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Recombinant human IL-7 administration in mice affects colony-forming units-spleen and lymphoid precursor cell localization and accelerates engraftment of bone marrow transplants

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Abstract: Murine reconstitution assays were used to investigate the effects of recombinant human interleukin-7 (rhIL-7) on myeloid and lymphoid precursors and on bone marrow engraftment. Reconstitution with bone marrow from rhIL-7-treated mice results in a 3.4-fold decrease in total colony-forming unit-spleen (CFU-S) activity (day 9) and an 18.1- and 11.9-fold decrease in its ability to generate thymocytes and splenic B lineage cells, respectively. In contrast, after reconstitution with splenocytes from rhIL-7-treated mice, CFU-S activity increased 23.6-fold (day 9) and the thymocyte and splenic B lineage cell regenerative capacity increased by 4.0- and 3.2-fold, respectively. In addition, CD40low, B220low+ cells that contain pre-pro-B cells and pro-B cells were expanded two- to threefold and IgM−, B220+, CD2− and IgM+, B220+, CD2+ B lineage cells were expanded approximately 10-fold and 10- to 45-fold (depending on the tissue examined), respectively, after rhIL-7 treatment. Administration of rhIL-7 to irradiated mice transplanted with bone marrow resulted in accelerated T cell and B cell reconstitution by up to 2-4 weeks. Thus, rhIL-7 administration affects the distribution of myeloid and lymphoid precursors. Moreover, rhIL-7 administration accelerates murine bone marrow cell engraftment and therefore may be useful in reducing the engraftment time in bone marrow transplant patients. J. Leukoc. Biol. 58: 151-158; 1995.

Key Words: hematopoietic precursor mobilization · lymphoid reconstitution · lymphopoiesis · myelopoiesis

INTRODUCTION

The differentiation of cells of the various hematopoietic lineages involves a complex and as yet incompletely understood series of events. In recent years there have been advances in isolating and expressing cytokines known to be involved in hematopoiesis. Granulocyte-macrophage-colony-stimulating factor (CSF), interleukin-3 (IL-3), granulocyte-CSF, CSF-1, and IL-6 have been shown to be directly stimulatory to myeloid progenitor cells [1, 2]. In contrast, the factors that regulate lymphopoiesis at the progenitor cell level are largely unknown, despite a large body of evidence showing significant effects of various ILs on mature T cells and B cells.

The development of a long-term B lineage culture system allowed the demonstration of sustained lymphopoiesis in vitro [3, 4], whereby populations of pro-B and pre-B cells developed over a period of several months. By using this technique IL-7 was originally described as a bone marrow stromal cell-derived factor that could support the proliferation of B cell precursors from Whidlock-Witte bone marrow cultures [5], thus demonstrating its growth/ regulatory function in lymphopoiesis. Murine and human IL-7 cDNA have both been cloned, sequenced, and expressed [6, 7] and there is 60% homology at the amino acid level. Northern blot analysis of various murine and human tissues showed that the IL-7 gene is transcribed not only in the bone marrow, but also in the spleen and the thymus [6].

IL-7 was initially shown to be a B cell lineage growth factor with preferential stimulating effects on the proliferation of B cell precursors [6, 8, 9]. Subsequently, it was found that IL-7 increased the number of CD3+ cells in bone marrow cultures [10] and also supported the growth of thymocytes [11-13], in particular the CD4CD8−, CD4+CD8+, and CD4CD8− subsets [14]. IL-7 also was shown to act as a co-stimulator for peripheral T lymphocytes, in concert with mitogen or antigen in vitro [15, 16]. In vitro, IL-7 has been shown to enhance CSF-induced proliferation of myeloid colony formation [17].

The in vivo effects of IL-7 administration using murine systems also have been examined. Normal mice treated with recombinant murine IL-7 (rmIL-7) have increased lymphocyte numbers [18] and cyclophosphamide-treated mice were able to reconstitute the lymphocyte compartment more rapidly with rmIL-7 treatment [19]. Further-

Abbreviations: IL-3, interleukin-3; rmIL-7, recombinant murine IL-7; rhIL-7, recombinant human IL-7; CFU-c, colony-forming units-culture; CFU-GEMM, colony-forming units-granulocyte, erythroid, monocyte, and megakaryocyte colonies; CFU-S, CFU-spleen; NMS, normal mouse serum; FCA, flow cytometric analysis; CSF, colony-stimulating factor; HBSS, Hanks’ balanced salt solution; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate.

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more, administration of recombinant human IL-7 (rhIL-7) to sublethally irradiated mice resulted in accelerated repopulation of T and B lymphocytes and some immature myeloid lineage cells [20]. Our laboratory has previously demonstrated that administration of rhIL-7 to mice induced exportation of single lineage colony-forming units-culture (CFU-c) and multilineage CFU-granulocyte, erythroid, monocyte, and megakaryocyte (CFU-GEMM) colony-forming myeloid progenitors from the bone marrow to the spleen that resulted in a 90% decrease in bone marrow myeloid progenitors and a fivefold increase in the spleen [21, 22]. We also have shown that the administration of rhIL-7 resulted in a three- to fivefold increase in leukocytes in spleen and lymph nodes due to an increase in all major mature leukocyte subsets (B, T, and natural killer cells and macrophages) with a disproportionate increase in pre-B cells and CD8+ T cells [23]. Furthermore, T cells in rhIL-7-treated mice had enhanced and accelerated abilities to respond to various stimuli [23].

In this study we examined the effects of rhIL-7 administration on early myeloid and lymphoid precursors by using in vivo reconstitution assays. Our data demonstrate that administration of rhIL-7 to mice results in a relocalization of the earliest (CFU-spleen [CFU-S]) generating myeloid progenitors and of lymphoid precursors from the bone marrow to the spleen. These data also demonstrate an increase in pre-pro-B cells and pro-B cells and an increase in CD21+ and CD25+ B cell precursors after rhIL-7 treatment. In addition, administration of rhIL-7 to lethally irradiated mice after bone marrow transfer was able to accelerate bone marrow engraftment by up to 2-4 weeks. Thus, these data demonstrate that IL-7 has profound effects on both myeloid and lymphoid precursors and suggest that IL-7 may have a valuable role in accelerating bone marrow engraftment in transplant patients.

MATERIALS AND METHODS

Mice

Six to 10-week-old female C57BL/6 (Ly 5.2) or C57BL/6-Ly 5.1 congenic mice were obtained from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD) and were maintained under specific pathogen-free conditions.

Cytokine treatment

Recombinant human IL-7, generously supplied by Sterling Winthrop Inc. (Collegeville, PA), had a specific biological activity of 2-5 × 107 U/mg, as measured by the proliferation of a murine pre-B cell line [7]; the endotoxin levels were <2 endotoxin U/mg of rhIL-7. Mice were injected intraperitoneally twice daily with Hanks' balanced salt solution (HBSS) containing 0.1% normal mouse serum (NMS) as a vehicle control or with 5 or 10 μg/injection of rhIL-7 in HBSS and 0.1% NMS at 0.5 ml/injection. This regimen was based on observations from our previous studies [21, 23].

Cell suspension preparation

Bone marrow cells, splenocytes, and thymocytes were prepared as previously described [21, 23]. Erythrocytes in the bone marrow and splenocytes were rapidly lysed (5 s) with distilled water. After a final wash with HBSS, the cells were either injected intravenously into irradiated recipients or labeled with antibodies for flow cytometric analysis (FCA).

CFU-S assay

The CFU-S assay was performed as described by Till and McCulloch [24]. Briefly, 6-week-old recipient mice were lethally irradiated (900 rads) by using a MARK 1 Irradiator affixed with a 302 attenuator (J.L. Shepherd, San Fernando, CA) containing a 137Cs source emitting 229 rads/min, rested 3-4 h, then reconstituted intravenously with 2 × 106 bone marrow cells or 5 × 106 splenocytes. Nine and 12 days later their spleens were removed, fixed in Bouin's fixative, and scored for the presence of macroscopic colonies.

Reconstitution assay

Recipient mice were sublethally (750 rads) or lethally (900 rads) irradiated 3-4 h before reconstitution. Recipients were injected intravenously with varying numbers of donor-origin bone marrow cells or splenocytes. At various times after reconstitution the recipients were euthanized and their thymi and spleens were removed for further analysis.

Immunofluorescence labelling and FCA

Three mice per group were used for each time point. Single cell suspensions were labeled with antibody and FCA was performed as previously described [21]. Donor-origin and host-origin cells were detected with the monoclonal antibodies (mAb) reactive against Ly 5.1 (mouse IgG2α, clone A20-1.7; Ref. 25) and Ly 5.2 (mouse IgG2α, clone 104.2.1; Ref. 25), respectively, and developed with either fluorescein isothiocyanate (FITC)-conjugated, affinity-purified goat anti-mouse IgG and IgM (heavy and light chain specific) antibody (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) for thymocytes or phycoerythrin-conjugated, affinity-purified goat anti-mouse IgG2α (Caltag Laboratories, South San Francisco, CA) for splenocytes. Donor-origin splenic myeloid cells were detected in two-color analysis by using the biotin-conjugated mAb clone RB6-8C5 (rat IgG2a, Pharmingen, San Diego, CA) developed with streptavidin-RED680 (GIBCO-BRL, Life Technologies, Inc., Gaithersburg, MD) combined with the anti-Ly 5.1 mAb developed as indicated above. The mAb RB6-8C5 detects the RB6-8C5 myeloid differentiation antigen, which is expressed on all granulocytes in increasing amounts as cells mature. It may also be transiently expressed on cells undergoing macrophage differentiation [26].

Donor-origin splenic T cells were detected in two-color analysis by using the anti-CD8 mAb 500A2 (hamster IgG; Ref. 27) developed with a FITC-conjugated, affinity-purified goat anti-hamster IgG (heavy and light chain specific and mouse serum absorbed) antibody (Caltag Laboratories) combined with the anti-Ly 5.1 mAb developed as indicated above. Donor-origin splenic B lineage cells (B220+) and mature B cells (surface Igμ+) were detected in three-color analysis by using FITC-conjugated, affinity-purified goat anti-mouse IgM (μ heavy chain specific) antibody (FisherBiotech; Fisher Scientific, Orangeburg, NY) combined with phycoerythrin-conjugated anti-B220 mAb (rat IgG2α, clone RA3-6B2; Pharmingen) and anti-Ly 5.1 mAb conjugated with biotin in our laboratory and developed with streptavidin-RED680. Two sets of antibodies were used to examine immature B lineage cells. The first combination included FITC-conjugated anti-CD43 mAb (rat IgG2a, clone 57; originally produced by Gullely et al. [28] and conjugated by Randy Fischer, Biological Response Modifiers Program, National Cancer Institute, Frederick, MD) combined with phycoerythrin-conjugated anti-B220 mAb and the second combination included FITC-conjugated anti-CD2 mAb (rat IgG2α, clone RM-35; Pharmingen) combined with phycoerythrin-conjugated anti-B220 mAb and biotin-conjugated, affinity-purified goat anti-mouse IgM (μ heavy chain specific) antibody (Vector Laboratories, Inc., Burlingame, CA) developed with streptavidin-RED680 (GIBCO-BRL). All antibodies used were titrated to determine optimal working dilutions and isotype-matched controls were used to determine the amount of nonspecific binding. All polyclonal antibodies were pre-tested and found to be highly specific with no detectable cross-reactivities to Ig's of other species, isotypes, or subclasses.

Statistical analysis

Results from representative experiments are expressed as the mean ± s.d. The significance of alterations in the numbers of leukocytes obtained from various groups was determined by Student's t-test.

RESULTS

Effect of rhIL-7 on the frequency of CFU-S in bone marrow and spleen

Previous work in our laboratory demonstrated that rhIL-7 administration to normal mice decreased the number of CFU-c and CFU-GEMM myeloid progenitors in the bone marrow and increased the number of these progenitors...
in the spleens by exportation from the bone marrow to the spleen [21, 22]. To investigate the effect of rhIL-7 on even more immature erythro/myeloid progenitor cells, the in vivo CFU-S assay was performed. Donor C57BL/6-Ly 5.1 mice were injected intraperitoneally with HBSS and 0.1% NMS or 10 μg of rhIL-7, respectively, twice a day for 7 days. After cessation of treatment donor bone marrow cells or splenocytes were injected intravenously into lethally (900 rads) irradiated C57BL/6 (Ly 5.2) recipients per group. On days 9 or 12 after transfer the spleens were removed from the recipients and the number of CFU-S were enumerated. Lethally irradiated recipient controls that were injected only with HBSS had no CFU-S on days 9 or 12. Data represent the mean ± SD.

<table>
<thead>
<tr>
<th>Donor treatment (organ)</th>
<th>Number of CFU-S/recipient</th>
<th>Total No. of CFU-S generated by donor cells on a per organ basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS-bone marrow</td>
<td>Day 9: 15 ± 1, 9 ± 3</td>
<td>Day 9: 1080 ± 61, 675 ± 201</td>
</tr>
<tr>
<td></td>
<td>Day 12: 2 ± 2, 3 ± 3</td>
<td>Day 12: 320 ± 320, 575 ± 403</td>
</tr>
<tr>
<td>rhIL-7-bone marrow</td>
<td>Day 9: 3 ± 1, 4 ± 1</td>
<td>Day 9: 278 ± 109, 577 ± 129</td>
</tr>
<tr>
<td></td>
<td>Day 12: 13 ± 1, 12 ± 2</td>
<td>Day 12: 6552 ± 504, 5846 ± 982</td>
</tr>
<tr>
<td>HBSS-spleen</td>
<td>Day 9: 278 ± 109, 577 ± 129</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 12: 6552 ± 504, 5846 ± 982</td>
<td></td>
</tr>
</tbody>
</table>

Donor C57BL/6-Ly 5.1 mice (10–55/group) were injected intraperitoneally with HBSS and 0.1% NMS or 10 μg of rhIL-7, respectively, twice a day for 7 days. After cessation of treatment donor bone marrow cells or splenocytes were injected intravenously into five lethally (900 rads) irradiated C57BL/6 (Ly 5.2) recipients per group. On days 9 or 12 after transfer the spleens were removed from the recipients and the number of CFU-S were enumerated. Lethally, irradiated recipient controls that were injected only with HBSS had no CFU-S on days 9 or 12. Data represent the mean ± SD.

Results indicate the number of cells from a pool of mice divided by the number of mice.

These data were calculated by multiplying the number of CFU-S per recipient spleen by the total mean cellularity of the donor organ (column 2) and dividing by the number of donor cells injected.

Significantly different from HBSS-treated controls (P<0.01).}

TABLE 1. Administration of rhIL-7 to Mice Alters the Capacity of Spleen and Bone Marrow Cells to Generate CFU-S

<table>
<thead>
<tr>
<th>Donor treatment (organ)</th>
<th>Cellularity of donor organ after treatment (x10^6)</th>
<th>No. of donor cells injected (x10^5)</th>
<th>Number of CFU-S/recipient</th>
<th>Total No. of CFU-S generated by donor cells on a per organ basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS-bone marrow</td>
<td>14.6</td>
<td>2</td>
<td>15 ± 1, 9 ± 3</td>
<td>1080 ± 61, 675 ± 201</td>
</tr>
<tr>
<td>rhIL-7-bone marrow</td>
<td>32.0</td>
<td>2</td>
<td>2 ± 2, 3 ± 3</td>
<td>320 ± 320, 575 ± 403</td>
</tr>
<tr>
<td>HBSS-spleen</td>
<td>49.6</td>
<td>5</td>
<td>3 ± 1, 4 ± 1</td>
<td>278 ± 109, 577 ± 129</td>
</tr>
<tr>
<td>rhIL-7-spleen</td>
<td>252.0</td>
<td>5</td>
<td>13 ± 1, 12 ± 2</td>
<td>6552 ± 504, 5846 ± 982</td>
</tr>
</tbody>
</table>

Lymphocyte regeneration after transfer of bone marrow cells or splenocytes from rhIL-7-treated mice into irradiated recipients

To determine the effects of rhIL-7 on lymphoid progenitors in the bone marrow and spleen, lethally (750 rads) irradiated C57BL/6 (Ly 5.2) mice were injected intravenously with 5 × 10^6 bone marrow cells or 40 × 10^6 splenocytes from C57BL/6-Ly 5.1 mice that had been treated intraperitoneally with HBSS and 0.1% NMS or rhIL-7 (10 μg/injection) twice a day for 7 days. On day 15 after transfer the thymi and spleens were removed from the recipients. The generation of donor-origin cells in the thymus and donor-origin B lineage cells in the spleen was determined by FCA. The results in Table 2 demonstrate that the bone marrow from rhIL-7-treated mice had a 21- and 13.7-fold decrease in the ability to reconstitute the donor-origin thymocytes and B220+ splenocytes, respectively, early after transfer when compared with cells from vehicle control-treated mice. The fold decrease in the relative number of precursors on a per organ basis in bone marrow from rhIL-7-treated mice compared with HBSS and 0.1% NMS-treated mice was similar (18.1-fold for thymocytes and 11.9-fold for B220+ splenocytes). In contrast, the reconstituting activity of splenocytes from rhIL-7-treated mice was not significantly different from splenocytes obtained from untreated mice in their ability to reconstitute the thymus of irradiated mice compared with controls.

A decrease was observed in the ability of splenocytes from rhIL-7-treated mice to generate donor-origin B220+ cells (2.4-fold) in the spleens of recipient mice compared with controls (Table 2). However, when the reconstituting ability of splenocytes from rhIL-7-treated mice was calculated on a per organ basis, as shown in Table 2, there was actually an increase in the ability of these splenocytes to reconstitute the thymus (4.0-fold) and B220+ splenocytes (3.2-fold) when compared with the reconstituting ability of splenocytes from HBSS and 0.1% NMS-treated control mice.

rhIL-7 administration results in increased cell numbers at several stages of B cell differentiation

As previously reported, rhIL-7 administration expands pre-B cell and mature B cell numbers, up-regulates BP-1, and increases the numbers of cytoplasmic μ-chain-positive cells and the frequency of colony-forming cell–pre-B cells [18, 29]. To extend these observations, bone marrow cells and splenocytes from HBSS- or rhIL-7-treated mice were examined to quantitate specific stages of B cell differentiation by using FCA. The phenotypic markers B220 and CD43 were used to identify pre-pro-B cells and pro-B cells, which have been reported to express low levels of both of these markers [29]. The profiles in Figure 1A show the distribution of bone marrow cells and splenocytes from HBSS- or rhIL-7-treated mice based on CD43 expression and the box within each profile indicates the area that contains pre-pro-B cells and pro-B cells. The percentage and numbers of cells within each box are shown in Table 3 in the column labeled B220^low+.
CD43low+. Bone marrow and spleens from rhIL-7-treated mice had an approximately two- to threefold increase in the numbers of these cells compared with controls. The expression of CD2 in combination with B220 and surface Ig^i expression was also examined on the bone marrow cells and splenocytes from HBSS- or rhIL-7-treated mice. All surface Ig^i-positive B cells expressed CD2 in both HBSS- and rhIL-7-treated mice (data not shown). Work by Sen et al. [30] showed that CD2 is expressed not only on mature B cells but also on pre-B (surface Ig^j; B220+ ) cells expressing cytoplasmic μ-chain. Therefore, the expression of CD2 on surface Ig^j-, B220+ cells was also quantitated. The profiles shown in Figure 1B illustrate the distribution of B220 and CD2 on surface Ig^j-negative cells from the bone marrow and spleen of HBSS- or rhIL-7-treated mice. The corresponding percentages and numbers given in Table 3 demonstrate that less than 5% of total bone marrow cells from HBSS-treated mice are surface Ig^j, B220+, CD2+ cells. However, bone marrow cells from rhIL-7-treated mice have an 11.6-fold increase in the numbers of these cells. Spleen cells from HBSS-treated mice have virtually no cells of this phenotype. However, 9.99% (26.55 x 10^6) of total splenocytes are surface Ig^j, B220+, CD2+ after rhIL-7 administration.

Pre-pro-B cells and pro-B cells are contained in the surface Ig^j, B220+, CD2+ cells. When the percentage of B220low+, CD43low+ cells that contain pre-pro-B cells and pro-B cells is subtracted from the surface Ig^j, B220+, CD2+ cell percentage, 36% (13.78 x 10^6) of total bone marrow cells from rhIL-7-treated mice remain. This demonstrates that the majority of the cell expansion in the surface Ig^j, B220+, CD2+ subset was not due to pre-pro-B cells or pro-B cell expansion but does not the disproportional expansion of another B lineage stage, most likely pre-B cells that do not express CD2. Similar results were obtained for splenocytes from rhIL-7 mice. After rhIL-7 administration, there was a 10.8- and 45.8-fold increase in the numbers of cells in the surface Ig^j, B220+, CD2+ B lineage cell subset in the bone marrow and spleen, respectively, compared with HBSS controls.

rhIL-7 accelerates lymphoid regeneration in lethally irradiated mice given a bone marrow transfer from normal mice

C57BL/6 (Ly 5.2) mice were lethally (900 rad) irradiated and injected with 10 x 10^6 bone marrow cells from untreated C57BL/6-Ly 5.1 congenic mice. After transfer, recipient mice were examined by FCA to determine the amount of donor-origin myeloid reconstitution in the spleen. Administration of rhIL-7 resulted in a minor enhancement in myeloid reconstitution compared with bone marrow recipients treated with HBSS and 0.1% NMS (Fig. 2).

In a similar experiment, FCA was used to determine the total number of donor-origin cells generated and the amount of donor-origin T cell and B cell reconstitution in the spleen. The generation of donor-origin splenocytes was accelerated in recipient mice that received rhIL-7 treatment (Fig. 3). Recipient mice treated with HBSS and 0.1% NMS took up to 4 weeks longer to reach equivalent levels of reconstitution. Donor-origin subset analysis in the recipients’ spleens was performed using FCA. Examination of donor-origin B cells in numbers in Figure 4 and donor-origin T cell numbers in Figure 5 demonstrate that treatment of bone marrow recipient mice with rhIL-7 greatly enhanced the speed of reconstitution of both the B cell and T cell compartments by up to 4 and 2 weeks, respectively, with bone marrow recipients treated with the vehicle control. CD4/CD8 analysis indicated that both the helper CD4+ T cells as well as the cytotoxic CD8+ T cells underwent this expansion (Table 4). However, administration of rhIL-7 appeared to preferentially expand the CD8+ T cells, which we have previously described when normal mice are treated with rhIL-7 [20, 23].

**Table 2.** Administration of rhIL-7 to Mice Alters the Capacity of Spleen and Bone Marrow Cells to Regenerate Lymphoid Cells in Irradiated Recipients

<table>
<thead>
<tr>
<th>Donor treatment (organ)</th>
<th>Cellularity of donor organ after treatment (x10^6)a</th>
<th>No. of donor cells injected</th>
<th>No. of donor thymocytes generated/thymus (x10^6)b</th>
<th>No. of donor B220+ splenocytes generated/spleen (x10^6)c</th>
<th>Total no. lymphoid cells generated by donor cells on a per organ basis (x10^6)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS-bone marrow</td>
<td>19.4 ± 6.5</td>
<td>5</td>
<td>60.9 ± 19.1</td>
<td>59.1 ± 13.8</td>
<td>231.5 ± 72.7</td>
</tr>
<tr>
<td>rhIL-7-bone marrow</td>
<td>22.1 ± 5.4</td>
<td>5</td>
<td>2.9 ± 3.5</td>
<td>4.3 ± 0.3</td>
<td>12.8 ± 15.5</td>
</tr>
<tr>
<td>HBSS-spleen</td>
<td>55.3 ± 20.6</td>
<td>40</td>
<td>41.4 ± 19.6</td>
<td>14.9 ± 4.4</td>
<td>56.9 ± 26.9</td>
</tr>
<tr>
<td>rhIL-7-spleen</td>
<td>420 ± 113</td>
<td>40</td>
<td>21.7 ± 9.4</td>
<td>6.3 ± 0.3</td>
<td>227.5 ± 98.5</td>
</tr>
</tbody>
</table>

Donor C57BL/6-Ly 5.1 mice (5-25/group) were treated as described in Table 1. After treatment donor bone marrow or spleen cells were injected intravenously into sublethally (750 rads) irradiated C56BL/6 (Ly 5.2) recipients. Fifteen days after transfer the thymi and spleens from 3 mice/group were assayed by using FCA to determine the number of donor-origin cells in the thymus or the number of donor-origin B220+ cells in the spleen of recipient mice. Data represent the mean ± SD.

bSignificantly different from HBSS-treated controls (P<.01).

cSignificantly different from HBSS-treated controls (P<,05).

dSignificantly different from HBSS-treated controls (P<001).

eResults indicate the number of cells from a pool of mice divided by the number of mice.

*These data were calculated by multiplying the number of donor thymocytes or B220+ splenocytes per recipient spleen by the total mean cellularity of the donor organ (column 2) and dividing by the number of donor cells injected.
Fig. 1. Administration of rhIL-7 to mice results in expansion of several B lineage subsets. C57BL/6 Ly5.1 mice were injected intraperitoneally twice a day for 7 days with HBSS or 10 μg of rhIL-7 per injection. The day after cessation of treatment the spleen and bone marrow cells were removed and labeled for FCA with FITC-CD43 and phycoerythrin-B220 (A) or FITC-CD2, phycoerythrin-B220, and biotin anti-mouse IgM antibody developed with streptavidin-RED700 (B). The profiles in B illustrate the expression of B220 and CD2 on IgM+ cells. Cells were prepared from a pool of 3 tissues/group.

DISCUSSION

The effects of rhIL-7 on lymphoid and myeloid progenitors and on lymphoid and myeloid lineage repopulation were investigated by using C57BL/6 congenic mice. Recombinant human IL-7 treatment markedly influenced the ability of bone marrow and spleen cells to generate CFU-S when transferred into irradiated recipients resulting in a 3.4-fold reduction or a 23.6-fold increase in the capacity of bone marrow or spleen cells, respectively, to form day 9 CFU-S generated from precursors thought to be committed to a particular lineage [31]. Furthermore, day 12 CFU-S-generating capacity that arises from uncommitted precursors [31] was increased 15.5-fold in splenocytes from rhIL-7-treated mice. Although our previous data [21] demonstrated that localization of myeloid progenitors capable of generating CFU-c and CFU-GEMM is affected by rhIL-7 administration, the results presented here extend these observations to show that the numbers and localization of the earliest detectable erythro/myeloid progenitors also are affected by in vivo rhIL-7 treatment.

Because in vivo administration of rhIL-7 had pronounced effects on various stages of myeloid progenitors, we also examined its effects on lymphoid progenitors. The results presented here are the first demonstration that rhIL-7 administration to mice also affects localization of lymphoid precursors. Specifically, on a per organ basis, bone marrow from mice treated with rhIL-7 for 7 days had an 11.9- and 18.1-fold decrease in the ability to reconstitute the B and T lineages, respectively, in irradiated recipients. However, the spleens from rhIL-7-treated mice had a three- to fourfold increase in their ability to repopulate the lymphoid lineages. These effects were reversible and dependent on the length of rhIL-7 treatment (data not shown). Thus, in vivo administration rhIL-7 significantly affects the localization and numbers of both lymphoid and myeloid precursors and, moreover, recent results from our laboratory suggest that similar effects of rhIL-7 administration on the stem cell compartment occur as well [32].

The mechanisms by which IL-7 exerts its effects are not clear, however, these data combined with previously published results suggest that IL-7 may act (directly or indirectly) first by mobilizing precursor cells to extra medullary sites. This hypothesis is supported by the substantial IL-7-induced reduction in precursor cells in the bone marrow that coincide with an increase in those cells in the spleen. The second component of the IL-7-induced effect is probably due to the induction of differentiation and expansion of the number of precursors that result in increased numbers of mature lymphoid and myeloid lineage cells. Additional experiments are in progress to address these issues.
TABLE 3. Administration of rhIL-7 Expands B Cell Lineage Subsets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HBSS-bone marrow</th>
<th>rhIL-7-bone marrow</th>
<th>HBSS-spleen</th>
<th>rhIL-7-spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B220&lt;sup&gt;low&lt;/sup&gt;</td>
<td>CD43&lt;sup&gt;low&lt;/sup&gt;</td>
<td>Ig&lt;sup&gt;+&lt;/sup&gt;, B220&lt;sup&gt;+&lt;/sup&gt;, CD2&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Ig&lt;sup&gt;+&lt;/sup&gt;, B220&lt;sup&gt;+&lt;/sup&gt;, CD2&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group</td>
<td>%</td>
<td>No. (x10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>%</td>
<td>No. (x10&lt;sup&gt;6&lt;/sup&gt;)</td>
</tr>
<tr>
<td>HBSS-bone marrow</td>
<td>2.79</td>
<td>0.81</td>
<td>4.69</td>
<td>1.36</td>
</tr>
<tr>
<td>rhIL-7-bone marrow</td>
<td>5.00</td>
<td>1.93</td>
<td>40.71</td>
<td>15.71</td>
</tr>
<tr>
<td>HBSS-spleen</td>
<td>2.79</td>
<td>1.59</td>
<td>0.24</td>
<td>0.14</td>
</tr>
<tr>
<td>rhIL-7-spleen</td>
<td>2.18</td>
<td>5.79</td>
<td>9.99</td>
<td>26.55</td>
</tr>
</tbody>
</table>

The FCA profiles illustrated in Figure 1 were analyzed to determine the percentage of cells bearing indicated phenotypic markers. The data presented here show the percentages and also the calculated cell numbers for each subset. The percentages listed indicate the percentage of a particular subset based on the total splenocyte or bone marrow cell population.

In addition to documenting the effects of rhIL-7 administration on the ability of progenitors to repopulate cells of the B lineage, we also examined the effect of rhIL-7 on specific stages of B cell differentiation, as defined by phenotypic markers using FCA. The results presented here demonstrate that pre-pro-B cells and pro-B cells expand in number by two- to threefold in the spleen and bone marrow of rhIL-7-treated mice. This supports the work of Hardy et al. [29], demonstrating in vitro that CD43<sup>low</sup> cells respond to IL-7. The expansion of cells in other B lineage stages after rhIL-7 treatment is even more dramatic. Pre-B cells (surface Ig<sup>+</sup>, B220<sup>+</sup>) that expressed CD2 expanded 10.8- to 45.8-fold, depending on the organ examined. After rhIL-7 treatment surface Ig<sup>+</sup>, B220<sup>+</sup>, CD2<sup>+</sup> cells expanded by 11.6-fold in the bone marrow and to 26.53 x 10<sup>6</sup> cells in the spleen. This expansion of surface Ig<sup>+</sup>, B220<sup>+</sup>, CD2<sup>+</sup> cells cannot be accounted for by the expansion of the pre-pro-B and pro-B cell populations also contained in this subset. It is probable that the cells that are responsible for the majority of the expansion are in a stage between the pro-B cell and the classic pre-B cell, which expresses cytoplasmic <i>µ</i>-chain and CD2. The exact placement of this cell within the stages of B cell differentiation will most likely require the examination of its status in terms of Ig gene rearrangement. Because this cell subset is present in low numbers in normal bone marrow cells, the use of this IL-7 model may be important in better understanding the complexities of B cell differentiation. Thus, it appears that rhIL-7 administration either directly or indirectly results in expansion of cells at several stages of B cell differentiation from the pre-pro-B cells through to B cells.

In comparing the reconstitution data with the FCA data, it is quite clear that although rhIL-7 administration expands B lineage stages from the pre-pro-B cells and onward in the bone marrow, the precursor responsible for B cell reconstitution is not increasing in the bone marrow (Table 2). There are several possible explanations to account for this. One possibility is that precursors responsible for reconstitution have migrated from the bone marrow to the spleen and this has been addressed above. A second possibility is that the cells are rendered inactive by IL-7 either by functional inhibition or death. This is unlikely based on our results demonstrating that rhIL-7 accelerates B cell reconstitution after normal bone marrow transplantation. A third possibility is that the cells...
TABLE 4. Both CD4+ and CD8+ Donor-Origin T Cells Expand in Number in Irradiated Recipients After Bone Marrow Transfer and rhIL-7 Administration

<table>
<thead>
<tr>
<th>Treatment of recipient</th>
<th>Days after transfer</th>
<th>CD4+ (x10^6)</th>
<th>CD8+ (x10^6)</th>
<th>CD4:CD8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td>13</td>
<td>0.4 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.6:1</td>
</tr>
<tr>
<td>rhIL-7</td>
<td>13</td>
<td>2.4 ± 0.6a</td>
<td>4.7 ± 0.8a</td>
<td>0.5:1</td>
</tr>
<tr>
<td>HBSS</td>
<td>17</td>
<td>0.9 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>1.3:1</td>
</tr>
<tr>
<td>rhIL-7</td>
<td>17</td>
<td>2.9 ± 0.2b</td>
<td>5.7 ± 0.9b</td>
<td>0.5:1</td>
</tr>
<tr>
<td>HBSS</td>
<td>23</td>
<td>1.6 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.2:1</td>
</tr>
<tr>
<td>rhIL-7</td>
<td>23</td>
<td>4.8 ± 1.1a</td>
<td>7.0 ± 2.8c</td>
<td>0.7:1</td>
</tr>
<tr>
<td>HBSS</td>
<td>41</td>
<td>17.5 ± 7.1</td>
<td>7.2 ± 1.7</td>
<td>2.4:1</td>
</tr>
<tr>
<td>rhIL-7</td>
<td>41</td>
<td>16.7 ± 7.9</td>
<td>15.4 ± 6.7</td>
<td>1.1:1</td>
</tr>
</tbody>
</table>

Donor-origin splenic T cells shown in Figure 5 were further analyzed by FCA to determine the number of cells contained in the CD4+ and CD8+ subsets. Data represent the mean ± SD of 3 mice/group.

*aSignificantly different from HBSS-treated controls (P<0.01).

*bSignificantly different from HBSS-treated controls (P<0.001).

*cSignificantly different from HBSS-treated controls (P<0.05).

that are capable of repopulating the B cell lineage have been used to support the dramatic expansion of pre-pro-B cells to mature B cells and are thus depleted. The reconstitution data support this possibility in that these precursors appear to be essential for the B cell expansion in bone marrow-transplanted mice.

Because IL-7 generated a leukocytosis in normal mice [18, 23] and accelerated lymphocyte repopulation in cyclophosphamide-treated mice [19] and sublethally irradiated mice [20], its potential application in accelerating myeloid and/or lymphoid reconstitution after bone marrow transplantation was studied in the C57BL/6 Ly 5 congenic mouse model. Lethally irradiated mice that had received normal donor-origin bone marrow cells and were treated with rhIL-7 had somewhat enhanced myeloid reconstitution in mice treated with rhIL-7. However, lymphoid reconstitution was both accelerated and enhanced for both donor-origin T cells and B cells. By 13 days after bone marrow transfer there was a 3.4-fold increase in B cell reconstitution in recipients treated with rhIL-7 compared with those that had received only HBSS and 0.1% NMS. There was also a fourfold increase in the number of T cells generated in recipient mice treated with rhIL-7 compared with controls until at least the 3rd to 4th week after transfer when reconstitution of T cells in control mice began to approach that of the rhIL-7-treated group. Furthermore, there was no observable toxic effect of long-term (6 weeks) rhIL-7 treatment in normal adult mice.
these experiments, unlike the study of Fraser et al. [33] in which 23% of lethally irradiated recipient mice that had received bone marrow infected with a retrovirus expressing the mIL-7 gene were moribund within 4–16 weeks after transplantation. These data illustrate that rhIL-7 administration is able to accelerate bone marrow engraftment after transplantation.

The results presented here demonstrate that IL-7 has potent effects on lymphoid and myeloid mobilization and accelerates bone marrow engraftment in lethally irradiated recipients. Thus, IL-7 administration may be important in the enhancement and acceleration of lymphoid and myeloid regeneration in myeloablated cancer patients who subsequently receive bone marrow transplants.

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