The Second Example of Anti-Duffy

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A ten-year-old black girl with sickle cell disease developed anti-Fy² antibody. She received one unit of Fy² positive blood and had a delayed hemolytic transfusion reaction four days later. In addition she had anti-E, anti-C and anti-K antibodies. A clinical picture of autoimmune hemolytic anemia evolved with a strongly positive direct antiglobulin test and strong auto anti-I antibodies. She improved after a course of steroid therapy.

The Duffy blood group system was first described by Cutbush, Mollison and Parkin. Five antibodies belonging to this system have been described. Anti-Fy⁵ was reported by Colledge et al. Very little is known of the clinical significance of Fy² antibodies because the patient died shortly after the antibody was discovered. The report here describes a patient who had a delayed hemolytic transfusion reaction probably caused by anti-Fy⁵ antibodies.

Case Report

A ten-year-old black girl was admitted to Kings County Hospital Center because of symptoms for three days of headache and dizziness. The patient was known to have sickle cell disease (Hb electrophoresis: Hb S 96.5%, Hb A: 3.5%) and had been hospitalized several times. She had been transfused twice in the past. The last transfusion was three weeks prior to the present admission. She tolerated the transfusions well. On admission she was found to have a low grade temperature of 38.2 C and to be anemic (Hb 5.4 gm/dl).

The patient was found to be group B, D positive, e positive, e positive, C negative and E negative. Her posttransfusion serum showed weakly reacting anti-E and anti-C by the antiglobulin technique in addition to anti-Fy². The unit of blood transfused to her on admission was found to be positive for Fy² as well as for E and C antigens. Repeated cross-matching of the patient’s pretransfusion serum sample with the cells from the first transfused unit showed incompatibility. The direct antiglobulin test on the patient’s posttransfusion blood sample was positive. An ether eluate prepared from these cells showed anti-Fy² activity only. It seemed likely that the patient’s delayed hemolytic transfusion reaction was probably due to anti-Fy² although what role the anti-E and anti-C played could not be determined.

The patient continued to have spiking fever up to 40 C. She also had generalized bone and joint pains with swelling of her face, lips and abdomen. She was given steroids (prednisone 2 mgm/kg/day) for five days. This regimen resulted in gradual improvement of her facial swelling, fever and joint

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pain. Numerous cultures taken from her blood, throat and urine were negative. Serologic tests for viral infections, including mycoplasma, cytomegalovirus infections and febrile agglutinins were negative.

Six days after cessation of steroid therapy she again had fever of 40°C and bone pains. Her hematocrit had progressively dropped after the two exchange transfusions and on the 30th she was transfused with one unit of blood which was negative for Fy\(^a\), E and C. She tolerated the transfusion well.

Spiking temperature continued around 40°C and her bone pains were more severe, especially in the legs. Bone scan revealed increased radioactive uptake. Gram stains and culture of an aspiration bone biopsy specimen were negative. Culture taken from a small pustule of her right leg yielded pseudomonas and she was treated with Nafcillin and Ampicillin.

On the 37th hospital day she was found to have anti-K in addition to the anti-Fy\(^a\), anti-E, and anti-C by the indirect antiglobulin technique. The direct antiglobulin test was only positive microscopically. A unit of blood was transfused (negative for Fy\(^a\), E, C, K). There was satisfactory rise in hematocrit the next day after the transfusion. However, two days later it dropped rapidly from 23 to 12 percent. Her serum was icteric and hemoglobinuria was detected. At that time the direct antiglobulin test was 4+ with anti-IgG and broad spectrum antiglobulin reagents. An ether eluate prepared from her cells showed a panagglutinin with no specificity. Her serum was found to contain anti-C, anti-E, anti-K and strong auto anti-I reactive at room temperature, 37°C and by antiglobulin technique. The anti-Fy\(^a\) antibody had become only weakly reactive by antiglobulin technique. She was treated with prednisone, 2 mg/kg and one unit of blood (negative for Fy\(^a\), K, E, C) was transfused to her after passing the blood through a warming coil at 37°C. She tolerated the transfusion well. She required two more blood transfusions (prewarmed units negative for Fy\(^a\), E, C, K). Her fever and general condition gradually improved with appropriate antibiotic therapy and steroids. She was well upon discharge, 72 days after admission.

**Materials and Methods**

Most of the blood samples were clotted specimens. Anticoagulated blood (EDTA) was used in all elution studies and all elutions were done with the Rubin's ether method. Antibody identification was done by the indirect antiglobulin technique and, where appropriate, enzyme-treated cells were used. The methods were in conformation with standard procedures and procedures recommended by manufacturers.

**Discussion**

Anti-Fy\(^a\) was first described in an 11-year-old Negro boy who later died of leukemia.\(^4\) Anti-Fy\(^a\) is not a simple mixture of anti-Fy\(^a\) and anti-Fy\(^b\). It is one antibody directed against an antigen in the Duffy blood group system as is proved by absorption and elution studies. Its reactions are unaffected by enzymatic treatment of red blood cells. It will not react with Rh\(_{null}\) cells which have normal expression of their Duffy antigens. The anti-Fy\(^a\) in this report had all these characteristics. It is interesting to note that both patients were black and they were about the same age (one 10 years and the other 11 years old). Whether this is related to the increased usage of blood and components in the pediatric age group or that the Fy\(^a\) antigen is strongly antigenic is not clear.

About 70 per cent of American blacks are of the phenotype Fy (a−b−) but they generally do not form antibodies against the Duffy blood group system antigens.\(^12\) Issitt and Issitt\(^6\) have postulated that there are two genetic pathways in the production of the phenotype Fy (a−b−). One is due to presence of Fy\(^a\) Fy\(^b\) genes and the other due to the inability to form Duffy precursor substance (FYPS) because of the ff genes. They also postulate that Fy\(^a\) is made from CDE material under the control of Fy\(^a\) or Fy\(^b\) genes at the Duffy locus. Our patient apparently falls into the latter type.

The clinical significance of anti-Fy\(^a\) in the original report was not clear, as the patient died shortly after the new discovery. It was possible that on admission, our patient might have had a delayed transfusion reaction. She had anti-Fy\(^a\) in her serum and the weakly positive direct antiglobulin test was probably a mixed field agglutination. Unfortunately the cell sample was not sufficient for elution studies. After receiving a unit of Fy\(^a\) positive blood, the patient developed a hemolytic
transfusion reaction and disseminated intravascular coagulation. Anti-Fy\textsuperscript{a} was eluted from her posttransfusion blood sample and presumably it was the underlying cause for the reaction. However, the patient also had anti-C and anti-E in her posttransfusion blood sample and the transfused unit was positive for E and C. What role these two antibodies played in the reaction remains to be answered. Cr\textsuperscript{51} survival studies of Fy\textsuperscript{a} positive blood to this patient were not done. Anti-Fy\textsuperscript{a} disappeared almost completely at the time of her discharge from the hospital.

Autoimmune hemolytic anemia occurred in the later part of her hospital course. Serologically, autoimmune hemolytic anemia (AIHA) can be divided into the warm type where the antibodies are usually IgG and have Rh specificity, and the cold type where the antibodies are IgM or IgG and are usually anti-I, anti-P or Donath-Landsteiner antibodies.\textsuperscript{6,7,13} AIHA is a rare disease in children. In the 28 cases reported by Zuelzer \emph{et al.},\textsuperscript{14} there was no association with lymphomas or other conditions commonly associated with AIHA in adults, such as disseminated lupus erythematosus, ulcerative colitis or typhoid fever. There was a high association with cytomegalovirus infection. The cases of AIHA in his series were of the warm type and the effectiveness of steroids was variable.

Our patient probably had osteomyelitis as evidenced by the high fever, generalized severe bone pain and good response to appropriate antibiotic therapy. Repeated serologic tests for evidence of viral infections were negative. Her AIHA was of the cold type. The relationship of the clinically apparent osteomyelitis and the AIHA is not clear. She did improve with appropriate antibiotic therapy and steroid therapy. She tolerated two transfusions without evidence of hemolysis, possibly because the blood was warmed at 37°C before transfusion. The role the steroids played in her clinical course was unknown. In most patients with AIHA of the cold type high doses of steroid are ineffective in stopping the hemolysis. The fact that our patient improved clinically might be due to the fact that her infection was under control with appropriate antibiotic therapy.

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References