Effects of Recombinant Human Interleukin-11 on Hematopoietic Reconstitution in Transplant Mice: Acceleration of Recovery of Peripheral Blood Neutrophils and Platelets

By X.X. Du, T. Neben, S. Goldman, and D.A. Williams

We have examined the effects of recombinant human interleukin-11 (rhIL-11) on the recovery of peripheral blood cell counts and proliferation of progenitors and hematopoietic stem cells (day 12 colony-forming units-spleen–CFU-S12) in vivo using a mouse bone marrow (BM) and spleen cell transplantation model. Recovery of leukocytes was accelerated in animals receiving daily administration of rhIL-11 (100 μg/kg/d) and reached normal levels by day 14 posttransplantation. This increased total leukocyte count reflected mainly an increase in neutrophils. Neutropenia (absolute neutrophil count [ANC] < 1,500) was present in control transplant mice for 14 to 15 days, while in the rhIL-11–treated group, neutrophils recovered to normal by days 8 to 10 and continued to increase until day 19. Animals treated with rhIL-11 had only 1 day with ANC demonstrated <500. Correspondingly, rhIL-11 treatment increased granulocyte-macrophage progenitors (CFU-GM) derived from both spleen and BM cells. Higher doses of IL-11 increased CFU-GM nearly threefold and CFU-Mix fourfold to fivefold, while increasing burst-forming units-erythroid to a lesser degree. BM and spleen cellularity were both increased in IL-11–treated mice, but no increase in CFU-S12 was noted. In addition, in vivo daily administration of IL-11 increased peripheral platelet counts by threefold over control transplant mice at day 10 posttransplantation during the postirradiation platelet nadir. Further treatment led to platelet counts higher than normal 18 days posttransplantation when control animals had just attained normal platelet counts. IL-11 can accelerate the recovery of the peripheral blood leukocytes, mainly neutrophils, and platelets in transplant mice, effects that may be clinically useful in future applications for BM transplantation and chemotherapy-related cytopenias.

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MATERIALS AND METHODS

BM transplantation (BMT) and IL-11 administration. BM was harvested by gentle flushing from the hind limbs of normal 8- to 10-week-old male C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) using a minimum essential medium (α-MEM; Gibco, Grand Island, NY) containing 5% fetal calf serum (FCS; Gibco). For some experiments, donor mice were pretreated with rhIL-11 (obtained from Genetics Institute, Boston, MA) by subcutaneous injection at 100 μg/kg/d or 0.1% bovine serum albumin (BSA) (0.2 mL/mouse-control; see below) for either 5 or 10 days before harvesting BM for reinfusion. After total body irradiation with 10.8 Gy (137Cs at 126 cGy/min, split dose with a minimum of 3 hours between doses), 1 × 10⁶ BM cells and 1 × 10⁶ spleen cells were injected intravenously (IV) into 10-week-old mice. This dose of irradiation is lethal to 100% of mice without BM infusion.

rhIL-11 was diluted in Hank's Balanced Salt Solution (HBSS; Gibco) containing 0.1% BSA (wt/vol; Boehringer-Mannheim, Indianapolis, IN) and 0.025 mol/L HEPES solution. rhIL-11 (100 to 500 μg/kg body weight) was injected subcutaneously in 0.2-mL vol daily starting on the same day as irradiation and infusion of donor BM. Control mice received the same volume of HBSS/0.1% BSA. Cytokine administration continued for 18 to 28 days posttransplantation. In some experiments, IL-11 was administered once per day, while in the majority of experiments described here IL-11 was administered twice per day. Mice were weighed every 4 to 6 days during the posttransplantation period. For sublethal irradiation experiments, mice received 6.0 Gy irradiation in one dose and were started on rhIL-11 (using the protocol above) administration the same day of the irradiation dose. In these animals, no BM was infused postirradiation. This dose of irradiation resulted in ~10% mortality to the irradiated mice.

Hematologic analysis. Hematologic analysis of leukocyte cell counts and platelet counts were performed on tail vein bleeds on a Coulter Model ZM (Coulter Electronics, Hialeah, FL) using a 100-μm aperture for leukocyte determinations and a 50-μm aperture for platelet determinations. Red blood cells (RBCs) were lysed using Zapoglobin (Coulter Electronics) according to the manufacturer's recommendations. Automated counts were randomly checked with manual counts throughout the experimental period. Blood smears were stained with the Wright-Giemsa using standard methods and examined at 100× for differential analysis. The absolute neutrophil count (ANC), absolute monocyte count (AMC), absolute eosinophils count (AEC), and absolute lymphocyte count (ALC) in the peripheral blood was calculated by multiplying the total leukocyte count by the percentage of each cell type in the corresponding differential analysis. Peripheral blood hematocrits were performed by spinning capillary tubes for 5 minutes in a Model MB Micro-Capillary Centrifuge (IEC, Boston, MA).

Progenitors and day 12 colony-forming units-spleen (CFU-S12) assays. Transplanted mice were killed at days 12, 20, and 28 following transplantation by cervical dislocation. BM cells (harvested from the hind limbs of all animals) and spleen cells from individual animals were analyzed for cellularity and progenitor content (CFU-granulocytes and macrophages, CFU-GM; burst-forming units-erythroid, BFU-E; multilineage colony forming units, CFU-Mix; and megakaryocyte colony-forming cell, Meg-CFC). One hundred thousand pooled BM cells or low-density spleen cells (the interface cells following a 30-minute centrifugation at 1,500 rpm on Histopaque-1119 [Sigma Chemical Co, St Louis, MO]) were plated in 1-mL aliquots of α-MEM/methylcellulose (Fluka, Hauppauge, NY), 30% FCS (Sigma), 1% penicillin/streptomycin, 2 × 10⁻² mol/L L-glutamine (all Gibco), 10⁻³ mol/L β-mercaptoethanol (Sigma). Growth factors used included 3 U/mL erythropoietin and 50 ng/mL stem cell factor (SCF; Amgen, Thousand Oaks, CA), and 10 U/mL murine IL-3 (Genzyme, Cambridge, MA). Progenitor cocktails were plated in 1-mL cultures (in duplicates) in 10 × 35 mm tissue culture plates (Lux; Mile Scientific, Naperville, IL) and placed in 5% CO₂ at 37°C. Colonies were counted (>50 cells) at day 14 of cultures.

For Meg-CFC assays, marrow was flushed from the femurs with CATCH buffer (0.38% sodium citrate, 2 × 10⁻² mol/L theophylline, 1 × 10⁻³ mol/L adenosine in HBSS, pH 7.2) supplemented with 1 μg/mL prostaglandin E₁ (Sigma) and 3% BSA (Sigma) (supplemented CATCH) using a 25-gauge needle on a 10-ml syringe. A single cell suspension was made by passing the marrow through an 18-gauge needle three times. Cells were then centrifuged at 1,200 × g (350g) for 5 minutes. The pellets were resuspended in 10 mL of BCA lysis buffer (0.14 mol/L NaCl, 17 mmol/L Tris-HCl, pH 7.2) and incubated for 10 minutes at room temperature. The cells were then washed once in supplemented CATCH buffer and counted on a Baker 9000 hematology analyzer (Allentown, PA). Spleen pieces were teased between two forceps into 5 mL of CATCH buffer (no supplements). Large tissue pieces were removed from the suspension by allowing them to settle, and the cells were passed through an 18-gauge needle three times to form a single cell suspension. The spleen cells were centrifuged at 350g for 5 minutes. The pellets were resuspended in Iscove's modified Dulbecco's Modified Eagle Media (DME) (IMDM; Gibco) supplemented with 10% fetal bovine serum and counted on the Baker hematology analyzer. Meg-CFC were quantitated in semi-solid medium. Meg-CFC were established by the addition of 1 × 10⁵ marrow cells or 1 × 10⁶ spleen cells to a mixture of 0.325% agar (Difco, Detroit, MI), 1% IMDM supplemented with 10% FBS, 10% Wehi-3b conditioned medium (as a source of IL-3), and 30 U/mL of rhIL-11. Control wells contained the same volume of medium in place of conditioned medium and IL-11. Gels were allowed to form in 35-mm wells for 10 minutes before incubation for 6 days at 37°C in 5% CO₂, 95% humidified air. The gels were then air dried and stained for the presence of acetylcholinesterase. A megakaryocyte colony was defined as a positively stained group of three or more megakaryocytes.

For CFU-S₁₂ determinations, 1 × 10⁵ BM cells harvested from transplant recipient mice were injected IV into lethally irradiated mice (as above). Spleens were harvested and fixed in Bouin's solution at days 12 to 14 posttransplantation and colonies were counted using an inverted dissecting microscope.

RESULTS

Hematologic response after low-dose rhIL-11 administration in normal and sublethally irradiated mice. After administration of rhIL-11 (100 μg/kg/d) for 5 to 12 days to normal mice, total leukocyte counts and platelet counts in peripheral blood were slightly increased (10% higher than BSA group, 9,700 ± 2,600 vs 8,800 ± 1,450, mean ± SD, n = 4) while the percentage of neutrophils and ANC were 62% and 86% higher than BSA-treated groups (28.5% vs 17.6% and 3,000 ± 2,200 vs 1,600 ± 700, mean ± SD), respectively. These changes were not statistically significant compared with control groups. However, there was a higher percentage of 64N modal megakaryocyte ploidy (as determined by two-color flow cytometry) in IL-11–treated mice than in control mice (21% vs 16%) and a lower percentage of 32N megakaryocytes (40% vs 49%) (the modal megakaryocyte ploidy of normal C3H mice was 64N).

Table 1 shows the hematologic responses of rhIL-11 administration in sublethally irradiated mice. As expected with this dose of irradiation, animals became cytopenic shortly after irradiation. rhIL-11 administration blunted thrombo-
cytopenia in sublethally irradiated mice. With the daily administration of rhIL-11 (250 μg/kg/mouse) for 5 days after irradiation, platelet counts were 50% higher than control irradiated mice (690,000 ± 460,000/mm³). Total leukocyte counts were increased twofold. No difference in hematocrits was observed in these mice. In addition, there was no difference in BM or spleen cellularity or CFU-GM and BFU-E content of BM of sublethally irradiated mice treated with rhIL-11 compared with control mice (data not shown).

Effect of rhIL-11 on the recovery of peripheral blood cell counts in transplant mice. Table 2 shows the effect of daily administration of 250 μg/kg/d rhIL-11 on the recovery of peripheral total leukocyte counts and specific types of white blood cells (WBCs) in transplant-recipient mice. Total leukocyte counts in control mice decreased to as low as 600 cells/mm³ by 4 days after transplantation. Although total leukocyte counts gradually increased in control mice, normal levels were still not attained by 28 days after transplantation, reaching 50% of normal at the endpoint of the experiments (not shown). However, in mice treated with rhIL-11, the recovery of total leukocytes was enhanced, reaching a level of threefold or fourfold higher than control transplant mice at day 10 posttransplantation and fourfold to fivefold higher than control at day 18. The large standard deviation seen in treated mice was caused by isolated animals that developed extremely high WBC counts after repeated doses of IL-11.

The main stimulatory effect of rhIL-11 administration was on the neutrophil compartment (Table 2 and Fig 1). While no significant effects were seen on ALC, AMC, or AEC at 250 μg/kg/d (Table 2), at the lowest dose of rhIL-11 tested (100 μg/kg/d administered in a single dose), the percentage of neutrophils increased from 53% to 69% of the total leukocyte count in control transplant groups to 81% to 91% of the total leukocyte count in mice receiving daily treatment of rhIL-11 after transplantation. ANCs were higher by 4 days posttransplantation and were significantly higher by day 8 posttransplant. ANCs were normal by day 10 posttransplant in IL-11–treated mice and continued to increase until day 19 (Fig 1). After reaching a peak between days 18 and 20 posttransplantation, ANCs subsequently returned toward normal, even with continued IL-11 treatment reaching ANC of 5,600/mm³ at day 28 posttransplantation (data not shown). Radiation-induced neutropenia that occurred in control transplant mice continued until day 15, while in IL-11–treated mice the ANC returned to normal by day 15 (Fig 1). The percentage of neutrophils in IL-11–treated mice was 53% to 69% of the total leukocyte count, compared with 43% to 57% in control mice, which was not significantly different. The percentage of monocytes and lymphocytes was also not significantly different between the two groups. The percentage of eosinophils was lower in IL-11–treated mice compared with control mice, but this difference was not statistically significant.
groups, ANC < 500 was demonstrated only once during the posttransplant period (mean = 463 at day 4 in IL-11–treated group). No difference in neutrophil recovery was seen between mice receiving 150, 250, and 500 μg/kg/d of IL-11 (data not shown) and these data were similar to that obtained with doses of IL-11 of 100 μg/kg/d (Fig 1).

Figure 2 shows the effect of rhIL-11 administration in vivo on the recovery of platelet counts in transplanted mice. The platelet count nadir occurred at days 8 to 10 in control mice and platelet counts recovered to normal (10^9/mm^3) at day 18 after transplantation. Platelet counts were statistically higher than controls in mice treated with either 150, 250, or 500 μg/kg/d of rhIL-11 on days 10, 14, and 18, except in mice receiving the lower dose on day 18. At all three doses of IL-11, platelet counts were normal by day 14, 4 days before control mice attaining normal platelet counts. In mice receiving 300 μg/kg/d of rhIL-11 platelet counts were significantly higher at day 18 than the platelet counts in both control mice and mice receiving 150 μg/kg/d. The increase in platelet count was associated with increased numbers of megakaryocytes present in BM and spleen sections in mice treated with higher doses of IL-11 (see below, Fig 3). In contrast to the effects of low-dose IL-11 on neutrophil recovery, at the lowest dose of IL-11 (100 μg/kg/d) tested, no statistically significant changes in peripheral platelet counts were seen (data not shown) until day 20 posttransplantation when IL-11–treated mice showed increased platelet counts compared with the normal platelet counts in the control mice (952,000 v 1,106,000/mm^3, P < .01, n = 6 in each group). Although no difference was seen in peripheral platelet counts in mice receiving 100 μg/kg/d of IL-11 until late after transplantation, an increase in the concentration and total number of Meg-CFC in the spleens of these mice was noted on both day 12 and day 20 posttransplant, with the values reaching significance at day 20 (Table 3). rhIL-11 elicited negligible effects on hematocrit in this strain and these changes also were not statistically significant (data not shown).

**Influence of rhIL-11 on hematopoiesis in transplant mice.** Table 4 shows no significant difference in BM or spleen cellularity of mice treated with low-dose IL-11 (100 μg/kg/d) compared with control mice. However, pronounced changes were observed in mice treated with higher doses of IL-11 (>150 μg/kg/dose) after transplantation (Table 4 and Fig 3). Treatment of rhIL-11 led to significantly increased spleen and BM cellularity compared with control mice killed at day 18 posttransplantation. The increase in spleen cellularity was also reflected in low-density mononuclear cells (spleen mono) following separation by density gradient centrifugation and therefore represented increase in cell populations other than neutrophils in the spleen. There was no further increase in spleen cellularity with the continuation of IL-11 treatment past day 18 (data not shown). Spleen cellularity recovered to normal at day 28 posttransplantation in control transplant mice. At higher doses of IL-11 (>250 μg/kg/d) a marked increase in megakaryocytes was observed in the BM of treated mice (Fig 3c).

BM and low-density spleen cells from transplant mice were analyzed for in vitro progenitor and in vivo CFU-S12 cell content. Figure 4 and Table 5 show that rhIL-11 significantly increased CFU-GM progenitors derived from BM cells of treated mice. Higher doses of IL-11 increased CFU-GM in BM cells nearly threefold and CFU-Mix fourfold to fivefold (dose at 500 μg/kg/d) (Table 5 and Fig 4). Increased to a lesser degree were BFU-E progenitors. BM cells were also assayed for stem cell content and, as seen in Table 5, rhIL-11 did not result in a significant increase in CFU-S12 content since the concentration of CFU-S decreased as BM cellularity increased. Animals that were transplanted with BM harvested from mice pretreated with IL-11 for 6 days before BM harvest (no posttransplant treatment with rhIL-11) demonstrated transiently increased peripheral leukocyte counts between days 4 and 8 posttransplantation (data not shown). These data suggest that the myeloid precursor compartment was expanded in these pretreated mice.

**DISCUSSION**

Hematopoiesis occurs both in vitro and in vivo in close association with a complex group of cells termed the hematopoietic microenvironment (HM). Based on analysis of murine hematopoiesis in the BM and splenic environments and in clonal-derived hematopoietic colonies arising in transplanted tissue, Wolf and Trentin postulated that the HM plays an “inductive” role in stem cell differentiation (hematopoietic inductive microenvironment). Although the biochemical and molecular basis for this local control over hematopoiesis remains largely unknown, these observations have led to attempts at defining specific factors produced by cells making up this HM that have direct effects on hematopoiesis. One approach to studying the cellular interactions involved in the HM control of hematopoiesis has been immortalization and cloning of specific cell types that reside in HM of adult and fetal tissues. One cell line derived for this purpose, PU-34, was immortalized from LTMC established from the medullary cavity of a nonhuman primate and supports the growth and differentiation of both primate and human hematopoietic cells in vitro and growth factor production by this cell line has been analyzed in detail. The ability of conditioned media from PU-34 cells to stimulate the proliferation of a variety of progenitor cell types, not all of which were expected based on known growth factors...
Fig 3. Effect of IL-11 on BM cellularity in transplant recipient mice. Arrowheads denote megakaryocytes, original magnification ×200. (a) Control; (b) 150 μg/kg/d; (c) 250 μg/kg/d.
produced by PU-34, led to the cloning of a previously unknown hematopoietic cytokine, IL-11. Subsequently, IL-11 has been shown to be a multifunctional hematopoietic regulator and to be identical to a protein that inhibits the differentiation of a pre-adipocytic cell line in vitro. Studies in vitro have shown synergy between rhIL-11 and IL-3 in the stimulation of megakaryocyte colony (CFU-Mk) formation in murine cultures and BFU-Mk in human cultures and the apparent shortening of Go of blast colony forming cells. Previous studies have also demonstrated that rhIL-11 increases the number of Ig-secreting B cells, a response that is dependent on the presence of T cells in murine cultures and IL-1-induced IL-11 mRNA and protein induction by PU-34 suggest that IL-11 may play a physiologic role in stress situations. We have concentrated our studies on administration of IL-11 during stress hematopoiesis. In the present report we have examined the effect of in vivo administered IL-11 on the recovery of peripheral cell counts and the proliferation of more primitive progenitor and stem cells in mice after sublethal irradiation and syngeneic BMT after lethal total body irradiation.

The most prominent hematologic effect of IL-11 in these models is to promote the recovery of peripheral blood leukocytes, especially the recovery of neutrophils, following lethal irradiation and BMT. In addition, rhIL-11 resulted in a large increase in peripheral platelet counts. These results are in contrast to the effects of rhIL-11 in vivo in normal mice where the most prominent hematologic effect is on peripheral platelet counts and neutrophils are unaffected. The data obtained from the in vivo treatment of posttransplant mice are consistent with the observed synergy of IL-11 with other growth factors in the production of myeloid colony types in vitro and suggest that specific synergistic factors induced in the irradiated animal are important in the activity of the growth factor in vivo. The return toward normal of leukocyte counts after 18 to 20 days in spite of continued treatment with IL-11 may also represent this dependence on other cytokines, because several aspects of hematopoiesis are normalizing during this period in transplanted mice (spleen and BM cellularity, peripheral blood counts). Because BM injury is a common feature of both chemotherapy and radiation therapy, IL-11 might well provide cycle-specific stimulation of neutrophils during cancer therapies.

### Table 3. Effect of rhIL-11 on CFU-Mk Production in BMT Mice

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<thead>
<tr>
<th>Day 12</th>
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<tr>
<td></td>
<td>BM</td>
<td></td>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>CFU-Mk/2 × 10⁶ Cells</td>
<td>CFU-Mk/Hind Limb</td>
<td>CFU-Mk/1 × 10⁶ Cells</td>
<td>CFU-Mk/Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.5 ± 6.4 (2)</td>
<td>4,020.5 ± 2,079.6 (2)</td>
<td>5.3 ± 3.0 (3)</td>
<td>782.5 ± 778.2 (3)</td>
<td></td>
<td></td>
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<tr>
<td>IL-11*</td>
<td>13.8 ± 5.9 (2)</td>
<td>4,528 ± 2,378.7 (7)</td>
<td>13.2 ± 5.9 (3)</td>
<td>1,395.3 ± 836.1 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 20</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>7.4 ± 2.5 (3)</td>
<td>1,226.4 ± 208.5 (3)</td>
<td>1.9 ± 0.9 (3)</td>
<td>173.2 ± 61.7 (3)</td>
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<tr>
<td>IL-11*</td>
<td>9.9 ± 1.9 (3)</td>
<td>1,463.2 ± 364.7 (3)</td>
<td>5.1 ± 0.8 (3)</td>
<td>471.6 ± 59.9 (3)</td>
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</table>

( ), No. of animals.
* 100 μg/kg/d administered in one dose.
† v control, Student's t-test.

### Table 4. Effect of rhIL-11 on BM and Spleen Cellularity

| IL-11 Treatment* | Control | 100 μg | 250 μg | | | | |
|-----------------|---------|-------|-------|---|---|---|
| BM* | 2.9 ± 0.6 (12) | 2.6 ± 0.6 (8) | 4.1 ± 0.4 (5) | | | |
| Spleen§ | 9.1 ± 2.6 (12) | 10.1 ± 3.1 (8) | 13.3 ± 3.3 (5) | | | |
| Spleen Mono II | 1.6 ± 1.2 (10) | 1.7 ± 1.2 (6) | 4.9 ± 1.6 (5) | | | |

( ), No. of animals, NS, no statistical difference, Student's t-test.
* μg/kg/d (100 μg single dose, 150 and greater in two divided doses).
† Mean ± SD (×10⁷ cells)/2 hind limbs, all animals examined 18 days after transplantation.
§ Mean ± SD (×10⁷ cells)/whole spleen.
II Mean ± SD (×10⁷ low-density mononuclear cells)/spleen.
The development of lymphoid cells. According to these data, IL-11 may also play a key role in similar effects of IL-1 when administered in vivo in normal mice and mice made lymphopenic with cyclophosphamide. Yin et al have demonstrated transplantation of mice was also associated with more rapid platelet recovery than comparable doses in transplanted mice. In sublethally irradiated mice, rhIL-11 treatment (100 μg/kg/mouse) for 7 days was associated with higher platelet counts than control, irradiated mice, whereas transplanted mice had minimal changes in platelet counts at this dose. In contrast, the acceleration of neutrophil recovery was seen even at the lowest dose tested in transplant recipients. These data suggest that IL-11 may produce differing effects depending on the cellular and/or cytokine constituents present in the immediate HM. IL-6 has also been shown to synergize with IL-3 in vitro in the stimulation of megakaryocyte progenitors and has been demonstrated to increase murine peripheral platelet counts when administered in vivo. This similarity in response of platelets is intriguing because IL-11 was cloned based on stimulation of an IL-6-dependent cell line.

Our studies have shown that the prompt recovery of peripheral blood leukocytes (mainly neutrophils) was induced by in vivo rhIL-11 administration in lethally irradiated BM transplant recipient mice. In addition, IL-11 shortened the period of postirradiation thrombocytopenia in both transplant recipients and sublethally irradiated, nontransplant mice. At higher doses, IL-11 increased BM and spleen cellularity as well as the content of clonogenic progenitors in BM. Day 12 CFU-S were not significantly increased. These results suggest that IL-11 may mediate differentiation and expansion of early progenitors, including multilineage progenitors. IL-11 may be clinically useful in shortening the period of cytopenias after irradiation or other ablation used in BMT and chemotherapy protocols.

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REFERENCES


Table 5. Effect of rhIL-11 on Stem Progenitor Compartment

<table>
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<th>Control</th>
<th>250 μg</th>
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</thead>
<tbody>
<tr>
<td>CFU-S/1 × 10^6</td>
<td>11.8 ± 1.5 (5)</td>
</tr>
<tr>
<td>CFU-S/2 hind limbs</td>
<td>3,503.4</td>
</tr>
<tr>
<td>CFU-GM/1 × 10^6</td>
<td>51.5 ± 8.4 (4)</td>
</tr>
<tr>
<td>CFU-GM/2 hind limbs</td>
<td>15,290.4</td>
</tr>
<tr>
<td>BFU-E/1 × 10^6</td>
<td>1.3 ± 1.0 (4)</td>
</tr>
<tr>
<td>BFU-E/2 hind limbs</td>
<td>371.1</td>
</tr>
<tr>
<td>CFU-mix/1 × 10^6</td>
<td>0.8 ± 1.0 (4)</td>
</tr>
<tr>
<td>CFU-mix/2 hind limbs</td>
<td>222.7</td>
</tr>
<tr>
<td>Total progenitor/1 × 10^6</td>
<td>53.5 ± 9.3 (4)</td>
</tr>
<tr>
<td>Total progenitor/2 hind limbs</td>
<td>15,884.2</td>
</tr>
</tbody>
</table>

( ), No. of animals; all animals examined 19 days after transplantation. * μg/kg/d administered in two divided doses.
† P < .001, compared with control group, Student’s t-test.

The changes in peripheral blood counts during reconstitution were associated with increased myeloid progenitors in vivo, specifically CFU-GM, CFU-Mix, and Meg-CFC. BFU-E were not significantly increased, which correlates with the lack of effects IL-11 had on peripheral hematocrits. IL-11 had no effect on CFU-S content because the concentration of CFU-S decreased while BM cellularity increased. The lack of expansion of the CFU-S suggests that the profound increase in peripheral cell counts seen is the result of expansion of progenitor and precursor compartments either caused by proliferation of progenitors or increased commitment from the stem cell compartment. The transient increase in leukocyte counts in mice transplanted with BM harvested from mice pretreated with IL-11 is consistent with this view.

The in vitro studies of Musashi et al showed that IL-11 synergized with IL-3 to support the formation of primitive blasts colonies. Mapping studies demonstrated that the basis for this synergy was the shortening of the dormant period of stem cells in this culture system. More recently Tsuji et al showed that IL-11 belongs to the group of synergistic factors (including IL-6 and granulocyte colony-stimulating factor) that affect multilineage colony formation. Taken together, the in vivo data presented here, it appears that a primary effect of IL-11 may be to promote the commitment of dormant stem cells into more differentiated progenitors. Recent studies have also demonstrated that IL-11 promotes differentiation of human B lymphocytes in vitro in a T-cell–dependent fashion. In addition, Yin et al have shown similar effects of IL-11 when administered in vivo in normal mice and mice made lymphopenic with cyclophosphamide. According to these data, IL-11 may also play a key role in the development of lymphoid cells.

Although IL-11 has no inherent megakaryocyte colony stimulating activity, in vitro it does potentiate the action of IL-3 in promoting murine CFU-Mk in murine and BFU-Mk in humans. Administration of rhIL-11 in normal mice resulted in stimulation of megakaryocytopoiesis and increased peripheral platelet counts. Administration of rhIL-11 in transplant mice was also associated with more rapid platelet recovery, with most dramatic changes at doses greater than 150 μg/kg/d. However, at a lower dose of IL-11, the response of sublethally irradiated, nontransplanted mice was characterized by significantly larger increases in platelet recovery than comparable doses in transplanted mice. In sublethally irradiated mice, rhIL-11 treatment (100 μg/kg/mouse) for 7 days was associated with higher platelet counts than control, irradiated mice, whereas transplanted mice had minimal changes in platelet counts at this dose. In contrast, the acceleration of neutrophil recovery was seen even at the lowest dose tested in transplant recipients. These data suggest that IL-11 may produce differing effects depending on the cellular and/or cytokine constituents present in the immediate HM. IL-6 has also been shown to synergize with IL-3 in vitro in the stimulation of megakaryocyte progenitors and has been demonstrated to increase murine peripheral platelet counts when administered in vivo. This similarity in response of platelets is intriguing because IL-11 was cloned based on stimulation of an IL-6-dependent cell line.

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