Thrombocytopenia and neutropenia remain as dose-limiting consequences after high-dose irradiation or cytotoxic drug exposure. Cytokines such as recombinant human granulocyte and granulocyte-macrophage colony-stimulating factor have been effective in reducing the duration of neutropenia after radiation- or drug-induced marrow aplasia in preclinical1,8 and clinical protocols.3-9 Several cytokines have shown therapeutic efficacy in modulating the recovery of platelets after radiation,6,10 or drug-induced marrow aplasia.11-13 Interleukin-3 (IL-3) has shown a modest yet consistent efficacy in preclinical models of myelosuppression,6,11 but has demonstrated variable efficacy in modulating thrombocytopenia in the clinical situation.14-18 More recently, IL-6 has displayed efficacy in reducing thrombocytopenia and enhancing recovery of platelets in murine,19,21 canine,22 and primate23-26 models of either radiation- or drug-induced thrombocytopenia. Results from clinical trials with IL-6 have shown it to be efficacious in modulating drug-induced thrombocytopenia.27,28 IL-11 has also been shown to enhance the recovery of platelets in several murine models of myelosuppression.29,30 Yet another cytokine, leukemia inhibitory factor (LIF), has recently been shown to promote megakaryocyte maturation in vitro31,32 and to increase circulating levels of platelets in normal mice33 and nonhuman primates.34 The purpose of this study was to investigate the therapeutic efficacy of LIF in a high-dose, sublethal, primate model of radiation-induced marrow aplasia.

MATERIALS AND METHODS

Animals. Domestic male rhesus monkeys (Macaca mulatta; mean weight, 4.3 ± 0.59 kg) were housed in individual stainless steel cages in conventional holding rooms at the Armed Forces Radiobiology Research Institute (AFRRI) in a facility accredited by the American Association for Accreditation of Laboratory Animal Care. Monkeys were provided 10 air changes/h of 100% fresh air conditioned to 72°F ± 2°F with a relative humidity of 50% ± 20% and were 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.35

Recombinant cytokine. The recombinant human LIF was provided by the Cytokine Development Unit of Sandoz Pharmaceuticals Corp (East Hanover, NJ). The nonglycosylated protein was extracted from Escherichia coli cells expressing the LIF cDNA from a plasmid vector. The in vitro biologic activity of the recombinant human LIF (rhLIF) was analyzed in a proliferation assay of the DA1 murine leukemia cell line and was approximately $13 \times 10^6$ U/mg protein. The units were calculated on the basis that 1 U of LIF was able to induce 50% of maximal proliferation. The endotoxin content was 10.6 EU/mg of LIF as determined by the Limulus assay37 (Limulus amoebocyte lysate assay; Bio Whittaker, Inc, Walkersville, MD).

Irradiation. After a prehabitation period, each monkey was placed in an aluminum restraining chair and subjected to posterior-to-anterior total-body irradiation from the AFRRI Mark-F TRIGA nuclear reactor. The torso of each monkey was shielded from the intense gamma radiation emitted by placing a 2.5-cm lead shield between the core of the reactor and the exposure position. Each monkey received a pulse of 4.5 Gy ($<500$ milliseconds) free-in-air total mixed neutrons and gamma rays with a precision of ±2.5% (%SD, n = 16) and a statistical accuracy of 10%. The neutron dose to the total dose (neutron + gamma) was 0.50% ± 2.3% (%SD, n = 17), with a statistical accuracy of 14%. All exposures were actively monitored by ionization chambers and passively monitored by sulfur activation tablets.

Study design. Each animal was irradiated on day 0 and randomly assigned to receive either LIF (n = 4) or human serum albumin (HSA; n = 5; Miles Inc, Cutter Biological, Elkhart, IN). LIF or HSA were administered as a single, subcutaneous injection at a dosage of 15 µg/kg/day. The dosage and administration route were based on results shown in normal rhesus primates by Mayer et al,16 in which efficacy at platelet production without toxicity was evident at 10 µg/kg/d for 14 days.

Support. An antibiotic regimen was initiated prophylactically when the white blood cell count was less than 1,000/µL and continued daily until the white blood cell count was greater than 1,000/µL for 3 consecutive days. Gentamicin (Lyphomed, Deerfield, IL; 1.5 mg/kg, twice daily) and rocephin (Roche, Nutley, NJ; 100 mg/kg/d) were administered subcutaneously. Fresh, irradiated (1,500 cGy Co-60) whole blood from a random donor pool (monkeys...
weighing >10 kg) was administered when the platelet count was less than 30,000/\mu L and the hematocrit was less than 20%. Whole blood transfusions and antibiotics were required to ensure 100% survival in all control HSA-treated animals (unpublished results).

**Hematologic evaluations.** Peripheral blood was obtained from the saphenous vein to assay complete blood (Model S Plus II; Coulter Electronics, Hialeah, FL) and differential counts (Wright-Giemsa stain; Ames Automated Slide Stainer, Elkhart, IN). Baseline levels were obtained before irradiation. These parameters were monitored for 60 days after irradiation and the degree of anemia and the durations of neutropenia (absolute neutrophil count <1,000/\mu L) and thrombocytopenia (platelet count either <30,000 or 20,000/\mu L) were assessed.

**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) and exact P values were obtained. The Mann Whitney test was used to evaluate the statistical difference between the nadirs.

**RESULTS**

**Platelet and neutrophil recovery.** LIF administration induced recovery of circulating platelets to baseline values, without lessening the nadir, earlier than did HSA treatment (day 22 v day 32, respectively; Fig 1A). LIF administration, compared with HSA treatment, also significantly decreased the duration of thrombocytopenia either at a platelet count less than 30,000 or 20,000/\mu L (P < .05) to 9 days or 6.3 days v 12 days or 10.2 days, respectively (Fig 1A and Table 1). Although neither the duration of neutropenia nor the recovery of neutrophils in the LIF-treated monkeys differed from those in HSA-treated monkeys, the period of absolute neutropenia was modified by LIF administration (Fig 1B and Table 1).

**Anemia.** LIF did not exacerbate the radiation-induced anemia noted in HSA-treated monkeys (Fig 2). Mean hemoglobin (HGB) values were lower in the HSA-treated monkeys, although there was no significant difference in the respective HGB nadirs. The HGB values in the LIF-treated monkeys returned to within baseline levels more quickly (day 48) than in the HSA-treated monkeys (day 70). All animals did not require transfusions; the HSA-treated animals required an average of two transfusions per animal, whereas the LIF-treated animals received less than one transfusion per animal.

**DISCUSSION**

We have shown that the therapeutic administration of LIF, as compared with HSA, to sublethally irradiated monkeys can induce an earlier recovery of circulating platelets to normal levels and can significantly reduce the duration of thrombocytopenia. The return to normal circulating platelet levels is the result of the apparent increase in production rate of platelets during week 3 of LIF administration. This finding is concordant with results in normal primates receiving LIF in which the onset of platelet production occurred at the end of a 14-day administration period. The number and size of megakaryocytes in the bone marrow of the normal primates did not change. However, recombinant murine LIF administered to normal mice increased both megakaryocyte and progenitor cells in marrow and spleen and resulted in an almost twofold increase in the circulating platelet count.

Using the same radiation model as reported here, we recently described the therapeutic efficacy of IL-3 and IL-6 in promoting platelet and neutrophil production. In our previous report, IL-3 and IL-6 reduced the duration of thrombocytopenia from 12 days in the controls to 6.6 days and 5 days, respectively. IL-3 and IL-6 were used at the same dose (15 \mu g/kg/d) and protocol as reported here.

The therapeutic efficacy of LIF was suggested by data showing the shared gp130 signal transducing component
LIF THERAPY IN IRRADIATED PRIMATES

... Metcalf et al. showed that LIF increased megakaryocyte colony formation and megakaryocytes when used in combination with IL-3. LIF alone had no demonstrable direct effect on these parameters despite the presence of LIF receptors on both immature and mature murine megakaryocytes. Burstein et al. compared LIF with IL-6 and IL-11 and found that LIF promoted megakaryocyte maturation in both liquid murine and human marrow cultures but did not induce an increase in megakaryocyte number in the absence of IL-3. This LIF-induced response did not appear to be facilitated through IL-6 action because the presence of an anti-IL-6 antibody had no influence on the LIF-induced increase in ploidy. However, LIF has been shown to induce synthesis and release of biologically active IL-6 from monocytes. Leary et al. also showed that LIF in combination with IL-3 enhanced human marrow-derived blast-cell colony formation.

The lack of an LIF-induced production of neutrophils noted in this model of radiation-induced marrow aplasia agrees with that observed in normal animals. Daily injections of LIF into normal mice or primates had no influence on total and differential leukocyte counts. In a previous study of radiation-induced marrow aplasia in the primate, we showed that neither IL-6 nor IL-3 significantly altered the duration of neutropenia or the recovery of neutrophils but, as currently shown with LIF, did modify the neutropenic nadir, relative to HSA-treated controls. These results show that LIF may be effective in the treatment of radiation-induced thrombocytopenia. This effect may be mediated in part through the concomitant presence of endogenous IL-3, IL-6, or other complementary cytokines in the postirradiation marrow environment.

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Therapeutic efficacy of recombinant human leukemia inhibitory factor in a primate model of radiation-induced marrow aplasia

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