Commentary

O-acetylation of GD3: An Enigmatic Modification Regulating Apoptosis?

Helen Y. Chen and Ajit Varki

Glycobiology Research and Training Center, Department of Medicine and Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA 92093

Glycosphingolipids (GSLs) are amphipathic molecules with a polar glycan chain as a head group and a hydrophobic sphingosine-containing ceramide tail, which is typically embedded in the outer leaflet of the plasma membrane (1). GSLs are no longer thought to be mere physical components of the membrane. They often cluster in "glycosignaling domains" (GSDs; reference 2), sometimes along with other sphingolipids, glycosylphosphatidylinositol (GPI)-anchored proteins, and cholesterol, forming "lipid rafts" (3). These microdomains are thought to regulate signal transduction via cis interactions with signal transducer molecules. A GSL called GD3 has recently been shown to be involved in Fas-mediated apoptosis in hematopoietic cells, causing the loss of mitochondrial potential and the release of apoptotic factors (for reviews, see references 4 and 5). In this issue, Malisan et al. (6) show that a naturally occurring modification of GD3 (O-acetylation) can reverse its apoptotic effects, suggesting a way for cells to avoid the fate of GD3-induced apoptosis. To understand this interesting story better, it is first necessary to review some general information about GD3 and O-acetylation.

Structure, Biosynthesis, and Tissue Distribution of GD3. Gangliosides are GSLs with one or more sialic acid (Sia) residues. GD3 is a ganglioside with two Sias linked to a lactosylceramide core common to many GSLs (see Fig. 1). Sia is a generic name for members of a family of 9-carbon sugars typically found at the termini of glycan chains on vertebrate glycoproteins and glycolipids. Many endogenous or exogenous receptors recognize Sias, mediating or modulating processes such as cell adhesion, differentiation, signal transduction, pathogen invasion, or toxin action (for a review, see reference 7). The biosynthesis of GD3 is traditionally thought to occur in the ER-Golgi pathway, by the sequential addition to ceramide of a glucose, a galactose and two Sia residues, each step being catalyzed by distinct glycosyltransferases (see Fig. 1). The last three of these enzymes have their active sites oriented toward the lumen of Golgi compartments. Such newly synthesized gangliosides are delivered to the outer leaflet of the plasma membrane and eventually turned over via endocytosis and lysosomal degradation (1; see Fig. 1).

GD3 is expressed at high levels in the embryonic brain, with marked decreases during postnatal development (8). In normal adult humans, GD3 is only detectable in a few tissue types (9), and on some T lymphocytes (10). In contrast, GD3 expression is highly elevated in melanomas, neuroblastomas, small cell carcinomas, and certain leukemias (11, 12).

Modified Forms of GD3. The biological specificity of Sias can be modulated by substitutions or modifications at the 1, 4, 5, 7, 8, or 9 positions (7). O-acetyl esters are most commonly found at the 9-carbon position. Such ester groups can first be added at the 7-position and then slowly migrate to the 9-position under physiological conditions (7). The outermost Sia residue of GD3 can be 9(7)-O-acetylated to a varying extent in various cell types. 9-O-Acetyl-GD3 (9AcGD3) was first discovered as a surface marker for germinal cells of the central nervous system (13), and then structurally characterized from human melanoma cells (11). Interestingly, the distribution of GD3 and 9AcGD3 is not identical in some regions of the developing nervous system (13–15). Elimination of 9AcGD3 expression in the retina and adrenals of transgenic mice expressing an Influenza C Sia-specific 9-O-acetylesterase gave variable abnormalities in development (16). While many biological roles for 9AcGD3 have been proposed, the evidence is indirect, consisting of the effects of the viral 9-O-acetylesterase (16, 17), or of anti-9AcGD3 antibodies (18, 19).

Postnatally, 9AcGD3 expression becomes restricted to the retina and cerebellum, and the only nonneural expression known is in the adrenal medulla, kidney glomeruli in rats, and some human lymphoid cell types (9, 10). High expression of 9AcGD3 is also seen in human basal cell carcinomas (20), and in melanomas from many species (21). The less common 7-O-Acetyl-GD3 has been identified in hamster melanomas, and in human T cell lymphocytes (22, 23). In T lymphocytes, AcGD3 species appear to be the epitopes for anti-CD60 antibodies (10), although a similar terminal structure on glycoprotein glycans also contributes (24). Antibodies against GD3 and AcGD3 have been used in melanoma immunotherapy, so far with limited success (25–28).

Address correspondence to Ajit Varki, CMM-E, Rm. 1065, Mail Code 0687, University of California, San Diego, La Jolla, CA 92093-0687. Phone: 858-534-3296; Fax: 858-534-5611; E-mail: avarki@ucsd.edu

J. Exp. Med. © The Rockefeller University Press • 0022-1007/2002/12/1529/5 $5.00
Volume 196, Number 12, December 16, 2002 1529–1533
http://www.jem.org/cgi/doi/10.1084/jem.20021915

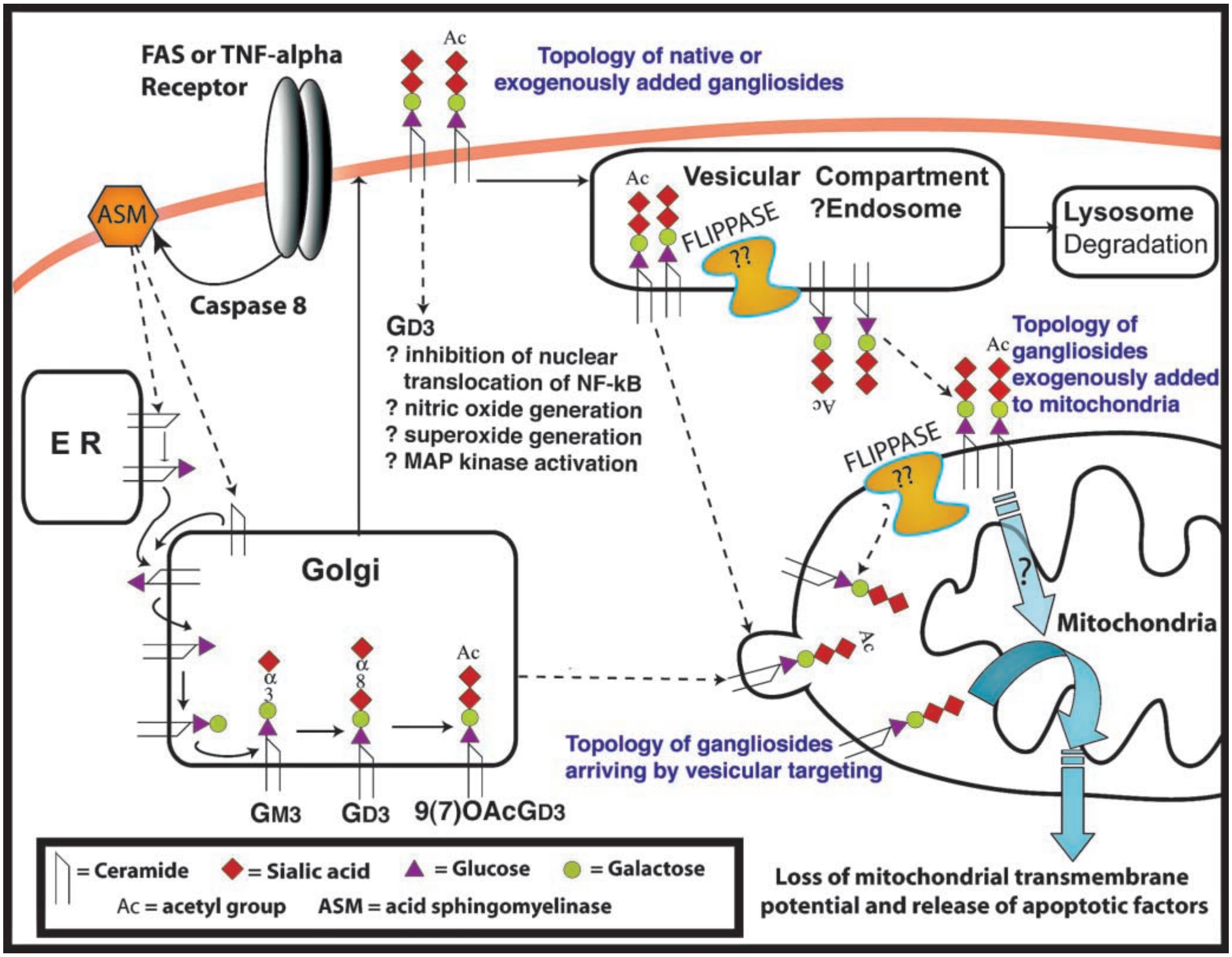

Figure 1. Subcellular compartments potentially involved in the biosynthesis, trafficking, and turnover of GD3 and AcGD3 are shown. Complexities involving 7- versus 9-AcGD3, and the possible action of O-acetylesterases are omitted. Various pathways for GD3-induced apoptosis are indicated, especially in relation to the proposed production and translocation of GD3 to mitochondrial membranes. Some of the unresolved topological issues are emphasized. Topological issues regarding ASM are not shown. See text for further discussion.

GD3-induced Apoptosis. GD3 synthesis is induced during Fas (APO-1/CD95)-mediated apoptosis, and is then thought to mediate the apoptotic effect by accumulating in mitochondria (an unconventional subcellular location for a ganglioside; reference 29). These phenomena are inhibited by blocking GD3 synthase expression, indicating that de novo synthesis of GD3 is required (30). In an apparently related process, apoptotic signaling is thought to occur through Fas-mediated activation of membrane-associated acidic sphingomyelinase (ASM), generating free ceramides (31, 32). It is proposed that these ceramides are converted to GD3 by returning to Golgi-like compartments (see Fig. 1). A similar ASM-GD3 pathway has been recently implicated in TNF-α–mediated apoptosis as well (33, 34). Independent work from others indicates that GD3 can be a component of the apoptotic response in cell types such as neurons (35), aortic smooth muscle cells (36), and keratinocytes (37). In keratinocytes, the structurally related ganglioside GT1b also has a proapoptotic effect, but unlike GD3, works in a fibronectin-dependent manner, apparently involving an integrin-linked kinase (37). In another study involving lymphoblastoid cells, GD3 association with the ezrin cytoskeleton protein was proposed to be involved in apoptosis (38). Also, low doses of GD3 stimulated superoxide generation and MAP kinase activation in human aortic smooth muscle cells (36).

Some of the above studies took advantage of the fact that when pure gangliosides (which are in the form of micelles in aqueous solution) are added to cells or organelles in vitro, they can become incorporated as integral components of the outer leaflet of their membranes (39). Addition of GD3 to intact cells induced apoptosis, and addition to isolated mitochondria gave a loss of mitochondrial transmembrane potential, along with release of apoptogenic factors such as cytochrome c and caspase-9. Gangliosides structurally related to GD3 did not exhibit these effects in most systems (29, 30, 40). Taken together, all these data constitute a strong case for a proapoptotic role for GD3. However, the mechanisms of GD3 action remain obscure, and there are puzzling topological issues (see below). Also, as GD3 is only found in a minority of adult cell types, this may represent a specialized subset of apoptosis control pathways. Indeed, on initial study, GD3 synthase null mice apparently did not exhibit gross developmental pathologies, nor show any differences from wild-type controls in Fas-mediated apoptotic reaction of thymocytes (41).

Is 9-O-acetyl GD3 an Antiapoptotic Factor? If GD3 is expressed in some normal as well as many cancer cells, how do they escape its apoptotic effects? Writing in this issue, Malisan et al. (6) offer an intriguing explanation. As many cells expressing GD3 also have 9AcGD3, the authors postulated that 9-O-acetylation could rescue the cell from GD3-induced apoptosis. Indeed, in striking contrast to GD3, 9AcGD3 did not induce apoptosis when added to intact cells, nor did it affect transmembrane potential, or cause the release of apoptotic factors when added to isolated mitochondria. The effects were restored when 9AcGD3 was chemically de-acetylated back to GD3, showing that the findings were not due to an inhibitory contaminant. De-O-acetylation of endogenous 9AcGD3 in intact cells was also achieved by transfecting cells with the viral 9-O-acetylesterase mentioned above. Cells that either express GD3 synthase endogenously or by transfection became apoptotic when the viral esterase was also present, and this correlated with a reduction in 9AcGD3. The authors conclude that by turning part of pro-apoptotic GD3 into ‘harmless’ 9AcGD3, 9-O-acetylation acts as an effective antiapoptotic mechanism. Together with the action of a putative endogenous 9-O-acetylesterase, an acetylation-deacetylation cycle is suggested as a subtle yet elegant means of regulating the apoptotic potential of GD3. Consistent with this concept is our recent finding that the ganglioside 9-O-acetylation machinery is directly induced by the expression of GD3 synthase (unpublished data). However, there are many unresolved issues.

What About the GD3:9AcGD3 Ratio? A substantial amount of GD3 continues to be present alongside 9AcGD3 in all the situations examined. Also, in our own work using CHO cells (42), expression of GD3 synthase was accompanied by 9-O-acetylation of only a minor fraction of the GD3, and yet no obvious apoptosis was observed. A dominant effect of 9AcGD3 over GD3 could explain such findings. However, mixing experiments by Malisan et al. appear to rule this out (6). It is possible that the ratios of GD3 and 9AcGD3 in whole cell extracts are misleading, and that 9AcGD3 is selectively enriched in a critical cellular subcompartment involved in mediating in proapoptotic effects of GD3. This could be checked by immunoelectron microscopy using the available antibodies. However, our own work with melanoma cells indicated a similar distribution for GD3 and 9AcGD3 in melanoma cells, with a novel intracellular distribution only for another Sia variation called de-N-acetyl GD3 (9).

Other Unresolved Subcellular and Topological Issues. How does the newly synthesized GD3, which is normally found in the ER-Golgi-plasmalemma pathway reach the mitochondria? Perhaps this occurs via vesicular trafficking associated with the cytoskeleton (33, 38), or through the proposed mitochondria-associated membranes bridging to ER/Golgi elements (43). Regardless, as GD3 is synthesized within the lumen of the Golgi and is then embedded in the outer leaflet of the plasma membrane, either of the above delivery systems would cause GD3 incorporation into the inner leaflet of the outer mitochondrial membrane (see Fig. 1). In contrast, the experiments with isolated mitochondria involve added GD3, which should be incorporated with its polar glycan head-group facing outward, in the cytosol-facing leaflet of the outer mitochondrial membrane (see Fig. 1). It is hard to imagine that these two topologically unique forms of GD3 mediate the same biological actions. Thus, one must postulate a “flippase” for gangliosides in the mitochondria and/or in the ER-Golgi-plasmalemma pathway that could transfer the polar headgroup between the two leaflets.

A Receptor for GD3? A more basic question is why GD3 is active in this pathway, but not other gangliosides with closely related structures, including 9AcGD3. This implies the existence of a receptor that specifically recognizes the structure of GD3, including the outer Sia residue that becomes O-acetylated in 9AcGD3. Perhaps this proposed receptor is the same as the putative “flippase” protein? In this regard, it is interesting that there are studies describing “glycolipid transfer proteins” in the cytosol (44, 45). Another potential candidate is Bid, a proapoptotic cytosolic factor of the Bcl-2 family, which is cleaved by FAS-induced caspase 8 (46). Truncated Bid associates with lipid membranes, has an affinity toward acidic phospholipids, and is thought to be involved in membrane lipid transfer to mitochondria (47). It is thought that Bid affects the structural state of multidomain antiapoptotic Bcl-2 proteins in the outer membrane by changing the lipid environment in mitochondria. Perhaps Bid or another Bid-like proapoptotic factor could be the putative GD3 flippase as well? This would fit with the earlier observation that enforced expression of Bcl-2 attenuated GD3-induced apoptosis. Yet another possibility is that a transient de-N-acetylation of GD3 could cause it to associate strongly with phospholipids (48). Such complexes might then be flipped over by the previously well-known phospholipid transfer proteins.

Other Missing Pieces of the Puzzle. In addition to the mechanism of “flipping,” the immediate downstream effectors of GD3 that induce the mitochondrial changes need to be elucidated. Cloning of the putative GD3:9(7)O-acetyl transferase(s) and esterase(s) would also help in understanding the regulation of 9(7)-O-acetylation in relation to GD3 expression in different cell types and within different subcellular compartments. Finally, what about the effects of the less common intermediate form 7AcGD3, which cannot be deacetylated by any known O-acetylesterase? Until a clearer picture emerges regarding all these issues, the biological significance and precise mechanisms of GD3-induced apoptosis remains somewhat of a mystery, and O-acetylation of GD3 remains an enigmatic modification in continued search of definitive functions.

A. Varki is supported by US Public Health Service (USPHS) or National Institutes of Health grant R01-GM32373.

Submitted: 29 October 2002
Revised: 8 November 2002
Accepted: 12 November 2002

References

1. Kolter, T., R.L. Proia, and K. Sandhoff. 2002. Combinatorial ganglioside biosynthesis. *J. Biol. Chem.* 277:25859–25862.
2. Hakomori, S. 2002. The glycosynapse. *Proc. Natl. Acad. Sci. USA.* 99:225–232.
3. Simons, K., and E. Ikonen. 1997. Functional rafts in cell membranes. *Nature.* 387:569–572.
4. Tomassini, B., and R. Testi. 2002. Mitochondria as sensors of sphingolipids. *Biochimie.* 84:123–129.
5. Ferri, K.F., and G. Kroemer. 2001. Organelle-specific initiation of cell death pathways. *Nat. Cell Biol.* 3:E255–E263.
6. Malisan, F., L. Franchi, B. Tomassini, N. Ventura, I. Condo, M.R. Rippo, A. Rufini, L. Liberati, C. Nachtigall, B. Kniep, and R. Testi. 2002. Acetylation suppresses the proapoptotic activity of GD3 ganglioside. *J. Exp. Med.* 196:1535–1541.
7. Angata, T., and A. Varki. 2002. Chemical diversity in the sialic acids and related alpha-keto acids: an evolutionary perspective. *Chem. Rev.* 102:439–470.
8. Yu, R.K., L.J. Macala, T. Taki, H.M. Weinfield, and F.S. Yu. 1988. Developmental changes in ganglioside composition and synthesis in embryonic rat brain. *J. Neurochem.* 50: 1825–1829.
9. Chammas, R., J.L. Sonnenburg, N.E. Watson, T. Tai, M.G. Farquhar, N.M. Varki, and A. Varki. 1999. De-N-acetyl-gangliosides in humans: unusual subcellular distribution of a novel tumor antigen. *Cancer Res.* 59:1337–1346.
10. Kniep, B., W.A. Flegel, H. Northoff, and E.P. Rieber. 1993. CDw60 glycolipid antigens of human leukocytes: Structural characterization and cellular distribution. *Blood.* 82:1776–1786.
11. Cheresh, D.A., R.A. Reisfeld, and A. Varki. 1984. O-Acetylation of disialoganglioside GD3 by human melanoma cells creates a unique antigenic determinant. *Science.* 225:844–846.
12. Merritt, W.D., J.T. Casper, S.J. Lauer, and G.H. Reaman. 1987. Expression of GD3 ganglioside in childhood T-cell lymphoblastic malignancies. *Cancer Res.* 47:1724–1730.
13. Levine, J., L. Beasley, and W. Stallcup. 1984. The D1.1 antigen: a cell surface marker for germinal cells of the central nervous system. *J. Neurosci.* 4:820–831.
14. Constantine-Paton, M., A.S. Blum, R. Mendez-Otero, and C.J. Barnstable. 1986. A cell surface molecule distributed in a dorsoventral gradient in the perinatal rat retina. *Nature.* 324: 459–462.
15. Sparrow, J.R., and C.J. Barnstable. 1988. A gradient molecule in developing rat retina: Expression of 9-O-acetyl GD3 in relation to cell type, developmental age, and GD3 ganglioside. *J. Neurosci.* 21:398–409.
16. Varki, A., F. Hooshmand, S. Diaz, N.M. Varki, and S.M. Hedrick. 1991. Developmental abnormalities in transgenic mice expressing a sialic acid-specific 9-O-acetylesterase. *Cell.* 65:65–74.
17. Birkle, S., S. Ren, A. Slominski, G. Zeng, L. Gao, and R.K. Yu. 1999. Down-regulation of the expression of O-acetyl-GD3 by the O-acetylesterase cDNA in hamster melanoma cells: effects on cellular proliferation, differentiation, and melanogenesis. *J. Neurochem.* 72:954–961.
18. Mendez-Otero, R., and J.E. Friedman. 1996. Role of acetylated gangliosides on neurite extension. *Eur. J. Cell Biol.* 71: 192–198.
19. Santiago, M.F., M. Berredo-Pinho, M.R. Costa, M. Gandra, L.A. Cavalcante, and R. Mendez-Otero. 2001. Expression and function of ganglioside 9-O-acetyl GD3 in postmitotic granule cell development. *Mol. Cell. Neurosci.* 17:488–499.
20. Heidenheim, M., E.R. Hansen, and O. Baadsgaard. 1995. CDw60, which identifies the acetylated form of GD3 gangliosides, is strongly expressed in human basal cell carcinoma. *Br. J. Dermatol.* 133:392–397.
21. Felding-Habermann, B., A. Anders, W.G. Dippold, W.B. Stallcup, and H. Wiegandt. 1988. Melanoma-associated gangliosides in the fish genus Xiphophorus. *Cancer Res.* 48: 3454–3460.
22. Ren, S., T. Ariga, J.N. Scarsdale, Y. Zhang, A. Slominski, P.O. Livingston, G. Ritter, Y. Kushi, and R.K. Yu. 1993. Characterization of a hamster melanoma-associated ganglioside antigen as 7-O-acetylated disialoganglioside GD3. *J. Lipid Res.* 34:1565–1572.
23. Kniep, B., C. Claus, J. Peter-Katalinic, D.A. Monner, W. Dippold, and M. Nimtz. 1995. 7-O-acetyl-GD3 in human T-lymphocytes is detected by a specific T-cell-activating monoclonal antibody. *J. Biol. Chem.* 270:30173–30180.
24. Fox, D.A., X. He, A. Abe, T. Hollander, L.L. Li, L. Kan, A.W. Friedman, Y. Shimizu, J.A. Shayman, and K. Kozarsky. 2001. The T lymphocyte structure CD60 contains a sialylated carbohydrate epitope that is expressed on both gangliosides and glycoproteins. *Immunol. Invest.* 30:67–85.
25. Houghton, A.N., D. Mintzer, C.C. Cordon, S. Welt, B. Fliegel, S. Vadhan, E. Carswell, M.R. Melamed, H.F. Oettgen, and L.J. Old. 1985. Mouse monoclonal IgG3 antibody detecting GD3 ganglioside: a phase I trial in patients with malignant melanoma. *Proc. Natl. Acad. Sci. USA.* 82:1242–1246.
26. Bajorin, D.F., P.B. Chapman, G. Wong, D.G. Coit, J. Kunicka, J. Dimaggio, C. Cordon-Cardo, C. Urmacher, L. Dantes, M.A. Templeton, et al. 1990. Phase I evaluation of a combination of monoclonal antibody R24 and interleukin 2 in patients with metastatic melanoma. *Cancer Res.* 50:7490–7495.
27. Kirkwood, J.M., R.A. Mascari, R.D. Edington, M.S. Rabkin, R.S. Day, T.L. Whiteside, D.R. Vlock, and J.M. Shipe-Spotloe. 2000. Analysis of therapeutic and immunologic effects of R24 anti-GD3 monoclonal antibody in 37 patients with metastatic melanoma. *Cancer.* 88:2693–2702.
28. Ritter, G., E. Ritter-Boosfeld, R. Adluri, M. Calves, S.L. Ren, R.K. Yu, H.F. Oettgen, L.J. Old, and P.O. Livingston. 1995. Analysis of the antibody response to immunization with purified O-acetyl GD3 gangliosides in patients with malignant melanoma. *Int. J. Cancer.* 62:668–672.
29. De, M.R., L. Lenti, F. Malisan, F. D'Agostino, B. Tomassini, A. Zeuner, M.R. Rippo, and R. Testi. 1997. Requirement for GD3 ganglioside in CD95- and ceramide-induced apoptosis. *Science.* 277:1652–1655.
30. Rippo, M.R., F. Malisan, L. Ravagnan, B. Tomassini, I. Condo, P. Costantini, S.A. Susin, A. Rufini, M. Todaro, G. Kroemer, and R. Testi. 2000. GD3 ganglioside directly tar-

gets mitochondria in a bcl-2-controlled fashion. *FASEB J.* 14:2047–2054.

31. Cifone, M.G., M.R. De, P. Roncaioli, M.R. Rippo, M. Azuma, L.L. Lanier, A. Santoni, and R. Testi. 1994. Apoptotic signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. *J. Exp. Med.* 180:1547–1552.
32. De, M.R., M.R. Rippo, E.H. Schuchman, and R. Testi. 1998. Acidic sphingomyelinase (ASM) is necessary for fas-induced GD3 ganglioside accumulation and efficient apoptosis of lymphoid cells. *J. Exp. Med.* 187:897–902.
33. Garcia-Ruiz, C., A. Colell, A. Morales, M. Calvo, C. Enrich, and J.C. Fernandez-Checa. 2002. Trafficking of ganglioside GD3 to mitochondria by tumor necrosis factor-alpha. *J. Biol. Chem.* 277:36443–36448.
34. Colell, A., A. Morales, J.C. Fernandez-Checa, and C. Garcia-Ruiz. 2002. Ceramide generated by acidic sphingomyelinase contributes to tumor necrosis factor-alpha-mediated apoptosis in human colon HT-29 cells through glycosphingolipids formation. Possible role of ganglioside GD3. *FEBS Lett.* 526:135–141.
35. Copani, A., D. Melchiorri, A. Caricasole, F. Martini, P. Sale, R. Carnevale, R. Gradini, M.A. Sortino, L. Lenti, M.R. De, and F. Nicoletti. 2002. Beta-amyloid-induced synthesis of the ganglioside Gd3 is a requisite for cell cycle reactivation and apoptosis in neurons. *J. Neurosci.* 22:3963–3968.
36. Bhunia, A.K., G. Schwarzmann, and S. Chatterjee. 2002. GD3 recruits reactive oxygen species to induce cell proliferation and apoptosis in human aortic smooth muscle cells. *J. Biol. Chem.* 277:16396–16402.
37. Wang, X.Q., P. Sun, and A.S. Paller. 2001. Inhibition of integrin-linked kinase/protein kinase B/Akt signaling: mechanism for ganglioside-induced apoptosis. *J. Biol. Chem.* 276: 44504–44511.
38. Giammarioli, A.M., T. Garofalo, M. Sorice, R. Misasi, L. Gambardella, R. Gradini, S. Fais, A. Pavan, and W. Malorni. 2001. GD3 glycosphingolipid contributes to Fas-mediated apoptosis via association with ezrin cytoskeletal protein. *FEBS Lett.* 506:45–50.
39. Schwarzmann, G., B.P. Hoffmann, J. Schubert, K. Sandhoff, and D. Marsh. 1983. Incorporation of ganglioside analogues into fibroblast cell membranes. A spin-label study. *Biochemistry.* 22:5041–5048.
40. Kristal, B.S., and A.M. Brown. 1999. Apoptogenic ganglioside GD3 directly induces the mitochondrial permeability transition. *J. Biol. Chem.* 274:23169–23175.
41. Okada, M., M. Itoh, M. Haraguchi, T. Okajima, M. Inoue, H. Oishi, Y. Matsuda, T. Iwamoto, T. Kawano, S. Fukumoto, et al. 2002. b-series ganglioside deficiency exhibits no definite changes in the neurogenesis and the sensitivity to Fas-mediated apoptosis but impairs regeneration of the lesioned hypoglossal nerve. *J. Biol. Chem.* 277:1633–1636.
42. Shi, W.X., R. Chammas, and A. Varki. 1996. Linkage-specific action of endogenous sialic acid O-acetyltransferase in Chinese hamster ovary. *J. Biol. Chem.* 271:15130–15138.
43. Rusinol, A.E., Z. Cui, M.H. Chen, and J.E. Vance. 1994. A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. *J. Biol. Chem.* 269:27494–27502.
44. Lin, X., P. Mattjus, H.M. Pike, A.J. Windebank, and R.E. Brown. 2000. Cloning and expression of glycolipid transfer protein from bovine and porcine brain. *J. Biol. Chem.* 275: 5104–5110.
45. Mattjus, P., A. Kline, H.M. Pike, J.G. Molotkovsky, and R.E. Brown. 2002. Probing for preferential interactions among sphingolipids in bilayer vesicles using the glycolipid transfer protein. *Biochemistry.* 41:266–273.
46. Gross, A., X.M. Yin, K. Wang, M.C. Wei, J. Jockel, C. Milliman, H. Erdjument-Bromage, P. Tempst, and S.J. Korsmeyer. 1999. Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. *J. Biol. Chem.* 274:1156–1163.
47. Esposti, M.D., J.T. Erler, J.A. Hickman, and C. Dive. 2001. Bid, a widely expressed proapoptotic protein of the Bcl-2 family, displays lipid transfer activity. *Mol. Cell. Biol.* 21: 7268–7276.
48. Sonnenburg, J.L., H.H. van, and A. Varki. 2002. Characterization of the acid stability of glycosidically linked neuraminic acid - use in detecting de-N-acetyl-gangliosides in human melanoma. *J. Biol. Chem.* 277:17502–17510.