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Interleukin-4 inhibits the differentiation of mouse myeloid leukemia M1 cells induced by dexamethasone, D-factor/leukemia inhibitory factor and interleukin-6, but not by 1α,25-dihydroxyvitamin D_3

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The effects of interleukin-4(IL-4) on the growth and differentiation of mouse myeloid leukemia M1 cells induced by various differentiation inducers were investigated IL-4 alone did not have any significant effect on the growth or differentiation of M1 cells, but inhibited their differentiation induced by dexamethasone, D-factor/leukemia inhibitory factor, or interleukin 6 IL-4 also restored the proliferation of M1 cells after growth inhibition during their induction of differentiation by inducers In contrast, IL-4 enhanced inhibition of growth and induction of differentiation of M1 cells by 1α,25-dihydroxyvitamin D_3 These results indicate that modulation of differentiation of M1 cells by IL-4 depends on the differentiation inducer

Interleukin-4, Differentiation, Growth, Myeloid leukemia cell, M1 cell

1. INTRODUCTION

Interleukin-4 (IL-4) was first designated as a B cell stimulating factor-1 from its stimulatory activity on B cells [1]. Subsequently, many effects of IL-4 on various hematological cell types have been reported [2–6]. IL-4 enhances granulocyte colony formation by hematopoietic progenitors in the presence of granulocyte colony-stimulating factor (G-CSF), but inhibits macrophage colony formation supported by interleukin-3 (IL-3) and macrophage colony-stimulating factor (M-CSF) [6,7] Peck and Bollag [8] reported that IL-4 slightly enhanced retinoid-induced differentiation of human promyelocytic leukemia HL-60 cells, but the effects of IL-4 on the growths and differentiations of other myeloid leukemia cells have scarcely been investigated

Mouse myeloid leukemia M1 cells [9] can be induced to differentiate into macrophage-like cells by various substances including dexamethasone (Dex) [10], D-factor/Leukemia inhibitory factor (D-factor/LIF) [11], interleukin-6 (IL-6) [12] and 1α,25-dihydroxyvitamin D_3 (VD3) [13]. In this study, we examined whether IL-4 modulates growth and differentiation of M1 cells.

2. MATERIALS AND METHODS

2.1 *Chemicals*

Recombinant mouse IL-4 was purchased from Genzyme (Cambridge, MA) Dex was obtained from Sigma Chemical Co Recombinant human D-factor/LIF was purified to homogeneity from conditioned medium of CHO cells transfected with a plasmid containing cDNA encoding the D-factor/LIF [14] Human recombinant IL-6 and VD3 were obtained from Ajinomoto (Kawasaki, Japan) and Chugai Pharmaceutical Co (Tokyo, Japan), respectively

2.2 *Cell line and cell culture*

Mouse myeloid leukemia M1 (clone S-2 and clone T22-3) cells [15,16] were cultured in suspension in Eagle's minimum essential medium with twice the normal concentrations of amino acids and vitamins, and supplemented with 10% heat-inactivated calf serum

2.3 *Assay of properties of differentiated cells*

Phagocytic activity was assayed as reported previously [17] The percentage of cells that were morphologically similar to macrophages was determined in May–Gruenwald-Giemsa-stained smears

3 RESULTS

3.1. *Effects of IL-4 on growth and differentiation of M1 cells*

We first examined the effect of recombinant mouse IL-4 on growth of M1 cells. After 4 days culture of M1 cells seeded at 4×10^4 cells/ml with and without various concentrations (3–300 U/ml) of IL-4, cell numbers were essentially similar (data not shown). Moreover, IL-4 did not induce any morphological change (Table I), phagocytic activity (Fig. 1) or lysozyme activity (data not shown) of M1 cells

3.2. *Effect of IL-4 on differentiation of M1 cells induced by various inducers*

Next, we examined whether IL-4 modulated the induction of differentiation of M1 cells by various indu-

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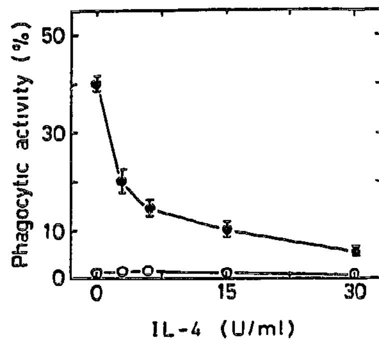

Fig 1 Effect of IL-4 on the induction of phagocytic activity of M1 cells by Dex M1 clone S-2 cells (2×10^5 cells/ml) were cultured with different concentrations of IL-4 in the presence (●) or absence (○) of 2×10^{-8} M Dex for 2 days Bars, SD (*n*=3)

cers M1 cells were cultured with Dex, a potent chemical inducer of differentiation for M1 cells, in the presence or absence of IL-4 for 4 days (Table I). IL-4 clearly inhibited Dex-induced morphological differentiation. This differentiation was accompanied by a decrease in cell growth Interestingly, this growth inhibition induced by Dex was also inhibited by IL-4 (Table I). IL-4 also caused dose-dependent inhibition of Dex-induced phagocytic activity, a typical functional marker of differentiation, of M1 cells (Fig 1) When M1 cells were cultured with 2×10^{-8} M Dex for 2 days, the concentration of IL-4 for 50% inhibition of phagocytic activity was 3 U/ml IL-4 (15 U/ml) exerted more than 60% inhibition of Dex-induced phagocytic activity of M1 cells throughout the incubation period (day 1 to day 3) (data not shown) IL-4 also inhibited lysozyme activity, a typical biochemical marker of differentiation of M1 cells, induced by Dex (data not shown)

We next examined whether IL-4 inhibited the differentiation of M1 cells induced by other differentiation inducers M1 cells were cultured with 0 2 ng/ml D-factor/LIF, a proteinous inducer of M1 cells, in the presence or absence of various concentrations of IL-4 for 4 days As shown in Fig 2, IL-4 also caused dose-dependent inhibition of D-factor/LIF-induced morphological differentiation of M1 cells The decrease in cell growth caused by D-factor/LIF was also inhibited dose-dependently by IL-4 IL-4 inhibited the D-factor/LIF-induced phagocytic activity of M1 cells (data not shown). Moreover, it also inhibited the phagocytic activity induced by IL-6, another proteinous inducer, (Fig 3) and by EG-6, an ethyleneglycol-type non-phosphorus alkyl ether lipid [18] (data not shown) The inhibitions of growth of M1 cells by IL-6 and EG-6 as well as by Dex and D-factor/LIF were counteracted by IL-4 (data not shown)

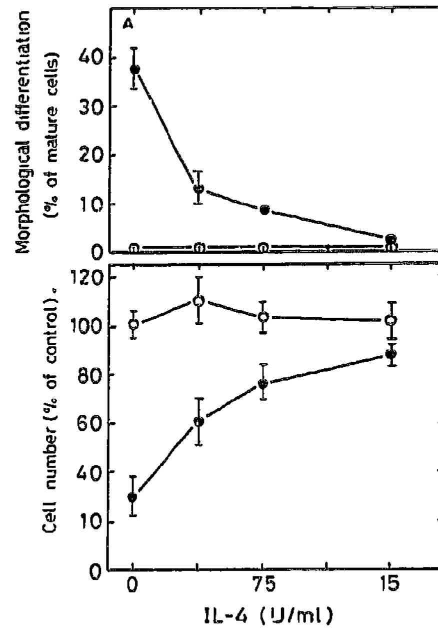

Fig 2 Effect of IL-4 on the induction of morphological differentiation of M1 cells by D-factor/LIF M1 clone T22-3 cells (3×10^4 cells/ml) were cultured with different concentrations of IL-4 in the presence (●) or absence (○) of 0 2 ng/ml D-factor/LIF for 4 days Then morphological change (A) and the number of cells (B) were assayed The number of cells in control cultures with or without D-factor/LIF were 2 4×10^5 cells/ml and 8 4×10^5 cells/ml, respectively Bars, SD (*n*=3)

Table I

Effect of IL-4 on induction of differentiation of M1 cells by Dex

Treatment	No of cells ($\times10^4$ cells/ml)	Cell type (%)		
		Blasts	Cells in Intermediate	Macrophages
None	131 ±8	99 0 ±1 0	1 0 ±1 0	0
IL-4	135 ±6	98 7 ±0 6	1 3 ±1 0	0
Dex	39 ±5	56 0 ±4 2	26 0 ±2 3	18 0 ±2 0
Dex + IL-4	85 ±8	90 0 ±3 6	8 7 ±3 0	1 3 ±0 6

M1 clone S-2 cells (3×10^4 cells/ml) were cultured with 2×10^{-8} M Dex in the presence or absence of 7 5 U/ml IL-4 for 4 days Values are means ± SD (*n*=3)

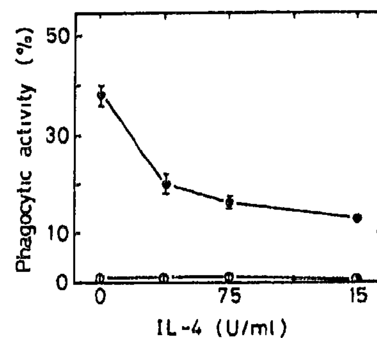

Fig 3 Effect of IL-4 on the induction of phagocytic activity of M1 cells by IL-6 M1 clone T22-3 cells (2×10^5 cells/ml) were cultured with different concentrations of IL-4 in the presence (●) or absence (○) of 50 U/ml IL-6 for 2 days Bars, SD (*n*=3)

3.3. *Effect of IL-4 on VD3-induced differentiation of M1 cells*

During examination of the effect of IL-4 on the induction of differentiation of M1 cells by different types of inducers, we found that IL-4 enhanced VD3-induced differentiation of the cells (Table II). IL-4 alone did not induce differentiation of M1 cells, but it enhanced VD3-induced morphological differentiation (Table II) and phagocytic activity of the cells (data not shown). Furthermore, the growth inhibition by VD3 was markedly enhanced by IL-4, which alone did not inhibit the growth of M1 cells (Table II). Treatment of M1 cells with IL-4 and VD3 were not toxic, more than 90% of the cells remaining viable. Thus IL-4 enhanced the VD3-induced differentiation and VD3-induced growth inhibition of M1 cells, whereas IL-4 inhibited the induction of differentiation and suppression of growth of M1 cells induced by the other differentiation inducers examined.

4. DISCUSSION

Although IL-4 itself had no significant effect on the growth or differentiation of M1 cells, it suppressed the induction of differentiation of M1 cells induced by Dex, D-factor/LIF, IL-6 and alkyl ether lipid EG-6, and partially restored the differentiation-associated inhibition of cell growth. IL-4 inhibited the induction of differentiation by these inducers despite their structural differences, suggesting that it may have a direct effect on M1 cells rather than acting on the differentiation inducers and/or their specific receptors.

The mechanisms of the inhibitory effect of IL-4 on the induction of differentiation of M1 cells are still unknown. Te Velde et al. [19] reported that IL-4 inhibited secretion of IL-6 by human monocytes. Miyaura et al. [20] reported that, although the production of IL-6 was stimulated by D-factor/LIF and VD3, the amounts of IL-6 secreted into the culture medium did not appear to be enough to induce differentiation of M1 cells and that the anti-IL-6 antibody did not suppress the differentiation of M1 cells induced by D-factor/LIF or VD3. Therefore, IL-6 does not appear to mediate the D-factor/LIF- or VD3-induced differentiation of M1 cells. Marked differentiation of M1 clone S-2 cells was induced by Dex (Table I) but not by IL-6 [21]. These results suggest that IL-6 does not mediate Dex-induced differentiation of M1 cells. In contrast, we found that IL-4 enhanced VD3-induced differentiation of M1 cells. From these findings, it is unlikely that the inhibition of induction of differentiation of M1 cells by IL-4 is due to suppression of IL-6 production in inducer-treated M1 cells.

There are reports [15,16,22,23] that some proteinous substances inhibit the induction of differentiation of M1 cells. I-factor [22] purified from conditioned medium of variant M1 clone cells, transforming growth factor-β1 (TGF-β1) [15,23] and interferon-γ (IFN-γ) inhibited the differentiation of M1 cells induced by Dex, VD3, D-factor/LIF, or IL-6. These inhibitory effects on differentiation of M1 cells were not associated with resumption of cell growth. Furthermore, the differentiation of M1 cells was not inhibited by other cytokines such as IL-1, IL-2, IL-3, IL-5, G-CSF, M-CSF and GM-CSF (data not shown). When M1 cells were cultured with Dex in the presence of both IL-4 and TGF-β1, the inhibitory effect on the Dex-induced phagocytic activity of M1 cells was more than cumulative (data not shown). These results indicate that IL-4 is a unique cytokine that inhibits differentiation of myeloid leukemia cells and that its actions on growth and differentiation of M1 cells are different from those of I-factor, TGF-β1 and IFN-γ.

The effect of IL-4 on the differentiation of M1 cells did not vary with the clone of M1 cells used. IL-4 inhibited Dex or D-factor/LIF-induced differentiation of all clones of M1 cells used, whereas IL-4 enhanced VD3-induced differentiation of all the clones of M1 cells used (Tables I and II, Fig. 2 and unpublished data). These results suggest that the effect of IL-4 on differentiation of M1 cells is dependent on the differentiation inducer, rather than the clone of M1 cells used.

The present results suggest that there are at least two distinct pathways of monocytic differentiation of M1 cells. One pathway involves several inducers other than VD3-induced differentiation and is inhibited by IL-4, and the other pathway involves VD3-induced differentiation and is stimulated by IL-4. Alternatively, VD3 may induce monocytic differentiation by a unique pathway(s). McInnes and Rennick [24] reported that IL-4 induced fusion of bone marrow cells and alveolar monocytes to form giant multinucleated cells which were associated with granulomatous lesions formed in response to foreign bodies, viruses, and bacteria. VD3, but not other differentiation inducers, also induced fusion of alveolar monocytes to form giant multinucleated cells [25]. Tanaka et al. [26] reported that several common proteins of alveolar monocytes were induced by

Tabel II

Effect of IL-4 on induction of differentiation of M1 cells by VD3

Treatment	IL-4 (15 U/ml)	No. of cells ($\times 10^4$ cells/ml)	Macrophages (%)
None	–	189 ± 9	0
	+	198 ±14	0
VD3 (0.25 ng/ml)	–	188 ± 5	0
	+	124 ±16	4.0 ±0.6
VD3 (0.5 ng/ml)	–	164 ±10	5.3 ±2.6
	+	74 ±11	17.6 ±3.0
VD3 (1 ng/ml)	–	104 ±11	16.3 ±5.5
	+	34 ± 4	30.5 ±2.1

M1 clone S-2 cells (3×10^4 cells/ml) were cultured with VD3 in the presence or absence of IL-4 for 4 days. Values are means ±SD (n=3).

IL-4 and VD3 but not other macrophage activators. Therefore, VD3 and IL-4 may induce monocytic differentiation to a specific subtype(s) of macrophages

In this study, we found that IL-4 modulates growths and differentiations of M1 cells induced by several compounds. IL-4 may act on a particular step(s) in the process of induction of differentiation of M1 cells. Therefore, IL-4 should be useful for studying the mechanisms of monocytic differentiation of myeloid leukemia cells.

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