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SELECTIVE GANGLIOSIDE SHEDDING FROM MOUSE ASCITES SARCOMA 37 CELLS

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Glycolipids, which are specific components of plasma membranes, undergo qualitative and quantitative changes during malignant transformation of cells. Under these circumstances, in particular, certain types of glycolipids such as G_{D3} of human melanoma [11], ceramide-trihexoside of Burkitt's lymphoma [10], fucose-containing monosialogangliosides in neoplasms of the gastrointestinal tract [9], ganglioside G_{M2} in neuroblastoma [12], and so on, which are specific markers of neoplasms, accumulate in tumors. We also know that gangliosides have the property of being continuously shed from the surface of tumor cells and certain normal cells into the extracellular space [1, 2, 13], where individual types of gangliosides may accumulate as the result both of shedding of products of "incomplete" synthesis and also of selective shedding of gangliosides by tumor cells.

This paper described the study of the shedding of gangliosides by cells of mouse ascites sarcoma 37.

EXPERIMENTAL METHOD

Experiments were carried out on male SHY/Kv mice weighing about 25 g with an intraperitoneally implanted ascites sarcoma 37. On the 8th day after transplantation of $5 \cdot 10^5$ tumor cells, the ascites fluid was withdrawn. The number of cells in the ascites fluid of carcoma 37 was $7.9 \cdot 10^7$/ml.

All subsequent procedures were carried out at a temperature of 0-4°C. The cell suspension was centrifuged at 800 g for 15 min. The supernatant (S_1) was removed and the cells resuspended in Hanks' solution and centrifuged as described above, to yield a supernatant (S_2) and cells (C_1). Supernatants S_1 and S_2 were centrifuged separately at 150,000g for 60 min. This yielded supernatants S_1' and S_2' and residues R_1 and R_2.

To determine galglioside shedding *in vitro*, carcoma 37 cells were sedimented by centrifugation at 800 g, washed twice as described above, and suspended in Hanks' solution. The number of cell suspensions was $7.7 \cdot 10^7$/ml. The number of viable cells, determined by the trypan blue test, was 85-90%. The cell suspension was incubated, with constant mixing, at 37°C for 60 min and centrifuged at 800g for 15 min, yielding cells (C_2 and supernatant S_3).

Gangliosides were separated from the tumor cells and lyophilized supernatants [6]. To determine the level of lipid-bound sialic acids (LSA) and to identify the gangliosides, micro-

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TABLE 1. Chemical Composition of Sarcoma 37 Cells and of Extracellular Fractions (M ± m)

Fraction	Protein, %	Lipid phosphorus		Lipid-bound sialic acids	
		%	μg/mg protein	%	μg/mg protein
Original sarcoma 37	100	100	—	100	—
C_1	66,2±3,5	87,8±4,8	3,3±0,7	79,9±5,1	0,14±0,05
R_1	0,8±0,2	4,4±0,9	0,76±0,14	1,1±0,4	0,12±0,03
R_2	0,1±0,04	0,5±0,03	0,88±0,23	0,2±0,03	0,08±0,01
C_1'	29,1±1,2	5,9±0,5	0,54±0,19	16,4±1,3	0,07±0,02
S_2'	3,8±0,6	1,4±0,3	0,97±0,35	2,4±0,5	0,07±0,01
Cells C_2	98,2±0,9	99,3±0,4	3,5±0,8	98,4±1,1	0,17±0,06
S_3	1,8±0,9	0,7±0,4	0,36±0,11	1,6±1,1	0,08±0,02

Legend. Mean results of 4-5 experiments shown.

TABLE 2. Ganglioside Profile of Cells and Extracellular Fractions of Sarcoma 37 (M ± m)

Fraction	Gangliosides, % of total						
	I	II	III	IV	V	VI	VII
Homogenate	10,3±1,5	9,1±1,3	36,6±2,1	16,9±2,3	10,5±1,4	14,4±1,5	2,2±1,0
C_1	8,6±1,2	12,1±1,9	32,2±2,4	18,1±2,3	9,2±0,8	17,1±2,2	2,7±0,6
S_1'	5,1±1,2	7,2±1,4	69,8±3,3	12,2±2,0	—	2,5±0,9	3,2±0,9
R_1	3,3±1,0	7,5±2,2	35,8±1,9	14,1±1,8	12,8±2,1	17,0±2,4	9,5±1,1
C_2	7,8±1,5	13,1±2,6	31,3±2,7	17,4±2,0	10,6±1,1	16,7±1,8	3,1±0,8
S_3	2,7±0,9	9,4±1,3	75,2±4,0	10,2±1,7	—	—	2,5±0,6

Legend. Mean results of 3-4 experiments shown.

thin-layer chromatography on silica-gel by the method in [3] was used. Animal gangliosides of known structures were used as standards. The relative content of the components in the mixture of gangliosides was determined on a Chromoscan-200 scanning densitometer (Joyce-Loebl, Great Britain). Treatment of the gangliosides with neuraminidase from NAG vibrio (activity 800 units/ml) was done by the method in [4]. Lipid phosphorus was determined by the method in [5] and protein by Lowry's method [8].

EXPERIMENTAL RESULTS

The chemical composition of the principal fractions obtained by centrifugation of the sarcoma 37 cells is given in Table 1. One-fifth of the gangliosides of sarcoma 37 and one-third of its protein are contained in the extracellular medium. Both the absolute and the relative ganglioside content in residue R_1 was very small. This means that ganglioside shedding with fragments of plasma membranes takes place only to a very limited degree in this tumor. Most of the extracellular gangliosides (about 19% of the total content in the tumor) are concentrated in fractions S_1' and S_2', i.e., they are bound with water-soluble proteins. During incubation of carcoma 37 cells *in vitro* only about 2% of the protein and gangliosides is shed.

The ganglioside profiles of supernatants S_1' and S_3 were very similar and differed considerably from the ganglioside profiles of the tumor cells C_1 and C_2 and the residue R_1 of high-speed centrifugation (Table 2). Gangliosides of the homogenate, cells C_1, and residues R_1 contained 31-37% of a component with chromatographic mobility similar to that of G_{M2} (fraction III), but components corresponding to NeuAc-G_{M3}, NeuGc-G_{M3}, G_{D3}, and G_{D2} (fractions I, II, IV, V, and VI) also were present in considerable quantities, as well as G_{D1b} (fraction VII) in residue R_1. By the action of neuraminidase on the gangliosides in C_2 fractions corresponding in chromatographic mobility to G_{M2} and B_{M1} were obtained in the ratio of 5:1. Fractions S_1' and S_3 were characterized by considerable simplification of their ganglioside composition: G_{M2} accounted for 70-75% of the total, whereas the remaining components either were not found or were present in very small quantities.

The experiments thus showed that the composition of the gangliosides shed in the form of complexes with water-soluble proteins differs considerably from the composition of the intracellular gangliosides of sarcoma 37, and also from that of gangliosides shed with fragments of plasma membranes. Shedding of gangliosides by sarcoma 37 cells *in vitro* takes place only to a very limited degree, but the composition of the extracellular gangliosides under these circumstances agreed completely with that observed during shedding *in vivo*. It can be concluded from the facts described above that accumulation of ganglioside G_{M2} in the extracellular space is the result of its selective shedding by ascites sarcoma 37 cells and is not due to shedding products of "incomplete" synthesis of the intracellular gangliosides. It was postulated previously [7] that gangliosides shed by tumor cells may participate in the defense of these cells against immune surveillance by the host. Further investigations are needed to solve the problem of the species-specificity of the effect of shed gangliosides on the cytotoxic activity of the effector cells of the tumor carrier.

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