Brief Definitive Report

Mobilization of Long-Term Reconstituting Hematopoietic Stem Cells in Mice by Recombinant Human Interleukin 7

By Krzysztof J. Grzegorzewski,* Kristin L. Komschlies,* Sten E. W. Jacobsen,‖ Francis W. Ruscetti,‡ Jonathan R. Keller,* and Robert H. Wiltrout§

*From *Biological Carcinogenesis and Development Program, Program Resources, Inc./DynCorp, and ‡Laboratory of Leukocyte Biology, §Laboratory of Experimental Immunology, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201; and ‖Department of Immunology, The Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway*

Summary

Administration of recombinant human interleukin 7 (rh)IL-7 to mice has been reported by our group to increase the exportation of myeloid progenitors (colony-forming unit [CFU]-c and CFU-granulocyte erythroid megakarocyte macrophage) from the bone marrow to peripheral organs (blood, spleen[s], and liver). We now report that IL-7 also stimulates a sixfold increase in the number of more primitive CFU-S day 8 ($CFU\text{-}S_8$) and day 12 ($CFU\text{-}S_{12}$) in the peripheral blood leukocytes (PBL) of mice treated with rhIL-7 for 7 d. Moreover, >90% of lethally irradiated recipient mice that received PBL from rhIL-7–treated donor mice have survived for >6 mo whereas none of the recipient mice that received an equal number of PBL from diluent-treated donors survived. Flow cytometry analysis at 3 and 6 mo after transplantation revealed complete trilineage (T, B, and myelomonocytic cell) repopulation of bone marrow, thymus, and spleen by blood-borne stem/progenitor cells obtained from rhIL-7–treated donor mice. Thus, IL-7 may prove valuable for mobilizing pluripotent stem cells with long-term repopulating activity from the bone marrow to the peripheral blood for the purpose of gene modification and/or autologous or allogeneic stem cell transplantation.

Autologous bone marrow (BM) transplantation has been used for support of high-dose chemotherapy in the treatment of various neoplastic diseases (1). Under steady-state conditions, the majority of primitive hematopoietic stem/progenitor cells reside in the bone marrow, and only a low number of these cells can be isolated from the peripheral blood. However, additional peripheral blood progenitor cells (PBPC) and peripheral blood stem cells (PBSC) can be mobilized by treatment with myelosuppressive agents (2), or growth factors (3, 4). Recent studies have shown that PBPC and PBSC exhibit enhanced potential for engraftment as compared with BM transplantation (4, 5).

Recently our group demonstrated that administration of recombinant human (rh)IL-7 to mice mobilized myeloid progenitor cells from the BM to the spleen(s) (6), blood, and liver (7). Administration of rhIL-7 to irradiated mice also increased the number of megakaryocytes and immature granulocytes in the spleen in vivo (8). In vitro rhIL-7 enhanced CSF-induced myeloid colony formation from primitive $Lin^-Sca\text{-}1^+$ murine BM progenitor cells, without effecting proliferation of committed $Lin^-Sca\text{-}1^-$ myeloid progenitors (9). These findings, in addition to the well-known effects of IL-7 on lymphopoiesis (10), and the documented expression of receptors for IL-7 on myeloid cells (11), suggested that this cytokine also may play an important role in myelopoiesis. The present studies were performed to determine whether rhIL-7 could mobilize PBSC with long-term reconstitution potential in lethally irradiated recipients.

Materials and Methods

Mice. C57BL/6 (Ly 5.2) and their congenic C57BL/6-Ly 5.1 mice (12) were obtained from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center, maintained in a specific pathogen-free environment, and used between 8 and 10 wk of age.

Reagents. rhIL-7 was purchased from PeproTech (Rocky Hill, NJ), and had a sp act of 2×10^7 U/mg as measured by proliferation of a murine pre-B cell line (IxN/A6). The endotoxin level was <0.1 ng/ml. Lyophilized material was diluted in citrate buffer, pH 6.0, to a concentration of 1 mg/ml. Mice received injections of rhIL-7 or diluent HBSS without Ca^{2+}, Mg^{2+} and phenol red

[Cellgro, Washington, DC], supplemented with 0.1% normal mouse serum [NMS]).

Enrichment and Purification of Peripheral Blood Leukocytes (PBL). Peripheral blood from C57BL/6-Ly 5.1 mice that had been treated with 5 μg of rhIL-7 or diluent twice daily for 7 d was collected by cardiac puncture and placed into EDTA-containing tubes. Blood was pooled by group, layered over Lymphocyte Separation Media gradients of 1.077–1.080 g/ml (Organon Teknika, Durham, NC) and centrifuged at 800 *g* for 20 min at 20°C. Low density (LD) PBL from the interface were collected, washed, resuspended in plain HBSS, and then used for transplantation.

Preparation of BM, Spleen Cells, and Thymocytes. BM from both femurs and tibiae, spleen cells, and thymocytes were isolated from recipient mice (6, 7, 13) and resuspended in HBSS plus 1% BSA (GIBCO/BRL, Bethesda, MD) for flow cytometric analysis (FCA). BM cells used for transplantation were resuspended in cold HBSS.

Transplantation. C57BL/6 (Ly 5.2) recipient mice were exposed to a total of 11.0 Gy ^{137}Cesium irradiation (dose rate, 23.2 cGy/min) delivered in two equal doses of 5.5 Gy given 3 h apart. The CFU-S assay was performed by the i.v. injection of 2×10^5 LD PBL or BM cells from rhIL-7–treated or HBSS-treated C57BL/6-Ly 5.1 donor mice into irradiated recipients. On days 8 and 12 after transplantation, the number of macroscopically visible surface colonies (CFU-S) on fixed spleens was counted (14). Long-term survival and reconstitution studies were done by injecting various numbers of LD PBL or BM cells from rhIL-7–treated or HBSS-treated C57BL/6-Ly 5.1 donors intravenously into lethally irradiated C57BL/6 (Ly 5.2) recipients.

Surface Phenotype Analysis. Hematopoietic reconstitution was determined by two- to three-color immunofluorescence labeling followed by FCA (6). Donor-derived (Ly 5.1$^+$) or host-derived (Ly 5.2$^+$) cells were detected using the mAb clones A-20-1.7 or 104-2.1 (15), respectively, and developed with FITC-conjugated affinity-purified goat anti-mouse IgG2a (Fisher Scientific, Orangeburg, NY) or they were biotinylated and developed with Streptavidin-RED$_{670}$ (GIBCO BRL). B-lineage cells and granulocytes were detected using PE-conjugated RA3-6B2 (B220) or PE-conjugated RB6-8C5, respectively (PharMingen, San Diego, CA). CD4$^+$ or CD8$^+$ T-lineage cells were detected using PE-conjugated GK 1.5 (L3T4) or biotin-conjugated 53-6.7 (Ly 2) developed with Streptavidin-RED$_{670}$, respectively (Becton Dickinson, San Jose, CA). T cells were detected using 500A2 (CD3) as previously described (6).

Statistical Analysis. All statistical evaluations were performed using the computer software Instat ver. 2.02 or GraphPad Prism for Windows ver 1.0 (GraphPad Software, San Diego, CA). Statistically significant differences based on absolute numbers were determined by two-tailed, Student's *t* test (16). Results from survival experiments were analyzed by log-rank nonparametric test and expressed as Kaplan-Meier survival curves.

Results and Discussion

Administration of IL-7 Increases the Number of Circulating CFU-S. Lethally irradiated C57BL/6 (Ly 5.2) recipient mice were injected intravenously with 2×10^5 LD PBL or BM cells from rhIL-7 (5 μg i.p./twice daily for 7 d) or HBSS-treated C57BL/6-Ly 5.1 donors. A statistically significant increase in the total number of CFU-S$_8$ ($p < 0.001$) and CFU-S$_{12}$ ($p < 0.001$) was observed after transfer of PBL from mice treated with rhIL-7 (day 8: 700 ± 87, day 12: 633 ± 29) (Fig. 1 *A*) compared with PBL from donors injected with HBSS (day 8: 128 ± 34, day 12: 118 ± 51). Thus, in addition to its established ability to mobilize single-lineage CFU-c and multi-lineage CFU-granulocyte erythroid megakarocyte macrophage (GEMM) (7), IL-7 potently mobilized primitive CFU-S from the BM to the blood.

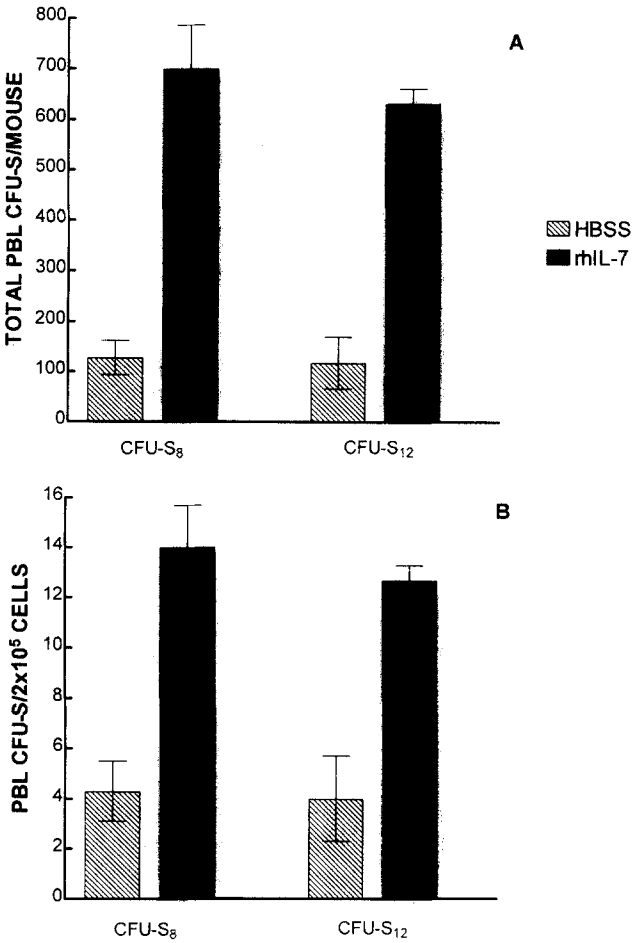


Figure 1. Effect of systemic administration of rhIL-7 on progenitors for CFU-S in peripheral blood. Lethally irradiated recipient mice ($n = 6$) were injected intravenously with 2×10^5 PBL obtained from rhIL-7 (5 μg/twice daily for 7 d)– or HBSS-treated (control) mice. After 8 and 12 d, mice were killed, and colonies on the surface of the spleens were counted. The results are presented as the mean number from three mice per group (at each time point) ± SD multiplied by the total number of cells obtained from donors and divided by 2×10^5 (the number of cells plated; *A*) or as the frequency of CFU-S obtained per 2×10^5 injected PBL (*B*).

PBL Isolated from rhIL-7–treated Donors Rescue Lethally Irradiated Mice. To determine if rhIL-7 could mobilize pluripotent stem cells, lethally irradiated C57BL/6 (Ly 5.2) recipients were transplanted with various numbers of PBL from C57BL/6-Ly 5.1 donors that were pretreated with rhIL-7 (5 μg/twice daily). As shown in Fig. 2 *A*, 10^6 PBL obtained from rhIL-7–treated donors rescued 90% of the irradiated recipients, whereas the same number of PBL isolated from control donors produced no survivors. A higher number of PBL (5×10^6 and 1×10^7) transplanted from rhIL-7–treated donors rescued 100% of the recipients, whereas these cell doses of PBL from control mice were much less efficient (5×10^6, 10%

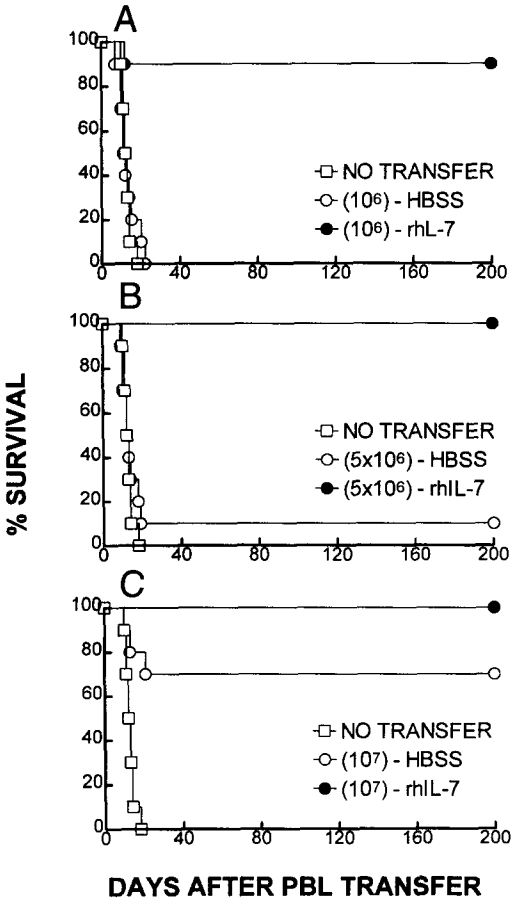


Figure 2. Survival of lethally irradiated recipients (Ly 5.2) transplanted with PBL isolated from donors (Ly 5.1) treated with rhIL-7 or HBSS. Lethally irradiated recipient mice (n = 10) were injected intravenously with 10^6, 5 × 10^6 or 10^7 PBL from rhIL-7 (5 μg/twice daily for 7 d)- or HBSS-treated mice. The survival of mice was monitored for up to 200 d. The results are presented as a Kaplan-Meier survival curve.

Table 1. *Cellularity of Various Organs after Reconstitution with PBL and BM from Animals Treated In Vivo with rhIL-7*

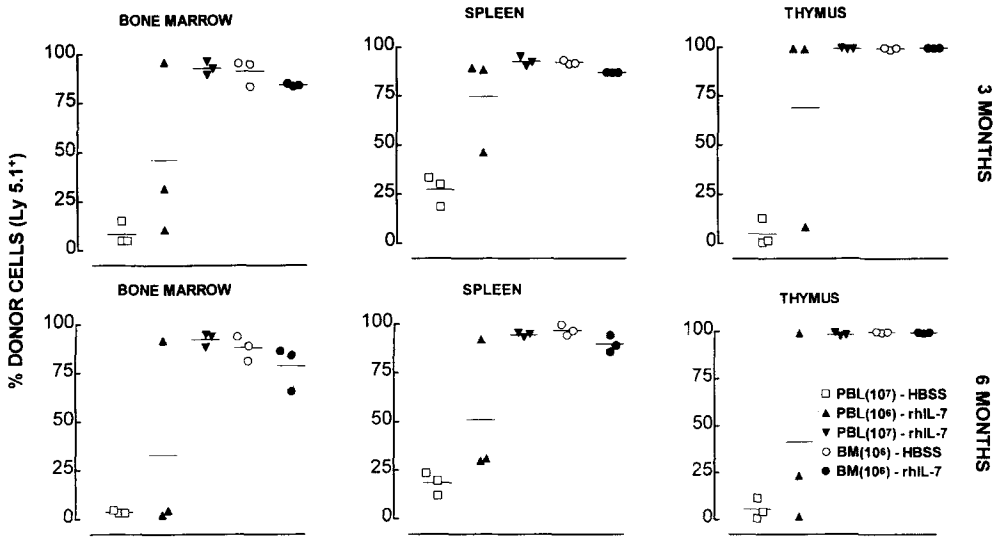
Recipient organ	Donor cells	Cellularity (× 10^6) 3 mo	6 mo
BM	PBL (10^7 cells) - HBSS	8 ± 3	17 ± 6
	PBL (10^6 cells) - rhIL-7	28 ± 1	22 ± 3
	PBL (10^7 cells) - rhIL-7	23 ± 6	22 ± 4
	BM (10^6 cells) - HBSS	16 ± 5	20 ± 6
	BM (10^6 cells) - rhIL-7	20 ± 4	21 ± 3
Thymus	PBL (10^7 cells) - HBSS	2 ± 2	23 ± 38
	PBL (10^6 cells) - rhIL-7	106 ± 17	24 ± 21
	PBL (10^7 cells) - rhIL-7	106 ± 8	37 ± 24
	BM (10^6 cells) - HBSS	80 ± 10	54 ± 9
	BM (10^6 cells) - rhIL-7	105 ± 10	65 ± 15
Spleen	PBL (10^7 cells) - HBSS	39 ± 32	42 ± 8
	PBL (10^6 cells) - rhIL-7	81 ± 11	52 ± 16
	PBL (10^7 cells) - rhIL-7	85 ± 2	77 ± 11
	BM (10^6 cells) - HBSS	74 ± 19	76 ± 8
	BM (10^6 cells) - rhIL-7	59 ± 6	65 ± 2

Lethally irradiated recipient mice (n = 6) were injected with 10^6 or 10^7 i.v. PBL obtained from rhIL-7 (5 μg/twice daily for 7 d)-treated mice, 10^7 PBL from HBSS-treated mice (control) or 10^6 BM cells obtained after either treatment. At 3 and 6 mo after transplantation, three mice from each group were euthanized and tibias, femurs, spleens, and thymi were collected individually from each group. BM cells, thymocytes, and splenocytes were isolated as described in Materials and Methods and counted. The results are presented as the mean of three mice per group ± SD.

and 1 × 10^7, 70%). These results demonstrated that PBL from rhIL-7–treated mice were much more efficient in long-term rescue of irradiated recipients, suggesting the mobilization of pluripotent stem cells with long-term marrow repopulating activity.

PBL from Mice Treated with rhIL-7 Contain Long-term Reconstituting Stem Cells that Repopulate all Leukocyte Lineages in Lethally Irradiated Recipient Mice. To determine if the rescue of lethally irradiated recipients by rhIL-7 mobilized PBL was associated with reconstitution by stem/progenitors cells transplanted from donor mice, the percentage of donor (Ly 5.1^+) vs host (Ly 5.2^+) repopulation in BM, thymus, and spleen of surviving recipient mice was assessed by FCA at 3 (short-term repopulation) and 6 mo (long-term repopulation). The analysis of data at 3 mo revealed that 10^7 PBL from rhIL-7–treated donors reconstituted BM, thymus, and spleen cellularity to the same degree as did 10^6 BM cells from control or rhIL-7–treated donors (Table 1). Analysis of short-term host vs donor reconstitution in recipient mice transplanted with 10^7 PBL from rhIL-7–treated donors (Fig. 3, *3 MONTHS*) demonstrated that the majority of the cells in various organs were of donor origin (the percentage of donor Ly 5.1^+ cells ranged from 89 to >99). These percentages were significantly (p <0.001) greater than those obtained from reconstitution by 10^7 PBL from control mice which showed only 8% donor reconstitution of the bone marrow and 5% donor reconstitution of the thymus in recipient mice. After 3 mo, reconstitution of the spleen with 10^7 PBL was somewhat better (27 ± 8%), but was still significantly lower than the reconstitution achieved by 10^6 (p <0.05) or 10^7 (p < 0.001) PBL from rhIL-7–treated mice. Thus, the repopulation that occurred in the group that received 10^7 PBL from control donors was largely of recipient origin by 3 mo. The transfer of 10^6 PBL from rhIL-7–treated donors also resulted in better mean levels of percent donor repopulation than did 10^7 transferred normal PBL, but the variability was higher among the responding mice (Fig. 3, *3 MONTHS*).

Because the 3-mo survival assay is considered to be a good measurement for only short-term repopulating stem cells (17), repopulation was also analyzed at 6 mo, which measures repopulation by totipotential stem cells. As shown in Table 1, all of the recipients had considerable repopulation by 6 mo; however the reconstitution of the spleen by 10^7 PBL



PERCENTAGE OF DONOR-DERIVED LEUKOCYTES DETECTED WITHIN EACH SUBSET AT 6 MO

DONOR PBL	BONE MARROW			SPLEEN			THYMUS	
	8C5+	*CD3+*	*B220+*	*8C5+*	*CD3+*	*B220+*	*CD4+*	*CD8+*
CONTROL	1 ± 1	35 ± 8	11 ± 9	25 ± 5	27 ± 3	14 ± 13	4 ± 4	11 ± 14
rHIL7	95 ± 5	90 ± 2	86 ± 2	91 ± 4	90 ± 3	96 ± 1	99 ± 1	99 ± 1

Figure 3. Percentage of donor cells (Ly 5.1+) repopulating various organs of lethally irradiated recipient (Ly 5.2+) mice transplanted with PBL and BM cells from rhIL-7– or HBSS-treated donors. Lethally irradiated recipient mice (n = 6) were injected intravenously with 10^6 or 10^7 PBL obtained from rhIL-7 (5 μg/twice daily for 7 d)–treated mice, 10^7 of PBL from HBSS-treated mice (control), or with 10^6 BM cells obtained from either treatment. At 3 and 6 mo after transplantation, three mice from each group were killed, and tibiae, femurs, spleen, and thymi were collected individually from each group. BM cells, thymocytes, and splenocytes were isolated and counted. Cells were labeled with anti-Ly 5.1 or anti-Ly 5.2 to determine whether they were of donor or host origin as described in the Materials and Methods. The inset table demonstrates multiple-lineage reconstitution determined by the percentage of total RB6-8C5+ (*8C5+*), CD3+, B220+, CD4+, or CD8+ cells that were of donor origin. The results are presented as the mean of three mice per group ± SD.

from control mice was significantly lower (p <0.05) when compared with PBL (10^7) from the rhIL-7–treated mice and BM from normal or rhIL-7–treated mice. The percentages of donor cells in surviving mice at 6 mo were similar to those noted at 3 mo (Fig. 3). The efficiency of overall donor-origin cell repopulation in some organs was less in mice that received 10^7 PBL from control mice versus those that received 10^7 PBL from rhIL-7–treated donors (BM, 4 ± 1% vs 92 ± 4%; thymus, 5 ± 6% vs 98 ± 1%; and spleen, 18 ± 6% vs 94 ± 1%). In fact, the efficiency of donor-origin cell repopulation by 10^7 PBL from rhIL-7–treated donors was as good as that achieved by 10^6 normal BM cells. Mice transplanted with 10^6 PBL from rhIL-7–treated donors at 6 mo exhibited higher variability in terms of the percentage of donor cells for various organs (BM, 33 ± 51%; thymus, 41 ± 51%; spleen, 51 ± 36%), but those mean percentages tended to be much higher than those attained by 10^7 PBL from normal donors.

These results show that administration of rhIL-7 can mobilize progenitor and stem cells required for both short- and long-term repopulation. Successful repopulation with donor cells was dependent on the number of PBL transplanted, with a dose of 10^6 from rhIL-7–treated donors being borderline for complete survival and repopulation of irradiated recipients, whereas 10^7 PBL or 10^6 BM cells were able to efficiently repopulate recipient mice. Interestingly, 10^7 PBL from control donors also were able to rescue some mice for 6 mo, however ultimately repopulation was largely of host origin. Thus, normal peripheral blood contains adequate numbers of short-term repopulating cells (STRC) (18) to allow survival of irradiated mice; however, there are few long-term repopulating cells (LTRC), which predominantly contribute to both 3- and 6-mo survival. The frequency of STRCs in normal PBL must be low since 10^6 PBL from control mice failed to rescue any recipients. In fact, as shown in Fig. 1 (*B*), there were only 4 ± 1 CFU-S_8 and 4 ± 2 CFU-S_{12} per 2 × 10^5 cells detected in the blood of control mice, whereas the rhIL-7–treated mice showed a significant increase (p <0.001) in the frequency of these progenitors (e.g., 14 ± 2 CFU-S_8 and 13 ± 1 CFU-S_{12}). This increase might contribute to the observation that 10-fold fewer PBL from IL-7–treated mice were able to fully rescue mice compared with PBL from normal mice. Although 10^6 PBL from rhIL-7-treated donors and 10^7 PBL from control mice were able to rescue the lethally irradiated recipients, only PBL from mice treated with rhIL-7 could successfully repopulate various organs with donor-derived cells, further suggesting that PBL from control mice contain STRCs supporting early BM recovery, followed by repopulation with host-originated LTRCs that survived irradiation. In contrast, PBL from rhIL-7–treated donors provide both STRCs for initial recovery and LTRCs that sustain long-term hematopoiesis. This is further emphasized by the data shown in Fig. 3, *inset table*, where complete trilineage (T and B cell, and myelomonocytic cell) reconstitution is demonstrated in various organs. A similar trend also was observed by 3 mo (data not shown).

Interestingly, BM cells from rhIL-7–treated mice were almost equal to BM cells from control mice in repopulating

activity. Our previously published results demonstrated that mature progenitors were reduced in BM from mice treated with rhIL-7 (CFU-C and CFU-GEMM) (6, 7), suggesting that the ability of such BM to repopulate lethally irradiated mice might also be diminished. The data presented herein suggest that at least some threshold number of short- and long-term repopulating cells are retained in BM from rhIL-7-treated mice. This is in agreement with previous data that various progenitors detected by different colony-forming assays do not always equate to an ability to reconstitute short- and long-term hematopoiesis in irradiated mice (19).

Several hematopoietic growth factors (HGF) have been studied as mobilizing agents, alone or in combination, in preclinical studies or clinical trials (e.g., G-CSF, GM-CSF, IL-3/Epo, IL-1, stem cell factor [SCF], IL-11) (4, 20). Some HGFs have their activity restricted to myelopoiesis whereas others stimulate lymphopoiesis and myelopoiesis (21). To date, IL-7 had been reported to primarily stimulate lymphopoiesis (13, 22), with a pronounced increase in the number of mature T lymphocytes, particularly $CD8^+$ T cells, as well as T cell-mediated responses (13). Additionally, we found that IL-7 also influenced early myeloid progenitors (7). In this regard, IL-7 has been shown to synergize with GM-CSF and SCF to enhance in vitro myeloid colony formation of Lin^- $Sca\text{-}1^+$ murine BM progenitor cells (9).

In summary, rhIL-7 has novel functions in vivo because of its ability to mobilize myeloid stem cells/progenitors from the BM to periphery, its ability to accelerate regeneration of donor B and T lineages after transfer to irradiated hosts (Boerman, O. C., T. A. Gregorio, K. Grzegorzewski, C. R. Faltynek, R. L. Wiltrout, and K. L. Komschlies, manuscript submitted for publication), and documented potent effects on lymphocyte proliferation and T cell function (13). The mechanism of action for the stem cell mobilizing effects of rhIL-7, as well as those for G-CSF and SCF, remains unknown and may be at least partially indirect through induction of other mobilizing cytokines (23) or through inhibition of negative regulators of hematopoiesis (24). A comparison of the mobilizing ability of rhIL-7 with other cytokines and its ability to synergize with other stem cell mobilizers is in progress. Ultimately, rhIL-7 may prove useful for increasing the efficiency of stem cell mobilization into the peripheral blood for gene transfer studies, and for autologous or allogeneic stem cell transplants.

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Address correspondence to Dr. Robert H. Wiltrout, NCI-FCRDC, Building 560, Room 31-93, Frederick, MD 21702-1201.

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References

1. Cheson, B.D., L. Lacerna, B. Leyland-Jones, G. Sarosy, and R.E. Wittes. 1989. Autologous bone marrow transplantation. Current status and future directions. *Ann. Intern. Med.* 110: 51–65.
2. To, L.B., M.L. Davy, D.N. Haylock, P.G. Dyson, D. Thorp, and C.A. Juttner. 1989. Autotransplantation using peripheral blood stem cells mobilized by cyclophosphamide. *Bone Marrow Transplant.* 4:595–596.
3. Briddell, R.A., C.A. Hartley, K.A. Smith, and I.K. McNiece. 1993. Recombinant rat stem cell factor synergizes with recombinant human granulocyte colony-stimulating factor in vivo in mice to mobilize peripheral blood progenitor cells that have enhanced repopulating potential. *Blood.* 82:1720–1723.
4. Molineux, G., Z. Pojda, I.N. Hampson, B.I. Lord, and T.M. Dexter. 1993. Transplantation potential of peripheral blood stem cells induced by granulocyte colony-stimulating factor. *Blood.* 76:2153–2158.
5. Gianni, A., M. Bregni, S. Siena, S. Villa, G.A. Sciorelli, F. Ravagvnani, G. Pellegris, and G. Bonadonna. 1989. Rapid and complete hemopoietic reconstitution following combined transplantation of autologous blood and bone marrow cells. A changing role for high dose chemo-radiotherapy. *Hematol. Oncol.* 7:139–148.
6. Damia, G., K.L. Komschlies, C.R. Faltynek, F.W. Ruscetti, and R.H. Wiltrout. 1992. Administration of recombinant human interleukin-7 alters the frequency and number of myeloid progenitor cells in the bone marrow and spleen of mice. *Blood.* 79:1121–1129.
7. Grzegorzewski, K., K.L. Komschlies, K. Kaneda, N. Usui, C.R. Faltynek, J.R. Keller, F.W. Ruscetti, and R.H. Wiltrout. 1994. Administration of recombinant human interleukin-7 to mice induces the exportation of myeloid progenitor cells from

the bone marrow to peripheral sites. *Blood.* 83:377–385.
8. Faltynek, C.R., S. Wang, D. Miller, E. Young, L. Tiberio, K. Kross, M. Kelley, and E. Kloszewski. 1992. Administration of human recombinant IL-7 to normal and irradiated mice increases the numbers of lymphocytes and some immature cells of the myeloid lineage. *J. Immunol.* 149:1276–1282.
9. Jacobsen, F.W., O.P. Veiby, C. Skjønsberg, and S.E.W. Jacobsen. 1993. Novel role of interleukin 7 in myelopoiesis: stimulation of primitive murine hematopoietic progenitor cells. *J. Exp. Med.* 178:1777–1782.
10. Takeda, S., S. Gillis, and R. Palacios. 1989. In vitro effects of recombinant interleukin 7 on growth and differentiation of bone marrow pro-B- and pro-T-lymphocyte clones and fetal thymocyte clones. *Proc. Natl. Acad. Sci. USA.* 86:1634–1638.
11. Park, L.S., D.J. Friend, A.E. Schmierer, S.K. Dower, and A.E. Namen. 1990. Murine interleukin 7 (IL-7) receptor. Characterization on an IL-7-dependent cell line. *J. Exp. Med.* 171:1073–1089.
12. Morse, H.C., F.W. Shen, and U. Hämmerling. 1987. Nomenclature for foci controlling mouse lymphocyte antigens. *Immunogenetics.* 23:71–78.
13. Komschlies, K.L., T.A. Gregorio, M.E. Gruys, T.C. Back, C.R. Faltynek, and R.H. Wiltrout. 1994. Administration of recombinant human IL-7 to mice alters the composition of B-lineage cells and T cell subsets, enhances T cell function, and induces regression of established metastases. *J. Immunol.* 152:5776–5784.
14. Lord, B.I. 1993. In vivo assays for multipotential and marrow repopulating cells. *In* Haematopoiesis: A Practical Approach. N.G. Testa and G. Molineux, editors. Oxford University Press, Oxford. 1–19.
15. Shen, F.W. 1981. Monoclonal antibodies to mouse lymphocyte differentiation alloantigens. *In* Monoclonal Antibodies and T Cell Hybridomas: Perspectives and Technical Advances. J. Hämmerling, U. Hämmerling, and J. F. Kearney, editors. Elsevier/North Holland and Biomedical Press, Amsterdam/New York/Oxford, 25–31.
16. Gosset, W.S. 1908. The probable error of the mean. *Biometrika.* 6:1–22.
17. Jones, R.J., P. Celano, S.J. Sharkis, and L.L. Sensenbrenner. 1989. Two phases of engraftment established by serial bone marrow transplantation in mice. *Blood.* 73:397–401.
18. Ferrero, D., H.E. Broxmeyer, G.L. Pagliardi, S. Venuta, B. Lange, S. Pessano, and G. Rovera. 1983. Antigenically distinct subpopulations of myeloid progenitor cells (CFU-GM) in human peripheral blood and marrow. *Proc. Natl. Acad. Sci. USA.* 80:4114–4118.
19. Ploemacher, R.E., and N.H.C. Brons. 1989. Separation of CFU-S from primitive cells responsible for reconstitution of the bone marrow hemopoietic stem cell compartment following irradiation: evidence for a pre-CFU-S cell. *Exp. Hematol.* 17:263–266.
20. Eaves, C.J. 1993. Peripheral blood stem cells reach new heights. *Blood.* 82:1957–1959.
21. Heyworth, C.M., S.J. Vallance, A.D. Whetton, and T.M. Dexter. 1990. The biochemistry and biology of the myeloid haemopoietic cell growth factors. *J. Cell. Sci. Suppl.* 13:57–74.
22. Tushinski, R.J., I.B. McAlister, D.E. Williams, and A.E. Namen. 1991. The effects of interleukin 7 (IL-7) on human bone marrow in vitro. *Exp. Hematol.* 19:749–754.
23. Alderson, M.R., T.W. Tough, S.F. Ziegler, and K.H. Grabstein. 1991. Interleukin 7 induces cytokine secretion and tumoricidal activity by human peripheral blood monocytes. *J. Exp. Med.* 173:923–930.
24. Dubinett, S.M., M. Huang, D. Dhanani, J. Wang, and T. Beroiza. 1993. Down-regulation of macrophage transforming growth factor β messenger RNA expression by IL-7. *J. Immunol.* 151:6670–6680.