EDUCATIONAL COMMENTARY – ANTI-K AND THE K ANTIGEN

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Learning Objectives

On completion of this exercise, the participant should be able to

• discuss the characteristics and clinical importance of the anti-K antibody;
• identify blood bank reagents that denature the K antigen;
• discuss blood bank testing methods used to detect anti-K and the K antigen; and
• describe the unique form of anemia anti-K can produce in cases of hemolytic disease of the fetus and newborn.

Introduction

The K antigen is a low-prevalence red blood cell (RBC) antigen that belongs to the Kell blood group system. The antibody directed against it, anti-K, is frequently identified in routine blood bank testing and is the most commonly encountered antibody of the Kell blood group system. Owing to the clinical significance of anti-K, management techniques should be used to improve patient outcomes. This review provides a general overview of anti-K and the K antigen, with an emphasis on their clinical importance in transfusion medicine and in cases of hemolytic disease of the fetus and newborn (HDFN).

History

In 1946, Mrs. Kelleher delivered her second child, who had HDFN.1 Coombs and coworkers2 had recently developed antihuman globulin (AHG) testing for use in routine blood bank testing. These AHG tests, also known as the indirect antiglobulin test (IAT) and the direct antiglobulin test (DAT), are based on the principle that AHG binds to human IgG or complement antibodies that are RBC bound and nonagglutinating.2,3 Using these tests, it was determined not only that Mrs. Kelleher’s antibody was binding to the newborn’s RBCs, but also that her serum was reactive toward the RBCs of her older child and husband.1,2 Mrs. Kelleher was the first producer of anti-K (originally named anti-Kell) to be identified, making this the first antibody to be discovered after the introduction of the AHG tests, and the Kell blood group system was named after her.2 Since its initial discovery, the Kell blood group system has grown to include 36 antigens, of both low and high prevalence, with three additional antigens whose category and name have become obsolete.4,5 Of those in the system, the K antigen and anti-K are still considered the most significant to transfusion medicine and HDFN.1
K Antigen

The K antigen is often referred to by its original name, Kell, but the correct terminology is K or the International Society of Blood Transfusion (ISBT) symbol KEL1. The antigen, consisting of intracellular, transmembrane, and extracellular regions, is located on the membrane glycoprotein CD238 and comprised of 731 amino acids. The intracellular region is denoted as the N-terminal domain. The large, external C-terminal domain is folded by many disulfide bonds, and linked to the Xk protein by 1 disulfide bond. The presence or absence of the Xk protein on the RBC determines whether the K antigen will be expressed. K-antigen expression occurs on fetal precursor cells and mature fetal RBCs beginning as early as 10 weeks’ gestation, with full development at birth. Its prevalence can range from 3,500 to 18,000 antigen sites per RBC. Although this is considered low compared with other blood groups, it ranks as the second most immunogenic non-ABO antigen in transfusion medicine.

The K antigen is a low-frequency antigen, occurring in 9% of persons of European ethnicity and 2% of persons of African ethnicity. It is rarely present in persons of East Asian ethnicity. The antithetical antigen to the K antigen is the high-prevalence k antigen (ISBT symbol, KEL2), formerly known as Cellano, which occurs in 99.8% of persons with European ethnicity and 100% of persons with African ethnicity. The k antigen was discovered 3 years after the K antigen, and the two antigens differ by a single nucleotide polymorphism in exon 6.

In blood bank testing, the K antigen shows resistance when treated with reagent enzymes such as ficin, trypsin, papain, and α-chymotrypsin. However, when trypsin and α-chymotrypsin are used together, the antigen is destroyed. Thiol-reducing agents, such as dithiothreitol (DTT), 2-aminoethylisothiouronium bromide (AET), ZZAP, and 2-mercaptopethanol (2-ME), denature the K antigen. It is also destroyed by EDTA-glycine acid (EGA) treatment.

To determine K-antigen expression on patient and donor RBCs, most transfusion services use serologic typing methods. This process is known as phenotyping, and it is a simple, inexpensive, and time-efficient way to determine the presence or absence of the antigen. The RBCs are tested with antisera specific for the K antigen, and if the antigen is expressed on the RBC, hemagglutination should be observed. Another method that can be used is DNA-based testing, also known as genotyping, which is used to predict the probable K phenotype. These molecular methods detect the genes that encode the antigen on the RBC but take longer and are more costly than serologic typing methods.
EDUCATIONAL COMMENTARY – ANTI-K AND THE K ANTIGEN (cont.)

Anti-K

Anti-K is the most commonly encountered of all of the non-ABO, non-Rh antibodies in blood bank testing.\(^2\) It accounts for one-third of antibody identifications of non-Rh antibodies and is formed after pregnancy or transfusion, as a result of K-antigen exposure.\(^4\) Once formed, the antibody can usually be detected in the serum for many years. Most anti-K antibodies are of the IgG immunoglobulin class, mainly subclass IgG1, and show best reactivity at the AHG phase of routine blood bank testing.\(^2,4\) There are rare reported examples of naturally occurring IgM anti-K antibodies, linked to bacterial infections.\(^2\) Autoantibodies directed against the K antigen have also been identified, and cases of autoantibodies that mimic an anti-K antibody have been reported.\(^2\)

Although some examples of anti-K have shown direct agglutination of RBCs in saline, the IAT should be used when performing routine blood bank testing, because it is the most reliable method for detection.\(^2,4\) Use of a potentiating medium, such as polyethylene glycol (PEG), has been shown to increase anti-K reactivity. Anti-K has shown poor reactivity with low-ionic-strength solution (LISS) procedures and with some automated blood bank testing methods.\(^2,5\)

Transfusion Considerations

Anti-K is considered a clinically significant antibody that can cause mild to severe acute or delayed hemolytic transfusion reactions.\(^5,7\) It is capable of binding complement, but most commonly, RBC destruction occurs extravascularly.\(^2\) If a patient possesses an anti-K antibody and an RBC transfusion is required, donor units that have tested negative for the K antigen should be selected. These units should be crossmatched with the patient’s serum using the IAT procedure and determined to be compatible before transfusion. Because the frequency of the K antigen is low in the general population, finding an antigen-negative donor is easy for most transfusion services.

Hemolytic Disease of the Fetus and Newborn

During pregnancy, the presence of anti-K is considered clinically significant and can cause HDFN. Compared with other antibodies implicated in HDFN, anti-K can produce a unique form of anemia in the fetus and newborn. If detected in pregnancy, an ongoing, comprehensive approach should be taken to diagnose, monitor, and, if necessary, treat the patient.

Pathophysiology

Hemolytic disease of the fetus and newborn, also referred to as *erythroblastosis fetalis*, occurs when maternal IgG antibodies cross the placenta and bind to the corresponding antigen on the fetal RBCs.\(^8\) Maternal RBCs lack the antigen, but it is expressed on fetal RBCs through paternal inheritance. Once
these fetal antigens are bound by maternal IgG alloantibodies, the fetal RBCs become attached to macrophage Fc receptors and phagocytosis occurs, mainly in the spleen. Maternal IgG antibodies are the only immunoglobulin class that will cross the placenta during pregnancy, beginning during the second trimester and lasting until birth. Antibodies of the IgG1 and IgG3 subclasses, compared with IgG2 and IgG4 subclasses, are more effective at causing this hemolysis.

The fetal bone marrow responds to the hemolysis by rapidly trying to produce more RBCs. If the bone marrow cannot compensate, RBC production is increased in the fetal liver and spleen, resulting in enlargement of both organs. This liver enlargement decreases production of plasma proteins, decreases plasma oncotic pressure, creates edema and ascites, and, if not treated, can result in fetal death. In addition, hemoglobin that is released as a result of fetal RBC hemolysis is metabolized into bilirubin. Before the fetus is born, this bilirubin is transported across the placenta to the maternal liver and is ultimately excreted. After birth, owing to the immaturity of the newborn’s liver pathways, the neonate cannot metabolize the bilirubin on its own. This can result in the accumulation of elevated levels of bilirubin, called kernicterus, which can cause irreversible damage to the neonate’s brain.

As previously stated, the clinical features seen with anti-K differ from those of other antibodies implicated in HDFN. This is because of the location of the K antigen and early antigen expression during erythropoiesis. Anti-K not only causes hemolysis of the mature circulating fetal RBCs, but also causes destruction of the RBC precursor cells in the bone marrow, beginning at an early gestational age. This destruction suppresses the formation of RBCs, and severe fetal anemia can occur. Essentially, HDFN from anti-K is more a result of the suppression of RBC formation than of hemolysis of circulating RBCs. Owing to the lack of hemoglobin in the destroyed precursor cells, increased levels of hemolysis and hyperbilirubinemia are not seen in the fetus or newborn. In these cases, amniotic fluid bilirubin levels may not correlate with the level of hemolysis that is occurring. In addition, also owing to precursor-cell destruction, increased reticulocytosis and erythroblastosis are generally not seen either.

Diagnosis and Monitoring

During pregnancy, patient history should be obtained, including previous transfusions and previous pregnancies and pregnancy outcomes. If HDFN has occurred in a previous pregnancy, the risk can increase with each subsequent pregnancy, and, in some severe cases, can begin at only 18 to 20 weeks’ gestation. In addition to patient history, it is recommended that an antibody screen be performed during the first trimester. The IAT should be used to detect clinically significant IgG antibodies, and if the test shows reactivity, the antibody must be identified.
When a clinically significant antibody is present in the maternal serum, fetal risk assessment and management for HDFN should occur. Maternal antibody titers may be used to determine if the fetus is inducing continuous (i.e., constant) immune stimulation of the mother. The relative concentration of the antibody is determined by preparing serial dilutions (e.g., 1:1, 1:2, 1:4, 1:8, 1:16, etc.) of maternal serum, then testing each dilution against RBCs known to possess the corresponding antigen. The AABB recommends performing titers using a saline-tube AHG method incubated for 60 minutes at 37°C and performed simultaneously with the mother’s previous titer sample for comparison. The reciprocal of the highest dilution showing agglutination is the titration of the antibody (e.g., 1:512 is reported as 512). Typically, a titer of 16 is considered critical and a titer of 32 is an indication that Doppler ultrasonography should be used to assess anemia in the fetus. In cases of anti-K HDFN, owing to the possible combination of destruction of fetal circulating RBCs and precursor cells, titration studies may not accurately correlate with the degree of HDFN that is occurring. Stillbirth has been reported with maternal anti-K titrations as low as 64. Owing to its unique form of anemia, an anti-K titer of 8 is considered critical, although some centers view any presence of the antibody during pregnancy as critical.

Other methods used to determine the risk and management of HDFN include paternal antigen typing, genotyping of fetal DNA, Doppler fetal ultrasound, cordocentesis, intrauterine transfusion, and delivery. Maternal therapeutic plasma exchange and intravenous immunoglobulin (IVIG) may also be used to manage HDFN. Unfortunately, there are currently no prophylactic immune globulins available to prevent K-antigen exposure during pregnancy.

**Treatment**

Cases of HDFN can range in severity from no impact on the newborn to fetal death. After delivery, a cord blood sample can be tested to investigate cases of HDFN, and this testing should include a DAT using an anti-IgG AHG reagent. A positive DAT confirms that maternal antibody has crossed the placenta and is binding to the neonate’s RBCs, but the serologic strength of the reaction may not actually correlate with the level of HDFN that is occurring. Therefore, the neonate’s hemoglobin and bilirubin levels should also be monitored.

Depending on the severity, different approaches may be used to treat the neonate’s bilirubin levels and hemolysis. Phototherapy may be used to induce oxidation of bilirubin, which is then removed through the urine. Intravenous immunoglobulin may also be given to limit hemolysis, and thereby control bilirubin levels. Small-volume RBC transfusions are usually used to treat anemia when phototherapy and IVIG have corrected the bilirubin level in the neonate. Generally reserved for severe cases, double-volume
EDUCATIONAL COMMENTARY – ANTI-K AND THE K ANTIGEN (cont.)

exchange transfusion uses techniques that remove a considerable amount of the neonate’s blood volume and replace it with a whole-blood product. Benefits to the neonate include reduction of maternal antibody, reduction of bilirubin level, and replenishment with compatible RBCs. A double-volume exchange transfusion should replace 85% to 90% of the neonate’s blood volume and reduce the bilirubin level by 50%.

In both types of transfusions, the RBC units given should be antigen tested and found to be negative for the implicated antigen and also crossmatched using the IAT procedure and found to be compatible. The crossmatch should be performed using the neonatal and/or maternal specimen, depending on the hospital’s transfusion service policy. This RBC selection allows for longer survival of the transfused cells, owing to the lack of antigen expression for the maternal antibody to bind with and destroy. Most transfusion centers use type O RBCs that are cytomegalovirus-risk reduced, irradiated, and hemoglobin S negative.

Summary

In blood bank testing, anti-K is the most frequently encountered antibody from the Kell blood group system. In transfusion, it is a clinically significant antibody owing to the severity of transfusion reactions it can produce. Because K is a low-frequency antigen, it is relatively easy for most transfusion services to identify an anti-K antibody and to find compatible antigen-negative RBC donor units for transfusion. When present in pregnancy, anti-K is considered clinically significant owing to the unique type of fetal anemia it can produce in the fetus and newborn. Not only can anti-K destroy mature fetal RBCs, it can also destroy fetal precursor cells. In essence, anti-K can prevent the fetus from producing its own RBCs, resulting in severe anemia or even fetal death. In both instances, when anti-K is present, clinical possibilities should be considered and a collaborative approach among health care professionals should be implemented to treat patients and manage their outcomes.

References


EDUCATIONAL COMMENTARY – ANTI-K AND THE K ANTIGEN (cont.)


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