Acute hemolytic transfusion reaction due to anti-Le\textsuperscript{b}

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BACKGROUND: Anti-Le\textsuperscript{b} is usually a clinically insignificant antibody of immunoglobulin M subclass most often found in the sera of pregnant women or individuals that are Le(a\textsuperscript{–}b\textsuperscript{–}). We report a case of an acute hemolytic transfusion reaction due to a hemolytic anti-Le\textsuperscript{b} that was not seen in the pretransfusion antibody detection test, but was strongly reactive in posttransfusion testing.

CASE REPORT: A 30-year-old African-American woman with metastatic renal cell carcinoma was receiving chemotherapy. She was anemic with hemoglobin (Hb) of 7.2 g/dL and had a negative antibody detection test by the solid-phase red blood cell adherence method. She was transfused with 2 RBC units without incident. Nine days later her Hb was 7.9 g/dL again with a negative antibody detection test. Transfusion of an additional RBC unit was begun. During the transfusion she developed chills, nausea, hypertension, and red-brown urine. The posttransfusion sample plasma was grossly hemolyzed with a strongly positive direct antiglobulin test (DAT) by gel. By comparison the pretransfusion plasma was normal appearing and the DAT was weaker. The eluate was negative on both occasions. Anti-Le\textsuperscript{b} was detected in the posttransfusion sample by MTS gel (Ortho Diagnostics). Both RBC units she had received before the RBC unit that caused the reaction were Le(b\textsuperscript{+}) as was the implicated RBC unit.

CONCLUSION: This case illustrates that anti-Le\textsuperscript{b} which is usually clinically insignificant can occasionally cause severe hemolytic transfusion reactions. Only three other reported cases of anti-Le\textsuperscript{b} causing hemolytic transfusion reactions could be found in the literature, two of which were in abstract form only.

Lewis antibodies are usually clinically insignificant antibodies most often found in the sera of pregnant women or individuals that are Le(a\textsuperscript{–}b\textsuperscript{–}). Both anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} are usually “naturally occurring” and of the immunoglobulin (Ig)M class, although IgG anti-Le\textsuperscript{a} has been reported. These antibodies generally are reactive from 4 to 37°C. Anti-Le\textsuperscript{a} and anti-Le\textsuperscript{b} may rarely cause hemolysis in vivo but have not been implicated in hemolytic disease of the fetus or newborn because the Lewis antigens are poorly formed on fetal and neonatal red blood cells (RBCs) and also because of their preponderance of IgM class.

Lewis blood group system antigen expression is made possible by two different genes: the Le gene on Chromosome 19, which codes for a fucosyltransferase (FUT3), and the Se (secretor) gene on the same chromosome, which encodes a different fucosyltransferase (FUT2). Both genes are expressed in glandular epithelia and have dominant alleles (Le and Se, respectively) coding for enzymes with fucosyltransferase activity and recessive alleles (le and se, respectively) that are nonfunctional. The Le gene enzyme (FUT3) adds a fucose moiety to the subterminal sugar of a precursor substance oligosaccharide to create the Le\textsuperscript{a} antigen. If the Se gene is also present another fucose is added to the Le\textsuperscript{a} substance producing the Le\textsuperscript{b} antigen. Unlike most other blood group systems, the Lewis blood group system antigens are secreted into the plasma and then adsorbed onto the surface of RBCs.

Hemolytic transfusion reactions are rare with anti-Le\textsuperscript{b}. We report a case of an acute hemolytic transfusion reaction due to anti-Le\textsuperscript{b}.

ABBREVIATIONS: IRL = immunohematology reference laboratory; SPRCA = solid-phase red blood cell adherence assay.
reaction due to a hemolytic anti-Le\textsuperscript{b} that was not seen in the pretransfusion antibody detection test when a solid-phase method (solid-phase RBC adherence assay [SPRCA]) was used, but was strongly reactive in posttransfusion testing using a gel method.

**CASE REPORT**

A 30-year-old African-American woman presented in her last month of a twin pregnancy with flank and abdominal discomfort. Patient chart indicated at least one previous pregnancy and no previous history of transfusion. A CT scan performed postpartum showed a large mass in the left kidney with left supraclavicular lymphadenopathy. After delivery she underwent a nephrectomy and lymph node dissection, which showed renal cell carcinoma with metastases to the supraclavicular and intraabdominal lymph nodes. While receiving treatment with radiation and chemotherapy, she developed anemia (hemoglobin [Hb] 7.2 g/dL) and a transfusion was requested. Pretransfusion testing (two separate samples tested) showed a blood type of B, D\textsuperscript{1} with a negative antibody detection test (Capture-R Ready-Screen, Immucor, Norcross, GA) and she received 2 electronically cross-matched B, D\textsuperscript{1} RBC units without incident. She received a better-than-expected RBC increment with the transfusion (Hb 11.0 g/dL the following day). However, 9 days later her Hb was 7.9 g/dL and she was weak and fatigued. Another RBC transfusion was requested and testing on a new sample again showed her blood type as B, D\textsuperscript{1} with agglutination noted with the B cells in the reverse grouping. This was thought to be due to a cold agglutinin and no further investigation was performed. Her antibody detection test by the same method was negative and again RBC units were electronically cross-matched for her. The second unit was being infused she developed chills, nausea, hypertension, and brown urine, suggestive of an acute hemolytic transfusion reaction. The RBC transfusion was discontinued and a blood sample was sent to the laboratory for transfusion reaction work-up.

**RESULTS**

The posttransfusion sample plasma was grossly hemolyzed (see Fig. 1) with a strongly positive DAT by gel (ID-MTS, Ortho Clinical Diagnostics, Raritan, NJ), IgG = 1+, C3 = 3+. By comparison the pretransfusion plasma was normal in appearance (see Fig. 1) with a weaker DAT, IgG = 1+, C3 = 2+. The eluate was negative on both occasions (see Table 1).

Anti-Le\textsuperscript{b} was detected in the pre- and posttransfusion sample by MTS gel. Using the SPRCA method both the pre- and posttransfusion reaction samples were equivocal in Cell 1 of the two-cell screen but were found to be negative when the antibody identification panel was used. Both RBC units she had received before the RBC unit that caused the reaction were Le(b+) as was the implicated RBC unit. Since a hemolytic transfusion reaction to anti-Le\textsuperscript{b} is extremely rare, the sample was sent to the immunohematology reference laboratory (IRL) for investigation of possible drug-dependent antibodies against cefepime, an antibiotic she received that day. Drug-dependent antibody studies were negative. The IRL confirmed the anti-Le\textsuperscript{b}. The anti-Le\textsuperscript{b} reacted at all phases of testing by low-ionic-strength saline and also by polyethylene glycol and by gel methods. By tube methods the anti-Le\textsuperscript{b} showed in vitro hemolysis. Evaluation of the patient’s 0.01 mol/L dithiothreitol-treated plasma suggested that the anti-Le\textsuperscript{b} was IgM class only, with a titer of 256. In addition anti-Le\textsuperscript{a} optimally reactive after 20-minute room temperature incubation was detected (tube method). The anti-Le\textsuperscript{a} was not detected at immediate spin in tube or by gel methods. The patient recovered from this episode and subsequently received Le(b–) RBCs without incident.

**DISCUSSION**

The following evidence strongly suggests that this patient’s hemolytic transfusion reaction was caused by anti-Le\textsuperscript{b}: 1) The antibody was hemolytic in vitro, a property almost all Lewis antibodies that are clinically significant possess. 2) The units she received at the time of the first transfusion were positive for Le\textsuperscript{b} as was the unit implicated in the hemolytic transfusion reaction. 3) Her phenotype was Le(a–b–), the most common phenotype that produces Lewis antibodies. 4) Drug-induced antibodies to an antibiotic (cefepime) she was receiving concomitantly were excluded. It is possible that she was sensitized to Le\textsuperscript{b} at the time of the first transfusion and then developed an
anamnestic response to the implicated unit. This is likely because the pretransfusion sample (just before the reaction but after the initial transfusion) also showed the implicated antibody by gel that was not detected by SPRCA. Although she did have anti-Lea, an antibody that could also be implicated in a hemolytic transfusion reaction, this antibody was not hemolytic in vitro, and the units transfused were negative for the antigen. The SPRCA system is not designed to detect IgM antibodies; therefore, when investigating unusual results it is important to know the limitations of the test system(s) being used.

A hemolytic transfusion reaction caused by anti-Leb is extremely rare. We could only find three other cases in the literature. All three previously reported were acute and delayed hemolytic transfusion reactions, although undoubtedly others have been detected (J. Moulds, personal communication, 2012). It is of note that in a recent review of the literature, only three published hemolytic reactions to anti-Lea were cited, even though the perception is that hemolytic reactions caused by anti-Lea are more common than those caused by anti-Leb. In addition to showing that anti-Leb can be hemolytic and cause a transfusion reaction, this case illustrates that antibody detection with one method is not always comprehensive, and a negative antibody detection test does not always exclude incompatibility and possible hemolysis. It also shows the importance of thoroughly investigating ABO typing discrepancies since the antibody thought to be a cold agglutinin that was not further investigated by the hospital laboratory was almost certainly the hemolytic anti-Leb and not the anti-Lea, as the anti-Lea that was later detected by the IRL was not reacting in tube at the immediate-spin phase.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES