COMPARATIVE ANALYSIS OF THE ACTION OF THYMUS AND BRAIN GD_3 GANGLIOSIDES ON TUMOR CELL SENSITIVITY TO NATURAL SPLENIC EFFECTORS

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The writers showed previously that incorporation of thymus gangliosides GM_3 and GD_3, and also of lactoside-ceramide in the membrane of tumor cells significantly enhances their sensitivity to the membrane-toxic action of natural splenic effectors [5]. The greatest effect was given by thymus ganglioside GD_3.

Later work showed that the group of glycosphingolipids of brain origin gives the directly opposite effect [4]. However, they did not contain gangliosides GD_3 and GM_3 of brain origin. It was therefore not clear whether the differences in the action of brain and thymus glycosphingolipids on tumor cells are connected with the structure of their carbohydrates or of their ceramide moiety.

The aim of the present investigation was to shed light on this problem by comparing the action of gangliosides GD_3 of thymus and brain origin.

EXPERIMENTAL METHOD

Ganglioside GD_3 was isolated from the brain of a patient with Tay-Sachs disease [7]. It was investigated by ascending thin-layer chromatography on KSK silica-gen with particle size of 5-10 μ. As the solvent, a mixture of chloroform—methanol—2.5 N NaOH (in the ratio of 60:35:9) was used. Other thymus and brain glycosphingolipids (Fig. 1) were isolated in Professor L. D. Bergel'son's Laboratory (Institute of Bioorganic Chemistry, Academy of Sciences of the USSR).

Liposomes were prepared from ovolecithin by sonication on a UZDN-1 ultrasonic generator. Glycosphingolipids were inserted into the tumor cell membrane as described previously [5]. Liposomes from ovolecithin were inserted into the membrane by treatment of P-815 cells with a suspension of liposomes in culture medium (lecithin concentration 200 μg/ml) for 2 h at 37°C, followed by washing 3 times. Lactosideceramide (LacCer) and brain GD_3 or lecithin lysosomes and brain GD_3 were incorporated into the target cell membrane alternately.

Leukemia YAC cells, maintained by intraperitoneal passage through A/Sn mice were used as target cells. In some experiments mastocytoma P-815 cells, maintained in DBA/2 mice, were the target. Splenocytes from BALB/c and (DBA/2 × BALB/c)F_1 mice were used as effectors.

Membrane-toxicity of the splenic effectors (i.e., reversible increase of membrane permeability of the target cell for ribonuclease molecules) was tested as described previously [3]. Immunologic properties of brain and thymus ganglioside GD_3 were compared by immunoenzyme analysis [6]. Gangliosides were precipitated in wells of flat-bottomed 96-well plates (Flow Laboratories, England) by drying from 96° ethanol in a quantity of 0.05 μg per well. The wells were then treated with a 1% solution of bovine serum albumin (Koch Light, England) and washed 6 times with a 0.05% solution of Tween-20. Next, monoclonal antibodies against brain GD_3, obtained in the writers' laboratory by N. A. Starosvetskaya and M. N. Boltovskaya (clone A3.b1.f8) for 60 min at 37°C, and this was followed by washing 6 times. As the 3rd layer a conjugate of rabbit antibodies against mouse IgG with horseradish peroxidase (Serva, West Germany), with the addition of 1% bovine serum albumin (1 h at 37°C), was used. After

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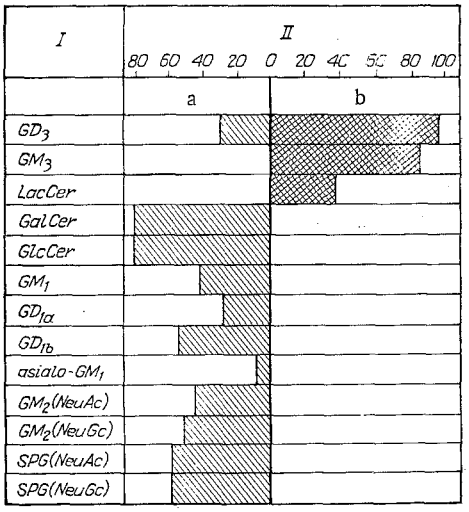

Fig. 1. Action of brain (a) and thymus (b) glycosphingolipids (0.5 μg/ml, 37°C, 20 min) on sensitivity of YAC cells to natural splenic effectors (error of method 10%). I) Glycosphingolipid, II) membrane-toxicy (in %) after treatment of target cells.

incubation, followed by washing 6 times, a 0.08% solution of 5-aminosalicyclic acid was added to the wells. The intensity of the stain was assessed on a Multiscan (Flow Laboratories) instrument.

EXPERIMENTAL RESULTS

Thymus gangliosides and LacCer, incorporated into the target tumor cell membrane, increased its sensitivity to the membrane-toxic action of splenic effectors (Fig. 1). Unlike thymus ganglioside GD_3, ganglioside GD_3 of brain origin (with small traces of gangliosides GM_2, GD_{1B}, and GT_{1B}, significantly reduced the sensitivity of the tumor target cell to splenic effectors. Brain GD_3 thus acts in the same way as certain other brain glycosphingolipids listed in Fig. 1. There are two possible explanations of these facts. First, it can be tentatively suggested that the functional differences are connected with structural differences in the carbohydrate head of the brain and thymus gangliosides GD_3. Second, it can be postulated that functional differences between them can be explained by differences in the ceramide moiety of the molecules.

We know that the carbohydrate heads of thymus and brain GD_3 differ in their acyl and glycosyl radicals. As a first approximation, this may account for their opposite action on tumor cell sensitivity to splenic effectors. In the present experiments thymus and brain gangliosides GD_3 were immunologically indistinguishable. On immunoenzyme assay optical density in wells with brain GD_3, with monoclonal antibodies in a dilution of 1:2, was 0.282 optical density unit (O.d.u.), whereas in a dilution of 1:12 it was 0.147 o.d.u., and the corresponding values for thymus GD_3 were 0.247 and 0.112 o.d.u.

Thymus gangliosides and LacCer, which differ significantly in the structure of their carbohydrate head, increased the sensitivity of the tumor cell to splenic effectors.

The above facts suggested that the sensitizing action of thymus GD_3 and the inhibitory action of brain GD_3 are connected with differences in the structure of their ceramide moiety. The two components of ceramide, namely sphingosine and fatty acid, are known to vary in their number of carbon atoms and their degree of saturation [2]. The content of unsaturated fatty acids falls with an increase in the phylogenetic age of the animals [1].

To test our hypothesis experiments were carried out, which showed that insertion of brain GD_3 together with thymus LacCer into the tumor cell membrane caused, not a decrease, but an increase in tumor cell sensitivity to the splenic effectors (Table 1). This could be connected with the properties of the fatty acids or sphingosine in thymus LacCer. Experiments with

TABLE 1. Enhancement of Sensitivity of P-815 Tumor Cells to Splenic Effectors from (DBA/2 × BALB/c)F_1 Mice after Insertion of Brain Ganglioside GD_3, in Combination with Thymus GD_3 or Ovolecithin, into Tumor Cell Membrane

Treatment of P-815 cells with lipids	Index of membrane-toxicity, %		Increase in sensitivity of tumor cells	
	effectors/target ratio			
	50/1	100/1	50/1	100/1
Not treated	37	60	—	—
Lecithin liposomes	47±14	66±1,6	32	13
Brain GD_3 and lecithin liposomes	71±12	90±1,7	86	50
LacCer	53±2,1	68±1,0	27	15
Brain GD_3 and LacCer	67±0,8	85±1,0	83	45

incorporation of brain GD_3 in combination with egg phosphatidyl choline showed that fatty acids (in this case, oleic and linolenic) were responsible for this change in the lipid matrix of the tumor cell membrane, whereby its sensitivity to splenic effectors was enhanced. In these experiments the carbohydrate head of GD_3, and not only LacCer and ovolecithin, was responsible for increased sensitivity of the tumor cell to the effectors.

For the reasons given above, a more penetrating investigation of the function of thymus glycosphingolipids and of the role of their carbohydrate and ceramide moieties in the immune system is indicated.

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