In Vivo Effects of Recombinant Interleukin-11 on Myelopoiesis in Mice

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Purified recombinant human interleukin-11 (rhuIL-11) was assessed for its in vivo effects on the proliferation and differentiation of hematopoietic progenitors as well as its capacity to accelerate the recovery of a drug-suppressed hematopoietic system. Dosage and time sequence studies demonstrated that administration of IL-11 to normal mice resulted in increases in absolute numbers of femoral marrow and splenic myeloid (granulocyte-macrophage colony-forming unit [CFU-GM]), burst-forming unit-erythroid [BFU-E], CFU-granulocyte, erythroid, macrophage, megakaryocyte progenitor cells and in stimulation of these progenitors to a higher cell cycling rate. This was associated with increased numbers of circulating neutrophils. Administration of IL-11 to mice pretreated with cyclophosphamide decreased the time required to regain normal levels of neutrophil and platelet counts in peripheral blood. In addition, IL-11 accelerated reconstitution to normal range of myeloid progenitors from bone marrow and spleen of myelosuppressed mice. These data suggest that IL-11 may play an important role in the regulation of hematopoiesis, and the application of this novel cytokine may have clinical therapeutic benefits.

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HEMATOPOIESIS is a dynamic developmental process regulated by a complex network of cytokines that can stimulate or inhibit the growth and development of hematopoietic cells.1 Proliferation and differentiation of hematopoietic progenitors also depend in part on cell-cell interactions between hematopoietic stem and progenitor cells and the hematopoietic microenvironment.2-4 The cDNA encoding interleukin-11 (IL-11), a recently identified lymphokine-hematopoietic cytokine, was cloned from a primate bone marrow-derived stromal cell line PU-34 and was able to support the growth of hematopoietic cells in long-term bone marrow cultures.5,6 The human homologue of the primate IL-11 gene was subsequently isolated from a human fetal lung fibroblast cell line MRC5.5

Previous studies have demonstrated that recombinant human IL-11 is able to stimulate the proliferation of an IL-6-independent murine plasma mycoma cell line T1165.5 IL-11 synergizes with IL-3 to enhance the proliferation of early hematopoietic progenitors by shortening the Go period of in vitro blast colony-forming cells,7 enhances cycling of later progenitors,8 and stimulates the proliferation and maturation of megakaryocyte colonies in vitro.9,10 In addition, IL-11 enhances in vitro and in vivo antigen-specific antibody responses in normal and cyclophosphamide (CYC)-immunosuppressed mice.11 Recent studies also have shown that IL-11 can act as an adipo genesis inhibitory factor (AGIF) to inhibit lipoprotein lipase activity and adipocyte differentiation in 3T3-L1 cells.12 IL-11, therefore, represents another cytokine with pleiotropic effects that may play an important role in hematopoiesis.

In the present report, we demonstrate the in vivo effects of purified recombinant human (rhu) IL-11 on normal myelopoiesis and on the reconstitution of hematopoiesis in mice pretreated with CYC.

MATERIALS AND METHODS

Mice. Female C57/HEJ mice, 8 to 12 weeks old, were purchased from the Jackson Laboratory (Bar Harbor, ME). These mice are relatively insensitive to the hematologic effects of endotoxin.

Cyclophosphamide. CYC (Sigma Chemical Co, St Louis, MO) was dissolved in pyrogen-free saline and injected intraperitoneally to mice at a dose of 200 mg/kg body weight at the beginning of the experiment.

rhuIL-11. Purified rhuIL-11, produced in Escherichia coli, had a specific activity of 2.5 x 10^9 U/mg protein as assessed in the T1165 cell proliferation assay.7 The IL-11 preparations contained less than 1 ng of endotoxin per milligram of protein as assessed by Limulus amebocyte lysate assay and were diluted in sterile pyrogen-free saline.

rhuIL-11 or control diluent (sterile pyrogen-free saline) was administered to mice twice daily, once subcutaneously and once intraperitoneally. This differential administration was used previously11 in order to optimize the chances of seeing an effect of IL-11 and was shown to be successful in terms of enhancing other in vivo activities. Experiments where mice were first pretreated with CYC, the first rhuIL-11 injection was performed 24 hours after the administration of CYC and continued twice daily for the duration of the experiment. At various time points mice were killed, bone marrow (BM) and spleen cells were enumerated, and these cells were used to set up colony assays for myeloid progenitor cells.

Clonal growth assay for granulocyte-macrophage progenitor cells (colony-forming unit granulocyte-macrophage [CFU-GM]). BM cells (7.5 x 10^4 to 1.0 x 10^5) and 1 x 10^4 spleen cells were plated in 35 mm standard tissue culture dishes (Corning Glass Works, Corning, NY) in 1 mL of 0.3% agar (DIFCO, Detroit, MI) culture medium with enriched McCoy’s 5A medium (GIBCO, Grand Island, NY) in 10% heat-inactivated fetal calf serum (FCS; Hyclone, Logan, UT) as described.13 Cultures were stimulated with 200 U/mL recombinant (r) murine (mu) granulocyte-macrophage colony-stimulating factor (rhuGM-CSF) alone and in combination with 50 ng/mL rmu steel factor (SLF, a c-kit ligand).14 Both were kind gifts from Dr Douglas E. Williams (Immunex Research and Development Corporation, Seattle, WA). Cells were incubated at 37°C in a humidified atmosphere flushed with 5% CO2 at lowered pH by a reciprocating shaker. Colonies were counted only if they reached a minimum of 50 cells per colony.

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Clonal growth assay for erythroid (BFU-E) and multipotential (CFU-granulocyte, erythroid, macrophage, megakaryocyte [CFU-GEMM]) progenitor cells. These were carried out as previously reported.\textsuperscript{16,17} Briefly, 1 mL of 7.5 × 10^4 to 1 × 10^5 BM or 1 × 10^6 spleen cells in enriched Iscove's modified Dulbecco's medium (IMDM; GIBCO) containing 30% non-heat-inactivated FCS were plated in 35-mm tissue culture dishes (Corning) with a final concentration of 1% methylcellulose (Fisher Scientific Co., Fair Lawn, NJ). Cells were cultured in the presence of 2 U/mL Epogen (Amgen, Thousand Oaks, CA) and 100 U/mL IL-3 contained in WEHI-3B cell line conditioned medium (CM) or 5% pokeweed mitogen mouse spleen cell CM (PWMSCM). Erythroid bursts and multipotential cell colonies were enumerated using an inverted microscope after 8 days of incubation at 37°C in 5% CO\textsubscript{2} at lowered (5%) oxygen tension.

Measurement of percentage myeloid progenitor cells in S-phase. The fraction of CFU-GM, BFU-E, and CFU-GEMM in DNA synthesis, S-phase of the cell cycle, was determined by exposing BM and spleen cells to 50 μCi tritiated thymidine (\textsuperscript{3}HdTdR) for 20 minutes at 37°C prior to washing and plating.\textsuperscript{16,17} The reduction in the number of colonies after exposure of cells to \textsuperscript{3}HdTdR, compared with the control, estimates the proportion of cells in S-phase.

Peripheral blood cell counts. Hematologic analysis of white blood cell counts (WBC) and platelet counts were carried out from tail vein bleeds of mice using a Coulter Counter model ZM (Coulter Electronics Ltd., Luton Beds, UK). Differentials of WBC were done enumerating 200 cells on Wright-Giemsa-stained peripheral blood smears.

Statistical analysis. Each animal was assessed individually with three to four plates per point assayed. Results are expressed as mean ± SD and the levels of significance for comparison between groups were determined with use of Student's t-test (two-tailed).

RESULTS

Effects of IL-11 on proliferation of myeloid progenitor cells in normal mice. IL-11 was first assessed for effects in previously untreated mice. These mice were injected with IL-
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CFU-GM (GM-CSF)  CFU-GM (GM-CSF + SLF)  BFU-E (Epo + PWMSCM)  CFU-GEMM (Epo + PWMSCM)

3 hr
Control
IL-11

24 hr
Control
IL-11

48 hr
Control
IL-11

72 hr
Control
IL-11

96 hr
Control
IL-11

% Progenitors in S-phase

CFU-GM and splenic CFU-GM were not significantly (P > .05) enhanced. No significant effect (P > .05) was observed on absolute numbers of CFU-GM, BFU-E, or CFU-GEMM per femur or per spleen (combined results of two experiments for a total of nine mice/group). Upon dose-response analysis (Fig 1) in which mice were inoculated with either 0.5, 1, 2, 4, or 8 μg IL-11/injection and killed 24 hours later, as little as 1 μg/injection IL-11 significantly (P at least <.05) enhanced the cycling rates of femoral marrow CFU-GM (stimulated in vitro with GM-CSF plus SLF), BFU-E, and CFU-GEMM (stimulated in vitro with Epo plus PWMSCM). Significant enhancement (P at least <.05) was also seen in the absolute numbers of CFU-GM, BFU-E, and CFU-GEMM in the marrow of mice inoculated with 4 to 8 μg IL-11/injection (Fig 1). Injection of 2 μg IL-11/injection

11 or control diluent two times in 1 day and the mice killed 24 hours after the first and about 10 hours after the second injection. In initial studies, mice were inoculated with 1 μg IL-11/injection, a dosage that was previously shown to have significant, but not optimal, enhancing effects in vivo in mice on primary and secondary immune responses.11 In this set of experiments, the percentage of progenitor cells in S-phase was significantly enhanced (P < .005) for marrow CFU-GM (stimulated in vitro with granulocyte-macrophage colony-stimulating factor [GM-CSF]) from 36.2 ± 2.1 to 54.5 ± 1.1 and for splenic BFU-E and CFU-GEMM (stimulated in vitro with Epo plus WEHI-3BCM), respectively, from 24.0 ± 0.5 to 41.6 ± 5.3 and from 23.0 ± 0.3 to 44.0 ± 2.1 (combined results of three experiments from a total of 12 mice/group). In these experiments, the cycling rates of marrow BFU-E and CFU-GEMM and splenic CFU-GM were not significantly (P > .05) enhanced. No significant effect (P > .05) was observed onabsolute numbers of CFU-GM, BFU-E, or CFU-GEMM per femur or per spleen (combined results of two experiments for a total of nine mice/group). Upon dose-response analysis (Fig 1) in which mice were inoculated with either 0.5, 1, 2, 4, or 8 μg IL-11/injection and killed 24 hours later, as little as 1 μg/injection IL-11 significantly (P at least <.05) enhanced the cycling rates of femoral marrow CFU-GM (stimulated in vitro with GM-CSF plus SLF), BFU-E, and CFU-GEMM (stimulated in vitro with Epo plus PWMSCM). Significant enhancement (P at least <.05) was also seen in the absolute numbers of CFU-GM, BFU-E, and CFU-GEMM in the marrow of mice inoculated with 4 to 8 μg IL-11/injection (Fig 1). Injection of 2 μg IL-11/injection
slightly, but significantly, enhanced absolute numbers of marrow CFU-GEMM (Fig 1). Based on the results of this dose-response analysis, a time sequence study was performed with mice injected two times daily with 4 μg IL-11/injection. As shown in Fig 2A and B, the cycling rates of femoral marrow and splenic CFU-GM, BFU-E, and CFU-GEMM were significantly (P at least <.05) enhanced as early as 3 hours after the first injection of IL-11 for most compartments, and this enhancing effect was sustained for all compartments at 24, 48, 72, and 96 hours during administration of IL-11. Significant enhancing effects were also noted on absolute numbers of myeloid progenitors in the marrow (Fig 3A) and spleen (Fig 3B), but these effects, which were first apparent at 24 hours, were not as consistent as the enhancing effects seen for progenitor cell cycling rates (Fig 2A and B). Evaluation of the effects of IL-11 on circulating leukocyte counts in the mice used for the studies in Figs 2 and 3 demonstrated significant increases (P at least <.05) in neutrophilic granulocytes (PMNs), but not other leukocyte compartments, as early as 3 hours after the first injection. Respective values for PMN/mL × 10^6 at 3, 24, 48, 72, and 96 hours for mice given control diluent versus 4 μg IL-11/INjection were 2.6 ± 0.6 versus 5.9 ± 0.4, 2.5 ± 1.1 versus 3.9 ± 0.9, 3.0 ± 1.3 versus 4.8 ± 0.8, 3.8 ± 0.4 versus 8.0 ± 1.1, and 3.8 ± 0.9 versus 9.2 ± 2.0.

Effects of IL-11 on proliferation of myeloid progenitor cells in CYC-pretreated mice. Based on the enhancing effects of IL-11 on the proliferation of myeloid progenitor cells noted above in previously untreated mice, a more in-depth analysis of the effects of IL-11 (1 μg/INjection) was undertaken in mice recovering from CYC. As seen in Fig 4, 24 hours after CYC administration the absolute numbers of marrow and

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Fig 3. Time sequence study of the effects of rhuIL-11 (4 μg/INjection, two times daily) on the absolute numbers of femoral marrow (A) and splenic (B) myeloid progenitor cells. These results (mean ± SD) are from the same animals evaluated in Figure 2. *P at least <.05.
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Fig 4. Effects of rhuIL-11 (1 µg/injection, two times daily) on absolute numbers of marrow and splenic myeloid progenitors in mice pretreated with CYC. Data show the combined results of three experiments (mean ± SD) with 9 to 12 animals per group. Control animals received a similar number of injections of control diluent (pyrogen-free saline). a, P < .005; b, P < .05; c, P < .1 (= not significant). [ ■] Control; [ □] CYC; [ ●] CYC + IL-11.

spleenic myeloid progenitor cells were significantly depleted and then with time increased to normal or above-normal levels. Although the time sequence varied for the different progenitor cell compartments in marrow and spleen, IL-11 significantly enhanced the absolute numbers of marrow and spleen myeloid progenitor cells in these CYC-pretreated mice and in a number of cases accelerated recovery of these cells (Fig 4).

Effects of IL-11 on the recovery of peripheral blood cell counts in CYC-pretreated mice. We first evaluated effects in mice given 1 µg/injection, two times daily. The results from one of two reproducible experiments are shown in Fig 5. Total leukocyte counts decreased drastically 24 hours after the administration of CYC (Fig 5, upper panel). The counts then increased gradually but did not return to normal levels until 7 days later. In mice pretreated with CYC and injected daily with IL-11, the recovery of total leukocytes was only slightly enhanced. Significant increases of leukocyte counts were observed at 7 and 14 days of IL-11 treatment compared with the controls and with the animals injected with CYC alone. As seen in Fig 5 (lower panel), in mice injected with CYC alone, platelet counts decreased to the lowest level by 3 days and then gradually increased and attained normal levels by 14 days. In mice injected with IL-11, recovery of platelet counts to normal levels was accelerated with normal platelet levels attained by 7 days. At 14 days after CYC, mice treated with IL-11 still showed significantly increased numbers of platelets. In another set of similar experiments, mice were injected two times daily with 4 µg IL-11/injection (Fig 6). IL-11 again accelerated recovery of platelets, with the higher dosage of IL-11 shown in Fig 6 having an apparently greater effect in enhancement of platelet numbers than that noted for the lower dosage of IL-11 in Fig 5. Effects on recovery of peripheral blood leukocytes was the same using 4
µg IL-11/injection (Fig 6) versus 1 µg IL-11/injection (Fig 5). As shown in Fig 6, the significant IL-11–enhancing effects on peripheral blood leukocytes were entirely because of increases in PMNs. There was no difference in recovery of lymphocytes (Fig 6) or monocytes (data not shown). Circulating erythrocyte values were only slightly decreased by CYC at day 3 from control values, and there were no significant differences in erythrocyte counts in CYC-treated mice given control diluent versus IL-11 (data not shown).

DISCUSSION

The cDNA encoding IL-11 was originally isolated from a primate BM-derived stromal cell line based on its ability to stimulate the proliferation of an IL-6–dependent mouse plasmacytoma cell line. IL-11 has subsequently been shown to have pleiotropic effects on the regulation of hematopoiesis in both murine and human in vitro culture systems. We have previously demonstrated that IL-11 significantly enhanced in vitro antigen-specific antibody responses and augmented antigen-specific antibody responses in both normal and immunosuppressed mice. In this report, we evaluated the capability of IL-11 to accelerate hematopoietic reconstitution of myelosuppressed C3H/HeJ mice following CYC pretreatment.

IL-11 appears to have no effect on the in vitro proliferation of hematopoietic progenitors when added to the cultures alone. In our present studies, the most remarkable in vivo effect of IL-11 administered to normal mice was to promote marrow and splenic CFU-GM, BFU-E, and CFU-GEMM to a higher proliferative state. This is consistent with the previous observation that IL-11, like other blast cell growth factors such as IL-6, G-CSF, and c-kit ligand, can bring the early hematopoietic progenitors out of the G0 stage of the cell cycle to respond to other growth factors. A significant enhancement of absolute numbers of marrow and splenic myeloid progenitor cells was observed in normal mice (although these results were not as consistently significant as the cycling studies) and in mice pretreated with CYC and administered with IL-11. These data plus the observed synergistic interactions of IL-11 with IL-3 in vitro suggest that the in vivo effects of IL-
IL-11 may be caused by cooperation of this cytokine with other endogenous biomolecules produced in mice. Dose-response and time sequence studies in normal mice demonstrated that cycling effects on progenitors are seen within 3 hours, but increases in absolute numbers of progenitors are seen by 24 hours. That IL-11 enhanced neutrophil release from these mice as early as 3 hours after administration suggests that IL-11 may also induce release of neutrophils. This early induced release is not likely because of endotoxin contamination because little or no endotoxin was in the IL-11 preparations and we used C3H/HeJ mice, which do not respond well to endotoxin, as recipients. Recent studies demonstrating that IL-11 synergizes with IL-3 to stimulate the proliferation and maturation of megakaryocytes, along with our current study in which IL-11 accelerated the recovery of platelet counts in CYC-pretreated mice, suggest that IL-11 may play an important role in the regulation of megakaryocytogenesis in vivo.

In human BM transplant patients, reconstitution of the immunohematopoietic system proceeds slowly over a period of months because of histocompatibility mismatching, the presence of graft versus host disease, or the persistence of viral infection. The results presented in this report and our recent studies on the effects of IL-11 on antigen-specific antibody responses suggest that IL-11 administration may have therapeutic benefit in accelerating the recovery of a drug-suppressed immunohematopoietic system.

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