Volume 234, number 1, 17–21 FEB 05963 July 1988

Recombinant human interleukin 6 (B-cell stimulatory factor 2) is a potent inducer of differentiation of mouse myeloid leukemia cells (M1)

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Received 22 March 1988

Recombinant human interleukin 6 (IL-6), a lymphokine involved in the final differentiation of activated B-cells into antibody-forming cells, greatly suppressed proliferation and induced differentiation of murine myeloid leukemia cells (M1) into mature macrophage-like cells. When M1 cells were treated with IL-6, their growth was completely arrested as early as on day 2, and they were induced to differentiate morphologically into macrophage-like cells. Differentiation-associated properties such as phagocytic activity, adherence to the dish surface, Fc and C3 receptors, were also induced within 24 h by IL-6, and they reached their respective maximal levels on day 2 or 3. The potency of IL-6 in suppressing proliferation and inducing differentiation was much greater than that of 1α,25-dihydroxyvitamin D_3 one of the most potent inducers of M1 cells. The present report indicates that IL-6 is involved in the differentiation of not only B-cells but also myeloid leukemia cells.

B-cell stimulatory factor 2; Interleukin-6; Vitamin D_3; Growth inhibition; Differentiation; (Mouse myeloid leukemia cell)

1. INTRODUCTION

The myeloid leukemia cell line (M1), originally established by Ichikawa [1] from an SL mouse with myeloid leukemia, can be induced to differentiate into macrophages and granulocytes in vitro by several compounds such as protein inducers, bacterial lipopolysaccharides, polyribonucleotides, alkyl-lysophospholipids, glucocorticoids and 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$]. Of these compounds, 1α,25$(OH)_2D_3$ is one of the most potent inducers of M1 cells in vitro [2]. Administration of 1α,25$(OH)_2D_3$ or its synthetic analogue, 1α-hydroxyvitamin D_3, significantly enhanced the survival time of mice inoculated with M1 cells in vivo [3].

Recently, much attention has been focused on the nature of the biological protein inducers called differentiation-inducing factors (DIFs). Various DIFs derived from conditioned media of tumor cells and mitogen-stimulated T-lymphocytes have been purified [4–6]. Gearing et al. [7] reported the molecular cloning of the cDNA of a murine DIF. The amino acid sequence of the DIF was different from any other hematopoietic cytokines identified to date. In addition, Tomida et al. [8] have shown that recombinant human granulocyte colony-stimulating factor (G-CSF) induces the macrophage differentiation of M1 cells. The relationship of these protein factors is a matter of debate.

B-cell stimulatory factor 2 (BSF-2) was originally found in the supernatants of mitogen- or

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

antigen-stimulated mononuclear cell cultures [9,10], and it subsequently was identified as a T-cell derived lymphokine involved in the final differentiation of activated B-cells into antibody-forming cells [11]. Recently, the cDNA for BSF-2 was cloned [12] and the mutual identity of the amino acid sequence in BSF-2 and other cytokines such as 25 kDa-protein, interferon β_2 and plasmacytoma growth factor was established [13–15]. Moreover, it has been demonstrated that BSF-2 acts on T-cells [16–18], hepatocytes [19,20] and hematopoietic stem cells [21]. These results indicate that BSF-2 has an important role not only in the immunoglobulin production of B-cells but also in the regulation of growth and differentiation of various other cells. It is now proposed that BSF-2 be called interleukin 6 (IL-6).

We report here that recombinant human IL-6 is a potent inducer of differentiation of M1 cells into mature macrophages. IL-6 was much more potent than $1\alpha,25(OH)_2D_3$ in suppressing growth and inducing differentiation of M1 cells.

2. MATERIALS AND METHODS

2.1. *Drugs*

Recombinant human IL-6 (rIL-6) was prepared by expressing a cDNA for BSF-2 [12] in *E. coli*, followed by further purification. The specific activity was determined as 5.2×10^9 U/g by using the IL-6 responsive human B lymphoblastoid cell line, SKW6-CL4 [11]. $1\alpha,25(OH)_2D_3$ was the generous gift of Dr I. Matsunaga, Chugai Pharmaceutical Co., Tokyo.

2.2. *Cells and cell culture*

M1 cells, clone T22, were kindly donated by Dr M. Hozumi (Saitama Cancer Center Research Institute, Saitama, Japan). They were cultured at 37°C under 5% CO_2/95% air in Eagle's minimal essential medium supplemented with twice the normal concentrations of amino acids and vitamins and 10% heat-inactivated calf serum (Chiba Serum Institute, Chiba, Japan). They were transferred every 2–3 days.

2.3. *Measurement of phagocytic activity*

After M1 cells were washed with serum-free medium, they were incubated for 4 h with polystyrene latex particles (2 μl/ml, average diameter 0.81 μm; Difco) at 37°C under 5% CO_2 in air. After incubation, the cells were washed three times with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline [PBS(−)], and the number of phagocytic cells among at least 200 cells was counted with a hemocytometer.

2.4. *Detection of Fc and C3 receptors*

Cells with Fc or C3 receptors were determined by the method of Lotem and Sachs [22]. M1 cells (5×10^5) were mixed with 2.5×10^7 rabbit anti-sheep erythrocyte antibody-coated erythrocytes or antibody and mouse complement-coated erythrocytes in 0.5 ml of serum-free medium. After incubation for 30 min at 37°C, the percentage of rosette-forming cells with 4 or more erythrocytes per cell was counted with a hemocytometer. At least 200 cells were counted.

2.5. *Determination of adherent cells*

After culture for the indicated time, non-adherent cells were collected. The cell-layer was gently rinsed with pre-warmed serum-free medium and the recovered cells were combined with the non-adherent cells. Adherent cells were collected by pipetting with ice-cold PBS(−). Adherent cells were expressed as the percentages of total cells by counting cell numbers of both adherent and non-adherent cells.

2.6. *Determination of growth of non-adherent M1 cells pretreated with either rIL-6 or $1\alpha,25(OH)_2D_3$*

Cells were seeded at 1×10^5/ml and incubated for 3 days with 100 U/ml of rIL-6 or 12 nM $1\alpha,25(OH)_2D_3$. On day 3, the respective non-adherent cells were collected as described above (2.5), washed 3 times with serum-free medium, and reseeded at 1×10^5/ml in the control medium without either inducer. The number of viable cells was determined on each day by counting the cells which were not stained with trypan blue.

3. RESULTS

When M1 cells were cultured for 3 days with 1–100 U/ml of rIL-6, cell growth was strikingly inhibited in time- and dose-dependent manners. The remaining cells were attached to the dish surface. Most of the untreated control cells were myeloblastic with a large round nucleus (fig.1A). With the treatment with rIL-6 for 3 days, the cells changed their morphology into macrophage-like cells with abundant cytoplasm (fig.1B). Fig.2 shows time courses of change in the expression of typical markers for macrophage differentiation by rIL-6. Treatment with 100 U/ml of rIL-6 greatly induced phagocytic activity within 24 h, and 90% of the cells exhibited the activity on day 2. Formation of Fc and C3 receptors was also increased by rIL-6 in a time-dependent manner. The percentage of adherent cells in the culture treated with 100 U/ml of rIL-6 attained a plateau of 70% on day 2. Addition of polymyxin B did not affect the induction of differentiation by rIL-6, indicating that the influence of endotoxin that might have been contaminated in this culture system can be excluded (not shown).

We have reported that 0.12–120 nM $1\alpha,25(OH)_2D_3$ markedly suppresses growth and induces differentiation of M1 cells dose-dependently [2]. $1\alpha,25(OH)_2D_3$ is one of the most potent

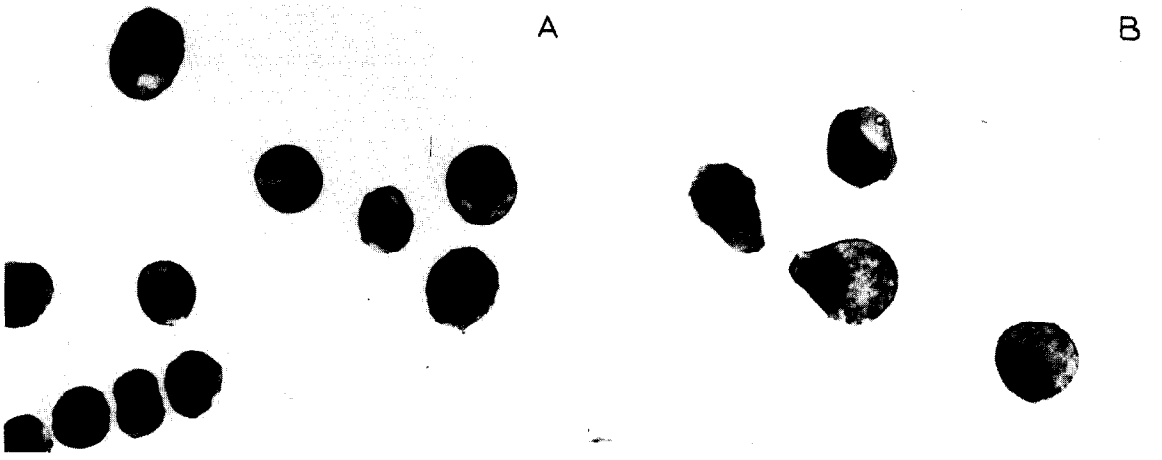

Fig.1. Morphology of M1 cells incubated for 3 days without (A) or with (B) 100 U/ml of rIL-6. Stained with May-Grünwald-Giemsa; × 600.

known inducers of M1 cells [2]. Therefore, we compared the effects of rIL-6 and $1\alpha,25(OH)_2D_3$ on growth and differentiation of M1 cells (table 1). rIL-6 at 1–100 U/ml inhibited growth of M1 cells dose-dependently. Phagocytic activity was significantly increased by the treatment with as little as 1 U/ml of rIL-6, and it attained a maximal level of 90% at 100 U/ml. Significant increases in Fc and C3 receptors were induced by 33 U/ml of rIL-6, and more than half of the cells exhibited the respective receptors at 100 U/ml. $1\alpha,25(OH)_2D_3$ at 1.2–120 nM also suppressed cell growth and induced differentiation in a dose-dependent manner (table 1). The maximal inhibition of cell growth and stimulation of differentiation by rIL-6 were always much greater than those induced by $1\alpha,25(OH)_2D_3$ (table 1).

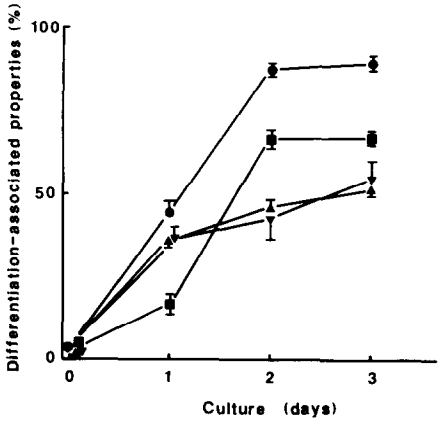

Fig.2. Time course of change in the differentiation-associated properties of M1 cells incubated with 100 U/ml of rIL-6. On the indicated culture days, percentages of the cells expressing phagocytic activity (●), Fc receptor (▲), C3 receptor (▼) and adherence to the dish surface (■) were determined as described in section 2. Data are the means ± SE of three independent experiments.

Finally, we examined whether non-adherent M1 cells after pretreatment with rIL-6 or $1\alpha,25(OH)_2D_3$ can proliferate in the control medium without either inducer. Non-adherent cells collected from the culture medium and also by washing adherent cell layers after preculture with 12 nM $1\alpha,25(OH)_2D_3$ proliferated again, whereas those after preculture with 100 U/ml of rIL-6 did not grow at all (fig.3). This indicates that rIL-6 affected both adherent and non-adherent cells.

4. DISCUSSION

The present study clearly demonstrates that IL-6 is involved not only in the final differentiation of activated B-cells into antibody-secreting cells, but also in the growth and differentiation of myeloid leukemia cells. It is likely that IL-6 inhibits growth and induces differentiation of M1 cells by a receptor-mediated mechanism. Taga et al. [23] have reported that IL-6 receptors are present not only in the activated B-cells but also in some established cell lines including human promyelo-

Table 1

Dose-response effects of rIL-6 and $1\alpha,25(OH)_2D_3$ on the growth and differentiation of M1 cells

Compounds added	Concentration	Cell number	Phagocytic activity (%)	Fc receptor (%)	C3 receptor (%)
Ethanol (control)	0.1%	100	1.4 ± 0.4	0.3 ± 0.2	0.6 ± 0.3
rIL-6	1 U/ml	81.4 ± 0.5*	8.4 ± 0.4*	0.3 ± 0.2	0.7 ± 0.5
	10 U/ml	50.0 ± 5.3*	16.8 ± 1.6*	1.3 ± 0.5	1.9 ± 0.3**
	33 U/ml	37.4 ± 3.3*	50.8 ± 2.4*	20.3 ± 3.8*	30.5 ± 4.0*
	100 U/ml	21.7 ± 1.3*	90.8 ± 1.7*	52.5 ± 2.7*	53.6 ± 6.4*
$1\alpha,25(OH)_2D_3$	1.2 nM	64.1 ± 2.6*	19.6 ± 4.2**	1.8 ± 0.6	2.9 ± 1.3
	12 nM	52.7 ± 2.0*	53.4 ± 5.1*	19.2 ± 2.8*	20.6 ± 0.9*
	120 nM	50.4 ± 2.1*	52.5 ± 3.6*	20.4 ± 3.6*	22.7 ± 1.5*

Values are expressed as the means ± SE of 3–6 experiments. After culture for 3 days, the cell number and the percentages of phagocytic activity and the cells with Fc or C3 receptors were determined as described in section 2. The cell number was expressed as percentages of the control culture. The statistical significance of the difference from the control was analysed by Student's t-test. * $p<0.005$; ** $p<0.05$

cytic leukemia cells (HL-60). Although the exact mechanism of the IL-6 action in the myeloid leukemia cell differentiation has to be elucidated in the future, the marked in vitro effects of IL-6 on the growth and differentiation of M1 cells suggest the possibility that this cytokine will be useful for the treatment of leukemic animals and humans.

It has been reported that there are some similarities in the amino acid sequence between IL-6 and G-CSF [12]. Like IL-6, G-CSF has been found to induce differentiation of M1 cells [8]. Ikebuchi et al. [21] also reported that IL-6 controls proliferation of multipotent hematopoietic progenitors. These results further support the hypothesis that IL-6 is involved in the regulation of growth and differentiation of hematopoietic cells.

Recently, several protein factors that induce differentiation of M1 cells (DIFs) have been purified from conditioned media of tumor and normal cells. We reported that one of the DIFs purified from conditioned media of mouse spleen cell cultures treated with concanavalin-A exhibited po-

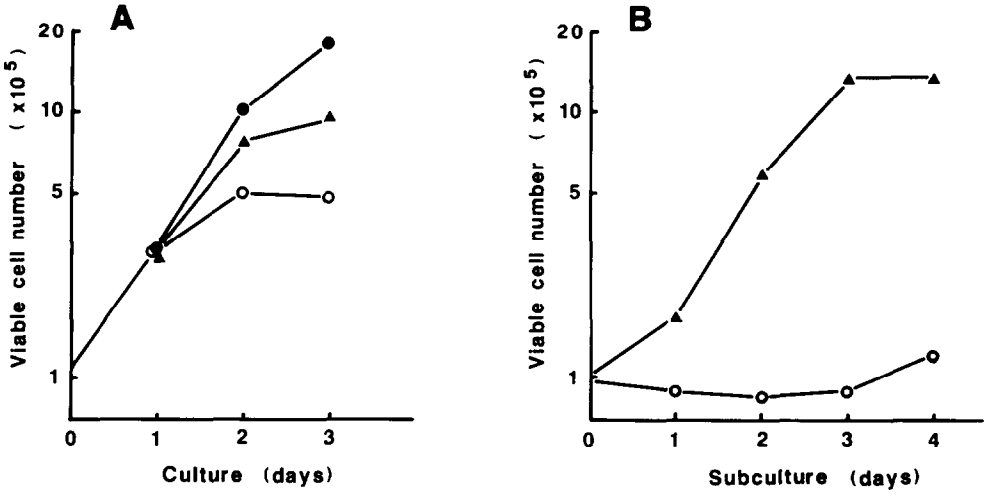

Fig.3. Comparison of the growth activity of non-adherent M1 cells after pretreatment with either rIL-6 or $1\alpha,25(OH)_2D_3$. [A] M1 cells were incubated for 3 days with vehicle (●), 100 U/ml of rIL-6 (○) or 12 nM $1\alpha,25(OH)_2D_3$ (▲). On the days indicated, viable cells were counted with a hemocytometer. [B] On day 3 of the culture in [A], non-adherent cells were collected from the cultures treated with rIL-6 (○) or $1\alpha,25(OH)_2D_3$ (▲), washed thoroughly, and subcultured for 4 days in the control medium without either inducer. Data are the means of three wells. Each SE was smaller than the size of the symbol.

tent bone-resorbing activity [6]. Thus, IL-6 may have an important role in the differentiation of osteoclast progenitors into multinucleated osteoclasts. This possibility is currently under investigation in our laboratories.

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