Immune Reconstitution Following Bone Marrow Transplantation: Comparison of Recipients of T-Cell Depleted Marrow With Recipients of Conventional Marrow Grafts


The reconstitution of hematopoietic cells and in vitro assays of immunologic function have been followed in leukemic patients after conventional bone marrow transplantation (BMT) \((N = 34)\) and T-cell depleted BMT \((N = 52)\) from human leukocyte antigen (HLA)-identical sibling donors. No effects of the T-cell depletion could be seen on the recovery of myeloid or lymphoid cells as measured by the day to engraftment or by the absolute number of cells through day 100. Normal numbers of lytically active natural killer cells returned the earliest and were rapidly followed in both groups of patients by the appearance of circulating B cells and normalization of the responses to B-cell mitogens. However, the recovery of normal T-cell proliferative responses were more delayed in recipients of T-cell depleted grafts. Significant quantitative differences were seen only during the first 3 months after transplantation. Neither the number of CD3 T cells nor the ratio of CD4:CD8 positive cells differed markedly between the two transplant groups. Mitogen-induced immunoglobulin production by peripheral blood lymphocytes (PBL) from patients following T-cell depleted BMT was quantitatively less than that of conventional marrow recipients through the first year, with low normal IgM production reached by 4 to 6 months in both groups. IgG production reached low normal 7 to 9 months after conventional BMT but did not remain at this level until 1 year following either type of transplant. Assessment of the incidence of infections from the day the absolute neutrophil count reached 500 until day 180 after transplant revealed no significant differences between the two groups; indeed, the overall nonleukemic mortality was higher in the recipients of conventional bone marrow. Thus, in our series, the removal of mature cells from the marrow graft did not affect the rate or degree of recovery of myeloid and lymphoid cells but did affect the regeneration of in vitro T-cell dependent functions. We noted early quantitative differences and a delay in the normalization of the T-cell functions measured rather than prolonged absolute deficiencies. The in vitro deficiencies did not result in significantly clinically apparent differences between the two groups.

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Immunocompetent donor-derived cells transfused with the marrow graft in recipients of T-cell depleted grafts.\(^5,10\) Other studies in murine\(^12\) and in human\(^13,15\) systems have suggested that the hematopoietic and immunologic reconstitution in recipients of T-cell depleted marrow is not impaired.

One of the more effective T-cell depletion methods currently in use is the technique developed by Reisner et al\(^14\) that entails fractionation of donor marrow with the lectin soybean agglutinin followed by removal of rosette-forming cells with sheep erythrocytes (SBA-E-BMT). This technique consistently results in a 2.5-3.0 log depletion of clonable T cells. The first 127 recipients of human leukocyte antigen (HLA) identical SBA-E-BMT have shown an incidence of grades I or II acute GVHD of 7% and 5% respectively with no clinically apparent chronic GVHD without the addition of posttransplant GVHD chemophrophylaxis.\(^6,15\) In previous separate studies we have assessed immune function in recipients of conventional or T-cell depleted grafts during different time periods and had noted some differences relative to production of and response to interleukin-2 (IL-2).\(^16,17\) In this comparative study we have measured the recovery of granulocytes, natural killer (NK) cells, T cells, and B cells in leukemic patients transplanted during the same time period with HLA-identical marrow both with and without T-cell depletion. We have measured the effect of the marrow manipulation on the degree or rate of reconstitution of these cells and their functional capacities.

MATERIALS AND METHODS

Patients and controls. Durabley engrafted patients who had received transplants from HLA-identical sibling donors for chronic or acute leukemia were included in this study. The recipients of T-cell depleted marrow consisted of 29 men and 23 women (median
age, 28.3 yr; range, 3-48 yr), who had received transplants for chronic myelocytic leukemia (CML) in first or second chronic phase (N = 30), acute myelocytic leukemia (AML) in first, second, or third remission (N = 16), or acute lymphoblastic leukemia (ALL) in first, second, or third remission (N = 6). Patients receiving conventional BMT included 19 men and 15 women (median age, 13.7 yr; range, 2-37 yr), and included patients who had received transplants for AML in first, second, or third remission (N = 12), ALL in first, second, or third remission (N = 17), CML (N = 4), and one patient with biphenotypic leukemia. All of the patients were conditioned with either 1,440 or 1,320 rads of hyperfractionated total body irradiation followed by treatment with cyclophosphamide (60 mg/kg/day for two days). None of the recipients of T-cell depleted BMT received GVHD chemoprophylaxis and none developed chronic GVHD or higher than grade II acute GVHD. Patients receiving conventional marrow transplants received short-course methotrexate \(^{19}\) with or without cyclosporine posttransplant as GVHD prophylaxis. \(^{19}\) Twelve of the conventional marrow recipients tested had \(\geq\) grade II acute GVHD and six patients developed chronic GVHD. Control patients consisted of 56 normal laboratory personnel or bone marrow donors. These studies were performed after informed consent was obtained under protocols approved by the Human Subjects Review Committee of Memorial Hospital.

**Cell preparations.** Peripheral blood lymphocytes (PBL) were separated from heparinized blood on Ficoll-hypaque density gradients was obtained under protocols approved by the Human Subjects Review Committee of Memorial Hospital.

**Proliferative assays.** PBL were resuspended in culture medium additionally supplemented with 10\(^{-5}\) mmol/L 2-mercaptoethanol for proliferation and immunoglobulin (Ig) production assays. The cells were plated in flat bottomed, 96-well microtiter trays at 10\(^5\) cells per well with optimal concentrations of mitogens. These were: 0.01% *Staphylococcus aureus* Cowen strain A (SAC) (Pansorbin, Calbiochem), rabbit antibody to human IgM coupled to polyacrylamide beads (anti-Mu) (Immunobeads, Bio-Rad, Richmond, CA) at 4, 2, and 1 \(\mu\)g/mL, phytohemagglutinin (PHA; Difco, Detroit) at 5-10 \(\mu\)g/mL, or pokeweed mitogen (PWM) (Gibco, Grand Island, NY) at a final culture dilution of 1:100. The cultures were maintained for 72 hr at 37\(^\circ\)C in a humidified atmosphere of 5% CO\(_2\) and labeled the last 24 hr of culture with 1 \(\mu\)Ci well of \(^{3}H\)-thymidine (6.8 \(\mu\)Ci/mM) (New England Nuclear, Boston) prior to harvest. The results are expressed as the mean counts per minute (cpm) of triplicate cultures minus the unstimulated medium control.

**Immunoglobulin production assays.** Ig synthesis was induced by the coculture of 2 \(\times\) 10\(^5\) PBL per well for seven days at 37\(^\circ\)C in a humidified atmosphere of 5% CO\(_2\) with a synergistic combination of PWM (1:4,000) and SAC (0.004%). \(^{26}\) in a total volume of 200 \(\mu\)L. Ig secreted into the supernatant was measured by an inhibition enzyme-linked immunosorbent assay (ELISA) based on a method described by Voller et al. \(^{21}\) The optical density (OD) at 405 nm was measured and the amount of Ig in the test sample was calculated from the regression line of the Ig standard. The sensitivity of the assay was approximately 10 ng/mL of Ig. Control cultures of T cells alone, highly purified B cells alone, and irradiated PBL (3,000 rads) consistently produced <10 ng/mL of Ig.

**NK cell assay.** The cytolytic activity of freshly isolated PBL was measured in a standard four hour \(^{51}Cr\) release assay using as a target the NK-sensitive cell line K562 as previously described. \(^{25}\) Data was expressed as the percentage of specific lysis at a given E:T ratio calculated from the equation:

\[
\text{Specific Lysis} = \frac{\text{Experimental CPM} - \text{Spontaneous CPM}}{\text{Max CPM} - \text{Spontaneous CPM}} \times 100
\]

**Immunofluorescence analysis.** One- and two-color immunofluorescence analyses were performed by standard techniques, using a whole blood lysis method or PBL isolated as described above. A pan leukocyte antigen, HLe-FITC, served as a control to gate out residual RBCs. Each two-color fluorescence study included a double negative control (MsIgG-FITC/MsIgG-PE), a positive FITC/ negative PE control (HLe-FITC/MsIgG-PE), and a negative FITC/positive PE control (MsIgG-FITC/Leu4 or B1-PE). The monoclonal antibodies used in this study were purchased from Becton Dickinson (Mountain View, CA) and include markers of T cells (Leu4 (CD3), Leu3 (CD4), and Leu2 (CD8)), B cells (Leu16 (CD20)), monocytes (Mo2), and NK cells (Leu19, Leu11 (CD16)). For the determination of the percentage of CD8\(^+\) T cells, only brightly fluorescent cells that coexpressed CD3 were included. Immunofluorescence samples were analyzed on an EPICS-C cytofluorograph cell sorter (Coulter Immunology, Hialeah, FL).

**Statistics.** The time to recovery of granulocytes and lymphocytes was found to be normally distributed in each of the two transplant groups; therefore, the data were compared using Student’s t-test. The assays of T and B cell function included multiple observations on each patient made at differing times posttransplant. As a result, the observations were grouped into time intervals and the median observation was used if a patient was tested more than once during a given interval. Each parameter tested was analyzed separately. Proliferation and immunoglobulin production data were not usually normal distribution. For these analyses, the data were compared using statistical tests of nonparametric data (Kruskal-Wallis, Mann-Whitney).

**Table 1. Recovery of Granulocytes and Lymphocytes Following Conventional and T-Cell Depleted BMT**

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Conventional BMT (\text{(N = 33)})</th>
<th>T-Cell Depleted BMT (\text{(N = 52)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC 500/(\mu)L</td>
<td>Mean day 21.2 ± 5.1(^{7}+)</td>
<td>19.8 ± 6.3</td>
</tr>
<tr>
<td>Median day</td>
<td>22.0</td>
<td>18.5</td>
</tr>
<tr>
<td>Range</td>
<td>11-30</td>
<td>11-35</td>
</tr>
<tr>
<td>ANC 1,000/(\mu)L</td>
<td>Mean day 25.8 ± 7.2</td>
<td>27.1 ± 9.9</td>
</tr>
<tr>
<td>Median day</td>
<td>26.0</td>
<td>26.0</td>
</tr>
<tr>
<td>Range</td>
<td>13-40</td>
<td>12-58</td>
</tr>
<tr>
<td>ALC 500/(\mu)L</td>
<td>Mean day 30.7 ± 24.9</td>
<td>26.4 ± 8.9</td>
</tr>
<tr>
<td>Median day</td>
<td>26.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Range</td>
<td>14-154</td>
<td>13-58</td>
</tr>
</tbody>
</table>

**NOTE.** The data show the mean, median, and range of the posttransplant day to recovery of absolute neutrophil counts (ANC) of 500 and 1,000/\(\mu\)L and absolute lymphocyte counts (ALC) of 500/\(\mu\)L following conventional and T-cell depleted BMT.

\(^{7}+\) Data are expressed as the mean ± SD; there were no significant differences in the time to recovery of ANC or ALC between the two groups as measured by Student’s t-test.

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of in vivo infectious complications differed between the two groups to be considered different. Tests to determine if the incidence of differences in the levels for the two treatment groups. As a result of found not to be normally distributed; thus, the nonparametric Wilcoxon rank sum test which required a P-value of in the time to neutrophil and lymphocyte engraftment (Table 1). The mean day to recovery of an absolute granulocyte count of 1,000/μL following conventional BMT was day 25.8 and the recipients of T-cell depleted grafts reached this same level by day 27.1 (P = .54). The engraftment of 500/μL lymphocytes occurred slightly later after conventional BMT (30.7 days, range 26–140 days) than after T-cell depleted BMT (25.4 days, range 13–58 days), but this difference was not significant (P = .17). The absolute number of granulocytes and lymphocytes were followed for the first 100 days posttransplant in the majority of patients and continued to show no marked transplant-related differences (Fig 1). Phenotypic analysis of PBL from a subset of these patients (25 conventional marrow recipients and 41 recipients of T-cell depleted BMT) (Figs 2 and 3) indicated that the total number of and percentage of lymphocytes, consisting predominantly of CD8+CD3+ cells (bright CD8), was slightly higher in conventional marrow recipients during the first month posttransplant. The number of CD8+ T cells was above normal in three of six conventional marrow recipients tested between 13 and 18 months (two of whom had chronic GVHD) whereas most T-cell depleted graft recipients had normal during the first 180 days assumed that the number of infections per individual followed a Poisson distribution.

RESULTS

Recovery of granulocytes, lymphocytes and lymphocyte subsets. There was little difference between recipients of T-cell depleted BMT who achieved durable engraftment and conventional marrow recipients in the time to neutrophil and lymphocyte engraftment (Table 1). The mean day to recovery of an absolute granulocyte count of 1,000/μL following conventional BMT was day 25.8 and the recipients of T-cell depleted grafts reached this same level by day 27.1 (P = .54). The engraftment of 500/μL lymphocytes occurred slightly later after conventional BMT (30.7 days, range 26-140 days) than after T-cell depleted BMT (25.4 days, range 13-58 days), but this difference was not significant (P = .17). The absolute number of granulocytes and lymphocytes were followed for the first 100 days posttransplant in the majority of patients and continued to show no marked transplant-related differences (Fig 1). Phenotypic analysis of PBL from a subset of these patients (25 conventional marrow recipients and 41 recipients of T-cell depleted BMT) (Figs 2 and 3) indicated that the total number of and percentage of lymphocytes, consisting predominantly of CD8+CD3+ cells (bright CD8), was slightly higher in conventional marrow recipients during the first month posttransplant. The number of CD8+ T cells was above normal in three of six conventional marrow recipients tested between 13 and 18 months (two of whom had chronic GVHD) whereas most T-cell depleted graft recipients had normal
numbers of CD8+ cells during and after this period. CD4+ cells, in contrast, were well below normal through the first 6 months in both groups and reached the normal range in the majority of patients 7 to 9 months following either type of transplant. The ratio of CD4+ to CD8+ cells was below the normal range (1.5-2.5) through the first 6 months in both patient groups. By 13 to 18 months most patients had a normal CD4:CD8 ratio and six of nine T-cell depleted marrow recipients who were tested between 25 and 35 months posttransplant had CD4:CD8 ratios >1.5. In contrast to CD4+ T cells, B lymphocytes recovered by the second month and greatly exceeded normal from 7 months throughout the longest period studied in both groups. NK cells as measured with Leu11 (CD16) (not shown) and NKH1 tended to be higher throughout the study period in the T-cell depleted BMT recipients but were above the normal range as early as the first month posttransplant in patients from both groups.

Proliferative response to T-cell mitogens. The functional capacity of PBL from recipients of HLA-identical marrow was tested in proliferation assays following stimulation with the T cell mitogens PHA and PWM. The median responses to PHA by PBL from conventional marrow recipients were higher than those from recipients of T-cell depleted grafts at all but the 10 to 12 month interval. This difference was statistically significant only during the first two intervals tested (0.5 weeks to 1 month and 2-3 months) \((P < .002,\) Wilcoxon rank sum test). The responses of most conventional BMT recipients were within the lower range of normal by 4 to 6 months whereas the median proliferative responses to PHA did not reach this level for 10 to 12 months in recipients of T-cell depleted grafts. PBL from 17 recipients of T-cell depleted grafts were tested between 19 and 35 months and five conventional BMT recipients were tested between 35 and 88 months after transplant. These patients did not show proliferative responses that were significantly higher than the patients tested at 16 to 18 months; therefore, for the purpose of comparison only the results through the first 18 months posttransplant are shown in Fig 4.

The recovery of proliferative response to PWM was similar. PBL from conventional graft recipients showed higher responses than PBL from recipients of T-cell depleted grafts during the first 6 months, but were significantly higher only during the first posttransplant month \((P < .002)\) (median, 5,700 and 1,039 cpm respectively). By month two the median had fallen to well below normal in conventional BMT recipients and both groups gradually recovered to normal values at 10 to 12 months posttransplant (data not shown). PBL from 8 of 11 recipients of conventional transplants who were examined during the first as well as the second or third posttransplant month showed a decline in the proliferative response to PHA and PWM. In those patients who were serially monitored, this response was found to subsequently increase. This early proliferative response to T-cell mitogens may be due to mature T cells transfused with the marrow graft since it was not seen in serially tested recipients of T-cell depleted marrow. The serially monitored responses to PWM are shown for four representative patients in Fig 5.

Proliferative responses to B cell mitogens. The majority of patients in both groups had PBL responses to SAC that
were nearly normal by the second posttransplant month and responses to anti-Mu that recovered to normal by 4 months (Fig 6). Proliferative responses to B-cell mitogens could be detected as early as 6 weeks, in parallel with the appearance of CD20+ B cells in peripheral blood. The median SAC response by PBL in both patient groups was above that of the normal controls by 4 months and remained higher throughout the study. The level of anti-Mu response by PBL from T-cell depleted marrow recipients also exceeded the normal control range for an extended period posttransplant. In contrast, the response to anti-Mu by cells from most conventional marrow recipients was below the median of the normal controls during this same period. Since many patients had increased numbers of peripheral B cells after transplant, we correlated the level of SAC response with the percentage of CD20+ cells in individual patients to determine if this increased responsiveness might result from a higher number of B cells in the assay. The correlation was significant in both patient groups: conventional BMT recipients, $r = 0.54 \ P = 0.001$ and T-cell depleted BMT recipients, $r = 0.53 \ P = 0.009$, thereby suggesting that the B cells themselves were not inherently more responsive.
**Immune Reconstitution of Post BMT**

*In vitro immunoglobulin production posttransplant.* As a measure of the cooperative functional capacity of T and B cells, PBL from these same patients were also tested in in vitro Ig production assays. Following 7 days of stimulation with a synergistic combination of PWM and SAC,20 Ig secretion into the culture supernatants was measured by ELISA. Although the T-cell independent B cell proliferative response to SAC and anti-Mu returned early, the ability to differentiate and produce Ig was delayed, with IgM production preceding IgG in both patient groups. Ig production by B cells from conventional BMT recipients exceeded that of T-cell depleted BMT recipients through the first year although this difference reached significance only for IgM production during the 7 to 9 month interval (P < .002, Wilcoxon rank sum test). Normal levels of IgM were produced by cells from the majority of patients in both groups by 4 to 6 months after transplant. In vitro IgG production in most conventional BMT recipients reached the low normal range by 7 to 9 months (low normal = 1.4 μg/mL, median = 6.1 μg/mL), but four of five patients tested from 10 to 12 months fell below this range and the median did not recover to low normal until the 13 to 15 month interval (Fig 7). Four of the 5 long-term conventional BMT recipients produced normal amounts of IgG (median = 4.2 μg/mL). The IgG production by B cells from T-cell depleted BMT recipients first reached the low normal range 13 to 15 months after transplant and was above normal in three of four T-cell depleted BMT recipients tested between 25 and 35 months (median = 11.8 μg/mL) (data not shown).

**NK cell activity.** We have previously reported the early appearance of normal NK cell and LAK activity in recipients of T-cell depleted BMT.23 For this study we compared the lysis of NK sensitive targets (K562) by PBL from 11 conventional marrow recipients and 24 recipients of T-cell depleted marrow during the first 6 months posttransplant. Despite GVHD chemoprophylaxis, normal levels of NK activity could be detected in both groups as early as 3 weeks after transplant and remained normal or slightly above normal through 6 months. The mean percent lysis of K562 targets at a PBL effector to target cell ratio of 100:1 during this period was 40.1 ± 17.9% for conventional marrow recipients and 48.4 ± 18.6% for T cell depleted BMT recipients versus 48.1 ± 18.8% for 34 normal control patients.

**Infectious complications and nonleukemic mortality.** Chart reviews were performed for the assessment of infectious complications from the day the absolute neutrophil count reached 500 through day 180; the period during which differences in the development of immune functions were noted. The charts were evaluated for the incidence of culture positive sepsis, localized infections (sinusitis, otitis, cellulitis), pneumonia (viral, bacterial, and fungal), Herpes...
Zoster, and Candida mucositis. Of the 86 patients in the in vitro study, 72 were followed closely enough to permit evaluation, including 24 of the 34 recipients of conventional marrow grafts and 48 of the 52 recipients of T-cell depleted marrow. As shown in Table 2, the incidence of broviac catheter-related sepsis was higher in recipients of T-cell depleted marrow, although this difference was not statistically significant. The incidence of sepsis, local infections, and candida mucositis were similar; however, the incidence of pneumonia was somewhat higher in recipients of conventional marrow grafts ($P = 0.18$). Furthermore, when nonleukemic mortality was evaluated, there was found to be an overall higher incidence of death due to infectious events in recipients of conventional marrow grafts. Five of the 25 conventional marrow recipients died 6 to 21 weeks after transplant of infectious complications (sepsis, cytomegalovirus pneumonia, pneumocystis pneumonia, interstitial pulmonary infiltrates [2 patients]), while none of the 34 recipients

Table 2. Incidence of Posttransplant Infectious Episodes in Recipients of T-Cell Depleted and Conventional BMT

<table>
<thead>
<tr>
<th>Infection</th>
<th>T-Cell Depleted BMT (N = 48)</th>
<th>Conventional BMT (N = 24)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture positive sepsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broviac related</td>
<td>14/0.29</td>
<td>3/0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Not broviac related</td>
<td>2/0.04</td>
<td>2/0.08</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>Localized infections (sinusitis, otitis, cellulitis)</td>
<td>5/0.10</td>
<td>2/0.08</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>Pneumonia (fungal, viral, idiopathic)</td>
<td>3/0.06</td>
<td>4/0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Herpes Zoster (localized)</td>
<td>5/0.10</td>
<td>0/0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>Candida mucositis</td>
<td>4/0.08</td>
<td>1/0.04</td>
<td>&gt;0.20</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of infections/rate per patient. The incidence of the indicated infections were determined by retrospective chart reviews beginning on the day the absolute neutrophil count reached 500 and continuing through day 180 after transplant. Four patients in the conventional group and four patients in the T-cell depleted group either died or relapsed before day 180. A correction was used that assumes that the number of infections per patient follows a Poisson distribution such that each individual is followed for a 180-day period.
of a T-cell depleted graft died of infection ($P < .01$). A subset of patients in each group (four conventional and five T-cell depleted marrow recipients) have had episodes of sinusitis, otitis, and/or bronchitis from 7 to 14 months after transplant. T-cell proliferative responses and IgM production were normal at or near the time of the episode in six of the nine patients. In contrast, IgG production was normal in only three of the patients, all more than one year after transplant.

**DISCUSSION**

Treatment of bone marrow with soybean agglutinin and sheep erythrocytes removes not only T cells but also functionally mature B cells and NK cells from the donor marrow, making it unlikely that either cellular or humoral donor immunity can be directly transferred with the marrow graft. The development of a donor-derived immune system in recipients of marrow depleted of T cells by this method, therefore, likely occurs from precursors of immunocompetent cells within the marrow graft. Furthermore since neither prophylactic drugs for GVHD were used nor did this group of patients suffer from GVHD, the immune reconstitution in these patients was not influenced by these factors. We wished to determine the effect of the depletion of mature cellular elements from the donor marrow on in vivo hematologic and immunologic reconstitution and on an immunologic recovery as measured by assays of T and B cell populations and their functional capacities. We have limited this study to include only durably engrafted leukemic patients transplanted with marrow from HLA-identical sibling donors either with or without T cell depletion in order to minimize the effects of variations in pretransplant therapies or histocompatibility on the parameters measured. The results have shown that there are modest quantitative and temporal differences in the reconstitution of some, but not all immune functions following T-cell depleted BMT as compared with conventional BMT which did not appear to result in marked differences in the incidence or types of posttransplant infectious complications.

The physical recovery of myeloid and/or lymphoid cells and their subsets after various types of transplants have been reported by numerous groups, sometimes with conflicting results. With rare exception these studies were performed on blood from recipients of either conventional or T-cell depleted transplants but not both and often utilized different panels of monoclonal antibodies. In contrast to the results reported by Prisch and Maki, we did not find a delay in the time to engraftment and level of recovery of lymphoid and myeloid cells in recipients of T-cell depleted marrow. Indeed, after the first month the absolute number of lymphocytes was slightly higher in the recipients of T-cell depleted marrow. It has also been reported that there is an overshoot of CD8+ T cells after conventional BMT which is less apparent in recipients of T-cell depleted marrow. Although we saw individual patients in both groups with abnormally high numbers of CD8+ T cells, the median number of cells during each posttransplant interval tested was within the normal range. Rather than fewer CD8+ T cells in the patients receiving T-cell depleted marrow, we found that during the first year, the absolute number slightly exceeded that of the conventional patients. These results are likely explained by the inclusion in the previously published studies of dimly fluorescent CD8+ cells that coexpress markers of NK cells which have also been found to be increased following BMT. In determining the proportion of CD8+ T cells we included only brightly fluorescent cells that coexpressed CD3. There was, however, a similar slow recovery of CD4+ cells in both groups rather than the more delayed recovery due to T-cell depletion reported by Janossy et al. The low number of CD4+ cells during the first 6 months posttransplant, coupled with the normal to high number of CD8+ cells resulted in CD4:CD8 ratios that were below normal through this period in both patient groups. The early recovery of NK cell numbers and functional capacity seen after both types of transplants was in agreement with previously reported studies. We observed a higher than normal absolute number as well as percentage of NK cells throughout the study period, which was consistently highest in the recipients of T-cell depleted BMT.

The functional capacity of the engrafting cells was measured using T and B cell specific mitogens. The recovery of proliferative responses by PBL to B cell mitogens (SAC and anti-Mu) did not appear to be affected by the type of transplant and were detected concurrently with the appearance of CD20+ B cells in the periphery (approximately 6 weeks). The above normal responses to SAC seen after the third month (Fig 6) appeared to be due to the larger percentage of B cells in the PBL and not to a greater degree of reactivity. Indeed, the results reported by Matsue et al suggest that purified B cells isolated after the third month posttransplant from patients without GVHD proliferate normally in response to SAC. Compared to conventional recipients, there was a somewhat higher proliferative response to anti-Mu by PBL from the T-cell depleted patients tested 4 to 24 months posttransplant. This may reflect differences in the subsets of B cells present, since a differential response to SAC versus anti-Mu has been described. T-cell responses required longer to normalize than did those of B cells, and recurred earlier in recipients of conventional grafts. The greatest differences were seen during the early period posttransplant when the response to both PHA and PWM was higher in PBL from conventional BMT recipients. High, early T-cell responses were shown to subsequently decline (Fig 5), suggesting that this early proliferation may possibly reflect the activity of mature T cells transfused with the marrow graft. This hypothesis is further supported by the observation that PBL from some conventional marrow recipients had higher numbers of CD3+ T cells consisting primarily of CD8+ cells during the first month after transplant. The subsequent decline of these cells could be due to treatment of conventional marrow recipients with cyclosporine as GVHD chemoprophylaxis during this period. Both the lymphocyte counts and percentages of CD3+ cells in conventional marrow recipients increased after the first 6 months when cyclosporine dosage was tapered.

Both T helper and B-cell function was measured in the mitogen (PWM plus SAC)-stimulated Ig production assays. IgM production recovered to low normal by the 4 to 6 month
interval in both groups, although similar to the results seen with T-cell mitogen responses, recipients of conventional grafts produced higher amounts of IgM through the first year. IgG production was more delayed than IgM, and first reached normal 7 to 9 months following conventional BMT and 13 to 15 months after T-cell depleted BMT. There was some suggestion that low IgG production might be associated with episodes of sinusitis and otitis after 7 months in patients in both groups.

The basis for the immune deficiencies seen and the differences between the two types of transplant may be ascribed to a combination of factors including the proportion of the types of cells present, the absence of functionally mature cells, or active suppression. Our phenotypic analysis of T and B cells during this period have shown that the predominant cell types present early after transplantation, when T-cell responses to PHA were low, are CD3+ CD8+ T cells and NK cells. Further studies have shown that the majority of the CD3+ CD8+ cells coexpressed DR, CD11(Leu15) and/or Leu7 and of the relatively infrequent CD4+ cells the majority were of the helper inducer subset (CD4+ CD8-)(Small et al, manuscript in preparation). It is known that helper inducer cells and NK cells respond poorly to T-cell mitogens.40,41 Moreover, CD11+ CD8+ T cells can inhibit IL-2 production and proliferation to mitogens.42 We have additionally seen increased numbers of unusual populations of B cells (CD38+, CD1c+, CD5+) and high levels of cells with known capacity to inhibit PWM-induced Ig production (CD8+ Leu7+ T cells).43 The disproportionate representation of these populations, therefore, likely explains the observed defects and a difference in the relative proportions of these cells may explain the faster recovery of responses by recipients of conventional bone marrow.

The majority of the recipients of conventional grafts who were available for study were younger (mean age, 14.0 yr) than the recipients of T-cell depleted BM (mean age, 28 yr). A preliminary analysis of the in vitro data from the T-cell depleted group separated by age showed that those patients below the age of 17 (N = 7) had a pattern of recovery similar to the older patients suggesting that the type of transplant rather than the age of the recipient has the strongest effect.

Although the use of standard assays of immune function may in some cases underestimate the extent of the deficiencies present,11,46,47 it is during the first 6 months after transplant when in vitro mitogen responses are low that patients are most susceptible to infectious complications.4 Therefore, we were encouraged to find that despite the extreme depletion of T cells and other mature marrow cells that occurs during the SBA and E rosetting treatments (approximately 6% of the starting nucleated cell number), there were relatively minimal differences in hematopoietic or immunologic reconstitution of patients who were successfully engrafted. The defects seen in vitro were not absolute and subsequently rose to normal levels.

Evaluation of infectious complications during the period when differences in immune function were maximal documented a somewhat higher incidence of broviac-related infections in recipients of T-cell depleted grafts, whereas, the incidence of other bacterial or fungal infections was similar. The basis for this difference is unclear, but may reflect variation in broviac management in the older patient group, or other unrelated factors. Recent studies have suggested that neutrophils developing early after T-cell depleted marrow grafts may be functionally abnormal, a factor that might contribute to this observation.48

Overall, there did not appear to be a significant deleterious effect of the immune deficiencies seen on the incidence, types, or severity of posttransplant infectious complications in the recipients of T-cell depleted marrow. Indeed, infectious mortality was lower in the recipients of T-cell depleted marrow, probably reflecting the reduced incidence of pneumonia in this patient group. However, this clinical finding must be considered preliminary, since the study was not conducted in the context of a prospective trial with patients matched for age, disease, and disease stage. Such a study of appropriately matched groups is currently in progress to more fully address this issue.

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Immune reconstitution following bone marrow transplantation: comparison of recipients of T-cell depleted marrow with recipients of conventional marrow grafts

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