Prevention of Graft Rejection Following Bone Marrow Transplantation

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Bone marrow transplantation from an HLA-identical sibling is increasingly used in the treatment of severe aplastic anemia. One major problem with this approach is graft rejection that occurs in 25%-60% of patients conditioned for transplantation with cyclophosphamide. At most transplant centers it has been difficult to accurately identify patients at high risk for graft rejection. We studied a conditioning regimen of cyclophosphamide (200 mg per kg) and low-dose total body irradiation (3 Gy; equivalent to 300 rad) in 23 consecutive unselected patients with aplastic anemia followed for a minimum of 6 mo. There was only one episode of graft rejection. Graft-versus-host disease and interstitial pneumonitis were not increased by the more intensive conditioning regimen. Actuarial survival was 61% at 1 yr and 49% at 2.5 yr. Cyclophosphamide and low-dose total body irradiation is an effective conditioning regimen in patients with aplastic anemia. It may be particularly useful when accurate predictive tests of graft rejection are not available as is the case in most transplant centers.

GRAFT REJECTION is a major problem following bone marrow transplantation for aplastic anemia. Several centers have reported 25%-60% rejection rates among HLA-identical graft recipients conditioned with cyclophosphamide at a dose of 200 mg per kg.15 Indirect data including studies in dogs6 and in untransfused patients2 suggest that graft rejection in this setting results from immunity to minor histocompatibility antigens.

Several centers have analyzed factors associated with graft rejection. These data are summarized in Table 1. Several investigators have reported correlations between in vitro tests of recipient antidonor immunity including the relative response index in mixed lymphocyte culture, lymphocyte-mediated cytotoxicity, and the antibody dependent cellular cytotoxicity test and subsequent graft rejection.811 An inverse relationship between the marrow dose and the likelihood of graft rejection has also been noted.10 Critical analysis of this data is complicated by several factors. First, most of these analyses were retrospective. Second, in some studies patients received different conditioning regimens including cyclophosphamide, procarbazine, antithymocyte globulin, and total body irradiation alone or in combination. Finally, as indicated in Table 1, these predictive indicators have not been readily translatable to other transplant centers. These disparate results may relate to several factors including technical differences in the tests or in the size or composition of patient study group. For example, studies from UCLA and Baltimore could not confirm the correlation between relative response index and graft rejection.12 Both analyses involved fewer patients than the Seattle series, and the tests were performed differently at the three centers. Similar problems have complicated other studies. We reported a correlation between pretransplant lymphocytotoxins and graft rejection.11 This observation could not be confirmed by two other transplant teams nor could we reproduce it when coded sera from one center were retested at UCLA.1415

These conflicting data suggest that most transplant centers are unable to accurately identify patients at high risk for graft rejection. It is important, therefore, to develop conditioning regimens which will prevent graft rejection in unselected patients. We report results using a conditioning regimen of cyclophosphamide and low-dose total body irradiation in 23 consecutive unselected bone marrow transplant patients with aplastic anemia followed for 6 mo to 2.5 yr. This regimen resulted in a low incidence of graft rejection without increasing the incidence of graft-versus-host disease (GVHD) or interstitial pneumonia.

MATERIALS AND METHODS

Study Group

Twenty-three consecutive patients with severe aplastic anemia entered the trial between March 1, 1977, and December 1, 1979. Data were analyzed as of June 1, 1980. Criteria for severe aplasia included: granulocytes ≤ 0.5 x 109/liter, platelets ≤ 20 x 109/liter.

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THE UCLA BONE MARROW TRANSPLANT TEAM

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Table 1. In Vitro Tests Reported to Predict Graft-Rejection Following Bone Marrow Transplantation

<table>
<thead>
<tr>
<th>Test</th>
<th>No. Centers Reporting Predictive Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative response index (RRI)</td>
<td>Yes 10 12 Reference 10 12</td>
</tr>
<tr>
<td>Cell mediated immunity (CMI)</td>
<td>Yes 10 12 Reference 10 12</td>
</tr>
<tr>
<td>Lymphocytotoxins (LCT)</td>
<td>Yes 10 12 Reference 10 12</td>
</tr>
<tr>
<td>Antibodies to CFU-C</td>
<td>Yes 10 12 Reference 10 12</td>
</tr>
<tr>
<td>Non-HLA antibodies</td>
<td>Yes 10 12 Reference 10 12</td>
</tr>
<tr>
<td>Migration inhibition factor (MiF)</td>
<td>Yes 10 12 Reference 10 12</td>
</tr>
<tr>
<td>Inhibition of leukocyte migration (SLM)</td>
<td>Yes 10 12 Reference 10 12</td>
</tr>
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</table>

Table 2. Study Group Characteristics

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>17 (3-43)</th>
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<tbody>
<tr>
<td>Sex (male/female)</td>
<td>17/6</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
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</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Therapy</td>
<td>Androgens</td>
</tr>
<tr>
<td>Drug</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td>Unknown</td>
<td>20</td>
</tr>
<tr>
<td>Diagnosis to transplant (m)</td>
<td>13</td>
</tr>
<tr>
<td>&lt;2</td>
<td>13</td>
</tr>
<tr>
<td>&gt;4</td>
<td>5</td>
</tr>
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</table>

Transplantation

Recipients were conditioned for transplantation with cyclophosphamide, 50 mg/kg/day, for 4 days (day -5 to -2), and total body irradiation, 3 Gy GCo-single source; 0.05-0.07 Gy Gmin on day -1. Bone marrow was infused intravenously on day 0. The mean dose of nucleated marrow cells infused was 3.22 × 10^6 per kg (range 1.3-6.0). Fifteen recipients received greater than and 8 less than 3.0 × 10^6 nucleated marrow cells per kg. Methotrexate, 10-15 mg/m^2/day was given intravenously on days 1, 3, 5, 6, 11, and weekly thereafter to day 102 to modify GVHD.25 Engraftment was documented by increasing blood counts, marrow morphology, chromosome analysis, red cell antigens, and red cell and leukocyte isoenzymes.23 There was one or more informative chromosome or genetic marker in each case. GVHD and interstitial pneumonitis were evaluated by previously reported criteria.22 Patients without GVHD were classified as grade 0, those with mild GVHD as grade 1, and those with moderate to severe GVHD as grades 2 to 4. Patients with a grade 2 GVHD received high-dose corticosteroids or antithymocyte globulin. Seven patients received transfusions of cytomegalovirus immune plasma (CMVIP) to prevent or modify interstitial pneumonitis.31

Patients were managed in reverse isolation and received oral nonabsorbable antibiotics. Documented or suspected infections were treated with carbencillin and an aminoglycoside antibiotic. Fungal infections were treated with amphotericin. Patients were randomized to receive "prophylactic" granulocyte transfusions or not.24 All blood products given post-transplant were irradiated with 15 Gy to prevent their engraftment.

Statistics

Survival data were analyzed by means of a product limit method using program BMDP12 of the UCLA Health Sciences Computing Facility.27 Data were analyzed as of June 1, 1980, with a minimum observation period of 6 mo.

RESULTS

Twenty-three consecutive patients with severe aplastic anemia entered the study. Clinical features are indicated in Table 2. Seventeen patients were male and 6 were female, with the median age being 17 yr (range 3-43 yr). Thirteen were ≤21 yr (57%) and 10 > 21 yr (43%). Aplastic anemia developed in association with hepatitis in two patients and was associated with the exposure to chloramphenicol in one. No etiology was identified in the remaining 20 patients. All patients had received extensive transfusions of blood products. Ten patients received 1-10 transfusions; seven, >10-100; and six, >100 transfusions; no patient was untransfused. Median interval from diagnosis to transplant was 1.5 mo (range, 1-120 mo). Eighteen patients were transplanted within 4 mo of diagnosis, including 13 within 2 mo. Five patients were transplanted 6, 8, 9, 84, and 120 mo following diagnosis.

A series of in vitro tests were performed in these patients in an attempt to detect sensitization. These included: relative response index (RRI),8 complement dependent cytotoxicity to donor lymphocytes;16 direct cell mediated and antibody dependent cellular cytotoxicity (ADCC);28 pretransplant lymphocytotoxins;27 and antibodies to granulocyte-macrophage progenitor cells (CFU-C).28 The results of these studies are summarized in Table 3.

Twelve patients are currently alive 6.5 mo to 2.5 yr following transplantation. Actuarial survival is 61% at 1 yr (95% confidence interval (CI), 41%-81%), and 49% at 2.5 yr (95% CI, 21%-71%) (Fig. 1). Eleven

Table 3. In Vitro Tests of Recipient Anti-Donor Immunity

<table>
<thead>
<tr>
<th>Test</th>
<th>No. Tested</th>
<th>No. Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative response index (RRI)</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Cell-mediated immunity (CMI)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Antibody dependent cellular cytotoxicity (ADCC)</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytotoxins (LCT)</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Complement dependent cytotoxicity (CDC)</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Antibodies to CFU-C</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>
MARROW TRANSPLANTS IN SENSITIZED RECIPIENTS

![Graph showing actuarial survival following bone marrow transplantation in patients with aplastic anemia.]

Patients died 1 day to 20 mo following transplantation (Table 4). Causes of treatment failure included infection and graft rejection in one patient each, GVHD in three patients, GVHD and interstitial pneumonia in four patients, and chronic GVHD and infection in two patients. There were no deaths from interstitial pneumonia alone. The patient who rejected her graft received $3.5 \times 10^8$ bone marrow cells per kg.

Twenty-one patients were at risk to develop GVHD. Fourteen patients had absent or mild GVHD including 12 with no GVHD and 2 with grade 1 GVHD. Seven patients developed moderate to severe GVHD; 4 had grade 2 and 3. Four additional patients developed chronic GVHD without preceding acute GVHD. All patients with ≥2 acute GVHD died; 3 of GVHD alone and 4 of GVHD in association with interstitial pneumonia. Two of the 4 patients with chronic GVHD died.

Interstitial pneumonia developed in 6 patients and was fatal in 3. Three cases were related to CMV, 1 to herpes simplex, and 1 to adenovirus and CMV. No etiology was identified in one case. The three patients who died of interstitial pneumonia all had ≥ grade 2 GVHD. There were no fatal cases of interstitial pneumonia in patients without GVHD.

**DISCUSSION**

Bone marrow transplantation from an HLA-identical sibling is an effective form of therapy in patients with severe aplastic anemia. Several centers have reported 30%–78% long-term disease-free survival in previously transfused patients.1–29

Graft rejection has been a major problem in these patients accounting for 25%–59% of treatment failures in patients conditioned with cyclophosphamide alone. Several centers have attempted to prospectively identify patients at high risk for graft rejection (Table 1). Results of these studies have been contradictory and difficult to reproduce, and few investigators feel that they can accurately predict graft rejection in transfused patients. No prospective analyses addressing this important problem have been reported. We studied a conditioning regimen of cyclophosphamide and low-dose total body irradiation that we hoped would prevent graft rejection in unselected transfused patients. This approach was successful with graft rejection occurring in only 1 of 23 consecutive patients. Furthermore, the incidence and severity of GVHD and interstitial pneumonitis were not increased despite more intensive immunosuppression.

Survival results following conditioning with cyclophosphamide and low-dose total body irradiation are encouraging with 12 patients alive for periods of 6 mo to 2.5 yr. These data compare favorably with other reports of transplantation of multiple transfused patients, particularly in adults.

There are presently two approaches to preventing graft rejection at transplant centers that lack accurate predictive tests. The first is to add additional immunosuppressive agents to cyclophosphamide such as total body irradiation as in the present study, lymphoid irradiation29 or procarbazine and antithymocyte globulin (ATG).30 A second approach to preventing graft rejection involves supplementing the marrow graft with peripheral blood leukocytes. Storb and coworkers have reported a 14% rejection rate in “sensitized” patients who received “buffy-coat” transfusions.31 Presumably, this rate would be lower in unselected patients.

One concern about regimens that employ radiation in patients with aplastic anemia is the risk of developing malignancies. This has not been observed in patients with Ewing’s sarcoma who received comparable doses of total body irradiation.32

It is not presently known which of these approaches

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**Table 4. Transplant Outcome**

<table>
<thead>
<tr>
<th>Complications</th>
<th>Alive</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejection</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>GVHD (≥2)*</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>GVHD + IP†</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>IP‡</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*Acute graft-versus-host disease (≥ grade 2).
†Interstitial pneumonia.
‡Chronic graft-versus-host disease.
to preventing graft rejection is preferable and controlled trials may be necessary to resolve this question. Regardless of which approach is adopted it should now be possible to substantially lower the incidence of graft rejection following HLA-identical bone marrow transplantation.

REFERENCES

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32. Storb R, for the Seattle Marrow Transplant Team: Decrease in the graft rejection rate and improvement in survival after marrow transplantation for severe aplastic anemia. Transplant Proc 11:196–198, 1979

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