Therapeutic Efficacy of Recombinant Interleukin-6 (IL-6) 
Alone and Combined With Recombinant Human IL-3 
in a Nonhuman Primate Model of High-Dose, 
Sublethal Radiation-Induced Marrow Aplasia

By Thomas J. MacVittie, Ann M. Farese, Myra L. Patchen, and Laurie A. Myers

Using a nonhuman-primate model of radiation-induced bone marrow aplasia, we examined whether the single, concomitant, or sequential administration of recombinant human interleukin-3 (IL-3) and IL-6 would promote bone marrow regeneration measured by an increase in circulating platelets (PLT) and neutrophils (PMN). Rhesus monkeys were irradiated at 450 cGy and were randomly assigned to one of five treatment protocols, receiving IL-6; IL-3; combined IL-6 and IL-3; sequential IL-3 and IL-6; or human serum albumin (HSA) as a control. Cytokines or HSA were administered at total dosages of 15 µg/kg/day. Complete blood counts and white blood cell differentials were monitored for 60 days postirradiation. Both IL-3 and IL-6 significantly enhanced the regeneration of PLTs and decreased the duration of thrombocytopenia (P = .005) without affecting PMN recovery. The radiation-induced anemia that was observed in the HSA-treated controls was less severe and resolved more quickly in the IL-6 treated animals. Sequential IL-3/IL-6 significantly increased the production of PLTs when compared with the HSA-treated controls (P = .003) and monkeys receiving concomitant IL-3/IL-6 (P = .041) but did not alter PMN levels significantly (P = .80). Coadministration of IL-6 and IL-3 did not enhance PLT but improved PMN recovery over IL-6 alone. In this primate model of marrow aplasia, IL-6 significantly enhanced the regeneration of PLTs but had no significant effect on PMN production, and did not exacerbate radiation-induced anemia. Furthermore, the use of sequentially administered IL-3 and IL-6 may improve PLT recovery as compared with concurrent IL-3/IL-6 administration, although this protocol is not significantly different in effect than either cytokine alone. This is a US government work. There are no restrictions on its use.

Two of the key objectives in developing therapeutic protocols that use either single or combinations of recombinant cytokines continue to be prevention of both hemorrhage and infectious episodes. Cytokines such as recombinant human granulocyte-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been effective in reducing the radiation-induced neutropenia and drug-induced marrow aplasia in preclinical and clinical protocols. When administered as single-agent protocols, interleukin-3 (IL-3) and IL-6 have been implicated in enhanced platelet (PLT) production. In vitro studies have shown IL-3 to directly stimulate primitive bone marrow progenitor cells and immature megakaryocytes. Moreover, IL-3 stimulates hematopoiesis at multiple levels, as shown in vitro by directly stimulating not only the primitive progenitor cells but also the growth and differentiation of both multilineage and single-lineage colony-forming cells. Preclinical studies by Gillio et al showed that IL-3 enhanced myeloid recovery in cyclophosphamide-treated dogs. Preclinical studies using IL-6, both alone and in combination with IL-3, have shown enhanced thrombopoietic effects in normal mice and dogs, and non-human primates. Use of IL-6 in animal models of either drug- or radiation-induced marrow aplasia has repeatedly shown its efficacy in the reduction of both the depth and duration of neutropenia. Furthermore, the use of sequentially administered IL-3 and IL-6 may improve PLT recovery as compared with concurrent IL-3/IL-6 administration, although this protocol is not significantly different in effect than either cytokine alone. This is a US government work. There are no restrictions on its use.

From Experimental Hematology, Armed Forces Radiobiology Research Institute, Bethesda, MD; and the Cytokine Development Unit, Sandoz Pharmaceutical Corp, East Hanover, NJ. Submitted January 26, 1994; accepted June 15, 1994.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

This is a US government work. There are no restrictions on its use.

BLOOD, VOL 84, NO 8 (OCTOBER 15), 1994; PP 2515-2522

2515
duration of thrombocytopenia. The purpose of this study was to determine the therapeutic efficacy of IL-3 and IL-6 on platelet and neutrophil production when administered as single agents or combined either sequentially or concurrently in a nonhuman primate model of high-dose, sublethal radiation-induced marrow aplasia.

MATERIALS AND METHODS

Animals. Domestic born male rhesus monkeys, Macaca mulatta (mean weight 3.9±0.2 kg), were housed in individual stainless steel cages in conventional holding rooms at the Armed Forces Radiobiology Research Institute in an animal facility accredited by the American Association for Accreditation of Laboratory Animal Care. Monkeys were provided 10 air changes per hour of 100% fresh air, conditioned to 72±2°F with a relative humidity of 50±20% and maintained on a 12-hour light/dark full spectrum light cycle, with no twilight. Monkeys were provided with commercial primate chow, supplemented with fresh fruit and tap water ad libitum. Research was conducted according to the principles enunciated in the Guide for the Care and Use of Laboratory Animals.

Irradiation. Monkeys placed in an aluminum restraining chair, after a prehabitation period, were total body irradiated (TBI) in a posterior-anterior direction using the AFRRI TRIGA reactor. They were exposed to a pulse (≤500 msec) of mixed (1:1, free in-air) fission neutron and gamma radiation to a total free-in-air (skin surface dose) of 450 cGy. All exposures were monitored using ionization chambers, sulfur activation foils, and radioluminescent glass and silicon diodes.

Recombinant cytokines. IL-6 and IL-3 were Escherichia coli-derived and were provided by Sandoz Pharmaceuticals Corp (East Hanover, NJ). The specific activities of IL-6 and IL-3 were 9.8×10⁷ U/mg protein and 6.6×10⁶ U/mg protein, respectively. The cytokines or the control protein, human serum albumin (HSA; Miles Inc, Cutter Biological, Elkhart, IN), were administered by subcutaneous injection at a dosage of 15 μg/kg/day.

Study design. The animals were randomly assigned to one of five treatment groups composed of four to six animals. Each animal was irradiated on day 0. On day 1, groups of animals received cytokine therapy for 23 consecutive days with one of the following: IL-6 alone, IL-3 alone, or concomitant IL-6 and IL-3. Another cohort of monkeys received IL-3 and IL-6 in a sequential regimen of IL-3 administered for 7 days (d 1 through 7), followed by IL-6 for 17 days (d 7 through 23). The control group received HSA for the 23-day treatment period. IL-6 was administered once daily; IL-3 was administered as a divided dose twice daily when administered alone, sequentially, or concomitantly with IL-6. These studies were performed at a single dose for each cytokine. Dose selection was based on previous reports of IL-6 use in normal nonhuman primates.

Maximal efficacy of cytokine protocols are most likely dependent on cytokine dose, combination, and protocol.

Clinical support. An antibiotic regimen was initiated prophylactically when the white blood cell count (WBC) was less than 1,000/μL and continued daily until the WBC was greater than 1,000/μL.

Fig 1. Hemoglobin values (g/dL) for rhesus monkeys treated with HSA or IL-3/IL-6 cytokine protocols administered for 23 consecutive days after sublethal irradiation as described in the Materials and Methods.
for 3 consecutive days. Gentamicin (Lyphomed, Deerfield, IL; 10 mg/d) and Rocephin (Roche, Nutley, NJ; 250 mg/d) were administered subcutaneously. Fresh, irradiated (1,500 cGy Co-60) whole blood (approximately 30 mLtransfusion) from a random donor pool (monkeys of >10 kg) was administered when the PLT count was less than 30,000/mL and the hematocrit was less than 20%. Transfusions and antibiotics were required to ensure 100% survival (unpublished results).

Hematologic evaluations. Peripheral blood was obtained from the saphenous vein to assay complete blood (Model S Plus II; Couter Electronics, Hialeah, FL) and differential counts (Wright-Giemsa Stain; Ames Automated Slide Stainer, Elkhart, IN). Baseline levels (BL) were obtained before irradiation. These parameters were monitored for 60 days after irradiation and the durations of neutropenia (absolute neutrophil count [ANC] <1,000/mL) and thrombocytopenia (PLT <30,000/mL) were assessed. Whole blood transfusions could have possibly altered the ANC and PLT count; therefore, when determining the durations of neutropenia and thrombocytopenia, ANC and PLT counts needed to be maintained for 3 consecutive days above threshold levels after the first increase for a true recovery to be noted.

Statistical analysis. The Normal Scores Test was used to make pairwise comparisons of the durations of neutropenia and thrombocytopenia. The test was performed using the software package StatXact (Cytel Software Corp, Cambridge, MA). The Mann Whitney test was used to evaluate the statistical significance between the nadirs. The exact P values were obtained for both analyses.

RESULTS

Anemia. Radiation-induced anemia developed in all HSA-treated controls and cytokine-treated groups within 2 to 3 weeks after exposure (Fig 1). Treatment with IL-6 alone decreased the requirement for transfusions, lessened nadir of the radiation-induced anemia (Hgb = 7.9 g/dL on day 13 v 5.4 g/dL on day 26 for HSA controls) and induced an earlier recovery (day 35 v 70 for HSA controls) to BL levels (13.0 g/dL) (Fig 1). All other cytokine-treated groups exhibited an intermediate degree of anemia that was not significantly different from the HSA-treated control group. There was no evidence of clinical bleeding in any particular group. All animals did not require transfusions: the HSA-treated control animals for an average of 12.2 days (day 9 through 20) after 450 cGy TBI (Fig 2A and Table 1). Administration of IL-3 or IL-6 significantly decreased the requirement for transfusions, lessened nadir from that noted with IL-3. IL-6 administration also significantly reduced the requirement for transfusions, lessened nadir relative to HSA controls for an average of 12.2 days (day 23) after 450 cGy TBI (Fig 2B and Table 1). However, the effect on the duration of thrombocytopenia observed with IL-6 administration was not significantly different (P = .39) from that noted with IL-3. IL-6 administration also significantly decreased the nadir of thrombocytopenia when compared with the control animals (P = .028; Fig 2A). The administration of either IL-3 or IL-6 accelerated platelet recovery, with preirradiation levels of circulating platelets attained within approximately 25 days, whereas HSA-treated animals required approximately 32 days (Fig 2A).

The effects on platelet recovery of IL-3 and IL-6 alone were compared with the combined effects of IL-3/IL-6 administered in a concurrent or sequential fashion. The concomitant administration of IL-3/IL-6 did not decrease the platelet nadir nor accelerate the rate of platelet recovery relative to IL-3 (P = .88) or IL-6 (P = .07) alone (Fig 2B). This concurrent combination did decrease the duration of thrombocytopenia relative to HSA-treated control animals (P = .055; Fig 2C and Table 1). The sequential IL-3/IL-6 protocol did not accelerate platelet recovery to normal circulating levels nor did it decrease the platelet nadir relative to IL-6 or IL-3 alone or coadministered (Fig 2C and D). However, the duration of thrombocytopenia was significantly reduced with sequential administration (3.2 days) rela-
Fig 3. Composite figure showing regeneration of the circulating platelet counts after sublethal irradiation of rhesus monkeys treated with HSA or IL-3/IL-6 cytokine protocols. Cytokines or HSA were administered over a 23-day protocol by subcutaneous injections as described in the Materials and Methods.

Table 1. Neutropenia and Thrombocytopenia in Sublethally Irradiated and Cytokine-Treated Rhesus Monkeys: Duration and Mean Days of Cytopenia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neutropenia (Days (duration))</th>
<th>Thrombocytopenia (Days (duration))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSA</td>
<td>17.2 (5.0-21.0 d)</td>
<td>12.2 (9.0-20.0 d)</td>
</tr>
<tr>
<td>IL-6</td>
<td>18.1 (5.0-22.6 d)</td>
<td>5.0 (11.0-15.0 d)*</td>
</tr>
<tr>
<td>IL-3</td>
<td>18.8 (6.0-21.0 d)</td>
<td>6.6 (12.0-17.5 d)*</td>
</tr>
<tr>
<td>IL-3 and IL-6 (d 1-23)</td>
<td>18.8 (5.0-20.6 d)</td>
<td>8.0 (10.0-17.0 d)*</td>
</tr>
<tr>
<td>IL-3 (d 1-7), IL-6 (d 7-23)</td>
<td>18.2 (4.0-19.6 d)</td>
<td>3.2 —*†</td>
</tr>
</tbody>
</table>

Monkeys who had been whole-body irradiated with 450 cGy of mixed fission:neutron gamma radiation were treated with control protein (HSA) or cytokines according to protocol. Neutropenia is an ANC of less than 1,000/μL. Thrombocytopenia is a platelet count of less than 30,000/μL.

* Statistically significant difference from the HSA-treated controls.
† Statistically significant difference from the concomitantly administered IL-3/IL-6.

Effect on granulocytes. The HSA-treated control animals required antibiotic support for an average 17-day (days 5 through 21) period of neutropenia (Fig 4A and Table 1). Treatment of irradiated animals with IL-3 (P = .27), IL-6 (P = .057), or the concurrent (P = .76) or sequential (P = .80) IL-3/IL-6 protocols did not significantly change either the recovery of neutrophils to normal baseline levels or the durations of neutropenia relative to HSA-treated controls (Fig 4A and D and Table 1). However, only the HSA-treated controls experienced absolute neutropenia and all cytokine protocols reduced the neutrophil nadir (≥0.1) relative to the HSA-treated controls in a similar manner (Figs 4A through D and 5). There was no significant increase in eosinophils in cytokine-treated groups relative to HSA-treated controls (data not shown). Recovery of eosinophils from irradiation was similar in all treatment groups, reaching baseline levels within 40 days after exposure, a marked delay relative to neutrophil recovery. The basophil recovery was variable and delayed with respect to neutrophils, with no noted basophilia (data not shown).

DISCUSSION

Infectious and hemorrhagic complications result from radiation-induced marrow aplasia. The role of hematopoietic growth factors in accelerating recovery from the hematopoie-
The present study was designed to test the in vivo efficacy of two regimens combining IL-3 and IL-6 in a primate model of radiation-induced marrow aplasia. The protocols included either the simultaneous or the sequential administration of IL-3 and IL-6, and the respective controls of IL-3 or IL-6 alone. In this study, these cytokines and protocols were shown to have lineage-specific effects on platelet production without any significant modulation of neutrophil production relative to HSA-treated controls.

We showed that the single-cytokine protocols of IL-6 or IL-3 were each capable of significantly decreasing the duration of thrombocytopenia relative to HSA-treated controls. Neither cytokine affected the duration of neutropenia. Our data also showed the comparative efficacy, albeit of a single dose of IL-6 and IL-3 in the same model. Although IL-6 reduced the thrombocytopenic duration by an average 1.6 days (5.0 vs 6.6 days relative to IL-3), the difference was not significant. Recent preclinical data confirmed the efficacy of IL-3 and IL-6 on platelet production. Gillio et al11 showed the efficacy of IL-3 in a 5-FU-induced model of myelosuppression, whereas Herodin et al13 and Zeidler et al16 using primate models of radiation-induced marrow aplasia, showed the efficacy of IL-6 in reducing the duration of thrombocytopenia. Burstein et al10 showed an IL-6 dose-related acceleration of platelet recovery in an irradiated canine model. Herodin et al13 and Burstein et al10 also confirmed the lack of an IL-6 effect on neutrophil recovery. Takatsuki et al19 also showed that IL-6 did not accelerate WBC recovery in a murine model of 5-FU-induced myelosuppression. In contrast, Gillio et al11 reported that IL-3 was capable of reducing both the neutrophil nadirs and durations of neutropenia in cyclophosphamide- and 5-FU-treated primates. However, we could not demonstrate efficacy of IL-3 in reducing the duration of neutropenia in this or a previous study6 using the same model of radiation-induced marrow aplasia as described in this report.

The sequential administration of IL-3 and GM-CSF,24,25,46 and IL-3 and IL-611 in the normal primate have been shown to modulate the production of neutrophils and/or platelets either synergistically or better than the single agents alone. We recently showed that the coadministration of IL-3 and GM-CSF was significantly better in reducing both the durations of neutropenia and thrombocytopenia in the same irradiated monkey model reported herein relative to IL-3 or GM-CSF alone or the sequential protocol of IL-3 and GM-CSF.5 The IL-3/GM-CSF fusion protein, PXY321, was also shown to promote earlier regeneration of both platelets and neutrophils.49 This was in contrast to what would have been predicted from results observed in normal primates (Farese et al, unpublished data).

Geissler et al13 showed that the sequential protocol of IL-3 followed by IL-6 significantly increased production of platelets relative to IL-3 or IL-6 alone in normal primates. In a murine model of 5-FU-induced marrow aplasia, Carrington et al47 demonstrated that the combination of IL-3 and IL-6 decreased the platelet nadir but not the duration of thrombocytopenia relative to IL-3 or IL-6 alone. In this study of radiation-induced marrow aplasia, we showed that, although the sequential IL-3/IL-6 protocol further reduced the
duration of thrombocytopenia relative to IL-3 or IL-6 alone, it was not statistically significant. However, the sequential IL-3/IL-6 protocol was significantly better in reducing the thrombocytopenic period than the concurrent IL-3/IL-6 protocol. Winton et al. observed that, in a primate model of hepsulfam-induced marrow aplasia, the concurrent administration of IL-3/IL-6 was no better than IL-6 alone in reducing the period of thrombocytopenia. Geissler et al. showed that IL-3 but not IL-6 increased the concentration of megakaryocyte progenitors. The time-dependent ability of IL-3 to increase IL-6 responsive megakaryocytes may account for the efficacy of the sequential relative to concomitant protocol. Wognum et al. have recently shown that the IL-6 receptor has a variable distribution on rhesus monkey CD34+ bone marrow cells. These immature marrow progenitors contain subsets that do not express detectable IL-6 receptors. The irradiated marrow may contain even fewer IL-6-responsive cells, whereas IL-3 may act to increase the fraction of surviving marrow cells that express IL-6 receptors or induce IL-6 receptor upregulation on surviving marrow progenitor cells.

IL-6-induced anemia has been reported by several groups. Burstein et al. noted a dose-dependent decrease in hematocrit in normal and all irradiated and IL-6-treated canines relative to controls. Geissler et al. and Asano et al. noted significant decreases in red blood cell (RBC) counts and hematocrit, respectively, in IL-6-treated normal primates. Our data in normal primates would concur with these findings (data not shown). Herodin et al. reported that the observed radiation-induced decline in RBC parameters was not changed by IL-6 administration in their baboon model. Our data suggest that the administration of IL-6 improved the radiation-induced anemia observed in the HSA-treated animals. Patchen et al. observed a similar effect in the irradiated mouse administered IL-6. Although the pathophysiology of the IL-6-induced anemia remains to be determined, the cytokine-induced production of platelets in the irradiated animals may contribute to the prevention of bleeding episodes.

The modulation of both neutrophil and platelet production continues to present a critical challenge in the treatment of the marrow-ablated patient. This study also suggests that combined protocols of IL-6 and G-CSF or GM-CSF may provide the desired effect of enhancing multilineage hematopoietic recovery. Recently, Patchen et al. showed enhanced recovery of both neutrophils and platelets using IL-6 and G-CSF in a rodent model of radiation-induced marrow aplasia. The concurrent administration of IL-3 and GM-CSF3 or the PIXY321 fusion protein has also shown efficacy in enhancing neutrophil and platelet recovery.
REFERENCES


31. Quesenberry PJ, McGrath HE, Williams ME, Robinson BE, From www.bloodjournal.org by guest on October 3, 2017. For personal use only.


40a. The Institute of Laboratory Animal Resources, National Research Council: Guide for the Care and Use of Laboratory Animals. Washington, DC, National Institutes of Health, publication no. 86-23


Therapeutic efficacy of recombinant interleukin-6 (IL-6) alone and combined with recombinant human IL-3 in a nonhuman primate model of high-dose, sublethal radiation-induced marrow aplasia

TJ MacVittie, AM Farese, ML Patchen and LA Myers