Delayed hemolytic transfusion reaction caused by a primary immune response

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Delayed hemolytic transfusion reactions usually occur as a result of a secondary immune response with maximal hemolysis occurring seven days posttransfusion. We report a delayed hemolytic transfusion reaction in which hemoglobinuria, anemia, and reticulocytosis developed four weeks after transfusion. The incriminated antibody, anti-C, was first detected eight weeks posttransfusion using enzyme-treated red blood cells. We conclude, that in all likelihood, this hemolytic transfusion reaction was due to a primary immune response. This case illustrates the importance of sequential testing in cases of suspected transfusion reactions. **TRANSFUSION** 1982;22:248–250.

DELAYED HEMOLYTIC transfusion reactions usually become apparent between three and 14 days posttransfusion[1] at a time when the rapidly rising antibody titer produces sufficient red blood cell destruction to cause anemia, fever, jaundice, or hemoglobinuria. There may be a further delay of three to 11 days after symptoms appear before the incriminated antibody can be identified.[2-5] This sequence is consistent with a secondary or anamnesic immune response. Almost all patients have a history of prior transfusion, pregnancy, or both. We report an unusual delayed hemolytic transfusion reaction in which hemoglobinuria developed four weeks after transfusion and the offending antibody could not be identified for an additional four weeks. This delay and the lack of prior transfusion or pregnancy strongly suggest that the reaction was due to a primary immune response.

Case Report

A 26-year-old woman delivered a healthy seven-pound male infant after an uneventful first pregnancy. The child typed as O Rh_o(D) positive. The direct antiglobulin test on the cord blood was negative, as was the mother's indirect antiglobulin test. Two hours after delivery, she developed profuse vaginal bleeding and her blood pressure became unobtainable. The patient was resuscitated with intravenous fluids. A large clot was evacuated from the vagina, the episiotomy site was resutured, and a vaginal pack was inserted. Three hours later, because the bleeding continued, she was examined under anesthesia. The uterus was empty, but a large vaginal wall hematoma and continued bleeding from the episiotomy site were found. The latter was repaired. Hematuria occurred (red cells too numerous to count on microscopic examination). A diagnosis of disseminated intravascular coagulation was made (prothrombin time 14.6 seconds, control 10.4 seconds; partial thromboplastin time 30.8 seconds, control 20.8 seconds; fibrinogen 84 mg/dl; fibrin split products $>$ 160 but $<$ 320, platelet count 74,000 per μl. The etiology of the disseminated intravascular coagulation was felt to be hypovolemic shock. Twelve units of red blood cells, six units of fresh frozen plasma, and eight units of cryoprecipitate were given. The pretransfusion antibody screen was negative, and the major and minor crossmatches were compatible. The history of prior transfusion was negative. On the day after transfusion the hemoglobin concentration rose to 10.0 g/dl, bleeding had stopped, coagulation studies were normal, the bilirubin was 0.3 mg/dl and the LDH was 300 mU/ml. The hemoglobin concentration remained stable, and was 10.4 gm/dl when the patient was discharged from the hospital on the fifth postpartum day.

Four weeks after delivery, the patient noted tea-colored urine and felt weak. Investigation revealed anemia (hemoglobin 8.0 g/dl), hemoglobinuria (3 plus hemoglobin and only 2 to 3 red blood cells per high power microscope field on urinalysis), bilirubin 1.3 mg/dl, SGOT 34 IU/ml, and SGPT 8 IU/ml. LDH, plasma hemoglobin, and haptoglobin were not done. The direct antiglobulin test of red blood cells from an EDTA sample of blood was weakly positive due to complement. An eluate was not done. Anti-Le^b was detected in the serum.

Five days later she was found to have a hemolytic anemia (hemoglobin 8.9 g/dl, reticulocyte count 17 percent, microspherocytes on peripheral blood smear, LDH 611 mU/ml). The direct antiglobulin test was negative. Anti-Le^b was again noted in the serum. The patient typed as A Rh_o(D) positive, probable genotype of R^2r. She typed as negative for Le^a, Le^b, C, Fy^a, Jk^b, and K. No mixed-field reactions were noted. Her hemoglobin remained stable, hemoglobinuria cleared, and she was discharged three days later.

Six weeks postpartum anti-Le^a was also identified in her serum. Eight weeks postpartum the patient's hemoglobin was 13.5 g/dl. Anti-C was noted, reacting 1+ with enzyme treated cells at 37°C. Incubation of the patient's serum with dithiothreitol failed to destroy this reactivity. Studies 11 weeks postpartum revealed microscopic agglutination of C-positive red blood cells in the antiglobulin phase. Six months postpartum the anti-Lewis antibodies were no longer detected but anti-C was now reactive at 37°C in albumin and 1+ in the antiglobulin phase (titer 2).

The husband typed as O Rh_o(D) positive, probable geno-

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type of R^1R^1. Although an Rh phenotype was not determined, the child is presumed to be heterozygous for C.

Discussion

In animal models, antibody will be detected approximately two weeks following an initial injection of antigen. The first, primary response, antibody produced is IgM, and is followed shortly by production of IgG. This IgG antibody usually has low avidity and is of low titer. A second challenge with antigen will lead to synthesis of a higher concentration of high avidity IgG antibody beginning after a lag of about two days and peaking at about one week. A much smaller dose of antigen is required to provoke this secondary or anamnestic immune response.

The immunologic events which follow an initial exposure to red blood cell transfusion in humans are less clear. Mollison[6] noted a collapse curve beginning as early as 10 days in as many as 30 percent of recipients of small aliquots of red blood cells. In contrast, only about 5 percent of recipients of a unit of whole blood develop a collapse curve, the onset again occurring as early as 10 days. However, not all of these recipients with shortened survival of transfused red blood cells develop antibody, nor can an anamnestic response be shown following a repeat challenge. On the other hand, red blood cell survival may be normal as late as six weeks after an initial transfusion even in recipients who develop alloantibody as a result of this initial challenge.[6] Antibodies may be undetectable until two to six months after a primary challenge[7] or may not be evident until after a second challenge is given.

Clinically, it is often difficult to differentiate between a primary and secondary response. The history may be unreliable or may be equivocal. In our patient there is a chance that sensitization could have occurred from C positive fetal cells crossing the placenta during the third trimester of pregnancy. Primary sensitization to the D antigen occurring during pregnancy accounts for some of the so-called Rh immune globulin failures. However, since C is far less immunogenic than D, the development of sensitization during pregnancy due to C would be expected to be quite rare. In addition, the long interval between transfusion and the development of hemolysis (four weeks) and the long interval between hemolysis and antibody detection (an additional four weeks) speak against an anamnestic response following transfusion. Also, the detection of antibody with enzyme-treated red blood cells several weeks before it is demonstrable in the antiglobulin phase is a pattern seen after primary immunization to the D antigen.

The time interval between transfusion and symptoms does not always permit distinction between a primary and secondary immune response with certainty. While most delayed hemolytic transfusion reactions occurring as a result of an anamnestic response, are recognized within 14 days of transfusion, reactions occur as late as 21 days in previously transfused individuals[8] and as early as nine to 15 days in persons without prior history of transfusion.[2] Three of 40 patients with delayed hemolytic transfusion reactions reported by Croucher[1] had anti-D first detectable at 38, 50, and 58 days posttransfusion. Although no comment was made regarding prior exposure to red blood cell antigens, the antibodies presumably were the result of a primary immune response. The presence of IgM antibody is not essential for the diagnosis of a primary immune response. Not all individuals who develop anti-D can be shown to go through an initial phase of saline reacting IgM antibody production.[6,9]

There are several reasons why delayed hemolytic transfusion reactions following a primary immune response are rarely detected clinically. The antibody initially produced is of low titer and low avidity. The rate of red blood cell destruction may be slow and prevent observable symptoms from occurring. Many of the transfused red blood cells may no longer be circulating when appreciable quantities of hemolytic antibody are produced. This would be particularly true in patients receiving only a small number of transfused red blood cells. Most patients are not followed closely after the initial beneficial effects of transfusion have occurred.

At the time the patient presented to us, she had destroyed all twelve transfused red blood cell units since she typed as negative for C, Fy^a, Jk^b, and Kell without any evidence of mixed-field reaction. Only eight of the 12 units would be expected to be C-positive by chance alone. However, we were not able to test the donor units. Apart from anti-Lewis antibody no other antibodies were detected. It seems unlikely that the anti-Lewis antibodies could be incriminated as the cause of the reaction. One would expect Lewis substance to be eluted from the transfused red blood cells during the storage, leaving donor cells Le(a−b−) and the eluted antigen to neutralize the Lewis antibody as it was produced. We are unable to explain why the patient's red blood cells were weakly and transiently coated with complement at the time of hemolysis. Although Rh antibodies which fix complement have only rarely been reported,[10,11] six of six Rh antibodies studied by Frank[12] were able to cause complement sensitization of red blood cells. Thus, it is possible that anti-C was responsible for causing complement coating of the patient's red blood cells at the time of hemolysis. Unfortunately, an eluate was not done.

Until recently, anti-C was thought to be an infrequent cause of the delayed hemolytic transfusion

reaction. Howard[13] and Pineda[8] failed to see an example in their series of patients. Pickles[14] *et al.* recently reported five cases of delayed hemolytic transfusion reactions due to anti-C occurring five to nine days posttransfusion. The case reported here is similar in that hemoglobinuria occurred in spite of low levels of antibody. It differs in that a long latent period preceded the reaction. Croucher[1] noted that ten percent of delayed hemolytic transfusion reactions were due to anti-C and in almost all cases there was hemoglobinuria. Hemoglobinuria accompanying delayed hemolytic transfusion reactions is far more common than is appreciated, occurring in 30 to 50 percent of all cases.[8,13] The presence of hemoglobinuria suggests that complement fixation may be occurring even when antibodies which are not usually considered capable of fixing complement, such as anti-C, are involved.

Unexplained anemia, fever, jaundice, or hemoglobinuria occurring even several weeks following transfusion should prompt an investigation for a delayed hemolytic transfusion reaction. The importance of performing sequential tests to identify the causative antibody in such cases is well-illustrated by our case, since anti-C was not demonstrable until four weeks after the episode of hemolysis which was eight weeks posttransfusion.

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