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Synergy of interleukin 1 and granulocyte colony-stimulating factor: *In vivo* stimulation of stem-cell recovery and hematopoietic regeneration following 5-fluorouracil treatment of mice

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ABSTRACT **The human bladder carcinoma cell line 5637 produces hematopoietic growth factors [granulocyte and granulocyte/macrophage colony-stimulating factors (G-CSF and GM-CSF)] and hemopoietin 1, which synergizes with CSFs to stimulate colony formation by primitive hematopoietic stem cells in 5-fluorouracil-treated mouse bone marrow. Molecular and functional properties of hemopoietin 1 identified it as identical to interleukin 1α (IL-1α). When bone marrow cells from 5-fluorouracil-treated mice were cultured in suspension for 7 days with recombinant human IL-1α and/or G-CSF, it was found that the two factors synergized to enhance recovery of myelopoietic cells and colony-forming cells of both high and low proliferative potential. G-CSF alone did not sustain these populations, but the combination had greater-than-additive stimulating capacity. *In vivo*, 5-fluorouracil (150 mg/kg) produced profound myelosuppression and delayed neutrophil regeneration for up to 2 weeks in C3H/HeJ mice. Daily administration of recombinant human G-CSF or recombinant human IL-1α accelerated recovery of stem cells, progenitor cells, and blood neutrophils by up to 4 days in 5-fluorouracil-treated C3H/HeJ and B6D2F$_1$ mice. The combination of IL-1α and G-CSF acted synergistically, reducing neutropenia and accelerating recovery of normal neutrophil numbers by up to 7 days. This was accompanied by accelerated regeneration of spleen colony-forming units and erythroid, myeloid, and megakaryocytic progenitor cells in marrow and spleen, with enhanced erythroid and granulocytic differentiation. These results indicate the possible therapeutic potential of combination therapy with IL-1 and hematopoietic growth factors such as G-CSF in the treatment of chemotherapy- or radiation-induced myelosuppression.**

Four major groups of hematopoietic growth factors have been identified as interacting to control the production and function of granulocytes and macrophages. One of these, granulocyte-colony-stimulating factor (G-CSF), acts predominantly on mature neutrophils and their progenitors to stimulate proliferation, differentiation, and functional activation (1–4). G-CSF and granulocyte/macrophage (GM)-CSF have been purified from medium conditioned by the human bladder carcinoma cell line 5637 (2, 3, 5) and G-CSF from this source has been molecularly cloned (6). Because G-CSF is species-crossreactive, the *in vivo* action of recombinant human G-CSF (rhG-CSF) purified from *Escherichia coli* has been studied in mice (7), hamsters (8), and monkeys (9). The predominant response in all species involved a rapid and sustained neutrophil leukocytosis. In mice, the major expansion of neutrophil production elicited by chronic G-CSF treatment was met by a rapid onset of splenic granulopoiesis with an associated increase in erythropoiesis and megakaryocytopoiesis and elevated numbers of pluripotential stem cells and progenitors of all hematopoietic lineages (7). The ability of G-CSF to significantly shorten the period of chemotherapy-induced bone marrow hypoplasia has been documented in cyclophosphamide-treated monkeys (9) and in 5-fluorouracil-treated mice (10). Granulopoietic recovery after injury by 5-fluorouracil appeared to occur in two stages: early marrow regeneration, which was relatively uninfluenced by G-CSF treatment; then regeneration of CFU-GM (granulocyte/macrophage precursor cells, or "colony-forming units") in the spleen, which was significantly enhanced by G-CSF (10). Stem cells surviving a single injection of 5-fluorouracil are more primitive than the average normal stem cell and have greater self-renewal potential (11, 12). Bradley and Hodgson (13) described an *in vitro* assay of marrow from 5-fluorouracil-treated mice; in this assay, primitive stem cells with high proliferative potential formed large (>0.5-mm diameter) macrophage colonies in the presence of macrophage-CSF (CSF-1) and an obligatory synergistic activity found in human spleen or placenta conditioned medium. An *in vitro* radioreceptor assay using post-5-fluorouracil marrow showed that the synergistic activity, in concert with CSF-1, induced the generation of cells bearing CSF-1 receptors (14). Using this assay, a synergistic activity termed hemopoietin 1 was purified from the conditioned medium of the 5637 cell line (5637CM) (15). Hemopoietin 1 acted on the most primitive hematopoietic cells yet shown to proliferate and differentiate in culture, enabling them to respond to hematopoietic growth factors such as CSF-1 (14, 15) and interleukin 3 (IL-3) (16). We have shown (17) that hemopoietin 1 is distinct from the G-CSF and GM-CSF species produced by 5637 and is functionally, biochemically, and antigenically identical with IL-1α, which is constitutively produced by this cell line.

Here we show that rhIL-1α can stimulate *in vitro* proliferation and differentiation of bone marrow from 5-fluorouracil-treated mice, inducing expansion of stem cells with high and low proliferative potential (HPP- and LPP-CFU) in suspension culture and synergizing with rhG-CSF in generating neutrophils. In addition, we demonstrate a major *in vivo* role for IL-1 in promoting hematopoietic regeneration after 5-fluorouracil treatment, and a synergy with G-CSF in accelerating neutrophil regeneration.

MATERIALS AND METHODS

Mice. C3H/HeJ and (C57BL/6 × DBA/2)F$_1$ (B6D2F$_1$) mice were purchased from The Jackson Laboratory.

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Abbreviations: CSF, colony-stimulating factor; CSF-1, macrophage-CSF; G-CSF, granulocyte-CSF; GM-CSF, granulocyte/macrophage-CSF; IL-1 and IL-3, interleukins 1 and 3; rh-, recombinant human; 5637CM, conditioned medium from human bladder carcinoma cell line 5637; CFU, colony-forming unit(s); CFU-c, CFU assayed in culture; CFU-s, CFU assayed in spleen; CFU-GM, granulocyte/macrophage-CFU; CFU-Meg, megakaryocyte-CFU; CFU-GEMM, mixed-CFU; BFU-E, erythroid burst-forming unit; HPP, high proliferative potential; LPP, low proliferative potential.

Growth Factors. rhIL-1α (specific activity 6.6×10^6 units/mg) was kindly supplied by P. Lomedico (Hoffmann–La Roche). rhCSF-1 was a gift from P. Ralph (Cetus Corporation, Emeryville, CA). rhG-CSF (specific activity 10^8 units/mg) was supplied by Amgen Biologicals (Thousand Oaks, CA). Murine IL-3 was purified to homogeneity from WEHI-3 conditioned medium by procedures described by Ihle *et al.* (18). GM-CSF was partially purified from mouse lung conditioned medium. 5637CM was the source of synergistic activity (15).

Drug Treatments. rhIL-1α and rhG-CSF were diluted in Earle's balanced salts solution buffered to pH 7.2 with 10 mM Hepes and containing bovine serum albumin (100 μg/ml) as carrier. Four hours before the initiation of growth factor therapy, mice received a single i.v. injection of 5-fluorouracil (150 mg/kg of body weight). Mice were injected i.p. twice daily with 2 μg of rhG-CSF, 0.2 μg of rhIL-1α, or a combination of both agents. Mice were bled retroorbitally for determination of hematocrit and total and differential leukocyte count.

ΔCFU Assay. Femoral bone marow cells taken from $B6D2F_1$ mice 24 hr after a single i.v. injection of 5-fluorouracil (150 mg/kg) were incubated in Iscove's modified Dulbecco's medium (IMDM; GIBCO) with 20% fetal bovine serum (FBS; KC Biologicals, Lenexa, KS) in 24-well cluster plates (Corning) containing test stimuli. Each well received a total of 1 ml containing 2.5×10^5 cells. After 7 days of incubation, cells were recovered for morphological assessment and for CFU-s and CFU-c assays.

Spleen Colony Assay. Cells were injected intravenously into groups of five irradiated (9.5 Gy) syngeneic mice, and spleen colonies (CFU-s) were scored at 8 or 12 days.

LPP-CFU and HPP-CFU Assays. These CFU-c (CFU, culture) assays used a modification of the double-layer agar technique (13). In brief, underlayers (0.5% agarose in IMDM/20% FBS) were prepared in 35-mm Petri dishes (previously charged with test stimuli). Cell suspensions ($2.5–5 \times 10^4$ cells) in 0.5 ml of IMDM/20% FBS containing 0.36% agarose were used as second layers. Cultures were scored after incubation for 7 days under low oxygen tension (5% O_2/5% CO_2/90% N_2). All colonies estimated to contain >50 cells were scored, and colonies with diameters >0.5 mm were scored as HPP-CFU.

BFU-E, CFU-Meg, and CFU-GEMM Assays. Bone marrow cells (10^5) or spleen cells (2×10^5) were resuspended in 1 ml of IMDM/0.8% methylcellulose/30% FBS/0.2 mM hemin containing 100 units of pure IL-3 and 1 unit of recombinant human erythropoietin (Amgen). Dishes were incubated in a fully humidified, low-oxygen atmosphere. After 7 days of incubation, colonies were scored on the basis of gross morphology as erythroid burst (BFU-E), megakaryocyte (CFU-Meg), or mixed (CFU-GEMM).

RESULTS

ΔCFU Assay of Bone Marrow from 5-Fluorouracil-Treated Mice. Bone marrow cells were obtained from $B6D2F_1$ mice 24 hr after injection of 5-fluorouracil. Suspension cultures (2.5×10^5 cells in 1 ml) were established in the presence of various stimuli: rhG-CSF (2000 units), rhIL-1α (100 units), rhG-CSF plus rhIL-1α, endotoxin (0.1 μg; *Escherichia coli* lipopolysaccharide, Difco), or 5637CM (5%), which contains high levels of G-CSF (2) and IL-1α (17). After 7 days, cells were recovered for morphological assessment and for CFU assay. In the absence of added factors, cellularity fell to 12% of input by 7 days, with only macrophages recovered, and few or no CFU-s or CFU-c were detected in subsequent clonal assay (Table 1). After suspension culture with G-CSF alone, 24% of cells were recovered, mostly macrophages. Clonogenic assay of this population detected no CFU-s; low numbers of CFU-c responsive to G-CSF; a 4- to 40-fold increase in HPP-CFU responsive to GM-CSF, IL-3, or CSF-1; and a 2- to 3-fold increase in LPP-CFU over input values. Comparable results were obtained in cultures exposed to endotoxin (data not shown). Suspension culture with IL-1 alone increased the total number of cells 1.6-fold, with a 25-fold increase in blast cells and early granulocytes. Assay after 7 days of suspension culture with IL-1 revealed a major increase (Δ) in CFU. ΔCFU was greatest for HPP-CFU stimulated by IL-1 plus CSF-1 (>500-fold); smaller increases were seen for HPP-CFU stimulated with IL-1 plus G-CSF (>20-fold), GM-CSF (88-fold), or IL-3 (260-fold). LPP-CFU were maximally increased in colony assays with G-CSF alone or in combination with IL-1 (>900-fold); smaller increases were produced by IL-1 in combination with GM-CSF (27-fold), IL-3 (34-fold), or CSF-1 (140-fold). Day-12 CFU-s were slightly higher than input, and day-8 CFU-s, absent in the input marrow, were detected. Synergism between IL-1 and G-CSF was clearly seen in the colony assay but was less evident when the two factors were combined in the suspension preculture: recovery of total cells and early granulocytes was twice that predicted by addition of cells recovered after

Table 1. Day-7 suspension culture of bone marrow from 5-fluorouracil-treated $B6D2F_1$ mice

Suspension-culture stimulus*	Cells recovered,† no. $\times 10^{-4}$				CFU-c recovered (HPP, LPP) per well‡				CFU-s recovered per well (day 12, day 8)§
	Total	EG	LG	Mϕ	GM-CSF	IL-3	CSF-1	G-CSF	
None	3.0	0.0	0.0	3.0	3 ± 1, 10 ± 0	0 ± 0, 0 ± 0	7 ± 1, 6 ± 0	0 ± 0, 0 ± 0	0 ± 0, 0 ± 0
G-CSF	6.0	0.9	0.1	5.0	40 ± 2, 210 ± 9	13 ± 1, 100 ± 0	40 ± 2, 190 ± 4	0 ± 0, 7 ± 0	0 ± 0, 0 ± 0
IL-1	40.0	20.0	9.6	10.4	440 ± 40, 2200 ± 120	770 ± 40, 1200 ± 100	500 ± 20, 2500 ± 140	20 ± 3, 910 ± 40	20 ± 0, 12 ± 3
5637CM	25.0	17.6	4.4	3.0	380 ± 12, 2900 ± 20	380 ± 30, 810 ± 64	210 ± 13, 3600 ± 200	0 ± 0, 450 ± 40	44 ± 4, 0 ± 0
G-CSF + IL-1	95.0	39.0	12.4	43.6	720 ± 20, 3900 ± 80	490 ± 220, 1600 ± 440	990 ± 300, 1100 ± 360	30 ± 3, 1300 ± 190	40 ± 6, 10 ± 2

Bone marrow cells were obtained 24 hr after injection of 5-fluorouracil and cultured in suspension for 7 days. Initial cell no. was 2.5×10^4 per 1-ml culture. For morphology and CFU content of input marrow prior to culture, see text.

*Stimulus added to 1-ml suspension cultures: 2000 units of rhG-CSF, 100 units of rhIL-1α, or 5% 5637CM.

†Cells recovered after 7 days of incubation in suspension: EG, early granulocytes (blast, promyelocyte, myelocyte); LG, late granulocyte (metamyelocytes), Mϕ, macrophages.

‡Suspension cultures were assayed for HPP-CFU (upper line) and LPP-CFU (lower line) in quadruplicate agarose cultures stimulated by IL-1 plus the CSF species indicated.

§CFU-s were scored as spleen colonies in groups of four or five mice at day 8 or 12.

incubation with either factor alone; recovery of day-12 CFU-s was twice the additive value; and recovery of HPP- and LPP-CFU responsive to GM-CSF or G-CSF was 1.5 times that predicted by simple addition of recoveries from suspension cultures with IL-1 or G-CSF alone.

***In Vivo* Action of G-CSF and IL-1 in 5-Fluorouracil-Treated Mice.** G-CSF therapy accelerated neutrophil recovery in 5-fluorouracil-treated C3H/HeJ mice, with normal levels achieved in 9 days in contrast to 14 days required in controls (Fig. 1). Supranormal numbers of neutrophils were produced between 9 and 14 days of G-CSF therapy. Neutrophil numbers peaked at $\approx 10^5$ per mm^3, 1–3 orders of magnitude higher than in the 5-fluorouracil-treated mice not given G-CSF. IL-1 (given for 10 days) alone or combined with G-CSF had comparable consequences on neutrophil counts for the first 9 days, differing from G-CSF alone in three respects. An accelerated decrease in neutrophils occurred 1–3 days after 5-fluorouracil treatment. At no stage in IL-1-treated mice were neutrophils absent from the circulation. The exponential increase in neutrophil counts with IL-1 or IL-1 plus G-CSF preceded by 1–2 days that produced with G-CSF alone and preceded by 7 days the recovery after 5-fluorouracil alone. Synergy between IL-1 and G-CSF was evident between 7 and 9 days, since the combination induced the production of more neutrophils than either agent alone (Fig. 1).

Total numbers of hematopoietic cells (marrow, spleen, blood) were comparable in all groups at day 4 after 5-fluorouracil injection, with higher numbers of late granulocytes in all factor-treated groups (Fig. 2). By day 10, total cell numbers were comparable in the 5-fluorouracil and 5-fluorouracil plus G-CSF groups, but the latter group had 2–3 times more granulocytic cells, with increased myelopoietic activity in the spleen. The response of the mice to IL-1 or to IL-1 plus G-CSF was comparable, with a 3.5-fold increase in total cellularity and increases of 2- to 2.5-fold in erythroid elements, 5- to 6-fold in early granulocytes, and 12- to 13-fold in late granulocytes (Fig. 2).

Recovery of progenitor cells in the marrow 4 days post-5-fluorouracil showed significant augmentation of regeneration in groups receiving IL-1 alone or in combination with G-CSF (Fig. 3). HPP-CFU and LPP-CFU responsive to CSF-1 were increased 2.8-fold in IL-1-treated mice relative to control (5-fluorouracil alone), and the increase in CFU responsive to the other factors was 3.2-fold for IL-3, 2-fold for GM-CSF, and 5-fold for G-CSF. CFU-s were increased 6-fold in the IL-1-treated group and 3-fold in the IL-1 plus G-CSF group. G-CSF treatment alone augmented HPP- and LPP-CFU by a factor of 1.1–2.7 and CFU-s by a factor of 1.1. An intermediate level of progenitor augmentation (1.9- to 3.5-fold) was found in mice receiving IL-1 plus G-CSF. By day 7, progenitor and stem-cell regeneration was still confined to the marrow in the control mice, but extensive splenic repopulation with all classes of progenitors was seen with G-CSF or IL-1 treatment, and G-CSF and IL-1 in combination produced a 5–10 times greater incidence of splenic progenitors

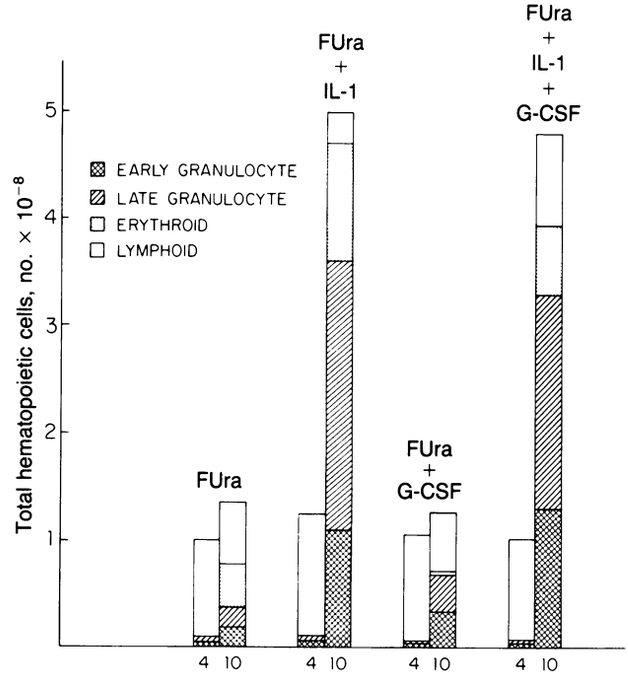

FIG. 2. Total hematopoietic cells (marrow, spleen, blood) and cell morphology in C3H/HeJ mice 4 or 10 days after injection of 5-fluorouracil (FURa; 150 mg/kg) alone or followed by i.p. injections of rhIL-1α and/or rhG-CSF as described for Fig. 1. Total cellularity was determined as total femoral marrow cellularity (multiplied by 20, to approximate total marrow cellularity) plus total spleen cellularity plus total blood nucleated cells (assuming a blood volume of 1.5 ml).

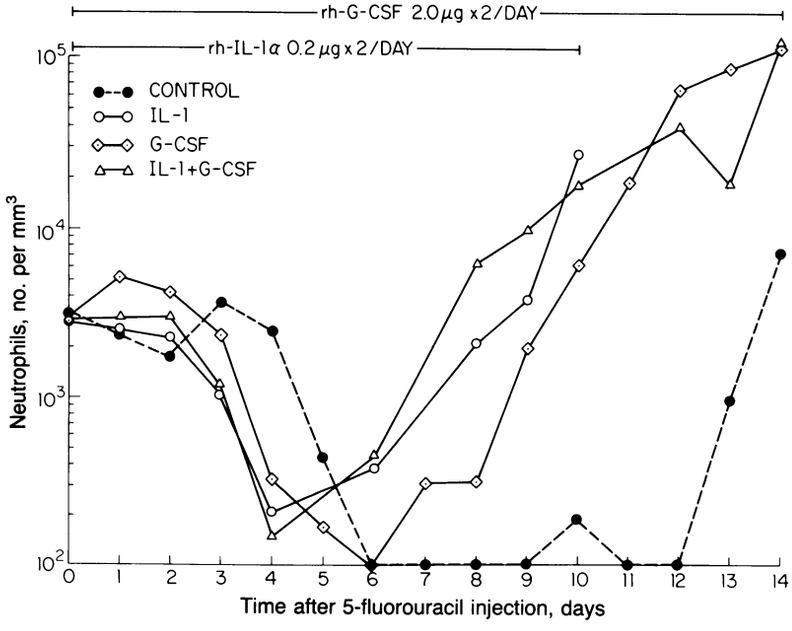

FIG. 1. Neutrophil counts in mice receiving 5-fluorouracil (150 mg/kg) as a single dose i.v. (on day 0) and either (*i*) 0.2 μg of rhIL-1α i.p. twice daily for 10 days, (*ii*) 2 μg of rhG-CSF i.p. twice daily for 14 days, or (*iii*) a combination of therapies *i* and *ii*. Factor injections were begun 4 hr after treatment with 5-fluorouracil. Control mice received 5-fluorouracil only. Results are for groups of four C3H/HeJ mice. Note logarithmic scale on the ordinate. Control mice had no detectable neutrophils between days 6 and 9.

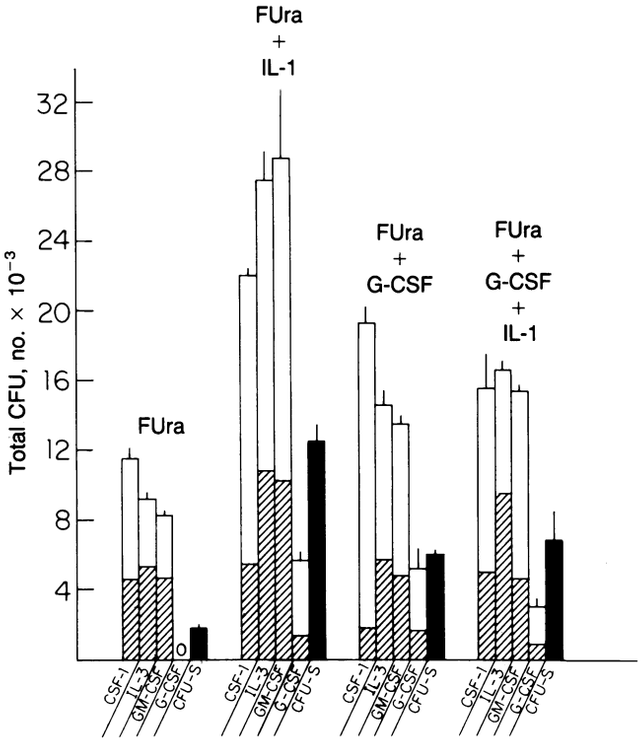

FIG. 3. Total marrow and spleen HPP-CFU (hatched column) and LPP-CFU (open column) and day-12 CFU-s (black column) (+ SEM) in mice 4 days after a single i.v. injection of 5-fluorouracil (FUra; 150 mg/kg). Total numbers were calculated by determining incidence in one femur, multiplying by 20, and adding the total per spleen. HPP-CFU and LPP-CFU assays were done with quadruplicate cultures (2.5×10^4 cells per ml) stimulated by CSF-1, IL-3, GM-CSF, and G-CSF in the presence of rhIL-1α. CFU-s colonies were scored in groups of five recipients. Each data point is based on pools of four femurs and two spleens from two mice in each group. Groups received rhIL-1, rhG-CSF, or both as described in the legend to Fig. 1. Mice were killed 16 hr after the last injection.

than treatment with either agent alone. Total numbers of CSF-1-responsive HPP- and LPP-CFU were increased 23-fold with IL-1 treatment, 11-fold with IL-1 plus G-CSF treatment, and 9-fold with G-CSF alone (Fig. 4). Comparable increases were seen in progenitors responsive to G-CSF, and lesser increases were found in progenitors responsive to GM-CSF and IL-3, where the increase over 5-fluorouracil treatment alone was 7- to 11-fold for IL-1 treatment, 6-fold for G-CSF, and 5- to 7-fold for IL-1 plus G-CSF. By 10 days post-5-fluorouracil, HPP-CFU and LPP-CFU recoveries were augmented to similar degrees for IL-1- and IL-1 plus G-CSF-treated mice, with the response to CSF-1 up by a factor of 4.3–9.3; to IL-3, 3.6–5.4; to GM-CSF, 5.6–8.1; and to G-CSF, 3.4–3.9 (Fig. 4). CFU-s were augmented by 2.5, CFU-Meg by 1.2–1.8, CFU-GEMM by 6–19, and BFU-E by 22–39. Treatment with G-CSF alone had little or no effect on the number of CFU-s and of HPP- and LPP-CFU responsive to various CSFs (<1.3-fold increase), but CFU-GEMM and BFU-E were increased 3.3-fold and 3.9-fold, respectively.

DISCUSSION

These studies show that IL-1 can act synergistically with hematopoietic growth factors both *in vivo* and *in vitro*. HPP colonies developing in the presence of IL-1 and CSFs were of a frequency and morphological type that was determined by the CSF species, with CSF-1-stimulated HPP colonies being most frequent and entirely macrophage, and G-CSF-stimulated HPP colonies being least frequent and having the greatest neutrophil component. The ΔCFU suspension-culture studies confirmed the enhanced self-renewal capacity of HPP-CFU, first demonstrated in serial colony recloning studies (16), and showed it to be profoundly influenced by IL-1. The greatly enhanced recovery of both HPP- and LPP-CFU following 7 days of culture in the presence of IL-1 alone or in combination with G-CSF indicates an IL-1 action on self-renewal of early stem cells, as well as an action promoting differentiation into directly CSF-responsive LPP-CFU compartments. Previous work (17) showed that CSF-1, like G-CSF, is incapable of supporting HPP-CFU proliferation and differentiation; however, IL-3 and GM-CSF were capable of doing so to some degree, probably due to their ability to interact directly with subpopulations of pluripotent stem cells and so to enhance self-renewal, differentiation, or survival (1, 19). In no case, however, were CSFs or IL-3 capable of acting directly upon the most primitive stem cells, which remain absolutely dependent upon IL-1 for survival, proliferation, and receptor up-regulation, at least within the time frame of the studies reported here.

In the HPP clonal assay, IL-1 alone had no direct colony-stimulating ability; in suspension culture, it stimulated significant myelopoiesis and production of both HPP- and LPP-CFU. The most probable explanation for this is that the cells in the suspension assay were cultured at a 10-fold higher concentration than in the clonal assay. Marrow accessory-cell populations such as fibroblasts, endothelial cells, and macrophages under such conditions are capable of G- and GM-CSF production when exposed to low concentrations of IL-1 (20–23). The possibility that the action of IL-1 on early stem cells is also indirect, perhaps involving induction of endogenous CSF by accessory cells, is excluded by the following observations. (*i*) In the HPP-CFU assay, colony formation was linearly related to the number of cells plated, down to limiting dilutions (17). (*ii*) In suspension culture, the addition of G-CSF, GM-CSF, CSF-1, and IL-3, alone or in various combinations, at concentrations up to 10-fold plateau concentrations for maximal LPP colony formation did not potentiate HPP- and LPP-CFU recovery to the extent seen with IL-1 alone or with IL-1 plus supraadditive concentrations of CSFs (17).

The identification of hemopoietin 1 in 5637CM as IL-1α significantly extends the known biological activity of this pleiotropic, nonspecific, hormone-like cytokine. Its action in hematopoiesis involves activation of neutrophils with increased lactoferrin release, neutrophil chemotaxis, and release of neutrophils from bone marrow stores into the blood (24). To all that we must now add the ability of IL-1 to induce CSF production (20–23) and stimulate early stem cells to display CSF receptors and to synergize with various CSFs to stimulate hematopoietic cell production.

Pretreatment of mice with IL-1 provides a dose-dependent protection from the lethal effects of ionizing radiation (25). An explanation for this protection could be a direct action of IL-1 on the proliferation and differentiation of early stem cells.

The action of G-CSF alone in 5-fluorouracil-treated mice clearly led to accelerated recovery of neutrophils, hematopoietic cells, and all classes of progenitors and stem cells, but recovery was much slower than that seen after treatment with high doses of cyclophosphamide (7, 9). In contrast, IL-1 given twice daily for 4 or 10 days had a major impact on myelopoietic regeneration, accelerating the appearance of all classes of stem cells and progenitors in marrow and spleen, stimulating regeneration of differentiating erythroid and granulocytic cells, and accelerating neutrophil recovery to an extent exceeding that seen with G-CSF alone. In view of the *in vitro* evidence that IL-1 alone does not support myeloid or erythroid differentiation, the most plausible explanation for the *in vivo* effects implicates a dual role for this cytokine—

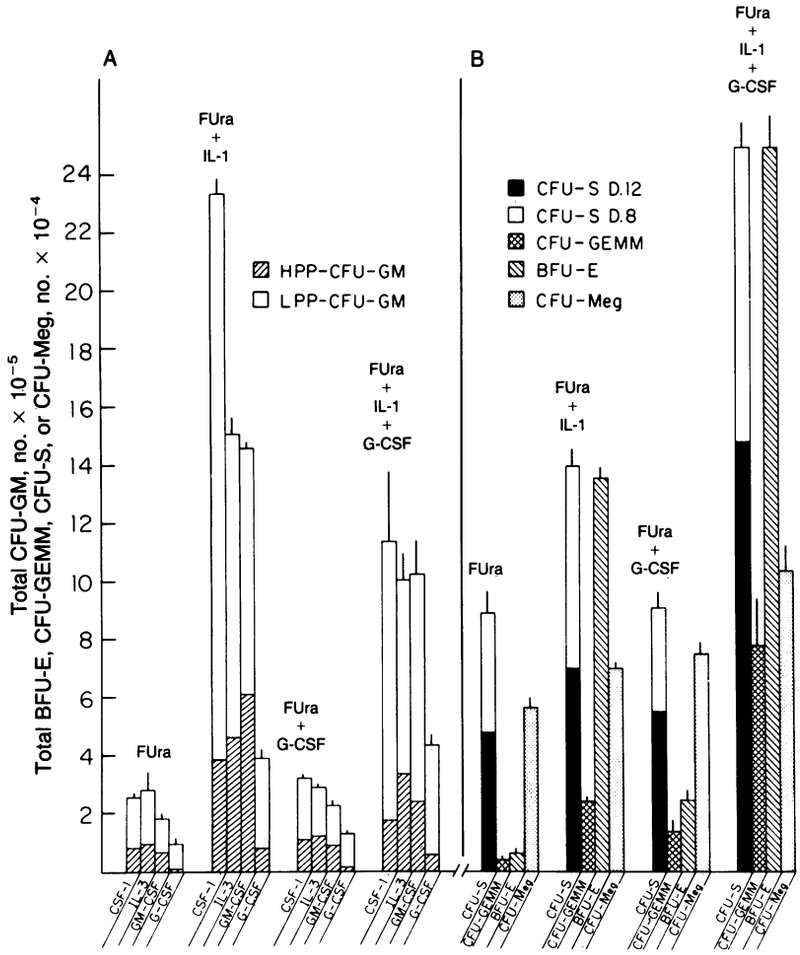

FIG. 4. Total stem-cell and progenitor populations in C3H/HeJ mice 10 days after injection of 5-fluorouracil (FUra). As indicated, mice received rhIL-1α, rhG-CSF, or a combination therapy as described for Fig. 1. Control mice received no therapy. (*A*) Total marrow and spleen HPP-CFU and LPP-CFU responsive to various CSFs in agarose culture were determined as for Fig. 3. (*B*) Total day-8 (two mice per group) or day-12 (four mice per group) CFU-s were scored. CFU-GEMM, BFU-E, and CFU-Meg were scored in triplicate methylcellulose cultures stimulated with IL-3 and recombinant human erythropoietin. Data are based on pools of four femurs and two spleens from two mice in each group.

induction of increased production of G- and GM-CSF by an action on host accessory cells and direct stimulation of early stem cells resulting in CSF-receptor up-regulation. The greater-than-additive effects of the combination of IL-1 and G-CSF on *in vivo* granulopoiesis and total neutrophil number strongly suggests that G-CSF production, even in response to IL-1, is less than optimal and that the combination of the two factors is the most effective way to promote maximal hematopoietic regeneration.

The *in vivo* results that we have obtained point to an important role for IL-1 in combination with specific hematopoietic growth factors in counteracting chemotherapy- or radiation-induced myelosuppression.

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1. Metcalf, D. (1986) *Blood* **67,** 257–267.
2. Welte, K., Platzer, E., Lu, L., Gabrilove, J., Levi, E., Mertelsmann, R. & Moore, M. A. S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1526–1530.
3. Platzer, E., Welte, K., Gabrilove, J., Lu, L., Harris, P., Mertelsmann, R. & Moore, M. A. S. (1985) *J. Exp. Med.* **162,** 1788–1801.
4. Nicola, N. A., Metcalf, D., Johnson, G. R. & Burgess, A. W. (1979) *Blood* **54,** 614–627.
5. Gabrilove, J., Welte, K., Harris, P., Platzer, E., Lu, L., Levi, E., Mertelsmann, R. & Moore, M. A. S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2478–2482.
6. Souza, L., Boone, T., Gabrilove, J., Lai, J., Zsebo, K., Murdock, D., Chazin, V., Bruszewski, J., Lu, H., Chen, K., Barendt, J., Platzer, E., Moore, M. A. S., Mertelsmann, R. & Welte, K. (1986) *Science* **232,** 61–65.
7. Moore, M. A. S., Welte, K., Gabrilove, J. & Souza, L. (1987) *Blut*, in press.
8. Zsebo, K., Cohen, A., Murdock, D., Boone, T., Inoue, H., Chazin, V., Hines, D. & Souza, L. (1987) *Immunobiology*, in press.
9. Welte, K., Bonilla, M. A., Gillio, A. P., Boone, T. C., Potter, G. K., Gabrilove, J. L., Moore, M. A. S., O'Reilly, R. J. & Souza, L. M. (1987) *J. Exp. Med.* **165,** 941–948.
10. Shimamura, M., Kobayashi, Y., Yuo, A., Urabe, A., Okabe, T., Komatsu, Y., Itoh, S. & Takaku, F. (1987) *Blood* **69,** 353–355.
11. Rosendaal, M., Hodgson, G. S. & Bradley, T. R. (1979) *Cell Tissue Kinet.* **12,** 17–22.
12. Van Zant, G. (1984) *J. Exp. Med.* **159,** 679–690.
13. Bradley, T. R. & Hodgson, G. S. (1979) *Blood* **54,** 1446–1450.
14. Bartelmez, S. H. & Stanley, E. R. (1985) *J. Cell. Physiol.* **122,** 370–378.
15. Jubinsky, P. T. & Stanley, E. R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2764–2768.
16. Stanley, E. R., Bartocci, A., Patinkin, D., Rosendaal, M. & Bradley, T. R. (1986) *Cell* **45,** 667–674.
17. Moore, M. A. S., Warren, D. & Souza, L. (1987) in *Leukemia: Recent Advances in Biology and Treatment*, eds. Gale, R. P. & Golde, D. W. (Liss, New York), in press.
18. Ihle, J. N., Keller, J., Henderson, L., Klein, F. & Palaszynski, E. (1982) *J. Immunol.* **129,** 2431–2436.
19. Bartelmez, S. H., Sacca, R. & Stanley, E. R. (1985) *J. Cell. Physiol.* **122,** 362–369.
20. Lovhaug, D., Pelus, L. M., Nordlie, E. M., Boyum, A. & Moore, M. A. S. (1986) *Exp. Hematol.* **14,** 1037–1042.
21. Bagby, G. C., Dinarello, C. A., Wallace, P., Wagner, C., Hefeneider, S. & McCall, E. (1986) *J. Clin. Invest.* **78,** 1316–1323.
22. Zucali, J. R., Dinarello, C. A., Oblon, D. J., Gross, M. A., Anderson, L. & Weiner, R. S. (1986) *J. Clin. Invest.* **77,** 1857–1863.
23. Fibbe, W. E., van Damme, J., Billiau, A., Voogt, P. J., Duinkerken, N., Kluck, P. M. C. & Falkenburg, J. H. F. (1986) *Blood* **68,** 1316–1321.
24. Oppenheim, J. J., Kovacs, E. J., Matsushima, K. & Durum, S. K. (1986) *Immunol. Today* **7,** 45–56.
25. Neta, R., Douches, S. & Oppenheim, J. J. (1986) *J. Immunol.* **136,** 2483–2485.