Retarded Recovery of Functional T Cell Frequencies in T Cell–Depleted Bone Marrow Transplant Recipients

By John P. Daley, Marta K. Rozans, Brian R. Smith, Steven J. Burakoff, Joel M. Rappeport, and Richard A. Miller

We have studied the effect of removing donor T cells by treatment with the monoclonal antibody Leu-1 and complement before marrow transplantation on the regeneration of functionally competent T lymphocytes in the blood at selected times after transplant. Using sensitive limiting-dilution methods that allow us to enumerate helper, cytotoxic, and proliferating T lymphocyte precursors, we report that regeneration of a functional T cell compartment is more severely impaired for the first 180 days after transplantation in those patients given T cell–depleted bone marrow than in recipients of untreated marrow. After this first 6 months, however, patients given T cell–depleted bone marrow had blood T cell frequencies comparable to those observed in patients given untreated marrow. Diminished frequencies of reactive T cells in recipients of depleted marrow could leave them more susceptible to infection or to the recurrence of neoplastic cells.

© 1987 by Grune & Stratton, Inc.

MATERIALS AND METHODS

Patient population and clinical protocol. Thirty-eight patients were studied, 11 of whom received T cell–depleted marrow. All patients were advised of the procedures to be used and the attendant risks, in accordance with institutional guidelines, and all gave informed consent. Table I presents a summary of patient characteristics. A variety of preparative regimens were used, depending on the underlying disorder and clinical condition. The leukemic patients in general received cytosine arabinoside (500 mg/kg/d as a continuous infusion for seven days), cyclophosphamide (60 mg/kg/d for two days), and total body irradiation (either a 1,000-rad midline dose in a single treatment or 1,100 to 1,400 rad in six to eight fractions over three to four days). The patients with aplastic anemia received cyclophosphamide (50 mg/kg/d for four days), procarbazine (12.5 mg/kg/d for three days), and rabbit antithymocyte serum (0.2 mL/kg/d for three days).

Thirty-two patients had HLA-compatible donors, either siblings or, in one case, an HLA-identical father. Seven of these HLA-matched individuals received T cell-depleted marrow because they were felt to be at high risk for severe, acute, and/or chronic GvHD in that they were either >30 years old and/or sex mismatched with the prospective donor. Four other patients received depleted marrow because they lacked HLA-matched donors. Of these four donors, two were haploidentical (ie, completely matched for one haplotype and mismatched for the other), and two others had identity at one haplotype and partial identity at the other.

For those patients who were to receive T cell-depleted marrow, 15 to 20 mL of donor marrow was harvested per kilogram of recipient body weight and the leukocyte fraction concentrated on an IBM Blood Cell Processor. Mononuclear cells were then isolated on Ficoll-Hypaque sedimentation gradients. The mononuclear cells were then placed in conical tubes at 20 × 10^6 cells/mL, 25 mL/tube, to which was added 10 µg/mL anti-Leu-1 monoclonal antibody (Becton Dickinson Immunocytometry Systems, Mountain View, CA); after a 30-minute incubation at 4°C, the cells were pelleted and brought up in 25 mL of diluted (1:3) newborn rabbit complement (Pel-Freez Biologicals, Rogers, AR). The cells remained in the complement for 45 minutes at 37°C, were washed once in medium, and in six cases (determined by surviving cell number) were treated with two additional cycles of antibody and complement. After completion of the depletion treatment, the remaining marrow cells were resuspended in 50 mL of medium and infused intravenously into the recipient over 10 to 30 minutes.

It is noteworthy that engraftment, as indicated by recovery of...
T CELL FREQUENCIES AFTER T-DEPLETED BMT

Table 1. Summary of Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>T-Depleted Marrow</th>
<th>Nondedepleted Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Male sex</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>24 ± 12</td>
<td>17 ± 9</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>AGvHD</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>CGvHD</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Disease recurrence</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Died</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviations: AGvHD, acute GvHD; CGvHD, chronic GvHD.

Methods to estimate the frequencies of mitogen-responsive T cell frequencies after T-depleted BMT cell depletion protocol also removes functional helper T lymphocyte (pHTL) and cytotoxic T lymphocyte precursor (pCTL) cells.

Measurement of functional T cell frequencies by LD analysis. Methods to estimate the frequencies of mitogen-responsive precursors for interleukin-2 (IL-2) secreting helper cells (pHTL), proliferating cells (pPTL), and cytotoxic cells (pCTL) have been described in detail elsewhere.17 18

Statistical analyses. Because the distribution of LD frequencies within each tested population is nonnormal, simple between-group comparisons (by Student’s t test) are carried out on log-transformed frequencies, and the resulting geometric means and error factors are presented in the Tables. Some patients had been tested more than once; in these cases we selected the test(s) carried out closest to 6 and 12 months after transplantation for analyses. Step-wise multiple regression analyses to examine the independent effects of age, sex, acute and chronic GvHD, days after transplantation, and marrow depletion on LD results were also performed on log-transformed data.

RESULTS

Delayed regeneration of pCTL and pPTL in recipients of T cell–depleted bone marrow. To see whether T cell depletion from transplanted marrow retarded the recovery of functional T lymphocytes in the blood of bone marrow transplant (BMT) patients, we performed a series of LD tests on 11 individuals who had received marrow treated with anti-Leu-1 antibodies and complement and compared their status to 27 contemporaneous recipients of nondepleted marrow and to healthy young adult values assayed in our lab over the same period of time. We performed independent analyses for data obtained within the first 6 months after transplantation and for data obtained after the first 6-month interval. The results are presented in Fig 1, and summary statistics are shown in Table 2.

We have previously reported17 that levels of pHTL, pCTL, and pPTL are greatly below normal values for nearly all patients within the first posttransplant year and remain low for most patients for many years after transplantation. The amount of deficit, however, was found to be even more severe for recipients of depleted marrow than for recipients of untreated marrow. Deficits of pHTL, pCTL, and pPTL for control patients were 11-, 7-, and 22-fold compared to normals, respectively, within the first 6 months, but were 20-, 40-, and 100-fold, respectively, for the recipients of T-depleted marrow. The differences between the two groups of transplant recipients could not be attributed to underlying differences in lymphocyte counts (1,107 ± 222...

Fig 1. (A) Frequencies of concanavalin A–reactive precursors for IL-2–secreting helper T cells (pHTL) in the blood of normal controls and BMT recipients at selected times after transplantation expressed as responding cells per 10⁶ peripheral blood mononuclear cells (PBMC). (B) Frequencies of PHA/IL-2–reactive pCTL. (C) Frequencies of PHA/IL-2–reactive pPTL.
SEM, N = 19, for recipients of undepleted marrow at 91 to 180 days post-BMT as compared with 854 ± 157, N = 11, for recipients of depleted marrow; P > .1) but reflect instead differences in the fraction of the blood lymphocytes that responded in culture.

To see whether the effect of marrow treatment could be attributed to differences between the two patient groups in characteristics that could potentially influence T cell regeneration, we performed a step-wise multiple regression analysis to seek independent effects of five such variables in addition to marrow treatment: recipient age, recipient sex, time after transplantation, and the presence of acute or chronic GvHD. In this model, the independent effects of marrow treatment on responder cell frequencies before 6 months post-BMT were found to be significant for pPTL at the level of P < .03 and for pCTL at the P < .001 level. The trend toward lower pHTL values in recipients of treated marrow was not found to be statistically significant.

The discrepancy in pPTL and pCTL levels between depleted and nondepleted groups diminished in the second half of the first posttransplant year: for tests performed at times more than 6 months after transplantation (Fig 1 and Table 2), there was no significant difference between these two patient populations. Although there is a suggestion, from the limited data available, of continued impairment of pHTL function in recipients of treated marrow, the difference does not reach statistical significance (P > .05).

T cell frequencies in the first posttransplant month. It is unclear to what extent functional T cells present in the marrow inoculum contribute to immune function in the transplant recipient during the immediate posttransplant period. To address this question, we have determined precursor frequencies within the first 40 days post-BMT in a small number of patients given T-depleted bone marrow compared with those given untreated marrow. These results for pHTL and pPTL are presented in Table 3; there were too few available pCTL tests within this interval to provide useful information. Although our sample sizes are quite small, precursor frequencies within this early time period seem to mirror those observed at later times within the first 6 posttransplant months (Table 2). Patients given T-depleted marrow had a sevenfold diminution in pPTL within this time period compared with those given untreated marrow (P < .02). Helper cell frequencies, though lower in the former patients, were not statistically different from the pHTL frequencies for patients given untreated marrow.

**DISCUSSION**

The reestablishment of a normally functioning immune system is crucial to the disease-free survival of BMT recipients. We have previously demonstrated that LD methods are extremely sensitive in their ability to detect long-term functional deficits in the helper and cytotoxic T cell compartments of BMT patients. Our present study was undertaken to determine whether the removal of donor T cells from the marrow before transplantation, in an attempt to reduce the incidence of GvHD, had an effect on the level of T cell function seen in the transplant patient in the first 6 months post-BMT. Our findings demonstrate that the level of T cell function (as measured by the pPTL and pCTL tests) within this period is significantly reduced in those patients given T cell–depleted donor bone marrow compared with those given untreated bone marrow. Although we cannot rule out the presence of suppressor cells in the transplant recipients, the linear dose-response curves and the absence of suppressive interactions in mixing experiments (not shown) provided no evidence that the diminished frequencies recorded were due to suppressor cell activity. The LD methods provide only minimal estimates of frequencies, and it is possible that different culture conditions might elicit functional responses from a higher proportion of the cells, but our findings are consistent with and suggest a potential basis for diminished protective immune function in transplant recipients.

How can one account for the especially severe deficit in T cell precursor frequencies seen in recipients of depleted marrow? One possibility is that higher number of immunocompetent T cells seen in recipients of nondepleted marrow
might simply represent survival, after transplantation, of T cells present in the marrow inoculum. A rough calculation suggests that this idea is plausible only if the infused cells are all assumed to survive and to restrict themselves to the blood rather than to the peripheral lymphoid system as a whole. Our “average” patient received $10^8$ nucleated marrow cells/kg body weight, or $5 \times 10^8$/50-kg patient; these $5 \times 10^8$ nucleated marrow cells are expected to contain $10^8$ pPTL cells. If all of these were to survive and to localize entirely in the blood, they would generate by themselves a pPTL frequency of approximately $10/10^3$ (calculated as $10^8$ pPTL/$10^9$ total blood lymphocytes), similar to the frequency actually observed in the first 40 days after transplantation (Table 3). If, however, we make the more likely assumptions of incomplete survival and distribution within the 20-fold larger pool of peripheral T cells, the observed blood frequencies fall to $<0.5 \, \text{pPTL}/10^3 \, \text{PBMC}$, substantially below the observed values.

We suspect, therefore, that most of the pPTL seen in BMT recipients even in the early posttransplantation period have developed in the recipients, either from mature T lymphocytes by antigen-driven clonal expansion or from less mature cells. There is evidence that specific humoral immune responses can be transferred from donor to recipient by BMT, but these responses are often transient in the absence of renewed sensitization. The development of antiviral responses (determined serologically) to herpes simplex and to cytomegalovirus has been shown not to depend on the pretransplant immune status of the donor, although virus-reactive cells in the transplant recipient are predominantly donor derived. These studies suggest that, although mature, antigen-specific immunocytes can be transferred with marrow to BMT recipients, reestablishment of recipient immune competence depends not on transferred mature cells but on in situ regeneration of new T cells from donor-derived precursors.

Whether these precursors are themselves Leu-1+ post-thymic T cells and therefore likely to be damaged by the depletion protocol used in our study is unknown.

Differences between recipients of depleted and nondepleted marrow might in addition reflect trophic effects of transferred mature T cells on the development of other, less mature cell types. This would be consistent with delayed recovery of nonlymphoid lineages in recipients of T-depleted marrow. An alternate explanation, that the depletion method might inadvertently damage a cell type required for rapid lymphoid recovery, should also be considered despite evidence that the method used does not impair function of CFU-GM, CFU-GEMM, or BFU-E.

Finally, the observed differences in T cell function might reflect differences in the two patient groups rather than an effect of depletion per se. For ethical reasons, treatment was restricted to those patients who were thought, on historical grounds, to be at higher risk for development of GvHD; as anticipated, however, the incidence of chronic GvHD was lower in the recipients of depleted marrow. Although there was, in our small sample, no independent, statistically significant correlation between observed precursor frequency and either GvH status or genetic or sex disparity, it would be rash to exclude these factors themselves as potential influences on the pace of T cell regeneration.

Because immunodeficiency after BMT is typically more severe in recipients with GvHD, it is hoped that T cell depletion, by diminishing GvH severity, might lead to improved immune function. Our present findings, however, suggest that the recipients of depleted marrow may, for a limited time, be more severely immunocompromised than patients who receive nondepleted marrow. Following studies using LD methods will be needed to document the late effects of marrow depletion and genetic disparity on regeneration of functional T cells.

REFERENCES

clonal antibody OKT3 to prevent acute graft-versus-host disease in allogeneic bone-marrow transplantation for acute leukaemia. Lancet 1:700, 1982


Retarded recovery of functional T cell frequencies in T cell-depleted bone marrow transplant recipients

JP Daley, MK Rozans, BR Smith, SJ Burakoff, JM Rappeport and RA Miller