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.

RAFT 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. Indirect data including studies in dogs and in untransfused patients 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. An inverse relationship between the marrow dose and the likelihood of graft rejection has also been noted. 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. 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. 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.

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 10^9/liter, platelets ≤ 20 x 10^9/liter, anemia.
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>
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</thead>
<tbody>
<tr>
<td>Relative response index (RPI)</td>
<td>1, 8, 10, 2, 12, 13</td>
</tr>
<tr>
<td>Cell mediated immunity (CMI)</td>
<td>1, 9, 10, 1, 1, 12, 13</td>
</tr>
<tr>
<td>Lymphocytotoxins (LCT)</td>
<td>1, 12, 2, 14, 15</td>
</tr>
<tr>
<td>Antibodies to CFU-C</td>
<td>2, 28, 33</td>
</tr>
<tr>
<td>Non-HLA antibodies</td>
<td>1, 5, 16</td>
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<tr>
<td>Migration inhibition factor (MIF)</td>
<td>1, 12</td>
</tr>
<tr>
<td>Inhibition of leukocyte migration (SLM)</td>
<td>1, 12</td>
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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>
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<tr>
<td>Etiology</td>
<td></td>
</tr>
<tr>
<td>Hepatitis</td>
<td>2</td>
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<tr>
<td>Drug</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>20</td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td>Androgens</td>
<td>7</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>9</td>
</tr>
<tr>
<td>Transfusions</td>
<td>1-10</td>
</tr>
<tr>
<td>1-10</td>
<td>10</td>
</tr>
<tr>
<td>11-100</td>
<td>7</td>
</tr>
<tr>
<td>&gt;100</td>
<td>6</td>
</tr>
<tr>
<td>Diagnosis to transplant (m)</td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>13</td>
</tr>
<tr>
<td>≤4</td>
<td>18</td>
</tr>
<tr>
<td>&gt;4</td>
<td>5</td>
</tr>
</tbody>
</table>

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>

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 (60Co-single source; 0.05-0.07 Gy min) on day -1. Bone marrow was infused intravenously on day 0. The mean dose of nucleated marrow cells infused was 3.22 x 10^7 per kg (range 1.3-6.0). Fifteen recipients received greater than and 8 less than 3.0 x 10^7 nucleated marrow cells per kg. Methotrexate, 10-15 mg/m2/day was given intravenously on days 1, 3, 5, 6, 11, and weekly thereafter to day 102 to modify GVHD.28 Engraftment was documented by increasing blood counts, marrow morphology, chromosome analysis, red cell antigens, and red cell and leukocyte isoenzymes.38 There was one or more informative chromosome or genetic marker in each case. GVHD and interstitial pneumonitis were evaluated by previously reported criteria.21,22 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.39

Patients were managed in reverse isolation and received oral nonabsorbable antibiotics. Documented or suspected infections were treated with carbencilin and an aminoglycoside antibiotic. Fungal infections were treated with amphotericin. Patients were randomized to receive “prophylactic” granulocyte transfusions or not.41 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 BMDP2L 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;37 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


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patients died 1 day to 20 mo following transplantation (Table 4). Causes of treatment failure included infec-
tion and graft rejection in one patient each, GVHD in
two patients, GVHD and interstitial pneumonia in
four patients, and chronic GVHD and infection in two
patients. There were no deaths from interstitial pneu-
monia 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 includ-
ing 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 $\geq 2$ acute GVHD died; 3 of GVHD alone
and 4 of GVHD in association with interstitial pneu-
monia. 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 $\geq$ grade 2
GVHD. There were no fatal cases of interstitial pneu-
monia in patients without GVHD.

DISCUSSION

Bone marrow transplantation from an HLA-identi-
cal 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 fail-
ures 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 address-
ing 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 cyclo-
phosphamide 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 immuno-
suppressive agents to cyclophosphamide such as total
body irradiation as in the present study, lymphoid
irradiation29 or procarbazine and antithymocyte glob-
ulin (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 develop-
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 $\leq 2$</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>IP†</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cause of Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>GVHD</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>GVHD + IP</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*Acute graft-versus-host disease ($\geq$ 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.

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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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