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Interleukin 3 or interleukin 1 abrogates the reconstituting ability of hematopoietic stem cells

(hematopoiesis/growth factors)

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ABSTRACT **Because of their known myelopoietic activities, both interleukin (IL)-3 and IL-1 are often used in combination with other cytokines for *in vitro* (*ex vivo*) expansion of stem cells. We have investigated the effects of IL-3 and IL-1 on *in vitro* expansion of murine hematopoietic stem cells with long-term engraftment capabilities, using a highly purified progenitor population. Lineage-negative, Ly-6A/E$^+$, c-*kit*$^+$ bone marrow cells from male mice were cultured in suspension in the presence of stem cell factor, IL-6, IL-11, and erythropoietin with or without IL-3 or IL-1. Kinetic studies revealed an exponential increase in total nucleated cells and about 10-fold enhancement of nucleated cells by IL-3 during the initial 10 days. Addition of IL-3 hastened the development but significantly suppressed the peak production of colony-forming cells. Addition of IL-1 also significantly suppressed the numbers of colony-forming cells. The reconstituting ability of the cultured cells was tested by transplanting the expanded male cells into lethally irradiated female mice. The cells expanded from enriched cells in the absence of IL-3 and IL-1 revealed engraftment at 2, 4, 5, and 6 months, whereas addition of IL-3 or IL-1 to the cultures significantly reduced the reconstituting ability. The results suggest that these cytokines may have a modulatory role on the self-renewal of stem cells and further indicate that the use of IL-3 and IL-1 for *in vitro* expansion of human stem cells needs to be cautiously evaluated.**

Currently one of the major emphases in experimental hematology is *in vitro* (*ex vivo*) expansion of hematopoietic stem cells and progenitors. If it becomes possible to expand stem cells in culture, it would have a profound impact on clinical bone marrow transplantation and gene therapy. Already, investigators in a number of laboratories have shown that it is possible to increase the numbers of hematopoietic cells and progenitors in culture by using combinations of recombinant cytokines (1–14). Attempts to expand the population of stem cells with long-term reconstituting ability have not met with much success (5–7). In general, the cytokines employed by investigators are selected from the early acting cytokines such as stem cell factor (SCF, c-*kit* ligand), interleukin (IL)-1, IL-3, IL-6, IL-11, granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, and IL-3 (15). Because of the known myelopoietic effects of IL-3 and IL-1, all protocols contained either IL-3 or IL-1, and many contained both factors.

Recently, we developed a two-step methylcellulose culture assay for murine lymphohematopoietic progenitors that are capable of B-cell and myeloid lineage expression (16) and observed that, while SCF-based cytokine combinations support the proliferation and differentiation of lymphohematopoietic progenitors, the addition of IL-3 or IL-1 (α or β) to the permissive cytokine combinations abrogates the B-lymphoid potential of the progenitors (17). Subsequently, we obtained preliminary information that the T-cell potential of lymphohematopoietic progenitors may also be inhibited by IL-3 and IL-1 (18). These observations raised the possibility that IL-3 and IL-1 may be stage-specific negative regulators and that they may suppress the earliest process of hematopoiesis, i.e., self-renewal of the stem cells. Consequently, we tested the effects of IL-3 and IL-1 (α and β) on the *in vitro* expansion of stem cells with long-term engrafting capability.

MATERIALS AND METHODS

Growth Factors. The source of murine IL-3 was a medium conditioned by Chinese hamster ovary cells that had been genetically engineered to produce murine IL-3 in high titer (70,000 units/ml) provided by T. Sudo of Biomaterial Research Institute, Yokohama, Japan. According to a titration study (17), 200 units/ml of this preparation was equivalent to 1 ng/ml of purified recombinant IL-3, producing 90–95% inhibition of B-cell potential of primitive progenitors. Recombinant rat SCF was produced in *Escherichia coli* and purified (19). Purified human IL-6 was prepared as described previously (20). Purified human erythropoietin (Ep) was provided by F.-K. Lin, Amgen Biologicals. Recombinant human IL-11 was provided by P. Schendel, Genetics Institute, Cambridge, MA. Purified recombinant human IL-1α and IL-1β were provided by Y. Hirai, Otsuka Pharmaceutical, Tokushima, Japan. Concentrations of the cytokines used in this study were as follows: IL-3, 200 units/ml; SCF, 100 ng/ml; IL-6, 100 ng/ml; Ep, 2 units/ml; IL-11, 20 ng/ml; IL-1α, 1 ng/ml; IL-1β, 1 ng/ml.

Cell Preparations. Two- to 5-month-old male BDF1 mice (Charles Rivers, Raleigh, NC) and male C57BL/6 mice (The Jackson Laboratory) that are congenic for Ly5 allotypes were used for transplantation. 5-Fluorouracil (5-FU) (Adria Laboratories) was administrated i.v. through the tail veins of mice at 150 mg/kg of body weight, and bone marrow cells were harvested 2 days later. Single-cell suspensions were prepared from pooled femurs and tibias, and cells with a density between 1.0631 and 1.0770 g/ml were collected with gradients of metrizamide (Accurate Chemicals Westbury, NY). The cells were further enriched for progenitors by negative immunomagnetic selection with a mixture of lineage-specific antibodies (21). Lineage-negative (Lin$^-$) cells were incubated with fluorescein isothiocyanate-conjugated monoclonal antibody D7 (anti-Ly-6A/E) (22) and biotin-conjugated monoclonal ACK4 (anti-c-*kit*) (23) for 15 min on ice. Isotype controls were fluorescein isothiocyanate-conjugated rat IgG2a and biotin-

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Abbreviations: SCF, stem cell factor; IL, interleukin; Ep, erythropoietin; 5-FU, 5-fluorouracil; Lin$^-$, lineage-negative; BSA, bovine serum albumin; TNCC, total nucleated cell counts; CFU-C, colony-forming units in culture; CFU-GEMM, colony-forming units, granulocyte/erythrocyte/macrophage/megakaryocyte.

conjugated rat IgG2a. The cells were then washed twice with Ca^{2+}, Mg^{2+}-free phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (fraction V; Sigma) and incubated with R-phycoerythrin-conjugated streptavidin (Jackson ImmunoResearch) for 15 min on ice. The cells were washed twice, resuspended in PBS/BSA, and kept on ice until cell sorting. Flow cytometric analysis and cell sorting were performed on a FACStar^{PLUS} (Becton Dickson).

Suspension and Clonal Cell Cultures. Sorted Lin^- Ly-6A/E^+ c-*kit*$^+$ cells were incubated in each well of a 24-well plate (Falcon) in suspension culture. The culture medium contained α-medium, 20% (vol/vol) fetal calf serum (Intergen, Purchase, NY), 1% deionized fraction V BSA, 1×10^{-4} M 2-mercaptoethanol (Sigma), and combinations of cytokines. At each time of replating, aliquots were analyzed for colony formation in 35-mm suspension culture dishes (Falcon) containing α-medium, 1.2% 1500-centipoise methylcellulose (Shinetsu Chemical, Tokyo, Japan), 30% fetal calf serum, 1% BSA, 1×10^{-4} M 2-mercaptoethanol, SCF, IL-3, IL-11, and Ep. Colony types were determined on day eight of incubation by *in situ* observation on an inverted microscope according to the criteria described previously (24, 25).

Reconstitution Experiments. Female BDF1 mice were irradiated with single 950-cGy total body irradiation from a 4-MV linear accelerator, and the female C57BL/6 mice were irradiated with 850 cGy. After total body irradiation of the recipient mice, sorted fresh male marrow cells or cultured male cells were injected into the tail veins of female recipients together with 4×10^5 compromised female marrow cells in a final volume of 0.2 ml of PBS containing 0.1% BSA. Compromised cells had been subjected to two previous rounds of transplantation and regeneration in female mice (26). Peripheral blood (50–100 μl) was obtained from the retro-orbital venous plexus using heparin-coated micropipettes (Drummond Scientific, Broomall, PA) 2, 4, 5, and 6 months after transplantation. Fifty μl of peripheral blood was lysed by 0.747% NH_4Cl in Tris (pH 7.4). The samples were then used for flow cytometric analysis of donor-derived cells by staining with anti-Ly5.1 (A20–1.7) or for PCR analysis of donor-derived male cells as presented in the next paragraph.

PCR Analysis. Genomic DNA was extracted from lysed peripheral blood cells by using the phenol/chloroform method (27). A 100-ng DNA sample was added to a 50-μl PCR mixture consisting of 10 mM Tris·HCl (pH 8.3), 2.5 mM $MgCl_2$, 200 μM each deoxynucleoside 5′-triphosphate, and 300 ng of each primer. The sequences of the Y chromosome-specific primers (pY2) were 5′-GCATTTGCCTGTCAGAGAGAG-3′ (sense) and 5′-ACTGCTGCTGCTTTCCAACTA-3′ (antisense). The sequences of control primers for Ep receptor were 5′-CACATAGCCGGGATGCAGAGG-3′ (sense) and 5′-TTGGCCTCAAAGCCCAGGCCA-3′ (antisense). The reaction mixture was covered with mineral oil, heated at 95°C for 5 min, cooled to 80°C, and mixed with 0.2 unit of Tfl thermostable DNA polymerase (Epicenter Technologies, Madison, WI). The PCRs for pY2 consisted of 26 cycles of denaturation for 1 min at 95°C, annealing for 2 min at 62°C, and polymerization for 3 min at 72°C and were carried out in a DNA Thermal Cycler (Perkin–Elmer/Cetus). The PCR conditions for Ep receptor were 1.5 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase (GIBCO/BRL), 94°C, 55°C, and 72°C for 1 min, 2 min and 4 min, respectively. The amplified DNA was analyzed by agarose gel electrophoresis and stained with ethidium bromide.

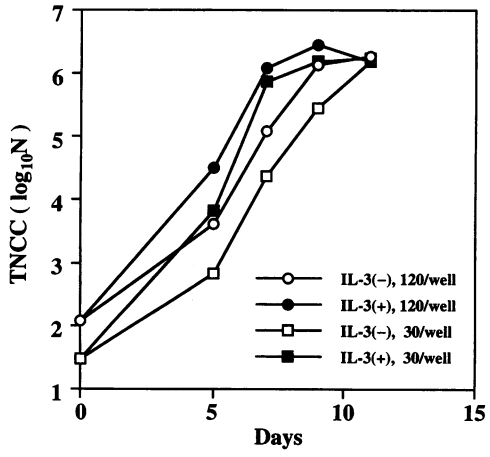

FIG. 1. Expansion of TNCC in suspension cultures. Thirty or 120 enriched cells were cultured with SCF, IL-6, IL-11, and Ep in the presence or absence of IL-3.

RESULTS AND DISCUSSION

In order to develop the optimal conditions for *in vitro* expansion, we first examined the expansion of the progenitors that are capable of colony formation in culture. We used Lin^- Ly-6A/E^+ c-*kit*$^+$ cells that had been prepared from bone marrow of 5-FU-treated BDF1 mice (21, 28). This subpopulation was found to represent 0.04% of the unfractionated marrow cells and was enriched approximately 600-fold for multipotential progenitors. This population of cells has been shown to be capable of hematopoietic reconstitution in lethally irradiated mice (29). Our laboratory has extensively characterized the cytokine response of these cells. When stimulated by combinations of early-acting cytokines, they routinely exhibit 50% colony-forming efficiency. Initially we analyzed the

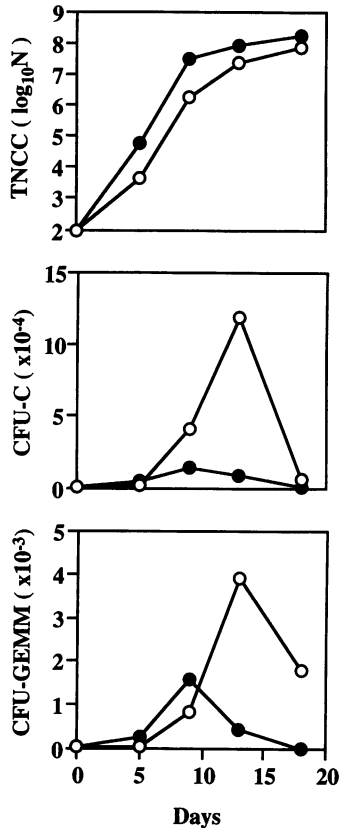

FIG. 2. Expansion of TNCC, CFU-C, and CFU-GEMM in suspension cultures with or without IL-3. One hundred enriched cells were cultured with SCF, IL-6, IL-11, and Ep in the presence (●) or absence (○) of IL-3. On days 5, 9, 13, and 18 of incubation, the cultured cells were washed and diluted in freshly prepared media, and the incubation was continued in order to maintain the cell concentrations below 2×10^6 cells per ml. At each time of replating, aliquots were analyzed for colony formation.

Table 1. Expansion of Ly-6A/E$^+$ c-*kit*$^+$ cells and progenitors in suspension culture

	Additional cytokine	Day 7 TNCC	Day 7 Ly-6A/E$^+$ c-*kit*$^+$ cells	Day 7 CFU-C	Day 14 TNCC	Day 14 Ly-6A/E$^+$ c-*kit*$^+$ cells	Day 14 CFU-C
Experiment 1	None	2.4×10^5	48,960	20,910	1.7×10^7	854,000	81,980
	IL-3	2.0×10^6	94,000	4,700	2.0×10^7	120,000	360
Experiment 2	None	2.2×10^5	39,450	17,110	4.6×10^7	1,210,000	82,600
	IL-3	1.5×10^6	40,200	3,880	6.4×10^7	88,700	180
	IL-1α	4.8×10^5	5,860	340	6.4×10^5	11,300	10
Experiment 3	None	1.0×10^5	30,250	15,500	2.9×10^7	860,000	61,060
	IL-3	7.0×10^5	103,670	13,200	1.3×10^8	320,000	250
	IL-1β	7.1×10^5	28,400	7,100	1.2×10^6	44,000	190

100 Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ cells were cultured in the presence of SCF, IL-6, IL-11, Ep, and a designated additional cytokine. On day seven, the cultured cells were resorted, and only the Ly-6A/E$^+$ c-*kit*$^+$ cells were replated in freshly prepared media; incubation continued for 7 more days. On both day 7 and day 14 of culture, TNCC, total number of Ly-6A/E$^+$ c-*kit*$^+$ cells, and total number of CFU-C were analyzed.

kinetics of the proliferation of the total nucleated cell counts (TNCC) by plating 30 or 120 enriched cells in suspension culture in the presence of SCF, IL-6, IL-11, and Ep with or without IL-3. As shown Fig. 1, a log–linear increase in TNCC and augmentation of TNCC by IL-3 were observed until cell concentration reached 2×10^6 cells per ml on day 11 of culture when plateauing began, probably due to exhaustion of nutrients and/or accumulation of waste products.

In order to avoid the negative effects of higher cell concentrations, we employed two approaches in subsequent experiments. In the first series of experiments, we serially diluted the cultures on days 5, 9, 13, and 18 and continued incubation of cells in freshly prepared media. Each time the cultures were diluted, aliquots of the cells were assayed for colony formation *in vitro*. A representative comparison of the cultures carried out with SCF, IL-6, IL-11, and Ep, in the presence or absence of IL-3, is shown in Fig. 2. Again IL-3 augmented TNCC. IL-3 hastened the development but significantly suppressed the peak production of colony-forming units in culture (CFU-C) and multipotential progenitors, CFU-granulocye/erythrocyte/macrophage/megakaryocyte (CFU-GEMM). In the second series of experiments, 100 enriched bone marrow cells were cultured in the presence of SCF, IL-6, IL-11, and Ep, in the presence or absence of IL-3, IL-1α or IL-1β. On day seven aliquots of the cultured cells were analyzed for Ly-6A/E and c-*kit* expression. The results of analyses are shown in Table 1. Again IL-3 enhanced production of TNCC but appears to have significantly suppressed CFU-C production in two of three experiments. IL-1α and IL-β independently suppressed CFU-C production. The remainder of the samples were resorted on the FACStar^{PLUS}, and Ly-6A/E$^+$ c-*kit*$^+$ cells were replated in freshly prepared media in the 24-well plates at 500 cells per ml and incubated for 7 more days. On day 14 of culture, the total numbers of Ly-6A/E$^+$ c-*kit*$^+$ cells and CFU-C were determined again (Table 1). Again IL-3 augmented TNCC, but its effect was weaker than on day seven. IL-1α and IL-β individually caused significant reduction in the number of TNCC and Ly-6A/E$^+$ c-*kit*$^+$ cells. Both IL-3 and IL-1 caused striking reduction in CFU-C numbers on day 14.

We then tested the inhibitory effects of IL-3 and IL-1α on the *in vivo* reconstituting ability of cultured cells. The suspension cultures were initiated with Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ male BDF1 cells. On day seven of incubation, the cultured cells were sorted again, and the Ly-6A/E$^+$ c-*kit*$^+$ cells were incubated in freshly prepared media for 7 more days. We then transplanted the whole sample or fractions of the sample of cells expanded from 100 enriched cells into lethally irradiated female BDF1 mice. As a control, we also injected 100 fresh enriched marrow cells. To detect the presence of male cells, we used a PCR-based assay that utilizes oligonucleotide primers (30) derived from a segment (pY2) of the murine Y chromosome. First we analyzed the sensitivity of the PCR and determined that PCR amplification of the pY2 region of 100 ng of murine DNA for 26 cycles allows detection of 0.1% male cells. We then exam-

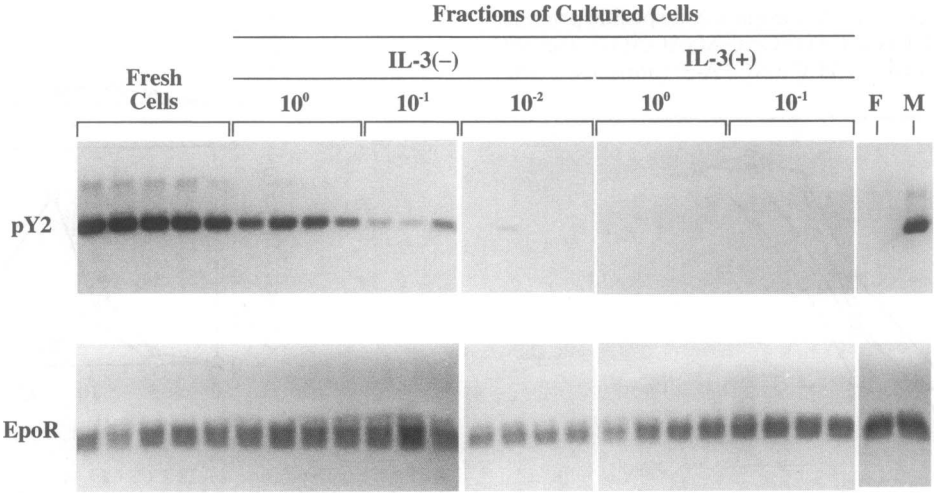

FIG. 3. Representative PCR analyses of peripheral blood cells of individual female BDF1 mice showing the presence of donor male cells. Suspension cultures were initiated with Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ cells of male BDF1 mice in the presence or absence of IL-3. On day seven of incubation, the cultured cells were sorted again, and the Ly-6A/E$^+$ c-*kit*$^+$ cells were incubated in freshly prepared media for 7 more days. All or a fraction of the cells expanded from 100 enriched cells or 100 fresh sorted cells were injected i.v. into lethally irradiated BDF1 mice. Five months later, 50 μl of blood was harvested from individual mice and analyzed for the presence of male cells by PCR. F, peripheral blood cells from normal female BDF1 mice; M, peripheral blood cells from normal male BDF1 mice.

Table 2. Repopulating ability of fresh bone marrow and day 14 cultured cells

Additional cytokine	TNCC (% Ly-6A/E$^+$ c-*kit*$^+$ cells)	Fraction of cells injected	Analysis of repopulation: 2 months, Positive animals by PCR*	2 months, % donor PB cells by Ly5.1†	6 months, Positive animals by PCR*	6 months, % donor PB cells by Ly5.1†
Fresh	100 (100.0)	1	5/5	66.4 ± 8.7	3/3	70.0 ± 2.3
None	1.0×10^7 (4.1)	1	5/5	11.1 ± 5.3	4/4	9.4 ± 5.6
		1/10	5/5	2.0 ± 0.4	4/4	2.2 ± 1.7
		1/100	3/5	1.0 ± 0.4	2/4	1.1 ± 0.8
IL-3	1.1×10^7 (0.5)	1	0/4	0.7 ± 0.2	0/4	0.4 ± 0.3
		1/10	0/5	0.8 ± 0.4	0/4	0.3 ± 0.2
IL-1α	3.6×10^5 (4.6)	1	0/5	0.6 ± 0.2	0/5	0.4 ± 0.3
		1/10	0/3	0.3 ± 0.1	0/3	0.5 ± 0.2

Irradiated recipients (five mice per group) were transplanted with 100 fresh Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ cells (Fresh) or with all or fractions of a day 14 culture that had been initiated with 100 Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ cells. These cells had been expanded in culture in the presence of SCF, IL-6, IL-11, Ep, and the designated additional cytokine; 4×10^5 "compromised" cells were co-injected.

*Number of mice showing male cells/number of surviving mice.

†Mean ± SD of percent donor-derived nucleated peripheral blood cells tested with anti-Ly5.1. Background (peripheral blood cells of nontransplanted female mice) staining revealed 0.5% ± 0.2%.

ined engraftment by cultured cells and control fresh marrow cells by using this PCR assay. The results are presented in Fig. 3. Similar to the results reported by other investigators (29), 100 Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ cells from 5-FU-treated mice were clearly capable of *in vivo* reconstitution. Transplantation of all or 1/10th of the cells that were expanded from 100 enriched cells in the absence of IL-3 revealed reconstitution 5 months later. One of four mice transplanted with 1/100th fraction of the cultured cells also revealed engraftment. In contrast, neither all cells nor a 1/10th fraction of the cells expanded in the presence of IL-3 revealed signs of engraftment.

In the next experiment, we cultured enriched bone marrow cells of male C57BL/6-Ly5.1 mice and transplanted into female C57BL/6-Ly5.2 mice. Blood was analyzed for signs of engraftment 2 and 6 months after transplantation (Table 2). Similar to the results presented in Fig. 3, only blood from mice transplanted with 100 fresh marrow cells or fractions of cells cultured in the absence of IL-3 and IL-1α revealed signs of donor cell engraftment upon PCR analysis. Studies of the Ly5 allotype markers confirmed the results of the PCR analysis and documented the presence of approximately 10% donor cells in the peripheral blood of mice transplanted with all cells expanded from 100 enriched cells. Incubation with SCF, IL-6, IL-11, and Ep for 14 days does not appear to have expanded the population of stem cells, since the engraftment levels by the entire sample of cultured cells were about 10% compared with near 70% levels achieved by transplantation of 100 fresh marrow cells. Transplantation of 1/10th fraction of the cultured cells revealed the presence of 2.0% and 2.2% male cells 2 and 6 months later, respectively. Donor cells of the monocyte, granulocyte, and T- and B-lymphocyte lineages were detected (data not shown). In contrast, transplantation with the cells expanded in the presence of IL-3 or IL-1α failed to reveal signs of engraftment.

The studies by Rebel *et al.* (5) suggested that in *in vitro* expansion, there may be discrepancies between the number of long-term engrafting cells and the number of cells with stem cell phenotypes. It was possible that we have lost some of the long-term reconstituting cells by resorting the day seven expanded cells for Ly-6A/E$^+$ c-*kit*$^+$ cells. Alternatively, both IL-3 and IL-1 may have simply caused kinetic alterations in production of long-term engrafting cells. In order to exclude these possibilities we cultured Ly-6A/E$^+$ c-*kit*$^+$ only for 7 days and tested the *in vivo* engrafting abilities of the cultured cells without resorting of the samples. The results are presented in Table 3. Incubation with SCF, IL-6, IL-11, and Ep for 7 days appears to have maintained the engrafting capability of the cultured cells. In contrast, incubation in the presence of IL-3 or IL-1α for 7 days significantly reduced the reconstituting abilities of the expanded cells. These observations indicated that the suppression by IL-3 and IL-1 of the reconstituting abilities of cultured cells is not an artifact of phenotypic changes of stem cells. Furthermore, the study excluded the possibility that the apparent negative effects of IL-3 and IL-1 are due solely to kinetic alterations of progenitor production.

It has been shown that IL-3 as a single factor can support proliferation of actively cycling progenitors. At all dates of analyses in the kinetic study shown in Figs. 1 and 2, IL-3 induced about a 10-fold augmentation of TNCC. Even though IL-3 decreased the peak CFU-C and CFU-GEMM numbers, the emergence of the peaks was hastened by IL-3. These results indicate that IL-3 is a positive regulator of early myelopoietic processes. While we did not observe a corresponding stimulation by IL-1 with the concentration used, we cannot exclude the possibility that a careful dose titration would produce a

Table 3. Repopulating ability of fresh bone marrow and day 7 cultured cells

Additional cytokine	TNCC	Fraction of cells injected	Analysis of repopulation, % donor PB cells by Ly5.1*: 2 months	4 months
Fresh	500	1	71.3 ± 17.8	73.7 ± 22.4
		1/5	41.0 ± 27.2	44.2 ± 29.7
		1/25	2.1 ± 0.3	1.8 ± 0.9
None	8.0×10^5	1	69.5 ± 17.5	61.7 ± 25.3
		1/5	38.2 ± 13.2	32.6 ± 13.3
		1/25	31.5 ± 6.9	31.0 ± 9.7
IL-3	3.3×10^6	1	19.3 ± 11.1	12.5 ± 4.1
		1/5	1.7 ± 1.0	1.9 ± 0.2
		1/25	2.2 ± 1.7	1.9 ± 0.8
IL-1α	1.2×10^6	1	4.2 ± 3.0	4.0 ± 2.2
		1/5	2.5 ± 2.6	1.8 ± 1.2
		1/25	1.2 ± 0.5	1.5 ± 0.3

Irradiated recipients (four mice per group) were transplanted with 500, 100, and 20 fresh Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ cells or with all or fractions of a day 7 culture that had been initiated with 500 Lin$^-$ Ly-6A/E$^+$ c-*kit*$^+$ cells. These cells had been expanded in culture in the presence of SCF, IL-6, IL-11, Ep, and the designated additional cytokine; 4×10^5 "compromised" cells were co-injected.

*Mean ± SD of percent donor-derived nucleated peripheral blood cells tested with anti-Ly5.1. Background (peripheral blood cells of nontransplanted female mice) staining revealed 0.5% ± 0.2%.

similar effect. On the basis of analysis of the distribution of spleen colony-forming cells in individual spleens and computer simulation of self-renewal and differentiation, Till *et al.* (31) proposed that the choice of the stem cell to self-renew or become committed to differentiation is a stochastic process. The observations presented in our paper suggest the possibility that IL-3 and IL-1 influence this early decision-making process in hematopoiesis.

Because of the well-known myelopoietic effects of IL-3 and IL-1, all preclinical protocols currently used for *in vitro* expansion of murine (2–7) and human (8–14) hematopoietic progenitors and stem cells include one or both of these cytokines. Recently 10 patients with advanced cancers were transplanted with peripheral blood progenitors that had been expanded in cultures containing IL-3 and IL-1β (32). While restoration of hematopoiesis after high dose chemotherapy was similar to that in historical control patients who received either mononuclear cells or CD34-positive cells, no information was provided regarding long-term effects on the recipients' hematopoiesis. We do not know whether or not IL-3 and IL-1 negatively affect human stem cells. We are attempting to test this question, using xenogeneic transplantation as an assay for human stem cells. Meanwhile, the use of IL-3 or IL-1 may need to be cautiously evaluated in attempts to expand *in vitro* human hematopoietic stem cells with reconstituting capabilities.

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