Although antibodies to the Di\textsuperscript{b} antigen are generally considered to be of potential clinical significance, we know of no reports assessing the clinical significance of anti-Di\textsuperscript{b} (in vivo or in vitro). We report on an 88-year-old Japanese male gastrectomy patient who had alloanti-Di\textsuperscript{b}. After transfusion of two Di(b–) units, three Di(b+) units had to be transfused, and there were no clinical signs of acute hemolysis. Di(b+) RBC survival was followed retrospectively by flow cytometry. On days 1, 7, and 10, the percent of circulating Di(b+) RBCs was determined to be 39, 30, and 11 percent, respectively, compared to an expected 49, 43, and 41 percent based on calculations. The Di(b+) RBCs appear to have been tolerated for about 6 days, then were removed from the circulation. Direct anti-IgG tests were 1–2+ mixed field with all post-transfusion samples. Monocyte monolayer assays (MMAs), which have been reported to predict the clinical significance of alloantibodies, gave borderline positive results. MMA results using sera from days 0, 3, and 9 were 2.7 and 5.5, 0.8 and 4.8, and 3.0 and 3.7 percent, respectively, without and with added fresh normal serum as a source of complement (clinical significance = > 3% reactivity). The subclass of the anti-Di\textsuperscript{b} was IgG1. This is the first documentation of the clinical significance of an anti-Di\textsuperscript{b}. Immunohematology 1997;13:93–96.

Key Words: anti-Di\textsuperscript{b}, red cell survival, flow cytometry, transfusion reaction

Anti-Di\textsuperscript{b} was first described by Thompson et al.\textsuperscript{1} in the serum of two unrelated Mexican-Indian women. Both women had delivered multiple infants without clinical evidence of hemolytic disease of the newborn (HDN). One of these patients had an apparent delayed hemolytic transfusion reaction one week after receiving three units of serologically compatible blood. Although many other examples of anti-Di\textsuperscript{b} have been found, and there have been reports of mild to moderately severe HDN,\textsuperscript{2–5} there have been no detailed reports of transfusion reactions due to anti-Di\textsuperscript{b}. We are reporting the first assessment of the survival of Di(b+) red blood cells (RBCs) in a patient with anti-Di\textsuperscript{b} whose urgent clinical condition necessitated the transfusion of incompatible RBCs.

RBC survival studies using radioisotopes, such as Cr\textsuperscript{51}, are not easy to arrange even in large metropolitan areas. As an alternative, flow cytometric methods have been utilized to measure RBC destruction.\textsuperscript{5,8} Flow cytometric methods rely on the antigenic differences between the recipient and transfused donor RBCs in order to quantitate the survival of the transfused RBCs. Flow cytometry has been used to measure the survival of antigen-positive RBCs in the presence of the corresponding antibody (e.g., anti-c\textsuperscript{8}, anti-Jk\textsuperscript{a},\textsuperscript{9} anti-Ge1,\textsuperscript{2} anti-Js\textsuperscript{b},\textsuperscript{11} anti-Lu\textsuperscript{6},\textsuperscript{12} and anti-B\textsuperscript{13}). It should be emphasized that seven of the eight flow cytometry studies cited above were carried out in only two laboratories (the American Red Cross in Los Angeles\textsuperscript{6,9,11,13} and at the New York Blood Center\textsuperscript{7,10,12}).

Case Report

An 88-year-old Japanese male with gastric cancer was admitted for a gastrectomy. At the hospital, the patient’s RBCs typed group B, D+. All antibody screening and panel identification cells were strongly reactive by the antiglobulin test. The direct antiglobulin test (DAT) was negative. Our reference laboratory identified anti-Di\textsuperscript{b} in the patient’s serum. The patient’s RBCs typed Di(a+b–). Two Di(b–) units were requested through the American Red Cross Rare Donor Registry. Due to a shortage of Di(b–) RBCs in the United States, the units were obtained from the Canadian Red Cross.

The patient’s preoperative hemoglobin was 9.3 g/dL. Both Di(b–) units were transfused during surgery. The next morning, the patient’s hemoglobin was 5.8 g/dL. As additional Di(b–) units were not immediately available, three units of Di(b+) RBCs were transfused and the patient showed no clinical signs of acute hemolysis. Posttransfusion samples were collected for retrospective survival studies by flow cytometry. The patient’s posttransfusion hemoglobin and total bilirubin levels are shown in Table 1. There were no signs of renal failure. On the 11th day after the transfusion of the Di(b+) units, the patient received 1 Di(b–) unit. Six days later, the patient’s hemoglobin was 8.3 g/dL and he was subsequently discharged.

Materials and Methods

Standard serologic tests were performed as previously described.\textsuperscript{14} Direct and indirect antiglobulin tests were performed with anti-IgG (The American National Red
Flow cytometry studies

Flow cytometric studies were performed as previously described. Control RBCs included Di(b+) RBCs, the patient's pretransfusion Di(b–) RBCs, and mixtures of these Di(b+) and Di(b–) RBCs. One hundred μL of control and the patient's posttransfusion RBCs were incubated with 200 μL of anti-Dib (the patient's pretransfusion plasma) at 37°C for 30 minutes. After washing \( \times 4 \) with phosphate-buffered saline (PBS), pH 7.3, the RBCs were resuspended in 100 μL of PBS and transferred to a clean tube. Ten μL of fluorescein isothiocyanate (FITC)–conjugated goat Fab anti-human IgG (Organon Teknika Corporation-Cappel Research Products, Durham, NC), diluted 1:10, were added and the RBCs were incubated for 30 minutes at room temperature, washed, and resuspended in PBS for analysis on a FACSort flow cytometer (Becton Dickinson, San Jose, CA). Prior to analysis, each RBC suspension was mixed using a fine bore pipet and by vortex. Ten thousand events were collected using logarithmic amplification. The percentage of Di(b+) RBCs in each sample was determined by electronically setting a marker on the green fluorescence histogram between the two populations of RBCs.

Calculations for RBC survival assessment

To assess the survival of the transfused Di(b+) RBCs, the flow cytometry results were compared to results that were calculated assuming that the transfused RBCs survived normally. From the patient's height and weight, an estimate of the patient's blood volume was determined. An estimate of the patient's RBC volume was determined by multiplying the number of units transfused \( \times \) the approximate RBC volume in a unit of packed cells (180 mL). The amount of RBCs transfused was then corrected for survival over the sampling period using a normal Cr\( ^{51} \) survival curve. Finally, the percentage of transfused RBCs expected to be present in each sample was determined by dividing the estimated amount of transfused RBCs by the patient's estimated RBC volume.

Monocyte monolayer assay

The monocyte monolayer assay (MMA) was performed as described previously. Di(b+) and Di(b–) RBCs were sensitized with the patient's serum, with and without the addition of fresh normal serum (FNS) as a source of complement, and then incubated with a monolayer of monocytes. Reactivity of monocytes with sensitized RBCs was expressed as the percentage of adherence and phagocytosis when 600 monocytes were counted.

IgG subclass determination

IgG subclassing was performed by a capillary antiglobulin test. Anti-IgG1, anti-IgG2, anti-IgG3, and anti-IgG4 were obtained from the Netherlands Red Cross and were standardized as reported previously.

Results

The DAT and anti-Dib titration results are shown in Table 1. The DAT went from negative on the pretransfusion sample to 1–2+ mixed field on all posttransfusion samples. Evaluation of the posttransfusion DAT was not helpful in determining the survival of the transfused Di(b+) RBCs. The titer (score) of the serum anti-Dib increased significantly from 2 (7) on day 3 to 64 (43) on day 6. This coincides with an increase in total bilirubin from 1.4 mg/dL on day 3 to 3.4 mg/dL on day 6.

Flow cytometry studies

As seen in Figure 1, good separation was obtained on the green fluorescence histogram between the Di(b+) RBC population on the right and the Di(b–) RBC population that constitutes the peak on the left. In Table 2, the percentage of transfused Di(b+) RBCs that was measured by flow cytometry is compared to the expected percentage that was calculated. The percentage of transfused Di(b+) RBCs held steady up to day 6; starting on day 7, decreased survival of these RBCs was noted. We have no explanation for the difference between the measured 39 percent and the expected 49 percent survival of Di(b+) RBCs on day 1. However, the expected values are based upon estimates and calculations.

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Table 1. Laboratory results on blood samples drawn from the patient with anti-Dib, prior to and up to 17 days after transfusion of Di(b+) RBCs

<table>
<thead>
<tr>
<th>Day</th>
<th>Hb (g/dL)</th>
<th>T. bili (mg/dL)</th>
<th>LDH (U/L)</th>
<th>DAT anti-IgG Titer/Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>5.8</td>
<td>0</td>
<td>4/14</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.3</td>
<td>1.4</td>
<td></td>
<td>1+mf†</td>
</tr>
<tr>
<td>2</td>
<td>8.5</td>
<td>1.4</td>
<td></td>
<td>1+mf</td>
</tr>
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<td>6</td>
<td>9.2</td>
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<td>673</td>
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</tr>
<tr>
<td>7</td>
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<td>5.9</td>
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<tr>
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<td></td>
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<tr>
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</tr>
<tr>
<td>16</td>
<td>7.8</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>8.3</td>
<td></td>
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</tbody>
</table>

*The day three Di(b+) units were transfused (results from that day are pretransfusion)
†Mixed field

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Cross, Washington, D.C.). Antibody titers were scored using a scale of 1–10.
Survival of Di(b+) red blood cells

MMA and IgG subclass

MMA results on samples from days 0, 3, and 9 indicated clinical significance only when FNS (as a source of complement) was added to the patient’s serum during the RBC sensitization phase. Results without addition of FNS were 2.7%, 0.8%, and 3%, respectively (a total reactivity of > 3% = potential clinical significance). After addition of FNS, results were 5.5%, 4.8%, and 3.7%, respectively. Previous reports have suggested that such weakly positive (borderline) results indicate potential shortened RBC survival. The subclass of the anti-Di(b+) was IgG1.

Discussion

This case provides the first documentation of the clinical significance of anti-Di(b+) for transfusion. Although this patient initially tolerated the transfusion of incompatible Di(b+) units, the decreased hemoglobin and increased bilirubin after 6 days correlated with the shortened RBC survival measured by flow cytometry. Previously, we reported an anti-Yt(b) that had a borderline MMA result and normal Cr51 RBC survival at 1 and 24 hours but a reduced Cr51 half life. The interpretation of a borderline positive MMA result seemed similar to the interpretation of a 1-hour Cr51 RBC survival, which suggested that while the patient may not experience an acute hemolytic episode, incompatible RBCs may not have normal survival. The borderline positive MMA results in this case correlated with the patient’s clinical course.

Anecdotal reports of patients with more serious, acute hemolytic transfusion reactions due to anti-Di(b+) are in contrast to this patient who had a mild, delayed hemolytic reaction. More studies need to be performed to determine which outcome is more common. We have performed MMAs on two other examples of anti-Di(b+), both of which gave strongly positive results (73% and 83% reactivity); both of these were IgG3 (unpublished observations). Neither patient received Di(b+) blood, but we would expect more severe reactions than reported here.

This case illustrates, as has been previously reported, that in cases where compatible, antigen-negative blood is difficult to obtain and the patient requires urgent transfusion, incompatible transfusion should not be withheld. Although the transfused Di(b+) units did not have normal survival, the patient did not experience an acute hemolytic transfusion reaction and the transfused RBCs helped the patient clinically. Of course, when the alloantibody involved is generally considered to be clinically significant, all efforts should be made to obtain antigen-negative blood (local blood centers, Rare Donor Registry, siblings) and transfusion should always be avoided unless it is clinically necessary, but on occasion (such as in this case), that is not possible.

<table>
<thead>
<tr>
<th>Table 2. Results of survival studies by flow cytometry</th>
</tr>
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<tbody>
<tr>
<td>Day</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>0</td>
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<tr>
<td>1</td>
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<td>7</td>
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<tr>
<td>9</td>
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<td>10</td>
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</table>

*The day that 3 Di(b+) units were transfused (sample was drawn prior to transfusion)*

References


