Red Blood Cell Alloimmunization

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This case study focuses on the differences in blood types and one possible medical complication that can arise during pregnancy or blood transfusion. Alloimmunization is an allergic reaction to red blood cell antigens and is a significant challenge for physicians caring for women of child-bearing age. Exposure to foreign red blood cell antigens during transfusion or pregnancy leads to the development of antibodies. After alloimmuinzation has occurred if during a subsequent fetus bears that antigen, maternal antibodies may attack the fetal red blood cells causing red cell destruction and clinically significant hemolytic disease of the fetus and newborn. In addition to complications during pregnancy, alloimmunization is also a significant clinical concern for chronically transfused patients such as those hemoglobopathies (as in sickle cell disease and thalassemia), myelodysplastic syndromes (as is acquired aplastic anemia or acute myeloid leukemia), and chronic renal disease. Alloimmunization can also complicate organ transplantation and other therapies.

Blood Types: There are three primary types of blood cells. Platelets are small cells responsible for clotting. White blood cells are a diverse group of blood cells and are responsible for fighting infections. Red blood cells actively transport oxygen to tissues in the body and actively removes and carbon dioxide. The outer surfaces of erythrocytes, Red Blood Cells (RBC), and many eukaryotic cells are covered with complex carbohydrates. These carbohydrates are components of the glycoproteins and glycolipids of the cell membrane. The carbohydrates form a thick, fuzzy outer layer on the cell called the glycocalyx and serve as markers for various cell recognition processes. These features of the cell surface act as antigens. An antigen is substance which induces an immune response in the body, especially the production of antibodies. An antibody is a blood protein produced in response to and counteracting a specific antigen. Antibodies are produced when an antigen binds to the surface of a B-cell. B-cells can secrete antibodies or the antibodies can remain bound to the surface of the B-cell. In placental mammals there are five antibody isotypes: IgA, IgD, IgE, IgG, and IgM. Human erythrocytes have over 300 genetically distinct surface antigens. These surface antigens have been used to define approximately 36 different blood group systems. The two most clinically important are the ABO System, discovered by Karl Landstiener in 1901, and the Rhesus (Rh) Blood Group System, discovered by Karl Landsteiner and Alexander S. Wiener in 1937. The ABO and Rh blood group systems are the basis of modern blood typing. Several of the other blood group systems and RBC surface antigens are used for other clinical applications, most commonly in paternity testing.

ABO Blood Group System: The ABO surface antigens are variations in carbohydrates attached to glycoproteins and glycolipids on the surfaces of red blood cells. These carbohydrate chains, called oligosaccharides, project above the RBC surface. Each RBC shares a base oligosaccharide. (**Figure 1.**) This sugar polymer is composed of a glucose molecule anchored to the cell wall, followed by a galactose molecule, a N-acetyl-glucosamine molecule, another galactose molecule, and finally a molecule of fucose. This basic oligosaccharide is the O blood antigen. The A blood antigen is produced when an N-acetyl-galactosamine is added in an α1-3 glycosidic bond. Similarly, the B blood antigen is produced when a galactose molecule is added in the same manner. The difference between the A and B blood antigens is merely the presence/absence of an N-acetyl

group on the added galactose. The ABO blood type is clinically important for blood transfusion and organ matching. When a mismatch between donor and recipient occurs IgM antibodies to the foreign blood antigen are produced. For this reason, ABO blood types of donor and recipient must match to avoid a potentially life-threatening reaction. Individuals with type A cannot, for example donate to recipients with types B or O. In the same manner, individuals with type B cannot donate to recipients with types A or O. Naturally occurring Anti-A antibodies are found in the serum of people with blood groups O and B. Anti-B antibodies are found in the serum of people with blood groups O and A. When a recipient receives incompatible blood, the anti-A or anti-B antibodies bind to RBCs. The foreign RBCs are then eliminated in two ways. First, B cells can coat and sequester the foreign RBCs. Second, the presence of anti-A/anti-B antibodies activates the compliment cascade, which lyses the RBCs while they are still in the circulation (intravascular hemolysis). Most deaths caused by blood transfusion are the result of transfusing ABO-incompatible blood.

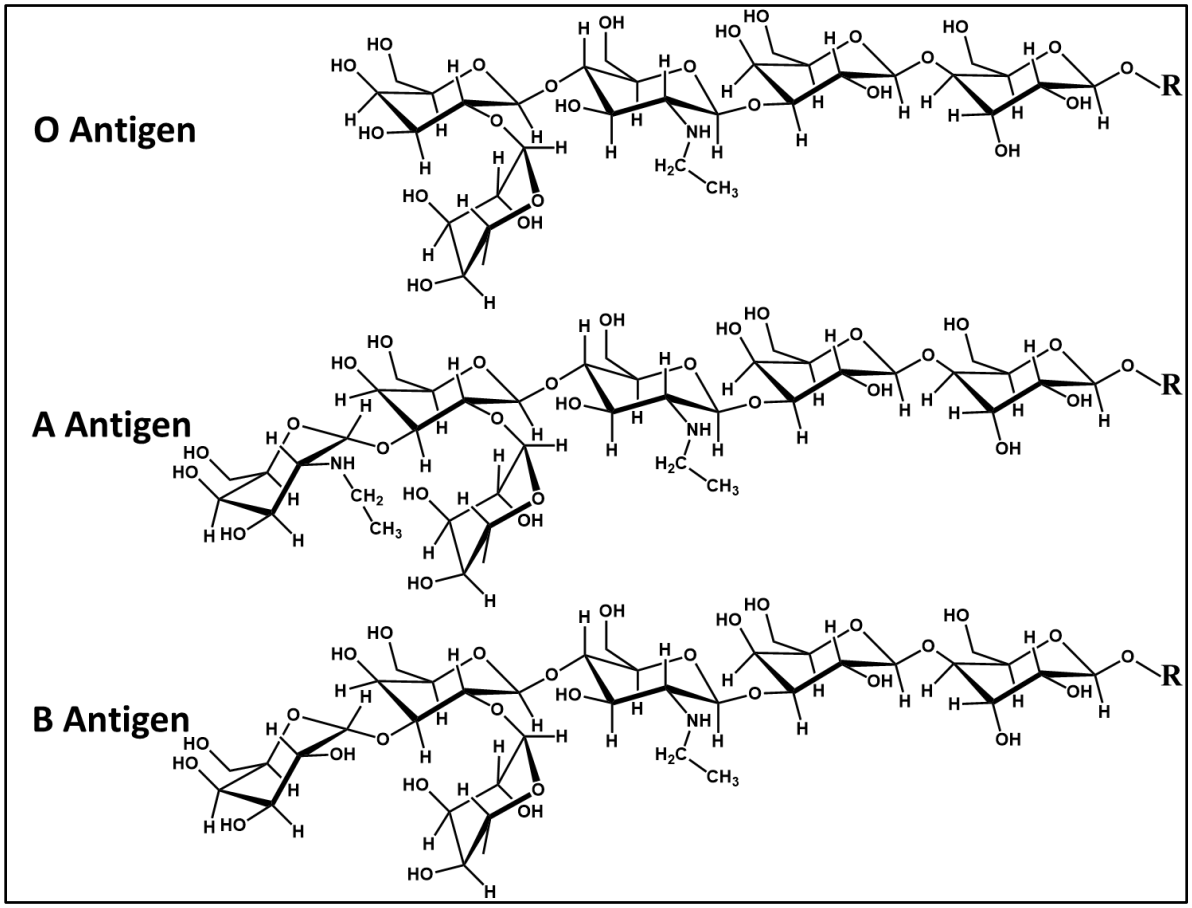

Figure 1. ABO Blood Group Antigens: The O blood antigen (top) is composed of (from right to left) a glucose covalently bound to the cell surface (R) followed by a galactose (β1-4 linkage), a N-acetyl - glucosamine (β1-3 linkage), another galactose (β1-4 linkage), and finally a molecule of fucose (α1-2 linkage). This oligosaccharide is the basis of the ABO blood group system. The A blood antigen is produced when a N-acetyl-galactosamine is added to the terminal galactose (α1-3 linkage). The B blood antigen is produced when another galactose is added to the terminal galactose (α1-3 linkage). The difference between the A and B blood antigens is merely the presence/absence of an N-acetyl group on the added galactose. This small difference is the basis for a potentially life-threatening immune response.

Blood type O is the exception to this rule. Because of the O antigen is the basis of all three blood types, it is accepted without production of antibodies. Individuals with blood type O can donate to O, A, and B recipients. Naturally occurring Anti-A antibodies are found in the serum of people with blood groups O and B. Anti-B antibodies are found in the serum of people with blood groups O and A. In pregnancy the ABO system is not as clinically unimportant. In general, the IgM antibodies produced due to a mismatch between donor/recipient or mother/fetus do not cross the placental barrier and therefore cannot be the cause of alloimmunization during pregnancy. However, some cases of alloimmunization during pregnancy due to ABO blood group are known. In these cases, the mother has first encountered the incompatible blood group prior to pregnancy. While the initial immune response to ABO mismatch is via the IgM route, the long-term immune response is a switch to IgG antibody production. This is called the secondary immune response. The initial IgM antibodies bind the foreign antigen weakly, but subsequent IgG antibodies are, generally, much better targeted. Production of IgG antibodies can remain high for years after an exposure event. In such cases, mother/fetus alloimmunization due to ABO blood groups resulting in fetus/newborn hemolytic disease can occur. Even in such cases, alloimmunization is rare because of the number and volume of fetal tissues expressing the ABO antigens. The concentration of maternal IgG antibodies does reach a critical level.

Rhesus Blood Group System: The Rh blood group system is often considered the second most clinically important blood group system after the ABO blood group system. However, for alloimunnization during pregnancy, mismatches of Rh blood group system between mother and fetus is the primary cause of alloimmunization and hemolytic disease of the fetus or newborn. The Rh antigens are portions of a transmembrane protein that extends beyond the surface of the cell. There are actually five main antigens in the Rh blood group system: D, C, E, c, and e. These antigens are encoded on two adjacent genes. The first gene, RHD, encode for the Rh D protein. The second gene, RHCE, encodes for the C, E, c, and e variants. The presence or absence of the Rh D variant is the determinant of Rh (+) or Rh(-) status, and thus is often considered the only clinically relevant antigen in this blood group system. Over 85% of the human population is thought to be Rh(+). (85% Caucasian descent, 92% African descent, 99% Asian descent) Rh(-) status is usually due to deletion of the entire RHD gene. When a mismatch between donor and recipient occurs IgG antibodies to the foreign blood antigen are produced. An Rh(-) recipient will produce antibodies to the Rh D antigen. An Rh(-) mother will produce antibodies to the Rh(+) antigens of a fetus. The IgG antibodies then mark the foreign RBCs for destruction. Since IgG antibodies do cross the placental barrier, Rh mismatches between mother and fetus can result in serious complications during pregnancy. Conversely, an Rh(+) recipient of Rh(-) blood donation, or an Rh(+) mother carrying an Rh(-) fetus, pose no clinical risk.

Alloimmunization: If an Rh(-) person is exposed to Rh(+) blood or tissue IgG antibodies are produced in response to the foreign blood antigen. In the case of transfusion or transplant, these antibodies cause rejection of the foreign tissue. If during a blood transfusion an Rh(-) individual is given Rh(+) blood production of antibodies can be prevented by administration of Rho(D) immune globulin, commonly known as RhoGAM. RhoGAM binds to the Rh antigens present on the surface of the RBC and prevents the host immune system from recognizing it. Without this drug, the recipient would develop antibodies to the foreign Rh(+) blood. During pregnancy an Rh(-) mother and an Rh(+) father will have a high chance of producing Rh(+) offspring. If the father is Rh D homozygous, i.e. a carrier of two RHD alleles, then there is 100% chance of Rh(+) offspring. If the father is Rh D heterozygous, i.e. a carrier of only one RHD allele, then there in

only a 50% chance of Rh(+) offspring. (The Rh(-) mother lacks the RHD gene on both alleles.) If an Rh(-) mother carries an Rh(+) fetus then antibodies to the Rh D antigen will be produced. Generally, this is without clinical impact during the first pregnancy, but during subsequent pregnancies the pre-existing antibodies typically result in a significant immune response to the Rh D antigen of the fetus. The existence of these pre-existing IgG antibodies is called alloimmunization. If left untreated this condition can be fatal to a fetus or newborn. Treatment of the Rh(-) mother during pregnancy with RhoGAM will prevent this problem. In countries without Rho(D) immune globulin protocols, as many as 14% of affected fetuses are stillborn and 50% of live births result in neonatal death or brain injury.

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Post-Case Questions:

1. Define the term “antigen”.

2. What are antibodies?

3. How are antibodies produced?

4. Define Alloimmunization.

5. What antibodies are responsible for recognizing ABO blood group antigens?

6. What is the difference between A, B, and O blood antigens?

7. What kind of antibodies are produced in response to the Rhesus blood antigen? Why are these antibodies dangerous to the fetus during pregnancy?

8. Why don’t mismatches in ABO antigens between mother and infant cause hemolytic disease in newborns?

9. What are the chances of an Rh(+) offspring between an Rh(+) father and an Rh(-) mother?

10. What drug is used to treat/prevent alloimmunization?