Case report: mixed-field agglutination in a patient with a weak D antigen presenting as a possible fetal-maternal hemorrhage

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A gravida 1, para 0, 17-year-old black female was found on prenatal testing to be group O, D^+. The latter test showing many unagglutinated cells. Because of the mixed-field appearance, the patient was thought initially to have had a fetal-maternal hemorrhage. Additional red cell typings were performed, but no other apparent mixed-field reactions were observed. The Kleihauer-Betke test and hemoglobin electrophoresis indicated that the mixed-field agglutination was not due to a fetal-maternal hemorrhage. Thus, the finding of a mixed-field D typing could be explained best by a weak D antigen. Immunohematology 1992;8:77-78.

Mixed-field reactions may occur when there is more than one population of red blood cells (RBCs) present. This can be seen in genetic or transfusion-induced chimeras, and in cases where there has been a fetal-maternal bleed. Mixed-field reactions may also occur in D-variant individuals or in cases where there is a low affinity between antigen and antibody, i.e., when there are too few antigen sites available to combine with the corresponding antibody or when the antibody is low titer.1-4

Weakened forms of the D antigen can be due to the following: C and D position effect (i.e., C in trans position to D, resulting in a weakened D antigen expression); genetically transmitted D^+; and, finally, the unusual D-variant individuals whose red cells show weak reactivity with anti-D reagents and may appear to be similar to the low grade D^0.2-5

Case Report

A blood sample from a 17-year-old black prenatal patient (nine months gestation) was referred to our reference laboratory because of mixed-field reactions in D typing. Tests with four slide and modified tube anti-D reagents demonstrated weak mixed-field agglutination in the antiglobulin phase of testing, as did tests with two monoclonal anti-D reagents. (Monoclonal anti-D reagents currently available are mixtures of one monoclonal IgM anti-D and a pool of polyclonal IgG antibodies. Since the mixed-field agglutination observed was only in the antiglobulin phase, reactivity with the monoclonal reagents was most likely due to the presence of the polyclonal anti-D.)

Materials and Methods

Red cell studies

All reagents were used according to manufacturers' instructions. Routine phenotyping tests were performed on the direct antiglobulin test-negative sample using reagents from several sources (Immucor, Norcross, GA; Gamma Biologicals, Houston, TX; and Ortho Diagnostic Systems, Inc., Raritan, NJ). Positive and negative controls were run in parallel. All tests were read microscopically to identify mixed-field agglutination. Autologous cells were harvested by microhematocrit centrifugation6 and retested with the same anti-D reagents that were originally used. Cell separation with anti-D was performed at 37°C using differential agglutination coupled with sedimentation.3 The patient's RBCs were tested with five sera from patients who have been previously classified as D-variants (categories III, IV, V, and VI). The limited quantity of each serum was insufficient for adsorption and elution tests.

The Kleihauer-Betke test and hemoglobin electrophoresis were performed according to standard procedures.2,7
Adsorption and elution procedures

Adsorptions were performed using one volume of the patient's ficin-treated RBCs and one volume of commercial anti-D diluted 1:4 with 6 percent bovine albumin at 37°C for 60 minutes. The adsorptions were performed by two technologists using different anti-D reagents. The cells were washed x12, changing tubes in between each wash. Eluates were prepared using ether and Gamma Elu-kit II procedures. The elutes were tested using two different panels of reagent red cells, as were negative control supernates from the last wash prior to elution.

Results

The patient's phenotype was group O, D+, C−E-c-e+, M+N+S+s+, Fy(a+b+), Jk(a+), and I+. No mixed-field reactions were observed except for the indirect antiglobulin test for D. Cells separated by differential agglutination demonstrated the same mixed-field reactions that were noted previously when retested with the original anti-D reagents. The reactions appeared slightly enhanced when the cells were enzyme-treated, but some mixed-field appearance was still noted. No fetal hemoglobin was detected by either the Kleihauer-Betke test or by hemoglobin electrophoresis. Ficin-treated red blood cells were weakly reactive with two anti-D sera from category III and category V D-variant individuals, and were nonreactive with sera from one category IV and two category VI D-variant individuals. Positive and negative controls reacted as expected.

Discussion

This patient was referred for investigation of the mixed-field reactions in D typing. Initially, a fetal-maternal bleed was suspected. This was ruled out by the following tests:

1. Additional phenotyping demonstrated no mixed-field typings. (This is strong evidence unless the fetus has the same red cell phenotype as the mother in all but the D testing.)
2. After cell separation by differential centrifugation, both populations showed mixed-field agglutination with anti-D.
3. Differential agglutination tests with anti-D, followed by sedimentation, yielded a single population of cells that continued to demonstrate a mixed-field appearance when tested with anti-D.

4. The Kleihauer-Betke test and hemoglobin electrophoresis demonstrated that no fetal hemoglobin was present.

From the above information, we conclude the patient has a weak D antigen that cannot be attributed to a fetal-maternal bleed or any other form of chimerism. The quantity of sample available did not permit further testing. Since the patient was lost to follow-up, we were unable to classify the weak D antigen or to repeat testing after delivery.

If a fetal-maternal hemorrhage cannot be excluded, then quantitation of the fetal cells present would be very important. The point of interest in this paper is not the nature of the weak D antigen but the fact that the mixed-field appearance with anti-D could have been misinterpreted as a fetal-maternal hemorrhage. We therefore recommend that this point be kept in mind when evaluating the serology of pregnant patients presenting with mixed-field D typing.

References


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