rule out the existence of antibodies in the recipient's serum, which could react with transfused red cells.

To establish the ABO and Rh compatibility between donor and recipient, both the recipient and the blood to be transfused are typed. The most direct way to detect antibodies in the recipient's serum that could cause hemolysis of the transfused red cells is to test the patient's serum with the donor's cells (major cross-match). The minor cross-match, which consists of testing a patient's cells with donor serum is of little significance and rarely performed, since any donor antibodies would be greatly diluted in the recipient's plasma, and rarely, it causes clinical problems.

Universal recipient: It is an ABO blood group individual whose red blood cells express antigens A and B, but whose serum does not contain anti-A and anti-B antibodies. Thus, red blood cells containing any of the ABO antigens, i.e., from an individual with type A, B, AB, or O, may be transfused to the universal recipient without inducing a hemolytic transfusion reaction.

It is best if the universal recipient is Rh positive, i.e., has the RhD antigen on the erythrocytes to avoid developing a hemolytic transfusion reaction. However, blood group systems other than ABO may induce hemolytic reactions in a universal recipient. Thus, it is best to use type-specific blood for transfusions.

Universal donor: It is a blood group O RhD-negative individual whose erythrocytes express neither A nor B surface antigens. This type of red blood cell fails to elicit a hemolytic transfusion reaction in recipients with blood group A, B, AB, or O. However, group O individuals serving as universal donors may express other blood group antigens on their erythrocytes that may induce hemolysis. It is preferable to use type-specific blood for transfusions, except in cases of disaster or emergency.

Complications of Blood Transfusion

Transfusion reaction is the major immunological complication following incompatible blood transfusion. Other transfusion reactions may be caused due to factors other than incompatibility, such as a person being hypersensitive to some allergens present in the blood. Transmission of infectious agents through blood is the most important complication. These include:

- Viruses, such as human immunodeficiency viruses I and II (HIV I and II), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and cytomegalovirus (CMV);
- Bacteria, such as *Treponema pallidum* and *Leptospira interrogans*; and
- Protozoa, such as *Toxoplasma gondii, Leishmania donovani,* and *Plasmodium* species.
- Transmission of HIV I and II, HBV, and HCV, which is a major concern.

Hemolytic Disease of Newborn (Erythroblastosis Fetalis)

Hemolytic disease of the newborn, also known as HDN, is an alloimmune condition. It develops in a fetus, which contains the IgG antibodies that have been produced by the mother and have passed through the placenta. These antibodies subsequently attack and lyse the red blood cells in the fetal circulation, resulting in reticulocytosis and anemia. This condition in fetus varies from mild to very severe, and fetal death may occur due to heart failure ***(hydrops fetalis)***. When the disease is moderate or severe, many erythroblasts are present in the fetal blood, and this form of the disease is called ***erythroblastosis fetalis***.

Immunological destruction of fetal and/or newborn erythrocytes is likely to occur when IgG antibodies are present in the maternal circulation directed against the antigen(s) present on the fetal red blood cells. This is because only IgG antibodies can cross the placenta and reach the fetal circulation. Anti-D and anti-A or anti-B are the two types of antibodies most usually involved in hemolytic disease of the newborn. Anti-A or anti-B antibodies are usually IgM, but, in some circumstances, IgG antibodies may develop (usually in group O mothers). This can be secondary to immune stimulation (some vaccines contain blood group substances or cross-reactive polysaccharides), or may occur without apparent cause for unknown reasons.

Antibodies are produced when the body is exposed to an antigen foreign to the make-up of the body. If a mother is exposed to an alien antigen and produces IgG (as opposed to IgM which does not cross the placenta), the IgG will combine with the antigen, if present in the fetus, and may affect it *in utero* and persist after delivery (Fig. 22-2).

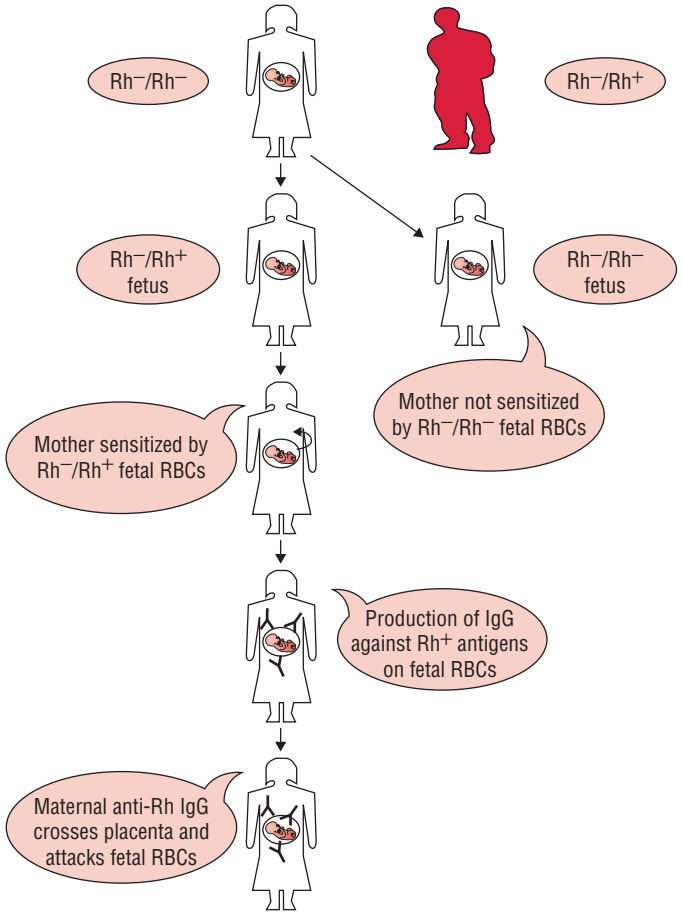

FIG. 22-2. Hemolytic diseases of newborn.

The three most common ways in which a woman becomes sensitized (i.e., produces IgG antibodies against) toward particular blood types are as follows:

1. Fetal–maternal hemorrhage can occur due to trauma, abortion, childbirth, ruptures in the placenta during pregnancy, or medical procedures carried out during pregnancy that breach the uterine wall. In subsequent pregnancies, if there is a similar incompatibility in the fetus, these antibodies then cross the placenta into the fetal bloodstream, combine with the red blood cells, and finally cause hemolysis. In other words, if a mother has anti-RhD (D being the major Rh antigen) IgG antibodies as a result of previously carrying an RhD-positive fetus, these antibodies will only affect a fetus with RhD-positive blood.
2. The woman may receive a therapeutic blood transfusion with an incompatible blood type. ABO blood group system and Rh blood group system typing are routine prior to transfusion. Suggestions have been made that women of childbearing age or young girls should not be given a transfusion with Rhc-positive blood or Kell-positive blood to avoid possible sensitization. However, it is considered uneconomical to screen for these blood groups.
3. The third sensitization model can occur in women of blood type O. The immune response to A and B antigens, which are widespread in the environment, usually leads to the production of IgM anti-A and IgM anti-B antibodies early in life. On rare occasions, IgG antibodies are produced. In contrast, Rhesus antibodies are generally not produced from exposure to environmental antigens.

A positive direct Coombs' (antiglobulin) test with cord RBC is invariably positive in cases of Rh incompatibility. In ABO incompatibility, the direct antiglobulin test is usually weakly positive and may be confirmed by eluting antibodies from the infant's red cells and testing the elute with A and B cells.

Before birth, treatment of the condition include intrauterine transfusion or early induction of labor when (*a*) pulmonary maturity has been attained, (*b*) fetal distress is present, or (*c*) 35–37 weeks of gestation have passed. The mother is also administered with plasma to reduce the circulating levels of antibody by as much as 75%.

After birth, treatment depends on the severity of the condition. These include temperature stabilization, phototherapy, transfusion with compatible packed red blood cells, administration of sodium bicarbonate for correction of acidosis, and/or assisted ventilation and exchange transfusion with a blood cells, type compatible with both the infant and the mother.

Rh-negative mothers who have had a pregnancy with or are pregnant with an Rh-positive infant are given Rh immunoglobulin (RhIG) during pregnancy and after delivery to prevent sensitization to the D antigen. The RhIG acts by binding any fetal red cells with the D antigen before the mother is able to produce an immune response and form anti-D IgG.

All the offsprings of Rh-incompatible marriages, however, do not suffer from hemolytic diseases of newborn. This may be due to either of the following causes:

1. Mother–fetus ABO incompatibility: When the mother and fetus possess the same ABO group, Rh immunization is more likely to occur. However, when Rh and ABO incompatibility coexist, Rh sensitization from the mother is very rare. In this condition, the fetal cells entering the maternal circulation are destroyed rapidly by the ABO antibodies before they can form the Rh antibodies.

2. Immune unresponsiveness to Rh antigen: Some Rh-negative individuals even after repeated injections of Rh-positive cells fail to form Rh antibodies. Such individuals are known as nonresponders. The exact reason for such immunological unresponsiveness, however, is not known.

3. Number of pregnancies: The risk of hemolytic disease of new born is more in second and successive child, but not in first child. This is because sensitization occurs only during the delivery; hence the first child escapes.

ABO Hemolytic Diseases

ABO hemolytic diseases occur in a very less number of cases, even though materno-fetal ABO incompatibility is very common. The condition is usually seen in O group mothers bearing blood group A or B fetus. It occurs largely in O group mothers because the isoantibodies are largely IgG in nature, which can cross the placenta. It does not occur in mothers with blood groups A or B because natural antibodies are mainly IgM in nature, which does not cross the placenta and sensitize the fetus.

Unlike hemolytic disease of the newborn, the ABO hemolytic diseases can occur even in first child even without prior immunization. This is because the ABO disease is caused by naturally occurring maternal isoantibodies.

ABO hemolytic disease is much milder condition than that of the Rh disease. The diagnosis of ABO incompatibility is made by a positive indirect Coombs' test but a negative direct Coombs' test. Peripheral blood smear characteristically shows spherocytosis.