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Effects of Interleukin-6 and Granulocyte Colony-stimulating Factor on the Proliferation of Leukemic Blast Progenitors from Acute Myeloblastic Leukemia Patients

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The effects of recombinant human interleukin-6 (rh IL-6), which has homology with rh granulocyte colony-stimulating factor (rh G-CSF) at the amino acid sequence level, and rh G-CSF on normal human bone marrow cells, fresh leukemic blast progenitors from 16 acute myeloblastic leukemia (AML) patients, and G-CSF-dependent human AML cell line (OCI/AML 1a) were investigated. rh G-CSF stimulated the proliferation of leukemic blast progenitors from 13 out of 16 AML patients tested. rh IL-6 stimulated the proliferation of blasts from eight AML patients and enhanced the G-CSF-dependent proliferation of the fresh AML blasts from two out of eight patients tested. On the other hand, rh IL-6 suppressed the blast colony formation from two AML patients and OCI/AML 1a cells and also reduced the G-CSF-dependent proliferation of the blast progenitors from one of the two patients and the cell line. rh IL-6 had no effect on the colony formation of normal granulocyte-macrophage colony-forming units (CFU-GM) with or without rh G-CSF. Differentiation-induction by rh IL-6 was not observed in the fresh AML blasts but was observed in OCI/AML 1a. The effect of IL-6 on the blast colony formation and G-CSF-dependent blast cell growth was complicated and heterogenous among the AML cases; IL-6 stimulated blast colony formation in some cases and suppressed it in others. The heterogeneity of the response was supposed to be derived from the heterogeneity of the characteristics of AML cells. Although G-CSF simply stimulated the blast colony formation, IL-6 had a bimodulatory effect on the proliferation of leukemic blast progenitors from AML patients. IL-6 might be involved in the regulation of the proliferation of AML cells *in vivo* as well as *in vitro*.

Key words: Interleukin-6 — Granulocyte colony-stimulating factor — Acute myeloblastic leukemia — Leukemic blast progenitor — CD 14

Acute myeloblastic leukemia (AML) is a highly lethal disease with progressive accumulation of leukemic blasts. Leukemic blast proliferation is supported by a minority of blast progenitors; blast progenitors can renew themselves and/or undergo terminal divisions. The growth of blast progenitors in AML has been considered to be regulated by multiple factors. Colony-stimulating factors (CSFs) have been reported to stimulate not only terminal divisions but also self-renewal of blast progenitors from the majority of AML patients, although their effects were heterogenous among the patients.[1-3] Interleukin-1 (IL-1) has been shown to stimulate the growth of blast progenitors, possibly via the induction of CSF production by leukemic cells.[4] In contrast, some cytokines such as INFγ,[5] TNFα,[5,6] and TGFβ[7] have been reported to suppress the growth of blast progenitors. These findings indicate that the proliferation of even malignant cells may be controlled by cytokines. Thus, it still remains to be determined why leukemic cells proliferate almost indefinitely in AML patients. To solve this problem, the effect of cytokines on leukemic blast progenitors should be thoroughly clarified.

IL-6 was initially detected as an interleukin that stimulates final differentiation of B cells into antibody-producing cells.[8] The structure of IL-6 has 25.7% homology with recombinant human granulocyte colony-stimulating factor (rh G-CSF) at the amino acid level, and therefore the two molecules may have been derived from a common ancestor.[8] IL-6 has various activities on multiple types of cells.[9] However, the role of IL-6 in normal and leukemic hemopoiesis still remains unclear. rh IL-6 has been reported to enhance G-CSF-dependent proliferation of the AML blasts in some cases.[10] On the other hand, rh IL-6 did not enhance the G-CSF-dependent colony formation of normal human bone marrow cells but reduced it in some cases.[11]

In order to clarify the effect of rh IL-6 on normal and leukemic hemopoiesis, we tested the effects of rh IL-6 on the proliferation of normal human bone marrow cells, leukemic blast progenitors from 16 AML patients, and a G-CSF-dependent human AML cell line, OCI-AML 1a. Since rh IL-6 has homology with rh G-CSF, we studied precisely the interaction between rh IL-6 and rh G-CSF.

MATERIALS AND METHODS

CFU-G assay Normal bone marrow cells were obtained from two healthy volunteers with their informed consents. Mononuclear cells were separated through a Ficoll-Hypaque density gradient (1.077 g/ml). Mononuclear cells were incubated with carbonyl iron for 30 min at 37°C and then iron-phagocytic cells were depleted by using magnet adhesion. Non-phagocytic bone marrow cells were plated at the concentration of 10^5 cells/ml in 0.1 ml of α-minimal essential medium (α-MEM) (Gibco, Grand Island, NY) with 0.8% methylcellulose, 20% fetal calf serum (FCS) (Gibco), and various concentrations of rh G-CSF (Kyowa Hakko, Tokyo) and/or rh IL-6 (Genzyme, Boston, MA), in a 96-microwell plate (Sumitomo Bakelite, Tokyo). After seven days of incubation under a humidified atmosphere of 5% CO_2 in air, colonies of more than 40 cells were counted as CFU-G. CFU-G were determined morphologically and cytochemically after Wright's staining, and α-naphthyl butyrate and naphthol ASD chloroacetate esterase staining.

AML blasts Peripheral blood samples were taken from 16 AML patients with their informed consents. Table I illustrates the patients' profile. Mononuclear cells were obtained through a Ficoll-Hypaque density gradient and T cells were depleted after E-rosette formation using sheep red cells.[12] Then phagocytic cells were depleted from the T-depleted mononuclear cells by carbonyl-iron ingestion as described above. Non-T, non-phagocytic blast cells were incubated at the concentration of 10^5 cells/ml in 0.1 ml of α-MEM with 0.8% methylcellulose, 20% FCS, and 0, 10, or 100 ng/ml of rh G-CSF and/or 0, 10, or 100 U/ml of rh IL-6 in a 96-microwell plate. In some cases, cells were incubated at the concentration of 10^5 or 4×10^5/ml in 35-mm Lux Petri dishes (Miles Lab., Naperville, IL). After seven days of incubation, colonies of more than 20 cells were counted. Cells of the colonies were picked up, stained by Wright's staining, and observed morphologically.

Table I. Patients' Characteristics

Patient No.	Age/Sex	FAB[a]	Peripheral blood	
			WBC (/μl)	% Blasts
1	34/F	M1	17,200	91
2	45/F	M1	82,000	90
3	54/M	M1	214,000	92
4	62/M	M2	20,200	57
5	64/F	M2	5,500	31
6	61/M	M2	30,900	93
7	50/M	M3	1,400	30
8	58/M	M3	1,300	45
9	18/M	M3	32,600	93
10	63/M	M4	127,400	100
11	70/M	M4	64,800	64
12	60/F	M4	52,900	83
13	54/F	M4	26,800	91
14	63/M	M4	13,900	80
15	37/M	M5	70,700	35
16	51/F	M5	119,500	97

a) FAB: French-American-British classification.[14]

G-CSF-dependent cell line G-CSF-dependent human AML cell line (OCI/AML 1a)[13] is a subline of OCI/AML 1 derived from an AML M4 patient (according to the FAB classification).[14] This subline has been kept in our laboratory for more than four years in the presence of G-CSF. OCI/AML 1a cells do not express G-CSF mRNA constitutively, as detected by Northern blot analysis. Cells of this subline respond to IL-3 or GM-CSF, but the responses are very weak compared to the response to G-CSF. M-CSF stimulates the colony formation of the line weakly in methylcellulose, but does not support the exponential growth of them in suspension culture.

OCI/AML 1a cells were cultured at the concentration of 10^5 cells/ml in 0.1 ml of α-MEM with 0.8% mthylcellulose, 20% FCS, and various concentrations of rh G-CSF and/or rh IL-6 in a 96-microwell plate. After seven days of culture, colonies of more than 20 cells were counted. Then cells were picked up, stained by Wright's staining, and analyzed morphologically.

OCI/AML 1a cells were also cultured at the concentraion of 10^6 cells/ml in 3 ml of α-MEM with 20% FCS, in the presence or absence of 10 ng/ml of rh G-CSF or 100 U/ml of rh IL-6 by the method of Nara and McCulloch.[15] After seven days of incubation, cells were harvested and counted (A). The cells were washed three times with α-MEM containing 10% FCS. Cells were cultured at the concentration of 10^5 cells/ml in 0.1 ml of α-MEM with 0.8% methylcellulose, 20% FCS, and 10 ng/ml of rh G-CSF. After seven days' incubation, colonies of more than 20 cells were counted (B). (A)×(B) gave the absolute number of clonogenic cells in the dish. The rest of the cells were re-cultured as described above. Repetition of these procedures gave the cumulative growth curve.

Surface marker analysis of OCI/AML 1a OCI/AML 1a cells were cultured with or without 10 ng/ml of rh G-CSF or 100 U/ml of rh IL-6 for two weeks with changes of medium and CSF every seven days. Each sample was studied with a panel of monoclonal antibodies: Mo 2 (Coulter, Hialeah, FL) and My 4 (Coulter) which belong to CD 14 and react specifically with monocytes/macrophages; My 7 (Coulter) which belongs to CD 13 and reacts with myelomonocytic lineage cells.

Monoclonal antibodies were used in a direct or indirect immunofluorescence technique, and the surface markers were analyzed by using flow cytofluorometry (FACS-440, Becton Dickinson, Mountain View, CA).

Northern blot analysis Cells of OCI/AML 1a were incubated at the concentration of 10^6 cells/ml in 40 ml of α-MEM with 20% FCS in the presence of 100 U/ml of rh IL-6 for 0, 1, 6, 24, and 48 h, or 100 U/ml of rh IL-6 plus 10 ng/ml of rh G-CSF for 1 h, 6 h, 24 h, 48 h, and 37 days. After incubation, cells were harvested and washed twice with phosphate-buffered saline on ice. Total RNA was extracted from each sample by the guanidium-cesium method using an ultracentrifuge.[16] Total RNAs from cells of human bladder carcinoma cell line 5637 and human small cell lung carcinoma cell line (GKT3-1.3V)[17] given an hour's exposure to rh GM-CSF (Sumitomo Pharmaceutical Company, Tokyo) were used as positive controls for the Northern blot analysis. The total RNAs (20 μg) were run on 1.0% agarose gel with 5.4% formaldehyde and transferred to nylon filters. Agarose gels were checked with ethidium bromide staining to confirm that almost equal amounts of RNAs were loaded and that no degradation occurred. The filters were prehybridized and then hybridized at lower stringency (presence of 40% formamide) with tumor necrosis factor α (TNFα) cDNA probe[18] (kindly supplied by Asahi Chemical Company, Tokyo) labeled with ^{32}P using a random primer method. Filters were washed and autoradiographed with an intensification screen at -80°C. After hybridization, membranes were washed and rehybridized with actin cDNA (Oncor, Gaithersburg, MD) to check that almost equal amounts of undegraded mRNAs were blotted on the nylon membranes.

Statistical analysis Group data were compared by the use of Student's t test. The data are presented as mean $\pm$SD of three to five replicated cultures.

RESULTS

CFU-G Colony formation by CFU-G was not observed in the absence of G-CSF. rh G-CSF (10 ng/ml) stimulated the growth of CFU-G significantly. No significant difference of stimulating effect was detected between 10 and 100 ng/ml of rh G-CSF. The results were consistent with our previous data.[2,3] Next, rh IL-6 was added to the culture system. rh IL-6 alone at the doses of 1–100 U/ml did not stimulate the growth of CFU-G in methylcellulose culture. Furthermore, rh IL-6 did not significantly alter the stimulatory effect of rh G-CSF on CFU-G when rh IL-6 was added in combination with rh G-CSF. Figure 1 illustrates the result from one donor. The results were similar in another donor.

AML blasts The effects of rh G-CSF and rh IL-6 on the growth of leukemic blast progenitors from AML patients

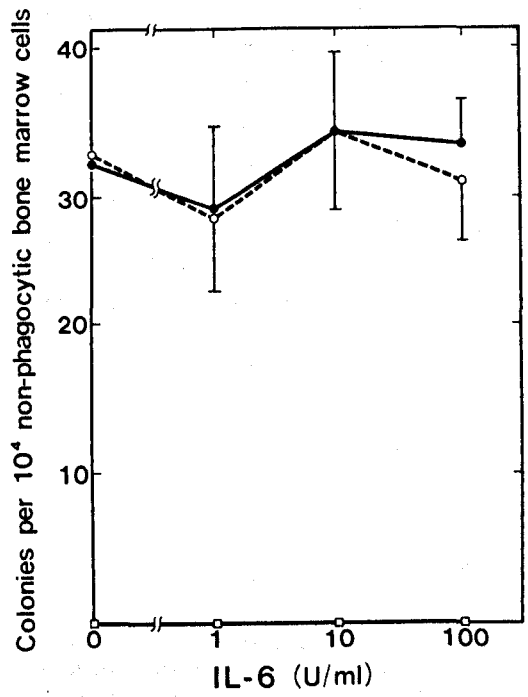

Fig. 1. Colony formation by CFU-G from 10^4 non-phagocytic normal bone marrow cells in methylcellulose culture. rh G-CSF at 100 ng/ml (●), 10 ng/ml (○), or 0 ng/ml (□) was added to the culture in combination with 100, 10, 1, or 0 U/ml of rh IL-6. rh IL-6 alone at the doses of 1–100 U/ml did not stimulate the growth of CFU-G at all. rh G-CSF at the doses of 10 or 100 ng/ml stimulated the growth of CFU-G and the effects of the two doses were almost equivalent. Without G-CSF, no colony formation was observed. rh IL-6 did not act significantly on the stimulating effect of rh G-CSF on CFU-G when rh IL-6 was added in combination with rh G-CSF.

are summarized in Table II. rh G-CSF stimulated significantly the proliferation of 13 out of 16 AML patients; this result was similar to that in our previous report.[2,3] rh IL-6 stimulated the proliferation of blasts from eight AML patients, while it suppressed the proliferation from two AML patients. In those two cases, spontaneous blast colony formation without adding cytokines was observed and was reduced in a dose-responsive manner by rh IL-6 (see Fig. 2C). In this study, we observed spontaneous blast colony formation in eight patients. rh IL-6 stimulated the growth of blasts in six of them but suppressed it in the other two. We further analyzed the combined effects of rh G-CSF and rh IL-6 on the proliferation of blast progenitors from eight AML patients. Patients were selected on the basis of positive responsiveness to G-CSF and availability of the cells. The combined effects of rh G-CSF and rh IL-6 were classified into three categories as illustrated in

Table II. Effects of Cytokines on Blast Colony Formation

Patient No.	No. of cells plated	Numbers of colonies			
		Medium	G-CSF	IL-6	G-CSF and IL-6
1	10^4	0	4.0±4.5[a]	0	15.8±11.3
2	10^5	1518±63	1706±94	1306±10[c]	ND
3	10^5	576±69	961±141[a]	1087±42[a]	ND
4	10^4	0	8.0±2.8[a]	0	17.8±2.7[b]
5	10^4	0.8±1.2	2.4±1.2	3.2±1.2[a]	ND
6	10^5	274±53	645±25[a]	372±22[a]	ND
7	4×10^5	156±25	11112±1956[a]	389±99[a]	9760±88
8	10^4	0	204.4±12.6[a]	1.6±0.8[a]	392.2±24.0[b]
9	10^5	0	49.3±15.5[a]	11.7±4.0[a]	ND
10	10^4	26.6±3.9	120.2±12.5[a]	35.0±5.8[a]	100.2±6.0
11	10^4	0	21.2±2.8[a]	0	15.4±1.4
12	10^5	326±17	443±34[a]	169±11[c]	316±26[d]
13	10^5	0	15.3±4.8[a]	0	ND
14	10^5	0	9.0±1.4[a]	0	ND
15	10^4	1.2±1.2	17.0±0.9[a]	3.8±1.3[a]	18.4±1.0
16	10^5	0	1.7±1.2	0	ND

ND: Experiment not done.
a) Blast colony formation was stimulated significantly compared to the medium ($P<0.05$).
b) Blast colony formation was stimulated significantly compared to G-CSF alone ($P<0.05$).
c) Blast colony formation was suppressed significantly compared to the medium ($P<0.05$).
d) Blast colony formation was suppressed significantly compared to G-CSF alone ($P<0.05$).

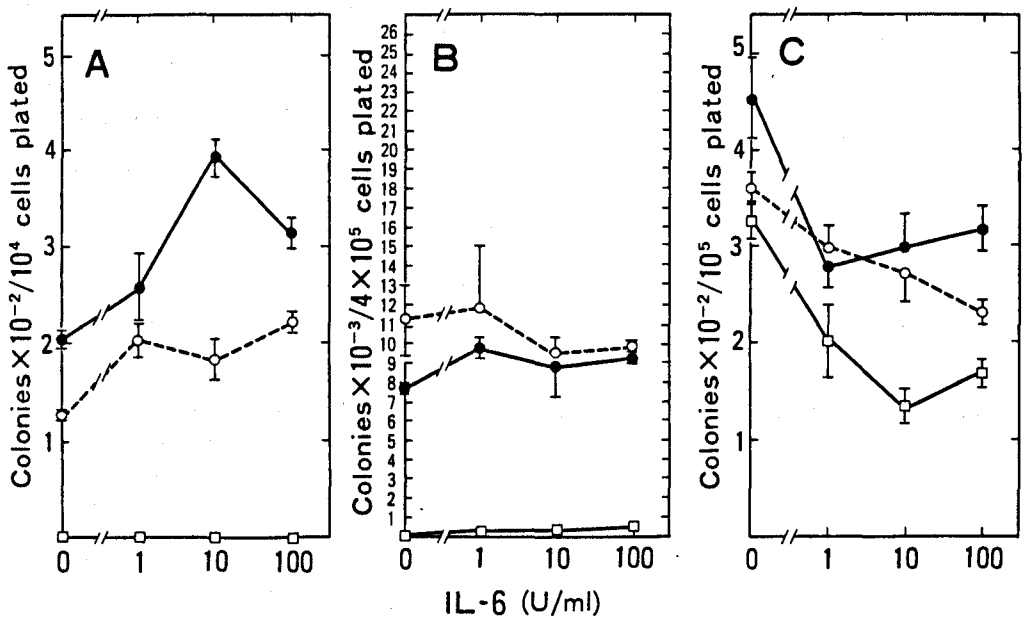

Fig. 2. The combination effects of rh G-CSF and rh IL-6 on the growth of non-phagocytic leukemic blast progenitors from seven patients were classified into three types. rh G-CSF at 100 ng/ml (●). 10 ng/ml (○), or 0 ng/ml (□) was added to the culture in combination with 100, 10, or 0 U/ml of rh IL-6. Figure 2A shows the result from patient 8. rh IL-6 enhanced the G-CSF-dependent blast colony formation in a dose-responsive manner. Figure 2B shows the result from patient 7. rh IL-6 did not act on the G-CSF-dependent blast colony formation. Figure 2C shows the result from patient 12. rh IL-6 alone suppressed the spontaneous blast colony formation in a dose-responsive manner. rh IL-6 also reduced the G-CSF-dependent blast colony formation in a dose-responsive manner.

Fig. 2. rh IL-6 stimulated the G-CSF-dependent proliferation of two cases (patients 4 and 8) ($P<0.05$). Figure 2A shows the result from patient 8. rh IL-6 suppressed the G-CSF-dependent proliferation of one case (patient 12) in a dose-responsive manner (Fig. 2C) ($P<0.05$). In the other cases, rh IL-6 did not act significantly on the G-CSF-dependent proliferation of the blasts. Figure 2B shows a typical result from patient 7. To determine whether rh G-CSF and rh IL-6 induce the differentiation of blast progenitors, we tested the morphological changes of blasts in colonies made in methylcellulose culture. Cells in the colonies were like blasts with or without G-CSF or IL-6. These two factors did not seem to induce differentiation of blast progenitors at least within seven days' culture.

OCI/AML 1a cell line Because fresh AML blasts consisted of heterogenous cell subpopulations, we carried out the following experiment to clarify the combined effects of G-CSF and IL-6 on more highly purified targets. That is, we tested the combined effects on the G-CSF-dependent cell line, OCI/AML 1a. rh G-CSF stimulated the colony formation of OCL/AML 1a cells in a dose-responsive manner. rh IL-6 reduced the G-CSF-dependent proliferation of OCI/AML 1a cells in a dose-responsive manner (Fig. 3); 1000, 100, and 10 U/ml of rh IL-6 reduced the colony formation to 29.1–51.9, 34.4–70.3, and 69.7–98.2% of the control, respectively, in the presence of 0.01–100 ng/ml of rh G-CSF. rh IL-6 alone did not stimulate the proliferation of OCI/AML 1a cells at all. In the presence of IL-6, some colonies appeared different morphologically from the colonies obtained

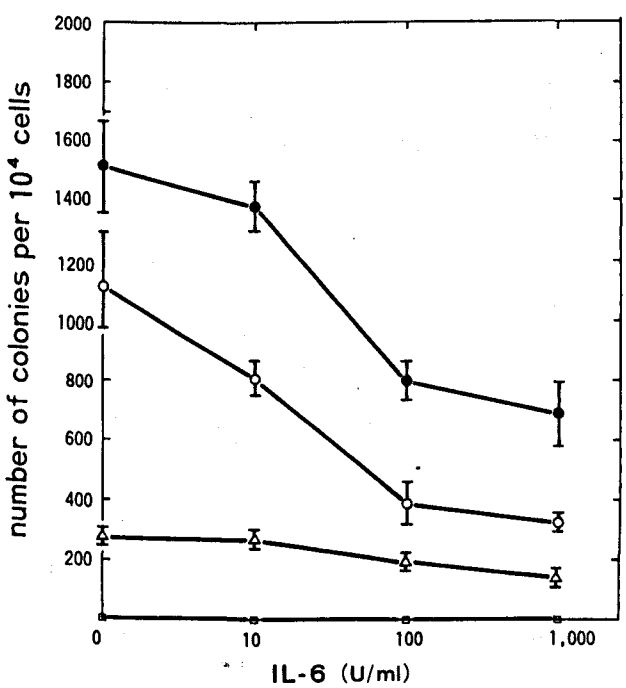

Fig. 3. Blast colony formation of 10^4 G-CSF-dependent OCI/AML 1a cells. rh G-CSF stimulated the colony formation in a dose-responsive manner. rh IL-6 by itself did not stimulate the proliferation of OCI/AML 1a cells at all. rh IL-6 reduced the G-CSF-dependent proliferation of OCI/AML 1a cells in a dose-responsive manner. Each symbol indicates the concentration of rh G-CSF as follows: □, 0 ng/ml; △, 0.01 ng/ml; ○, 1 ng/ml; ●, 10 ng/ml.

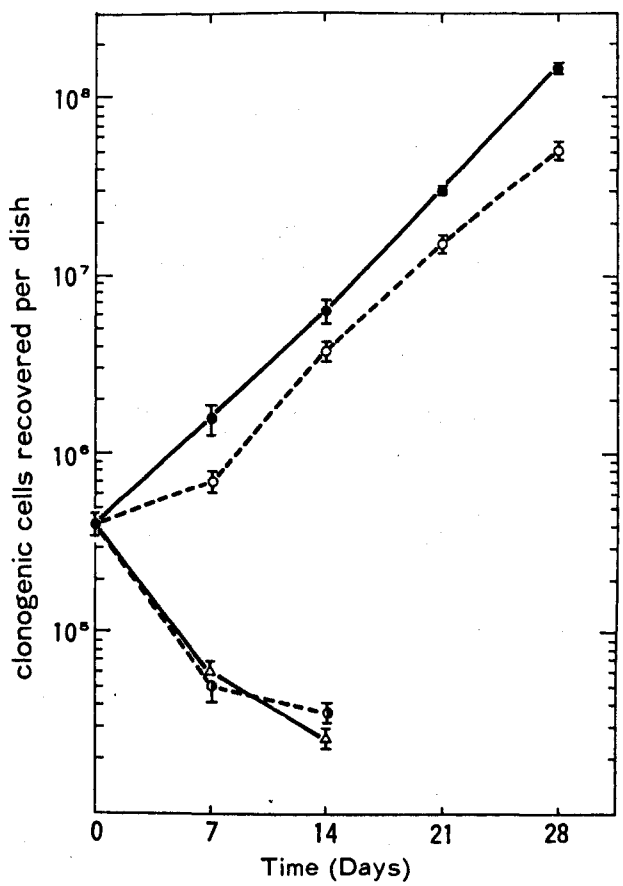

Fig. 4. Cumulative growth curve of OCI/AML 1a cells with or without 10 ng/ml of rh G-CSF or 100 U/ml of rh IL-6 in long-term suspension culture. rh G-CSF supported the exponential growth of OCI/AML 1a cells. In combination with rh G-CSF, rh IL-6 reduced significantly the cumulative G-CSF-dependent exponential growth, but the exponential growth had been maintained for more than four weeks. rh IL-6 alone or medium did not maintain the exponential growth of OCI/AML 1a cells. Each symbol indicates the cytokine(s) presented in the suspension culture as follows: ●, G-CSF alone; ○, G-CSF plus IL-6; ◑, IL-6 alone; △, medium alone.

Table III. Surface Marker Analysis of the G-CSF-dependent Cell Line

CD number	Monoclonal antibody	% Reactivity with monoclonal antibody			
		Medium	+G-CSF	+IL-6	+G-CSF +IL-6
CD 14	Mo 2	1.8	11.3	29.5	14.5
	My 4	6.7	12.8	29.2	12.5
CD 13	My 7	94.1	99.3	97.4	99.2

with G-CSF alone. They were compact and consisted of cells larger than those obtained with G-CSF. These colonies amounted to approximately 5% of all colonies. Such colonies were picked up, stained by Wright's staining and analyzed morphologically. These unusual colonies consisted of macrophage-like cells having a small nucleus with dense chromatin and broad cytoplasm enriched with vacuoles. These cells were different from cells growing in suspension, which had a large nucleus with a few nucleoli and scanty cytoplasm with some azurophilic granules. In order to rule out the possibility that rh IL-6 stimulated the contaminating macrophages in OCI/AML 1a cells to produce some suppressive cytokines, we tested the effects of rh IL-6 on the non-phagocytic cells of the OCI/AML 1a cells and obtained similar results to those illustrated in Fig. 3 (data not shown).

Figure 4 illustrates the cumulative growth of OCI/AML 1a cells in suspension culture. rh G-CSF supported the exponential growth of OCI/AML 1a cells. In combination with rh G-CSF, rh IL-6 reduced significantly the cumulative G-CSF-dependent exponential growth. However, the exponential growth of OCI/AML 1a cells had been maintained over four weeks. rh IL-6 alone or medium did not maintain the exponential growth of OCI/AML 1a cells.

Surface marker analysis of the OCI/AML 1a cell line rh IL-6 increased the expression of surface antigen specific for macrophage/monocytes (CD14) on OCI/AML 1a cells (Table III). Even in the presence of rh G-CSF, rh IL-6 increased Mo 2 expression on OCI/AML 1a cells, while rh IL-6 did not increase My 4 expression in the presence of rh G-CSF. The intensity of CD 14 expression was lower when the cells were stimulated by rh IL-6 in combination with rh G-CSF than when they were stimulated by IL-6 alone. CD 13, which reacts with myelomonocytic lineage cells, did not show any significant change regardless of the stimulating factor.

Northern blot analysis No TNFα mRNA gene expression was detected in the cells of OCI/AML 1a after 0, 1, 6, 24, and 48 h of exposure to IL-6, or after 1 h, 6 h, 24 h, 48 h, and 37 days of exposure to IL-6 plus G-CSF.

DISCUSSION

The present data show that responsiveness of blast progenitors to IL-6 is variable among the AML patients. IL-6 stimulated the growth in methylcellulose of blast progenitors from eight patients, suppressed that from two patients, and did not significantly affect that from the other six patients (Table II and Fig. 2). Although the present study included only 16 patients, the stimulating effect of IL-6 seems most prominent in FAB M2 and M3 cases. In the three M3 patients, blasts from all of them responded to IL-6 and proliferated significantly. The mechanism by which IL-6 stimulates growth of leukemic blasts remains unclear. IL-6 has been shown to have 25.7% homology with G-CSF at the amino acid sequence level, and this may suggest that IL-6 shares its biological activity at least in part with G-CSF. We have tested the effect of IL-6 on CFU-G. However, we did not find any significant effect of IL-6 on CFU-G (Fig. 1), while G-CSF significantly stimulated CFU-G in a dose-responsive manner. This finding indicates that IL-6 is not so active on granulopoietic precursors as G-CSF. Then, there is a possibility that IL-6 modulates the responsiveness of granulopoietic precursors or leukemic blast progenitors to other cytokines such as G-CSF. We have noted that IL-6 enhanced the responsiveness of blast progenitors to G-CSF in two out of eight patients. Hoang *et al.* reported that IL-6 acts synergistically with GM-CSF in three of five cases in blast colony formation.[10] Ikebuchi *et al.* reported that IL-6 shortens the G_0 phase of hemopoietic precursors and makes them more sensitive to IL-3.[19] These findings suggest that IL-6 modulates the biological properties of blast progenitors and makes blast progenitors more responsive to G-CSF or other cytokines. The stimulating effect on progenitors of IL-6 was not found in all patients but only in eight patients. Therefore, leukemic cells from some patients may be made more active cell-kinetically or more sensitive to other cytokines by IL-6, leading them to proliferate.

IL-6 suppressed the growth of blast progenitors in two patients: one was FAB M1 and the other was M4. A suppressive effect of IL-6 on primary human leukemic cells has not previously been reported. Therefore, these two cases may be unusual in terms of the responsiveness to IL-6. However, it is important to clarify the mechanism of the suppressive effect of IL-6. IL-6 also suppressed the growth of OCI/AML 1a cells (Figs. 3 and 4). We further studied the suppressive effect of IL-6 in OCI/AML 1a cells. When cultured with IL-6, OCI/AML 1a cells made colonies morphologically different from the ordinary colonies formed in the presence of G-CSF alone. Those colonies were composed of macrophages. Phenotypic analysis of the cells growing in suspension

culture in the presence of IL-6 revealed that monocytic cells increased. These findings suggest that IL-6 induces the monocytic differentiation of OCI/AML 1a cells. Since OCI/AML 1a cells have been derived from a FAB M4 patients, leukemic cells of monocytic lineage may be induced to differentiate to monocytic cells by IL-6. One of the two patients whose blast progenitors were suppressed by IL-6 was M4. In at least some AML patients of monocytic lineage, IL-6 may induce terminal differentiation to monocytic cells and reduce self-renewal capacity of leukemic blast progenitors. This being the case, IL-6 may represent a new approach to AML therapy. IL-6, however, stimulated the growth of blast progenitors in eight of 16 patients as described above. Therefore, we must be cautious in interpreting the effect of IL-6 on leukemic cells.

Cytokines show an indirect effect on target cells via modulating accessory cells as well as a direct effect. We have reported that IL-1 stimulates adherent cells to produce GM-CSF or G-CSF, which stimulates the growth of blast progenitors.[4] The major effect on blast progenitors of IL-1 is indirect rather than direct. GM-CSF has been reported to suppress the growth of U937 cell line.[20] The suppressive effect of GM-CSF is considered to be the production of TNFα or some other factors by U937 cells. Namely. GM-CSF makes U937 cells produce TNFα, which in turn suppresses the growth of U937 cells. These findings confirm that cytokines show complicated effects on target cells. In the present study, we checked the production of TNFα by OCI/AML 1a cells when cultured with IL-6. For this purpose, we studied the TNFα mRNA gene expression by Northern blot analysis in OCI/AML 1a cells after 0, 1, 6, 24, and 48 h of incubation with IL-6. We did not find TNFα mRNA gene expression. The suppressive effect on OCI/AML 1a cells of IL-6 can not be explained in terms of TNFα production.

As mentioned above, the effect on leukemic cells of IL-6 is complicated. The heterogeneity of the responsiveness of leukemic cells to IL-6 is considered to be derived from the heterogeneity of AML. Leukemic transformation has been considered to occur at some stage of hemopoiesis. When leukemia originates at a very immature stage of hemopoiesis, leukemic blast progenitors may behave as primitive stem cells. When leukemia cells are derived from more mature hemopoietic precursors, leukemic blast progenitors may be like more matured cells. Therefore, the growth patterns and growth requirements could be considered to be variable among AML patients. From this point of view, it is interesting that IL-6 stimulated the proliferation of all the M3 cases tested. This fact suggests that IL-6 plays some important role in the leukemogenesis of acute promyelocytic leukemia. To understand the mechanism of indefinite growth of leukemic cells, the effects of cytokines should be clarified well in each AML patient.

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