Effects of Interleukin-1 on Hematopoietic Progenitors: Evidence of Stimulatory and Inhibitory Activities in a Primate Model


The effects of recombinant human interleukin-1 beta (rhIL-1b) on hematopoietic recovery following chemotherapy in a primate model were investigated. Cynomolgus monkeys received 1 μg/kg/day rhIL-1b intravenously for 2, 7, and 14 days following 5-Fluorouracil (5-FU) treatment (75 mg/kg × 2 days). Compared with controls, a significantly shortened time to achieve an absolute neutrophil (ANC) count over 500/μL was observed in animals receiving 2- and 7-day courses of rhIL-1b (17 ± 30 days), while animals receiving a 14-day course of rhIL-1b achieved an ANC over 500/μL by 23 days. Concomitantly, a marked increase in granulocyte-macrophage colonies (CFU-GM) was observed at 14 days following 5-FU in animals receiving 2- and 7-day rhIL-1b courses. In animals receiving a 14-day rhIL-1b course, a significant increase in CFU-GM relative to control was not seen until 21 days post 5-FU.

INTERLEUKIN-1 (IL-1), a cytokine with multiple immunologic and inflammatory functions, has been implicated recently in hematopoietic regulation. The dual activity of IL-1 involves, on the one hand, activation of early hematopoietic progenitors, initiation of their proliferation, and upregulation of receptors to other colony-stimulating factors (CSFs), and on the other hand, induces the production of CSFs by accessory cells. Moore and Warren showed that IL-1 administered to mice pretreated with 5-Fluorouracil (5-FU) accelerated neutrophil and progenitor cell recovery. Similar studies done with high doses of cyclophosphamide and IL-1 also resulted in accelerated recovery of neutrophils. IL-1 administered to mice post sub-lethal irradiation has been shown to accelerate hematopoietic recovery and reduces the nadir in neutrophil count. The synergistic stimulatory activity of IL-1 with other growth factors has been demonstrated both in vitro and in vivo. In culture, IL-1 alone does not support growth of hematopoietic progenitors, but in combination with CSFs it enhances the recovery of colonies. In vivo, the combination of IL-1 with G-CSF following 5-FU resulted in an accelerated recovery of neutrophils that was greater than the additive effects of these cytokines when administered individually. In contrast to its stimulatory activity on early hematopoietic progenitors, IL-1 may induce hematopoietic inhibitory activities via the endogenous production of other cytokines. The induction of myelosuppressive cytokines, such as tumor necrosis factor (TNF) and interferon-gamma, has been reported after IL-1 exposure both in vitro and in vivo. Thus, the therapeutic utility of IL-1 in enhancing hematologic recovery following myelosuppressive therapy will depend on defining protocols that maximize myeloenhancing activities and minimize the myelosuppressive ones.

In this study we show that administration of recombinant human interleukin-1 (rhIL-1) beta (rhIL-1b) to primates over short periods of time (two or seven days) is myelostimulatory, whereas prolonged administration (14 days) is less effective due to the induction of a serum inhibitory activity identified as TNFa.

Utilizing a serum-free colony assay system, a 50% inhibition of normal marrow CFU-GM growth was observed with the addition of sera obtained on day 9 post 5-FU from animals receiving rhIL-1b for 14 days. Sera obtained at any time from animals receiving 2- and 7-day rhIL-1b treatment did not show any growth inhibition. Addition of antibodies to TNFa to the coculture assay abrogated the CFU-GM growth inhibition. TNFa levels in sera with the inhibitory activity were relatively high (918 pg/mL). Our data indicate that rhIL-1b enhances hematopoietic recovery following 5-FU if administered for short periods of time (<7 days), whereas prolonged administration has a counterproductive effect that is due in part to the induction of TNFa production.

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MATERIALS AND METHODS

Primates. Eight cynomolgus monkeys (Macaca Fascicularis, obtained from Halzeton Research Animals, Reston, VA) were included in this study. Animals were divided in four groups of two each including controls and animals treated with rhIL-1b (Syntex, Palo Alto, CA) for 2, 7, and 14 days. All animals were treated with 5-FU 75 mg/kg/d for two consecutive days (150 mg/kg total). Twenty-four hours later, the IL-1-treated animals received daily an intravenous bolus of 1 μg/kg of rhIL-1b for 2, 7, or 14 days. Daily complete blood counts (CBCs) were obtained for 45 days post 5-FU. To assess progenitor cell recovery, bone marrow aspirates were obtained at weekly intervals for clonogenic assays (CFU-GM). Primate whole blood was collected periodically and immediately placed on ice; serum was separated and frozen at –80°C.

CFU-GM assay. Primate bone marrow was aspirated primarily from femurs into heparinized syringes and erythrocytes were removed by differential sedimentation in 3% gelatin. Low-density mononuclear cells were isolated by separation on Ficoll-Hypaque, washed three times and resuspended in Iscove’s modified Dulbecco’s medium (IMDM) (GIBCO, Grand Island, NY) supplemented with...
20% fetal calf serum (FCS) (Hyclone, Logan, UT). The colony assay for CFU-GM was done as previously described. Briefly, cells were plated at the concentration of 1 x 10^5/mL in 35-mm tissue culture dishes containing a 1 mL mixture of IMDM, 0.36% Agarose (FMS, Rockland, ME), 20% FCS; in the presence of rhG-CSF (1,000 U/mL), rhGM-CSF (1,000 U/mL) (Amgen, Thousand Oaks, CA), or rhIL-3 (50 ng/mL) (Immunex, Seattle). Cultures were incubated at 37°C in humidified 5% CO2 in air, and CFU-GM colonies/clusters were scored on day 7.

Cocultivation assay. To investigate whether rhIL-1b administration could induce hematopoietic inhibitory activities, serum-free cocultures of normal primate bone marrow cells with serum samples obtained from rhIL-1b-treated primates were performed. The serum-free medium was prepared as previously described. The final concentrations of the mixture contained bovine serum albumin (30 mg/mL), cholesterol (5 µg/mL), low-density lipoprotein (50 µg/mL), iron-saturated human transferrin (300 µg/mL), and calcium chloride (90 µg/mL) (Sigma Chemicals, St Louis). Ten percent 5637 serum-free conditioned medium, 1,000 U/mL of rhGM-CSF, or 1,000 U/mL rhG-CSF were added as exogenous growth factors. Cultures were incubated at 37°C in humidified 5% CO2 in air, and CFU-GM colonies were scored on days 7 and 14.

To determine whether TNF present in sera of rhIL-1b-treated monkeys plays a role in CFU inhibition, a polyclonal antibody to human TNF-alpha (specific activity >10,000 neutralizing units/mL; Endogen Inc, Boston) was added to the coculture assay at saturating concentrations (200 U/mL), and day 7 CFU-GM frequency was determined.

Serum levels of TNF-alpha. An ELISA (Endogen Inc) specific for TNF alpha (no cross-reactivity with other cytokines such as TNFb, rhIL-1, rhIL-2, rhIL-6) was used to measure TNF concentrations in serum of primates receiving rhIL-1. Optic densities (ODs) at 405 nm were determined on an automated ELISA reader (Biotek, Inc, Rutherford, NJ) as directed. Test sensitivity was 10 pg/mL.

Statistical analysis. The paired Student’s T test was used to test for significant differences between the rhIL-1b courses.

RESULTS

Neutrophil recovery. Post 5-FU, the recovery of neutrophils above 500/µL following different rhIL-1b schedules is shown in Table 1. Animals treated for two or seven days showed neutrophil recovery by 17 days, whereas the controls and those treated for 14 days recovered by 30 and 23 days, respectively (P < .05).

CFU-GM frequency. rhGM-CSF-stimulated CFU-GM incidence per 1 x 10^5 bone marrow cells following 5-FU and rhIL-1b treatment are shown in Fig 1. Animals receiving a course of two or seven days of rhIL-1b after 5-FU had a significantly more rapid recovery of progenitors by day 14, whereas controls and animals receiving a 14-day rhIL-1b course had a further delay of seven days until their CFU-GM progenitors recovered (P < .05). Similar results were obtained when cells were stimulated with rhG-CSF or rhIL-3.

Suppressive activity due to TNF. The suppressive activity of sera obtained at different times after rhIL-1b administration is shown in Fig 2. In this serum-free coculture assay, sera obtained from animals treated for 14 days with rhIL-1b exhibited an inhibitory activity that appeared on day 9 of IL-1 therapy. In contrast, sera from controls and animals treated with rhIL-1b for two or seven days did not show this activity. The difference in inhibitory activity between sera
obtained on day 9 after short (two days) or prolonged (14 days) rhIL-1b therapy was statistically significant ($P < .05$). Pre-incubation of inhibitory sera with anti-TNFα antibody prior to the coculture assay restored colony growth to 91% of control in the presence of rhG-CSF (Fig 3).

Serum obtained from animals treated with rhIL-1b for 14 days showed high levels of TNFα on day 9 post 5-FU (918 pg/mL compared with <12 pg/mL of controls and monkeys treated with rhIL-1b for two or seven days).

**DISCUSSION**

This study suggests that administration of rhIL-1b over a relatively short period of time is sufficient to enhance hematopoietic recovery following myelosuppressive therapy. The delayed hematopoietic recovery associated with prolonged administration of rhIL-1b is in part due to the induction of hematopoietic inhibitors.

These data in primates, showing that administration of rhIL-1b over short periods of time accelerates hematopoietic recovery following 5-FU, confirms the results in murine models. Moore and Warren⁴ demonstrated that, in mice, injection of IL-1 for two days after 5-FU, either alone or with rhG-CSF, resulted in accelerated recovery of neutrophils.

Neta and Oppenheim⁶ have also demonstrated that a single injection of IL-1 administered to mice post sub-lethal irradiation resulted in improved survival.

The regulation of hematopoiesis involves a complex set of interactions between stimulatory and inhibitory activities. Stimulatory activities include the effects of different hematopoietic growth factors that induce proliferation and differentiation of hematopoietic progenitors.¹³ The main mechanisms that are involved in counteracting these activities and maintaining a physiologic balance between positive and negative effects include down-regulation of the receptors to the stimulating cytokine, the disappearance of the stimulating factor from the system, or the induction of suppressive molecules. Administration of IL-1, which synergizes with other CSFs to promote proliferation of early progenitors and also stimulates accessory cells to produce different CSFs, can be considered for enhanced hematopoietic recovery following myelosuppressive chemotherapy. However, its ability to induce suppressive activities might counteract its positive effect and result in a delayed recovery. Our data showing that rhIL-1b injection over 14 days was inferior to two- or seven-day courses indicate that prolonged administration of IL-1 is not necessarily better, and that stimulatory and inhibitory activities need to be taken into consideration when planning treatment regimens.

**Fig 2.** CFU-GM suppressive activity in sera of recipients of the 14-day course of rhIL-1b is demonstrated. Myelosuppressive activity was detectable in sera obtained on day 9 when added to normal marrow cells plated for CFU-GM. This activity was not present after rhIL-1b discontinuation and also was not detected in controls or recipients of rhIL-1b for 2- or 7-day courses. Results represent mean +/− SEM for duplicate experiments, two animals for each point; all CFU-GM were plated in triplicate. Differences between short (two days) and long (14 days) rhIL-1b administration were significant at $P < .05$ on day 9 of rhIL-1b therapy.

**Fig 3.** Identification of TNFα in sera of recipients of prolonged rhIL-1b administration. Pretreatment of recipient serum demonstrating CFU suppressive-activity with saturating amounts of anti-TNFα antibody markedly decreased the serum suppressive activity, and restored CFU-GM growth. Cocultures were performed utilizing the day-9 sera obtained from monkeys treated with rhIL-1b for 14 days.
Inhibitory activities can be separated by different schedules. In addition to TNFa, IL-1 can induce the production of other inhibitors. For instance, the prostaglandins, which are inhibitory to hematopoiesis, appear within a few hours after IL-1 administration. Although the administration of IL-1 induces production of inhibitors, its overall effect is myelostimulatory when given for short periods of time. In contrast, the overall effect of prolonged IL-1 administration is less stimulatory, mainly due to the induction of the production of other cytokines such as TNFa. The in vivo biologic effects of IL-1 are extremely complex with induction of inhibitory and stimulatory activities, and only the balance between these opposing activities determines the observed hematologic effects. Therefore, the design of clinical trials with IL-1 should take in consideration both the stimulatory and inhibitory activities and focus on schedules that optimize its myelostimulatory capacity.

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Effects of interleukin-1 on hematopoietic progenitors: evidence of stimulatory and inhibitory activities in a primate model

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