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Effect of Human G-CSF on Clonogenic Cells in Acute Myeloblastic Leukemia

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Abstract—*The effects of purified human native granulocyte colony-stimulating factor (G-CSF) on the growth of clonogenic leukemic blast cells from 10 Japanese patients with acute myeloblastic leukemia (AML) were studied, using an* in vitro *leukemic blast colony assay. The clonogenic leukemic blast cells from six patients with AML were stimulated to form colonies in viscous medium* in vitro *by the addition of* 100 ng/ml *G-CSF. The possibility that G-CSF may be a leukemic blast growth factor warrants further attention.*

INTRODUCTION

CLONOGENIC BLAST CELLS from the peripheral blood of patients with acute myeloblastic leukemia (AML) form colonies in cultures made viscous with methylcellulose and in the presence of appropriate growth factors [1]. These leukemic blast growth factors (LBGFs) are usually supplied by media conditioned by phytohemagglutinin-stimulated leukocytes (PHA-LCM) from normal donors [1] or media conditioned by culture of a human bladder carcinoma cell line, HTB9 (HTB9-CM) [2, 3].

Using human recombinant granulocyte macrophage-colony stimulating factor (GM-CSF), other workers found that GM-CSF stimulated proliferation of clonogenic cells in AML and GM-CSF was considered one of the LBGFs [4, 5]. In this paper, we report that granulocyte-colony-stimulating factor (G-CSF) was also proved to have activity towards leukemic blast growth, using human native G-CSF purified from media conditioned by culture of a squamous cell carcinoma cell line [6].

MATERIALS AND METHODS

Leukemic blast cells from 10 Japanese patients with AML were studied. The clinical information of the patients is shown in Table 1. The patients were diagnosed according to the French–American–British (FAB) classification of acute leukemia [7]. All patients had received no previous treatment. Mononuclear cells from the peripheral blood were obtained by density centrifugation [8], and T lymphocytes were removed by E rosetting [9]. The remaining cells were used for the leukemic blast colony assay.

The leukemic blast colony assay was a modification of the technique of Buick *et al.* [1]. Briefly, blast cells were plated in plastic 96-microwell plates in alpha medium (GIBCO, Grand Island, NY, U.S.A.) containing 0.88% methylcellulose and 10% fetal calf serum (FCS). Each well contained $2–4 \times 10^4$ cells in 0.1 ml of medium and a stimulator. The cultures were incubated for 6 days in a moist atmosphere containing 5% CO_2, and colonies containing more than 20 cells were counted using an inverted microscope. Individual colonies were picked and the cells placed directly on slides. After air drying and fixation in absolute methanol, they were stained with Giemsa solution.

The purified G-CSF ($2.5–5 \times 10^7$ units/mg protein) was obtained from Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan). This preparation had been purified to homogeneity from medium conditioned by a human CSF-producing tumor cell line, CHU-2 [6].

The bladder carcinoma cell line, HTB9, was provided by Dr. E.A. McCulloch (Ontario Cancer Institute, Toronto, Canada) [2]. The cells were grown in alpha medium supplemented with 5% FCS and were trypsinized at confluency and expanded five-fold. After 5 days of culture, the

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Table 1. Clinical characteristics of the patients

Case No.	Age	Sex	Diagnosis (FAB)*	Peripheral blood Leukocytes (/μl)	Peripheral blood Blasts (%)	Bone marrow NCC† (× 10^4/μl)	Bone marrow Blasts (%)
1	86	F	M2	27,300	82	49.8	93.2
2	30	M	M2	13,300	90	53.5	92.8
3	55	M	M2	5600	89	15.9	96.9
4	66	M	M2	28,600	79	48.2	84.4
5	32	M	M3	2500	58	4.5	51.6
6	61	F	M4	7400	93	Dry tap	>95
7	79	M	M4	65,800	92	10.2	68.4
8	38	M	M4	24,500	98	14.7	36.8
9	42	F	M5	8700	99	12.3	75.2
10	55	M	M6	4500	76	74.7	36.8

*The French–American–British (FAB) classification was determined as described [7].
†NCC: nuclear cell count.

conditioned medium was harvested and sterilized by filtration (Millipore, 0.22 μm).

RESULTS

The effects of G-CSF on the growth of clonogenic leukemic blast cells from 10 patients with AML were studied. A substantial number of colonies was formed when over 10 ng/ml of G-CSF was added to the culture, in the two cases shown in Fig. 1a. The colonies were compact and cells within the colonies were similar in morphology to leukemic blasts. The maximal stimulatory effect on colony formation was reached at a concentration of 50 ng/ml of G-CSF (Fig. 1a). All further experiments in this study were performed with the addition of 100 ng/ml G-CSF. As positive controls for leukemic colony assay, HTB9-CM was similarly tested. The maximal colony formation was observed by the addition of 5% HTB9-CM (Fig. 1b), and following experiments were done using 10% HTB9-CM.

The number of leukemic blast colonies formed on addition of 100 ng/ml G-CSF is shown in the extreme right column in Table 2. A substantial number of colonies was observed in seven of the 10 cases examined, though a small number of colonies did form, even in the absence of exogenous G-CSF in two cases. In both cases (case 6 and case 8), the patients were diagnosed as M4. In one (case 8) of the two cases in which spontaneous colonies were formed, the clonogenic blast cell growth was not

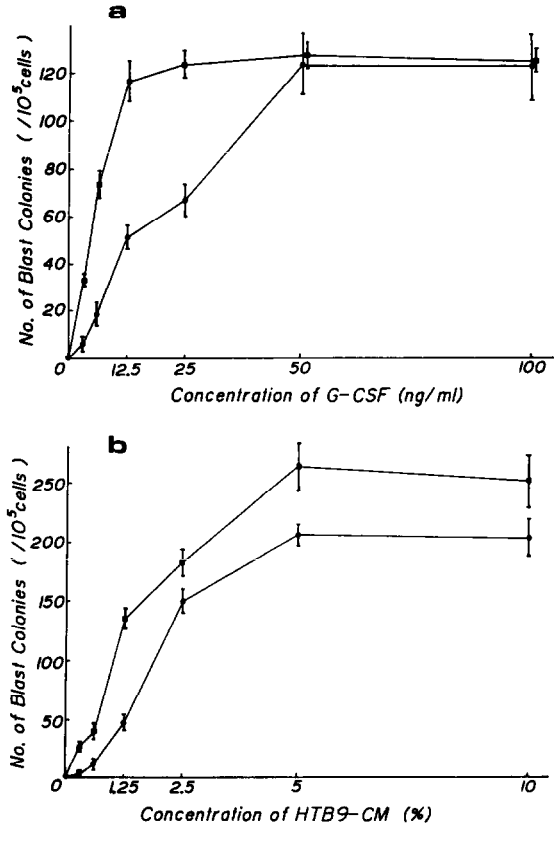

Fig. 1. Dose-response relationships between blast colonies formed and concentration of purified G-CSF (a: upper panel) and HTB9-CM (b: lower panel). Peripheral blood cells of two patients with AML, FAB classification M2 (●—●) and M4 (■—■) were tested. Each point and vertical bar represent the mean ± S.D.

*Table 2. Effects of purified G-CSF (100 ng/ml) and HTB9-CM (10%) on blast colony formation**

Case No.	Stimuli: Control	Stimuli: HTB9-CM	Stimuli: G-CSF
1	0	205 ± 12	0
2	0	179 ± 9	13 ± 6
3	0	203 ± 18	126 ± 14
4	0	75 ± 10	42 ± 2
5	0	333 ± 10	69 ± 17
6	33 ± 2	241 ± 22	173 ± 8
7	0	702 ± 45	0
8	68 ± 5	157 ± 4	69 ± 6
9	0	893 ± 125	49 ± 8
10	0	71 ± 3	0

*Numbers of blast colonies formed are expressed as mean ± S.D./10^5 cells.

further stimulated by the addition of G-CSF. In the other six cases, colonies did form and a substantial stimulating effect of G-CSF on the clonogenic blast cells was also observed (Table 2).

HTB9-CM was consistently a more effective stimulator for clonogenic blast cell growth than was G-CSF, despite patient to patient variations.

DISCUSSION

LBGFs are required for the growth of clonogenic cells from patients with AML, except some patients with M4 [10]. Hoang and McCulloch [2] suggested the presence of a novel growth factor in the HTB9-CM which potently and widely enhances growth of the clonogenic blast cells from patients with AML. Griffin *et al.* [4] demonstrated that recombinant GM-CSF, a hemopoietic growth factor, stimulated blast colony formation. Hoang *et al.* [5] confirmed this observation. The HTB9 cell line expressed GM-CSF mRNA [3]. As there is the possibility that other known hemopoietic growth factors might act as a LBGF, purified G-CSF, one of the four known hemopoietic factors, was studied using the blast colony assay system. It was reported that purified G-CSF could stimulate growth and differentiation of normal granulocyte progenitor cells [6] and that extremely large quantities of G-CSF (1×10^5 unit/ml) could induce differentiation in some leukemic cells, *in vitro* [11].

Clonogenic blast cells from the majority (six out of 10 patients) but not all patients with AML were stimulated to form colonies in the methylcellulose culture in the presence of G-CSF, as shown in Fig. 1 and Table 1. This growth stimulatory effect of human G-CSF on clonogenic blast cells *in vitro* does not necessarily mean that G-CSF modulates leukemic blast cell growth *in vivo*. The 100 ng/ml of G-CSF used in the experiments *in vitro* may be an extremely high concentration, although the actual level of G-CSF in humans remains unknown.

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