Lipid characteristics of Friend erythroleukaemia cells differentiated by dimethyl sulphoxide or hexamethylenebisacetamide, and of non-inducible clones treated with the inducers

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We studied the lipid changes in Friend erythroleukaemia (FEL) cells differentiated by dimethyl sulphoxide (DMSO) or hexamethylenebisacetamide (HMBA). We also examined clones of FEL cells non-inducible to differentiation by DMSO or HMBA for lipid changes not related to the differentiation process. A decrease in triacylglycerols was found in both DMSO- or HMBA-differentiated FEL cells, whereas a decrease in phosphatidylethanolamine was observed only in DMSO-differentiated FEL cells. The characteristics of the phospholipid fatty acid profiles of FEL cells, which proved to be similar to those of other transformed cells, were not significantly affected by erythroid differentiation.

INTRODUCTION

It has long been argued that the malignant phenotype in tumour cells may be related to an anomalous differentiation (Straus, 1981; Pierce & Wallace, 1981). This issue has been highlighted by the induction of terminal cell differentiation in different lines of haemopoietic transformed cells (Ichikawa, 1969; Friend, 1976; Hozumi, 1983; Myers & Siegel, 1984; Marks & Rifkind, 1984) exposed to various chemical agents (Marks & Rifkind, 1978) or biological stimulators (Hozumi, 1983). However, it is still unclear whether restoration of fundamental differentiation characteristics in transformed cells is accompanied by the regression of all the biological and biochemical features typical of the malignant phenotype.

In this study, we examined whether Friend erythroleukaemic (FEL) cells show characteristics in their lipid composition similar to those typically found in other transformed cells (Snyder *et al.*, 1969; Snyder & Snyder, 1975; Ruggieri & Fallani, 1973, 1979; Ruggieri *et al.*, 1979; Wood, 1975; Van Hoeven *et al.*, 1975; Yau *et al.*, 1976; Montaudon *et al.*, 1981). If so, the question arises whether these characteristics revert to a normal pattern when FEL cells are induced to erythroid differentiation by dimethyl sulphoxide (DMSO) (Friend *et al.*, 1971) or hexamethylenebisacetamide (HMBA) (Reuben *et al.*, 1976). We also tested two variants of FEL cells which were not inducible to differentiation by DMSO or HMBA for lipid changes independent of the differentiation process.

Some aspects of our experimental design have previously been investigated in other laboratories (Zwingelstein *et al.*, 1981, 1982; Rittmann *et al.*, 1982; Rawlyer *et al.*, 1983; Simon *et al.*, 1984; Van der Schaft *et al.*, 1987).

MATERIALS AND METHODS

Cells and culture conditions

FEL cells (clone 745A), kindly supplied by Dr. G. B. Rossi (Istituto Superiore di Sanità, Roma, Italy), were grown in our laboratory in suspension culture at 37 °C in a humidified atmosphere containing 5% CO₂ in RPMI 1640 medium (Gibco) with 5% (v/v) foetal-calf serum (Flow). Cells were routinely seeded at 1×10^5 cells/ml and passaged after 5 days, at which time cultures reached a saturation density of 2.5×10^6 cells/ml and contained more than 95% viable cells as tested by the Trypan-Blue-exclusion method. The extent of spontaneous erythroid differentiation, as tested by the benzidine reaction (Orkin *et al.*, 1975), was consistently lower than 1%.

Clones of FEL cells non-inducible by DMSO were isolated by growth in 1.4% (w/v) Methocel (Sigma) semi-solid medium containing 280 mM-DMSO (Merck) (Rovera & Bonaiuto, 1976); clones non-inducible by HMBA were selected by growing FEL cells in 5 mM-HMBA (Sigma) and then by cloning the surviving cells in HMBA-free 1.4% Methocel semi-solid media. Only clones with less than 1% inducible cells were used in this study.

Induction of differentiation

FEL cells were induced to erythroid differentiation by growth in media supplemented with 280 mM-DMSO or 5 mM-HMBA under the same culture conditions used for the routine cultivation of untreated cells. After 5 days of growth in the presence of DMSO or HMBA, cell cultures contained 70% and 95% benzidine-positive cells respectively, and their cellular viability was never less than 95%. The clones non-inducible by DMSO and HMBA were also exposed to the corresponding inducers for 5 days.

Abbreviations used: FEL, Friend erythroleukaemia; DMSO, dimethyl sulphoxide; HMBA, hexamethylenebisacetamide; PE, phosphatidylethanolamine.

Table 1. Changes in lipid composition of FEL cells upon differentiation with DMSO or HMBA, and in clones non-inducible by DMSO and HMBA treated with the corresponding inducer

Values represent percentages of total lipids and are means $\pm$ s.e.m. for five experiments. Phospholipids were calculated by multiplying by 25 the lipid P assayed on total lipids (Martin & Doty, 1959) after digestion with $H_2SO_4/HClO_4$ (3:2, v/v). Statistical significance as determined by Student's *t* test: **P* < 0.05, ***P* < 0.01, ****P* < 0.005, compared with the appropriate control.

Lipid class	FEL cells			Non-inducible clone (DMSO)			Non-inducible clone (HMBA)		
	Control	DMSO	HMBA	Control	DMSO		Control	HMBA	
Phospholipids									
Free cholesterol	80.6 $\pm$ 4.2	85.1 $\pm$ 7.1	78.5 $\pm$ 3.2	86.0 $\pm$ 10.1	87.4 $\pm$ 3.2		78.6 $\pm$ 7.8	82.0 $\pm$ 3.4	
Esterified cholesterol	13.0 $\pm$ 1.2	11.4 $\pm$ 0.5	15.8 $\pm$ 1.7	8.4 $\pm$ 0.5	10.0 $\pm$ 1.1		14.2 $\pm$ 2.5	12.9 $\pm$ 2.6	
Triacylglycerols	4.6 $\pm$ 0.6	1.5 $\pm$ 0.2***	4.0 $\pm$ 0.6	4.0 $\pm$ 0.4	1.2 $\pm$ 0.2***		5.6 $\pm$ 2.3	3.1 $\pm$ 0.4	
Non-esterified fatty acids	0.5 $\pm$ 0	0.2 $\pm$ 0.1**	0.3 $\pm$ 0*	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1		0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	
Alkyl-diacylglycerols	0.6 $\pm$ 0.1	0.8 $\pm$ 0.3	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1		0.7 $\pm$ 0.2	0.9 $\pm$ 0.2	
	0.7 $\pm$ 0.2	1.0 $\pm$ 0.2	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	0.6 $\pm$ 0		0.6 $\pm$ 0.1	0.8 $\pm$ 0.2	
Total lipids (pg/cell)	115.3	91.9	94.4	124.3	139.3		141.4	131.3	

Table 2. Changes in phospholipid composition of FEL cells upon differentiation with DMSO or HMBA, and in clones non-inducible by DMSO or HMBA treated with the corresponding inducer

Values represent percentages of total lipid P (Martin & Doty, 1949) and are means $\pm$ s.e.m. for five experiments. Statistical significance as determined by Student's *t* test: **P* < 0.05, ****P* < 0.005, compared with the appropriate control.

Lipid class	FEL cells			Non-inducible clone (DMSO)			Non-inducible clone (HMBA)		
	Control	DMSO	HMBA	Control	DMSO		Control	HMBA	
Diphasphatidylglycerol	7.9 $\pm$ 0.6	7.6 $\pm$ 0.8	6.0 $\pm$ 0.3	4.9 $\pm$ 1.0	6.2 $\pm$ 1.8		8.0 $\pm$ 0.7	7.6 $\pm$ 0.5	
Phosphatidylethanolamine (PE)	23.6 $\pm$ 1.1	18.3 $\pm$ 1.5***	22.6 $\pm$ 1.7	25.6 $\pm$ 0.5	21.8 $\pm$ 1.08		22.5 $\pm$ 0.9	24.2 $\pm$ 1.9	
Phosphatidylcholine	55.3 $\pm$ 0.5	59.8 $\pm$ 2.2	59.1 $\pm$ 2.7	56.9 $\pm$ 1.5	55.1 $\pm$ 1.3		55.7 $\pm$ 0.6	56.0 $\pm$ 1.9	
Phosphatidylinositol + phosphatidylserine	9.5 $\pm$ 0.2	10.8 $\pm$ 0.7	9.8 $\pm$ 0.8	9.1 $\pm$ 0.6	14.1 $\pm$ 1.9*		10.7 $\pm$ 0.8	10.1 $\pm$ 1.1	
Sphingomyelin	3.5 $\pm$ 0.4	3.5 $\pm$ 1.6	2.5 $\pm$ 0.5	3.5 $\pm$ 0.4	2.8 $\pm$ 0.7		3.1 $\pm$ 0.1	2.1 $\pm$ 0.3	

Table 3. Changes of PE fatty acid composition in FEL cells upon differentiation with DMSO and HMBA, and in clones non-inducible by DMSO or HMBA treated with the corresponding inducer

Values are percentages of total fatty acids, and are means $\pm$ S.E.M. for the numbers of experiments listed in parentheses.

Fatty acid	FEL cells (7)			Non-inducible clone (DMSO) (3)		Non-inducible clone (HMBA) (3)	
	Control	DMSO	HMBA	Control	DMSO	Control	HMBA
C _{16:0}	29.2 $\pm$ 1.3	28.6 $\pm$ 2.4	34.1 $\pm$ 3.4	33.2 $\pm$ 3.7	31.4 $\pm$ 2.6	26.9 $\pm$ 3.2	33.0 $\pm$ 3.4
C _{16:1}	4.1 $\pm$ 1.0	3.1 $\pm$ 0.3	4.0 $\pm$ 1.0	5.6 $\pm$ 0.8	4.5 $\pm$ 0.6	3.1 $\pm$ 0.5	4.5 $\pm$ 0.6
C _{18:0}	15.1 $\pm$ 1.2	15.6 $\pm$ 1.2	13.4 $\pm$ 1.6	22.7 $\pm$ 1.3	19.3 $\pm$ 3.4	14.6 $\pm$ 2.5	14.6 $\pm$ 1.9
C _{18:1}	34.2 $\pm$ 1.8	32.8 $\pm$ 2.0	34.4 $\pm$ 3.4	22.1 $\pm$ 5.0	33.2 $\pm$ 5.3	32.6 $\pm$ 1.3	26.7 $\pm$ 0.3
C _{18:2}	2.0 $\pm$ 0.3	2.2 $\pm$ 0.9	1.2 $\pm$ 0.1	0.7 $\pm$ 0.1	0.6 $\pm$ 0.2	4.7 $\pm$ 1.5	5.5 $\pm$ 0.1
C _{20:4}	8.4 $\pm$ 0.8	11.3 $\pm$ 0.7	4.8 $\pm$ 2.0	7.1 $\pm$ 0.8	7.7 $\pm$ 1.9	10.7 $\pm$ 2.7	11.7 $\pm$ 0.2
C ₂₂ (polyunsaturated)	6.0 $\pm$ 0.7	6.4 $\pm$ 2.1	7.8 $\pm$ 0.7	8.6 $\pm$ 0.9	3.2 $\pm$ 1.5	7.4 $\pm$ 1.3	18.4 $\pm$ 2.0

Table 4. Changes of phosphatidylcholine fatty acid composition in FEL cells upon differentiation with DMSO or HMBA, and in clones non-inducible by DMSO or HMBA treated with the corresponding inducer

Values are percentages of total fatty acids, and are means $\pm$ S.E.M. for the numbers of experiments listed in parentheses: tr., trace.

Fatty acid	FEL cells (7)			Non-inducible clone (DMSO) (3)		Non-inducible clone (HMBA) (3)	
	Control	DMSO	HMBA	Control	DMSO	Control	HMBA
C _{16:0}	35.5 $\pm$ 3.6	40.7 $\pm$ 2.5	47.2 $\pm$ 1.9	48.7 $\pm$ 4.6	40.7 $\pm$ 1.8	39.3 $\pm$ 1.7	45.0 $\pm$ 3.4
C _{16:1}	8.8 $\pm$ 0.8	9.0 $\pm$ 1.2	8.0 $\pm$ 1.0	5.1 $\pm$ 0.9	8.8 $\pm$ 1.5	8.2 $\pm$ 0.3	8.0 $\pm$ 1.3
C _{18:0}	5.8 $\pm$ 0.4	6.1 $\pm$ 0.7	3.3 $\pm$ 0.3	7.4 $\pm$ 0.9	5.9 $\pm$ 1.2	4.5 $\pm$ 1.1	3.8 $\pm$ 0.9
C _{18:1}	44.0 $\pm$ 2.6	42.2 $\pm$ 1.1	36.4 $\pm$ 2.7	35.4 $\pm$ 1.7	41.6 $\pm$ 1.7	44.7 $\pm$ 4.0	39.1 $\pm$ 2.9
C _{18:2}	1.8 $\pm$ 0.2	1.0 $\pm$ 0.4	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	1.5 $\pm$ 0.7	1.0 $\pm$ 0	0.8 $\pm$ 0.3
C _{20:4}	1.8 $\pm$ 0.2	1.0 $\pm$ 0.4	1.5 $\pm$ 0.3	0.9 $\pm$ 0.1	0.8 $\pm$ 0.3	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2
C ₂₂ (polyunsaturated)	1.6 $\pm$ 0.9	tr.	1.9 $\pm$ 0.8	1.5 $\pm$ 0.6	0.7 $\pm$ 0.5	1.4 $\pm$ 0.4	2.7 $\pm$ 0.9

Lipid analysis

FEL cells were washed twice by suspension and centrifugation (250 g, 10 min) in 0.1 M-phosphate-buffered saline (pH 7.2). The sonicated cell suspensions were extracted by the method of Folch *et al.* (1957). Neutral lipids and phospholipids were fractionated by silicic acid column chromatography and then separated into individual lipid classes as previously described (Ruggieri *et al.*, 1979).

Triacylglycerols, non-esterified fatty acids, free cholesterol and esterified cholesterol were determined by quantitative g.l.c. analysis under the conditions described previously (Ruggieri *et al.*, 1979). Alkyldiacylglycerols were converted into alkylglycerols by hydrogenolysis with sodium bis(2-methoxyethoxy)aluminium hydride (Aldrich Chemical Co., Steinheim, Germany) (Su & Schmidt, 1974) and then assayed by quantitative g.l.c. as previously reported (Fallani *et al.*, 1982).

RESULTS AND DISCUSSION

Lipids of undifferentiated FEL cells were mainly composed of phospholipids and free and esterified cholesterol, whereas non-esterified fatty acids, triacylglycerols and alkyldiacylglycerols were minor components (Table 1). The lipid composition of non-inducible

FEL cells did not differ from that of inducible FEL cells. The amounts of phospholipids and total cholesterol in FEL cells were comparable with those found by Montfoort & Boere (1978) in Rauscher erythroleukaemia cells. FEL cells differentiated by either DMSO or HMBA showed a decrease in triacylglycerols which was not found in non-inducible clones exposed to DMSO or HMBA. In DMSO-differentiated FEL cells there was a decrease in esterified cholesterol, also reported by Rittmann *et al.* (1982), which reflects an effect of DMSO on membrane lipids, since it also occurred in the clone non-inducible by DMSO treated with the inducer. Alkyldiacylglycerols were not changed in FEL cells differentiated by DMSO or HMBA.

The phospholipid composition of inducible and non-inducible FEL cells was similar to that found in other types of cell lines (Weinstein *et al.*, 1969; Wood & Falch, 1973; Ruggieri *et al.*, 1979) (Table 2). In FEL cells differentiated by DMSO, but not in those differentiated by HMBA, there was a significant decrease in phosphatidylethanolamine (PE). Zwingelstein *et al.* (1981) also reported a decrease in PE in DMSO-differentiated FEL cells, whereas Honma *et al.* (1980) found an increase in PE in a mouse myeloid leukaemia cell line induced to granulocyte-macrophage differentiation. Thus specific molecular patterns seem to be associated with cellular differentiation, according to the inducer and cell type.

Table 2 also shows that the amount of the phosphatidylinositol + phosphatidylserine fraction was increased in the clone non-inducible by DMSO after exposure to the inducer.

The PE fatty acid compositions of FEL cells were characterized by a high proportion of oleic acid ($C_{18:1}$) and low percentages of linoleic ($C_{18:2}$), arachidonic ($C_{20:4}$) and C_{22} polyunsaturated fatty acids (Table 3). These characteristics were still more pronounced in the phosphatidylcholine of FEL cells (Table 4). These characteristics in the phospholipid fatty acid pattern are reminiscent of those found in several types of transformed cells and tumours compared with their respective controls (Snyder *et al.*, 1969; Ruggieri & Fallani, 1973; Wood, 1975; Van Hoesen *et al.*, 1975; Yau *et al.*, 1976; Ruggieri *et al.*, 1979; Montaudon *et al.*, 1981).

The non-inducible clones of FEL cells had the same PE and phosphatidylcholine fatty acid profiles as those found in the inducible FEL cells. A higher proportion of monounsaturated fatty acids was found in non-inducible than in inducible clones of myeloid leukaemia cells (Simon, 1979) and of FEL cells (Simon *et al.*, 1984).

When FEL cells were differentiated by either DMSO or HMBA, their PE and phosphatidylcholine fatty acid profiles maintained the characteristics of the undifferentiated state. The higher value of $C_{20:4}$ acid in PE of DMSO-differentiated FEL cells compared with undifferentiated cells was not statistically significant (Tables 3 and 4). Zwingelstein *et al.* (1981), using the same clone of FEL cells as we did, reported instead a marked increase in $C_{20:4}$ acid, associated with a decrease in $C_{18:2}$ acid in phospholipids when erythroid differentiation was induced by DMSO. The discrepancy between the results of Zwingelstein *et al.* (1982) and ours raises the question of whether specific characteristics in phospholipid fatty acid composition may play a role in the induction of cellular differentiation.

The following conclusions can be drawn from this study. (1) One of the more typical lipid characteristics of malignant cells, namely a high proportion of $C_{18:1}$ associated with low percentages of $C_{20:4}$ and C_{22} polyunsaturated fatty acids, was unaffected in FEL cells when the block of differentiation caused by the Friend erythroleukaemia virus was removed by DMSO or HMBA (Friend, 1976); thus, in terms of our model, changes in the phospholipid molecular-species pattern of malignant cells do not seem to be indicative of an anomalous differentiation. (2) No specific pattern in the content of the major lipid components and in the fatty acid composition seems to be related to the biological characteristics of the non-inducible clones isolated from FEL cells.

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