Enhancement of Thymopoiesis After Bone Marrow Transplant by In Vivo Interleukin-7

By Ellen Bolotin, Monika Smogorzewska, Susan Smith, Michael Widmer, and Kenneth Weinberg

Bone marrow transplantation (BMT) is followed by a period of profound immune deficiency, during which new T lymphocytes are generated from either stem cells or immature thymic progenitors. Interleukin-7 (IL-7) induces proliferation and differentiation of immature thymocytes. We examined whether the in vivo administration of IL-7 to mice receiving BMT would alter thymic reconstitution. Lethally irradiated C57BL/6 mice received syngeneic BMT, followed by either IL-7 or placebo from days 5 to 18 post-BMT. At day 28, BMT recipients that had not received IL-7 had profound thymic hypoplasia (<5% of normal), with relative increases in the numbers of immature thymocytes, decreased numbers of mature peripheral (splenic) T lymphocytes, and severely impaired T- and B-cell function. In contrast, transplanted mice treated with IL-7 had normalization of thymic cellularity, with normal proportions of thymic subsets and T-cell receptor β variable gene (TCRβ) usage, normal numbers of peripheral CD4+ T lymphocytes, and improved antigen-specific T- and B-cell function. In the BMT–IL-7 mice, there was an eightfold increase in the number of immature CD3+CD4+CD8+ thymocytes in G1-M of the cell cycle, indicating that restoration of thymic cellularity was due to enhanced proliferation of immature thymic progenitors. Similar effects following IL-7 administration were also observed when donor bone marrow was depleted of mature T lymphocytes, indicating that IL-7 administration affected immature hematopoietic progenitors. IL-7 promotes thymic reconstitution after BMT and may be useful in preventing post-BMT immune deficiency.

ONE MARROW transplant (BMT) is followed by a variable period of profound immune deficiency. T-lymphoid abnormalities seen after BMT include T lymphopenia, abnormal proportions of CD4+ and CD8+ cells, diminished or absent mitogen proliferation, and absence of antigen-specific proliferation. B-lymphoid abnormalities include B lymphopenia, diminished primary antibody responses, and defective Ig class switching. Post-BMT immune deficiency is clinically significant because it contributes to transplant-related morbidity and mortality from a variety of infections normally controlled by functional lymphocytes. Despite neutrophil recovery, BMT recipients are at risk of serious infections with cytomegalovirus, herpes simplex virus, varicella zoster virus, fungi (candida and P. carinii), and encapsulated bacteria. Additionally, BMT patients are susceptible to lymphoproliferative disease caused by Epstein-Barr virus (EBV). Prevention or treatment of such infections with antivirals, antibiotics, and intravenous immune globulin are expensive and only partially effective.

The pathogenesis of post-BMT immune deficiency is multifactorial. In recipients of allogeneic BMT, graft-versus-host disease (GVHD) and the immunosuppressive drugs used to treat it, contribute to immune deficiency. However, recipients of allogeneic BMT who do not have GVHD, and recipients of autologous BMT, are also immune deficient. Therefore, post-BMT immune deficiency is not solely due to GVHD or its treatment.

There is evidence that immune ontogeny after BMT recapitulates normal fetal ontogeny. Normal human thymus forms at 7 to 9 weeks of gestation, but circulating mitogen responsive cells are not found until approximately 12 to 16 weeks of gestation. Similarities between peripheral blood T lymphocytes of BMT recipients and certain normal thymic subpopulations and fetal peripheral blood have been observed. Circulating cells with thymic phenotype have been observed both in newborn cord blood and BMT recipients. Like thymocytes, circulating T lymphocytes post-BMT have a proliferative defect in which mitogen responses are present only when exogenous cytokines, eg, interleukin-2 (IL-2), are added to the cultures. Taken together, these data suggest that a major reason for the delay in post-BMT T-lymphoid function is due to delayed ontogeny, secondary to the recapitulation of the maturational steps in which stem cells differentiate into thymocytes and then to functional mature T lymphocytes.

We have investigated whether post-BMT immune reconstitution can be enhanced by in vivo treatment of recipients with interleukin-7 (IL-7), a cytokine known to be important for T and B lymphopoiesis. IL-7 was originally identified as a soluble protein secreted by fetal liver, but is now known to be produced by both thymic and marrow stroma. IL-7 has both proliferative and differentiative effects on immature T lymphoid cells. Patients and dogs with X-linked severe combined immune deficiency (XSCID), which is caused by mutations in the γc chain of the IL-7 receptor, have profound defects in thymopoiesis.

Because of the known effects of IL-7 on immature T cells, we hypothesized that in vivo treatment of IL-7 following BMT would enhance T lymphocyte reconstitution. IL-7 administration to cyclophosphamide-treated or irradiated mice improves peripheral lymphocyte recovery. A syngeneic murine BMT model was used to avoid the confounding variable of GVHD. The study plan was to transplant the mice after ablative radiation, administer subcutaneous injections

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of either IL-7 or placebo for 14 days, and then analyze the animals for T-lymphocyte recovery. Immune reconstitution was assessed by T-lymphocyte number, phenotype, cell-cycle analysis, TCR/Vβ usage patterns, T-cell mitogen responsiveness, and specific antibody production in the transplanted animals and age-matched untransplanted controls. These analyses indicate that IL-7 treatment induced the proliferation of immature thymocytes, which then underwent normal thymic maturation and export to the periphery.

MATERIALS AND METHODS

Animals. Four- to six-week-old C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice were rested for 2 weeks after receipt and then used either as marrow donors (males) or recipients (females). Untransplanted normal control mice were littermates of the recipient mice and were the same age at the time of sacrifice. The animals were maintained in laminar flow cages with acidified water and antibiotics. Mice were killed with CO₂ narcosis. All of the work was performed in accordance with protocols approved by the Animal Care Committee of Childrens Hospital Los Angeles.

BMT procedure. Recipient female mice were prepared for transplant with 1,050 cGy of radiation given in two divided doses (600 cGy on day -1 and 400 cGy on day 0). The marrow from the male donor mice was obtained by perfusion of the femurs after sacrifice. A total of 2 × 10⁶ nucleated marrow cells were given per recipient. In some experiments, mature T lymphocytes were removed from the marrow before infusion, using rat anti-Thy-1.2 monoclonal antibody (Pharmingen, San Diego, CA) and immunomagnetic beads (Dynal, Great Neck, NY). Each recipient mouse received <2,000 Thy-1.2⁻ cells, as determined by fluorescence-activated cell sorting (FACS) of the infused marrow (<0.1% contamination). In each experiment, two mice received radiation, but no cells were infused; the death of these mice was used to verify that a marrow ablative dose of radiation had been given.

BMT-IL-7 mice were treated between day 5 to 18 post-BMT by subcutaneous injection of 500 ng recombinant human IL-7 twice daily. BMT mice received placebo injections of sterile saline, using the same protocol as for IL-7 treated mice.

Immunophenotyping. At sacrifice, thymus and spleen cells were obtained after teasing and total cell number determined. A total of 1 × 10⁶ cells were stained with optimal concentrations of fluorescein isothiocyanate (FITC) and/or phycoerythrin (PE)-labeled monoclonal antibodies. Cells were stained with antibodies directed against CD3, CD4, CD8, Thy-1.2, CD45RB, CD45R, CD2, CD4, CD8, and isotype control antibodies (Pharmingen). TCR Vβ usage was assessed by staining with biotinylated antibodies directed against TCR Vβ2, Vβ3, Vβ5.1, 5.2, Vβ6, Vβ7, Vβ8.1, 8.2, Vβ9, Vβ10, Vβ11, Vβ12, Vβ13, Vβ14, Vβ17, or an isotype control antibody (Pharmingen), followed by staining with streptavidin-tricolor (Caltag, South San Francisco, CA). After staining, cells were washed twice in phosphate-buffered saline and analyzed on the Becton Dickinson FACScan or FACS Vantage flow cytometers (Mountain View, CA). Five to 10,000 gated events were acquired and the number of cells positive for each antibody was determined by subtraction against the isotype control. The number of CD4⁻CD8⁻ double-negative (DN), CD4⁺CD8⁻ double-positive (DP), CD4⁺CD8⁻ single-positive (CD4⁺SP), and CD4⁻CD8⁻ single-positive (CD8⁻ SP) cells was determined after staining with anti-CD4-FITC and anti-CD8-PE. The number of CD3⁺CD4⁻CD8⁻ triple-negative (TN) thymocytes was determined by simultaneous staining with CD3-FITC and a cocktail of CD4-PE and CD8-PE antibodies. Data were analyzed with PC-Lysis and Cellfit software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Cell cycle analysis. To determine which subsets of thymocyte were proliferating, simultaneous three-color immunofluorescence was performed to assess two surface antigens and DNA content. Staining with antibodies directed at CD3, CD4, and CD8 were used to define the TN, DP and CD4⁺ SP, and CD8⁻ SP cells. Antibody staining, permeabilization with saponin and DNA staining with 7-amino actinomycin D (7-AAD) was performed exactly as described. Analysis was performed on either the FACScan or FACS Vantage flow cytometers, using a single 488 nm argon laser. Green fluorescence with the FITC-labeled antibodies was measured at 525 nm, orange fluorescence with the PE-labeled antibodies at 570 nm, and red fluorescence for 7-AAD at >650 nm.

Mitogen assays. Isolated splenocytes were incubated for 3 days in RPMI 1640 with 10% fetal bovine serum; 2-mercaptoethanol, 1 × 10⁻⁵ mol/L; L-glutamine, 2 mmol/L; penicillin, 50 U/mL; and streptomycin, 50 μg/mL with or without Concanavalin A (Con A), 4 μg/mL, in triplicate wells of 96-well microtiter plates with 10⁵ cells/well. Cells were pulsed with 1 μCi [³H]thymidine (5 μCi/mL) overnight before harvesting and then the incorporated radioactivity determined using a Beckman scintillation counter. Results were expressed as mean incorporated counts per minute (CPM) of stimulated wells − mean CPM from unstimulated wells, ± standard deviation (SD).

Sheep red blood cell (SRBC) immunizations. Mice were immunized with SRBC (Colorado Serum Co, Denver, CO) via intraperitoneal (IP) injection. After 2 weeks, blood was drawn for primary antibody response, and the mice were boosted with SRBC for secondary responses, which were measured 1 week later. The agglutinating antibody titers in the serum were determined by serial dilution and incubation with SRBC in 96-well V-bottom microtiter plates.

Statistical analyses. Differences between groups were analyzed by Student's t-test.

RESULTS

Following BMT, mice received injections of either IL-7 (BMT-IL-7 group) or placebo (BMT group). Initially, mice were killed at 2, 3, 4, and 8 weeks to determine the immune reconstitution of the BMT and BMT-IL-7 mice. Some mice were observed for long-term effects. In later experiments, mice were routinely analyzed between 28 to 35 days post-BMT (d28-35). In some experiments, T-cell depleted marrow was used to control for the possibility that the observed changes in thymic recovery were due to mature T cells present in the marrow inoculum.

Increased numbers of thymocytes in BMT-IL-7 group. Following BMT, the cellularity of the thymus was examined over 8 weeks following BMT. The rate of recovery of thymocytes was greater in the BMT-IL-7 animals as compared with control animals, such that normal numbers of thymocytes were present by week 4, while normal numbers of thymocytes were only found by 8 weeks in the BMT animals. At sacrifice on d28-35, mean thymocyte number in the BMT-IL-7 group was 79 × 10⁶ (P = .12), 6.04 × 10⁵ in the BMT group (P < .0007), and 114 × 10⁵ in the normal group (Fig 1).

Normalization of thymic subsets in BMT-IL-7 group. On d28 after BMT, the phenotypes of the cells present in the thymus of BMT control mice differed markedly from that of normal mice. There was a shift towards immaturity with an increased proportion of DN thymocytes and a con-
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The mean percentage of DN cells was 30% in the BMT mice, as compared with 2% in the normal mice (P < .005). The percentage of DP thymocytes, which is normally the predominant population, was 37% in the BMT mice as compared with 86% in the normal group (P < .0009). Similarly, the percentage of CD4⁺ and CD8⁺ SP thymocytes were 23% and 10% (normal = 7% for CD4⁺ SP thymocytes, P < .04; normal = 5% for CD8⁺ SP thymocytes, P < .04). Because the total number of thymocytes in the BMT group was reduced, the difference in the absolute number of DP and SP thymocytes was greater. The absolute number of DN, DP, CD4⁺ SP, and CD8⁺ SP thymocytes in the BMT mice was significantly reduced compared with the normal mice, respectively (Table 1).

The BMT-IL-7 group had thymic subpopulations that approximated those seen in the normal untransplanted mice. The mean percentage of DN, DP, CD4⁺ SP, and CD8⁺ SP thymocytes were 3%, 89%, 6%, and 2%, respectively, comparable to that of normal mice (Fig 2, Table 1). The absolute number of DN, DP, CD4⁺ SP, and CD8⁺ SP thymocytes was not different between the BMT-IL-7 and normal groups (P ≥ .1) (Table 1). The thymic reconstitution of the BMT-IL-7 mice following the transplantation of T-cell depleted marrow did not differ from that seen when unmanipulated bone marrow was used (data not shown).

Effect of IL-7 on cell-cycle distribution of thymocytes following BMT. Because of the abnormal phenotypic distribution of thymocytes in the BMT animals, we investigated which thymic subpopulations were proliferating after BMT. Normally, the immature (TN or DN) thymocyte population proliferates to produce DP thymocytes. CD4⁺ SP and CD8⁺ SP thymocytes are derived from DP cells that have undergone antigen-driven positive and negative selection. To examine the effects of IL-7 on the proliferation of specific thymic subsets, we simultaneously analyzed two different cell surface antigens and DNA content, using 7-AAD. The percentage of cells in G2-M phase was used to measure the proportion of cells proliferating within a defined phenotypic population (Fig 3).

Comparison of thymic subpopulations from BMT-IL-7, BMT, and normal mice demonstrates the major proliferative effects of IL-7 treatment (Table 2). The proliferation of the immature TN cells was increased nearly 8 times normal in the BMT-IL-7 group, as compared with only 3 times normal in the BMT group. Fewer DP cells were in cell cycle in both BMT groups than in normal mice. The percentages of the more mature CD4⁺ SP and CD8⁺ SP subsets in G2-M from both BMT groups were increased over normal. These results indicate that the major difference between the BMT-IL-7 and BMT groups was the increased proliferation of the immature TN thymocytes after IL-7 treatment. Decreased proliferation of DP cells and increased proliferation of SP cells was a general feature of BMT and was not significantly affected by IL-7 treatment.

Effects of IL-7 on TCR Vβ usage. The distribution of TCR Vβ gene segment usage in mice is determined by major histocompatibility complex (MHC) expression of the thymus. If IL-7 primarily affects the proliferation of immature TN cells, but not the DP cells which are undergoing TCR-MHC driven positive and negative selection, then the pattern of TCRVβ usage in the BMT-IL-7 group should be similar to normal C57BL/6 mice. TCRVβ usage in the thymus was analyzed by three-color immunofluorescent staining for
CD4, CD8, and the TCRVβ segment. The percentage of SP thymocytes positive for each Vβ was then determined. The distribution of TCRVβ usage on the mature SP thymocytes in the normal, BMT, and BMT–IL-7 mice was very similar (P > .10 for all comparisons) (Fig 4). The results of these experiments indicate that IL-7 did not significantly alter the process of thymic selection, a result that would be expected if IL-7 acted on the immature TN cells.

Increased peripheral (splenic) lymphocytes derived from immature progenitors. Differences in the absolute numbers of spleen cells were not significant between normal, BMT, and BMT–IL-7 mice. At d28, the mean spleen cell number of the BMT–IL-7 group was 92 × 10⁶ (P = .26), while the BMT group was 75 × 10⁶ (P = .16) and the normal group was 91 × 10⁶ (Fig 1). The number of CD4⁺ T lymphocytes was normal in the BMT–IL-7 mice, but was decreased in the BMT group (P < .04) (Table 3). The number of CD8⁺ T lymphocytes in both the BMT–IL-7 and BMT groups was significantly reduced, as compared with the normal mice (P < .01 for both). The BMT–IL-7 mice had normal numbers of B220⁺ B lymphocytes, as compared with normal mice, while the BMT mice had reduced numbers (P < .003).

We studied expression of CD45 isoforms and CD44 in the BMT–IL-7 mice that had received T-cell depleted marrow to confirm that the mature T cells in the spleen were derived from immature progenitors. Virgin T lymphocytes that develop in vivo after BMT from immature marrow progenitors express the CD45RB high molecular weight isofrom and low levels of CD44. In the BMT–IL-7 group, approximately 80% of the splenic CD4⁺ cells were CD45RB⁺ and CD44low (Fig 5). Thus, the majority of peripheral CD4⁺ T lymphocytes induced by IL-7 treatment were derived from

### Table 1. Thymocyte Numbers in Transplanted Mice

<table>
<thead>
<tr>
<th>Thymic Subpopulation</th>
<th>Normal (n = 10)</th>
<th>BMT (n = 9)</th>
<th>BMT–IL-7 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DN</td>
<td>2.1 ± 0.3 (2%)</td>
<td>0.65 ± 0.2 (30%)</td>
<td>1.9 ± 0.5 (3%) (P1 &lt; .01)</td>
</tr>
<tr>
<td>DP</td>
<td>83 ± 1 (86%)</td>
<td>0.69 ± 0.5 (37%)</td>
<td>62 ± 7 (89%) (P1 &lt; .00001)</td>
</tr>
<tr>
<td>CD4⁺ SP</td>
<td>6.6 ± 0.3 (7%)</td>
<td>0.44 ± 0.5 (23%)</td>
<td>4.2 ± 2 (6%) (P1 &lt; .01)</td>
</tr>
<tr>
<td>CD8⁺ SP</td>
<td>4.5 ± 0.1 (5%)</td>
<td>0.18 ± 0.2 (10%)</td>
<td>1.3 ± 0.3 (2%) (P1 &lt; .01)</td>
</tr>
</tbody>
</table>

The number of thymocytes in each analyzed developmental stage (DN = double negative, DP = double-positive, CD4⁺ SP = CD4⁺ single-positive, CD8⁺ SP = CD8⁺ single-positive) is shown for the normal, BMT, and BMT–IL-7 groups. All values are the mean number of cells × 10⁶ ± 1 SD. The mean percentage of thymocytes in each developmental stage is shown in parentheses. Differences between groups were analyzed by 2-tailed t-test with unequal distributions; P1, normal v BMT; P2, normal v BMT–IL-7; and P3, BMT v BMT–IL-7.

### Table 2. Percent of Thymocytes in G2-M

<table>
<thead>
<tr>
<th>Thymic Subpopulation</th>
<th>Normal (n = 10)</th>
<th>BMT (n = 5)</th>
<th>BMT–IL-7 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
<td>0.65 ± 0.18</td>
<td>2.0 ± 0.4</td>
<td>5.1 ± 1.1 (P1 &lt; .025)</td>
</tr>
<tr>
<td>DP</td>
<td>14.8 ± 2.8</td>
<td>3.4 ± 0.5</td>
<td>5 ± 0.6 (P2 &lt; .05)</td>
</tr>
<tr>
<td>CD4⁺ SP</td>
<td>1.4 ± 0.05</td>
<td>21 ± 3.2</td>
<td>17 ± 2.7 (P2 &lt; .003)</td>
</tr>
<tr>
<td>CD8⁺ SP</td>
<td>3.2 ± 0.21</td>
<td>11 ± 2.6</td>
<td>13 ± 2.6 (P2 &lt; .008)</td>
</tr>
</tbody>
</table>

The mean percentage of thymocytes from each group of mice at specific developmental stages, in the G₂-M phase of cell cycle is shown. The cells were stained as for Fig 3, and the percentage of positive cells ± 1 SD shown. Differences between groups were analyzed by 2-tailed t-test with unequal distributions; P1, normal v BMT; P2, normal v BMT–IL-7; and P3, BMT v BMT–IL-7.
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Fig 4. TCR Vβ usage after BMT. The distribution of TCR Vβ segments among the SP thymocytes in the normal, BMT, and BMT-IL-7 groups is shown. The mean percentage of SP thymocytes (CD4+ SP and CD8+ SP cells combined) using each of the indicated TCR Vβ segments is shown ±1 SD.

Development of T-cell-dependent antibody responsiveness. Because the BMT-IL-7 group developed mitogen responses sooner than the BMT group, the development of a T-cell dependent antibody response was tested. Mitogen responsiveness usually precedes antigen-specific responses in normal ontogeny. After immunization with SRBC, the primary and secondary antibody responses to SRBC were measured in recipients of TCD-BMT. The BMT group had no significant anti-SRBC titers after primary immunization at d28 and very low titers after secondary boost at d42 (Table 5). In contrast, the BMT-IL-7 group had titers after primary and secondary immunization, which were comparable to those observed in the normal group.

DISCUSSION

We report that the administration of IL-7 for 2 weeks following BMT profoundly enhances T lymphocyte reconstitution. The BMT-IL-7 group had normalization of peripheral T-lymphocyte numbers, thymic cellularity, and thymic subsets, whereas BMT control mice had profound T-lympho-

Table 3. Spleen Cell Numbers in Transplanted Mice

<table>
<thead>
<tr>
<th>Splenic Subpopulation</th>
<th>Normal (n = 10)</th>
<th>BMT (n = 9)</th>
<th>BMT-IL-7 (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>CD3-CD4+</td>
<td>8.3 ± 1.3</td>
<td>5.0 ± 0.98</td>
<td>7.1 ± 1.9</td>
</tr>
<tr>
<td>(P1 &lt; .04)</td>
<td>(P2 &lt; .45)</td>
<td>(P3 = .12)</td>
<td></td>
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<tr>
<td>CD3-CD8+</td>
<td>4.6 ± 1.5</td>
<td>2.4 ± 0.68</td>
<td>2.3 ± 0.62</td>
</tr>
<tr>
<td>(P &lt; .01)</td>
<td>(P2 &lt; .01)</td>
<td>(P3 &lt; .47)</td>
<td></td>
</tr>
<tr>
<td>B220+</td>
<td>23 ± 4.1</td>
<td>2.8 ± 7.5</td>
<td>27 ± 9.2</td>
</tr>
<tr>
<td>(P1 &lt; .003)</td>
<td>(P2 &lt; .36)</td>
<td>(P3 &lt; .002)</td>
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</table>

The number of splenocytes of each lineage (CD3-CD4+ = CD4+ T lymphocytes, CD3-CD8+ = CD8+ T lymphocytes, B220+ = B lymphocytes) are shown for the normal, BMT, and BMT-IL-7 groups. The total number of splenocytes from each animal was multiplied by the percentage of FACS-gated cells positive for each phenotype. All values are the mean number of cells × 10^6 ± 1 SD. Differences between groups were analyzed by 2-tailed t test with unequal distributions; P1, normal vs BMT; P2, normal vs BMT-IL-7; and P3, BMT vs BMT-IL-7.
cyte deficiency, thymic hypocellularity, and depletion of all thymic subsets. In the BMT control mice, thymocytes of the relatively mature DP and SP stages were most affected, resulting in a relative increase in the proportion of immature DN or TN cells. The enhanced thymopoiesis led to the early development of mitogen- and antigen-responsive peripheral T lymphocytes. The results differ from a recent report in which IL-7 only affected B-cell numbers. However, the dose, timing, and route of administration may have led to lower IL-7 levels than those achieved in our study.

Several lines of evidence indicate that the mature splenic T lymphocytes observed in the BMT-IL-7 group were derived from immature progenitors that had undergone differentiation in vivo. First, the results of the experiments with T-cell-depleted marrow and whole marrow were similar. If reconstitution occurred from a mature T-cell population in the marrow inoculum, T-cell depletion would be expected to significantly reduce the number or mitogen responsiveness of splenic T lymphocytes. Instead, the BMT-IL-7 mice that received T-cell-depleted marrow had near-normal levels of splenic T lymphocytes, indicating that the mature T cells were probably derived from an immature progenitor. The pattern of CD45RB$^+$ and CD44$^+$ expression on the splenic CD4$^+$ T lymphocytes in the BMT-IL-7 group was also consistent with the development of the peripheral T lymphocytes from an immature progenitor.

Finally, the cell cycle status of different thymic populations demonstrated that the proliferative effects of IL-7 were primarily exerted on the immature populations. The percentage of immature TN cells in G$_1$-M phase in the BMT-IL-7 group was nearly 3 times greater than in the BMT group. The effect on the immature thymocytes is consistent with previous studies of mice treated with anti-IL-7 antibodies, IL-7R knockout mice, and X-SCID which demonstrated defective maturation of TN cells in the absence of IL-7R-mediated signaling. We also observed decreased cycling in the DP population in both BMT groups, the basis for which is unknown. Although much has been learned about positive and negative selection of thymocytes, the regulation of the proliferation of DP thymocytes is poorly understood. Our data suggest that DP cell proliferation may be IL-7 independent, as it was not affected by IL-7 treatment. The normal distribution of TCRV$\beta$ usage is consistent with minimal effects of IL-7 on the DP thymocytes.

Our experiments do not establish that the defects in thymic reconstitution after BMT are secondary to deficient production of IL-7. In fact, we have found normal stromal production of IL-7 in BMT mice and increased plasma levels of IL-7 in patients undergoing BMT (E. Bolotin and K. Weinberg, unpublished observations, December 1995). It is possible that the pharmacologic dose of IL-7 used in the present experiments overcame resistance or insensitivity to IL-7 among the transplanted stem cell or progenitor cell populations. IL-7 resistance might result from abnormal expression or function of the IL-7 receptor. The suboptimal response to endogenously produced IL-7 after BMT could also be due to abnormalities of cytokine presentation in the microenvironment. Damage to the proteoglycans that bind cytokines in the thymic or marrow microenvironments might result in ineffective presentation of IL-7 and other cytokines. The results of IL-7 administration are similar to the effects of pharmacologic doses of granulopoietic cytokines, eg, granulocyte colony-stimulation factor, on myeloid regeneration after BMT or in congenital agranulocytosis. Although there are supranormal levels of endogenous growth factor production after BMT, increased granulopoiesis is observed with growth factor administration. The development of mitogen and antigen responsive T cells in the BMT-IL-7 mice indicates that functional T lymphocytes were generated. Abnormalities observed after clinical BMT, especially after TCD-BMT, have included defective mitogen responses of T cells and inability to make specific antibody. The delay in appearance of functional T lymphocytes has been associated with the high risk of infection in clinical TCD-BMT. As in clinical BMT, we observed abnormal mitogen and antigen responses, which were most depressed in the BMT-IL-7 mice. IL-7 treatment resulted in the early generation of mitogen and antigen responsive T lymphocytes, even after TCD-BMT (Tables 4 and 5). The normalization of anti-SRBC antibody production indicated that the T and B lymphocytes resulting from IL-7 treatment were able to cooperatively generate antigen-specific primary and secondary humoral responses.

Clinical strategies that have been described for preventing or treating post-BMT T-cell deficiency have included infusion of donor peripheral blood lymphocytes or donor-derived antigen-specific T lymphocyte clones. The infusion of per-

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<th>Table 4. Con A Responses in Transplanted Mice</th>
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<tr>
<td>Normal</td>
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<tr>
<td>BMT</td>
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<tr>
<td>TCD-BMT</td>
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<tr>
<td>BMT-IL-7</td>
</tr>
<tr>
<td>TCD-BMT-IL-7</td>
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*Con A responses of splenic T cells from normal and transplanted mice were measured on d28 after BMT. Mean $^3$H incorporation from triplicate wells of 10 mice from each group are shown. All values are mean CPM ± 1 SD. Alternation: TCD, T-cell depleted.*
Peripheral blood lymphocytes from donors previously infected with EBV has been successfully used to treat EBV-associated lymphoproliferative disease occurring after BMT. This strategy is complicated by the risk of GVHD. Donor cytotoxic T-cell clones have been used to successfully prevent cytomegalovirus infection after BMT. However, because adequate helper cell function may not be present in the recipient, the infused cytotoxic T lymphocytes (CTL) have a limited lifespan and do not produce lasting immunity. Furthermore, protection by CTL clones is limited to a single organism.

The method for correcting post-BMT immune deficiency that we describe here differs in several ways from strategies using infusion of mature T lymphocytes or T-lymphocyte clones. By enhancing the in vivo generation of mature T lymphocytes from immature progenitors, a representative antigenic repertoire may be generated. This is suggested by the normal distribution of Vβ usage in the peripheral T lymphocytes that we observed in the BMT-IL-7 group. The normalization of SRBC responses in the BMT-IL-7 mice also indicates that normal T-helper function is present. Further experiments demonstrating antigen-specific responses to a variety of pathogens are needed to verify whether BMT-IL-7 mice have a normal repertoire of T cells with functional activity. Another potential advantage of our strategy is that thymic selection of the T-lymphoid precursors is occurring in the recipient thymus. As a result, thymic selection may proceed normally at eliminating auto-reactive clones. Administration of IL-7 following allogeneic BMT will enable us to determine whether IL-7 exacerbates GVHD. In the present experiments, we used young donor and recipient mice to maximize the likelihood that the stem cells and thymic microenvironment would be normal. We are evaluating IL-7 treatment in BMT recipients that are thymectomized, congenitally athymic or aged to determine whether an intact thymus is necessary for the observed effects of IL-7.

The results of the present experiments may be of relevance to other situations, in which stimulation of postnatal thymopoiesis would be beneficial. Recently, conventional doses of cytotoxic chemotherapy have been shown to lead to persistent diminution of CD4+ T lymphocytes in proportion to patient age. We have found that the regeneration of T lymphocytes in patients after BMT is also age-dependent. The failure of older individuals to generate new T lymphocytes may also be a general feature of aging. In human immunodeficiency virus (HIV) infection, the progressive loss of CD4+ T lymphocytes ultimately overtakes any compensatory production of new T lymphocytes. Even if a successful antiviral strategy to arrest progression of HIV can be developed, recovery of immune function in infected patients will require postnatal thymopoiesis to generate new T lymphocytes. The BMT experiments indicate that IL-7 treatment can stimulate postnatal thymopoiesis and normalize T-lymphocyte recovery.

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