Synthetic sialyl glycolipids (sialo-cholesterol and sialo-diglyceride) induce granulocytic differentiation of human myelogenous leukemia cell line HL-60

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When HL-60 cells were cultivated with synthetic sialyl glycolipids, sialo-cholesterol and sialo-diglyceride, the cells were found to be differentiated into mature granulocytes on morphological and functional criteria. The differentiation of cells was accompanied by inhibition of cell proliferation. The differentiation-inducing activity of sialo-cholesterol was greater than that of sialo-diglyceride on a molar basis, and the α-anomer of each compound was more potent than the β-anomer, suggesting that the stereospecific structure of the compounds is important for the differentiation-inducing activity.

Sialo-cholesterol; Sialo-diglyceride; Granulocytic differentiation; Myelogenous leukemia; HL-60

1. INTRODUCTION

Recent studies indicate that sialyl glycolipids play an important role in the proliferation and the differentiation of various types of cells, including fibroblasts, lymphocytes, astroglial and leukemic cells [1-5]. We have recently demonstrated that neolacto series gangliosides induce granulocytic differentiation not only of the human myelogenous leukemia cell line HL-60 cells but also of a subline of HL-60 cells resistant to the differentiation induction by retinoic acid [4,5]. Taking into account that neolacto series gangliosides are natural components of granulocyte plasma membrane, these observations indicate that neolacto series gangliosides could be candidates for the differentiation therapy for certain types of myelogenous leukemia. Then, we searched for synthetic sialyl glycolipids mimicking the action of neolacto series gangliosides. In this paper, we show that synthetic sialo-cholesterol and sialo-diglyceride are able to induce granulocytic differentiation of HL-60 cells.

2. MATERIALS AND METHODS

2.1. *Cells and cell culture*

HL-60 cells (a wild-type, mycoplasma-free) were grown in a serum-free synthetic medium (DME/F12) as described [5]. For induction of differentiation, HL-60 cells were seeded at approximately 2×10^5 cells/ml and grown in the presence or absence of sialyl glycolipids. Cell viability was determined by the erythrosine B dye exclusion test.

2.2. *Chemicals*

Alpha-sialo-cholesterol (α-D-*N*-acetylneuraminyl cholesterol, α-sialo-diglyceride (3-*O*-α-D-*N*-acetylneuraminyl-1,2-di-*O*-tetracecylglycerol) and their β-anomers were synthesized as described [6]. Cytochrome *c* type III, *N*-formyl-methionyl-leucyl-phenylalanine (FMLP), phorbol myristate acetate (PMA) and superoxide dismutase were purchased from Sigma Chemical Co., St. Louis, MO.

2.3. *Determination of cell differentiation*

For morphological assessment of the cells, Cytospin slide preparations were prepared using a Schandon Cytospin centrifuge (Schandon Southern Instruments, Inc., Sewickley, PA) and stained with Wright-Giemsa. The morphological assessment was performed under a light microscope. Surface membrane antigens were assessed by indirect immunofluorescence and cytofluorometry in an Ortho Spectrum III (Ortho Diagnostic Systems, Westwood, MA) using anti-OKM1 (CD11) [7], anti-OKB2 (CD24) [8] (Ortho Diagnostic Systems) and anti-LeuM1 (CD15) [9] (Becton Dickinson Immunocytometry Systems, CA) monoclonal antibodies, and fluorescein isothiocyanate-conjugated goat $F(ab')_2$ anti-mouse IgG or IgM antibodies. Mouse IgG was used as a negative control and a gate was used. The activity of non-specific esterase and nitroblue tetrazolium reduction was determined as described [5]. Superoxide (O_2^-) release was assayed spectrophotometrically by superoxide dismutase-inhibitable reduction of ferricytochrome *c*, and the continuous assay was performed in a Hitachi 557 spectrophotometer (a double wavelength spectrophotometer, Hitachi Ltd., Tokyo) as described [10].

3. RESULTS AND DISCUSSION

When HL-60 cells were cultivated with α-sialo-cholesterol (0.5 μM), an apparent morphological change reflecting differentiation along the granulocytic

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Abbreviations: FMLP, N-formyl-methionyl-leucyl-phenylalanine; PMA, phorbol myristate acetate

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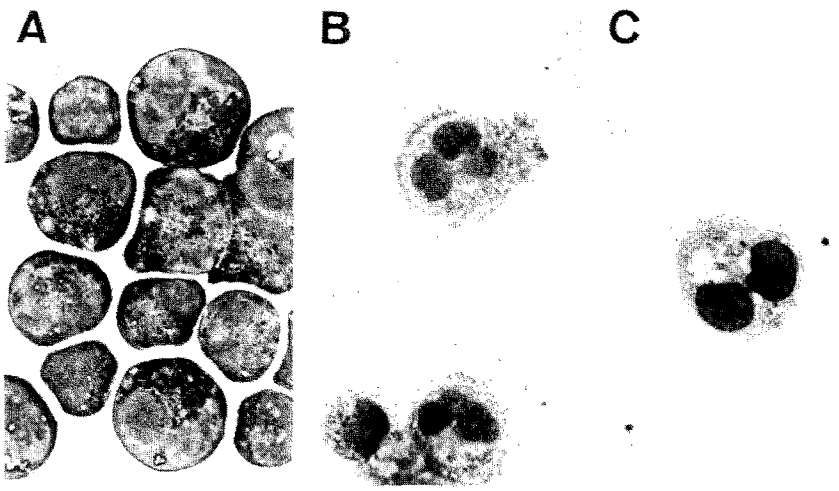

Fig. 1. Light microscopic pictures of HL-60 cells cultivated with (B) α-sialo-cholesterol (0.5 μM) or (C) α-sialo-diglyceride (15 μM) for 6 days. (A) control cells.

lineage was observed on day 5–6 (Fig. 1). Concomitantly with morphological changes, the number of cells strongly postive for the granulocytic lineage-specific naphthol AS-D chloroacetate esterase activity was increased (Table I). As shown in Fig. 2, HL-60 cells differentiated by α-sialo-cholesterol showed the increased expression of surface membrane antigens recognized by anti-OKM1 and anti-OKB2. Anti-OKM1 reacts on granulocytes and monocytes [7], and anti-OKB2 reacts on granulocytes but not on monocytes [8]. Thus, the findings with non-specific esterase activity and surface membrane antigens indicated that HL-60 cells differentiated by α-sialo-cholesterol were granulocytic cells. When HL-60 cells were maintained in a serum-free synthetic medium instead of a serum-containing medium, the cells were found to be differentiated functionally to some extent (data not shown), which may partly explain the increased expression of OKB2 on control cells used in the present experiments. LeuM1 was expressed on all immature HL-60 cells and the expression of LeuM1 was unchanged during the differentiation of HL-60 cells. The respiratory burst activity was increased in HL-60 cells differentiated by α-sialo-cholesterol when assessed

Table I

The activity of non-specific esterase and nitroblue tetrazolium reduction in HL-60 cells differentiated by α-sialo-cholesterol

	Naphthol AS-D chloroacetate esterase			α-Naphthyl butyrate esterase	Nitroblue tetrazolium reduction
	+	+ +	+ + +		
Control	20.0	58.2	9.6	12.2	12.5
α-Sialo-cholesterol	12.2	48.4	31.0	8.4	62.4

HL-60 cells were cultivated with 0.5 μM α-sialo-cholesterol for 5 days. The cells positive for naphthol AS-D chloroacetate esterase activity were divided into 3 classes according to the staining intensity; stained patchily (+), light blue (+ +) and dark blue (+ + +). Values represent the means of 4 experiments for the esterase activity and of two experiments for the nitroblue tetrazolium-reducing activity, and are expressed as the percentage of cells. The statistical analysis revealed that the number of cells strongly positive (+ + +) for the naphthol AS-D chloroacetate esterase activity was significantly ($p<0.01$) increased by the treatment with α-sialo-cholesterol

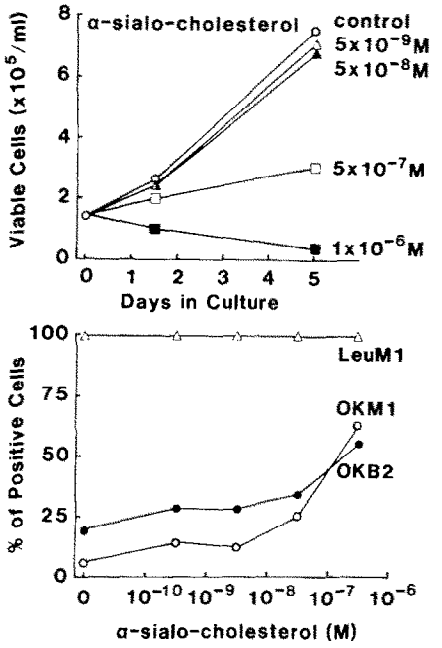

Fig. 2. Inhibition of cell proliferation and induction of mature granulocyte antigens by α-sialo-cholesterol. Upper: HL-60 cells were cultivated with various concentrations of α-sialo-cholesterol, and viable cells were counted on indicated culture days. Each point represents the mean of 3 experiments. Lower: HL-60 cells were cultivated with various concentrations of α-sialo-cholesterol for 5 days, and the surface membrane antigens were assessed by cytofluorometry. Each point represents the mean of 3 experiments.

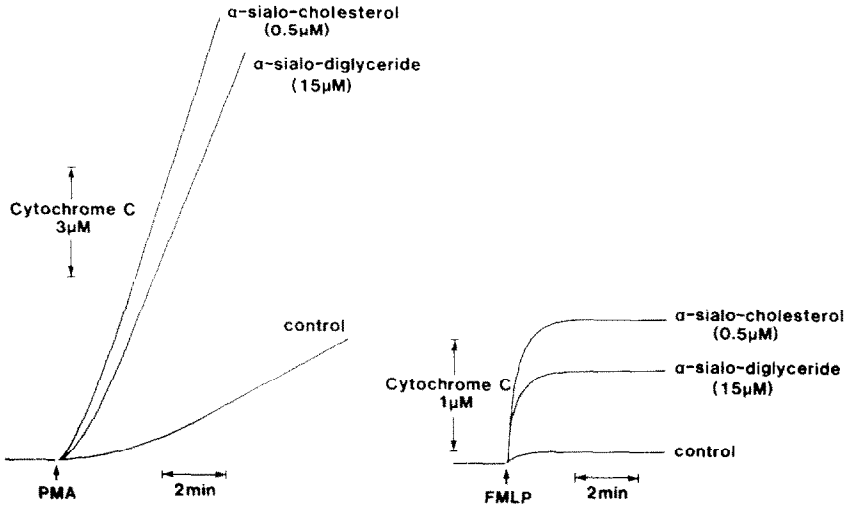

Fig. 3. Time courses of O_2^- release in HL-60 cells cultivated with α-sialo-cholesterol (0.5 μM) or α-sialo-diglyceride (15 μM) for 6 days. The cell suspension (5×10^5 cells/ml) in Hanks' balanced salt solution was added to a 1-ml cuvette containing 66 μM ferricytochrome c, and the absorbance change at 550–540 nm was followed on the recorder. PMA (100 ng/ml) and FMLP (1 μM) were used as stimuli.

by the nitroblue tetrazolium-reducing activity and the O_2^--releasing capacity (Table I, Figs 3 and 4). The time courses of O_2^- release in mature HL-60 cells stimulated by PMA or FMLP, a chemotactic peptide, were similar to those in normal granulocytes, and the amount of O_2^- release in mature HL-60 cells was almost comparable to that in normal granulocytes (Fig. 3) [10]. HL-60 cells were also differentiated into granulocytic cells morphologically and functionally when the cells were cultivated with α-sialo-diglyceride or the β-anomer of sialo-cholesterol or sialo-diglyceride for 5–6 days (Figs 1, 3 and 4; the pictures were not shown for the β-anomers). The differentiation-inducing activity of sialo-cholesterol was greater than that of sialo-diglyceride on a molar basis, and the α-anomer of each compound was more potent than the β-anomer (Fig. 4). These findings suggest that the stereospecific structure of the compounds is important for exhibiting the differentiation-inducing activity. The differentiation of HL-60 cells by all these sialyl glycolipids was accompanied by inhibition of cell proliferation (Fig. 2; data not shown for the other compounds). The higher concentrations of α-sialo-cholesterol (> 1 μM) were toxic to the cells. Neither free sialic acid (2 μM) nor cholesterol (2 μM) by itself induced the differentiation of HL-60 cells.

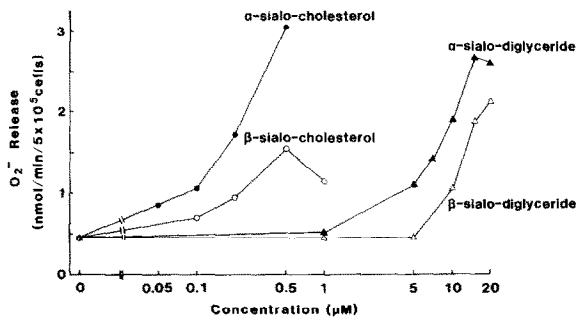

Fig. 4. O_2^- release in HL-60 cells differentiated by α- and β-anomers of sialo-cholesterol and sialo-diglyceride. HL-60 cells were cultivated with various concentrations of each compound for 6 days, and O_2^- release stimulated by PMA (100 ng/ml) was assayed.

The differentiation-inducing activity of sialo-cholesterol or sialo-diglyceride has been recently demonstrated in neuronal cells; a mouse neuroblastoma cell line (Neuro 2a) [11] and rat normal glioblasts [12] or astroblasts [13]. The present experiments demonstrated that sialo-cholesterol and sialo-diglyceride also acted on human hematopoietic cells and induced the differentiation of leukemic cells. Although the precise mechanisms by which these sialyl glycolipids induce the differentiation are unknown, the studies with rat glioblasts or astroblasts suggest that sialo-cholesterol, like gangliosides, acts directly on the plasma membrane [12,13]. The existence of protein kinases regulated by gangliosides and the ganglioside-specific binding protein is reported in guinea pig and rat brain [14–16]. Thus, it is possible that sialo-cholesterol and sialo-diglyceride may alter the membrane microenvironment to affect the protein kinase activity or may produce intracellular messengers that are not yet identified. The in vivo effects of these compounds on leukemic cells are now under investigation at our laboratory by using nude mice to which human leukemic cells are transplanted.

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