LOW RATE OF RHESUS IMMUNIZATION FROM RH-INCOMPATIBLE BLOOD TRANSFUSIONS DURING LIVER AND HEART TRANSPLANT SURGERY

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Transfusion of one unit or more of Rh-positive red blood cells normally causes circulating anti-D antibody to appear 2–6 months later in 80–95% of Rh− persons. We asked whether transplant immunosuppression with cyclosporine and corticosteroids affects Rh immunization. Nineteen Rh+ liver, heart, and heart-lung transplant recipients received RBCs at surgery and were tested for anti-D 2 months later. Three patients developed anti-D at 11–15 days; one may have had an unusually rapid primary immune response and two were secondary to previous exposure by pregnancy. None of the other 16 patients had anti-D when tested 2.5–51 months later (13 patients, >11.5 months). This low rate of Rhesus immunization in association with cyclosporine immunosuppression allows greater flexibility in meeting the transfusion needs of Rh− liver and heart transplant patients. Caution is still advised in young females and in patients who may have been previously exposed to Rh+ RBCs by transfusion or by pregnancy prior to the availability of perinatal Rh immune globulin twenty years ago. Other humoral immune responses to some vaccines or infectious agents may also be impaired in transplant patients.

The D antigen on red blood cells is highly immunogenic. As little as 30 μl of RBCs has caused Rh immunization, and after a single 1-mI injection of Rh+ RBCs, 10–40% of Rh− subjects develop anti-D (1). After transfusions of one or more Rh+ units, anti-D appears 2–6 months later in 80–95% of Rh− persons (2–4). Hemolytic disease of the newborn (HDN)* or hemolysis of Rh-incompatible RBC transfusions may then ensue.

Transplantation of livers, hearts, and heart-lungs can require large amounts of blood (5, 6). Because only 15% of white and 7% of black patients are Rh*, sufficient Rh− blood may not always be available for Rh− patients. In view of the setting of transplant immunosuppression, we studied our Rh− organ transplant recipients who received Rh+ RBCs during surgery to determine whether they subsequently formed anti-D at the normal high rate. The results are of interest with regard to both transplant transfusion management and the pathogenesis of Rhesus immunization.

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* Abbreviations: HDN, hemolytic disease of the newborn; RhIG, Rh immune globulin.

MATERIALS AND METHODS

Standard serologic methods (7) were employed in Central Blood Bank's transfusion service at the University of Pittsburgh, which supports adult organ transplants at Presbyterian-University Hospital and pediatric transplants at Children's Hospital of Pittsburgh. Blood bank records were reviewed for Rh− liver, heart, and heart-lung transplant patients from 1981 to early 1987, and all who received Rh+ RBCs during surgery were assessed in retrospect for evidence of subsequent anti-D. None of these patients had anti-D at our center prior to surgery. We then sought new samples from survivors for Rh antibody testing, including use of ficin-treated screening RBCs to enhance reactivity of Rh antibodies. We also reviewed a second group of patients with anti-D prior to surgery in order to assess the subsequent persistence of antibody. As is customary, we tested blood donors, but not patients, for the weak Rh*D phenotype present in 0.23–0.56% of all people (1), or about 1.5–3% of apparently Rh− patients.

For immunosuppression, our transplant programs have employed cyclosporine, corticosteroids, adjuvant rabbit antilymphocyte globulin, and—in recent years—OKT3 monoclonal antibody (6–12).

RESULTS

The proportions of Rh− transplant patients receiving intra-operative Rh+ RBCs because of rapid need and/or low Rh− inventory were as follows: liver, 19 of 109; heart, 8 of 51; and heart-lung, 2 of 8. None received Rh immune globulin (RhIG).

Eight of the 29 patients given Rh+ RBCs died within 2 months; three of these 8 had no anti-D by 7 weeks. Two other patients were not tested for RBC antibodies beyond one month after surgery. Thus, a total of 19 patients had sufficient follow-up for analysis.

Three liver transplant patients developed anti-D 11–15 days after receiving Rh+ RBCs. Two were women 64 and 53 years old, each with 3 past pregnancies and thus presumably immunized prior to the licensure of perinatal RhIG in the U.S.A. in 1968. One received 29 Rh+ units during two liver transplants over 12 days. Three days later, anti-D was present in the serum and on circulating RBCs; concurrently, the hematocrit dropped from 34% to 26%, and the total and indirect bilirubin levels rose from 8.1 and 3.4 mg/dl to 19.0 and 8.6 mg/dl. The other woman received 3 Rh+ units, the last of 13 in total. Anti-D appeared in the serum, but not on circulating RBCs, at 11 days, along with a hematocrit change from 28% to 18% and a rise in total and indirect bilirubin from 8.9 and 3.7 mg/dl to 20 and 6.2 mg/dl. Thus, both of these patients had evidence of mild delayed hemolysis at times when no bleeding was apparent.

The third patient was a 61-year-old man who was never known to have received Rh+ RBCs or platelets and had no RBC antibodies detected elsewhere 6 months before transplant.
After transfusion of 10 units of Rh- RBCs, anti-D and -E were found in the serum, but not on RBCs, at 11 days, but were undetectable at another hospital two months after surgery. There were no RBC antibodies found in the plasma of his blood donors. The organ donor was Rh+, virtually ruling out the graft as the origin of the anti-D. Evaluation for hemolysis was obscured by concurrent reoperation for biliary obstruction.

Of the other 16 patients, none had anti-D ≥2 months later, including 13 with followup ≥11.5 months (Table 1). Two were female patients 41 and 53 years old; the rest were male.

Two of these patients were later given Rh+ RBCs again, but died shortly thereafter. Case 1 received such units a few days before death, and case 16 was given more than 50 more Rh+ units during his 2.5-month survival period.

Four other liver transplant patients with preoperative anti-D had ≥1 month of follow-up antibody testing after surgery. In each case the anti-D was still detectable 1.5, 18, 48, and 52 months later. This suggested that immunosuppression did not interfere with the usual long-term persistence of preexisting anti-D (7).

DISCUSSION

In a previous retrospective blood bank study of 1000 consecutive liver transplants in 781 patients at our center (13), many RBC antibodies appeared 1-5 weeks later, but only 3 patients were found to have formed new significant RBC alloantibodies (anti-K and -E) after more extended follow-up (from 7 weeks to 4.5 months). However, the true rate of primary RBC alloimmunization was uncertain because most patients did not have long-term testing. In this study of 19 Rh- organ transplant patients tested ≥2 months after receiving numerous Rh+ RBCs, there was only one apparently primary anti-D, there were two rapid secondary immune responses, and in 16 patients no subsequent antibody was found. Lack of alloimmunization was further supported by negative results of additional testing in 10 patients when enzyme-treated screening cells were used to enhance Rh antibody detection.

In immunocompetent subjects receiving ≥1 Rh-incompatible unit of RBCs, 80-95% formed anti-D. Cook and Rush (3) tested 20 heart surgery patients who had received 12-33 units of RBCs; 19 (95%) had anti-D, although 7 antibodies were detected only by enzyme-treated test cells. After 500 ml of whole blood, 18 of 22 subjects (82%) studied by Pollack et al. (2) were alloimmunized, without any detection advantage in enzyme screening (14). Likewise, Urbaniak and Robertson (4) found that 24 of 28 subjects (86%) formed anti-D after 200 ml of RBCs. RhIG has prevented Rh immunization after up to 1000 ml of RBCs, but the dose required is massive—6.7 ml(10^9) ml RBCs, which in the U.S. must be given intramuscularly—and may cause symptomatic hemolysis of the Rh+ RBCs (1, 15). Anti-D generally does not appear until 2-6 months after exposure. In the 18 subjects tested monthly by Pollack et al. (2), antibody first appeared in 9 at 2 months, in 7 at 3 months, and in 2 at 4-5 months. Furthermore, anti-D is generally very persistent. We previously found that 86% of such antibodies were still present 1-60 months after detection (7). Therefore, anti-D would have been expected in most of our patients.

Our lone case of primary anti-D formation is similar in timing and transience to a case of anti-D after liver transplant observed by Blomqvist et al. (16). After 38 units of Rh+ blood, this antibody was detected by enzyme-treated RBCs at days 12-16 and subsequently disappeared in a previously untransfused male patient (Blomqvist BI, personal communication). However, the most rapid primary anti-D response previously reported was at 4 weeks (7). It is also possible that these two patients had previously undetected levels of "naturally occurring" anti-D; some of these antibodies can be stimulated by exposure (17, 18).

Our findings are of practical importance for transfusion management of Rh- organ transplant patients when Rh- blood is in short supply. Excluding our two secondary immune responses, the frequency of subsequently detectable primary anti-D was low (1/17) after multiple Rh+ RBCs. Perhaps immunosuppressive regimens could be devised for other settings of large-Rh-incompatible blood exposure. However, two cautions should be made. First, in transplant patients who may have been previously Rh-sensitized by transfusions or remote pregnancies, delayed hemolytic transfusion reactions can ensue after Rh+ RBC transfusions, as noted here and as we and others have observed with non-D RBC antibodies (13, 19). Second, only two of our patients have been rechallenged with Rh+ RBCs, and they died shortly thereafter. In the pre-RhIG era, some women sensitized by low levels of Rh+ fetal RBCs did not produce anti-D until the next Rh+ gestation. Therefore, it is possible that some of our patients have been silently immunized. None were girls or women of childbearing age (Table 1). Since successful pregnancies have occurred after cyclosporine transplantation (20), we still recommend avoidance of Rh+ RBCs in girls or young women, if feasible, pending further data.

In the setting of cancer therapy immunosuppression, repeated Rh-incompatible platelet and granulocyte transfusions containing small amounts of Rh+ RBCs have led to varying rates of anti-D formation. Two studies yielded 6-18% rates of Rh alloimmunization (21, 22), but another group reported only a 2% frequency (23). Our RBC data suggest that the risk of immunization by Rh+ platelets alone would be minimal in liver and heart transplantation.

Rh+ RBCs in kidney grafts occasionally have caused primary or secondary alloimmunization in Rh- transplant recipients.

### Table 1. Rh- transplant patients testing negative for anti-D ≥2 months after receiving Rh+ RBCs at surgery

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Organ</th>
<th>Units of Rh+ RBCs</th>
<th>Times of testing (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>Liver</td>
<td>5</td>
<td>3, 51*</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>M</td>
<td>Liver</td>
<td>10</td>
<td>45*</td>
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<tr>
<td>3</td>
<td>56</td>
<td>M</td>
<td>Heart</td>
<td>4</td>
<td>5, 31*</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>M</td>
<td>Heart-lung</td>
<td>6</td>
<td>24*</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>M</td>
<td>Liver</td>
<td>16</td>
<td>5, 24*</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>M</td>
<td>Heart</td>
<td>7</td>
<td>5, 22*</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>M</td>
<td>Heart</td>
<td>6</td>
<td>21*</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>F</td>
<td>Liver</td>
<td>12</td>
<td>3, 20*</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>M</td>
<td>Liver</td>
<td>10</td>
<td>20*</td>
</tr>
<tr>
<td>10</td>
<td>53</td>
<td>M</td>
<td>Heart</td>
<td>7</td>
<td>20</td>
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<tr>
<td>11</td>
<td>41</td>
<td>F</td>
<td>Liver</td>
<td>7</td>
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<td>43</td>
<td>M</td>
<td>Liver</td>
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<td>12*</td>
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<tr>
<td>13</td>
<td>22</td>
<td>M</td>
<td>Heart</td>
<td>19</td>
<td>3, 6, 11.5*</td>
</tr>
<tr>
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<td>32</td>
<td>M</td>
<td>Liver</td>
<td>153</td>
<td>3*</td>
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<tr>
<td>15</td>
<td>23</td>
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<td>Liver</td>
<td>138</td>
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<tr>
<td>16</td>
<td>29</td>
<td>M</td>
<td>Liver</td>
<td>86</td>
<td>2.5*</td>
</tr>
</tbody>
</table>

* Died.  
* Enzyme-treated RBCs used in final antibody testing.
Rh (3) 2-33 units of Rh-negative blood was given to 500 ml of Rh-negative blood, or transfused血 transfusion. The transfusion of Rh-negative blood to Rh-positive recipients was associated with the development of anti-D antibodies in up to 7% of cases. These antibodies can cause hemolytic reactions, which may be severe in cases of Rh sensitization.

However, several of these instances were associated with a high rate of graft rejection, suggesting that immunosuppression might have been insufficient. In bone marrow transplantation, Rh-negative recipients of Rh-positive grafts had a lower rate of graft rejection compared to those receiving Rh-positive grafts.

Our results in RBC transfusions may be related to experimental evidence in rats and dogs that cyclosporine can prevent transfusion-induced humoral alloimmunization to antigens of the major histocompatibility complex (27, 28). In humans, this drug has been reported to impair T cell-dependent antibody responses in bone marrow transplant recipients immunized with keyhole limpet hemocyanin (29), and also in renal transplant patients who were given influenza vaccine (30). Further work is needed to determine whether the RBC Rh protein might also be a T cell-dependent antigen.

Besides cyclosporine, other immunosuppressive measures used in our patients could have played a role in the effect observed. Primary antibody responses to other antigens such as vaccines or infectious agents may also be impaired at the time of organ transplantation.

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REFERENCES


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