Interleukin-1 (IL-11) is a bone marrow microenvironment-derived growth factor with pleiotropic effects on a variety of hematopoietic cells. To more accurately assess the effects of IL-11 on stem and progenitor compartments within the hematopoietic microenvironment (HM), we added recombinant human (rh) IL-11 to human and murine long-term bone marrow cultures (LTMC) and analyzed primitive (high proliferative potential-colony forming cells [HPP-CFC]), long-term culture-initiating cells (LTC-IC), and long-term reconstituting stem cells and progenitor (day 12 colony forming unit-spleen [CFU-S]), colony forming unit-megakaryocyte [CFU-Mk]) and colony forming unit-granulocyte/macrophage [CFU-GM]) compartments throughout the duration of the cultures. rhIL-11 (100 ng/ml) added twice weekly resulted in significantly increased nonadherent (NA) cellularity, CFU-GM, and CFU-Mk production in human LTMC. Addition of rhIL-11 to murine LTMC was associated with a 5- to 40-fold increase in CFU-GM and a four- to 20-fold increase in day 12 CFU-S in NA cells. However, IL-11 had no significant effect on total HPP-CFC concentration and decreased the size of the more primitive stem/progenitor compartment as evidenced by both decreased LTC-IC frequency in human LTMC and decreased frequency of long-term reconstituting stem cells in murine LTMC. These data suggest that IL-11 may increase commitment of stem cells into a multipotent progenitor compartment.

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Proliferation and differentiation of hematopoietic stem cells depend on interactions with a variety of cells, extracellular matrix proteins, and cytokines in the hematopoietic microenvironment (HM). These interactions may include signaling from one cell to a number of associated cells (including stromal cells) within the immediate vicinity, termed a local area network (LAN). Interleukin-11 (IL-11) is a stromal cell-derived multifunctional hematopoietic growth factor. Previous studies have demonstrated that IL-11, alone or with other cytokines, can stimulate megakaryopoiesis and thrombopoiesis, myelopoesis, lymphopoiesis, and erythropoiesis in vitro. In the absence of stromal cells, IL-11 acts synergistically with other cytokines, such as interleukin-3 (IL-3), stem cell factor (SCF), or the combination of IL-3 and granulocyte-colony-stimulating factor (G-CSF) to expand human primitive long-term culture-initiating cells (LTC-IC) populations that are capable of sustained in vitro production of large numbers of hematopoietic cells. IL-11 enhances the survival of murine primitive cells (day 28 cobble area forming cells, [CAFC]) in liquid culture when added together with SCF or IL-3. IL-11 with IL-3, interleukin-4 (IL-4), and SCF increases murine blast colony-forming cells and colonies derived from colony-forming unit-granulocyte / erythocyte / macrophage / megakaryocyte (CFU-GEMM) formation during the second post 5-fluorouracil (5-FU) marrow cells in vitro. These effects of IL-11 on both murine and human stem and/or progenitor progenitors may be due to the entry of dormant stem cells into the cell cycle.

However, IL-11 administration in vivo has little effect on day 12 colony forming unit-spleen (CFU-S) content in either the bone marrow (BM) or spleen of BM transplant (BMT) recipient mice. IL-11 significantly increases peripheral total leukocyte (especially neutrophil) and platelet counts, as well as total cellularity and the content of multilineage progenitor cells in BM and spleen of treated animals. These data suggest that increases in primitive cell numbers seen in vitro cultures with recombinant cytokines may not reflect hematopoietic microenvironment (HM) hematopoietic stem cell interactions occurring in vivo.

To more accurately determine the effects of IL-11 on different hematopoietic cell compartments within the HM, we added recombinant human IL-11 to human and murine long-term bone marrow cultures (LTMC) and analyzed stem and progenitor compartments for an extended period. We report that IL-11 significantly stimulates myeloid hematopoiesis, including myeloid and multipotential progenitors in both human and murine LTMC; however, IL-11 does not significantly increase the number of high proliferative potential-colony forming cells (HPP-CFC) in murine LTMC and appears to decrease the size of the more primitive stem cell compartment in both human and murine LTMC. Taken together with previous studies, these data suggest a role of IL-11 in commitment of primitive hematopoietic stem cells.

MATERIALS AND METHODS
Mice. All mice were purchased from the Jackson Laboratory (Bar Harbor, ME), housed five to a cage, and fed sterilized lab chow and acidified water (pH 2.4) ad libitum. The BM donor mice and the recipients for CFU-S; determinations were C57Bl/6J. The recipients for long-term repopulation assays were unirradiated WBB6F1-W/N.

BM cultures. Human BM was aspirated from the posterior superior iliac crest of normal volunteers in accordance with guidelines previously established by the Human Investigation Committee of the Indiana University School of Medicine. Aspirates were diluted 1:1 in Iscove's modified Dulbecco's medium (IMDM) supplemented
with 10% fetal calf serum (FCS; GIBCO, Grand Island, NY), 100 U/ml penicillin, and 100 μg/mL streptomycin (GIBCO). Human BM cells were layered on Ficoll-Paque (Pharmacia, Piscataway, NJ) and subjected to centrifugation at 1,500 rpm for 30 minutes at room temperature. Cells at the interface were collected and washed twice with IMDM and then counted on a Coulter Counter (Model ZM; Coulter Electronics, Hialeah, FL) with a 100-μm aperture, after the addition of Zapoglobin (Coulter) for red blood cell lysis (according to the manufacturer’s recommendations). Ten million (10⁶) low density mononuclear cells were seeded into 25-cm² culture flasks (Costar, Cambridge, MA) in human LTMC media containing IMDM supplemented with 10% horse serum (GIBCO), 10% FCS (GIBCO), 10⁻⁴ M hydrocortisone sodium succinate (Solu-Cortef; Upjohn, Kalamazoo, MI), 350 μg/mL sodium chloride (Sigma, St Louis, MO), 100 U/mL penicillin, and 100 μg/mL streptomycin.

Murine bone marrow cells from C57Bl/6J mice sacrificed by cervical dislocation were flushed from femurs and tibias using murine LTMC media containing Fisher media (GIBCO) supplemented with 20% horse serum (GIBCO), 10⁻³ M hydrocortisone sodium succinate (as above), 1 μg/ml sodium bicarbonate, 10⁻⁴ M 2-mercaptoethanol (BME, Sigma), 100 U/mL penicillin, and 100 μg/mL streptomycin. A single cell suspension was obtained by passing the bone marrow cell suspension through a 25-gauge needle several times. Murine BM cells were washed and counted as above. Ten million (10⁶) murine BM cells were seeded into 25-cm² cell culture flasks (as above). Both human and murine cultures were maintained at 33°C, 5% CO₂, and humidified atmosphere. Recombinant human (rh) IL-11 (Genetics Institute, Cambridge, MA) was added twice weekly at a final concentration of 100 ng/ml. Human and murine LTMC were fed following 70% and 50% depopulation, respectively, weekly.

**Human colony-forming units-megakaryocyte (CFU-Mk) assay.** All nonadherent (NA) cells from weekly feedings of human cultures were pooled and subsequently plated in the presence of rhIL-3 (Genzyme, Boston, MA) (10 ng/mL) and rh stem cell factor (SCF) (Amgen, Thousand Oaks, CA) (100 ng/mL) in a single serum-depleted, fibrin clot culture system at a cell density of 2 X 10⁵ cells/ml as previously described. Briefly, an IMDM-based serum substitute solution consisting of iron-saturated human transferrin (Sigma) (90 μg/mL), sodium bicarbonate (0.04%, wt/vol, GIBCO), bovine serum albumin (0.09%, wt/vol, BSA, Fraction V, Calbiochem, La Jolla, CA), L-asparagine (10⁻⁴ M, GIBCO), calcium chloride (CaCl₂) (280 μg/mL), and 55 mmol/L L-epsilon-amino-n-caproic acid (Sigma) were used. A semisolid clot matrix was achieved from the interaction of fibrinogen (0.3%, wt/vol, Kabi, Stockholm, Sweden) and 0.3 μg/mL thrombin (Sigma) diluted in phosphate-buffered saline (PBS). A final volume of 1 mL was plated per 35-mm grided tissue culture dish (Costar) and incubated for 12 to 14 days at 37°C, 5% CO₂ in an humidified atmosphere. After incubation, fibrin clots were fixed in situ in methanol-acetone (1:3) for 20 minutes, washed with PBS, and air dried. The fixed dishes were stained with IOE5, a murine monoclonal antibody against human platelet glycoprotein (gp)IIb-IIIa receptor complex (provided by Dr Barry S. Coller, Mt Sinai, New York) at a dilution of 1:400 in PBS for 1 hour at 4°C with gentle agitation. After washing in PBS, fluorescein isothiocyanate (FITC)-conjugated goat antiserum polyclonal antibody (Kirkegaard & Perry, Gaithersburg, MD) was added at a dilution of 1:20 for 1 hour at 4°C. After washing with PBS, nonmegakaryocyte colonies were counterstained using a 0.125% Evans Blue (Sigma) for 1.5 minutes at room temperature. Subsequently the 35-mm petri dishes were inverted, and the base area was completely scored at 100× using a fluorescence microscope (Axioskop, Zeiss) equipped with a band pass excitation filter at 450 to 490 nm (Zeiss). A CFU-Mk-derived colony was defined as a cluster of three or more FITC-positive cells (assayed in duplicates). The results are expressed as cumulative means of observable CFU-Mk-derived colonies determined weekly from two separate experiments. This represents the total number of CFU-Mk produced by the cultures during the time noted in the figure legend.

**Myeloid progenitor assays.** NA cells obtained weekly from LTMC were centrifuged and washed; the cell pellet was resuspended in IMDM (human) or α-MEM (murine) and counted as described above. Human NA cells (4 X 10⁵ cells) were plated in 1-ml aliquots of 1.1% methylcellulose (Fluka, Hauppauge, NY) in IMDM supplemented with 26% FCS (GIBCO), 10% human plasma (obtained from individual volunteers), 10⁻⁴ M 2-mercaptoethanol (BME, Sigma), 4 μg/ml erythropoietin (Epo; Amgen), 10 ng recombinant human IL-3 (Genzyme), 100 ng rhSCF, 100 U penicillin and 100 μg streptomycin (GIBCO). A total of 2 X 10⁵ murine NA cells were plated in 1-ml aliquots of 1% methylcellulose (Fluka) in α-MEM supplemented with 30% FCS, 10⁻³ M 2-mercaptoethanol (BME, Sigma), 2 mmol/L glutamine (GIBCO), 4 U Epo (Amgen), 20 U recombinant murine IL-3 (Genzyme), 100 ng recombinant rat (rr) SCF (Amgen), penicillin and streptomycin (as above). Triplicate or quadruplicate cultures were incubated at 37°C in 5% CO₂ in humidified 10 X 35-mm tissue culture dishes (Nunc Inc, Naperville, IL) for 12 to 14 days and colonies (>50 cells) were counted on an inverted microscope and scored as colony-forming units granulocyte and macrophage (CFU-GM), burst-forming units-erythroid (BFU-E), or multilineage colony-forming units (CFU-Mix).

**Day 12 CFU-S assay.** Donor cells from murine LTMC were injected via tail vein into lethally irradiated C57Bl/6J recipient mice (11 Gy, 96 cGy/min in two doses divided by at least 4 hours, using a 137Cs irradiator, Norland, Kanata, Canada). A minimum of five mice were used as recipients per time point per experiment. Twelve days after marrow infusion, the recipients were sacrificed by cervical dislocation, spleens were removed and fixed in Telly’s fixative (70% ethanol:acetic acid:formalin 20:1:1), and the macroscopic surface colonies present in each spleen were counted using a dissecting microscope.

**HPP-CFC assay.** Double-layer agar cultures were prepared as described. Recombinant hematopoietic growth factors including 100 ng rhSCF (Amgen), 1,600 U human macrophage colony-stimulating factor (M-CSF, Genetics Institute), 200 U murine IL-3 and 1,000 U murine IL-1α (Genzyme) were added to each 10 X 35-mm grided tissue culture dish. The underlayer contained 1 mL α-MEM media with 0.5% agar, 2 mmol/L glutamine, 0.224% (wt/vol) sodium bicarbonate, 20% FCS, penicillin, and streptomycin. Triplicate cultures of 5 X 10⁵ nonadherent or adherent cells from murine LTMC were plated in 0.5 mL α-MEM media (containing 0.33% agar, glutamine, sodium bicarbonate, FCS, penicillin, and streptomycin as in the underlayer) per dish with above cytokines and incubated in 5% O₂, 10% CO₂ in humidified atmosphere. After 14 days incubation, the dense compact macroscopic colonies greater than 0.5 mm were scored as HPP-CFC.

**Long-term repopulation assay.** All cells from 4-week-old murine LTMC established with bone marrow from C57Bl/6J mice (homozygous for single hemoglobin, Hbb/Hbb) were intravenously injected to WBB6F1-W/W⁺ mice (heterozygous for diffuse and single hemoglobin, Hbb⁺/Hbb⁻), at cell dilutions of 0.5 flask/mouse, 1 flask/mouse and 2 flasks/mouse, respectively. The presence of single hemoglobin and disappearance of diffuse hemoglobin were examined monthly posttransplantation as described. The presence of single hemoglobin without diffuse hemoglobin in W/W⁺ recipient mice at 4 months posttransplantation was considered evidence of reconstitution by donor stem cells present in LTMC.

**RESULTS**

**Effects of IL-11 on cellularity of LTMC.** IL-11-treated human LTMC demonstrated higher NA cellularity from
weeks 2 to 8. This increase in NA cell production reached statistical significance at week 4 of LTMC. However, after prolonged exposure of human LTMC to IL-11, treated cultures demonstrated lower NA cell production than control cultures (Fig 1A). In contrast to human LTMC, IL-11 added to murine LTMC resulted in more profound and persistent increases in NA cellularity (Fig 1B). IL-11 significantly increased NA cells from weeks 3 through 13 in murine LTMC. IL-11-treated murine cultures showed a 20-fold increase in NA cellularity compared with control cultures at week 7 of LTMC, which was the peak of NA cellularity of IL-11-treated cultures. These data are from one of three independent experiments showing similar results. We also examined the effect of IL-11 on the adherent cellularity of murine LTMC. In contrast to the effect on human LTMC, the addition of IL-11 was found to decrease the cellularity of adherent cells in murine LTMC, and this decrease only reached significance at weeks 15 and 17 of murine LTMC (data not shown).

Evaluation of progenitor compartments. Addition of IL-11 to human LTMC resulted in a fourfold increase in the cumulative production of CFU-Mk over 6 weeks of culture (Fig 2). Figure 3 shows the effects of IL-11 on myeloid progenitor production in both human and murine LTMC. At weeks 4 and 6 of human LTMC, the addition of IL-11 increased CFU-GM production by threefold and twofold, respectively, compared with control cultures (Fig 3A). However, by weeks 10 and 12, IL-11-treated human LTMC demonstrated significantly lower CFU-GM production (Fig 3A). In contrast to these effects in human LTMC, IL-11 resulted in a prolonged and dramatic increase in myeloid progenitor content in murine LTMC (Fig 3B). IL-11-treated murine cultures demonstrated significantly higher CFU-GM content than control cultures from week 4 to week 13. By week 7 of murine LTMC, IL-11 treatment resulted in more than a 40-fold increase in CFU-GM content in NA cells (24,534 ± 2,360 in IL-11 treated cultures vs 607 ± 67 in control cultures, mean ± SD) and more than 4-fold increase in CFU-GM content in adherent cells (8,370 ± 3,430 in IL-11 vs 1,969 ± 260 in control, mean ± SD).

Evaluation of less primitive multilineage stem cell compartments. Addition of IL-11 to murine LTMC enhanced the production of day 12 CFU-S in both nonadherent and adherent layers (Table 1). Although there were no significant differences in the number of CFU-S12 from the adherent layer at 3 weeks of LTMC, IL-11 markedly increased CFU-S12 content in the adherent layer at week 7. However, by weeks 13 and 15 of culture, the content of CFU-S12 in the adherent layer of IL-11-treated cultures became undetectable or much lower than in control cultures (Table 1). IL-11 significantly increased CFU-S12 numbers in the nonadherent cells from week 5 through week 10 (data from weeks 7 and 10 shown) of LTMC (Table 1). At week 7 of murine LTMC, IL-11-treated cultures demonstrated greater than a 27-fold increase in CFU-S12 compared with control cultures in nonadherent cells. Again, later in the culture period, IL-11-treated cultures showed diminished CFU-S12 numbers in the NA cell population compared with control, similar to the effect of IL-11 on CFU-S12 in the adherent cells. Table 1 also summarizes the effect of IL-11 on total day 12 CFU-S numbers from both nonadherent and adherent cells in murine LTMC. IL-11 addition increased total CFU-S12 at weeks 7 and 10, but decreased CFU-S12 numbers at weeks 13 and 15. The effect of IL-11 on more primitive HPP-CFC numbers was more complicated (Table 2). IL-11-treated cultures demonstrated consistently higher HPP-CFC numbers in non-
adherent cells compared with control cultures from week 5 to week 10 (week 5 data not shown). The increased HPP-CFC numbers in the NA cells were concurrent and proportional to decreased HPP-CFC numbers in the adherent cell layer (Table 2). The net effect of IL-11 addition to murine LTMC was no significant change in the number of HPP-CFC during the 15 weeks of culture (Table 2).

**Effects of IL-11 on more primitive stem cell compartments.** In contrast to the effects of IL-11 on committed and multilineage progenitor compartments in LTMC, IL-11-treated human LTMC demonstrated a decreased frequency of primitive hematopoietic stem cells compared with control cultures at week 4 and week 8 using limiting-dilution LTC-IC assay (Table 3). At week 4 of human LTMC, the frequency of LTC-IC in IL-11–treated cultures were almost twofold lower than control cultures. At week 8 of culture, LTC-IC in IL-11–treated cultures became undetectable, while LTC-IC were still measurable in control cultures. By week 12, no LTC-IC were detected in either control or IL-11–treated human LTMC. To more precisely determine the effect of IL-11 on reconstituting hematopoietic stem cells, we quantitated stem cells present in murine LTMC by an in vivo long-term repopulation assay (Fig 4). After 4 weeks of IL-11 treatment, the frequency of long-term reconstituting stem cells (assayed 6 months after transplantation) was decreased in murine LTMC compared with control cultures. None of 15 W/W’ recipients were reconstituted by infusion of 2 flasks per mouse from IL-11–treated LTMC. Mice infused with cells from vehicle treated (control) cultures demonstrated dose-dependent engraftment with 17% (one of six mice), 33% (two of six mice), and 75% (three of four) of mice reconstituted by infusion 0.5 flask, 1 flask, and 2 flasks per mouse, respectively (Fig 4).

**DISCUSSION**

Previous studies have shown that IL-11 has potent stimulatory activity on megakaryopoiesis both in vitro and in vivo. These data suggest that IL-11 stimulates a

| Table 1. Effect of IL-11 on CFU-Sₖ孵 Production in Murine LTMC |
|-----------------|-----------------|-----------------|-----------------|
|                 | Nonadherent     | Adherent        | Total           |
|                 | BSA             | IL-11           | BSA             | IL-11           |
| Week 3          | ND              | ND              | 24.8 ± 3.6      | 16.2 ± 7.5      |
| Week 7          | 75.7 ± 23.0     | 2,069.9 ± 404.8* | 122.0 ± 7.2    | 539.2 ± 101.2*  |
| Week 10         | 37.1 ± 9.5      | 434.5 ± 152.4*  | 74.5 ± 19.0     | 211.5 ± 62.9†   |
| Week 13         | 6.2 ± 4.2       | ND              | 23.4 ± 0.3      | ND              |
| Week 16         | 12.8 ± 19.4     | 2.0 ± 2.0*      | 57.5 ± 5.2      | 12.2 ± 6.5*     |

Mean ± SD from one of three experiments with five animals/group. Abbreviation: ND, not detected.

* P < .01 vs BSA groups.
† P < .05 vs BSA groups.
Table 2. Effect of IL-11 on HPP-CFC Production in Murine LTMC

<table>
<thead>
<tr>
<th></th>
<th>Nonadherent</th>
<th>Adherent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA</td>
<td>IL-11</td>
<td>BSA</td>
</tr>
<tr>
<td>Week 3</td>
<td>4.0 ± 5.8</td>
<td>6.4 ± 6.3</td>
<td>ND</td>
</tr>
<tr>
<td>Week 7</td>
<td>256.0 ± 143.6</td>
<td>1473.6 ± 705.6*</td>
<td>689.4 ± 279.6</td>
</tr>
<tr>
<td>Week 10</td>
<td>274.7 ± 56.1</td>
<td>1631.4 ± 578.8*</td>
<td>1415.0 ± 933.0</td>
</tr>
<tr>
<td>Week 13</td>
<td>124.0 ± 122.0</td>
<td>92.7 ± 34.4</td>
<td>ND</td>
</tr>
<tr>
<td>Week 15</td>
<td>0.4 ± 0.5</td>
<td>ND</td>
<td>9.1 ± 0.4</td>
</tr>
</tbody>
</table>

Mean ± SD from one of three experiments with five flasks per group performed in triplicate.

Abbreviation: ND, not detected.
* P < .01 vs BSA groups.
† P < .05 vs BSA groups.

Marrow cultures resulted in a significant increase in both cellularity and production of myeloid-restricted progenitor cells, including a consistent increase in the production of megakaryocyte progenitor cells, CFU-Mk. We have previously reported increased cellularity and myeloid progenitor production in human LTMC treated with IL-11, although our previous studies did not examine effects on CFU-Mk. Multilineage progenitors and less primitive stem/progenitor cells, such as CFU-Mix and day 12 CFU-S are also increased in the early phases of LTMC by addition of IL-11. Production of these multilineage progenitor cells appear to diminish as the culture period lengthens, perhaps as a result of effects of IL-11 on the stromal compartment or due to exhaustion of more primitive progenitors. We have yet examined whether this effect can be modulated by addition of smaller amounts of IL-11 in these cultures. Human IL-11 was detected at high concentrations before feeding in the treated cultures by enzyme-linked immunosorbent assay (ELISA) (X.X. Du and D.A. Williams, unpublished data), demonstrating that exogenous hIL-11 protein was stable in this in vitro culture system. We are currently examining the effects of IL-11 on the stromal cells in the HM, although our laboratory and other investigators have previously demonstrated potent inhibitory effects of IL-11 on differentiation of adipocytes.21–28

More primitive stem cell compartments were analyzed in murine cultures by long-term reconstitution in vivo and by the use of HPP-CFC cultures in vitro. No quantitative effects were seen on HPP-CFC numbers, although IL-11 clearly lead to mobilization of HPP-CFC from the adherent compartment to the nonadherent compartment of LTMC. Whether this mobilization from the stromal adherent layer is a similar phenomenon to mobilization of pluripotent progenitor cells from the BM to peripheral blood is unknown. IL-11 has been shown to be a potent mobilizer of these cells in vivo in both mice and humans (S. Goldman, personal communication, November 1994). The ability of cells to reconstitute hematopoiesis in vivo remains a strict definition of the hematopoietic stem cell. The ability of LTMC to reconstitute genetically anemic W/W* mice was clearly diminished by chronic exposure to IL-11, suggesting that the number of primitive stem cells were decreased by IL-11 treatment.

A current limitation of experimental analysis of human hematopoietic stem cell populations is the lack of a reconstituting assay system. Several in vitro assays have been developed, which measure primitive human hematopoietic cells, although the exact correlation of the cells scored in these assay systems with the cell responsible for long-term reconstitution in vivo remains unknown.29 Several investigators have hypothesized that the ability of cells to initiate long-term marrow cultures correlates with reconstitution capacity and quantitative analysis of this cell, termed long-term culture-initiating cells, as has been described.30,31

The chronic exposure of human LTMC to IL-11 appears to diminish the frequency of LTC-IC. Because murine and

Table 3. Effect of IL-11 on LTC-IC Frequency in Human LTMC

<table>
<thead>
<tr>
<th>Weeks of LTMC</th>
<th>Control*</th>
<th>IL-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.6 X 10^-4</td>
<td>2.6 X 10^-4</td>
</tr>
<tr>
<td>4</td>
<td>3.0 X 10^-4</td>
<td>5.6 X 10^-4</td>
</tr>
<tr>
<td>8</td>
<td>2.0 X 10^-5</td>
<td>Undetectable</td>
</tr>
<tr>
<td>12</td>
<td>Undetectable</td>
<td>Undetectable</td>
</tr>
</tbody>
</table>

* Frequency of LTC-IC determined by limiting dilution.30
† Frequency <2 X 10^-5.

Fig 4. Effect of IL-11 on long-term reconstituting stem cell in murine LTMC. Data represent percentage of W/W* mice reconstituted by infusion cells from 4-week-old LTMC. Animals were analyzed at 6 months posttransplantation. Data were pooled from two or three independent experiments.
human cultures were established under similar conditions and the effects of IL-11 on more differentiated progenitors in these cultures appears very similar, the correlation of the effects of IL-11 on LTC-IC in human cultures and reconstituting hematopoietic stem cells in murine studies may be significant.

Previous studies have demonstrated that IL-11 acts synergistically with other cytokines, such as IL-3 and SCF or G-CSF, in combination with IL-3 to expand primitive LTC-IC populations capable of generating HPP-CFC. These combinations of growth factors appear not to be functional in long-term marrow cultures, as no expansion of LTC-IC was seen during any time point of long-term cultures studied in this report. IL-11 with IL-3, IL-4, and SCF stimulates the entry of dormant hematopoietic blast colony forming cells into cell cycle. Taken together with the data presented in this report, it appears that IL-11 in combination with additional cytokines present in the LAN of the hematopoietic microenvironment may be capable of affecting commitment of primitive stem cells into the multilineage progenitor compartment.

These data suggest several possible clinical uses of IL-11. For instance, expansion of multilineage progenitors in vivo may prove advantageous in the treatment of pancytopenias associated with cancer therapies and congenital or acquired BM failure syndromes. Our laboratory has recently reported that IL-11 administered to mice with significant pancytopenia due to nitrosourea therapy leads to improved peripheral counts of all lineages and subsequent increased survival of mice. Delayed pancytopenia is a dose-limiting toxicity of nitrosoureas. In addition, IL-11 has been shown to hasten the recovery of neutrophils and platelets in murine BMT recipients and after cytoxan treatment in vivo. Recent reports of the initial phase I trial of IL-11 in women treated with cyclophosphamide and doxorubicin suggested that IL-11 has thrombopoietic effects in humans. Given data, which has accumulated in murine experiments, the effects of IL-11 on peripheral cytopoenias may vary depending on the specific chemotherapy regimen. Finally, stimulation of dormant primitive cells into cell cycle in vitro may provide important implications in the successful use of retroviral vectors to transduce primitive hematopoietic cells for genetic therapy of hematopoietic diseases.

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