ABID CASE #21, ANSWERS

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Indian Immunohematology Initiative

See ABID case #20 for additional explanation.

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

   The patient's serum gives reactions consistent with anti-D. As in the previous case (ABID case #20) although the patient appears to be D-positive since the direct antiglobulin test (DAT) is negative it appears to be an alloantibody, not an autoantibody. So again this patient might have a "partial D" protein lacking one or more D epitopes. 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 examples of anti-D made by other partial D individuals as shown in ABID case #20, or rare partial D RBCs from other individuals.

   Failure to react with an antibody made by another partial D person of the same type suggests that the patient's RBCs lack the epitope or epitopes against which the partial anti-D serum is directed and that the two individuals had one or more missing epitopes in common. This is how the previous partial D patient was demonstrated and subtyped.

   When a partial D individual makes anti-D (the usual way in which such individuals are detected) it should fail to react with partial D RBCs similar to those of the person who made the antibody. So in contrast to the previous case, the patient's antibody could be tested against multiple previously characterized partial D RBCs. If the patient's antibody failed to react with one type of partial D RBCs, it could be inferred the patient's cells were partial D of the same type.

   At the time of this case the laboratory didn't have access to either. However, 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 other test 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 might help to gauge whether the fetus is at risk of 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.

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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 occurred at titers of 64 or below in a study at the author's laboratory (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).

It is typically stated that the critical titer of anti-D for fetal anemia should be validated in all laboratories performing the test, but few laboratories encounter enough HDFN cases to do so today. 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 may not extrapolate 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, such as a fetal middle cerebral artery blood velocity determination by Doppler ultrasound, 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.