**Recombinant Human Interleukin-11 Stimulates Megakaryocytopoiesis and Increases Peripheral Platelets in Normal and Splenectomized Mice**

By Tamlyn Yee Neben, Jean Loebelenz, Lori Hayes, Kyle McCarthy, John Stoudemire, Robert Schaub, and Samuel J. Goldman

The effects of recombinant human interleukin-11 (rhIL-11) on in vivo mouse megakaryocytopoiesis were examined. Normal C57Bl/6 mice and splenectomized C57Bl/6 mice were treated for 7 days with 150 μg/kg rhIL-11 administered subcutaneously. In normal mice, peripheral platelet counts were elevated compared with vehicle-treated controls after 3 days of rhIL-11 treatment and remained elevated until day 10. Splenectomized mice treated with rhIL-11 showed elevated peripheral platelet counts that were similar in magnitude to normal rhIL-11–treated mice. However, on day 10 the platelet counts in rhIL-11–treated, splenectomized mice were no longer elevated. Analysis of bone marrow megakaryocyte ploidy by two-color flow cytometry showed an increase, relative to controls, in the percentage of 32N megakaryocytes in both normal and splenectomized animals treated with rhIL-11. In normal mice, the number of spleen megakaryocyte colony-forming cells (MEG-CFC) were increased twofold to threefold relative to controls after 3 and 7 days of rhIL-11 treatment, whereas the number of bone marrow MEG-CFC were increased only on day 7. The number of MEG-CFC in the bone marrow of rhIL-11–treated, splenectomized mice was increased twofold compared with controls on both days 3 and 7 of the study. These data show that in vivo treatment of normal or splenectomized mice with rhIL-11 increased megakaryocyte progenitors, stimulated endoreplication of bone marrow megakaryocytes, and increased peripheral platelet counts. In addition, results in splenectomized mice showed that splenic hematopoiesis was not essential for the observed increases in peripheral platelets in response to rhIL-11 administration.

**INTERLEUKIN-11 (IL-11)** was originally identified as a factor produced by IL-1–stimulated PU-34 bone marrow stromal cells that could stimulate proliferation of the IL-6–dependent T1165 plasmacytoma. This biological activity was used to screen a cDNA library generated from IL-1–stimulated PU-34 mRNA expressed in COS cells. A primate IL-11 probe was then used to screen a cDNA library generated from PMA and IL-1–stimulated MRC 5, a human fetal lung fibroblast cell line. The isolated human IL-11 cDNA contained a unique open reading frame of 579 nucleotides encoding a predicted polypeptide of 199 amino acids that included a putative protein secretory sequence of 20 amino acids.

Preliminary characterization of biological activity has shown that recombinant human (rh)IL-11 is a multifunctional hematopoietic cytokine. In addition to stimulating proliferation of the T1165 plasmacytoma, rhIL-11 could stimulate immunoglobulin-producing B cells in vitro and in vivo. It has also been shown to synergize with IL-3 and IL-4 in vitro to enhance the proliferation of early progenitors by shortening the G0 period. Secondary cultures of IL-3–generated blast colonies in the presence of rhIL-11 produced macrophage colonies.

A number of reports have described multiple effects of rhIL-11 on megakaryocytopoiesis in vitro. In serum-depleted murine bone marrow cultures, addition of rhIL-11 increased megakaryocyte size, megakaryocyte ploidy, and acetylcholinesterase activity. It has also been shown to synergize with IL-3 to stimulate murine and human megakaryocyte colony formation. Similar megakaryocytic stimulatory activities have been described for other cytokines, including IL-6 and leukemia inhibitory factor (LIF).

To date, little is known about the in vivo activities of rhIL-11. We have examined the effects of rhIL-11 on hematologic cell counts and megakaryocytopoiesis in normal and splenectomized mice. The results show that rhIL-11 increased peripheral platelet counts in normal mice. In addition, there was a stimulation of both early and later stages of megakaryocytopoiesis in bone marrow and spleen. The in vivo activity of rhIL-11 in splenectomized animals was very similar to the activity in normal mice, demonstrating that splenic megakaryocytopoiesis was not necessary for the observed increase in peripheral platelets that occurred in response to rhIL-11 treatment.

**MATERIALS AND METHODS**

*Mice.* Adult female C57Bl/6 mice, 8 to 12 weeks old were purchased from Jackson Laboratories, Bar Harbor, ME. The mice were housed five to a cage and fed standard rodent chow diet with water supplied ad libitum. The average weight of the animals was approximately 20 g. During the study, the animals were monitored daily for clinical signs of ill health, behavioral changes, or reaction to treatment. Splenectomies were performed as previously described except that methoxyflurane was used for anesthesia.

Recombinant cytokine. rhIL-11 purified to homogeneity from Escherichia coli was used in these studies. The protein was diluted with saline to appropriate concentration for injection supplemented with 0.5% homologous mouse serum. The control article was saline for injection (Abbott Laboratories, North Chicago, IL) supplemented with 0.5% homologous mouse serum. Mice were treated for 7 consecutive days with rhIL-11 at a dose of 75 μg/kg twice a day (150 μg/kg/d). The cytokine was administered by subcutaneous injection (intrascapular)."
lyophilized EDTA. Automated hematologic analyses were performed on a Baker 9000 hematology analyzer (Serono Baker Diagnostics, Allentown, PA) using mouse-specific discriminator settings. The analyses included: hematocrit, WBC counts, hemoglobin, red blood cell (RBC) counts, and platelet counts. Blood smears were prepared and stained with Wright-Geimsa for WBC differentials based on a 100-cell count.

**Histology.** Samples for histology, with the exception of the sternum, were fixed in 10% neutral buffered formalin. The sternum was simultaneously fixed and decalcified for 48 hours in Calci-X I1 (Fisher, Pittsburgh, PA). The tissues were then processed in a Tissue Tek vacuum infiltration processor (Miles Inc, Dallas, TX) for paraffin embedding. The samples were sectioned at 6 μm and stained with hematoxylin and eosin.

**Preparation of bone marrow cells for flow cytometry and colony-forming cell (CFC) assays.** The femurs were cleaned of any surrounding tissue and the marrow was flushed with the femurs with CATCH buffer (0.38% sodium citrate, 2 × 10⁻³ mol/L theophylline, 1 × 10⁻³ mol/L adenosine in Hank’s Balanced Salt Solution, pH 7.2) supplemented with 1 μg/mL prostaglandin E₂ (supplemented CATCH) and 3% bovine serum albumin (BSA) (Sigma Chemical Co, St Louis, MO) 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. The cells were centrifuged at 1,200 rpm for 10 minutes and resuspended in 5 mL of supplemented CATCH buffer, counted on the Baker hematology analyzer, and directly aliquoted for flow cytometric analysis. Marrow cells for colony assay were centrifuged at 1,200 rpm for 5 minutes. The pellets were resuspended in 10 mL of RBC lysis buffer (0.14 mol/L NH₄Cl, 17 mmol/L Tris-HCl, pH 7.2) and incubated for 10 minutes at room temperature. The cells were then washed once in CATCH buffer and resuspended in Iscove’s modified Dulbecco’s Modified Eagle’s (DME) supplemented with 10% heat-inactivated (HI) fetal bovine serum (FBS).

**Preparation of spleen cells for flow cytometry and CFC assays.** The spleen pieces were teased between two forceps into several 25 mL 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 1,200 rpm for 5 minutes. The pellets were resuspended in Iscove’s modified DME supplemented with 10% HI FBS and counted on the Baker hematology analyzer.

**Quantitation of megakaryocyte progenitors.** Megakaryocyte colony-forming cells (Meg-CFC) were quantitated in semisolid medium. Meg-CFC were established by the addition of 1 × 10⁶ marrow cells or 1 × 10⁶ spleen cells to a mixture of 0.325% agar (Difco, Detroit, MI), Iscove’s modified DME 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 the WEHI-3b and rhIL-11. Gels were allowed to form in 1-mL aliquots 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 cells.

**Determination of megakaryocyte frequency and ploidy.** Megakaryocyte ploidy was determined using a modification of the method of Corash et al. Bone marrow cells (1 to 4 × 10⁹/mL) in 1 mL of supplemented CATCH buffer plus 3% BSA were incubated with 200 μg/mL of rat Ig (to block F₃ receptor) and 1 to 2 μg/mL of the megakaryocyte-specific monoclonal antibody, 4A5 (12), on ice for 60 to 90 minutes. After incubation, samples were underlayered with 1 mL of supplemented CATCH buffer plus 5% BSA and spun at 1,200 rpm (350g) for 6 minutes. The supernatants were removed and discarded. To each sample, 500 μL of supplemented CATCH buffer plus 3% BSA containing 0.5 μg/mL of streptavidin fluorescein was added, and samples were incubated on ice for 30 to 40 minutes. After incubation, samples were underlayered with 750 μL of supplemented CATCH plus 5% BSA, centrifuged as above, and supernatants were discarded. To each sample, 500 μL of supplemented CATCH plus 3% BSA and 0.5% Tween 20 were added, samples were vortexed to resuspend the pellets and placed at 4°C for 30 to 40 minutes. After incubation, 500 μL of 1% paraformaldehyde in phosphate-buffered saline (PBS) containing 0.5% Tween 20 was added to each sample and samples were incubated at 4°C for an additional 10 minutes. Samples were spun at 1,200 rpm for 6 minutes and supernatants were discarded. Pellets were resuspended in 1 mL 0.5% citrate in PBS plus 1% BSA. For ploidy determinations, 50 μg/mL propidium iodide and 2 to 5 μg/mL of RNase were added to appropriate samples and incubated for 90 minutes at room temperature. All samples were filtered through 100 micron nytex before FACS analysis. A minimum of 1,000 megakaryocytes were analyzed for ploidy determinations and frequency determinations were based on 500 megakaryocytes.

**RESULTS**

The animals in both the rhIL-11-treated group and the vehicle-treated control group exhibited normal activity and seemed healthy throughout the study. There were no notable differences between the groups. The spleens were obtained by splenectomy on day 3, following which the animals were allowed to recover for 7 days. The spleen weights were measured on days 3 and 7 and compared between the control and treated groups using Student's t-test. The results showed a significant difference between the two groups, with the treated group having a higher spleen weight compared to the control group (P < 0.05). The spleen weights of the treated group were significantly greater than those of the control group on day 3 (P < 0.01) and day 7 (P < 0.01). Data are presented as mean ±SD, n = 5 for normal mice and n = 4 for splenectomized mice.
Hematology. Administration of rhIL-11 to normal mice resulted in a significant increase in peripheral platelet counts (Table 1 and Fig 1A). After 3 days of dosing, the platelet counts of rhIL-11-treated animals were 17% above vehicle-treated controls. The platelet counts in rhIL-11-treated animals were 31% above vehicle-treated controls on day 7. On day 10, peripheral platelets in rhIL-11-treated animals remained 33% above vehicle control, and on day 15 there was no significant difference in the number of peripheral platelets between treated and control animals. Comparing mice treated with rhIL-11 with vehicle-treated controls, there were no consistent changes during the course of the study in RBC, reticulocyte, or WBC counts (data not shown). There were no changes during the course of the study in the WBC differential in either group (data not shown).

Administration of rhIL-11 to splenectomized mice also resulted in significant increases in peripheral platelet counts compared with vehicle-treated controls (Table 1 and Fig 1B). The magnitude of the platelet responses in rhIL-11-treated, splenectomized animals was very similar to the responses observed in normal rhIL-11-treated mice during the first 7 days of the study (Fig 1). However, on day 10 the platelet counts in rhIL-11-treated, splenectomized mice were no longer elevated. This contrast with the result observed in normal mice treated with rhIL-11 in which the day 10 platelet counts were still elevated.

The WBC count in splenectomized mice was about twofold higher than in normal mice. There was no consistent change in the WBC count in vehicle-treated, splenectomized controls during the course of the study (Table 1). However, the WBC counts of rhIL-11-treated, splenectomized mice were significantly decreased compared with controls on days 3 and 7, and returned to control levels on day 10 (Table 1). There were no consistent changes in the WBC differential of either vehicle-treated control or rhIL-11-treated mice during the course of the study (data not shown). No other changes in hematology were observed.

Megakaryocyte frequency. Quantitation of megakaryocytes in the bone marrow by flow cytometry showed no changes in the frequency of megakaryocytes between rhIL-11-treated animals and vehicle-treated controls in either normal or splenectomized mice (Table 2). This observation was confirmed by histologic examination of bone marrow megakaryocytes (data not shown). However, histologic evaluation of megakaryocyte frequency in the spleen showed an increase in the number of splenic megakaryocytes in rhIL-11-treated normal mice (Fig 2). This trend was apparent after 3 days of dosing with rhIL-11 and persisted throughout the course of the study.

Megakaryocyte ploidy. The ploidy distribution of bone marrow megakaryocytes was determined by two-color flow cytometry. Normal mice and vehicle-treated control mice had a modal megakaryocyte ploidy of 16N (40% to 50% of megakaryocytes) with 15% to 20% of megakaryocytes in the 8N ploidy class and 10% to 15% of megakaryocytes at 32N (Fig 3). After 3 days of dosing with rhIL-11 there was a clear increase in 32N megakaryocyte ploidy in mice treated with rhIL-11 compared with vehicle-treated controls (28% compared with 13%). There was a corresponding decrease in the percentage of 8N megakaryocytes (9% in rhIL-11-treated mice compared with 17% in vehicle-treated controls) (Fig 4A). Stimulation of endoreplication of megakaryocytes to higher ploidy classes in rhIL-11-treated mice was also observed on day 7. The rhIL-11-treated mice had an average of 26% of megakaryocytes in the 32N ploidy class compared with 16% in vehicle-treated control. The percentage of 8N and 16N megakaryocytes on day 7 in rhIL-11-treated mice.
Table 2. Megakaryocyte Frequency in Bone Marrow of Normal Mice

<table>
<thead>
<tr>
<th></th>
<th>Megakaryocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>0.15 (0.03)</td>
</tr>
<tr>
<td>IL-1 treated</td>
<td>0.18 (0.01)</td>
</tr>
<tr>
<td>Splenectomized</td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>ND</td>
</tr>
<tr>
<td>rhIL-11 treated</td>
<td>ND</td>
</tr>
</tbody>
</table>

Bone marrow cells were processed and stained with the megakaryocyte-specific monoclonal antibody, 4A5, as described in Materials and Methods. Megakaryocytes were analyzed by flow cytometry and frequency determinations were based on collection of a minimum of 400 megakaryocytes per animal. Data are presented as mean (±SD). n = 5 for normal mice and n = 4 for splenectomized mice.

Abbreviations: ND, S/B not determined.

was 15% and 35%, respectively (Fig 4B). On day 10 there was no difference in the ploidy distribution between treated and control animals (data not shown).

A similar pattern of stimulation of bone marrow megakaryocyte endoreplication was observed in splenectomized mice. Vehicle-treated, splenectomized mice had a modal ploidy of 16N (55% to 60% of megakaryocytes) with 15% to 20% of megakaryocytes in the 8N ploidy class and about 10% of megakaryocytes at 32N. Figure 4 also shows that megakaryocyte endoreplication was stimulated by rhIL-11 treatment on both day 3 (Fig 4C) and day 7 (Fig 4D). The relative increase in 32N megakaryocytes was similar to the increase observed in normal mice (compare Fig 4A and B with C and D). At day 10 there was no difference in the ploidy distribution between treated and control splenectomized mice (data not shown).

Megakaryocyte progenitors. Treatment of normal or splenectomized mice with rhIL-11 resulted in stimulation of megakaryocyte progenitors. There was no difference in MEG-CFC in the bone marrow between vehicle-treated control and rhIL-11–treated normal mice on day 3 (Fig 5A). However, on day 7 there was a threefold increase, relative to vehicle-treated controls, in MEG-CFC in the bone marrow of rhIL-11–treated mice. In splenectomized mice there was a twofold increase, relative to vehicle controls, in bone marrow MEG-CFC in rhIL-11–treated animals on both days 3 and 7 (Fig 5B). On day 10 there were no differences in bone marrow MEG-CFC between vehicle-treated control and rhIL-11–treated animals in either normal or splenectomized mice.

In the spleens of rhIL-11–treated normal mice there was a threefold increase, relative to vehicle-treated controls, in MEG-CFC on day 3 (Fig 5C). After 7 days of rhIL-11 dosing, MEG-CFC in the spleens of rhIL-11–treated mice were still elevated compared with vehicle-treated controls. On day 10 there was no difference in spleen MEG-CFC between vehicle-treated control and rhIL-11–treated mice. Although there

![Fig 2](https://example.com/fig2.png) Histologically identifiable megakaryocytes in the spleen of control and rhIL-11–treated mice. (A) Paraffin embedded sections of the spleen from days 1.5, 3, 7, 10, and 15 were stained with hematoxylin and eosin. The average number of megakaryocytes per spleen section was determined by counting the number of histologically identifiable megakaryocytes in a total of four randomly chosen, nonoverlapping spleen sections per mouse (n = 5 mice/group): (■) vehicle-treated controls; (●) rhIL-11, 150 μg/kg/d.

![Fig 3](https://example.com/fig3.png) Bone marrow megakaryocyte ploidy in control mice. Megakaryocyte ploidy was quantitated by two-color flow cytometry as described in Materials and Methods. Megakaryocytes form discrete ploidy classes that were quantitated by setting markers in the valleys between peaks. The location of 2N and 4N ploidy classes was confirmed by examining unfractionated bone marrow. The histogram shown is representative of the ploidy distribution observed in vehicle-treated mice.
Fig 4. Megakaryocyte ploidy in control and rhIL-11–treated mice. Megakaryocyte ploidy was quantitated by two-color flow cytometry as described in Materials and Methods. A minimum of 1,000 megakaryocytes were collected for each animal and the percentage of cells in each ploidy class was determined as described in Fig 3. (A and B) Normal mice, days 3 and 7, respectively. (C and D) Splenectomized mice, days 3 and 7, respectively. Compared with vehicle-treated controls there were statistically significant increases ($P < .01$) in the percentage of 32N megakaryocytes in rhIL-11–treated normal mice and rhIL-11–treated splenectomized mice on days 3 and 7. Data are presented as the mean (±SD) $n = 5$ for normal mice and $n = 4$ for splenectomized mice: (○) vehicle-treated controls; (□) rhIL-11, 150 μg/kg/d.

were clear differences in spleen and bone marrow MEG-CFCs between vehicle-treated control and rhIL-11–treated normal mice on day 7 (Fig 5A and C), the absolute number of megakaryocyte colonies in vehicle-treated controls was lower (compared with days 3 and 10) in both groups of animals.

DISCUSSION

In this report we have examined the effects of in vivo administration of rhIL-11 to normal and splenectomized mice. Administration of rhIL-11 to normal mice resulted in a marked stimulation of megakaryocytopoiesis and a corresponding increase in peripheral platelet counts. The magnitude of the increase in peripheral platelets in these studies was modest (30% to 40%); however, platelet increases of this magnitude could be maintained throughout the dosing period when rhIL-11 was administered for 14 days (data not shown). In vivo administration of IL-6 in mice resulted in platelet increases of similar magnitude.21,22

There was no change in the frequency of bone marrow megakaryocytes in normal mice following rhIL-11 treatment. However, there were increased numbers of bone marrow MEG-CFC. Endoreplication of bone marrow megakaryocytes
was also stimulated by rhlL-11 treatment. These data suggest that early as well as later stages of megakaryocytopoiesis were affected by rhlL-11 treatment. In addition, there were a number of profound changes in the spleens of rhlL-11-treated animals. Spleen weights increased by 35% and there was a trend toward increased splenic cellularity. Assay of megakaryocyte progenitors in the spleen showed an increase in MEG-CFC and histologic examination of the spleens also showed a trend of increased numbers of morphologically identifiable megakaryocytes throughout the dosing period. There was also a modest (two- to threefold) increase in macrophage progenitors in the spleens of rhlL-11–treated animals (J. Loebelenz and S. Goldman, unpublished observation). These data suggested that rhlL-11 could stimulate splenic hematopoiesis and megakaryocytopoiesis in normal mice.

In normal mice, evidence suggests that the spleen does not play a role in platelet production. To address the role of the spleen in the in vivo response to rhlL-11 we examined the effect of rhlL-11 in splenectomized mice. Analyses of peripheral platelets, MEG-CFC, and megakaryocyte endoreplication produced results that were similar to the results observed in normal mice. In addition, there was a 15% to 25% increase in the cross-sectional area of mature bone marrow megakaryocytes in rhlL-11–treated, splenectomized mice compared with megakaryocytes from vehicle-treated, splenectomized controls (T. Neben, unpublished observation). These data indicated that splenic megakaryocytopoiesis was not essential for the observed increase in peripheral platelets in response to in vivo rhlL-11 treatment. The ability of rhlL-11 treatment to stimulate both early and later stages of megakaryocytopoiesis was also unaffected by splenectomy.

Some minor differences were observed between splenectomized mice and normal mice in the kinetics of the above responses to rhlL-11. Increased numbers of bone marrow MEG-CFC appeared earlier in splenectomized mice than in normal animals (day 3 v day 7, Fig 5), whereas the decline of peripheral platelets to control levels was faster in splenectomized mice than in normal animals (day 10 v day 15, Fig 1). The accelerated rate of decline in peripheral platelet counts...
in splenectomized mice is consistent with the suggestion that splenic megakaryocyteopoiesis may play a role in sustaining the platelet response after dosing with rhIL-11 has stopped. However, subsequent studies have shown that treatment of splenectomized mice with higher doses of rhIL-11 (ie, 250 \(\mu g/kg/d\)) resulted in sustained platelet increases on day 10 similar to those observed in normal mice (data not shown).

In vitro characterization of rhIL-11 has shown that this cytokine can exhibit multiple biological activities on hematopoietic cells from the myeloid, lymphoid, and erythroid lineages. In some of these cases rhIL-11 mediated effects may be indirect. For example, the ability of rhIL-11 to stimulate B cell immunoglobulin production was dependent on the presence of CD4 T cells. One possible explanation for rhIL-11 effects on B cells was suggested by the observation that rhIL-11 can stimulate CD4/CD45RA T cells to produce IL-6 mRNA. The observation that rhIL-11 could induce IL-6 mRNA raised the possibility that some of rhIL-11 effects on megakaryocytes may result, at least in part, from induction of IL-6. However, the ability of rhIL-11 to enhance in vitro megakaryocyte endoreplication in mouse and in human was not blocked by the addition of a neutralizing IL-6 antibody, nor did rhIL-11 induce IL-6 production in murine short-term liquid marrow cultures. In a preliminary experiment using the B9 bioassay for IL-6, we were unable to detect any serum IL-6 bioactivity on either day 3 or day 7 in mice that received subcutaneous injection of rhIL-11 (250 \(\mu g/kg/d\)) for 7 days (unpublished observation).

Most in vitro activities of rhIL-11 are seen as synergistic responses of rhIL-11 with other cytokines. One possible explanation for the apparent single-activity effects of rhIL-11 in vivo is that the complement of cytokines present in the bone marrow of normal mice may channel the synergistic activity of rhIL-11 toward the megakaryocyte lineage. However, it should be noted that stimulation of other lineages has been seen following in vivo rhIL-11 treatment in mice. Increased neutrophil and platelet counts have been reported in normal mice of a different strain (B6DF1) following rhIL-11 treatment. In addition, Du et al have shown that in vivo rhIL-11 treatment can accelerate both neutrophil and platelet recovery in lethally irradiated, bone marrow transplanted mice. These data are consistent with the suggestion that the synergistic interactions of rhIL-11 with other cytokines in vivo may differ in various animal models leading to different experimental outcomes.

In vivo treatment of normal mice affected predominantly cells of the megakaryocyte lineage. No consistent changes were observed in the RBC, reticulocyte, or WBC counts, or WBC differentials. In contrast to these results in normal mice, a transient decrease in the WBC count was observed in splenectomized mice during dosing with rhIL-11. The nature of this effect is unclear, although the observation that the WBC differential did not change in rhIL-11-treated, splenectomized mice suggests that this effect is global rather than targeted to a specific compartment of leukocytes. Further studies will be necessary to address the mechanism of this transient decrease in WBC count.

The data presented in this report show that treatment of normal or splenectomized mice with rhIL-11 stimulated megakaryocyte progenitors, endoreplication of bone marrow megakaryocytes, and increased peripheral platelet counts. The responses of normal and splenectomized animals were similar. These studies suggest that rhIL-11 may be useful in the management of thrombocytopenia associated with disease states or marrow ablative therapies.

ACKNOWLEDGMENT

We thank Dr Samuel Burstein for the generous gift of the 4A5 monoclonal antibody, Dr Shirley Ebbe for the use of the Zeiss Mop 3 image analysis system, and Andy Long for performing the B9 bioassay. rhIL-11 was provided by Ed LaVallie and Kathy Grant. We also thank Dr Katherine Turner for a critical reading of the manuscript.

REFERENCES

17. Ebbe S, Yee T, Phalen E: 5-Fluorouracil-induced thrombocytosis in mice is independent of the spleen and can be partially reproduced by repeated doses of cytosine arabinoside. Exp Hematol 17:822, 1989
Recombinant human interleukin-11 stimulates megakaryocytopoiesis and increases peripheral platelets in normal and splenectomized mice

TY Neben, J Loebelenz, L Hayes, K McCarthy, J Stoudemire, R Schaub and SJ Goldman