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COMMENTARY

GLUCOSPHINGOLIPIDS AS SITES OF ACTION IN THE CHEMOTHERAPY OF CANCER

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Searches for abnormalities in tumors have revealed a dismayingly large number of metabolic changes, both increases and decreases in the content of qualitatively normal substances. Substances having a novel chemical structure have been found only rarely. The possibility should be considered that these apparently new compounds, despite their variety and inconsistency of appearance, are somehow responsible for the unrestrained growth, malignancy, and invasiveness characteristic of cancer. A corollary possibility should also be considered: certain admittedly normal substances found in tumors in relatively high concentrations can, if they reach a triggering concentration, begin the cancerous process.

This paper presents selected clues about a class of compounds in the above categories, the glycosphingolipids based on GlcCer.‡ They are called "glucosphingolipids" in the title and "glycolipids" in the text.§ These substances are formed from *N*-acyl sphingols (ceramides) by a transferase which attaches glucose to the C-1 of the sphingol. Other transferases subsequently attach additional sugar groups to the GlcCer. The most-studied glycolipids are the ones containing sialic acid, the gangliosides. Acetyl groups can be present in amide and ester linkage, and glycolyl groups can be present in amide linkage.

Many researchers have come to believe that glycolipids are "closely involved" in one or more aspects of cancer cell metabolism and this article summarizes some of their reports. On the basis of literature reading and initial success with a drug that inhibits glycolipid synthesis in mouse Ehrlich ascites tumor cells, we have come to believe that glycolipids are *causally* involved in cancer. Our hypothesis starts with the recent discoveries that some types of cancer are apparently due to inactivating damage to, or deletion of, *both* copies of a specific gene on one chromosome or another, depending on the particular tumor [see, for example, Refs. 1 and 2]. The double defect can be of prenatal origin, of postnatal origin, or both. Thus, these—or all—forms of cancer may be due to a lack of a specific protein normally produced by the affected cells. The lack needs to occur in only one cell, and the missing protein apparently cannot be furnished by surrounding normal cells.

In the next stage of our hypothesis, we postulate that the missing protein acts in normal cells to hydrolyze a specific glycolipid that is normally present at a very low concentration. A cell unable to hydrolyze the lipid would then become a cancer cell, accumulating relatively large amounts of the glycolipid, which acts to initiate its fatal proliferation. Feedback mechanisms to block the accumulation of glycolipids apparently do not exist, judging by sphingolipidosis patients. The lipid may normally function in promoting growth or differentiation or cell adhesion; alternatively, it may have no normal or important function, just as (the nonenzymatically produced) creatinine and glucosylated hemoglobin seem to have no metabolic functions. Only when its concentration becomes elevated does the lipid take on a primary neoplastic role.

According to this hypothesis, cancer is a *glucosphingolipidosis of a single cell*, with a great variety of secondary effects which depend on the cell type and the specific gene deletion. The secondary effects could appear as physical changes in the properties of specific membranes and their receptors, as stimulatory or inhibitory effects of the accumulating lipid on the activities of several enzymes, as induction of the synthesis of various proteins, and as changes in the rates of synthesis or breakdown of sphingolipid-metabolizing enzymes. Widely varying secondary phenomena are characteristic of the genetic sphingolipidoses, despite the simplicity of the genetic defects, and it appears that each sphingolipid controls many different functions.

In the case of tumors caused by mechanical loss of the allelic genes via a chromosomal break, several additional proteins coded for by adjacent genes would also be missing, adding additional variety to the manifestations of the individual patient's tumor.

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‡ GlcCer = glucosylceramide or glucocerebroside.

§ The other major glycosphingolipid family consists of the galactolipids, particularly galactosylceramide and sulfatide. They have not yet been implicated in cancer.

Where radiation, a carcinogen, or a virus is involved, one could postulate that such agents can ultimately damage both genes in one particular cell. Cells with only one gene damaged would become cancerous if the companion gene was genetically defective, a type of genetic predisposition. The loss of hydrolytic activity need not affect the identical glycolipid in every type of tumor; there is evidence that different glycolipids can have similar roles in malignancy, depending on the cell type. One could propose that oncogenes act by inactivating the particular glycolipid hydrolase, an idea consistent with experiments cited below [3, 4].

A double deletion of this nature could, in some patients, involve the loss of an *activator protein* for the glycolipid hydrolase. Three activator proteins for known glycolipid hydrolases have already been found, and there are undoubtedly others to be discovered. Still other forms of cancer could arise from loss of a specific carrier protein, which normally may act to bring the lipid to a hydrolase. Glycolipid transport proteins are known to exist. One can imagine additional ways in which a specific hydrolase might become ineffective or in which the accumulated glycolipid might become more or less effective in inducing the neoplastic state.

While many glycolipids have been found to be associated with tumors, it is possible that only a few of them are causally involved. The actual neoplasm-producing compounds have perhaps not been correctly identified because of (1) the contaminating presence of other glycolipids that have similar chromatographic characteristics, (2) their complexity of structure, and (3) their low level of occurrence (even when found in relatively high concentrations). Glycolipid chromatography is still in a primitive state.

An important test of our hypothesis would be the demonstration that a glycolipid hydrolase is largely or completely absent from a tumor but present in the tissue of tumor origin. This could be done by seeking specific hydrolases, using natural substrates (preferably the novel or unusual glycolipids characteristic of the specific tumor being studied). The common practice of assaying for hydrolases with unnatural substrates, such as nitrophenyl glycosides, is unsatisfactory since they are probably each hydrolyzed by several different enzymes. The addition of bile salts as enzyme stimulators should not be relied on, since they may hide the lack of a natural hydrolase activator.

The following sections summarize—all too concisely—a few of the findings which lend support to this set of speculations. Some of the topics have been touched upon in greater depth in previous reviews. In our conclusion we propose chemotherapeutic approaches based on these ideas.

A. The proliferating effects of glycolipids

A major clue comes from patients with Gaucher disease, a genetic disorder characterized by a low level of GlcCer glucosidase activity and accumulation of very high levels of GlcCer. Reports have appeared pointing to an unexpectedly high incidence of leukemia and other disorders of B-cell proliferation [5–7]. The high content of tissue GlcCer in these patients, it has been suggested, somehow overstimulates B-cell proliferation with consequent malignant or benign transformation [6].

Gaucher patients not only accumulate high concentrations of GlcCer, but they also suffer from severe hypertrophy of the spleen and liver. These organs are the primary sites of blood cell destruction, a process which includes GlcCer hydrolysis, so they normally have a relatively high hydrolytic workload. When the hydrolase is defective, GlcCer accumulates. A similar phenomenon occurs in some patients with chronic leukemias, which apparently overload the capability of their glucosidase [8]. We suggest that the extra GlcCer somehow, in a relatively direct way, acts to produce splenic proliferation.

We have found that injecting young mice with emulsified or liposomal GlcCer causes rapid growth of the liver as well as marked uptake of GlcCer [9]. The growth is accompanied by a correspondingly increased formation of protein, lipid, and DNA and elevated levels of enzymes involved in DNA synthesis. Thus, the growth stimulation seems to be of a proliferative nature. The effect is stronger (up to 25% growth in 1 day) when we also inject conduritol B epoxide, which blocks the catabolism of GlcCer by inactivating GlcCer glucosidase. GlcCer or one of its metabolic products can be considered to be a mitogen which normally occurs at very low tissue concentrations.

In vitro, ganglioside stimulated axonal sprouting in neuronal cultures [10]. Conversely, an inhibitor of GlcCer synthesis *inhibited* axonal sprouting in retinal cultures.* A specific ganglioside, GQlb, was found to increase the number of neuroblastoma cells and their neurites [11]. The B subunit of cholera toxin, which specifically binds to endogenous ganglioside GM1, produced thymocyte proliferation, suggesting that activation of the binding site containing GM1 results in growth stimulation [12]. The proliferative action of the toxin/receptor complex may arise through its ability to raise local cyclic AMP levels [13]. A mixture of gangliosides was found recently to stimulate the growth and metastasis of human and rat tumors in nude mice [14]. The ability of weakly tumorigenic AKR lymphoma cells to proliferate after implantation into mice was enhanced greatly by adding the gangliosides which had been secreted by a highly tumorigenic cell line of the same strain [15]. Thus, the glycolipids that are more glycosylated than GlcCer can also exert a proliferative effect *in vitro* and *in vivo*.

We found that the growth of mouse ascites cells (Ehrlich carcinoma) could be enhanced >50% by injecting GlcCer intraperitoneally [16]. Conversely, an inhibitor of GlcCer synthesis blocked the growth of the cells. One might conclude, at least in the case of these cancer cells, that the tumor's speed of synthesizing this lipid is rate-limiting in the growth process.

Another similarity between Gaucher disease and cancer comes from the observation that the concentration of ferritin (the iron-storage protein) is very elevated in both kinds of tissue [17].

* Agranoff BW, Heacock AM, Vunnam RR and Radin NS, unpublished work.

B. The occurrence of new glycolipids

It has been known for many years that certain tumors contain normal glycolipids ("cytolipins G, H, K"), but at abnormally high levels. More recently, ganglioside GM1 or GM3 was found at a high level in human meningiomas when compared to normal meninges [18]. In a different study, human leukemia cells were found to contain ganglioside GD3 whereas normal leukocytes did not contain detectable amounts of this lipid [19]. A study of neuroblastomas from fifty-three patients concluded that the prognosis of a patient could be correlated with the tumor's content of ganglioside GT1b [20]. Thus, a tumor can contain a glycolipid that occurs in some normal tissues but not in the tissue of tumor origin.

Researchers on glycolipids have been reporting, at an accelerating rate, that tumors contain hitherto *completely unknown* glycolipids [21–28]. A recent report described four new glycolipids in oat cell carcinoma of human lung, compared with normal human lung [21]. The compounds differed from those of normal tissues mainly in the linkages between the different sugar and sialic acid moieties.

Other novel tumor-associated glycolipids have been found by using monoclonal antibodies, but their structures are still unknown.

C. Successful therapy of tumors via antibodies to glycolipids

The use of specific monoclonal antibodies, apparently directed against certain gangliosides, has proved to be an exciting approach [29–32]. Human melanomas produce unusual glycolipids, especially those containing ester groups. Certain antibodies, administered in high dosages, specifically seek out the cancer cells and help to destroy them.

Significantly, some tissues *do* contain low levels of the same gangliosides but appear to be undamaged by the antibody. As Hakomori has pointed out [33, 34], the glycolipids may be less exposed sterically in normal plasma membranes or a critical concentration must be exceeded before antibodies can be significantly damaging. A mixture of both cytotoxic and complement-dependent antibodies would be more useful than either one alone [35].

A similar application to human neuroblastoma cells has been made, based on a monoclonal antibody to ganglioside GD2 [36]. The antibody was tested in nude mice exposed to the human cells and found to suppress tumor establishment, as well as growth. A similar antibody was found to be very useful in a human patient.

Admittedly, this kind of clue indicates only that certain glycolipids occur at an unusually high concentration in the tumor plasma membrane, not that they play a causal role in malignancy. One might be able to achieve similar results with antibodies to any substance that occurs in high concentrations in the tumor surface provided that other tissues contain safely low concentrations. However they do support our suggestion that cancer cells suffer from *micro*sphingolipidoses.

D. Immunodefense by cancer cells due to glycolipids

A major characteristic of cancer is the difficulty that its victims have in generating an immuno-mediated defense against the tumor. The important suggestion has been made that certain glycolipids secreted by tumor cells protect them against the host's immune system by suppressing lymphocyte responses [37–41]. For example [41], murine lymphocytes respond to concanavalin A by proliferating, but the gangliosides isolated from the ascites fluid induced by lymphoma cells in mice were able to block the effect almost completely. The blockage was effective at less than 28 μM ganglioside, a concentration similar to that found in the ascites fluid. The same effect was produced by gangliosides isolated from the lymphoma cells themselves.

Another recent example is ganglioside GD1a, which is shed by a mouse fibrosarcoma and which enters the plasma. This glycolipid inhibited the T cell mitogen response as well as the proliferation of a T cell clone's response to interleukin-2 and the *in vitro* proliferation of spleen cells [42]. The tumorigenic effect of gangliosides on weakly tumorigenic cells, cited above [15], could be ascribed to an immunosurveillance blockage.

In a review of this important subject [43], Marcus concluded in 1984 that the evidence was so incomplete that one could not take the concept of glycolipid immunomodulation seriously. While the review was realistic in its call for more data, we feel that this strong skepticism was itself based on too many negative assumptions.

E. Glycolipid changes during malignant transformation and metastasis

Cells undergoing developmental or malignant or viral transformation undergo marked changes in the relative proportions of their glycolipids [3, 44–47]. A good example is the appearance of ganglioside GD3 in rat cells transfected with a transforming gene, while the pretransformed cells do not contain this glycolipid [3]. The introduction of oncogenes into rat 3Y1 cells resulted in the appearance of several gangliosides not normally seen in the cells [4]. Different malignancy states of human colonic tumors could be correlated with the expression of the glycolipids responsible for Lewis blood type groups, as revealed with monoclonal antibodies [48].

A study was made with melanoma cells, which progress *in vivo* through a series of stages of increasing malignancy [49]. As the cells changed, they produced higher and higher concentrations of 9-*O*-acetyl ganglioside GD3. Normal melanocytes and fibroblasts did not contain a detectable amount of this glycolipid. Another glycolipid, ganglioside GD2, appeared only in advanced tumors and, when the tumor cells at the later stages were cultured, was shed into the culture medium. The authors suggested that the appearance of the unusual gangliosides somehow explains the disappearance of T cells from advanced stage melanomas.

A study of tumorigenicity using human melanoma cells showed that cultured cells having a high level of ganglioside GM2 were able to form tumors in nude athymic mice, whereas cells having a low level of GM2 were not [50]. A graphed line relating tumor size to the *sum* of GM2 and GD2 concentrations had a remarkably high correlation coefficient, suggesting that GD2 also played some role in the phenomenon.

The glycolipids of three strains of a murine tumor, one of them *non*metastatic, were compared and the nonmetastatic one was found to have a much lower ganglioside content and a corresponding increase in the level of the precursor glycolipid, GlcCer. There were marked differences in ganglioside distribution [51].

Recently a *non*ionic minor glycolipid, not yet characterized, was found to be a general marker for proliferating cells, such as transformed human cells [52]. The lipid, apparently present only in human and closely-related primate cell lines, was detected by an antibody. Nonproliferating cells reacted only weakly or not at all.

Studies too numerous to mention, seeking to connect specific gangliosides with binding between cells and penetration of tissues by cells, have been very suggestive. A case could be made for the likelihood that gangliosides are components of the receptors for the adhesion proteins.

F. Glycolipids in plasma as a cancer test

If tumors do indeed shed novel glycolipids or high concentrations of rare lipids into extracellular fluid, one might hope to detect them in the blood stream. The longed-for diagnostic test for the presence of a tumor by demonstrating the presence in plasma of a strange compound, or an elevated amount of a compound, has been reported a few times. Each time the test is ultimately found to give too many false positive or negative answers, or to be sensitive only to advanced tumors, or to be specific for only a single type of tumor. Recent reports have indicated some success with tests for glycolipids [53–57]. These reports are consistent with the evidence showing that tumors shed copious amounts of glycolipids, which enter plasma quickly enough to make them detectable.

A complex containing much glycolipid (and RNA and polypeptides) has been isolated from the plasma of cancer patients [58]. Apparently it is absent from normal individuals and the authors suggested that the novel material comes from the tumor.

Iguro *et al.* [59] described a monoclonal antibody which detected a sialylated derivative of lacto-*N*-fucopentaose III (Lewis^x) in the blood of 26% of their cancer patients. None of their noncancerous patients yielded a positive response except for 17% of those with tuberculosis.

Another monoclonal antibody, against ganglioside GD2, reacted highly with almost every child with neuroblastoma but not with children bearing other types of tumors or with normal controls [60]. In one patient, the level of serum GD2 followed the regression of the tumor during therapy.

A recently described test for cancer [61] involved analysis of the plasma lipids with a nuclear magnetic spectrometer. The change in lipids during cancer development (and in the reverse direction during treatment) was not examined chemically so the nature of the abnormality is unknown, but the test was unusually accurate and specific. It is possible that the change in NMR spectrum was due to the presence of a glycolipid.

Ascitic fluids from patients with cancer contain glycolipids, some of which are not present in the plasma [62]. The latter are presumably shed by the tumor itself.

Since different tumors shed different glycolipids, a generalized test (such as total plasma sialic acid or lipid-bound sialic acid) might have only limited value. Chromatographic analysis, combined with monoclonal antibodies for individual plasma glycolipids, seems essential.

G. Abnormal levels of glycolipid-metabolizing enzymes in cancer

Since abnormal glycolipids occur in tumors, it is not surprising to find new enzymes or changed levels of the enzymes responsible for their metabolism.

Human leukocytes from myelogenous leukemia patients were found to have three times the normal level of glucosidase, the enzyme which degrades GlcCer [63]. A study of liver preneoplastic nodules induced by a carcinogen showed that a galactosyltransferase acting on ganglioside GM2 was somewhat higher in the nodules of grade II; about 4.6 times as active as normal liver [64]. Smaller nodules showed normal or subnormal specific activities.

Assay of a rat galactosyltransferase which acts on glycolipids showed that normal liver had about one-tenth of the activity when compared with hepatoma [65].

A study of various glycosidases in human colon and breast showed markedly higher values in malignant tissue for β-galactosidase, β-acetylgalactosaminidase, α-mannosidase and a neuraminidase [66]. The region considered to be normal was taken from the tissue adjacent to the tumor. Important control measures, to rule out indirect cancer effects, were made for DNA, protein, and (by others) mitotic rate. Some hydrolases were *not* elevated.

Examination of rats with metastasizing mammary tumors showed that the plasma level of a sialyltransferase was above normal and that the enzyme concentration in microsomes from the tumors was six times as high as in nonmetastasizing tumors [67]. The elevated plasma values indicate that tumors shed not only glycolipids but also some of their enzymes.

The induction of a fucosyltransferase and of a galactosyltransferase occurred in the precancerous liver of rats that were fed a carcinogen [68]. These changes were accompanied by the accumulation of certain glycolipids as the liver moved toward the hepatomic state. Similar appearance of a new *N*-acetylglucosaminyltransferase was seen in a human tumor [69].

The appearance of increased levels of glycolipid-metabolizing enzymes seems to contradict our suggestion that tumors lack a specific glycolipid hydrolase. However such a phenomenon has been observed in the case of sphingolipidoses, where a genetic defect in one hydrolase somehow produces increased levels of other sphingolipid hydrolases.

The ability of tumors to synthesize lipids *de novo* seems to be relatively low and they apparently have to requisition preformed lipids from the host. Tumors absorb triglycerides quite readily [70] and secrete lipotrophic factors which draw the host's fat into the blood, allowing it to be taken up by the tumor [71, 72]. The fatty acids thus stolen are pre-

sumably also available for the synthesis of glycolipids (both the sphingol and acyl moieties). It is possible that the host also furnishes *intact* glycolipids (such as GlcCer), which are found in the plasma lipoproteins. Uptake of host lipids may reflect the importance of shedding certain glycolipids to the tumor's survival.

H. Biological effects of glycolipids

Gangliosides of different types have been connected to many phenomena: complex formation with cholera toxin, Sendai virus, interleukin, and epidermal growth factor, promotion of neuronal growth, interaction with the peripheral nerve growth factor, cell adhesion, induction of growth or developmental changes, and protein kinase stimulation or inhibition.

A study with murine macrophages showed that addition to the medium of the primary glycolipid, GlcCer, caused the cells to form and release increased amounts of interleukin-1 [73]. By way of comparison for specificity, the authors showed that other sphingolipids had no such effect. Human blood monocytes behaved like macrophages. A similar increase in interleukin formation occurred when phorbol diester was added to the macrophage medium. The similarity between GlcCer and phorbol ester effects is intriguing although there seems to be no obvious structural similarity. Recently, our inhibitor of GlcCer synthesis (see Section I) was found to destroy the proliferative response of T cells to interleukin-2, apparently by eliminating glycolipids essential for the action of the IL-2 receptor [74].

Other reports have shown that glycolipids can be influenced by substances which enhance differentiation. Phorbol diester, added to cultured CEM-2 leukemia cells, produced an increased concentration of the nonionic glycolipids, hexosylceramide and lactosylceramide, as well as ganglioside GM3 [75]. Perhaps the increases were the result of a direct or indirect inhibition by diester of the enzyme, GlcCer glucosidase, or stimulation of the glucosyltransferase that makes GlcCer. Such a phenomenon would explain some stimulating effects of phorbol esters. Moskal *et al.* [76] have found phorbol ester to produce a >5-fold increase in sialyltransferase of NIL 8-HSV cells.

Gangliosides have also been shown to form a complex with interferon type I (not with type II), rendering the interferon unable or less able to block viral replication within mouse LY cells [77].

I. Therapeutic approaches through the glycolipids

This brief survey and set of hypotheses point to the need to synthesize drugs which can interfere with the glycolipids. The evidence cited above, particularly in Sections A, B, D, and E, gives strong support for the idea that cancer is the *result* of a microsphingolipidosis. While it is possible to hypothesize that the glycolipid changes and effects are epiphenomena, one must disregard many of the published reports.

The typical biological processes of the glycolipids are (i) synthesis from nonglycolipid precursors and from lower glycolipids, (ii) hydrolytic degradation of higher to lower glycolipids and then to ceramide, and (iii) binding by specific proteins for the formation of membrane-bound receptors, or binding by enzymes in order to modulate their activities, or binding by plasma membrane proteins to form recognition complexes or membrane stabilization complexes, or binding by transport proteins in the plasma and in cell cytosol. Blockage of these processes could be accomplished by the administration of synthetic analogs of the glycolipids, as needed for a particular kind of cancer.

We synthesized an inhibitor of GlcCer synthetase in order to block process "i" and found it to have good antitumor activity against the Ehrlich ascites tumor *in vivo* [16]. The effectiveness of the drug was reduced by co-injecting GlcCer, as one might predict. Moreover the injection of GlcCer into untreated mice stimulated tumor growth >50% (the closely related sphingolipid, galactosylceramide, had no effect). These observations support the hypothesis that glycolipid formation is essential for tumor proliferation. Preliminary data also indicate the ability to block the development of a human colonic tumor in athymic mice.*

Process "ii" should not be blocked, since it may lead to increased glycolipid *accumulation*. Another stage of glycolipid synthesis that might usefully be interfered with is prior to ceramide formation, either in the transfer of fat from the host or in its utilization to make sphingols or ceramide.

Some investigators have suggested that cancer cells produce strange or greatly increased amounts of glycolipids because the gene for a glycolipid synthetase is derepressed. If this is correct, it still makes sense to block those synthetases by an appropriate drug.

One may conclude from *in vitro* uptake studies that all cells have the ability to absorb glycolipids from the medium. In the case of cancer cells, the source would be plasma lipoproteins or ascites fluid. In order to starve the tumor, one should design inhibitors of glycolipid synthetases that act on human enzymes, as well as tumor enzymes.

One should aim not only to design a synthetase inhibitor but also, as is well known in pharmacology, to make an artificial substrate which might be converted enzymatically by the tumor to a glycolipid-like synthetase inhibitor or destabilizing component of a glycolipid-containing complex.

Even if a particular inhibitor of glycolipid synthesis is only partially effective, it could act synergistically with other drugs having a different mode of action. Mixtures of drugs are usually used in current anticancer therapy.

Few attempts, in laboratories other than this one, have been made to use specific drugs to directly lower the levels of tissue glycolipids and to look for changes in cell properties. Sundaram and Lev [78] have shown that an antibiotic, cycloserine, can block sphingosine biosynthesis *in vivo* and, therefore, block glycolipid synthesis. Various substances that block the biosynthesis of glycoproteins, such as tunicamycin or monensin, have been tested for their effects on glycolipid synthesis. For example, tuni-

* Boland CR, Deshmukh GD, Inokuchi J and Radin NS, unpublished work.

camycin inhibited ganglioside and neutral glycolipid synthesis in neuronal cells [79]. Monensin caused accumulation of GlcCer and lactosylceramide in human fibroblasts [80] while apparently inhibiting the synthesis of the higher glycolipids in other cells [81]. 2-Deoxy-D-glucose was found to inhibit GlcCer synthesis in BHK-21 cells [82], apparently by forming a deoxyglucose nucleotide which inhibited glucosyltransferase. These compounds may have merit in treating cancer if combined with a more specific inhibitor of glycolipid synthesis.

A designer of an inhibitor of glycolipid synthesis must also consider the "rescue" problem: how to treat a patient who has been overdosed with the inhibitor. In the case of a drug which blocks GlcCer synthesis, this could be done with an injection of GlcCer or conduritol B epoxide, which prevents the degradation of endogenous GlcCer. It might also be possible to help the patient without hindering chemotherapy against the tumor by injecting a rescue glycolipid, such as a ganglioside which is particularly needed by the host but which does not provoke tumor growth.

While the ideal anti-glycolipid drug should kill all the cancer cells, it is common for antineoplastics to leave some cryptic surviving cells. The second ideal property, in view of the evidence in Section D, is interference with glycolipid shedding and consequent freedom from the host's immunosurveillance system. An experimental test of the latter property could be run by giving surviving test animals a *second* inoculation of the same tumor cells. If the drug has helped the animal's immune response, the second set of cancer cells should not proliferate, even without drug treatment. We observed this kind of immunity against Ehrlich ascites cells with our drug [16]. Of course more specific measurements of immune response should be run.

The drug must not kill all of the tumor cells too quickly or the host will not have enough exposure time to develop antibodies. The complexity of this requirement is evident when one considers the different types of antigen and antibody, as well as the antibody concentration at the site of the tumor. A detailed study of L1210 cell rejection concluded that prolonged mouse survival required development of a high level of IgG_{2a} anti-tumor antibodies within an adequate time [83].

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