Graft Failure After T-Cell-Depleted Human Leukocyte Antigen Identical Marrow Transplants for Leukemia: I. Analysis of Risk Factors and Results of Secondary Transplants

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Risk factors for graft failure were analyzed in 122 recipients of an allogeneic T-cell-depleted human leukocyte antigen (HLA)-identical sibling marrow transplant as treatment for leukemia. In each case pretransplant immunosuppression included 1,375 to 1,500 cGy hyperfractionated total body irradiation and cyclophosphamide (60 mg/kg/d × 2). No patient received immunosuppression posttransplant for graft-versus-host disease (GVHD) prophylaxis. Nineteen patients in this group experienced graft failure. The major factors associated with graft failure were transplants from male donors and the age of the patient (or donor). Among male recipients of male donor derived grafts a low dose per kilogram of nucleated cells, progenitor cells (colony forming unit-GM) and T cells was also associated with graft failure. Additional irradiation to 1,500 cGy, high dose corticosteroids posttransplant, and additional peripheral blood donor T cells did not decrease the incidence of graft failure. In addition, type of leukemia, time from diagnosis to transplant, an intact spleen, or the presence of antidonor leukocyte antibodies did not correlate with graft failure. To ensure engraftment of secondary transplants, further immunosuppression was necessary but was poorly tolerated. However, engraftment and survival could be achieved with an immunosuppressive regimen in which antithymocyte globulin and high dose methylprednisolone were administered both before and after infusions of secondary partially T-cell-depleted marrow grafts.

RAFT-VERSUS-HOST disease (GVHD) is a major limitation to the success of bone marrow transplants (BMT) for the treatment of leukemia.2,3 This complication occurs in 50% to 70% of leukemic recipients of human leukocyte antigen (HLA)-identical sibling marrow transplants and is sufficiently severe (grades II to IV) to warrant treatment in 30% to 55% of patients, depending on the selection of pretransplant and posttransplant immunosuppressive agents.4,5 Several clinical trials with bone marrow depleted of donor T-lymphocytes indicate that both the incidence and severity of GVHD in engrafted patients are greatly reduced.6,9,10 Associated with these encouraging results in the reduction of GVHD, however, there has been an increased incidence of graft failure or graft rejection in recipients of T-cell-depleted transplants from either HLA-identical or HLA-nonidentical donors.4,6 In contrast to leukemic recipients of unmodified HLA identical marrow grafts, in which there is a 0.1% incidence of graft rejection5 and only 7% of the recipients require secondary transplants for poor graft function,10 the reported incidence of graft failure among recipients of T-cell-depleted HLA-identical transplants ranges from 10% to 30%.5,9 For patients transplanted with T-cell-depleted marrow from HLA-nonidentical donors, the incidence of rejection has been even higher.7,11 Previous analyses of T-cell-depleted marrow transplants have indicated that graft failure correlates with both the degree of HLA mismatch7 and the intensity of the pretransplant immunosuppression.12,13

In this report we have analyzed characteristics of the donor and recipient, including the cellular composition of the bone marrow graft, and the intensity of the pretransplant cytoreduction regimen used for their impact on the incidence of primary graft failure in recipients of T-cell-depleted HLA-identical marrow grafts. We have analyzed 122 patients with leukemia who received marrow grafts depleted of T cells by a modification of the technique of Reisner et al.14 In an accompanying paper results of in vitro analyses of peripheral blood mononuclear cells obtained from these patients at the time of graft failure are described.

We also present results of secondary transplants administered to 17 of the 19 patients with graft failure. The experience suggests that administration of equine antithymocyte globulin and high doses of methylprednisolone both before and after secondary partially T-cell-depleted grafts allows for reversal of primary graft failure and long-term survival in some patients.

MATERIALS AND METHODS

Patients and treatment. One hundred twenty-two patients received an HLA-identical sibling T-cell-depleted BMT for treatment of leukemia. Each patient received a marrow graft depleted of T cells with soybean agglutinin and sheep erythrocytes (SBA- E-).4,13 All patients received pretransplant immunosuppression with hyperfractionated total body irradiation (1,375 cGy or 1,500 cGy) administered over 4 days in 11 or 12 doses of 125 cGy at a dose rate of 8 to 20 cGy/min according to a modification of the technique described by Shank et al.16,17 followed by cyclophospha...
mide, 60 mg/kg for 2 days. Twelve patients received methylprednisolone in the early posttransplant period (5 mg/kg × 4 days beginning day + 3 with tapering over 8 days). Nine patients received donor peripheral blood mononuclear cells, calculated to give 0.5 × 10^7/kg clonable T cells. These were infused with the marrow inoculum on day 0. In a previous study a dose of 1 to 2 × 10^5 clonable T cells per kilogram was associated with grade I/II skin GVHD only. Accordingly, no posttransplant GVHD prophylaxis with 0.5/kg clonable T cells per kilogram was associated with grade I/II skin GVHD only.17 Accordingly, no posttransplant GVHD prophylaxis was given to these patients. Only the 12 patients who received methylprednisolone received immunosuppressive therapy in the posttransplant period for GVHD prophylaxis. The protocols were reviewed by the investigational review board of Memorial Sloan-Kettering Cancer Center and informed consent was obtained from each patient (or parent if the patient was a minor).

**Definition of graft failure.** The diagnosis of graft failure was made if the marrow and peripheral blood either failed to reveal any recovery of myeloid or erythroid elements from days 15 to 25, or, after initial partial or complete recovery, regressed to a state of pancytopenia with marrow aplasia. The day of graft failure was assigned to the day that the absolute neutrophil count declined to less than 1,000/mm^3 in the absence of acute GVHD. In the absence of hematologic recovery, day 15 posttransplant was considered the day of graft failure.

**Laboratory assays.** Residual T cells in the SBA^-E^- marrow grafts were quantitated by a microculture limiting dilution assay (LDA). The assay quantitates the frequency of phytohemagglutinin and interleukin-2 responsive T-lymphocytes in a given bone marrow sample.

Enumeration of committed myeloid colony-forming units (CFU-GM) in the unseparated and SBA^-E^- marrow fractions was carried out as previously described. Colonies and clusters were scored after 7 days of incubation at 37°C in humidified 5% CO2 atmosphere.

The donor and/or host origins of hematopoietic and lymphoid cells were established by cytogenetic analyses of spontaneously dividing marrow cells and phytohemagglutinin stimulated peripheral blood lymphocytes using autosomal polymorphisms distinguishable by quinacrine banding in sex-matched pairs, and the Y chromosome in sex disparate pairs.

HLA serotyping was performed by standard serologic techniques. Patient sera were screened to detect the presence of HLA, B-cell, and T-cell specific antibodies according to previously described methods.

**Statistical analysis.** The patient characteristics considered in this study were donor sex, recipient sex, donor age, recipient age, diagnosis, disease status at transplant, time from diagnosis to transplant, presence or absence of a spleen, presence or absence of antileukocyte antibodies, cytoreduction regimen, and the number of nucleated marrow cells, T cells (n = 80) and CFU-GM (n = 77) infused with the marrow inoculum as well as the number of each of these cell doses per kilogram. Initially, comparisons with respect to these individual characteristics were made between patients that engrafted and patients that experienced graft failure.

For discrete explanatory variables, Fisher's exact tests were performed to examine differences between groups defined by the response variable. The Wilcoxon rank-sum test was used to examine continuous explanatory variables in patients with or without graft failure. The Kruskall-Wallis test was used to determine the statistical significance between the results of cell doses obtained for each group of patients (engraft v graft failure).

Subsequently, multivariate analysis was performed to eliminate the redundancy among highly correlated characteristics, each of which may be individually significant. Because graft failure occurred within 60 days posttransplant in this patient population, the logistic regression model was applied. The logistic model is of the form:

\[
\log \left( \frac{P_i}{1 - P_i} \right) = \sum_{j=1}^{k} X_{ij} \beta_j
\]

where \(P_i\) is the probability of graft failure for the \(i^{th}\) patient. In addition, \(X_{ij}\) is the value of the \(j^{th}\) characteristic of the \(i^{th}\) individual, and \(\beta_j\) are the unknown regression coefficients. All covariates that added information to the model at the .05 level of significance as measured by the likelihood ratio test were included.

The probability of graft failure based on the sex of the donor was estimated using the Kaplan-Meier product-limit method. Because no patient experienced graft failure after day 60 posttransplant, the curves were terminated at day 70.

**RESULTS**

**Characteristics of patients and donors.** Pretransplant patient characteristics are summarized in Table 1. Of the 122 patients, four patients died early and four patients relapsed before day 60 during the course of hematopoietic recovery. One hundred fourteen consecutive patients were considered evaluable for durable engraftment. Nineteen of 114 evaluable marrow graft recipients experienced graft failure for an overall incidence of graft failure in this group of 17%.

**Patterns of graft failure.** After transplantation patients with durable engraftment showed prompt neutrophil recovery; an absolute neutrophil count of 500 cells/mm^3 was

<table>
<thead>
<tr>
<th>Table 1. Characteristics of Patients Evaluable for Graft Failure After an SBA^-E^- HLA Identical Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>CML</td>
</tr>
<tr>
<td>1-CP</td>
</tr>
<tr>
<td>2-CP</td>
</tr>
<tr>
<td>ACC-BL</td>
</tr>
<tr>
<td>AML</td>
</tr>
<tr>
<td>1 REM</td>
</tr>
<tr>
<td>2 REM</td>
</tr>
<tr>
<td>3 REM/REL</td>
</tr>
<tr>
<td>ALL</td>
</tr>
<tr>
<td>1 REM</td>
</tr>
<tr>
<td>2 REM</td>
</tr>
<tr>
<td>3 REM/REL</td>
</tr>
</tbody>
</table>
reached at a median of 19 days (range, 11 to 43 days). The pattern of graft failure varied considerably. Two patients failed to show any evidence of engraftment (Fig 1A). Four patients developed myeloid progenitors in the marrow and experienced an increment in peripheral blood neutrophil counts to between 300 and 3,300/mm³, which subsequently declined without a significant increment in the number of circulating lymphocytes (Fig 1B) and five patients developed early myeloid activity followed by an abrupt lymphocytosis between days 15 and 20 posttransplant identical to the pattern we have described in patients who have rejected HLA-nonidentical T-cell-depleted transplants (Fig 1C).²⁵

Eight patients reconstituted marrow cellularity and developed peripheral blood neutrophil counts in a manner similar to those patients who achieved sustained engraftment. Between days 31 and 60, absolute peripheral blood neutrophil counts declined to less than 1,000/mm³ and the patients developed marrow aplasia (Fig 1D). No patient developed graft failure after day 60.

**Cytogenetic studies.** In the group of 19 patients with graft failure, 8 of 14 evaluable patients had between 95% and 100% donor cells on their day 14 to 21 marrow samples. These patients were no different from those who achieved durable hematopoietic reconstitution: donor cells constituted between 27% and 100% (median 100%) of the marrow metaphases in 74 patients studied who had evaluable samples obtained between days 14 and 21 posttransplant.

In marked contrast to these cytogenetic findings in the marrow, the T cells obtained from the peripheral blood at the time of graft failure were 100% host origin in 12 of 14 patients, whereas among 33 engrafted patients whose T cells were evaluated between 14 and 70 days posttransplant, only one patient had 100% host T cells ($P < .001$).

**Recipient and donor characteristics associated with early or late graft failure.** Several donor and patient characteristics were considered for their association with graft failure. The sex of the donor was found to be strongly correlated with graft outcome. Recipients of male donor-derived grafts incurred a high risk for graft failure ($P = .002$). A Kaplan-Meier analysis of probability of durable engraftment among recipients of male donor grafts and female donor grafts is depicted in Fig 2. Overall, 96.3% of patients with female donors engrafted, whereas only 73.0% recipients of male donor marrow engrafted ($P = .001$).

The sex of the recipient did not, of itself, significantly affect the probability of graft failure (Table 2). Marrow grafts from male donors were at increased risk for failure in both male and female recipients (17% vs 15%, respectively). However, over and above male donor, male donor-female recipient pairs were at particular risk for graft failure following an initial apparent durable engraftment (Fig 1D, Table 2).

Among the continuous variables examined by the Wilcoxon rank-sum test (Table 3), the age of both the patient and donor correlated with graft failure ($P = .01, .005$, respectively). Because the age of the recipient was strongly correlated with the age of the donor ($r = .83$), it was not possible to discriminate between these two effects. Among 17 evaluable patients under the age of 15 years, no patient rejected his/her graft.

Diagnosis, status at the time of transplant, time from diagnosis to transplant, presence or absence of a spleen, presence or absence of antileukocyte antibodies, and specific cytoreduction regimen did not correlate with graft failure. In the course of this study, four preparative regimens were examined for their potential to limit the incidence of graft failure. Patients at the age of 15 years or older received altered regimens. Neither an additional fraction of irradiation (125 GY) pretransplant ($n = 61$) nor the administration of steroids ($n = 12$) in the posttransplant period altered the incidence of graft failure. The addition of donor peripheral blood mononuclear cells to the marrow inoculum successfully provided a dose of clonable T cells previously correlated with development of clinical signs of GVHD. For
the nine patients who received additional T cells, the median dose of T cells administered was $1.62 \times 10^5$/kg (range, 0.66 to $2.30 \times 10^5$/kg). Three of these nine patients developed grade II-IV GVHD; however, two of nine patients also suffered graft failure. Thus, in this small series, none of these approaches prevented or reduced the incidence of graft failure compared with hyperfractionated total body irradiation (1.375 cGy) and cyclophosphamide, 60 mg/kg for 2 days ($P > .9, = .62, = .66$, respectively).

No HLA antigen or haplotype was found more frequently among the patients with graft failure compared with those patients who achieved durable engraftment. Thus, in this series, the presence of specific HLA antigens was not associated with an altered risk of graft failure.

Because prior sensitization to donor HLA antigens has been associated with graft rejection among transfused patients with aplastic anemia, patients who suffered graft failure were also reviewed for exposure to sensitizing antigens through transfusion or pregnancy. Of the 19 patients who rejected their grafts, four had no history of either pregnancy or blood transfusions before transplant.

**Characteristics of the bone marrow graft associated with early or late graft failure.** To explain the increased sensitivity of male donor grafts to graft failure, we considered the possibility that the male donor grafts might have a lower number of total nucleated cells, marrow progenitors, or T cells. However, the doses of nucleated cells, CFU-GM, and clonable T cells in marrow grafts derived from male donors were actually significantly higher than those present in marrow grafts from female donors ($P = .03, .04, and .01$, respectively).

The total nucleated cell dose and the nucleated cell dose per kilogram were evaluated for each patient. The risk of graft failure tended to increase as the nucleated cell dose per kilogram decreased ($P = .08$) (Table 3). However, within the subgroup of male donor $\times$ male recipient transplants, graft failure was significantly related to a decrease in the nucleated cell dose per kilogram ($P = .03$) (Fig 3A). This degree of significance was not observed in the other sex paired transplants.

Because rejection of HLA-identical bone marrow transplants in leukemic patients is a phenomenon almost exclusively associated with T-lymphocyte depleted grafts, we also analyzed the number of T cells remaining in the inoculum after SBA- E$^+$ depletion as quantified by LDA. This sensitive assay has been used previously to document a correlation between the number of clonable T cells administered and the subsequent occurrence of GVHD in recipients of HLA-identical SBA- E$^+$ transplants. In the current study, 80 grafts were analyzed for residual T cells by LDA. Overall, there were no differences in the number of T cells received by patients with durable engraftment compared with those with early graft failure. However, the number of T cells per kilogram in male donor $\times$ male recipient grafts was also significantly correlated with graft failure ($P = .02$) (Fig 3B).

Seventy-seven of the T-cell–depleted grafts were analyzed for the number of CFU-GM. Overall, there were no differences in the CFU-GM content or CFU-GM/kg in grafts received by patients who engrafted and those whose grafts failed. However, again among the male donor $\times$ male recip-

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**Table 2. Effect of Donor/Recipient Sex Pairing on Graft Failure**

<table>
<thead>
<tr>
<th>Sex Donor/Recipient</th>
<th>Total Pairs</th>
<th>Fig 1 A, B, C $^*$</th>
<th>Fig 1D</th>
<th>Total GF</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F</td>
<td>33</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>F/F</td>
<td>24</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>M/M</td>
<td>28</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>F/M</td>
<td>29</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Abbreviation: GF, graft failure.

$^*$Patterns as defined in Fig 1.

$^\dagger$Estimate of graft failure at 70 days.

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**Table 3. Characteristics Associated With Graft Failure (P Value)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex of donor $^*$</td>
<td>.002</td>
</tr>
<tr>
<td>Age of donor $^\dagger$</td>
<td>.01</td>
</tr>
<tr>
<td>Age of patient $^\dagger$</td>
<td>.01</td>
</tr>
<tr>
<td>Nucleated cells/kg $^\dagger$</td>
<td>.08</td>
</tr>
</tbody>
</table>

*Fisher's exact test.

$^\dagger$Wilcoxon rank-sum test.
A

B

C

Fig 3. Absolute numbers (A) of nucleated cells (B), T-cells, and (C) CFU-GM expressed as cells per kilogram recipient body weight. F → F, female donor × female recipient. F → M, female donor × male recipient. M → F, male donor × female recipient. M → M, male donor × male recipient. (♀), Engrafted patients. (♂), Patients with graft failure.

Multivariate analysis. As a result of univariate analyses, four factors appeared to be predictive of graft failure (Table 3): donor sex, donor age, recipient age, and nucleated cell dose per kilogram. Multivariate analysis was undertaken to determine which factors were most important for predicting graft failure. In view of the binary response (graft failure or engraftment), a logistic regression model was applied to the data. Figure 4 shows the results of fitting this model. The model indicates that the odds of graft failure are higher if the patient has a male donor (coefficient β₁ = 2.44 ± 0.81 [SE]) or as the patient ages (coefficient β₂ = 0.067 ± 0.03 [SE]). Nucleated cell dose per kilogram and donor age were not included in the final model. However, due to the strong correlation between donor age and recipients age (r = .83), replacement of recipient age with donor age would produce a negligible change in the fit of the logistic regression model.

Results of secondary transplants. Seventeen of the 19 patients who developed graft failure received secondary marrow grafts. Four patients received a secondary infusion of marrow without additional immunosuppression. Each failed to engraft despite infusions of SBA-E− (1 patient) or unseparated (1 patient) marrow from the same donor or autologous cryopreserved marrow (2 patients).

Six patients received a secondary infusion of untreated marrow from the same donor following immunosuppression as outlined in Table 4. Partial or complete engraftment was seen in 3 of 4 patients who received both total body irradiation (TBI) and cytoxan (CTX), and neither patient who received total lymphoid irradiation (TLI) in combination with cytoxan engrafted. Although three of these six patients demonstrated partial or complete engraftment, no patient survived. Four patients died of infections (one with interstitial pneumonia), one patient died of grade IV GVHD, and one patient died following a third transplant. One additional patient who received irradiation and chemotherapy died of sepsis 5 days following an infusion of SBA-E− marrow and was therefore unevaluable for engraftment.

The results in this small group of patients suggested that immunosuppression was likely necessary to ensure engraftment of secondary unseparated marrow infusions following a primary graft failure but that further immunosuppression was poorly tolerated. Furthermore, the development of fatal GVHD in one patient suggested that engraftment of an unmodified secondary transplant to occur, prophylaxis for GVHD would be necessary.

Following our identification of host T cells specifically
<table>
<thead>
<tr>
<th>Patient (UPN)</th>
<th>Day Post</th>
<th>1st BMT</th>
<th>Day</th>
<th>Treatment</th>
<th>Posttransplant</th>
<th>Engraft</th>
<th>Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td>419 57</td>
<td>-12 -9</td>
<td>-7</td>
<td>-6</td>
<td>-5</td>
<td>TLI 720 cGy</td>
<td>CTX 50 mg/kg x 2 days</td>
<td>BMT</td>
</tr>
<tr>
<td>458 37</td>
<td>-</td>
<td>-</td>
<td>-3</td>
<td>-2</td>
<td>ARA-C 3 g/m² q12h x 2 days</td>
<td>TBI 300 cGy</td>
<td>CTX 50 mg/kg x 2 days</td>
</tr>
<tr>
<td>509 42</td>
<td>-</td>
<td>-</td>
<td>-5</td>
<td>-2</td>
<td>CTX 50 mg/kg x 2 days</td>
<td>TBI 600 cGy</td>
<td>BMT</td>
</tr>
<tr>
<td>560 52</td>
<td>ATG 40 mg/kg/d</td>
<td>TBI 300 cGy</td>
<td>CTX 40 mg/kg x 4 days</td>
<td>BMT</td>
<td>None</td>
<td>Yes</td>
<td>52 (104) Grade IV GVHD</td>
</tr>
<tr>
<td>581 37</td>
<td>-</td>
<td>-</td>
<td>-3</td>
<td>-2</td>
<td>TBI 300 cGy</td>
<td>CTX 40 mg/kg x 4 days</td>
<td>BMT</td>
</tr>
<tr>
<td>596 49</td>
<td>-</td>
<td>TLI 600 cGy</td>
<td>ATG 40 mg/kg/d x 4 days</td>
<td>BMT</td>
<td>MTX (d1, 3, 6, 11) CYA</td>
<td>No</td>
<td>19 (68) sepsis</td>
</tr>
</tbody>
</table>

*Days post secondary (primary) transplant.
GRAFT FAILURE AFTER T-CELL-DEPLETED BMT

suppressive for donor marrow progenitor cells in patients experiencing graft failure, seven patients received immuno-suppression with a protocol similar to that used successfully for treatment of aplastic anemia. Antithymocyte globulin (Atgam; Upjohn, Kalamazo, MI) and methylprednisolone were administered as outlined in Table 5 and patients were given infusions of marrow from the same donor. Two patients died of hemorrhagic complications (due to pre-existing refractory thrombocytopenia) after initiation of the protocol but prior to or on the day after marrow infusion and thus were not evaluable for engraftment. Of five patients in this series who could be evaluated for engraftment (Table 5), three patients engrafted with full hematopoietic reconstitution. Both patients who received partially T-cell-depleted marrow (SBA-) (1.0 to 1.5 log10 T-cell depletion) engrafted, and only 1 of 3 patients who received a full SBA-"E" engrafted. One of the five engrafted patients (UPN 655) is alive and disease-free at 875 + days. One patient with CML in second chronic phase (UPN 661) relapsed at 167 days posttransplant and survives in chronic phase at 854 + days. One splenectomized patient (UPN 633) died, in remission, of pneumococcal sepsis at 565 days.

DISCUSSION

The clinical and laboratory characteristics of 114 evaluable leukemic recipients of HLA identical sibling marrow depleted of T cells with soybean agglutinin and sheep erythrocytes were analyzed for their association with graft failure. The incidence was significantly higher among recipients of marrow derived from male donors. The risk of graft failure for a transplant from a male donor was significantly increased irrespective of the sex of the recipient.

Previous studies that have analyzed risk factors associated with graft failure have focused on series of patients transplanted for treatment of aplastic anemia. In two analyses grafts from male donors were more often rejected than those from female donors. In the Seattle experience this risk of failure was present regardless of the sex of the recipient, whereas the group at Johns Hopkins reported that the risk of graft failure was greatest among male donor-female recipient pairs. The International Marrow Transplant Registry and the European Marrow Transplant Group failed to demonstrate an increased risk of graft failure for recipients of male donor grafts. However, the marked variation in preparative cytoreduction and GVHD prophylaxis used by the different centers contributing to these analyses may have obfuscated such a trend.

Analyses of risk factors associated with graft failure in studies in which T-cell depletion techniques have been used to prevent GVHD have not identified male donor derived grafts to be at increased risk for failure. However, in one study of 52 patients there was a trend for patients with sex-mismatched donors to be at higher risk for graft failure than those with sex-matched donors.

The basis for the increased probability of graft failure among recipients of transplants from male donors in our series is unclear. In male donor x female recipient pairings, it is possible that the H-Y antigen could serve as an alloantigenic target for the female host immune system. The H-Y antigen is expressed on human hematopoietic progenitor cells. This antigen has been shown by Goulmy et al to be significant for marrow graft failure in the setting of transplantation for treatment of aplastic anemia. Furthermore, Voogt et al have recently shown that in the setting of graft rejection it is possible to generate cytotoxic T-cell lines with reactivity against this antigen that are HLA class I restricted. Both peripheral blood and marrow progenitor cells can serve as target cells.

An H-Y directed immune response cannot be invoked, however, to explain the observed increased sensitivity of male donor grafts in male recipients. The possibility that the total nucleated cell dose, progenitor cell, or T-cell dose administered in a male donor compared with a female donor graft is reduced was considered, but in this series the converse was actually the case. However, among male recipients of male donor transplants, graft failure was significantly correlated with a decrease in the total nucleated cell dose per kilogram, progenitor cell (CFU-GM) dose per kilogram, and T-cell dose per kilogram ($P = .02$).

A correlation between cell dose and engraftment has been observed in several murine models of marrow transplantation in the context of natural or hybrid resistance. With T-cell-depleted marrow, graft failure in mice is also a cell-dose-dependent phenomenon in syngeneic, semiallogeneic, and minor antigen disparate strain combinations. In humans low cell dose has been correlated with an increased risk of graft failure. However, the marked variation in preparative cytoreduction and GVHD prophylaxis used by the different centers contributing to these analyses may have obfuscated such a trend.

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Table 5. Secondary Transplants: High-Dose Methylprednisolone/Antithymocyte Globulin (HDMP/ATG)

<table>
<thead>
<tr>
<th>Patient (UPN)</th>
<th>Graft Failure (d)</th>
<th>Day Post 1st BMT</th>
<th>BM1</th>
<th>Engraft</th>
<th>Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td>633</td>
<td>52</td>
<td>78</td>
<td>SBA-&quot;E&quot;</td>
<td>Yes</td>
<td>487 (565) pneumococcal sepsis</td>
</tr>
<tr>
<td>639</td>
<td>52</td>
<td>80</td>
<td>SBA-&quot;E&quot;</td>
<td>No</td>
<td>19 (99) pseudomonas sepsis</td>
</tr>
<tr>
<td>633</td>
<td>29</td>
<td>43</td>
<td>SBA-&quot;E&quot;</td>
<td>No</td>
<td>19 (62) relapse</td>
</tr>
<tr>
<td>655</td>
<td>20</td>
<td>34</td>
<td>SBA-&quot;</td>
<td>Yes</td>
<td>875 +</td>
</tr>
<tr>
<td>661</td>
<td>20</td>
<td>42</td>
<td>SBA-&quot;</td>
<td>Yes</td>
<td>125 (167) relapse</td>
</tr>
</tbody>
</table>

ATG: 15 mg/kg (days − 5 through − 1, 5, 7, 9, 11, 13, 15, 17, and 19). Methylprednisolone: 20 mg/kg (days − 5 through − 3); 10 mg/kg (days − 2 through 1); 5 mg/kg (days 2 through 5); 2 mg/kg (days 6 through 20); 1 mg/kg (days 21 through 30); after day 30, slowly taper over the next 2 months.

*Days post secondary (primary) transplant.
rate of graft rejection only in patients with aplastic anemia who have been conditioned with cyclophosphamide alone. The effect of marrow cell dose was not detected in leukemic patients conditioned with irradiation. In our series the correlation between cell dose and engraftment was significant only for male donor × male recipient pairings. Female derived marrow failed in only two instances. We are exploring the possibility that T-cell-depleted marrow obtained from a female donor contains small populations of T cells that have been previously sensitized to minor alloantigens through prior pregnancies or sexual experiences. Sensitized T cells could overcome residual host resistance yet not be present in sufficient numbers to induce overt GVHD. Consistent with this concept are previous analyses of factors associated with GVHD following untreated allogeneic marrow transplants in adults, which have identified marrow transplants from multiparous female donors to be associated with an increased risk of GVHD irrespective of the sex of the recipient. The patterns of graft failure in our patients are similar to those reported by other investigations. However, in contrast to other reports, no patient in our series experienced graft failure after day 60. The absence of posttransplant immunosuppression with cyclosporine could account for this finding because discontinuation of cyclosporine has been associated with late graft failure among recipients of untreated marrow for treatment of aplastic anemia. Among durably engrafted recipients of T-cell-depleted marrow a high incidence of mixed chimerism in both the hematopoietic and lymphoid cell populations has been observed. This was true in our series as well. However, notably, 5 of 6 patients with graft failure after day 30 and evaluable marrow metaphases at days 14 to 21 had 100% donor metaphases, indicating that even patients with apparent full donor marrow engraftment early posttransplant are at risk for late graft failure. Thus, cytogenetic analyses of marrow obtained between days 14 and 21 posttransplant was not predictive of patients who developed either early or late graft failure. However, in the limited group studied, the origin of phytohemagglutinin responsive T cells strongly correlated with graft outcome. Further studies are in progress to examine the issue in a larger series of patients.

Several studies have suggested that the administration of either higher doses of irradiation or additional chemotherapy reduces the incidence of graft failure following a T-cell–depleted transplant. In our series increasing the dose of irradiation from 1,375 to 1,500 cGy did not alter the incidence of graft failure. Martin et al have demonstrated that posttransplant cyclosporine, methotrexate, or a combination of both did not decrease the incidence of graft failure. Similarly, in our series the addition of high dose methylprednisolone had no effect on the engraftment rate.

Our initial experience with secondary transplants suggests that further immunosuppression is necessary to ensure engraftment of both allogeneic and autologous cryopreserved marrow. The reason for the latter is unclear but suggests either the presence of a deficient host microenvironment or the possibility of the presence of residual donor lymphoid cells with antihost activity. Unfortunately, additional immunosuppression with protocols involving either total body or total lymphoid irradiation were associated with intolerable toxicity. However, patients in our series who received ATG and high dose methylprednisolone prior to and following a secondary transplant of partially T-cell–depleted (SBA−) marrow achieved engraftment with limited toxicity. The immunosuppressive activity of ATG may have promoted engraftment of these secondary transplants. However, it is of note that ATG has been reported to be both mitogenic to T-lymphocytes and stimulatory to marrow progenitor cells and, thus, may promote engraftment through several mechanisms. The successful use of ATG and methylprednisolone for preparation of patients in these secondary transplants suggests that incorporation of these agents into cytoreductive regimens for recipients of primary T-cell–depleted transplants may reduce the risk of graft failure in leukemic recipients of male donor marrow grafts.

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Graft failure after T-cell-depleted human leukocyte antigen identical marrow transplants for leukemia: I. Analysis of risk factors and results of secondary transplants

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