Recombinant Human Interleukin-11 Stimulates Multilineage Hematopoietic Recovery in Mice After a Myelosuppressive Regimen of Sublethal Irradiation and Carboplatin

By J.P. Leonard, C.M. Quinto, M.K. Kozitza, T.Y. Neben, and S.J. Goldman

Interleukin-11 (IL-11) is a novel multifunctional hematopoietic cytokine capable of stimulating cells of the myeloid, lymphoid, erythroid, and megakaryocytic lineages in vitro. We have tested the pleiotropic properties of this cytokine on the hematopoietic recovery of mice after a combined regimen of sublethal irradiation and carboplatin administration. This regimen results in severe myelosuppression, characterized by a prolonged period of thrombocytopenia and severe anemia. Administration of recombinant human IL-11 (rhlL-11; 250 μg/kg/d) had multilineage effects on bone marrow and spleen hematopoietic activity, increasing the number of megakaryocyte, erythroid, granulocyte, and macrophage progenitors compared with the vehicle-treated controls. This was reflected in the peripheral circulation by a reduction of both the platelet and hematocrit nadirs and a significantly reduced period of thrombocytopenia and anemia in the rhlL-11-treated mice. The results from this study support the broad spectrum of biologic activities that have been attributed to rhlL-11 in vitro and suggest that this cytokine may be an effective agent in the treatment of myelosuppression associated with cancer chemotherapy and bone marrow transplantation.

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MATERIALS AND METHODS

Myelosuppressive regimen and rhIL-11 administration. Female C57BL/6 mice, 10 to 12 weeks old (Jackson Laboratories, Bar Harbor, ME), were housed five to a cage and supplied standard rodent chow diet and water ad libitum. Mice received 500 cGy sublethal irradiation from a 137Cs source followed by a single injection of the chemotherapeutic drug Carboplatin (1.2 mg/mouse intraperitoneally [IP]; Bristol Myers Squibb, Cambridge, MA). rhIL-11 (Genetics Institute, Cambridge, MA) was purified from Escherichia coli and administered by subcutaneous injection. To determine an optimal dose of rhIL-11 for this study, naive mice were injected with increasing doses of rhIL-11 and peripheral platelet counts were measured. From this study, a dose of 250 μg/kg/d, which gave maximal platelet increase, was chosen for administration to myelosuppressed mice.

rhIL-11 was administered by subcutaneous injection in two doses of 125 μg/kg each (administered morning and afternoon) in sterile saline containing 0.5% homologous mouse serum. rhIL-11 treatment was started 1 day after myelosuppression and was continued for 20 days. Control animals received an equal volume of vehicle alone. In each experiment, 15 mice were assigned to an experimental group and 5 mice were bled from each group on designated days in an alternating manner such that no individual mouse was bled more than once a week. The results for the peripheral hematocrit represents pooled data from three separate experiments (n = minimum of 10 mice for each time point). Mice were killed on day 15 (during the period of severe myelosuppression) and on days 30 or 34 (when peripheral platelet and red blood cell [RBC] counts had recovered). Hematopoietic progenitor assays were performed on pooled samples from 5 mice from each experimental group. To allow for the inherent variation associated with hematopoietic progenitor assays, aged-matched naive mice were killed on the same day and progenitor assays set up in parallel for comparison with data from the myelosuppressed mice. The progenitor data shown are from one experiment and are representative of the results obtained from three separate experiments performed at nadir and two separate experiments after recovery. In additional experiments, administration of rhIL-11 was delayed until day 3 or day 7 after myelosuppression and the recovery of peripheral platelets compared with that seen in either mice administered rhIL-11 commencing on day 1 or to vehicle-treated controls.

Peripheral hematology. Peripheral blood was drawn from the retroorbital sinus into EDTA/Heparin-coated capillary tubes and immediately transferred into EDTA-coated blood tubes. Automated hematologic analysis was performed on 20 μL of blood using a Bayer 9000 hematology analyzer (Sereno Baker Diagnostics, Alentown, PA) with mouse-specific discriminator settings. The analyses performed were platelet counts, RBC counts, white blood cell (WBC) counts, and hematocrit. In one study (n = 5 mice for each time point), the percentage of circulating reticulocytes was measured by flow cytometry using Thiazole Orange staining by a previously published method.13

Quantitation of bone marrow and spleen progenitors. The number of burst-forming units-erythroid (BFU-E) and colony-forming units granulocyte/macrophage (CFU-GM) were determined using single-cell suspensions from pooled bone marrow or spleen samples in CATCH buffer consisting of 0.38% sodium citrate (Fisher, Fair Lawn, NJ), 2 × 10^-3 mol/L theophylline, and 1 × 10^-3 mol/L adenosine (Sigma, St Louis, MO) in Hanks' Balanced Salt Solution (HBSS), pH 7.2, supplemented with 3% bovine serum albumin (BSA) and 1 μg/mL prostaglandin E1 (Sigma). Either 5 × 10^5 bone marrow cells or 5 × 10^6 spleen cells were added to 0.9% methylcellulose supplemented with 30% fetal bovine serum (FBS), 1.25% L-glutamine, 1 U/mL erthropoietin, 100 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF), and 10% WEHI-3b conditioned medium in a final volume of 1 mL. Cultures were incubated for 7 days at 37°C in 5% CO2 and 95% humidified air. Colonies were defined as discrete clusters of cells containing greater than 50 cells.

Table 1. Dose Response to rhIL-11 in Normal Mice

<table>
<thead>
<tr>
<th>rhIL-11 μg/kg/d</th>
<th>Platelets (&lt;10^9/μL)</th>
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</thead>
<tbody>
<tr>
<td>Day 3</td>
<td>Day 7</td>
</tr>
<tr>
<td>Control</td>
<td>1,037 ± 58</td>
</tr>
<tr>
<td>12.5</td>
<td>1,122 ± 33</td>
</tr>
<tr>
<td>36</td>
<td>1,188 ± 67</td>
</tr>
<tr>
<td>100</td>
<td>1,187 ± 64</td>
</tr>
<tr>
<td>250</td>
<td>1,328 ± 83</td>
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<tr>
<td>500</td>
<td>1,539 ± 183</td>
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rhIL-11 was administered subcutaneously at the designated dosage for 7 days. Mice were bled and peripheral platelets were quantitated as described in Materials and Methods. Data are presented as mean ± SD (n = 5).

Abbreviation: ND, not determined.

RESULTS

Dose response to rhIL-11 in normal mice. We have previously shown that rhIL-11 administered subcutaneously at a dose of 150 μg/kg/d for 7 days increased peripheral platelet counts in normal mice. To determine whether this was the optimal dose of rhIL-11 for platelet stimulation, we examined the platelet response to a range of doses of rhIL-11 (Table 1). The data show that rhIL-11 administration resulted in a dose-dependent increase in peripheral platelet counts. The optimal dose for the platelet response was 250 μg/kg/d. These data are consistent with the work of Du et al.15 who showed that 250 μg/kg/d was the optimal dose of rhIL-11 for the stimulation of platelet recovery in a murine model of lethal irradiation and syngeneic bone marrow transplant. Based on these two data sets, we chose a dose of 250 μg/kg/d of rhIL-11 for administration to myelosuppressed mice.

Peripheral hematology after combined modality treatment. The combined regimen of sublethal irradiation and carboplatin resulted in severe myelosuppression with a prolonged period of thrombocytopenia. Circulating platelet counts in the vehicle-treated controls reached a nadir on day 12 (95 × 10^3/mL) and remained substantially reduced for a period of 6 to 8 days. Administration of rhIL-11 commencing 1 day after the myelosuppressive treatment diminished the platelet nadir (210 × 10^3/mL, day 10) and decreased the extent and duration of thrombocytopenia (Fig 1), with periph-
rhIL-11 IN MYELOSUPPRESSED MICE

Fig 1. The effect of rhIL-11 administration on the recovery of peripheral platelets after myelosuppression. The data represent the mean ± SE from three separate experiments, with a minimum of 10 mice for each time point. Mice were bled from the retroorbital sinus on various days after myelosuppressive treatment. Peripheral platelet counts were determined on a Baker 9000 hematology analyzer. In comparison to vehicle-treated controls (○), platelet counts in the rhIL-11-treated mice (●) were significantly higher (*P < .01) on days 10 through day 21 (unpaired Student's t-test).

Fig 2. The effect of rhIL-11 administration on hematocrit recovery after myelosuppression. The data represent the mean ± SE from three separate experiments, with a minimum of 10 mice for each time point. Blood was collected as described in Materials and Methods. In comparison to vehicle-treated controls (○), the hematocrit in the rhIL-11–treated mice (●) was significantly higher (*P < .01) on days 12 and 14 through 24.

RHI-11 IN MYELOSUPPRESSED MICE

Fig 1. The effect of rhIL-11 administration on the recovery of peripheral platelets after myelosuppression. The data represent the mean ± SE from three separate experiments, with a minimum of 10 mice for each time point. Mice were bled from the retroorbital sinus on various days after myelosuppressive treatment. Peripheral platelet counts were determined on a Baker 9000 hematology analyzer. In comparison to vehicle-treated controls (○), platelet counts in the rhIL-11-treated mice (●) were significantly higher (*P < .01) on days 10 through day 21 (unpaired Student's t-test).

The combination of sublethal irradiation and carboplatin also resulted in a steady decline in the hematocrit, which reached the nadir on day 18 (18%) and recovered to near normal by day 24 in the vehicle controls. In contrast, the hematocrit nadir in mice treated with rhIL-11 occurred earlier (day 14) and was less severe (25%) (Fig 2). Hematocrit values in these mice returned to normal by day 20. RBC counts in both groups of mice were consistent with the changes seen in hematocrit (data not shown). In one study, circulating reticulocytes were measured using Thiazole Orange staining and flow cytometric analysis. Results from these studies demonstrated an increase in circulating reticulocytes that preceded the hematocrit recovery and occurred approximately 1 week earlier in the mice treated with rhIL-11 than in the vehicle-treated controls (Fig 3).

All mice suffered from severe leukopenia that lasted for more than 1 month. Administration of rhIL-11 had no effect on either the nadir or the recovery time course of the WBC count.

rhIL-11 stimulates bone marrow and spleen hematopoiesis. On day 15, during myelosuppression and on days 30 or 34, when platelet counts and hematocrit had recovered, mice from each experimental group were killed and progenitor assays were performed as described in Materials and Methods. On day 15, there was a modest increase in bone marrow cellularity in the rhIL-11–treated mice relative to vehicle-treated controls (19.4 × 10⁶ cells/femur for naive mice, 11.3 × 10⁶ cells/femur for vehicle-treated mice, and 14.7 × 10⁶ cells/femur for rhIL-11–treated mice). In the rhIL-11–treated mice, there was a significant increase in the number of bone marrow BFU-E, CFU-GM, and CFU-MEG compared with that of vehicle-treated controls (Fig 4A, B, and C, respectively, *P < .01). By day 30, bone marrow cellularity in both experimental groups was comparable to that in naive mice (data not shown). However, although the number of CFU-GM and BFU-E in the bone marrow of vehicle-treated mice showed signs of recovery from values obtained on day 15, there remained a significant reduction in the number of myeloid and erythroid progenitors relative to naive mice (50% and 40%, respectively, *P < .01; Fig 4A and B, respectively). In contrast, recovery in the rhIL-11–treated mice was such that the number of bone marrow
ministration began 1 day after the combined modality regimen of sublethal irradiation and carboplatin chemotherapy. We examined whether delaying treatment had any effect on the ability of rhIL-11 to accelerate hematopoietic recovery in this model. The data in Fig 5 show that delaying treatment until day 3 postirradiation/chemotherapy reduced the ability of rhIL-11 to accelerate platelet recovery, with this effect being most apparent between days 9 and 15. Delaying treatment with rhIL-11 until day 7 completely abrogated platelet activity in this model. Delayed dosing had similar detrimental effects on the ability of rhIL-11 to accelerate the recovery of RBC count and hematocrit (data not shown).

**DISCUSSION**

IL-11 is a multifunctional hematopoietic cytokine whose major in vitro biologic activities result from synergistic interactions with other growth factors. Current experimental data suggest that rhIL-11 has a key role in the regulation of hematopoiesis, and is capable of stimulating cells from the myeloid, erythroid, and megakaryocytic lineages. In vivo administration of rhIL-11 to naive mice predominantly effects cells of the megakaryocyte lineage, increasing the number of bone marrow CFU-MEG, stimulating megakaryocyte endoreplication, and increasing peripheral platelet counts. 22 Although IL-6 has been shown to have similar in vivo megakaryocytic stimulatory activities, 16-21 IL-6 could not be detected in serum of naive mice treated with rhIL-11 (Neben et al 13 and C. Quinto, unpublished observation). Similarly, in mice transplanted with bone marrow cells infected with a recombinant retrovirus bearing the cDNA for human IL-11 and expressing high levels of rhIL-11 protein, peripheral platelet counts were elevated in the absence of detectable IL-6 mRNA in the spleen. 22 These results argue against a role for IL-6 in mediating the thrombopoietic activities of rhIL-11.

To evaluate further the activity of rhIL-11, we have studied the effects of this cytokine on the hematopoietic recovery of mice treated with a combined regimen of low-dose irradiation and carboplatin administration. Carboplatin was specifically chosen because it has been shown to cause marked thrombocytopenia in humans. 23 This regimen resulted in severe myelosuppression with a prolonged period of thrombocytopenia and anemia. A platelet nadir of less than 10% normal values was reached 12 days after the treatment and platelet counts remained less than 20% of control values for a period of 8 days. The severity of thrombocytopenia resulting from this regimen far exceeds that induced by the administration of 5-fluorouracil, which typically causes a modest (40% to 50%) reduction in platelet counts lasting 3 to 4 days. 16 The myelosuppressive effects of carboplatin were confirmed by administration of a higher dose (1.8 mg) after the sublethal irradiation. This resulted in such severe myelosuppression that the vehicle-treated controls failed to recover after being bled at the platelet nadir (day 12). Interestingly, the therapeutic potential of rhIL-11 was still apparent in this regimen, because the mice treated with rhIL-11 survived (J. Leonard, unpublished observation). For this comparative study it was necessary to use the lower dose (1.2 mg) of carboplatin. This combination provided us
with a prolonged period of thrombocytopenia in the absence of a rebound thrombocytosis, making the model especially attractive for assessing the biologic activity of a cytokine such as rhIL-11, which is known to be a potent stimulator of megakaryocytopoiesis.

Therapeutic treatment with rhIL-11 after sublethal irradiation and carboplatin administration had significant effects on hematopoietic recovery, reducing the extent and duration of both thrombocytopenia and anemia. Furthermore, rhIL-11–treated mice had increased numbers of bone marrow myeloid, erythroid, and megakaryocytic progenitors during the period of platelet and hematocrit recovery. There was also a dramatic increase in splenic hematopoiesis in mice treated with rhIL-11 15 days after myelosuppres-
Previous studies have shown that, after sublethal irradiation, mice display a burst of splenic hematopoietic activity that can be enhanced by the addition of cytokines. However, the importance of splenic hematopoiesis to peripheral hematologic recovery is not clear. Whether increased splenic hematopoiesis reflects the stimulation of resident hematopoietic stem cells in the spleen or the mobilization of cells from the bone marrow is difficult to determine rigorously. Nonetheless, in this study it was clear that the increase in splenic hematopoietic activity in the rhIL-11-treated mice was not at the expense of the bone marrow, as cellularity and bone marrow progenitors (BFU-E, CFU-GM, and CFU-MEG) were all increased compared with vehicle-treated controls. These findings are consistent with previous studies showing that the effect of rhIL-11 administration on megakaryocyte progenitors, endoreplication, and platelet production is similar in normal and splenectomized mice.

The ability of rhIL-11 to reduce the severity of the platelet nadir marks an apparent difference between the in vivo biologic activities of rhIL-11 and IL-6. Although administration of IL-6 has been shown to increase platelet counts in vivo, treatment with IL-6 after sublethal irradiation or 5-fluorouracil administration in mice or sublethal irradiation in dogs failed to reduce the platelet nadir, even though platelet recovery was accelerated in all cases. A number of studies have reported similar stimulatory effects for both rhIL-11 and IL-6 on CFU-MEG–derived colonies when used in combination with IL-3, suggesting equivalent activities at this stage of megakaryocyte development. In contrast, stimulation of the earliest recognizable megakaryocyte progenitor, the BFU-MEG, has only been demonstrated for rhIL-11. If one assumes that in cases of severe myelosuppression, early megakaryocyte differentiation is required to initiate the platelet response, the ability of rhIL-11 to regulate megakaryocytopoiesis at multiple levels of development may account for the reduced platelet nadir and accelerated platelet recovery observed in these mice. In this context, it is interesting to note that the combination of IL-3, a well-documented early acting megakaryocytic growth factor, and IL-6 was effective in reducing the platelet nadir after 5-fluorouracil administration. However, neither IL-3 alone nor IL-6 alone had any effect on the platelet nadir. The observation in this study that rhIL-11 administered as a single agent was capable of reducing the platelet nadir and accelerating platelet recovery supports a pivotal role for IL-11 in the regulation thrombopoiesis in vivo.

In separate experiments we examined the effects of delaying the start of rhIL-11 administration after myelosuppression on the subsequent recovery of peripheral platelets. The results from these experiments demonstrated that the ability of rhIL-11 to affect the platelet nadir was only seen when rhIL-11 administration began 1 day after myelosuppression, and not when rhIL-11 administration was delayed until day 3 or 7. These data indicate that rhIL-11 must be present early after myelosuppression to ameliorate the platelet nadir, and suggest that an early interaction with a population of responsive progenitors is essential to achieve this result. In this context, preliminary studies indicate that beginning treatment with rhIL-11 on the same day as combined modality regimen may provide some additional benefit; however, further studies will be necessary to determine if these effects are statistically significant.

In naive mice, administration of rhIL-11 at a dose that stimulates megakaryocytopoiesis and platelet production has no effect on either the hematocrit or the circulating reticulocyte count. Similarly, administration of rhIL-11 to lethally irradiated, bone marrow transplanted mice accelerates the recovery of peripheral platelet and neutrophil counts but has no effect on the hematocrit or RBC recovery. In this study, administration of rhIL-11 to myelosuppressed mice had significant effects on the erythroid lineage,
reducing the severity and duration of the anemia relative to vehicle-treated controls. Mice treated with rhIL-11 had increased numbers of erythroid progenitors in the bone marrow and spleen compared with the vehicle-treated controls 15 days after myelosuppression. In addition, quantitation of circulating reticulocytes by flow cytometric analysis demonstrated a profound reticulocytosis before the hematocrit recovery, which occurred approximately 1 week earlier in the rhIL-11-treated mice than in the vehicle-treated controls. These results suggest that the accelerated hematocrit recovery in mice treated with rhIL-11 is the result of enhanced erythropoietic activity. Similar effects of rhIL-11 on erythroid recovery have been reported in a murine model of bone marrow transplantation in which mice received syngeneic bone marrow that had been retrovirally infected with the human IL-11 cDNA.  

The results from this study demonstrate the ability of rhIL-11 administration to reduce the platelet nadir and accelerate peripheral platelet recovery after a severe myelosuppressive regimen. This recovery appears to be caused, at least in part, by the stimulation of the early stages of megakaryocytopoiesis. Furthermore, the effects of rhIL-11 in this study were not restricted to the megakaryocyte lineage. Mice that received rhIL-11 displayed signs of marked stimulation of erythropoiesis that reduced the severity of anemia and accelerated the recovery of the hematocrit. Although rhIL-11 had no effect on leukocyte recovery, stimulation of both bone marrow and spleen CFU-GM was apparent in the treated mice, suggesting a potential for combination therapy with G-CSF or GM-CSF. The results from this study support the broad spectrum of biologic activities that have been attributed to this cytokine in vitro and suggest that rhIL-11 may prove effective in the treatment of myelosuppression associated with cancer chemotherapy and bone marrow transplantation.

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