Severe intravascular hemolysis due to autoantibodies stimulated by blood transfusion

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Autoantibodies may cause severe hemolytic anemia, but only rarely are they the cause of a hemolytic transfusion reaction due to the destruction of transfused allogeneic blood. In two patients, autoantibody was detected shortly after blood transfusion. The first case was a D-negative patient who produced an autoanti-Ce and subsequently developed hemoglobinuria and hyperbilirubinemia. The second case was a patient who developed an autoanti-Wr\(^b\) that caused severe hemolysis that resulted in death. Immunohematology 1996;12:80–83.

Red cell hemolysis due to autoantibodies is a rare but dangerous phenomenon. Autoantibody specificities have been described within the ABO,\(^1\) RH,\(^2\) LW,\(^3\) I,\(^4\) MNS,\(^5,6\) KEL,\(^7,8\) FY,\(^9\) and GE\(^10\) blood group systems. However, the specificity of warm-reacting IgG autoantibodies is most frequently related to the Rh system.\(^2\) Autoanti-Wr\(^b\) has also been described, most often in combination with Rh autoantibodies.\(^11\) In this article, we report on two patients in whom the production of an autoantibody that became apparent shortly after transfusion caused severe intravascular hemolysis.

Case Report

Case 1

A 57-year-old man with a 9-year history of chronic lymphocytic leukemia was being treated with steroids and 2-chlorodeoxyadenosine (2-CDA) after becoming refractory to chlorambucil. He also was taking warfarin for a previous pulmonary embolus. His blood group was O, D–C–C\(^w\)–E–c+e+. He had had many previous transfusions with D–C–E– red blood cells (RBCs). Samples from the patient were sent to the Regional Transfusion Centre, National Blood Service, SW (NBS-SW) because of difficulties in crossmatching. The last transfusion was 2 weeks prior to this finding. No previous transfusion or serologic problems had been encountered apart from a weakly positive direct antiglobulin test (DAT).

Case 2

A 43-year-old woman was being investigated at a local hospital for severe menorrhagia. She had a history of idiopathic thrombocytopenic purpura and subsequently underwent a splenectomy. On two previous occasions (5 years and 4 years prior to this admission), the DAT was found to be negative at the NBS-SW, and crossmatches were compatible. Hemoglobin (Hgb) levels on those two occasions were 10.7 g/dL and 9.0 g/dL, respectively. On this admission her Hgb was 6.9 g/dL. Her blood group was A\(_2\), D+C+C\(^w\)–E–c+e+, and the DAT was strongly positive with anti-IgG. The serum contained a weak, nonspecific antibody detected versus bromelain-treated RBCs and by the indirect antiglobulin test (IAT). Anti-A\(_1\) was ruled out. An eluate prepared from the patient’s cells showed strong activity against all panel RBCs. Six units of group A, D+c– RBCs were crossmatched and found to be very weakly incompatible by the IAT. She was then slowly transfused with all six units of blood and was discharged the following day at her own request. Two days following transfusion, she was admitted as an emergency, having become very drowsy at home. She was markedly jaundiced and her Hgb was 5.8 g/dL. Her condition deteriorated rapidly and she died 4 hours later from acute renal failure secondary to severe hemolysis.

Materials and Methods

Antibody identification and crossmatching

Panel RBCs and antiglobulin reagent for antibody identification and crossmatching tube methods were obtained from the NBS-SW. The panel cells were ready for use either suspended in a low-ionic-strength preservative medium (LISS) or pretreated with bromelain and suspended in a standard preservative medium. Crossmatching was performed using a LISS IAT, incorporating a 37°C reading phase after incubation and before washing. Results for both IATs and DATs were graded as 1+ (very weak) to 5+ (very strong) for tube
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Manfacturers’ panel cells from DiaMed-GB, Ltd. (Dalkeith, Scotland), and Ortho Diagnostic Systems, Ltd. (Amersham, England), were also used in Case 1 for the ID gel12 and BioVue™ column agglutination13 methods, respectively. The presence of alloantibodies of common specificities was excluded in Case 1 using C-e+ and/or C-e− RBCs.

Identification of the specificity of the autoantibody in Case 2 was performed using a panel of reconstituted frozen RBCs negative for high-frequency antigens including the only known example (Fritz) of the Wr(a+b−) phenotype. The auto nature of the antibody was confirmed by testing the patient’s DAT-negative RBCs, which had been stripped of antibody using chloroquine diphosphate (CDP), for the Wrb antigen.14

Red cell studies

Monospecific antiglobulin reagents for the DAT were obtained from the NBS-SW and from Biotest UK, Ltd. (Solihull, England). Rh phenotyping was performed using saline and enzyme methods in 96-well microplates supplied by the NBS-SW, predispensed with reagents and controls. Reagents from the NBS-SW and Biotest were used to phenotype the patient’s pretransfusion RBCs in Case 2. Mouse IgG2a monoclonal anti-Wrb (BRIC 14) was obtained from the International Blood Group Reference Laboratory (Bristol, England).

Adsorption and elution studies

Case 1. Autoadsorption was performed by incubating bromelain-treated RBCs and serum from the patient at 37°C. After centrifugation, the supernate was incubated with a fresh aliquot of the patient’s bromelain-treated RBCs. The supernate, after a second centrifugation, was then harvested.

Case 2. An acid glycine method15 was used to prepare eluates (pre- and posttransfusion) from the patient’s RBCs. The last wash supernates from the cells were retained and tested as negative controls. The possible presence of additional antibodies, which could not be excluded by virtue of the negative reaction with Wr(b−) RBCs, was investigated in the posttransfusion serum by a seven-fold adsorption with selected RBCs (not enzyme-treated). These RBCs were Wr(a+b+) and had identical expression of D, C, E, c, e, M, N, s, P1, Lea, Leb, K, k, KpA, Fya, Fyb, Jka, Jkb, and Luα antigens to the Wr(b−) cells used.

IgG subclass determination

O, D+C−E+c−e−(R2R2) RBCs were sensitized using an acid glycine eluate prepared from the Wr(b+) cells that had been used to adsorb the posttransfusion serum in Case 2. The DAT on these cells was then investigated with monoclonal anti-IgG subclass antibodies (Unipath, Bedford, England) diluted to 1:20 by adding two volumes of anti-IgG1, -IgG2, -IgG3, or -IgG4 to one volume of the sensitized cells, centrifuging at 1,000 g for 30 seconds, and reading for agglutination grade.

Results

Case 1

The NBS-SW found a negative DAT and an auto- and panagglutinin active at 37°C versus bromelain-treated RBCs, but not by the LISS IAT. The referring hospital had found the antibody screen positive using a BioVue™ IAT. Autoadsorption using bromelain-treated cells removed all activity, and three group O, D−C−E− units of blood were found to be crossmatch-compatible with the unadsorbed serum using a LISS 37°C and IAT.

Two of the units were transfused uneventfully. During the transfusion of the third unit, the patient’s urine suddenly became dark red. The pulse and blood pressure remained normal, though his temperature was mildly elevated at 37.5°C. Serum haptoglobin was absent and free hemoglobin was present in the urine. RBCs from a posttransfusion sample were strongly DAT-positive, showing complement (C3d) coating only. All three units of blood were subsequently found to be compatible at LISS 37°C and IAT.

Serologic and biochemical findings over the following 61 days are summarized in Table 1. No alloantibodies could be demonstrated during this period using C-e+ and/or C-e− RBCs, but auto- and panagglutinins were. The autoagglutinins predominantly demonstrated anti-Ce specificity, but anti-C and anti–e−, as single specificities, were also demonstrated, but only with the BioVue™ test (Table 1). The patient was kept on an intravenous drip for two days because of mild renal impairment, which subsequently resolved. As he was symptomatic with his anemia, two units of group O, C–D−e− reconstituted frozen blood were transfused 16 days after the first incident without any problems. Twenty-seven days after the hemolytic episode, the DAT was 2+, anti-Ce was still detectable, but the bilirubin had dropped to 14 μmol/L. Sixty-one days after the
patients whose RBCs could be shown to be sensitized with autoantibody following a delayed hemolytic transfusion reaction. However, in these patients, alloantibodies (anti-C+c and anti-Jkα) could also be demonstrated and were assumed to be responsible for the hemolysis. No alloantibodies could be demonstrated in our two cases.

The pathophysiology underlying the brisk hemolytic process may be explained by the ability of some individuals’ immune systems to produce autoantibodies very rapidly and efficiently and, in the cases we described, the activation of the complement system (as indicated by the posttransfusion DAT) may also have been a contributing factor. The patient described in Case I was receiving treatment with 2-CDA, but there is no evidence that this drug caused the hemolysis.

In Case 1, the DAT performed on the patient’s RBCs was negative prior to transfusion, and an “enzyme-only” autoantibody was detected but presumed to be of no clinical significance. After transfusion, a “new” autoantibody was detected, which increased in potency after 5 to 27 days. The apparent specificity of this antibody was anti-C, anti-Ce, or anti-e, depending on the antiglobulin method in use and the time of testing. Even though the patient had not been transfused with C+ blood, the presence of anti-C and anti-Ce was consistent with other examples of autoantibodies in Rh-negative (cde/cde) patients with a positive DAT. Such autoantibodies have a mimicking specificity, and can be shown, using adsorption-elution experiments, to be reactive with cells that lack the antigen against which the antibody appears to be directed (using routine antibody identification procedures). This “broad” specificity of the antibody can be explained now that a greater understanding of the spatial relationships between Rh epitopes is being revealed by molecular biology techniques. Recently it has been shown that Rh C/c and E/e polymorphisms are carried on the same mRNA, suggesting that these antigens are carried by the same polypeptide chains (Rh30A or Rh30C).

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It is, therefore, not unreasonable to postulate that autoantibodies with a “broad” specificity may not distinguish between the C and e epitopes in serologic tests.

Issitt et al. reported that, in a study of 87 patients with autoimmune hemolytic anemia, the sera of two patients contained only autoanti-Wrβ, although this specificity could be found as a separate component in 32 other sera. In some of these patients, the autoanti-Wrβ was thought to be responsible for significant RBC

**Table 1. Serologic findings and serum bilirubin levels in Case 1 following initial transfusion of three units of O, D-negative blood**

<table>
<thead>
<tr>
<th>Days after transfusion</th>
<th>Direct antiglobulin test</th>
<th>Indirect antiglobulin test methods</th>
<th>Bilirubin μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tube</td>
<td>DiaMed</td>
<td>BioVuc</td>
</tr>
<tr>
<td>1</td>
<td>4+</td>
<td>(C3d)</td>
<td>Panagglutinin</td>
</tr>
<tr>
<td>5</td>
<td>5+</td>
<td>(C3d)</td>
<td>anti-Ce</td>
</tr>
<tr>
<td>15</td>
<td>2+</td>
<td>(C3d)</td>
<td>anti-Ce</td>
</tr>
<tr>
<td>27*</td>
<td>2+</td>
<td>(IgG)</td>
<td>anti-Ce</td>
</tr>
<tr>
<td>61</td>
<td>1+</td>
<td>(IgG)</td>
<td>NAD†</td>
</tr>
</tbody>
</table>

*Patient transfused with two units of O, C-c– blood
†No antibodies detected
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destruction. However, Case 2 is the first report, as far as we are aware, of an autoanti-Wr\textsuperscript{b} causing severe hemolysis leading to death. As in Case 1, there was a marked increase in the serologic activity of the autoantibody shortly after transfusion.

Clinicians need to remain alert to this additional, albeit very rare, complication of blood transfusion.

References

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