Brief Definitive Report

Novel Role of Interleukin 7 in Myelopoiesis: Stimulation of Primitive Murine Hematopoietic Progenitor Cells

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Summary

Interleukin 7 (IL-7) has been demonstrated to be an important regulator of the growth of B and T cell precursors as well as mature T cells, whereas IL-7 has been reported to have no direct myeloproliferative effects. Here we show that IL-7 potently and directly enhances colony stimulating factor–induced myeloid colony formation from $Lin^-Sca\text{-}1^+$ murine bone marrow progenitor cells, increasing the cloning frequency up to ninefold and cell numbers up to 50-fold, without affecting their ability to differentiate along the myeloid lineages In contrast, IL-7 has no effect on proliferation of committed Lin^- myeloid progenitors. Thus, in addition to its established lymphopoietic potential, this study implicates a novel role of IL-7 in early myelopoiesis.

The hematopoietic system is organized in a hierarchical fashion, with the infrequent pluripotent hematopoietic stem cell at the top, capable of replenishing all cell lineages in the blood (1–3). This pluripotent stem cell gives rise to intermediate multipotent, bipotent, and unipotent myeloid progenitors as well as unipotent B and T cell precursors. Recently, the existence of bipotential precursors of B cells and macrophages has been demonstrated as well (4). The proliferation and differentiation of this diversity of hematopoietic progenitors and stem cells are controlled by a complex system of cell–cell interactions and soluble hematopoietic growth factors (HGFs) (3, 5–7). Whereas single HGFs can induce proliferation and differentiation of committed hematopoietic progenitor cells, optimal proliferation of immature progenitor cells can only be achieved when multiple HGFs act in synergy (3). Even though a number of HGFs have been demonstrated to stimulate proliferation of bone marrow stem cells, no known growth factor combination is capable of inducing proliferation to the extent observed when stem cells are cocultured with stroma feeder layers (8). Stem cell to stroma cell interactions are thought to be important for stem cell proliferation, but it also seems likely that not yet identified HGFs might enhance the proliferative capacity of immature hematopoietic stem cells.

G-CSF, CSF-1 (M-CSF), and GM-CSF represent a group of CSFs capable of inducing growth of only myeloid progenitor cells, whereas most HGFs stimulating B cell lymphopoiesis (such as IL-3, -4, -5, -6, -11, and stem cell factor [SCF]) have myeloproliferative effects as well (9–13). In fact, IL-7 is the only HGF reported to stimulate proliferation of B cell precursors but not myeloid progenitors (14–18). Furthermore, IL-7 potently enhances the growth of T cell precursors (19) and mature T cells (20). The expression of IL-7 receptors on myeloid cells (21) as well as the effects of IL-7 on myelopoiesis in vivo (22, 23) suggest that IL-7 might also have myeloproliferative effects, however, the only reported in vitro effect of IL-7 on myeloid cells is its ability to induce tumoricidal activity and secretion of IL-1, IL-6, and TNF-α by monocytes (24). Since previous studies (14–18) reporting that IL-7 lacks myeloproliferative effects have not used populations of primitive hematopoietic progenitors, this study was designed to examine direct myeloproliferative effects of IL-7 in vitro on a population of highly enriched primitive murine hematopoietic progenitor cells, the $Lin^-Sca\text{-}1^+$ cells. As few as 100 of these cells can rescue 50% of lethally irradiated mice, and long-term, reconstitute all lineages in the blood (2, 25, and Veiby, O. P., unpublished results).

Materials and Methods

Growth Factors. Purified recombinant human (rh) IL-7 was a gift from Steven Gillis (Immunex Corp., Seattle, WA). rhG-CSF and r murine (rm) GM-CSF were generously supplied by Ian K. McNiece (Amgen Corp., Thousand Oaks, CA); rmIL-3 was purchased from Promega Corp. (Madison, WI); and rCSF-1 was kindly supplied by Michael Geier (Cetus Corp., Emeryville, CA).

Unless otherwise indicated, all growth factors were used at predetermined optimal concentrations: rmGM-CSF, 20 ng/ml; rhCSF-1, 50 ng/ml; rhG-CSF, 20 ng/ml; rmIL-3, 20 ng/ml; and rhIL-7, 200 ng/ml.

Enrichment and Purification of Bone Marrow Progenitors. Lin^- cells were isolated from normal C57BL mice, according to a previously described protocol (2). Briefly, light density bone marrow cells were obtained using lymphocyte separation medium (Lym-

phoprep Animal; Nycomed, Oslo, Norway). Cells were washed twice in IMDM (Gibco, Paisley, UK), and resuspended in IMDM supplemented with 20% FCS (Sera-Lab Ltd., Sussex, UK), 100 U/ml penicillin, and 3 mg/ml glutamine (complete IMDM). The cells were incubated at 4°C for 30 min in a cocktail of predetermined optimal concentrations of Abs, RA3-6B2 (B220 antigen; PharMingen, San Diego, CA), RB6-8C5 (GR-1 antigen; PharMingen), MAC-1 (Serotec, Oxfordshire, UK), Lyt-2 (CD8; Becton Dickinson & Co., Sunnyvale, CA), and L3T4 (CD4; Pharmingen). The cells were washed twice and resuspended in complete IMDM. Sheep anti-rat IgG (Fc) conjugated immunomagnetic beads (Dynal, Oslo, Norway) were added at a cell/bead ratio of 1:20, and incubated at 4°C for 30 min. Labeled cells (Lin^+) were removed by a magnetic particle concentrator (Dynal), and the Lin^- cells were recovered from the supernatant.

Lin^-Sca1^+ cells were purified as described by others (2, 25). Briefly, 4–6 × 10^7 Lin^- cells were resuspended per milliliter of complete IMDM. The cells were incubated for 30 min on ice with either FITC-conjugated anti-mouse Ly-6A/E Ab (Pharmingen) directed against the Ly6A/E antigen (Sca-1) or an isotype-matched control Ab. The cells were washed twice, and $Lin^-Sca\text{-}1^+$ cells were sorted on a cell sorter (Epics Elite; Coulter Electronics, Hialeah, FL) equipped with a 488-nm tuned argon laser set to give a power of 15 mW, with a rate of 1,500–2,000 cells/s. Lin^- cells falling into median right angle scatter and median to high forward scatter were analyzed for Sca-1 expression, and cells falling into both regions were selected. Light scatter was collected through a 488-nm band pass filter and the FITC fluorescence was collected through a 488-nm–long pass filter and a 525-nm band pass filter. The final recovery of $Lin^-Sca\text{-}1^+$ cells was 0.05–0.1% of the initial number of bone marrow cells.

High Proliferative Potential Colony-forming Cell (HPP-CFC) Assay. The HPP-CFC assay was performed as previously described by others (26). Alpha modification of Eagle's MEM (Gibco) supplemented with 20% FCS (Sera Lab) was used for all HPP-CFC assays. Cytokines were incorporated into a 1-ml underlayer in 35-mm Petri dishes at a maximum of 3% of the culture volume, and cells were incorporated into a 0.5-ml overlayer. The final concentration of seaplaque agarose (FMC BioProducts, Inc., Rockland, ME) was 0.5% in the underlayer and 0.3% in the overlayer. Cultures were gassed with 5% O_2/5% CO_2/90% N_2 and incubated at 37°C for 12–14 d. HPP-CFC colonies were scored as dense colonies with a diameter of >0.5 mm and containing >50,000 cells.

Single-cell Proliferation Assay. $Lin^-Sca\text{-}1^+$ cells were seeded in Terasaki plates (Nunc, Kamstrup, Denmark) at a concentration of one cell per well in a volume of 20 μl complete IMDM. The wells were scored for proliferation after 12 d of incubation at 37°C, 5% CO_2 in air.

Cell Phenotyping. $Lin^-Sca\text{-}1^+$ cells were seeded in complete IMDM at 500 cells per ml, or one cell per well, and incubated for 12 d with cytokines as indicated at 37°C, 5% CO_2 in air. Cell morphology was determined following Giemsa staining of cytospin preparations.

Results and Discussion

Proliferation of Committed Lin^- Progenitors Is Not Affected by IL-7. In agreement with previous studies (15) we found that IL-7 (0.02–200 ng/ml) did not affect CSF-1, GM-CSF, G-CSF, or IL-3–induced proliferation of committed Lin^- bone marrow cells plated individually, and no colony formation was observed in the presence of IL-7 alone (Table 1). Similarly, IL-7 had no effect on the colony growth from unfractionated bone marrow cells (data not shown).

CSF-induced Proliferation of Single $Lin^-Sca\text{-}1^+$ Cells Is Enhanced by IL-7. The $Lin^-Sca\text{-}1^+$ cells are highly enriched in immature murine hematopoietic progenitor cells (2, 25, 27, 28), and the CSFs have been demonstrated to induce in vitro myeloid colony formation of $Lin^-Sca\text{-}1^+$ cells (5–7). To investigate whether IL-7 directly could affect CSF-induced colony formation from primitive hematopoietic progenitor cells, single $Lin^-Sca\text{-}1^+$ cells were cultured with CSFs in the presence or absence of IL-7 (Table 2). In agreement with previously published reports (6, 7), the only single cytokine capable of inducing significant proliferation of $Lin^-Sca\text{-}1^+$ cells was IL-3. Whereas IL-7 alone induced no colony formation, potent synergistic effects were observed when IL-7 was combined with IL-3, increasing IL-3–responding progenitors threefold. Furthermore, whereas CSF-1 and GM-CSF as single factors induced no colony formation of $Lin^-Sca\text{-}1^+$ cells, significant numbers of colonies were formed when IL-7 was combined with CSF-1 or GM-CSF (mean 11 and 8, respectively; Table 2). In contrast, IL-7 did not synergize with G-CSF. Finally, IL-7 enhanced colony formation up to fourfold in response to several CSF combinations (Table 2). The IL-7–induced synergy on CSF-stimulated colony formation

Table 1. *Effects of rhIL-7 on the Proliferation of Single Lin^- Cells*

Growth factors	Positive wells/300
IL-7 200 ng/ml	0 ± 0
CSF-1	17 ± 3
CSF-1 + 200 ng/ml IL-7	18 ± 4
CSF-1 + 20 ng/ml IL-7	17 ± 3
CSF-1 + 2 ng/ml IL-7	16 ± 2
CSF-1 + 0.2 ng/ml IL-7	18 ± 3
CSF-1 + 0.02 ng/ml IL-7	16 ± 3
GM-CSF	17 ± 3
GM-CSF + 200 ng/ml IL-7	18 ± 4
IL-3	19 ± 3
IL-3 + 200 ng/ml IL-7	19 ± 3
G-CSF	8 ± 2
G-CSF + 200 ng/ml IL-7	8 ± 2
CSF-1 + GM-CSF	16 ± 3
CSF-1 + GM-CSF + 200 ng/ml IL-7	15 ± 3

Lin^- cells were isolated as described in Materials and Methods and plated in Terasaki plates at a concentration of one cell per well in 20 μl, in the presence or absence of purified rhIL-7 and predetermined optimal concentrations of purified recombinant CSFs. Wells were scored for colonies (>10 cells) after 7 d of incubation at 37°C, 5% CO_2 in air. Results presented represent the means ± SEM of four experiments.

Table 2. *Effects of rhIL-7 on the Proliferation of Single Lin⁻Sca-1⁺ Cells*

Growth factors	Degree of proliferation 1	2	3	4	5	Total No. colonies/ 300 wells
IL-7 200 ng/ml	0	0	0	0	0	0 ± 0
IL-3	2	2	3	1	0	9 ± 1
IL-3 + 200 ng/ml IL-7	6*	6	6	6*	3*	27 ± 3*
CSF-1	0	0	0	0	0	0 ± 0
CSF-1 + 200 ng/ml IL-7	3*	2*	3*	2*	0	11 ± 3*
GM-CSF	0	0	0	0	0	0 ± 0
GM-CSF + 200 ng/ml IL-7	1	1	2*	3*	1*	8 ± 2*
G-CSF	0	0	0	0	0	0 ± 0
G-CSF + 200 ng/ml IL-7	0	0	0	0	0	0 ± 0
CSF-1 + IL-3	2	3	7	15	5	31 ± 3
CSF-1 + IL-3 + 200 ng/ml IL-7	2	2	3	21	39*	67 ± 9*
GM-CSF + IL-3	2	2	5	2	3	14 ± 3
GM-CSF + IL-3 + 200 ng/ml IL-7	3	4	6	6*	26*	45 ± 3*
G-CSF + IL-3	7	8	10	5	2	32 ± 6
G-CSF + IL-3 + 200 ng/ml IL-7	4	8	6	9	13*	40 ± 7
CSF-1 + G-CSF	3	8	10	11	0	31 ± 4
CSF-1 + G-CSF + 200 ng/ml IL-7	3	3	10	21	9*	44 ± 4
CSF-1 + GM-CSF	2	2	2	2	0	8 ± 1
CSF-1 + GM-CSF + 200 ng/ml IL-7	5	2	3	10*	13*	33 ± 4*
CSF-1 + GM-CSF + 100 ng/ml IL-7	3	2	4	9*	12*	30 ± 5*
CSF-1 + GM-CSF + 50 ng/ml IL-7	4	3	2	6*	10*	25 ± 4*
CSF-1 + GM-CSF + 20 ng/ml IL-7	3	3	4	8*	7*	23 ± 4*
CSF-1 + GM-CSF + 2 ng/ml IL-7	2	3	2	1	2*	10 ± 2
CSF-1 + GM-CSF + 0.2 ng/ml IL-7	1	3	1	1	1	7 ± 2
CSF-1 + GM-CSF + 0.02 ng/ml IL-7	1	2	2	2	0	7 ± 1

The Lin⁻Sca-1⁺ cells were plated in Terasaki plates at a concentration of one cell per well in 20 μl IMDM supplemented with 20% FCS. Cultures were incubated in the presence or absence of rhIL-7 and predetermined optimal concentrations of the CSFs as indicated. Wells were scored for cell growth after 12-d incubation at 37°C, 5% CO_2 in air. Scoring criteria: 1, 50 cells-10% of well covered by cells; 2, cells covering 10–25% of the well; 3, cells covering 25–50% of well; 4, cells covering 50–95% of the well; 5, complete confluency of the well. The results presented represent the means (means ± SEM for total number of colonies) of at least three experiments, with a total of at least 900 wells scored per group. Statistical analysis was performed using Student's *t* test comparing colony formation in the absence and presence of rhIL-7.
*$p < 0.05$.

from single Lin⁻Sca-1⁺ cells was concentration dependent with maximum stimulation observed at 100–200 ng/ml, and an ED_{50} of 2–20 ng/ml (Table 2).

The size of the colonies generated by hematopoietic progenitors is considered to be indicative of the maturity of the progenitor cell investigated, with immature progenitor cells generating large colonies, and more committed progenitors forming smaller colonies or clusters (26, 29). Interestingly, when colonies formed by single Lin⁻Sca-1⁺ cells were scored based on the degree of cell proliferation (Table 2), it became apparent that IL-7 preferentially stimulated formation of large colonies, that is those covering >50% of the wells (three- to ninefold increase), and in particular colonies giving complete confluency of the wells (six- to ninefold increase). For instance, GM-CSF plus IL-3 stimulated formation of only three colonies completely covering the well, whereas 26 were observed upon addition of IL-7. Similarly, CSF-1 plus GM-CSF stimulated no colonies with complete

Table 3. *Effects of rhIL-7 on Lin⁻Sca-1⁺ HPP-CFC*

Growth factors	HPP-CFCs/400 Lin⁻Sca-1⁺ cells		
	− IL-7	+ IL-7 (200 ng/ml)	+ IL-7 (50 ng/ml)
None	0 ± 0	0 ± 0	ND
IL-3	1 ± 0	9 ± 1*	ND
CSF-1 + IL-3	14 ± 4	27 ± 2*	ND
CSF-1 + G-CSF	7 ± 1	20 ± 1*	ND
CSF-1 + GM-CSF	3 ± 1	19 ± 3*	16 ± 1*
CSF-1 + GM-CSF + anti-IL-7	4 ± 0	ND	5 ± 1

Lin⁻Sca-1⁺ cells were plated in triplicate in the HPP-CFC assay (400 cells/dish) as described in Materials and Methods. HPP-CFC colonies (tight colonies with a diameter >0.5 mm) were scored after 12 d of incubation at 37°C, 5% O_2, 5% CO_2 and 90% N_2. A neutralizing mouse anti-human IL-7 Ab (Genzyme Corp.) was used at 25 μg/ml. No effect on IL-7–induced growth of HPP-CFC was observed when an isotype-matched control Ab was used (data not shown). Results presented are the means ± SEM of at least three experiments. Statistical analysis was performed using Student's *t* test, comparing colony formation in the absence and presence of rhIL-7.
* $p < 0.05$.

confluency in the absence of IL-7, whereas 13 were observed in the presence of IL-7. In contrast, the number of small colonies was not increased by IL-7, suggesting that IL-7 preferentially enhances CSF-induced colony formation from immature hematopoietic progenitor cells.

IL-7 Synergizes with the CSFs to Increase HPP-CFC Colony Formation from Lin⁻Sca-1⁺ Cells. Although Lin⁻Sca-1⁺ bone marrow cells contain pluripotent hematopoietic stem cells, some heterogeneity has been demonstrated within the population (7, 25, 27, 28). Since the HPP-CFC assay detects some of the most immature hematopoietic progenitor cells measurable in vitro (26, 29), we next investigated the ability of IL-7 to affect the cloning frequency of HPP-CFCs. IL-7 increased the number of HPP-CFC colonies up to ninefold when Lin⁻Sca-1⁺ cells were stimulated with different combinations of CSFs (Table 3). The IL-7–induced enhancement of HPP-CFC colony formation was blocked in the presence of an anti–human IL-7 Ab (Genzyme Corp., Cambridge, MA), demonstrating that the observed synergy was induced specifically by IL-7, and not by possible contaminants (Table 3).

IL-7 Does Not Affect CSF-induced Myeloid Differentiation of Lin⁻Sca-1⁺ Cells. To determine whether IL-7 also could affect the myeloid differentiation induced by CSFs, Lin⁻Sca-1⁺ cells were grown in liquid culture with CSFs, in the presence or absence of IL-7. After 12–14 d of incubation, cytospin preparations were analyzed morphologically (Table 4). Only granulocytes and macrophages were observed in the cultures, and none of the investigated CSF combinations resulted in the formation of lymphoid progeny, either in the absence or presence of IL-7. Although IL-7 increased the total number of cells up to 50-fold, the relative contributions of granulocytes and macrophages observed were not significantly affected by IL-7 (Table 4). Furthermore, only a low number of blasts (<2%) were observed in the cultures, and their frequency was not influenced by the presence of IL-7 (data not shown). These observations in bulk liquid culture were further supported by isolation of single Lin⁻Sca-1⁺ cell clones, demonstrating absence of lymphoid colonies, and the relative proportions of CFU-G, CFU-M, and mixed CFU-GM colonies unaltered by IL-7. For instance, IL-3 alone induced

Table 4. *Effects of rhIL-7 on Differentiation of Lin⁻Sca-1⁺ Bone Marrow Cells*

Growth factors	Cells/ml	Fold increase	Percent granulocytes	Percent macrophages
IL-3	7.2×10^4		50 ± 4	50 ± 4
IL-3 + IL-7	1.1×10^6	15	48 ± 6	52 ± 6
CSF-1 + GM-CSF	5.0×10^4		16 ± 3	84 ± 3
CSF-1 + GM-CSF + IL-7	2.6×10^6	52	15 ± 2	85 ± 2

Lin⁻Sca-1⁺ cells were seeded in liquid culture at 500 cells/ml in IMDM with 20% FCS. Cultures were stimulated with the cytokines indicated at predetermined optimal concentrations and rhIL-7 was used at 200 ng/ml. Cells were harvested after 12 d of incubation at 37°C, 5% CO_2 in air, and cell morphology was determined after Giemsa staining of cytospin preparations. Results represent means ± SEM of three separate experiments.

18% CFU-G, 24% CFU-M, and 58% CFU-GM, whereas the parallel numbers in the presence of IL-3 plus IL-7 (200 ng/ml) were 12, 26, and 62%, respectively. Similarly, of the single-cell $Lin^-Sca\text{-}1^+$ clones induced by CSF-1 plus G-CSF (plus 200 ng/ml IL-7), 0% (0%) were CFS-G, 80% (84%) CFU-M, and 20% (16%) CFU-GM.

Since the in vitro conditions in the present study were optimized for myelopoiesis, it is possible that IL-7 under other conditions might stimulate $Lin^-Sca\text{-}1^+$ cells to lymphopoiesis as well. In support of this, a recent in vitro study demonstrated that individual $Lin^-Sca\text{-}1^+$ cells could differentiate along the myeloid as well as the B-lymphoid lineage (30).

This report demonstrates that IL-7 has potent stimulatory effects on early myelopoiesis in synergy with CSF-1, IL-3, or GM-CSF. Furthermore, the single-cell assays indicate that the effect of IL-7 is directly mediated, although autocrine mechanisms cannot be excluded. Two recently published in vivo studies (22, 23), support these in vitro data by demonstrating that IL-7, in addition to having pronounced effects on lymphopoiesis, also affects myelopoiesis. One of the studies (23) also reported an increase in peripheral myeloid cells after IL-7 administration. Furthermore, experiments on primitive human hematopoietic progenitor cells in vitro demonstrate that IL-7 enhances early human myelopoiesis as well (Jacobsen, F. W., unpublished data). Thus, in conclusion, IL-7 is a cytokine with effects on lymphopoiesis and myelopoiesis, and can no longer be regarded as a lymphoid lineage–restricted cytokine.

We thank Dr. Erlend B. Smeland for helpful discussions during this project.

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Received for publication 21 June 1993 and in revised form 16 August 1993.

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