Serum gangliosides in mice with metastatic and non-metastatic brain tumors

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Abstract The content of serum gangliosides was examined in VM and C57BL/6J (B6) mice that contained subcutaneous metastatic (VM) and non-metastatic (CT-2A) brain tumors, respectively. Gas-liquid chromatography (GLC) and high performance thin-layer chromatography (HPTLC) were used to analyze the serum gangliosides. N-glycolylneuraminic acid (NeuGc) accounted for greater than 90% of the total serum sialic acid content in each mouse strain (5.53 nmol and 2.05 nmol per ml serum, respectively). G_{M2}-NeuGc was the major serum ganglioside detectable in both the normal and tumor-bearing mice of each strain. Shedding of tumor gangliosides into the serum occurs in various murine non-neural tumors and in human gliomas and neuroblastomas, but has not been previously studied in murine brain tumors. Our results show that serum ganglioside concentration was reduced in VM mice bearing the metastatic VM tumor, but was increased in B6 mice bearing the non-metastatic CT-2A tumor. These changes in concentration, however, were not associated with marked changes in serum ganglioside distribution. As serum gangliosides are synthesized in the liver, the differences in serum ganglioside concentration in the tumor-bearing mice may arise more from changes in liver function than from differences in tumor shedding.—**Cotterchio, M., and T. N. Seyfried.** Serum gangliosides in mice with metastatic and non-metastatic brain tumors. *J. Lipid Res.* 1994. **35:** 10–14.

Supplementary key words G_{M2}-NeuGc • tumor metastasis • ganglioside shedding

Gangliosides are sialic acid-containing glycosphingolipids that are most heavily concentrated in neuronal membranes of the central nervous system (CNS). Much lower ganglioside concentrations are found in membranes of non-neural cells (1) and in mammalian serum, where the gangliosides are bound to lipoproteins (2–5). Most gangliosides in normal serum are synthesized in the liver and are secreted into the serum (6–8). Using a gas–liquid chromatographic procedure for sialic acid quantitation, Yu and Ledeen (9) found 11.3 nmol, 13.1 nmol, and 2.8 nmol of ganglioside sialic acid per ml of plasma in human, bovine, and rabbit, respectively. NeuAc was the predominant sialic acid species in human and rabbit, whereas bovine plasma gangliosides contained equal amounts of NeuAc and NeuGc sialic acids. The NeuGc and NeuAc sialic acids differ structurally in that the former has a glycol group attached to the nitrogen on carbon 5, whereas the latter has an acetyl group in that position (10). Although differences in serum ganglioside concentration occur among different mouse strains (11–13), the distribution of NeuAc and NeuGc sialic acid in mouse serum has not been previously described to our knowledge.

The shedding of glycolipids from tumor cells into serum may facilitate tumor metastasis and progression by altering host immune responses (14–16). Ganglioside shedding into serum was observed previously in humans with malignant gliomas, astrocytomas, neuroblastomas, melanomas, and head carcinomas (17–22). Ganglioside shedding has not been previously studied in murine brain tumors, but it has been described in various murine non-neural tumors (11, 13, 15, 23, 24).

In this study, we examined the composition of serum gangliosides in mice with metastatic and non-metastatic brain tumors that were grown subcutaneously in the flank. The metastatic VM tumor arose spontaneously as an intracranial mass in a 425-day-old mouse in our VM colony. Although the expression of spontaneous CNS tumors in the VM mouse strain is relatively high, i.e., 1.5% (25), ours is the only known brain tumor that can readily metastasize to multiple organ systems including brain, spinal cord, lung, spleen, kidney, and liver after subcutaneous innoculation of tumor tissue. This tumor was provisionally classified as a primary malignant lymphoma, but its origin as a primitive neuroectodermal tumor could not be excluded (26). The non-metastatic tumor, designated CT-2A, was generated by implantation of the chemical carcinogen, 20-methylcholanthrene (20-MC) into the brain of a B6 mouse. This tumor was provisionally classified as a highly malignant, poorly differentiated anaplastic astrocytoma based on histology

Abbreviations: GLC, gas–liquid chromatography; HPTLC, high performance thin-layer chromatography; NeuGc, N-glycolylneuraminic acid; 20-MC, 20-methylcholanthrene.

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(27). Similar to most 20-MC-induced brain tumors, the CT-2A tumor grows only in the subcutaneous area of inoculation and is not metastatic. Our results show that serum ganglioside concentration is markedly reduced in mice with the metastatic VM tumor, but is elevated in mice with the non-metastatic CT-2A tumor.

METHODS AND MATERIALS

Mice

The B6 strain was obtained from the Jackson Laboratory (Bar Harbor, ME). The VM strain was obtained as a gift from Dr. George Carlson of the McLaughlin Research Institute, Great Falls, MT. All mice were propagated in the Boston College animal care facility as previously described (28). Male and female mice between the ages of 2 months to 3 months were used as tumor recipients.

Tumors

Both the CT-2A and the spontaneous VM brain tumors are maintained in our laboratory via serial, subcutaneous flank passages into the respective B6 or VM mice according to the procedures of Zimmerman and Arnold (29). Tumor tissue was diced and suspended in 0.1 ml phosphate-buffered saline (PBS; pH 7.4) and was then inoculated subcutaneously into the flanks of the VM or B6 mice using an 18-gauge syringe.

Ganglioside analysis

Twenty-three days after innoculation with tumor cells, serum was collected and pooled from mice bearing the CT-2A or VM tumors. Sera from normal B6 and VM mice were used as controls. The gangliosides were isolated and purified from the dried serum by partitioning (30) and ion exchange chromatography as previously described (31, 32). The gangliosides were treated with mild base and desalted using Sephadex G-50 column chromatography (32, 33). The ganglioside sialic acid content of the serum was determined by the GLC method of Yu and Ledeen (34). With this procedure both the NeuAc and NeuGc ganglioside sialic acids can be analyzed simultaneously. The total content of NeuAc and NeuGc was expressed as nmol per ml serum. The distribution of individual serum gangliosides was analyzed using HPTLC (Silica gel 60, Whatman Scientific) and densitometric scanning according to the method of Ando, Chang, and Yu (35). The conditions for the HPTLC are given in Figs. 1 and 2. In addition to the neutral solvent systems in Figs. 1 and 2, we also analyzed serum gangliosides using the ammonia solvent system previously described (27). The HPTLC distribution of the CT-2A and VM serum gangliosides was also compared with that from the respective solid tumors. Gangliosides were isolated from the lyophilized solid tumors as described above and previously (27, 31). The mouse tumor and serum gangliosides were identified by comparison with brain gangliosides purified from beef. G_{M2}-NeuGc was purified from mouse liver and G_{M3}-NeuAc was obtained from Sigma Chemicals Co. (St. Louis, MO).

RESULTS

The total ganglioside sialic acid concentration in normal serum from the VM and B6 mice was approximately 5.53 nmol/ml and 2.05 nmol/ml, respectively (**Table 1**). In both strains, NeuGc accounted for greater than 90% of the serum sialic acid content. G_{M2}-NeuGc was the only major ganglioside detectable in the serum of each control mouse strain using either the neutral solvent system (Fig. 1 and Fig. 2) or the ammonia solvent system (data not shown). This was also the major serum ganglioside in the tumor-bearing mice of each strain.

TABLE 1. Serum ganglioside content in mice with metastatic (VM) and non-metastatic (CT-2A) brain tumors[a]

Serum	Ganglioside Neuraminic Acid Content[b]		
	Total	NeuAc	NeuGc
	nmol/ml serum		
VM mice			
Control	5.53 (5.07, 5.99)	0.48 (0.40, 0.55)	5.04 (4.74, 5.35)
VM tumor[c]	2.96 (2.80, 3.12)	0.39 (0.31, 0.47)	2.57 (2.30, 2.84)
B6 mice			
Control	2.05 (2.07, 2.03)	0.10 (0.10, 0.10)	1.96 (1.95, 1.98)
CT-2A tumor[c]	3.50 (3.20, 3.81)	0.30 (0.20, 0.40)	3.20 (2.90, 3.48)

[a] All brain tumors were grown in the flanks of the mice.
[b] The values represent the means of two independent samples (shown in parentheses). Each sample consisted of serum pooled from approximately 20 mice and was analyzed in duplicate.
[c] Serum was collected 23 days after tumor cell innoculation.

Both the VM and the CT-2A tumors were approximately 3000 mm³ in size at the time of serum collection. The metastatic VM brain tumor caused a 47% reduction in the serum ganglioside content in the host VM strain (Table 1). This reduction was seen for the content of both NeuAc and NeuGc. The reduced serum ganglioside content in mice bearing the metastatic VM tumor was not associated with any noticeable change in the serum ganglioside distribution (**Fig. 1**). In marked contrast to the VM tumor, the non-metastatic CT-2A tumor caused a 42% elevation in the serum ganglioside content in the host B6 strain. This elevation also involved both NeuAc and NeuGc. The elevated serum ganglioside content in these mice was associated with the trace appearance of a new ganglioside that migrated near the region of G_{DIa} (**Fig. 2**). As this minor ganglioside migrated with the major ganglioside in the G_{DIa} region of the CT-2A tumor, it may have been shed from the tumor into the serum. The double band appearance of the G_{M2}-NeuGc in both the VM and CT-2A tumor-bearing mice likely arises from heterogeneity in the ceramide region of the molecule (9, 31). The very small amount of serum gangliosides prevented further structural studies of this ganglioside.

DISCUSSION

The total concentration of serum gangliosides that we found in the VM and the B6 strains (5.53 nmol and 2.05 nmol per ml serum, respectively) was lower than that previously found in other mouse strains, i.e., AKR/J, C3H/HeJ, and Swiss (11 nmol, 15 nmol, and 24 nmol per ml serum, respectively) (11–13). These differences probably result from the different methods used for ganglioside quantitation. We quantitated sialic acid content using GLC analysis, whereas these previous studies employed colorometric analysis, which can be influenced by false chromagens (34). Our serum ganglioside values are similar to those found in rats (8) and other mammalian species using GLC (9). It is also apparent from our studies and those of others that strain differences exist for serum ganglioside sialic acid content among mice.

We found that NeuGc comprised greater than 90% of the ganglioside sialic acid in mouse serum. Furthermore, G_{M2}-NeuGc was the most abundant ganglioside in the serum of both the VM and B6 strains. Recent findings indicate that most gangliosides present in serum are synthesized by the liver and are secreted into the serum (8). Our findings in mice are consistent with the origin of serum gangliosides from the liver, since G_{M2}-NeuGc is the major liver ganglioside in B6 mice and most other mouse strains (36; T. N. Seyfried, unpublished data). It is also interesting that G_{M2}-NeuGc is the major ganglioside in mouse erythrocytes and that the ganglioside pattern in erythrocytes closely parallels that of liver (36). These findings suggest that some erythrocyte gangliosides may be derived from the liver via the serum.

Quantitative differences in serum ganglioside concentration (nmol/ml serum) were observed between mice bearing the metastatic and the non-metastatic brain tumors (Table 1). Most notably, serum ganglioside content was decreased in VM mice bearing the metastatic VM tumor, but was increased in B6 mice bearing the

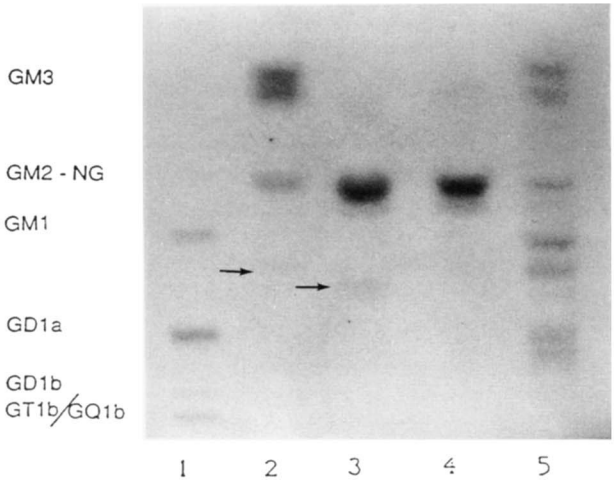

Fig. 1. High performance thin-layer chromatogram of gangliosides in the serum and the VM tumor of VM mice. Approximately 2.0 μg of gangliosides was spotted for each sample. The plate was developed in one dimension with chloroform–methanol–water 50:45:10 (by volume) that contained 0.02% $CaCl_2 \cdot 2H_2O$. The bands were visualized by the resorcinol spray (39). Lane 1, brain gangliosides purified from beef; lane 2, G_{M2}-NeuGc and G_{M3}-NeuAc standards; lane 3, serum from VM (control) mice; lane 4, serum from VM tumor-bearing mice; lane 5, VM tumor. The arrow indicates a yellow band which is not a ganglioside.

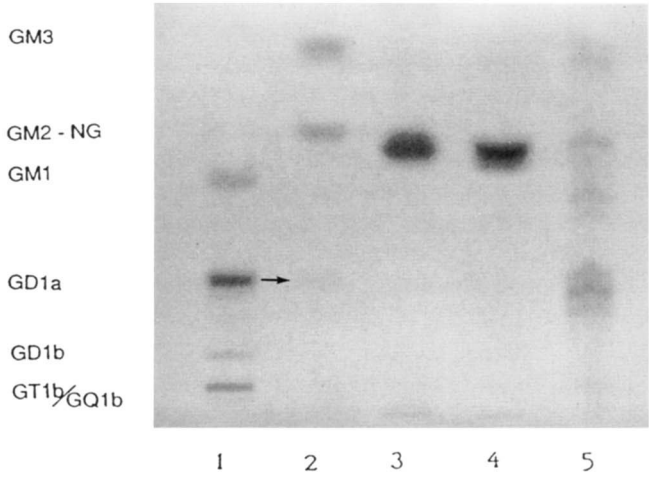

Fig. 2. High performance thin-layer chromatogram of gangliosides in the serum and the CT-2A tumor of B6 mice. Lane 1, brain gangliosides purified from beef; lane 2, G_{M2}-NeuGc and G_{M3}-NeuAc standards; lane 3, serum from B6 (control) mice; lane 4, serum from CT-2A-bearing B6 mice; lane 5, CT-2A tumor. The arrow indicates a yellow band which is not a ganglioside. Other conditions are as described in Fig. 1.

non-metastatic CT-2A tumor. These changes involved both NeuAc- and NeuGc-containing gangliosides. In contrast to the changes in ganglioside concentration, the qualitative distribution of serum gangliosides was not markedly altered in either of the tumor-bearing mouse strains. Trace amounts of serum gangliosides migrating near G_{Dla} were observed in the CT-2A-bearing mice. As this serum ganglioside migrated near a major ganglioside expressed in the CT-2A tumor, it may have been shed from the tumor into the serum. It is important to mention, however, that CT-2A tumor cells grown in vitro express only NeuAc-containing gangliosides and do not express G_{M2}-NeuGc (27). As G_{M2}-NeuGc was the major serum ganglioside expressed in the CT-2A-bearing mice, most of the ganglioside increase obviously involved this ganglioside. It is therefore unlikely that ganglioside shedding from the tumor is solely responsible for the serum ganglioside increase in these mice. Previous studies showed that liver diseases of various etiologies could alter ganglioside composition (6). Hence, an influence of the CT-2A tumor on some aspect of liver function in B6 mice may account for some of the increase in serum ganglioside content in these mice.

The decrease in serum ganglioside concentration in mice with the metastatic VM tumor was unexpected as previous studies in humans suggested an association between increased lipid-bound sialic acid in serum and tumor metastasis (37, 38). We suggest that the reduced serum gangliosides in the tumor-bearing VM mice may result from liver metastasis. The liver in VM tumor-bearing mice becomes heavily infiltrated with metastatic VM tumor cells (26). This could impair liver function and reduce the normal secretion of gangliosides into the serum.

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REFERENCES

1. Ledeen, R. W. 1983. Gangliosides. *In* Handbook of Neurochemistry. Vol. 3. A. Lajtha, editor. Plenum Publishing Corp., New York. 41–90.
2. Chatterjee, S., and P. O. Kwiterovich. 1976. Glycosphingolipids of human plasma lipoproteins. *Lipids.* **11:** 462–466.
3. Dawson, G., A. W. Kruski, and A. M. Scanu. 1976. Distribution of glycosphingolipids in the serum lipoproteins of normal human subjects and patients with hypo- and hyperlipidemias. *J. Lipid Res.* **17:** 125–131.
4. Marcus, D. M. 1984. A review of the immunogenic and immunomodulatory properties of glycosphingolipids. *Mol. Immunol.* **21:** 1083–1091.
5. Senn, H. J., M. Orth, E. Fitzke, J. Scholmerich, H. Wieland, and W. Gerok. 1989. Gangliosides in normal human serum. *Eur. J. Biochem.* **181:** 657–662.
6. Senn, H. J., M. Orth, E. Fitzke, J. Scholmerich, W. Koster, H. Wieland, and W. Gerok. 1990. Altered concentrations, patterns, and distribution in lipoproteins of serum gangliosides in liver diseases of different etiologies. *J. Hepatol.* **11:** 290–296.
7. Kivatinitz, S. C., A. Miglio, and R. Ghidoni. 1991. Ganglioside incorporation and release by the isolated perfused rat liver. *Biochem. J.* **274:** 581–585.
8. Senn, H. S., S. Sellin, E. Fitzke, T. Stehle, D. Haussinger, H. Wieland, and W. Gerok. 1992. Biosynthesis and excretion of gangliosides by the isolated perfused rat liver. *Eur. J. Biochem.* **205:** 809–814.
9. Yu, R. K., and R. W. Ledeen. 1972. Gangliosides of human, bovine, and rabbit plasma. *J. Lipid Res.* **13:** 680–686.
10. Schauer, R. 1985. Sialic acids and their roles as biological masks. *Trends Biochem. Sci.* **10:** 357–360.
11. Kloppel, T. M., T. W. Keenan, M. J. Freeman, and D. J. Morre. 1977. Glycolipid-bound sialic acid in serum: increased levels in mice and humans bearing mammary carcinomas. *Proc. Natl. Acad. Sci. USA.* **74:** 3011–3013.
12. Lengle, E. E. 1979. Increased levels of lipid-bound sialic acid in thymic lymphocytes and plasma from leukemic ARK/J mice. *J. Natl. Cancer Inst.* **62:** 1565–1567.
13. Saha, S., and U. Chattopadhyay. 1988. Changes in plasma gangliosides in relation to tumor growth and their tumor-enhancing effect. *Int. J. Cancer.* **41:** 432–435.
14. Kim, U., A. Baumler, C. Carruthers, and K. Bielat. 1975. Immunological escape mechanism in spontaneously metastasizing mammary tumors. *Proc. Natl. Acad. Sci. USA.* **72:** 1012–1026.
15. Ladisch, S., B. Gillard, D. J. Wong, and L. Ulsh. 1983. Shedding and immunoregulatory activity of YAC-1 lymphoma cell gangliosides. *Cancer Res.* **43:** 3808–3813.
16. Hoon, D. S. B., R. F. Irie, and A. J. Cochran. 1988. Gangliosides from human melanoma immunomodulate response of T cells to IL-2. *Cell Immunol.* **111:** 410–419.
17. Lo, H. S., E. L. Hogan, D. A. Koontz, and T. D. Traylor. 1980. Serum gangliosides in cerebral astrocytoma. *Ann. Neurol.* **8:** 534–538.
18. Ladisch, S., Z. Wu, S. Feig, L. Ulsh, E. Schwartz, G. Floutsis, F. Wiley, C. Lenarsky, and R. Seeger. 1987. Shedding of GD2 ganglioside by human neuroblastoma. *Int. J. Cancer.* **39:** 73–76.
19. Vinzenz, K. E. Schonthal, F. Zekert, and S. Wunderer. 1987. Diagnosis of head and neck carcinomas by means of immunological tumour markers. *J. Cranio-Maxillo-Fac. Surg.* **15:** 270–277.
20. Sela, B. A., D. Iliopoulos, D. Guerry, D. Herlyn, and H. Koprowski. 1989. Levels of disialogangliosides in sera of melanoma patients monitored by sensitive thin-layer chromatography and immunostaining. *J. Natl. Cancer Inst.* **81:** 1489–1492.
21. Valentino, L., T. Moss, E. Olson, H. Wong, R. Eloshoff, and S. Ladisch. 1990. Shed tumor gangliosides and progression of human neuroblastoma. *Blood.* **75:** 1564–1567.
22. Nakamura, O., M. Iwamori, M. Matsutani, and K. Takakura. 1991. Ganglioside GD3 shedding by human gliomas. *Acta Neurochir.* **109:** 34–36.
23. Shaposhnikova, G. I., N. V. Prokazova, G. A. Buznikov, N. D. Zvezdina, N. A. Teplitz, and L. D. Bergelson. 1984. Shedding of gangliosides from tumor cells depends on cell density. *Eur. J. Biochem.* **140:** 567–570.
24. Novikov, A. M., A. M. Kozlov, and L. S. Bassalyk. 1986. Selective ganglioside shedding from mouse ascites sarcoma 37 cells. *Biull. Eksp. Biol. Med.* **102:** 229–231.

25. Fraser, H. 1971. Astrocytomas in an inbred mouse strain. *J. Pathol.* **103:** 266–270.
26. Lee, M. C., M. El-Abbadi, T. N. Seyfried, C. G. Orosz, and A. J. Yates. 1993. Morphological characterization of a new transplantable brain tumor in the VM mouse. *J. Neuropathol. Exp. Neurol.* **52:** 323.
27. Seyfried, T. N., M. El-Abbadi, and M. L. Roy. 1992. Ganglioside distribution in murine neural tumors. *Mol. Chem. Neuropathol.* **17:** 147–167.
28. Flavin, H. J., A. Wieraszako, and T. N. Seyfried. 1991. Enhanced aspartate release from hippocampal slices of epileptic (El) mice. *J. Neurochem.* **56:** 1007–1011.
29. Zimmerman, H. M., and H. Arnold. 1941. Experimental brain tumors. I. Tumors produced with methylcholanthrene. *Cancer Res.* **1:** 919–938.
30. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226:** 497–509.
31. Seyfried, T. N., G. H. Glaser, and R. K. Yu. 1978. Cerebral, cerebellar, and brain stem gangliosides in mice susceptible to audiogenic seizures. *J. Neurochem.* **31:** 21–27.
32. Seyfried, T. N., T. Itoh, G. H. Glaser, N. Miyazawa, and R. K. Yu. 1981. Cerebellar gangliosides and phospholipids in mutant mice with ataxia and epilepsy: the Tottering/Leaning syndrome. *Brain Res.* **216:** 429–436.
33. Ueno, K., S. Ando, and R. K. Yu. 1978. Gangliosides of human, cat, and rabbit spinal cords and cord myelin. *J. Lipid Res.* **19:** 863–871.
34. Yu, R. K., and R. W. Ledeen. 1970. Gas–liquid chromatographic assay of lipid-bound sialic acids: measurement of gangliosides in brain of several species. *J. Lipid Res.* **11:** 506–516.
35. Ando, S., N. C. Chang, and R. K. Yu. 1978. High-performance thin-layer chromatography and densitometric determination of brain ganglioside compositions of several species. *Anal. Biochem.* **89:** 437–450.
36. Nakamura, K., Y. Hashimoto, T. Yamakawa, and A. Suzuki. 1988. Genetic polymorphism of ganglioside expression in mouse organs. *J. Biochem.* **103:** 201–208.
37. Erbil, K. M., J. D. Jones, and G. G. Klee. 1985. Use and limitations of serum total and lipid bound sialic acid concentrations as markers for colorectal cancer. *Cancer.* **55:** 404–409.
38. Plucinsky, M. C., W. M. Riley, J. J. Prorok, and J. A. Alhadeff. 1986. Total and lipid-associated serum sialic acid levels in cancer patients with different primary sites and differing degrees of metastatic involvement. *Cancer.* **58:** 2680–2685.
39. Svennerholm, L. 1957. Quantitative estimation of sialic acids. *Biochem. Biophys. Acta.* **24:** 604–612.