INVESTIGATION OF THE ACTION OF SOME PHOSPHOLIPIDS AND GANGLIOSIDES ON SENSITIVITY OF TUMOR CELLS TO THE CYTOSTATIC AND MEMBRANE-TOXIC ACTION OF SPLENIC EFFECTORS

I. V. Bogdashin, V. I. Shvets,
Yu. M. Krasnopol'skii, and B. B. Fuks

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Sensitivity of tumor cells to cytotoxic T lymphocytes has been shown to depend on the properties of the phospholipids and also on the original surface charge of the membrane [9]. Disturbance of biosynthesis of gangliosides [8] and of the properties of their ceramide moiety [7] is of great importance for sensitivity of tumor cells to natural killer cells.

The aim of this investigation was to determine which parts of the lipid molecule are most responsible for the increase in sensitivity of tumor cells to the membrane-toxic and cytostatic action of natural splenic effectors.

EXPERIMENTAL METHOD

Characteristics of the lipids used are given in Table 1. Liposomes were obtained with the aid of the UZDG-1 ultrasonic apparatus, as described previously [4]. Target cells (TC) were treated with a solution of gangliosides and (or) with liposomes containing phospholipids for 2 h at 37°C in medium RPMI-1640 with 10% fetal serum and 100 U/ml of penicillin and streptomycin. They were then washed three times with medium 199 with 10% bovine serum. According to [5], under those conditions lipids are incorporated into the cell membrane. The membrane-toxic test was carried out as described previously [1]. To test the cytostatic action of the splenocytes, a modified method [6] was used. Splenocytes from BALB/c mice, treated beforehand with actinomycin D to prevent incorporation of ^{3}H-uridine, were incubated with tumor cells of a P-815 mastocytoma of DBA mice for 4 h in 96-wall plates in medium RPMI-1640 with 10% fetal serum and 1 mM glutamine. Next, either ^{3}H-uridine or ^{3}H-thymidine (in a final concentration of 1 μCi per well) was added to each well. After 4 h the cells were transferred by means of a 12-channel harvester to "Flow" No. 1203 filters. Incorpora-

TABLE 1. Characteristics of Lipids Used

Lipids	Source	Fatty acids
Phosphatidylcholine	Hens' eggs	Position 1 — 16:0 — 70%; 18:0 — 24%; 18:1 — 5% Position 2 — 18:1 — 61%; 18:2 — 31%; 20:4 — 4%
Phosphatidylethanolamine	" "	Position 1 — 16:0 — 27,3%; 18:0 — 37,4%; 18:1 — 23,3% Position 2 — 18:1 — 40,7%; 18:2 — 22,4%; 20:4 — 13%
Cardiolipin	Bovine heart muscle	Position 1 — 18:1 — 9%; 18:2 — 84% Position 2 — 18:1 — 5,5%; 18:2 — 82%; 18:3—12%
1,2-Dipalmitoyl-glycero-3-phosphocholine (from Serva, West Germany)	Synthetic	Position 1 — 16:0 Position 2 — 16:0
Mixture of gangliosides (from Serva)	Bovine brain	14:0 — 0,5%; 16:0 — 3,1%; 18:0 — 88,6%

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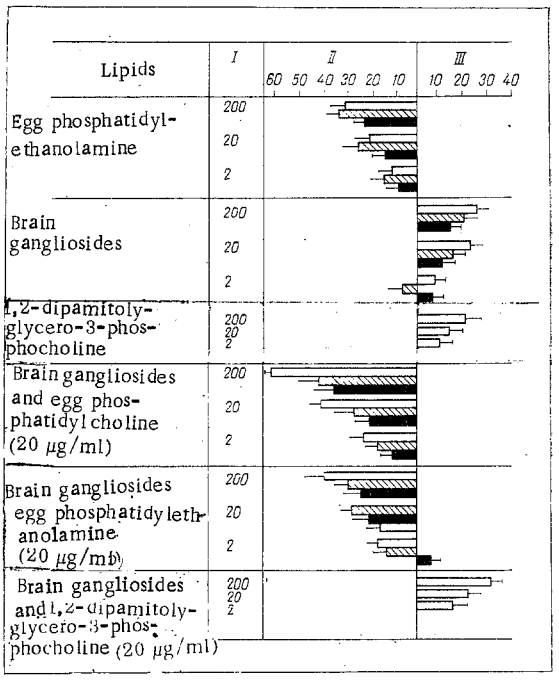

Fig. 1. Effect of lipids on sensitivity of target cells to membrane-toxic and cytostatic action of splenocytes. I) Concentration (in μg/mg), II) increase (in %), III) decrease (in %). Unshaded columns – membrane-toxicity, obliquely shaded – cytostasis with respect to RNA synthesis, black – cytostasis with respect to DNA synthesis.

tion of ^{3}H-thymidine and ^{3}H-uridine into TC was estimated on a Packard 3320 beta-counter. The cytostatic index (CI, in %) was calculated by the equation

$$CI = \left(1 - \frac{\text{counts/min in experimental wells} - \text{counts/min in wells with splenocytes}}{\text{counts/min wells with TC}}\right) \cdot 100.$$

In control wells splenocytes alone or TC alone were incubated with labeled nucleotides.

EXPERIMENTAL RESULTS

As will be clear from Fig. 1, treatment of TC with phosphatidylethanolamine caused an increase in their sensitivity in the membrane-toxic test with concentrations of lipid of 200 and 20 μg/ml. With these same concentrations an increase in sensitivity of the tumor cells also was observed in the cytostatic test. Cardiolipin and phosphatidylcholine increased the sensitivity of TC to splenic effectors neither in the membrane-toxic nor in the cytostatic test (data not given). Treatment of TC with preparations of brain gangliosides led to a marked decrease in their sensitivity to splenocytes. Dipalmitoylphosphatidylcholine reduced the sensity of TC.

It will be clear from Fig. 1 that the combinations of egg phosphatidylcholine – brain gangliosides used led to a marked increase in sensitivity of TC relative to effector cells. This effect depended on the dose of gangliosides.

The combination phosphatidylethanolamine – brain gangliosides increased the sensitivity of TC to splenocytes in the membrane-toxic test by a lesser degree than the combination phosphatidylcholine – brain gangliosides. In cytostatic tests the increase in sensitivity of TC when the combination phosphatidylethanolamine – gangliosides was used was less marked than when phosphatidylethanolamine alone was used. Treatment of TC with dipalmitoylphosphatidylcholine and brain gangliosides led to a greater decrease in sensitivity to splenic effectors than after treatment with dipalmitoylphosphatidylcholine alone.

The experiments thus showed that ovolecithin alone, introduced into the membrane of the tumor TC, did not affect its sensitivity to the cytostatic and membrane-toxic action of splenic effectors, whereas a mixture of brain gangliosides actually reduced the sensitivity of TC. Introduction of ovolecithin and a mixture of brain

gangliosides into the TC membrane significantly increased the sensitivity of TC to the cytostatic and membrane-toxic action of the effector cells.

It was shown previously [2] that brain gangliosides depress, whereas thymus gangliosides increase the sensitivity of tumor cells to the membrane-toxic action of splenic effectors. Further analysis [3] showed that the increase in sensitivity was mainly due to introduction of unsaturated fatty acids into the membrane, and partly to the difference in structure of the polar heads of the gangliosides. It can be postulated that the increase in sensitivity of the tumor cell in the present investigation to the cytostatic and membrane-toxic action of splenic effectors also was mainly due to a change in its membrane under the influence of unsaturated fatty acids of egg phosphatidylcholine, and that the properties of the carbohydrate heads of the brain gangliosides, inserted into the TC membrane, are the second essential condition for the action of effectors on TC. This is shown by the fact that treatment of TC with liposomes of dipalmitoylphosphatidylcholine,i.e., a phospholipid not containing unsaturated fatty acids, led to a decrease in sensitivity of TC. When the combination dipalmitoylphosphatidylcholine–brain gangliosides was used, an even more marked decrease in sensitivity of the tumor cell to the membrane-toxic action of splenocytes was observed.

It has been shown [9] that an increase in the content of phosphatidylcholine and sphingomyelin in the tumor cell membrane is accompanied by increase in the sensitivity of TC. However, the authors cited do not study changes in the fatty acid composition of phospholipids. There is evidence [8] that the concentration of certain gangliosides (GM_1, GD_{1a}, GD_{1b}, and GT) in the TC membrane correlates positively with its sensitivity to normal killer (NK) cells. The authors cited consider that these cells offer an alternative pathway of ganglioside synthesis. A contradiction exists between these data and the results obtained by other workers, who found that an increase in the sialic acid concentration on the surface of TC increases its resistance to NK cells. The ceramide part of the molecule was not studied by these workers. In [7] attention is drawn to a connection between the sensitivity of the tumor cell to NK cells and ceramide parts of the glycophingolipids of the tumor cell membrane, but fatty acids in the ceramides were not analyzed.

For further analysis of the role of the properties of tumor cell membrane phospholipids and the role of structure of the polar heads of the gangliosides in interaction between TC and effector cells, a more penetrating physicochemical analysis of membranes of the effector cells and targets on the same models is necessary.

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