Bone Marrow Transplantation for Paroxysmal Nocturnal Hemoglobinuria: Eradication of the PNH Clone and Documentation of Complete Lymphohematopoietic Engraftment

By Joseph H. Antin, David Ginsburg, Brian R. Smith, David G. Nathan, Stuart H. Orkin, and Joel M. Rappeport

Paroxysmal nocturnal hemoglobinuria (PNH) involves the proliferation of an abnormal and possibly premalignant hematopoietic stem cell. Successful treatment of PNH by marrow grafting requires that the PNH clone be eradicated by the pretransplant conditioning regimen. Four patients with PNH-associated marrow aplasia were transplanted with marrow from their HLA-matched, MLR-nonreactive siblings. Three patients were conditioned with cyclophosphamide, procarbazine, and antithymocyte serum (CTX/PCZ/ATS), and one was conditioned with busulfan/CTX/PCZ/ATS. Persistent complete engraftment of myeloid, lymphoid, and erythroid cell lines was demonstrated in all four patients by DNA sequence polymorphism analysis or cytogenetics, and RBC typing. There was no recurrence of the abnormal clone of cells for up to five years after transplantation despite the use of a conditioning regimen in three of them, which is not usually associated with permanent marrow aplasia. Bone marrow transplantation is a curative therapy in patients whose illness is severe enough to warrant the risk.

**MATERIALS AND METHODS**

Patients and transplantation procedures. The diagnosis of PNH was established by the demonstration of increased susceptibility of RBCs to complement-mediated lysis (acid-serum test or Ham test and sugar-water test). All four patients were pancytopenic, had evidence of in vivo hemolysis prior to transplantation, and were heavily transfused. Informed consent was obtained from all patients according to institutional review board guidelines. The transplant procedure and posttransplant care were as previously reported. In brief, the conditioning regimen used for three of the patients consisted of cyclophosphamide (CTX) 50 mg/kg/d on days −5 through −2, procarbazine (PCZ) 12.5 mg/kg on days −7, −5, −3, and rabbit antithymocyte serum (ATS) 0.2 mL/kg on days −6, −4, −2. These three patients all had marrow cellularity of <10%. The fourth patient (UPN 169) had severe pancytopenia by peripheral blood criteria, despite >30% marrow cellularity and grade 2 (grade 3 is normal) marrow erythropoiesis by 111Indium chloride scanning documented prior to transplantation. That patient received oral busulfan 3 mg/kg/d on day −9 through −6 in addition to the CTX/PCA/ATS described above. All four patients were transplanted from HLA-matched, MLR-nonreactive siblings. The dose of bone marrow infused ranged from 1.6 to 2.7 x 10⁸ nucleated cells per kilogram.

DNA sequence polymorphism analysis. For patients UPN 109, 145, and 169, engraftment was established with the use of DNA sequence polymorphism analysis as previously described. For UPN 145 and 167, informative DNA sequence polymorphisms which distinguish host and donor cells were identified by screening DNA prepared from pretransplant peripheral blood samples from patient and donor. For patient UPN 109, the patient's pretransplant genotype was determined by studying DNA prepared from cultured skin fibroblasts. At various times after transplantation (three months to two years) 10 to 20 mL of peripheral blood was collected in heparin. Granulocytes were prepared from the pellet after Ficoll-Hypaque centrifugation. For patient UPN 145, low-density mononuclear cells obtained from the Ficoll-Hypaque interface were further fractionated into T cells, B cells, and monocytes as previously described. In subsequent patients, further fractionation was only performed if mixed chimerism was demonstrated in the mononuclear fraction.

From the Department of Medicine, Division of Hematology, Brigham and Women's Hospital, and Department of Pediatrics, Division of Hematology/Oncology, Children's Hospital Medical Center, Harvard Medical School, Boston. Supported in part by Grants No. RR0088, HL06725, HD18661, and RR0128 from the National Institutes of Health. J.H.A. and D.G. are recipients of American Cancer Society Junior Faculty Clinical Fellowships.

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Address reprint requests to Dr Joseph H. Antin, Division of Hematology, Brigham and Women's Hospital, 75 Francis St, Boston, MA 02115.
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The low-density mononuclear cells are referred to as "lymphoid" cells, and the Ficoll-Hypaque pellets are referred to as "myeloid" cells. The genotype of the posttransplant cell fractions was compared with the pretransplant host and donor genotype. This method can detect 1% residual host elements.* Karyotype analysis was performed on patient UPN 079. Graft v host disease (GVHD) was graded according to the criteria established by Thomas and co-workers.11

Red blood cell analysis. Typing for 30 blood group antigens was performed in the Reference Laboratory of the Center for Blood Research, Boston, before transplantation on both patients and donors. Complete RBC typing was repeated after transplantation when >5 months had passed since the last packed RBC transfusion, and transfused cells were unlikely to be detectable. The sugar-water and acid-serum tests for PNH were performed by standard techniques.12

RESULTS

Three patients had uncomplicated transplant courses, and the fourth (UPN 169) had grade 3 acute GVHD (Table 1). None of the patients has chronic GVHD. All four patients had complete restoration of normal hematopoiesis, reversion of their sugar-water and acid-serum tests to normal, and conversion of their RBC surface antigen phenotypes to the donors' pattern (Table 1). Only one patient was transplanted from a donor of the opposite sex; UPN 079 was female but had a male marrow donor, and engraftment of both myeloid and lymphoid cell lines was documented by the presence of 100% male karyotypes in mitogenically stimulated peripheral blood lymphocytes and unstimulated bone marrow. In the other three patients, donor and recipient sex were the same. DNA sequence polymorphism analysis demonstrated complete engraftment of both lymphoid and myeloid series in all three cases (Fig 1). Both UPN 145 and UPN 169 were studied twice after transplantation and demonstrated complete engraftment on both occasions. Thus, all of our patients had evidence of complete lymphohematopoietic reconstitution with donor cells. In one of the patients (UPN 169), there were no unique recipient RBC antigens bone marrow transplantation prior to (BMT); thus, mixed erythroid chimerism cannot be strictly ruled out, however, the absence of demonstrable host lymphoid or myeloid cells and the normal acid-serum test makes isolated mixed erythroid chimerism unlikely.

DISCUSSION

This series of four patients with PNH treated by allogeneic bone marrow transplantation provides further clinical evidence of the efficacy of this approach to the disease. There are an additional eight patients described in the literature, raising the number of known transplanted patients to 12. Nine of 11 are known to be alive and well posttransplant (one of the previously reported patients was lost to follow-up). All of these patients had severe aplastic anemia with or without clinically unmanageable hemolytic disease as the indication for bone marrow transplantation. The question of when to transplant an individual with PNH remains a difficult one, especially for those patients who do not have overt aplasia, unmanageable hemolysis, or thrombotic problems. Although there is apparently an increased risk of leukemic transformation for these patients13-16 it is probably low; therefore, the current approach at our center for patients without a syngeneic donor is to perform a transplant only in those patients with life-threatening manifestations. Patients with an identical twin donor should probably be considered earlier for transplant; under those circumstances, the complications from the procedure can be expected to be low.

It is clear that some form of cytotoxic therapy is necessary to prepare patients with PNH for transplantation, since persistence of the PNH clone has been reported in two patients who received no cytotoxic or immunosuppressive preparation prior to syngeneic transplants.2-7 Because PNH

Table 1. Patient Characteristics and Clinical Outcome

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>UPN 079</th>
<th>UPN 109</th>
<th>UPN 145</th>
<th>UPN 169</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>CTX/PCZ/ATS</td>
<td>CTX/PCZ/ATS</td>
<td>CTX/PCZ/ATS</td>
<td>BU/CTX/PCZ/ATS</td>
</tr>
<tr>
<td>Acid-serum test*&lt;br&gt;Pretransplant</td>
<td>50%</td>
<td>30%</td>
<td>9%</td>
<td>7%</td>
</tr>
<tr>
<td>Posttransplant</td>
<td>&lt;0.2%</td>
<td>&lt;0.2%</td>
<td>&lt;0.2%</td>
<td>&lt;0.2%</td>
</tr>
<tr>
<td>RBC type&lt;br&gt;Recipient</td>
<td>AB,DcCe</td>
<td>A</td>
<td>DCe</td>
<td>Ns</td>
</tr>
<tr>
<td>Donor</td>
<td>O, Dce</td>
<td>O</td>
<td>Dce</td>
<td>NSs</td>
</tr>
<tr>
<td>Postransplant†&lt;br&gt;Recipient</td>
<td>0, Dce</td>
<td>0</td>
<td>Dce</td>
<td>NSs</td>
</tr>
<tr>
<td>Acute GVHD grade‡&lt;br&gt;Chronic GVHD&lt;br&gt;Clinical status&lt;br&gt;Follow-up</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Alive/well</td>
<td>Alive/well</td>
<td>Alive/well</td>
<td>Alive/well</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 yr</td>
<td>&gt; 3 yr</td>
<td>&gt; 2 yr</td>
<td>&gt; 1 mo</td>
<td></td>
</tr>
</tbody>
</table>

UPN, unique patient number; ATS, antithymocyte serum; BU, busulfan; CTX, cyclophosphamide; PCZ, procarbazine; GVHD, graft v host disease.

*Normal <5% hemolysis. UPN 145 and 169 were transfused prior to the performance of the acid-serum test.
†RBC typing was established more than five months after the last transfusion. Only the informative antigenic groups are shown.
‡Grading the acute GVHD was on a scale of 0 (no GVHD) to 4 (severe GVHD) by standard criteria.11
is characterized by the presence of a proliferating abnormal hematopoietic stem cell,\textsuperscript{18,19} we were initially concerned that a “nonablative” aplastic anemia preparatory regimen would eventually result in graft rejection or a mixed lymphohematopoietic chimeric state rather than complete engraftment. Regimens relying on large doses of CTX as pretransplant conditioning for aplastic anemia do not necessarily result in complete, irreversible injury to stem cells, as demonstrated by the 2\% to 8\% incidence of autologous recovery\textsuperscript{18} or mixed lymphohematopoietic chimerism.\textsuperscript{18,20} Although autologous recovery might be acceptable in de novo aplastic anemia, it is not acceptable in PNH, in which reestablishment of the PNH clone may result in symptomatic anemia or thrombosis, and because of the potential for the future malignant transformation of the abnormal stem cell. Furthermore, treatment of patients with hypocellular marrows due to “preleukemia” with CTX alone failed to prevent reemergence of the dysplastic clone.\textsuperscript{7} Although the number of patients studied is small, it appears that for most PNH patients, the CTX/PCZ/ATS conditioning regimen is adequate to eliminate the abnormal clone. DNA sequence polymorphism analysis, cytogenetics, and RBC typing have demonstrated that, in all of our patients, engraftment of the myeloid, lymphoid, and erythroid series has been complete and permanent. The absence of mixed erythroid chimerism is further supported by the normal acid-serum and sugar-water tests. Whether the latest patient in this series would have done well with only CTX/PCZ/ATS is not known. However, experience in the marrow transplantation of patients with genetic disorders\textsuperscript{21-23} and myelodysplasia\textsuperscript{7} suggests that for patients with cellular marrows some form of hematopoietic ablative therapy is necessary. On the other hand, pretransplant therapy with total body irradiation in patients with PNH has resulted in only two deaths in the 11 patients with follow-up.\textsuperscript{20} Both deaths were attributed to interstitial pneumonitis.\textsuperscript{5} The minimum treatment which will result in complete ablation is unknown. It is probable that CTX alone would be adequate in untransfused patients with marrow aplasia.\textsuperscript{2} However, there is a high rate of graft rejection observed in transfused patients with aplastic anemia who were conditioned with CTX as a single agent.\textsuperscript{2} Therefore, in our PNH patients, all of whom had received prior transfusions, we used a multiagent pretransplant conditioning regimen which was designed primarily for immunosuppression, and which allows successful engraftment in 90\% of transfusion-sensitized patients.\textsuperscript{8,24} We have had a very low complication rate attributed to the CTX/PCZ/ATS conditioning regimen used in a large series of patients with aplastic anemia: 1 of 40 patients developed fatal cardiomyopathy, and 2 of 40 patients developed interstitial pneumonitis (both secondary to herpes simplex, one fatal).\textsuperscript{24}

We conclude that allogeneic marrow transplantation using preparatory regimens that do not include irradiation is an effective and relatively safe therapy for individuals with PNH and associated marrow aplasia. The data suggest that the PNH clone is sensitive to CTX/PCZ/ATS, and that full engraftment of donor myeloid, lymphoid, and erythroid cells can occur with such regimens.
REFERENCES

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