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Proliferative Effects of a Recombinant Human Granulocyte Colony-Stimulating Factor (rG-CSF) on Highly Enriched Hematopoietic Progenitor Cells

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A. Introduction

Human multipotential and committed hematopoietic progenitor cells require the presence of specific glycoproteins, termed "colony-stimulating factors" (CSFs) for survival, clonal proliferation, and differentiation. Recently, a pluripotent G-CSF, constitutively produced by the human bladder carcinoma line 5637, has been purified to apparent homogeneity [1] and molecularly cloned with the complementary DNA copy of the gene expressed in *Escherichia coli* [2]. This factor induces terminal differentiation of the murine myelomonocytic cell line WEHI3B(D^+), the human promyelocytic cell line HL60, and leukemic cells from patients with certain forms of ANLL [2, 3] and has been shown to stimulate the growth of day-7 granulocyte colonies, erythroid bursts (BFU-E), and multilineage colonies (CFU-GEMM) from human bone marrow [1–3]. Despite its similarity with murine G-CSF [4], the latter does not support the proliferation of BFU-E and CFU-GEMM [5], biological activities shared by murine IL3 and GM-CSF [6–8]. However, IL3 has not been reported to induce differentiation of leukemic cells [9], no significant homology was found between the deduced amino acid sequence for hG-CSF and those for murine IL3 [10, 11] and murine and human GM-CSF [12, 13], and specific binding of radiolabeled hG-CSF was inhibited by an excess of unlabeled hG-CSF but not by hGM-CSF [2]. In these previous studies, accessory cell-mediated biological activities and direct effects on progenitor cells were not distinguished. To address these considerations, the effects of hG-CSF on accessory cell-free bone marrow populations highly enriched for hematopoietic progenitors were examined.

B. Methods

Low-density bone marrow separated by Ficoll-Hypaque density centrifugation was enriched 10- to 12-fold for progenitor cells by depletion of mature myeloid, monocytic, lymphoid, and erythroid cells using the mAbs Mol, MY8, MY3, N901, B4 (generously provided by J. Griffin, Boston), OKT4, OKT8, OKT11, and antiglycophorin by immunoadherence "panning" [14, 15] and complement-mediated cytotoxicity (LDAC-). By subsequent fluorescence-activated cell sorting, a nearly homogeneous blast population defined by low perpendicular and high forward light scatter (blast window) and expression of the HPCA-1 antigen (detected by the MY10 mAb), present on all hematopoietic progenitors [16], was isolated with a purity of 85%–95% and an overall plating efficiency of up to 30%. Alternatively to MY10, an anti-HLA-DR mAb (clone L243; both Abs a kind gift of N. Warner, Becton Dickinson) was used for positive selection of clonogenic cells [17]. DAD14 (d14) and DAY7 (d7) CFU-GM, BFU-E, and CFU-GEMM were assayed in agar and methylcellulose cultures respec-

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tively. Erythropoietin (EPO) was added on d3 to eliminate background growth of BPA-independent, EPO-responsive BFU-E [18]. In cultures of MY10+ and HLA-DR+ populations no spontaneous colony formation was observed.

C. Results and Discussion

As shown in Table 1, 100–2000 units of recombinant G-CSF (specific activity approximately 1×10^8 units per mg protein) stimulate proliferation of CFU-GM in a dose-dependent manner and by direct action on MY10+ progenitor cells. Aggregates that had developed by day 7 were uniformly small, rarely exceeding 20–30 cells even at high concentrations of G-CSF, and purely granulocytic, containing mature neutrophils as demonstrated by esterase stains. No significant differences in either number or size of aggregates were observed when equivalent concentrations of recombinant and highly purified G-CSF were compared (data not shown), indicating that lack of glycosylation of the recombinant material does not adversely affect its biological activity. CFU-GM scored after 14 days were predominantly granulocytic (64%–75%); in addition, the formation of a small number of mixed granulocyte/macrophage colonies (4% at 250 U/ml, 10% at 2000 U/ml) and macrophage clusters of 6–20 cells (20%–30% of total aggregates in several experiments) was stimulated. In contrast, growth of eosinophil colonies was not supported, even by high concentrations of G-CSF, as judged by Congo red stains of agar cultures. These data are consistent with results obtained previously with murine G-CSF [5].

Table 1. Stimulation by rhG-CSF of d7 and d14 CFU-GM from MY10+ bone marrow cells cultured in triplicate at 1000 cells/ml. Values (clusters: 4–50 cells; colonies: more than 50 cells) are expressed as mean ± standard deviation calculated for 1×10^5 cells

rhG-CSF U/ml	CFU-GM (colonies/clusters)	
	Day 7	Day 14
0	0/0	0/0
100	0/2780 ± 490	1600 ± 400/1600 ± 330
250	0/4890 ± 570	2660 ± 530/1400 ± 70
500	0/5130 ± 176	2900 ± 70/1130 ± 130
2000	0/5380 ± 550	3470 ± 130/1130 ± 200

The capacity of rG-CSF to stimulate BFU-E and CFU-GEMM was examined in a highly sensitive BPA assay, using sequentially purified progenitor populations (LDAC- and sorted HLA-DR+, MY10+ blasts) as target cells. As shown in Fig. 1 for MY10+ cells, 50–2000 U/ml rG-CSF failed to stimulate CFU-GEMM or increase BFU-E formation above background levels. Identical results were obtained in numerous experiments irrespective of the target population used. Comparison of highly purified and recombinant G-CSF at both 1000 and 5000 U/ml confirmed that the absence of BFU-E- and CFU-GEMM-stimulating activity of rG-CSF is not a consequence of the lack of glycosylation. Readdition of autologous, unstimulated OKT4- and OKT8-positive lymphocytes, isolated by cell sorting with 98% purity, to cultures of MY10+ cells did not augment colony formation in either the absence or the presence of G-CSF (data not shown). These results are at variance with earlier studies [1–3], which employed target populations depleted of accessory cells by plastic adherence and E-rosetting, techniques that do not facilitate the degree of progenitor enrichment and accessory cell depletion achieved by the immunological techniques used in this study. It is conceivable that the stimulation of erythroid and multilineage colonies observed in those reports was mediated by an as yet unidentified accessory cell.

To assess whether or not G-CSF is able to facilitate the survival and or initial proliferation of BFU-E and CFU-GEMM, delayed addition experiments were carried out (Table 2). The almost complete loss of BPA-responsive BFU-E and of CFU-GEMM caused by delaying the addition of BPA till day 4 of culture is not abrogated by the continuous presence of G-CSF. This result differs slightly from that obtained with murine fetal liver cells, where G-CSF appeared to stimulate the initial proliferation of a subpopulation of multipotential and erythroid precursors [5].

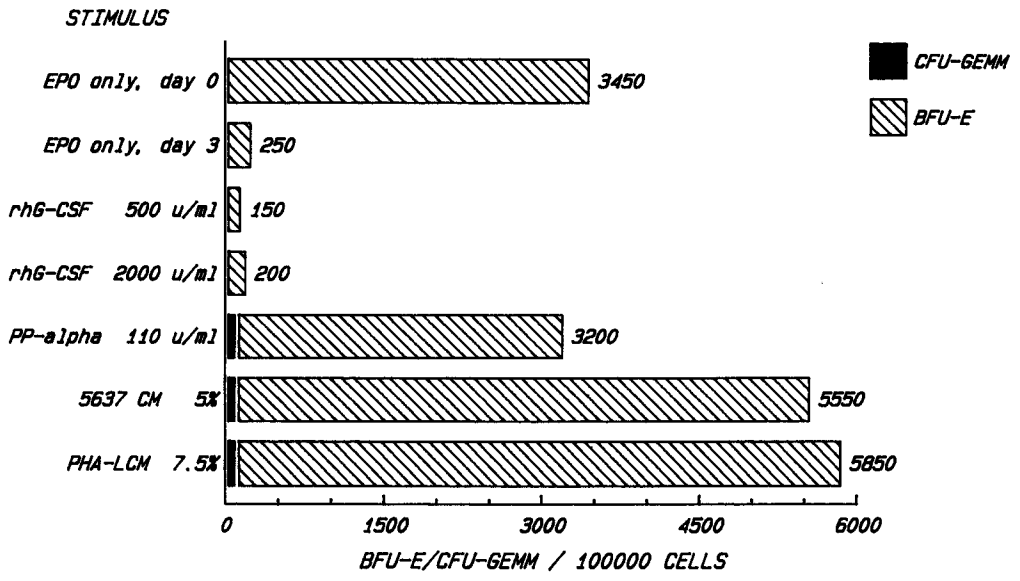

Fig. 1. Growth of BFU-E and CFU-GEMM in methylcellulose cultures of MY10+ cells cultured in duplicate at 1000 cells/ml. Comparison of rG-CSF with 5637 CM, PHA-LCM and PP-alpha (Pluripoietin-α), a GM-CSF-like activity purified from the bladder carcinoma line 5637 [20]. Recombinant EPO was added on day 3 of culture unless otherwise stated

Table 2. Delayed addition of burst-promoting activity (BPA) to methylcellulose cultures of enriched progenitor populations in the presence or absence of rhG-CSF. LDAC-, HLA-DR+, and MY10+ cells were plated at 1×10^4, 2.5×10^3, and 1×10^3 cells/ml respectively. 5637 CM was used as a source of BPA

Stimulus			Bone marrow fraction		
EPO	rhG-CSF	BPA	LD AC-	HLA-DR+	MY10+
Day of addition			Number of BFU-E/CFU-GEMM/10^5 cells		
3	–	–	90/ 0	40/ 0	350/ 0
3	–	0	700/10	1700/120	4800/420
3	0	–	160/ 0	200/ 0	200/ 0
3	–	4	210/ 0	180/ 0	300/ 0
3	0	0	140/ 0	0/ 0	400/ 0

In conclusion, it appears that the direct action of human G-CSF is restricted primarily to the granulocytic lineage, in which it supports the proliferation and differentiation of committed progenitors (d7 and d14 CFU-GM) and stimulates end-stage cells as determined by antibody-dependent cell-mediated cytotoxicity and induction of chemotactic peptide binding [2, 20].

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