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-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.
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; Phar-Mingen), 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 × 10⁷ 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⁻Sca1⁺ 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 long 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⁻Sca1⁺ cells was 0.05–0.1% of the initial number of bone marrow cells.

**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⁻ Sca1⁻ Cells Is Enhanced by IL-7.** The Lin⁻Sca1⁻ 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⁻Sca1⁻ cells (5–7). To investigate whether IL-7 directly could affect CSF-induced colony formation from primitive hematopoietic progenitor cells, single Lin⁻Sca1⁻ 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⁻Sca1⁺ 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⁻Sca1⁻ 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

| 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% CO₂ 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 | Total No. colonies/300 wells |
|---------------------------------------|-------------------------|------------------------------|
|                                       | 1                       | 2 | 3 | 4 | 5 |                              |
| 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% CO₂ 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 ED₅₀ 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<sup>−</sup>Sca-1<sup>−</sup> HPP-CFC

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

Lin<sup>−</sup>Sca-1<sup>−</sup> 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<sub>2</sub>, 5% CO<sub>2</sub> and 90% N<sub>2</sub>. 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.

Table 4. Effects of rhIL-7 on Differentiation of Lin<sup>−</sup>Sca-1<sup>−</sup> Bone Marrow Cells

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

Lin<sup>−</sup>Sca-1<sup>−</sup> 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<sub>2</sub> 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-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-1" cells to lymphopoiesis as well. In support of this, a recent in vitro study demonstrated that individual Lin-"Sca-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.

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