INDIAN IMMUNOHEMATOLOGY INITIATIVE
CASE OF THE MONTH: April, 2009
Case Study by Jim Perkins, MD(©2009)

Question:

1. What alloantibody or autoantibody appears to be present? Is this possible given the patient's blood type?

   The patient's serum gives reactions consistent with anti-D. Since the direct antiglobulin test (DAT) is negative, this appears to be an alloantibody, not an autoantibody. The patient is Rh(D) positive, so in the usual case we would not expect the patient to be able to make anti-D. However, rare individuals may have a "partial D" phenotype in which they only express a portion of the D protein lacking one or more of the epitopes on the complete D protein. If such an individual is exposed to the D antigen by pregnancy or transfusion he or she may make an antibody against the epitopes he or she lacks, and which therefore appear "not-self". Since the D positive RBCs on an antibody identification panel typically come from donors who express all of the normal D epitopes, antibodies made by partial D individuals have patterns of reactivity similar to the anti-D made by Rh negative individuals.

2. What additional workup would you do to prove your hypothesis?

   In the past, demonstration that an individual expressed a partial D antigen required rare antisera made by other partial D individuals. ***Failure to react*** *with such an antiserum suggested that the test RBCs lacked the epitope or epitopes against which the partial anti-D serum was directed and that the two individuals had one or more missing epitopes in common.*

   When a partial D individual makes anti-D, its characterization can suggest which type of partial D antigenthe patient has. Again, rare reagents were needed, in this case RBCs. The specificity of the antibody for partial forms of the D protein is demonstrated by ***failure of the serum to react*** *with RBCs of other, previously characterized, partial D individuals. An example of this type of investigation is given in antibody identification case study #11.*

   Today there are additional ways in which a partial D antigen can be demonstrated. The genetic basis of many partial D antigens has been demonstrated, so the patient's partial D genotype can be determined from the leukocytes in samples of blood. Also monoclonal antibodies have been developed that are directed against many of the D epitopes that are lacking in different forms of partial D. Failure of one or more of these antibodies to react with the patient's RBCs demonstrates the absence of the corresponding epitope(s). The pattern of reactivity can then be compared to that of known types of partial D.

3. What additional workup should be done on this pregnant woman?

   Anti-D made by partial D women may cause hemolytic disease of the fetus and newborn (HDFN), and titration of the antibody may give some idea as to the likelihood of clinically significant fetal anemia.

4. What does the monoclonal antibody panel demonstrate?

   The patient's RBCs failed to react with 3 of the 12 monoclonal antibodies directed against epitopes on the RHD protein in a pattern that is characteristic of the DOL form of partial D. This confirms the fact that the patient has a variant of D and is consistent with formation of anti-D from her prior pregnancies.

5. What is the antibody titer? Are there any special actions to be taken at this time based on the titer?

In this case the titer was 8 according to the procedure of the author's laboratory (100 µL serially diluted serum, 1 drop of a 3-5% suspension of R1r cells from one donor used for all tests, 1 hour incubation at 37°C, AHG test with monoclonal anti-IgG, titer interpreted as the reciprocal of the highest dilution causing macroscopic agglutination {w+}). Using this method a titer of 128 is strongly correlated with fetal anemia, and no cases of the latter have occurred at titers of 64 or below (Moise KJ, Perkins JT, Sosler SD, et al. The predictive value of maternal serum testing for the detection of fetal anemia in red cell alloimmunization. Am J Obst Gyne 172:1003-9, 1995). Note that the critical titer of anti-D for fetal anemia should be validated in all laboratories performing the test. Also note that this correlation has only been determined for anti-D made by Rh negative women. Just as data on the critical titer of anti-D cannot be extrapolated to other maternal blood group antibodies, strictly speaking they cannot be extrapolated to this case. In the absence of enough cases to determine such a correlation, the titer is less useful to predict anemia in utero, although if the titer increases significantly or is high, other tests for fetal anemia may be indicated. Determination of the middle cerebral artery velocity is very useful in this regard since it is a non-invasive test which, unlike amniocentesis or percutaneous umbilical blood sampling (PUBS), will not cause fetal maternal hemorrhage (FMH) which can stimulate increased synthesis of maternal antibody.