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CHANGES IN PHOSPHOLIPID COMPOSITION DURING DIFFERENTIATION OF CULTURED MOUSE MYELOID LEUKEMIA CELLS

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SUMMARY

Mouse myeloid leukemia cells(Ml) could be induced by various inducers to form Fc receptors, phagocytize, migrate in agar, produce lysosomal enzyme activities, and change into forms that were morphologically similar to macrophages and granulocytes. When Ml cells were cultured with inducer, the ratio of the percentage of phosphatidylethanolamine to that of phosphatidylcholine was increased about 2-fold. This ratio of the differentiated Ml cells was similar to that of peritoneal macrophages of normal mice or Mm-1 cells, which were established from spontaneously differentiated macrophage-like cells from Ml cells. These changes in phospholipid may be involved in the mechanisms of expression of the differentiation-associated phenotypic properties.

INTRODUCTION

Ml cells, established from an SL mouse, are myeloid leukemia cells that can be induced by various inducers to differentiate into forms that are functionally and morphologically similar to macrophages and granulocytes(1-4). The formation of mature macrophages and granulocytes is accompanied by the induction of Fc and C3 receptors on the cell surface, phagocytosis, locomotive activity, and secretion of lysozyme. Expression of these differentiation-associated properties may result from changes in structure of the cell membrane during differentiation of the cells.

Although several investigation have suggested that the cell surface plays an important role in mechanisms associated with differentiation of myeloid leukemia cells(5,6), there are few reports on changes of membrane components during differentiation of the cells. Sugiyama et al.(7) showed that the appearance of a glycoprotein with a molecular weight of 180,000 on the outer

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cell surface was associated with differentiation of the cells, but little is known about changes in other membrane components.

Changes in phospholipid composition could alter the change and fluidity of the membrane, and these changes could, in turn, affect various cellular functions in which the plasma membrane participates(8). In this work, therefore, we examined differentiation-associated changes in the phospholipid composition of mouse myeloid leukemia Ml cells.

MATERIALS AND METHODS

Cells; A myeloid leukemia cell line(Ml), established from cells of an SL strain mouse with myeloid leukemia, was grown in suspension in Eagle's minimum essential medium containing double the usual concentrations of amino acids and vitamins and supplemented with 10% calf serum(1). A macrophage-like cell line(Mm-1) developed spontaneously from Ml cells was also grown in the same medium(9).

Peritoneal macrophages were obtained from the peritoneal cavity of SL mice that had been treated for 3 days with 5% proteose peptone(Difco) and 5% starch(Merck), as reported previously(10).

Lipid extract and chromatography of phospholipids; After incubation with inducers, Ml cells were washed twice with phosphate-buffered saline(pH 7.4) and suspended in 1 mM EDTA(pH 7.0). Lipid extracts were prepared and washed by the method of Folch et al.(11). Two-dimensional thin-layer chromatography of the lipid extracts was performed on silica gel 60 plates(Merck). The plates were developed in the first dimension with chloroform/methanol/ammonia (65/25/5, by volume). Then they were dried for 30 min and developed in the second dimension with chloroform/acetone/methanol/glacial acetic acid/water (3/4/1/1/0.5, by volume). The lipids were located with iodine vapor, and areas corresponding to individual lipids were scraped into tubes for phosphorus analysis. Lipid phosphorus was determined by the method of Rouser et al.(12).

Materials; Proteinous inducer was prepared from the ascitic fluid of rats with hepatoma AH130, as previously reported(3,13). Dexamethasone and 5-fluoro deoxyuridine were obtained from Sigma Chemical Co. Lipopolysaccharide from Salmonella typhymurium was purchased from Difco Laboratories.

RESULTS AND DISCUSSION

Two types of Ml cells were used: sensitive Ml cells that could be induced to differentiate into macrophages and granulocytes by treatment with dexamethasone, proteinous inducer, or lipopolysaccharide, and resistant Ml cells that could not differentiate even with a high concentration of inducer(13). Some characteristics of the cells are shown in Table 1. Untreated sensitive and resistant Ml cells are non-phagocytic, and have no lysozyme activity or

Table 1. Differentiation-associated properties of Ml cells with and without inducer-treatment, and of Mm-1 cells

Cell type	Sensitive Ml cells		Resistant Ml cells		Mm-1 cells
Inducer(5×10^{-7}M dexamethasone)	-	+	-	+	-
Fc receptors (% of cells with rosette)	0.5	43	0	0	85
Phagocytosis(% of phagocytic cells)	1	53	0	0	98
Lysozyme activity (μg equivalent to egg white lysozyme /mg protein)	0.6	71	0	0	107

Fc rosette formation and phagocytosis were determined 2 days after seeding with the inducer by the procedures reported previously(6,18). Lysozyme activity was determined 4 days after seeding with the inducer, as previously reported(19).

Fc receptors. When the two types of cells were treated with dexamethasone, the sensitive, but not the resistant Ml cells became phagocytic, and developed Fc receptors and lysozyme activity. Mm-1 cells were phagocytic, and had Fc receptors and lysozyme activity even when cultured without inducers.

Table 2 shows the percentage phospholipid compositions of Ml cells, Mm-1 cells and peritoneal macrophages of normal mice. There was no significant difference between the phospholipid compositions of the sensitive and resistant Ml cells. Moreover, no difference was observed between non-confluent and confluent Ml cells. However, the percentage of phosphatidylcholine was less in differentiated Ml cells than in untreated Ml cells, while the percentage of phosphatidylethanolamine was more in differentiated Ml cells. Changes in the percentages of other phospholipids, such as lysophosphatidylcholine and sphingomyelin, during differentiation were slight. The phospholipid composition of differentiated Ml cells was similar to that of Mm-1 cells or macrophages. The ratio of phosphatidylethanolamine to phosphatidylcholine increased

Table 2. Phospholipid compositions of Ml cells, dexamethasone-treated Ml cells, Mm-l cells, and normal macrophages

Phospholipid	Untreated Ml cells	Dexamethasone-[a] treated Ml cells	Mm-l cells	Macrophages
Lysophosphatidylcholine	1.4 ± 0.2	6.1 ± 0.4	6.0 ± 0.3	7.0 ± 0.7
Sphingomyelin	2.7 ± 0.6	4.6 ± 0.2	4.8 ± 0.4	10.2 ± 0.9
Phosphatidylserine	5.6 ± 1.3	4.5 ± 0.3	3.9 ± 0.3	4.5 ± 0.4
Phosphatidylinositol	7.0 ± 0.9	5.3 ± 0.4	6.3 ± 0.5	4.2 ± 0.4
Phosphatidylcholine	59.6 ± 2.3	49.8 ± 1.4	45.6 ± 1.6	41.7 ± 1.1
Phosphatidylethanolamine	19.3 ± 1.6	25.6 ± 1.7	29.1 ± 1.9	27.8 ± 1.2
Others [b]	4.4 ± 0.4	4.1 ± 0.6	4.3 ± 0.4	4.6 ± 0.5
PE/PC [c]	0.32	0.51	0.64	0.67

Results are expressed as percentages of the total lipid phosphorus ± S.D. At least 4 different preparations of each cell type were analyzed. Recoveries of lipid phosphorus were more than 95%. a) Sensitive Ml cells were treated with 5×10^{-7}M dexamethasone for 4 days. b) Other phospholipids include lyso(bis)phosphatidic acid, diphosphatidylglycerol, and phosphatidylglycerol. c) PE/PC, ratio of phosphatidylethanolamine to phosphatidylcholine.

during differentiation of Ml cells and reached neary that of Mm-l cells or macrophages. This increase in the ratio was detectable within 3 days(Fig. 1).

Changes in phospholipid composition were also induced by other inducers of differentiation, such as lipopolysaccharide and proteinous inducer(Table 3), and there was no significant difference between the phospholipid composition of differentiated Ml cells induced by dexamethasone and those induced by other inducers. The inducers did not alter the phospholipid composition of resistant Ml cells(Table 3). Although growth of the sensitive Ml cells was inhibited by treatment with inducers, it is unlikely that the changes in phospholipid composition were due to inhibition of cell growth for two reasons. First, 5-fluorodeoxyuridine inhibited growth of the sensitive Ml cells, but did not induce differentiation of the cells, and the phospholipid compo-

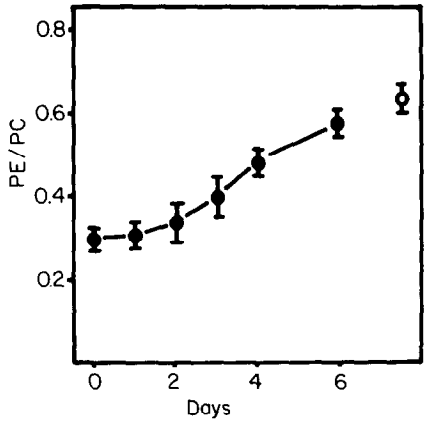

Fig. 1. Change in ratio of phosphatidylethanolamine to phosphatidylcholine by dexamethasone in Ml cells. Sensitive Ml cells were cultured with 2 X 10^{-7}M dexamethasone. Phospholipids were extracted and separated by chromatography on silica gel plates and the phosphorus in spots corresponding to phosphatidylethanolamine and phosphatidylcholine was determined. ●, Dexamethasone-treated Ml cells, ○, Mm-1 cells.

sition of the cells treated by 5-fluorodeoxyuridine was similar to that of untreated cells(Table 3). Second, the phospholipid composition of exponentially growing Mm-1 cells was similar to that of macrophages or differentiated

Table 3. Ratio of phosphatidylethanolamine to phosphatidylcholine in Ml cells treated by various inducers

Cell type	Treatment	No. of viable cells($\times 10^6$/ml)	$\frac{PE}{PC}$ (± S.D.)
Sensitive	None	2.9	0.31 ± 0.02
	Dexamethasone (5×10^{-7}M)	1.8	0.52 ± 0.03
	Proteinous inducer (5%,v/v)	2.0	0.54 ± 0.02
	Lipopolysaccharide (0.5 μg/ml)	1.7	0.54 ± 0.02
	5-Fluorodeoxyuridine (5×10^{-9}M)	1.5	0.33 ± 0.02
Resistant	None	3.4	0.32 ± 0.02
	Dexamethasone (5×10^{-8}M)	3.2	0.31 ± 0.02

Ml cells were incubated at 4×10^5 cells/ml with various inducers for 4 days. Average data from three separate experiments are shown in the Table. PE/PC, see footnote to Table 2.

Table 4. Effect of dexamethasone on phospholipid synthesis in sensitive Ml cells

Phospholipid	^{32}P incorporated(cpm/10^7cells) -Dexamethasone	+Dexamethasone	% inhibition
Total	64,791	37,050	43
Lysophosphatidyl-choline	259	182	30
Sphingomyelin	194	135	30
Phosphatidyl-serine	1,231	654	47
Phosphatidyl-inositol	22,482	5,562	75
Phosphatidyl-choline	17,882	10,841	39
Phosphatidyl-ethanolamine	20,862	18,811	10
Others	1,881	865	54

Sensitive Ml cells were cultured for 2 days with or without 2 X 10^{-7}M dexamethasone. The cells were labeled for 3 hours with ^{32}P-orthophosphate(50 μCi/ml). Average data from three separate experiments are shown in the Table.

Ml cells. These results suggest that changes in phospholipid composition are closely associated with cell differentiation.

The amounts of ^{32}P incorporated into different phospholipids of the cells in 3 hours are shown in Table 4. Most of the radioactivity was found in the fractions containing phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol. Phosphatidylinositol synthesis of the cells treated by dexamethasone was markedly inhibited, probably mainly owing to inhibition of cell growth, since phosphatidylinositol synthesis is correlated with cell growth(14,15). Moreover inhibition of the incorporation of ^{32}P into phosphatidylcholine was 39%. However, inhibition of the incorporation of ^{32}P into phosphatidylethanolamine was only 10%, suggesting that phosphatidylethanolamine synthesis is specifically maintained during differentiation of Ml cells

These changes in phospholipid composition could alter the structure and function of the membrane; in fact, a recent report(16) indicated that addition of membrane lipid components to Ml cell cultures modified the appearance of Fc receptors in the cell surface. Phosphatidylcholine can be synthesized by the stepwise methylation of phosphatidylethanolamine, and phospholipid methylation alters a variety of membrane functions(17). Studies on phospholipid methylation may provide fundamental information on the role of the cell membrane in mechanisms of cell differentiation.

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