The administration of recombinant human interleukin-7 (rhIL-7) to mice twice a day for 7 days does not appreciably change bone marrow (BM) cellularity, but does result in a threefold to fivefold increase in the total number of leukocytes in the spleen, an eightfold to 10-fold increase in the total number of nonparenchymal cells (NPC) obtained from the liver, and up to a 20-fold increase in the total number of peripheral white blood cells (WBC). This regimen of rhIL-7 administration also causes a profound reduction in the total number of progenitors in the BM for both single-lineage colony-forming units-culture (CFU-c) (>90%) and multilineage CFU-granulocyte, erythroid, monocyte, megakaryocyte (CFU-GEMM) (>99%) colonies. In contrast, mice treated with rhIL-7 exhibited increases in both CFU-c (20- to 40-fold, 20-fold, and 15- to 40-fold) and CFU-GEMM (8- to 10-fold, 30-fold, and 6- to 10-fold) cultured from the peripheral blood, spleen, and NPC, respectively. The increase in CFU in the NPC was accompanied by a fivefold increase in the number of MAC-1+ cells and a ninefold increase in the number of B220+ cells. Splenectomy of mice before the administration of rhIL-7 further increased the total number of WBC, NPC, and myeloid progenitors as compared with the rhIL-7-treated nonsplenectomized mice. Finally, selective depletion of the BM by intraperitoneal administration of 85Sr (98% reduction in BM cellularity and >99% reduction in BM myeloid progenitors) abrogated the rhIL-7-induced increases in cellularity and myeloid progenitor number in the peripheral blood, spleen, and NPC. These results show that the changes in myelopoiesis observed after in vivo administration of rhIL-7 to mice result largely from the emigration of myeloid progenitors from the BM through the blood to the spleen, liver, and, possibly, other peripheral organs. This is a US government work. There are no restrictions on its use.

From the Biological Carcinogenesis and Development Program, Program Resources, Inc/DynCorp, the Laboratories of Experimental Immunology and of Leukocyte Biology, Biological Response Modifiers Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD; and Sterling Winthrop, Inc, Collegeville, PA.

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colony-forming units in culture (CFU-c) and multilineage CFU-granulocyte, erythroid, monocyte, megakaryocyte (CFU-GEMM) in the BM. Concomitantly, we observed an increase in CFU-c and CFU-GEMM in the spleen, suggesting that rhIL-7 can differentially regulate myelopoiesis in the BM and spleen while stimulating lymphopoiesis. Thus, the aim of the present study was to determine if the observed changes in myelopoiesis induced by rhIL-7 are caused by progenitor exportation from the BM to peripheral sites or, alternatively, the expansion of extramedullary progenitor cells.

MATERIALS AND METHODS

Mice. C57BL/6 mice were obtained from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD). Mice were maintained in a specific pathogen-free environment and were used between 8 and 12 weeks of age. Animal care was provided in accordance with the procedures outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 86-23, 1985).

Cytokines and reagents. Recombinant human erythropoietin (Epo) was obtained from Amgen Inc (Thousand Oaks, CA). Recombinant murine IL-3 was purchased from PeproTech Inc (Rocky Hill, NJ). rhIL-7, which was produced in Escherichia coli and purified by Immunex Corp (Seattle, WA), was generously supplied by Sterling Winthrop, Inc (Collegeville, PA). The rhIL-7 had a specific activity of 2 to 5 x 10^5 U/mg, as measured by proliferation of a murine pre-B-cell line (IA/N/AO).15 and endotoxin levels were less than 0.1 ng/mL. Mice received injections of rhIL-7 or diluent (Hanks' Balanced Salt Solution [HBSS] without Ca^2+, Mg^2+, and phenol red, supplemented with 0.1% normal mouse serum [NMSI]). For enzymatic perfusion of the liver, collagenase P (lot no. 12445920-21; Boehringer Mannheim, Indianapolis, IN) was used.

Experimental design. Mice were randomly segregated, divided into treatment groups, and received intraperitoneal (IP) injections twice a day for 7 days of diluent or of rhIL-7 (10 μg/injection). This dose and schedule has been previously shown to be optimal for suppression of myelopoiesis in the BM and for increased myelopoiesis in the spleen. Administration of higher amounts of rhIL-7 or administration for more than 7 days does not appreciably increase these effects. In some experiments, mice were splenectomized (Spxl) 7 days before treatment. On days 8 through 14, the mice received IP injections twice a day of diluent or of rhIL-7. On day 15, all mice were euthanized, blood was collected, and the BM and liver were removed. Tissues also were removed from non-Spxl control and rhIL-7-treated mice.

Isolation of hepatic leukocytes. Two types of liver-associated leukocytes were obtained. Lavage cells (LC) were isolated by slow perfusion with lavage buffer ([10 mL of HBSS without Ca^2+ and Mg^2+] containing 0.5 mM/L, EGTA). Nonparenchymal cells (NPC) were isolated by enzymatic dissociation of the liver using a previously described and slightly modified method. Briefly, after the LC were obtained, livers underwent further slow perfusion with 20 mL of warm (37°C) perfusion buffer (HBSS without Ca^2+ and Mg^2+, containing 100 U/mL of collagenase P) for partial enzymatic digestion. The livers were then excised and incubated at an additional 20 minutes at 37°C to complete digestion. The tissue was then minced and the cell suspension filtered through 60-μm nylon mesh, washed once at 300g for 5 minutes, and further purified by fractionation on a metrizamide density gradient (Nyegaard, Oslo, Norway). The composition of this cell population was ascertained by examination of a Wright-Giemsa-stained cytospin preparation (>95% NPC). The cells were then resuspended either in HBSS with 0.1% sodium azide and 0.1% bovine serum albumin (BSA) for flow cytometry analysis (FCA) or with a medium (modified Eagle's minimum essential medium [EMEM]; Gibco/BRL, Grand Island, NY) for assessment of myeloid progenitor cells.

Isolation of peripheral blood mononuclear cells. Peripheral blood from mice was collected, resuspended in cold HBSS (ratio 1:2), and layered over a Lympholyte-M (Cedarlane, Ontario, Canada) density gradient (1.0875 g/mL). The gradients were centrifuged at 900g for 30 minutes to obtain white blood cells (WBC).

Preparation of BM and spleen cells. BM from the femurs and tibias of Spxl and non-Spxl mice that had received IP injections of rhIL-7 or diluent were obtained by lavage with cold HBSS. Spleens from non-Spxl mice were removed and mechanically dissociated. The single-cell suspensions from both organs were obtained by gentle forced passage through a fine mesh screen, suspended in cold HBSS, and further filtered through nylon mesh. The cells were washed twice and resuspended in a medium containing 20% heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT), 1% glutamine (Cellgro, Washington, DC), 1% penicillin-streptomycin mixture (Cellgro), and 5.6% sodium bicarbonate (Sigma, St Louis, MO).

Splenectomy. Mice were anesthetized with Metofane (Pitman-Moore, Washington Crossing, NJ). A transverse incision was made through the skin and through the peritoneal membrane at a point just under the rib cage on the left flank. The spleen and associated tissue were pulled gently from the peritoneal cavity, connective tissue attachments were cut, and blood vessels were clamped. The peritoneal membrane and skin were then joined with an autodocip. Splenectomized mice were rested for 1 week before they were used for further experimentation.

Administration of 89Sr. Mice were treated with 89Sr as previously described21 with some modifications. 89Sr was obtained from Amersham Corp (Arlington Heights, IL) as 89SrCl2 at a concentration of 190 μCi/mg of 89Sr. We previously reported that the IP administration of greater than 125 μCi/mouse was able to deplete BM cellularity (>90%) within 5 days in the absence of deleterious effects on spleen cellularity.22 Based on these studies, a single administration of 150 μCi/mouse was used as the standard dose for these experiments, and 7 days thereafter mice were treated with either rhIL-7 or HBSS + 0.1% NMSI.

Soft agar colony-formation assay. A total of 102 cells (BM) and 105 cells (WBC, NPC, and LC) were plated in 35-mm Lux petri dishes (Miles Laboratories, Inc, Naperville, IL) containing 1 mL of α medium that was supplemented with 20% FBS, 2 x 10^-4 mol/L hemin (Sigma), 5 x 10^-5 mol/L 2-mercaptoethanol (Sigma), 8 U/mL Epo, 100 U/mL rhIL-3, and 0.05 mg/mL gentamicin in 0.35% Seaplaque agarose (FMC Bioproducts, Rockland, ME). The petri dishes were incubated at 37°C in 5% CO2 for 10 days, at which time the number of colonies was determined. Colonies composed of more than 50 cells were enumerated and characterized as described by Metcalf.23 Multipotent colonies containing granulocyte, erythroid, megakaryocyte, and macrophage lineages were scored as CFU-GEMM, whereas monocyte (CFU-M), myeloid (CFU-G), erythroid (CFU-e and BFU-e), and myelomonocytic (CFU-GM) colonies were designated as CFU-e.

Surface phenotype analysis. The surface phenotype analysis was performed using immunofluorescence labeling followed by FCA, as previously described. Briefly, BM or spleen cells were quickly lysed with ACK lysis buffer (Quality Biologicals, Gaithersburg, MD) to remove red blood cells (RBC) and washed with HBSS without calcium, magnesium, and phenol red (Whittaker Bioproducts, Walkersville, MD). One million cells per sample were labeled with 10 μL of the optimal concentration of primary antibody, followed by the appropriate developing secondary reagent.
That could be isolated from the BM and spleen, respectively, as previously described.25 Reduced or increased the number of myeloid progenitor cells that the administration of rhIL-7 for 7 days maximally re-
duced or increased the number of myeloid progenitor cells that could be isolated from the liver or other sites in Splx mice; and (3) more myeloid progenitors detected in the peripheral blood during the course of rhIL-7 treatment. On the other hand, if rhIL-7 was expanding splenic hematopoietic progenitors in the absence of, or in addition to, an expansion of BM progenitors, splenectomy should reduce the myelostimulatory effects of rhIL-7 in the peripheral blood and liver.

The results from our initial studies showed that there were no obvious changes in BM cellularity between rhIL-7-treated and untreated mice (Table 1). However, administration of rhIL-7 to non-Splx mice (Table 1, experiment 1) produced a 20-fold increase in the WBC count, an eightfold increase in the number of hepatic NPC, and a fourfold increase in the number of hepatic LC (Table 1). These effects were all further increased in Splx mice such that, whereas the baseline number of NPC and LC in diluent-treated Splx mice was increased by $4.4 \times 10^6$ and $0.6 \times 10^6$ cells, respectively, over that seen in non-Splx mice, the number of NPC and LC in Splx mice increased by $40.5 \times 10^6$ and $2.4 \times 10^6$, respectively, after rhIL-7 administration. Replicate experiments showed similar effects (data not shown). Thus, removal of the spleen appreciably enhances the ability of rhIL-7 to increase the number of NPC and LC. This finding suggests that bloodborne leukocytes are accumulating in peripheral sites.

FCA of leukocytes obtained from Splx versus non-Splx mice. Because rhIL-7 induces a peripheral leukocytosis that results largely from an increase in pre-B cells and mature T cells (Komschlies et al., manuscript submitted for publication), FCA was performed to determine whether splenectomy actually increased the total number of leukocytes expressing a surface phenotype consistent with mature myeloid cells. These studies (Table 2) showed an 88% decrease in the number of BM cells expressing high-intensity 8C5(bsh) (mature granulocytes), whereas a 300% and 875%

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**Table 1.** Effect of rhIL-7 Treatment on the Total Cell Number in Different Tissues of Splx and Spl-Treated Mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tissue</th>
<th>In Vivo Pretreatment</th>
<th>Control</th>
<th>rhIL-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BM</td>
<td>None</td>
<td>55.3</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>Splx</td>
<td>63.0</td>
<td>53.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>None</td>
<td>0.3</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Splx</td>
<td>1.2</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BM</td>
<td>None</td>
<td>4.0</td>
<td>44.0</td>
</tr>
<tr>
<td></td>
<td>Splx</td>
<td>1.3</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>None</td>
<td>0.4</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Splx</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NPC</td>
<td>None</td>
<td>1.0</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>Splx</td>
<td>1.3</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>None</td>
<td>65.0</td>
<td>250.0</td>
</tr>
<tr>
<td></td>
<td>Splx</td>
<td>106.7</td>
<td>149.3</td>
<td></td>
</tr>
</tbody>
</table>

In experiment 1, mice were Splx on day 0. On day 8, mice were treated IP with rhIL-7 (10 pg/injection) or HBSS twice a day for 7 days. Twelve hours after the last injection of rhIL-7 or diluent, mice were euthanized, different tissues were recovered, and cells were isolated as described in the Materials and Methods. Each value represents the number of pooled cells $\times 10^6$ obtained from a particular organ, divided by the number of mice per group (n = 5). This is one representative experiment of three performed. In experiment 2, mice were injected IP with 150 $\mu$l of $^{38}$Sr. After 1 week, mice were treated IP with rhIL-7 (10 pg/injection) or HBSS twice a day for 7 days. Twelve hours after the last injection of rhIL-7 or diluent, mice were euthanized, different tissues were recovered, and cells were isolated as described in the Materials and Methods. Each value represents the number of pooled cells $\times 10^6$ obtained from a particular organ, divided by the number of mice per group (n = 5). This is one representative experiment of two performed.

Abbreviation: P8, peripheral blood.

The percentage of cells bearing a particular cell surface marker was determined using a Becton Dickinson (Mountain View, CA) FACScan flow cytometry analyzer using a 15-mW Argon-Ion laser with a 488 nm excitation beam. Myeloid cells were detected using a fluorescein-conjugated rat antimouse MAC-1 MoAb (clone M1/70, originally obtained from American Type Culture Collection [Rockville, MD] and provided by Dr James Kenny [PRI/DynCorp, NCI-FCRDC, Frederick, MD] in labeled form). Mature and immature granulocytes were detected using a biotin-conjugated rat antimouse granulocyte MoAb clone RB6-8C5 (8C5) (Pharmingen, San Diego, CA) developed with Streptavidin-Red 613 (GIBCO-BRL, Gaithersburg, MD).

Statistical analysis. Statistically significant differences based on absolute numbers were detected by two-tailed, Student’s t-test.26 Fold differences were compared by t-test using variances of the proportions, as previously described.27

**RESULTS**

Splenectomy enhances the rhIL-7-induced leukocytosis in the liver. Previous studies from our laboratory showed that the administration of rhIL-7 for 7 days maximally reduced or increased the number of myeloid progenitor cells that could be isolated from the BM and spleen, respectively.19 The present studies were designed to determine whether this result was caused by a large scale shift of progenitors from the BM to peripheral sites. Mice were Splx before the initiation of rhIL-7 or diluent administration, as described in the Materials and Methods. We hypothesized that if substantial numbers of myeloid progenitor cells were being exported to peripheral organs, then there should be (1) an increase in the total number of progenitors in normal spleen and liver; (2) an increase in the number of progenitors that could be isolated from the liver or other sites in Splx mice; and (3) more myeloid progenitors detected in the peripheral blood during the course of rhIL-7 treatment.

The results from our initial studies showed that there were no obvious changes in BM cellularity between rhIL-7-treated and untreated mice (Table 1). However, administration of rhIL-7 to non-Splx mice (Table 1, experiment 1) produced a 20-fold increase in the WBC count, an eightfold increase in the number of hepatic NPC, and a fourfold increase in the number of hepatic LC (Table 1). These effects were all further increased in Splx mice such that, whereas the baseline number of NPC and LC in diluent-treated Splx mice was increased by $4.4 \times 10^6$ and $0.6 \times 10^6$ cells, respectively, over that seen in non-Splx mice, the number of NPC and LC in Splx mice increased by $40.5 \times 10^6$ and $2.4 \times 10^6$, respectively, after rhIL-7 administration. Replicate experiments showed similar effects (data not shown). Thus, removal of the spleen appreciably enhances the ability of rhIL-7 to increase the number of NPC and LC. This finding suggests that bloodborne leukocytes are accumulating in peripheral sites.

**Table 2.** Phenotypic Analysis for Mature Myelomonocytic Markers

<table>
<thead>
<tr>
<th>Organ</th>
<th>Marker</th>
<th>Control rhIL-7 Change*</th>
<th>Control rhIL-7 Change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splen</td>
<td>8C5(bsh)</td>
<td>1.4</td>
<td>4.3</td>
</tr>
<tr>
<td>BM</td>
<td>8C5(bsh)</td>
<td>20.3</td>
<td>2.5</td>
</tr>
<tr>
<td>NPC</td>
<td>8C5(bsh)</td>
<td>0.4</td>
<td>3.5</td>
</tr>
</tbody>
</table>

| | | Splx (total cells $\times 10^6$) |
| | | Control rhIL-7 Change* |
| Splen | 8C5(bsh) | 1.4 | 4.3 | 2.9 | NA | NA |
| BM | 8C5(bsh) | 20.3 | 2.5 | 17.8 | 34.9 | 49.0 | 10.0 |
| NPC | 8C5(bsh) | 0.4 | 3.5 | 3.1 | 3.1 | 14.7 | 11.6 | 21.6 | 39.3 | 17.6 |

Values are expressed as the total number of pooled cells from groups of mice (n = 5) bearing the surface markers 8C5(bsh) or MAC-1. Abbreviation: NA, not applicable.

* Changes in the absolute number of cells expressing certain markers were calculated by subtracting the total cell number per mouse treated with rhIL-7 from the total number of cells per control mouse.
increase in the number of cells expressing this phenotype was observed in the spleen and liver of non-Splx mice treated with rhIL-7. The number of MAC-1+ cells (present on macrophages, mature granulocytes, and natural killer [NK] cells) isolated from the liver also was increased (by 4.7-fold). After treatment with rhIL-7, Splx mice showed similar changes in leukocyte subsets and NPC as those observed in non-Splx mice, but the changes in the number of cells expressing 8CSbrigh' or MAC-1 surface markers were further enhanced. However, because cells expressing these markers are mostly mature myelomonocytic cells, more detailed studies to evaluate changes in the number of myeloid progenitor cells were performed.

Effects of splenectomy on myeloid progenitor cells in extramedullary sites. Previous studies from our laboratory showed that rhIL-7 administration caused a substantial increase in myeloid progenitors in the spleen as part of a general extramedullary leukocytosis.19 The present studies using non-Splx mice confirmed a greater than 95% decrease in the total number of both CFU-c and CFU-GEMM in the BM (Fig 1A) and an 18-fold and 34-fold increase in spleen CFU-c and CFU-GEMM, respectively (data not shown). Splenectomy did not affect the ability of rhIL-7 to decrease myeloid progenitors in the BM (>94% in CFU-c and CFU-GEMM), but it did significantly increase the number of CFU-c and CFU-GEMM colonies generated from the peripheral blood (Fig 1B) and liver (Fig 1C and D). Specifically, in rhIL-7-treated non-Splx versus Splx mice, there was an absolute increase in (1) the total number of CFU-c in the peripheral blood from 465 to 600 (Fig 1B); (2) the total number of CFU-c in the NPC from 648 to 2,140 (Fig 1C); and (3) the total number of CFU-c in LC from 22 to 66 (Fig 1D). The results obtained for CFU-GEMM were similar. The absolute increase of CFU-GEMM was 24 versus 15 in the peripheral blood (Fig 1B), 57 versus 5 in the NPC (Fig 1C), and 2 versus 0 in the LC (Fig 1D) for Splx and non-Splx
HEMATOPOIETIC EFFECTS OF IL-7 IN VIVO

<table>
<thead>
<tr>
<th>Colony Type</th>
<th>Control</th>
<th>nL-7</th>
<th>Control</th>
<th>nL-7</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-c</td>
<td>60</td>
<td>195*</td>
<td>203</td>
<td>1,695*</td>
<td>24</td>
</tr>
<tr>
<td>CFU-GEMM</td>
<td>6</td>
<td>141†</td>
<td>1</td>
<td>53*</td>
<td>1</td>
</tr>
</tbody>
</table>

Values represent the mean difference in the total number of CFU-c and CFU-GEMM progenitors obtained from various organs in Splx versus non-Splx mice (n = 5). The symbols indicate the statistical significance between the absolute values in control and rhIL-7-treated mice and are calculated based on the data presented in Fig 1.

*P < .001.
†P < .01.
‡P > .05, not statistically significant.

mice, respectively. Electron microscopic analysis of histologic sections obtained from the livers of mice treated with rhIL-7 showed the presence of hematopoietic foci consisting of immature cells localized largely in the space of Disse (data not shown). Megakaryocytes were also observed in the liver parenchyma and in enlarged spaces of Disse (data not shown). These rhIL-7-induced morphologic changes were more impressive in Splx than non-Splx mice.

It also should be noted that splenectomy itself caused a statistically significant increase in the absolute number of progenitors present in the peripheral blood (P < .01), NPC (P < .01), and LC (P < .01) that were obtained from diluent-treated mice. However, these changes were quantitatively minor when compared with changes observed in rhIL-7-treated mice (Table 3) and practically undetectable in the liver using transmission electron microscopy (TEM), where we failed to detect the presence of the hematopoietic foci in Splx mice not treated with rhIL-7 (data not shown). Thus, the ability of rhIL-7 to increase the absolute number of single and multilineage myeloid progenitors in the blood (P < .001 and P < .01, respectively) and liver (P < .001 and P < .05, respectively) was significantly enhanced in Splx mice (P < .01), suggesting that the removal of a predominant localization site (the spleen) enhanced the uptake of bloodborne progenitors in another site (eg, the liver).

BM depletion abrogates rhIL-7-related changes in extramedullary myelopoiesis. The results of the studies described above supported, but did not prove, the hypothesis that rhIL-7 causes the exportation of progenitors from the BM to peripheral sites. Thus, experiments were performed to determine whether selective BM ablation abrogates rhIL-7-induced extramedullary myelopoiesis. In these experiments BM aplasia was selectively induced by bolus IP administration of 89Sr (150 μCi/mouse), a β-emitting, bone-seeking radionuclide that substitutes for calcium molecules in bone. Seven days thereafter, the standard 7-day course of rhIL-7 (10 μg/injection/twice a day) was initiated. These studies confirmed the selectivity of the 89Sr treatment that resulted in a 98% decrease in BM cellularity when 89Sr-treated mice were compared with control or rhIL-7-treated mice (Table 1, experiment 2). In contrast, baseline cellularity of the spleen, peripheral blood, and NPC was not decreased after 89Sr treatment (Table 1, experiment 2). Furthermore, pretreatment of mice with 89Sr profoundly inhibited the ability of rhIL-7 to increase spleen cellularity (39% vs 285%, respectively) and abrogated the rhIL-7-related increase in the cellularity of the blood and NPC compared with mice that were not exposed to 89Sr (Table 1, experiment 2).

Effects of BM ablation by 89Sr on the rhIL-7-induced increase in extramedullary myeloid progenitors. To specifically address the issue of progenitor redistribution, the effects of rhIL-7 treatment on CFU numbers in peripheral tissues were determined in normal and 89Sr-treated mice. The efficiency of this treatment for depletion of progenitor cells was confirmed by the data shown in Fig 2A, in which the total number of CFU-c generated from BM in 89Sr-treated mice was determined to be 67 ± 20 versus 30,267 ± 1,514 (P < .001) in mice that were not treated with 89Sr (a >99% reduction). 89Sr-treated mice that received rhIL-7 were completely devoid of progenitors for both CFU-c and CFU-GEMM in the BM (Fig 2A). Depletion of BM cellularity and myeloid progenitors by 89Sr also coincided with profound inhibition of the rhIL-7-induced increase in splenic CFU progenitors such that the total number of spleen CFU-c in rhIL-7-treated, BM aplastic mice was 2,389 ± 211 versus 17,667 ± 2,097 (P < .001) in rhIL-7-treated mice that were not pretreated with 89Sr. Similar effects were observed for splenic CFU-GEMM formation in which the rhIL-7–induced increase in the number of CFU-GEMM (to 976 in normal mice) was reduced to 149 (P < .05) in 89Sr-treated mice (Fig 2B). The augmenting effect of rhIL-7 on the total number of CFU-c progenitors in the peripheral blood and NPC also was dramatically reduced (Fig 2C and D). Specifically, although treatment with 89Sr and rhIL-7 did not change the total number of WBC as compared with rhIL-7 alone, it severely decreased CFU-c formation (90% inhibition, P < .001) from the peripheral blood and abrogated the appearance of CFU-GEMM (Fig 2C). This result is most likely caused by the removal of the BM as a continuous source of progenitor production. Thus, the small percentage of total peripheral blood leukocytes that are CFU progenitors was selectively removed by 89Sr treatment. The total number of CFU-c and CFU-GEMM generated from NPC isolated from 89Sr-treated mice was significantly increased (43 v 114; P < .01) and (0 v 4; P > .05, not significant), respectively, in comparison with normal mice, despite the fact that total cellularity was not affected by 89Sr treatment. This result occurs because of an increased frequency of progenitors per unit number of plated cells (data not shown). However, as in the spleen, 89Sr treatment abrogated the ability of rhIL-7 to increase the total number of CFU-c and CFU-GEMM in NPC (Fig 2D). Overall, the results from these experiments show that mice rendered selectively BM aplastic by 89Sr do not exhibit the rhIL-7–induced increase in extramedullary CFU, confirming that the increased extramedullary hematopoiesis induced by rhIL-7 is largely caused by an exportation of myeloid progenitor cells from the BM to the spleen.

DISCUSSION

In this study, we have investigated the mechanism by which rhIL-7 administration to mice causes a dramatic re-
duction in the number of progenitors for CFU-c and CFU-GEMM in the BM and a corresponding increase in these progenitors in the spleen, liver, and peripheral blood. Two approaches were used to address this issue. First, splenectomy, which removes the major site of rhIL-7-induced extramedullary hematopoiesis, resulted in an increased number of multilineage and single-lineage progenitors recovered from the peripheral blood and livers of rhIL-7-treated mice as compared with non-Splx mice. This result suggests that preexisting myeloid progenitors in the spleen are not a major contributing factor in the rhIL-7-induced increase in myelopoiesis detected in the blood and liver, and that CFU progenitors derived from the BM are retained in the blood and increasingly localize to the liver in the absence of their primary localization site (the spleen). This hypothesis was confirmed by studies in which the overall cellularity and CFU progenitor number was selectively depleted in the BM by 89Sr in the absence of similar effects in the periphery. Collectively, these results show that rhIL-7 induces an exportation of CFU progenitors from the BM to the spleen.

It must be emphasized that the rhIL-7-induced decrease in the number of myeloid progenitors in the BM occurs in the absence of a decrease in the total BM cellularity. While there is a significant decline in the number of BM cells expressing the myeloid marker 8C5 in mice treated with rhIL-7, there is a concomitant increase in the number of BM cells expressing the B220 marker of B-lineage cells. In addition, mice treated with rhIL-7 exhibit a profound systemic leukocytosis that is largely caused by an increase in B lineage cells and by mature T cells.

One possible hypothesis for the opposing effects of rhIL-7 on progenitor number in the BM versus the spleen is that rhIL-7 directly or indirectly inhibits preexisting myeloid progenitors in the BM, while stimulating those in the spleen. Alternatively, rhIL-7 may act to shift a BM progenitor common for lymphopoiesis and myelopoiesis preferentially to lymphopoiesis and that might trigger a compensatory burst of extramedullary hematopoiesis. Preliminary studies in our laboratory have failed to detect any direct effects of rhIL-7 on basal or IL-3-driven progenitors, a finding previously not observed.
reported by others, which suggests that the effects of rhIL-7 are indirect. Thus, we hypothesized that rhIL-7 induces progenitors for CFU-c, CFU-GEMM, and/or possibly even less mature progenitors or stem cells to exit the BM, traverse through the blood, and localize in the spleen and other peripheral sites. Therefore, we evaluated progenitor numbers in the blood, spleen, and the liver after rhIL-7 administration. The present studies extend previous observations from our laboratory by showing that administration of rhIL-7 significantly increases the number of myeloid progenitors that can be isolated from peripheral blood, spleen, and liver, strongly suggesting an rhIL-7-induced redistribution of myeloid progenitors from the BM to the periphery.

A second prediction of the exportation hypothesis is that removal of a major localization site (eg, the spleen) would elevate further the number of myeloid progenitors that could be recovered from the blood and the liver after treatment with rhIL-7. This prediction was confirmed in our studies in that significantly more CFU-c and CFU-GEMM were cultured from the peripheral blood, NPC, and LC in mice that were Splx before initiation of rhIL-7 administration. In particular, the increase in CFU generated from LC supports a bloodborne recruitment to the liver because the lavage procedure isolates cells from within the hepatic vasculature and not the hepatic parenchyma. However, TEM studies suggested that these bloodborne progenitor cells are indeed capable of organ colonization because hematopoietic foci are present in the liver of rhIL-7-treated mice but not of control mice. This issue is not trivial because hematopoiesis differs in mice versus humans. In healthy mice, the spleen plays an important role as a source of myelomonocytic progenitor cells, whereas in humans, normal splenic cell production is largely restricted to lymphopoiesis. This is confirmed in our model in that Splx itself caused a slight increase in the total number of BM cells, WBC, and leukocytes isolated from the liver (both NPC and LC). This effect was related to an Splx-induced compensatory increase in the number of progenitors produced by the BM. However, these changes are minimal compared with those obtained with rhIL-7, where rhIL-7 caused a pronounced increase in total cell number as well as in the number of both CFU-c and CFU-GEMM progenitors in the peripheral blood, NPC, and LC in Splx versus non-Splx mice. The most stringent test of the exportation hypothesis would be to selectively deplete the BM of progenitor cells and then determine whether the rhIL-7-induced increase in peripheral CFU is also eliminated. Through the use of the bone-seeking radionuclide 38Sr, we were able to perform such studies in mice in which the BM cellularity and BM CFU-forming potential were both virtually eliminated. It is important to note that this depletion of myeloid progenitors was specific for the BM because baseline hematopoiesis in the spleen and liver was not decreased by this treatment. Thus, when rhIL-7 was administered to 38Sr-treated mice, the number of progenitors for both CFU-c and CFU-GEMM in the spleen and liver did not increase as it did when normal mice were treated with rhIL-7. This result clearly shows that rhIL-7 causes most myeloid progenitor cells to exit from the BM and localize in the spleen, liver, and, perhaps, other organs.

The ability of rhIL-7 to cause a redistribution of myeloid progenitor cells is interesting for several reasons. First, mRNA for IL-7 has been detected in BM stromal cells, suggesting that production of IL-7 in the BM microenvironment could cause both the proliferation and maturation of pre-B cells and at the same time induce an efflux of myeloid progenitors to the periphery. mRNA for IL-7 also has been detected in the spleen. Thus, the overall rhIL-7-induced increase in myelopoiesis in the spleen is probably caused largely by an influx from the BM, but also could be partially a result of differentiation of some stem cells within this organ. Because we did not study the effect of BM ablation on lymphopoiesis in the spleen, we cannot conclude that the strong IL-7-induced lymphopoietic response in the spleen is solely attributed to lymphoid progenitor redistribution from the BM. However, the large magnitude of this increase and the dramatic increases in B220+ cells in the BM suggests that these effects are likely caused by exportation of lymphoid progenitors from the BM. All these data suggest that exportation of the progenitors from the BM is not restricted to those progenitors of the myelomonocytic lineage, but also may include progenitors within the lymphocytic lineage. At this point, we are not certain if the exportation could include a common progenitor for lymphopoiesis and myelopoiesis or, rather, is restricted to committed progenitors for both branches of hematopoietic differentiation. The ability to isolate an increased number of CFU-c and CFU-GEMM from blood indicates that some of the progenitors are committed at the time they leave the BM. Further studies investigating changes in the presence of bloodborne stem cells are in progress. In addition, it remains possible that IL-7 contributes to progenitor differentiation through indirect effects of other cytokines induced by rhIL-7. This possibility is supported by in vitro evidence that IL-7 is able to induce gene expression for several other cytokines (eg, IL-1, TNFα, IL-5, IL-6, IL-8, and TGFβ) (Grzegorzewski, unpublished observation) that have myelopoietic potential, such as for megakaryopoiesis (IL-6) and eosinophil colony formation (IL-5). However, because cytokine cascades are often induced in vivo, it also remains possible that non–lineage-commit ted progenitors exported from the BM to the spleen are preferentially directed toward lymphopoiesis or myelopoiesis by rhIL-7–induced factors.

In addition to documenting the ability of rhIL-7 to induce the exportation of myeloid progenitors from the BM to the spleen, our studies also show that there is a more general distribution of these exported cells. The detection of myeloid progenitors in the liver is interesting because it has been noted that the liver can be a source of myelomonocytic progenitors in mice, not only during fetal development but also in adults after administration of various factors. In some cases (eg, estradiol treatment), the kinetics of the hematopoietic response corresponds to the kinetics of activation of Kupffer cells that can produce Epo and other colony-stimulating factors under certain circumstances. Adult murine hepatocytes are capable of producing macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. Thus, both NPC and parenchymal cells may be beneficial for establishing a
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