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CONCANAVALIN A-MEDIATED AGGLUTINABILITY OF BALB/c3T3 CELLS GROWN IN MEDIA SUPPLEMENTED WITH DIFFERENT PHOSPHATIDYLCHOLINES[+].

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SUMMARY. Balb/c3T3 cells were grown near confluency in media supplemented with 10% fetal calf serum and than exposed for 24 hours to media containing different phosphatidylcholines bound to delipidated fetal calf serum. Compared to cells grown in regular media, 3T3 cells exposed to media containing dioleoyl-phosphatidylcholine dramatically increased their agglutinability by Concanavalin A. Exposure to several other phosphatidylcholines had no effect.

INTRODUCTION. Lectins are multivalent proteins which can bind to specific carbohydrate groups on cell surfaces and induce agglutination in different types of cells (1).In recent years, lectins have been used extensively to investigate the organization of cell surface (2). Interest in lectin-mediated agglutinability has been heightened by the observation that transformed cells are agglutinable by lower concentrations of lectins (e.g. Concanavalin A or wheat germ agglutinin) than are normal parental cells (3,4). The factors controlling agglutination properties of different types of cells remain uncertain and controversy persists regarding the changes responsible for the differential agglutination of transformed cells (4).

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Abbreviations : MEMx4, Eagle's minimal essential medium with fourfold concentration of vitamins and aminoacids; FCS, fetal calf serum; DFCS, delipidated fetal calf serum; PC, phosphatidylcholine; SV3T3 cells, SV40-transformed Balb/c3T3 cells; EGTA,ethyleneglycol--bis(β-aminoethylether)N,N'-tetraacetic acid; PBS,phosphate buffered saline; PE, phosphatidylethanolamine; EFA, essential fatty acids.

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To evaluate the role of cell surface lipids in lectin-mediated agglutination we explored the sensitivity of Balb/c3T3 cells to Concanavalin A when different exogenous phosphatidylcholines were incorporated into the cells. Previous studies have shown that the binding of Concanavalin A and the critical temperature for agglutination are influenced by the degree of unsaturation of the fatty acids supplemented to the growth media (5,6).

MATERIALS AND METHODS

Cells. The cells used in this study were Balb/c3T3 (clone A31) and SV40-transformed Balb/c3T3 cells (clone SVT2) from Aaronson (7). Cells were supplied by Dr. P.H. Black (Mass. General Hospital, Harvard Medical School, Boston) and routinely subcultivated in our laboratory under the conditions described in a previous paper (8).

Growth in manipulated media. Balb/c3T3 cells removed from tissue culture dishes by trypsinization were seeded in 10 mm Falcon dishes at $1x10^6$ cells per dish and grown for 3 days in MEMx4 supplemented with 10% fetal calf serum (FCS). The medium was then discarded and the cells were grown for 24 hours in the presence of MEMx4 supplemented with 10% delipidated FCS (DFCS) or MEMx4 supplemented with 10%DFCS enriched with each of the following phosphatidylcholines (PCs) : dipalmitoyl, palmitoyl-oleoyl, oleoyl-palmitoyl, palmitoyl--linoleoyl, dioleoyl, oleoyl-linoleoyl or palmitoyl-arachidonoyl. 3T3 cells grown for the entire 4-day period of culture in MEMx4 supplemented with 10% FCS were used as controls. In each experiment, SV3T3 cells grown in MEMx4 plus 10%FCS served both as a standard of agglutinability and as a measure of the modified agglutinability of 3T3 cells grown in manipulated media. All the cells were harvested by incubation in a 0.02% solution of EGTA in PBS, washed three times in PBS and diluted at $1x10^6$ cells/ml.

Agglutination assay. Con A (Con A type III from Sigma) was purified before use on Sephadex G-50 columns according to Agrawal and Goldstein (9). Aliquots of Con A solutions in 0.14 M NaCl at different concentrations (10-2500 μg/ml) were mixed with equal volumes of each cell suspension and incubated at 37°C for 30 min. In order to determine the degree of spontaneous agglutination and/or aggregation, each cell suspension was also incubated in the presence of NaCl 0.14 M solution without Con A.After incubation, cell suspensions were transferred onto microscope depression slides and agglutination was examined under the microscope. The degree of agglutination was calculated by comparing the number of non-agglutinated cells in the suspensions with and without Con A, respectively, and expressing this ratio as a percentage.

Preparation of DFCS-phosphatidylcholine complexes. The various PCs used in this study were prepared in our laboratory according to the procedure of Cubero-Robles and van Den Bergh (10). Delipidation of

FCS was obtained by multiple extractions in ethanol and diethyl ether according to the Scanu and Edelstein procedure reported by Horwitz and coll.(6).DFCS-PC complexes were prepared by mixing the dry powder derived from the delipidation of 10 ml of FCS with each phosphatidylcholine (0.2 mg) and cholesterol (0.1 mg), both in chloroform solution. Cholesterol was added so as to prevent the sterol depletion which may occur in cells incubated in the presence of phospholipid only (11). The mixture was stirred for a few minutes and, after the evaporation of the solvent, was diluted in bidistilled water and sonicated until a complete clarification of the suspension was obtained. The solution of the DFCS-PC complexes was then adjusted to restore the original volume of FCS.

Lipid analysis. Phospholipids of the sonicated cell suspensions were fractionated by TLC and the fatty acid methyl esters of PC and PE were submitted to gas-chromatographic analysis (8)in order to determine whether cells incorporated the various PCs and to evaluate the effects induced by exposure to DFCS on PC and PE fatty acid compositions.

RESULTS. The fatty acid compositions of PC and PE of Balb/c3T3 cells grown in regular media or exposed to media supplemented with DFCS or with DFCS-PC complexes are reported in Table 1. Compared to cells grown in media supplemented with FCS, Balb/c3T3 cells exposed to DFCS showed fatty acid changes in PC and PE (decrease of 20:4 and increase of 18:1 and 20:3 n-9) that are characteristic of EFA-deficiency (12).The rise of endogenous 18:1 due to exposure to DFCS probably interfered with the change in 18:1 level reflecting incorporation of certain exogenous oleoyl-containing PCs which were present in the growth media as DFCS complexes. However, the overall pattern of acyl chain changes found in Balb/c3T3 cells exposed to the different PCs is consistent with a certain degree of phospholipid incorporation into the cells. Moreover, as can be inferred from the data reported in Table 1, only in part PCs were incorporated into the cells as intact molecules, while a rather conspicuous amount was probably submitted to remodelling reactions (e.g. base exchange (13), deacylation-reacylation (14)), or, as in the case of palmitoyl-arachidonoyl-PC, underwent hydrolysis perhaps followed by degradation or utilization of the acyl group.

The effects on Con A-mediated agglutinability of Balb/c3T3 cells induced by exposure to media supplemented with DFCS or with DFCS-PC

TABLE 1 - Fatty acid composition of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from Balb/c3T3 cells grown in regular media (MEMx4+FCS) or exposed to media supplemented with delipidated FCS (DFCS) or with different DFCS-phosphatidylcholine (PC) complexes.

	% (by weight) of total fatty acids							
	16:0	16:1	18:0	18:1	18:2	20:3 9	20:4	22:5,6
				MEMx4+FCS				
PC	33.2	5.6	19.3	21.9	1.3	-	16.4	2.3
PE	13.8	3.7	26.2	14.0	1.3	-	24.5	16.5
				MEMx4+DFCS				
PC	43.4	6.6	11.7	29.0	1.5	0.1	5.2	2.5
PE	17.0	3.4	18.5	22.1	1.3	3.0	17.8	16.9
				MEMx4+DFCS-dipalmitoyl-PC				
PC	33.6	14.0	10.9	33.3	1.6	0.6	3.3	2.7
PE	16.8	5.9	19.0	27.3	1.8	5.2	15.3	8.7
				MEMx4+DFCS-palmitoyl-oleoyl-PC				
PC	36.0	10.2	10.1	33.9	1.1	1.2	2.1	5.2
PE	16.0	3.6	20.2	24.8	1.8	3.6	19.4	10.5
				MEMx4+DFCS-oleoyl-palmitoyl-PC				
PC	40.7	6.0	9.5	38.0	1.0	-	1.7	3.1
PE	16.1	2.9	19.1	28.5	1.0	0.8	17.6	14.0
				MEMx4+DFCS-dioleoyl-PC				
PC	33.3	9.4	11.3	38.8	2.1	1.0	3.8	1.4
PE	13.2	4.3	18.8	28.5	1.7	3.9	18.3	11.3
				MEMx4+DFCS-palmitoyl-linoleoyl-PC				
PC	43.8	10.4	8.4	18.6	9.4	0.9	4.4	4.1
PE	19.7	2.1	36.1	17.8	3.8	0.6	12.8	7.1
				MEMx4+DFCS-oleoyl-linoleoyl-PC				
PC	40.8	5.6	10.0	19.5	13.6	-	10.5	-
PE	22.3	4.7	16.1	15.8	5.8	-	19.8	15.6
				MEMx4+DFCS-palmitoyl-arachidonoyl-PC				
PC	60.6	5.3	14.8	12.7	0.5	-	5.7	0.4
PE	39.9	7.7	18.4	20.8	3.1	-	9.6	0.5

complexes are reported in Table 2. Balb/c3T3 cells exposed to DFCS exhibited a rather slight increase of agglutinability compared to cells grown in the presence of FCS for the entire 4-day period of culture. Exposure to dipalmitoyl-, palmitoyl-oleoyl- and oleoyl-palmitoyl-PC did not affect agglutinability of Balb/c3T3 cells. On the other hand, when Balb/c3T3 cells were exposed for 24 hours to media supplemented with dioleoyl-PC, they became agglutinable, even at the lowest concentrations of Con A. The degree of agglutinability of these cells was comparable to that shown by SV3T3 cells grown in regular media. Exposure to oleoyl-linoleoyl-PC, as well as to palmitoyl-linoleoyl-PC and palmitoyl-arachidonoyl-PC had also no effect on the Con A-mediated agglutinability of Balb/c3T3 cells.

DISCUSSION. The major finding of this study is that the agglutinability of Balb/c3T3 cells is increased only by incorporation of dioleoyl-PC.This effect is rather specific because the PCs used, even those with some similarity with dioleoyl-PC(e.g. oleoyl-linoleoyl--PC),do not produce changes in the agglutinability of Balb/c3T3 cells. In this light, it is possible to explain why Horwitz and coll. (8) found only minimal differences in agglutinability in Swiss 3T3 cells,both normal and transformed, grown in the presence of DFCS plus oleic acid bound to albumin. In fact, by using this procedure to manipulate fatty acid composition, the incorporated oleic acid may have been utilized for the synthesis of asymmetric phosphatidylcholines (e.g. palmitoyl-oleoyl-PC), which as shown in the present study, did not affect agglutinability by lectins. The specific effect of dioleoyl-PC on sensitivity of Balb/c3T3 cells to Con A is of particular interest in view of the abundance of this molecular species in different types of neoplastic cells (15,16). As to the mechanism by which dioleoyl-PC increased the sensitivity of Balb/c3T3 cells to Con A, it is possible to postulate that this particular PC may have perturbated the lipid environment of Con A receptors leading to their rearrangement so as to facilitate agglutination. It is worth noting that,in artificial model, Demel and coll.(17) found a lesser con-

TABLE 2 - Concanavalin A-mediated agglutinability of Balb/c3T3 cells grown in regular media (MEMx4+FCS) or exposed to media supplemented with delipidated FCS (DFCS) or with different DFCS-phosphatidylcholine (PC) complexes[+].

Media	Concanavalin A concentration(μg/ml)in the assay suspension					
	5	15	25	75	150	1250
MEMx4+FCS	0	0	0	0	0	+
MEMx4+DFCS	0	0	0	0	+	+
MEMx4+DFCS-dipalmitoyl-PC	0	0	0	0	0	+
MEMx4+DFCS-palmitoyl-oleoyl-PC	0	0	0	0	0	+
MEMx4+DFCS-oleoyl-palmitoyl-PC	0	0	0	0	0	+
MEMx4+DFCS-dioleoyl-PC	+++	+++	+++	+++	+++	+++
MEMx4+DFCS-palmitoyl-linoleoyl-PC	0	0	0	0	0	+
MEMx4+DFCS-oleoyl-linoleoyl-PC	0	0	0	0	0	0
MEMx4+DFCSpalmitoyl-arachidonoyl-PC	0	0	0	0	0	0
SV3T3 cells grown in MEMx4+FCS	++++	++++	++++	++++	++++	++++

[+] The degree of agglutination is indicated by the symbols 0, +, ++, +++, ++++, which correspond to 100, 100-75, 75-50, 50-25 and 25-0 per cent of the non-agglutinated cells (See Materials and Methods).

densing effect of cholesterol on dioleoyl phospholipids than on phospholipids composed of one oleoyl and one saturated chain. Enrichment of dioleoyl-PC in plasma membranes may also have affected the cytoskeleton-dependent transmembrane control on mobility of Con A receptors (18).

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