Glycoconjugate Journal 15, 627–636 (1998)

REVIEW

Biosynthesis and functions of gangliosides: recent advances

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Introduction

Gangliosides are a large group of sialylated glycosphingolipids widely expressed in mammalian tissues. This review will concentrate on haematosides (simple gangliosides) and ganglio-series gangliosides which form a complex family of interrelated compounds (Figure 1). Although particularly abundant in brain and nervous tissues, gangliosides are found in most or all tissues of the body where they are thought to perform specific functions. Recent progress in cloning the genes coding for some of the glycosyltransferases responsible for ganglioside biosynthesis and information that is emerging on their functions, make this a particularly appropriate time to review this topic. Earlier advances have been thoroughly reviewed by other investigators [1–4].

Biosynthetic pathways

Enzymology studies, mainly on brain and liver membranes, have shown that the biosynthesis of gangliosides proceeds in a step wise manner, starting from Lac-Cer, along four distinct pathways by the transfer of a sugar nucleotide to the appropriate acceptor (Figure 1). Although these pathways are generally accepted, many of the steps have not strictly been shown to occur in this sequence *in vivo* or by the enzymes indicated. In particular, questions concerning the number of enzymes involved and whether the same enzyme can carry out different steps are still under active investigation. Another important question being studied is which of the enzymes are glycolipid specific and which are active on both glycolipid and glycoprotein acceptors.

Molecular cloning of glycosyltransferases involved in ganglioside biosynthesis

Our understanding of ganglioside biosynthesis and expression has recently been greatly enhanced by the cloning of the genes for some of the transferases involved (Table 1). Cloning of cDNA coding for these enzymes has been accomplished by three approaches: (i) use of probes deduced from partial amino acid sequences of purified enzymes, (ii) expression cloning with detection of the glycolipid product with specific lectins or antibodies and (iii) in the case of sialyltransferase, PCR cloning using degenerate synthetic primers corresponding to the 'sialyl-motifs' known to occur in these proteins. As glycosyltransferases are notoriously difficult to purify, approaches (ii) and (iii) have been used most successfully. It should be noted that cloning of glycosyltransferases by expression approaches is more difficult than the cloning of other proteins as a recipient cell line must be found or created that expresses the acceptor glycolipid for the particular enzyme being studied.

Glucosylceramide synthase

A cDNA coding for this enzyme, which catalyzes the first step in the assembly of the sugar chains of gangliosides, was isolated by expression cloning after transfecting a human melanoma cell line cDNA library into a recipient cell line (GM-95) deficient in the enzyme and screening the transfectants with an antibody to G_{M3} [5]. The deduced amino acid sequence indicated a transmembrane protein with a Type II orientation similar to other glycosyltransferases, with, however, the presence of an additional hydrophobic sequence close to the C-terminal end of the protein. mRNA for this enzyme was found to be widely distributed in the body. This enzyme has also been purified from rat liver [6].

Lactosylceramide synthase (Gal-T-2)

This enzyme has not yet been cloned, but it has been purified from human kidney [7] and its enzymic properties have been studied [7, 8]. The kidney enzyme preparation (purified 440-fold), contained two components of 60,000 and

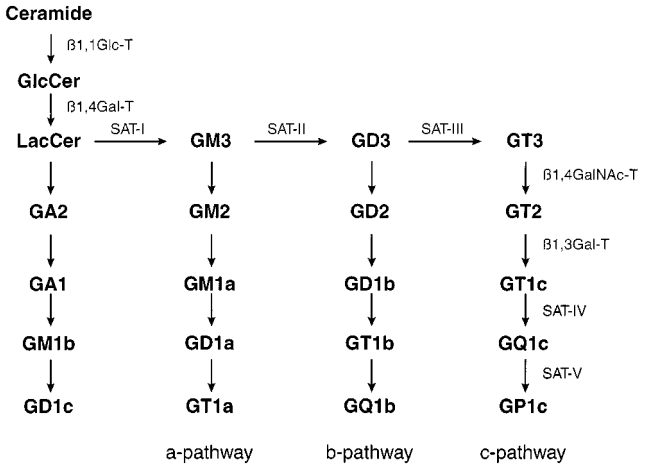

Figure 1. Proposed pathways for the biosynthesis of gangliosides, modified from refs. 4 and 12. The nomenclature is based on that of Svennerholm [120]. G_{M3}, G_{D3} and G_{T3} may be regarded as haematosides.

Table 1. Nomenclature and specificity of glycosyltransferases involved in ganglioside biosynthesis

Enzyme	*Enzyme Specificity*	*Nomen-clature*	*Abbreviation*[a]
Lactosyl ceramide synthase	β1,4 Gal-T	Gal-T-2	
G_{M3} synthase	α2,3 Sialyl-T	SAT-I	
G_{M2}/G_{D2} synthase	β1,4 GalNAc-T	GalNAc-T-1	
G_{D3} synthase	α2,8 Sialyl-T	SAT-II	ST8 Sia I
G_{T3} synthase	α2,8 Sialyl-T	SAT-III	ST3Gal IV
G_{M1a} synthase[b]	β1,3 Gal-T	Gal-T-3	
G_{T1b} synthase[c]	α2,3 Sialyl-T	SAT-IV	ST3 Gal II
G_{Q1b} synthase[d]	α2,8 Sialyl-T	SAT-V	ST8 Sia V

[a] Ref. 118.
[b] It has been proposed that the same enzyme produces G_{A1}, G_{M1a}, G_{D1b} and G_{T1c} [21].
[c] It has been proposed that the same enzyme produces G_{M1b}, G_{D1a}, G_{T1b} and G_{Q1c} [12].
[d] It has been proposed that the same enzyme produces G_{T1a}, G_{Q1b} and G_{D1c} [119].

58,000 daltons. This enzyme is different from the classical lactose synthase as it uses Glc-Cer as its preferred substrate. There is evidence that lactosylceramide synthase, as well as glucosylceramide synthase, is located on the cytoplasmic side of the Golgi apparatus [9].

G_{M2}/G_{D2} synthase

This β1,4GalNAc-transferase was the first glycolipid-specific glycosyltransferase to be cloned [10]. The approach used was to screen transfectants of a derivative of mouse B16 melanoma cell line (which expresses G_{M3}) using an antibody to the product of the enzyme (G_{M2}). Enzymatic and transfection [10, 11] studies with the cloned enzyme showed that it could utilize both G_{M3} and G_{D3} as acceptor, forming G_{M2} or G_{D2} respectively, as had been suggested earlier by Pohlentz *et al.* [12]. The predicted size of the β1,4GalNAc-transferase is 58,881, with three potential *N*-glycosylation sites. In transfected CHO cells the enzyme had a molecular weight of about 67,000 indicating that at least one of the glycosylation sites is used [13]. The cDNA identified had homology with another β1,4GalNAc-transferase which synthesizes the Sd[a] blood group structure, probably on glycoproteins [14]. The genomic organization of this gene has also been studied and the gene was shown to consist of at least 11 exons [15]. The corresponding mouse cDNA has also been identified [16, 17]. The distribution of β1,4GalNAc-transferase mRNA has been determined in a number of tumor cell lines and tissues by RT-PCR and Northern blotting [18, 19].

G_{M1} synthase (Gal-T-3)

G_{M1} is one of the most studied gangliosides, mainly because it is one of the major gangliosides in the vertebrate brain. The enzymology of G_{M1} synthase (β1,3 galactosyl transferase) has been studied extensively [8] and a cDNA coding for this enzyme has now been cloned from rat brain [20]. The cloned sequence represents a Type II membrane protein of 371 amino acids (Mr: 40 976) with little or no homology to other cloned proteins, including other galactosyltransferases. Analysis of the specificity of the expressed enzyme and transfected cells showed that the enzyme was capable of synthesizing G_{D1b} and G_{A1}, as well as G_{M1}, thus confirming earlier enzymological data [21]. In adult tissues, mRNA for the enzyme was most strongly expressed in rat kidney, testes, spleen and thymus. Relatively low levels were detected in adult brain but high levels were detected in embryonic brain, indicating that G_{M1} (and/or G_{D1b} or G_{A1}) plays an important role in the formation and development of brain tissues.

Sialyltransferases

A number of sialyltransferases acting on glycoproteins, glycolipids or both have recently been cloned and this topic has been reviewed [22–24]. All sialyltransferases cloned thus far have a Type II organization with a hydrophobic transmembrane region, a luminal 'stem' region and a large C-terminal catalytic domain (Figure 2). Moreover, the different sialyltranferases share two conserved domains termed 'sialylmotifs' [25]. Sialylmotif L consists of 44 or 45 amino acids with eight invariant residues, including one cysteine residue. Sialylmotif S (23 amino acids) is less well conserved but also has a conserved cysteine residue. The sialylmotif L appears to be involved in binding CMP-NeuAc [26]. Recently, Sasaki [24] pointed out that sialyltransferases with

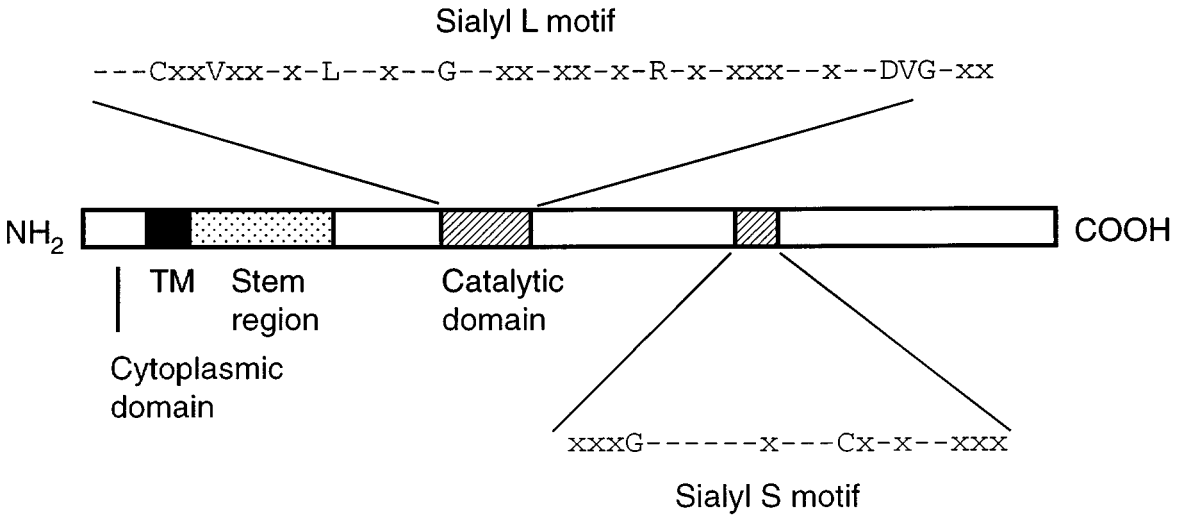

Figure 2. Schematic representation of structure of sialyltransferases showing topology, domain structure and sialyl motifs. Regions are not drawn to scale. TM: transmembrane region. In the sialyl motifs, completely conserved amino acids are shown in their single letter codes; x: highly conserved amino acid positions and —: non-conserved amino acid positions.

similar specificities, e.g., all 2,3Gal: sialyltransferases, have conserved residues in or close to the L motif characteristic of that specificity.

G_{M3} synthase (SAT-I)

The enzymatic properties of this enzyme have been studied extensively [8]. It is most active with Lac-Cer as substrate but it also has appreciable activity with Gal-Cer and G_{A2}. G_{M3} synthase has been purified from rat liver [27] and rat brain [28]. The apparent molecular weights of the two isolated enzymes were 60,000 and 76,000, respectively. A preliminary report on the molecular cloning of this enzyme has appeared [29].

G_{D3} synthase (SAT-II)

G_{D3} synthase (α2,8 sialyltransferase; SAT II) is one of the key enzymes in ganglioside biosynthesis, controlling, as it does, entry into the *b* and *c* pathways (Figure 1). cDNAs coding for this enzyme were isolated almost simultaneously by three groups in 1994 [30–32]. All three groups used expression cloning approaches using either anti-G_{D3} antibodies, or in the studies of Haraguchi *et al.* [30], an anti-G_{D2} antibody, after transfecting a cDNA library into a melanoma derivative expressing G_{M3} and G_{M2} but lacking the intermediate enzyme, G_{D3} synthase. The derived amino acid sequence of this enzyme is unusual in that it has an extra amino acid in the L motif. G_{D3} synthase has also been cloned from rat brain [33, 34]. There is some controversy as to the precise substrate specificity of SAT-II (see below), but all studies agree that G_{M3} is the preferred substrate for this enzyme. The expression of G_{D3} synthase mRNA has been studied in a range of human cancer and normal cell lines by RT-PCR and Northern blotting [35]. Melanoma cells and activated T-lymphocytes showed particularly strong expression.

G_{T3} synthase (SAT-III)

Expression cloning using COS-1 cells transfected with a melanoma cDNA library and an anti-G_{T3} antibody unexpectedly resulted in the identification of a cDNA identical to G_{D3} synthase (SAT-II) which appeared to be responsible for G_{T3} synthesis [36]. The authors suggest that the same enzyme can synthesize both disialyl and trisialyl sequences. Although SAT-II can use G_{D3} as a substrate at a low efficiency *in vitro* [36, 30] and when over-expressed in transfected cells *in vivo*, it is unclear whether it does so in cells naturally expressing G_{D3}. The fact that many cell lines have high levels of G_{D3} and no significant levels of G_{T3} suggests that another enzyme may be responsible for G_{T3} synthesis in untransfected cells or that there is another control mechanism for this effect. Furthermore, as pointed out by Tsuji [23] the substrate specificities of many sialyltranferases are rather broad *in vitro* and it is difficult to decide which enzyme is responsible for a particular step *in vivo*.

Polysialic acid synthases

Two other sialyltransferases that synthesize α2,8 NeuAc linkages have been identified [37–41]. These enzymes [STX(ST8Sia II) and PSA(ST8Sia IV)] are responsible for the biosynthesis of polysialic acid chains that are characteristic of N-CAM and a few other glycoproteins. The two enzymes have almost identical substrate specificities, however ST8Sia IV mRNA is strongly expressed in mouse lung, heart and spleen whereas STSia II mRNA is expressed mainly in the brain [26]. ST8Sia II is related to the rat STX cDNA which had been cloned earlier [37]. These enzymes

seem to have a specificity for NeuAcα2 → 3Galβ1 → 4GlcNAc- on N-linked chains and are unlikely to be involved in the synthesis of polysialic acid-containing gangliosides.

Other sialyltransferases

It is unclear at this time how many different sialyltransferases are involved in ganglioside biosynthesis. In addition to the confusion concerning G_{T3} discussed above, the identity of SAT-V which synthesizes G_{D1C}, G_{T1a} and G_{Q1b} is also in doubt. SAT-II can synthesize these compounds *in vitro* and in transfected cells [42], but another sialyltransferase (ST8Sia V) has been identified by Kono *et al.* [43] which also synthesizes the same gangliosides, as well as G_{T3}. A candidate cDNA for SAT-IV, which synthesizes G_{M1a}, G_{D1b} and G_{T1b} has also been reported (44). ST3 Gal II (SAT-IV) is probably involved in synthesizing α2,3NeuAc linkages on Galβ1 → 3GalNAc- in gangliosides and ST3Gal I may be involved in synthesizing this linkage in mucins. There may also be species differences in the substrate specificities of some sialyltransferases [45, 46].

O-Acetyltransferases

An important modification of gangliosides is the *O*-acetylation of sialic acid residues on C-9, C-7 or C-9 and C-7 observed in restricted cells and tissues. Based on its distribution in developing rat retina [47] and Purkinje cells [48] it has been suggested that *O*-acetylation of gangliosides serves as a cellular recognition signal. *O*-Acetylated gangliosides can also serve as tumor markers e.g., 9-*O*-AcG_{D3} in melanoma [49]. Recently an enzyme that apparently synthesizes *O*-acetyl G_{D3} from G_{D3} and acetyl CoA has been cloned from rat brain [50].

N-Glycolylneuraminic acid synthesis

Another modification of the sialic acids in gangliosides is the substitution of *N*-glycolyl for the more common *N*-acetyl group on C-3 of this sugar. As humans (and chickens) do not express *N*-glycolylneuraminic acid (NeuGc) this substituent serves as a 'heterophile antigen' known as the Hanganutziu-Deicher (H-D) antigen. It has been suggested the H-D specificity serves as a tumor antigen in some human tumors [51], although this finding has not been confirmed by all investigators [52]. The synthesis of NeuGc has recently been elucidated and appears to occur through hydroxylation of CMP-NeuAc utilizing both CMP-NeuAc hydroxylase and NADH-dependent cytochrome b_5 [53]. These two proteins have been cloned from mouse liver [54]. Analysis of the corresponding cDNA for CMP-NeuAc hydroxylase from human tissue indicated that it lacked approximately 100 amino acids from the N-terminal region and did not produce a functional protein [55]. This result again raises doubts as to whether human tumors can express *N*-glycolylneuraminic acid.

Regulation of ganglioside biosynthesis

A dramatic feature of ganglioside biology is the differential expression of gangliosides in different tissues and the changes observed in ganglioside expression during development and malignant transformation. These observations provide a rationale for believing that gangliosides serve specific functions in cells and tissues. On the other hand, factors that regulate ganglioside synthesis and expression in different cell types are poorly understood. Factors that play a role include the specificity and levels of the glycosyltransferases, the location and organization of the enzymes in the endoplasmic reticulum, Golgi apparatus and trans-Golgi network, and other factors such as intracellular pH. As a glycolipid may be an acceptor for more than one glycosyltransferase, the levels and efficiency of key enzymes is important. G_{M3}, G_{D3} and G_{T3} synthases play important roles, controlling entry into the *a*, *b* and *c* pathways (Figure 1). Recently, with the availability of cDNAs corresponding to some of these enzymes, new information has been derived on this point by transfecting cell lines with cloned cDNAs and examining the effect on ganglioside expression. In one study, transfection of cDNA for G_{M2}/G_{D2} synthase into a melanoma cell line expressing G_{M3} and G_{D3} converted the cell line into a transformant expressing only G_{M2} [14]. Thus, high levels of β1,4GalNAc-transferase subverted the biosynthetic pathway entirely and drove it into the *a* pathway (Figure 3). Two other studies [56, 57] examined whether G_{M2}/G_{D2} synthase can also synthesize G_{A2}, the corresponding neutral glycolipid. In most cell types, little or no G_{A2} is synthesized in the presence of G_{M3} synthase. In contrast, by transfection into cell lines expressing little or no G_{M3}, Yamashiro *et al.* [56] and Lutz *et al.* [57], were able to show efficient synthesis of G_{A2} by this enzyme *in vivo*. Transfection of G_{D3} synthase into a neuroblastoma cell line (SHSY5Y) expressing *a* series gangliosides (G_{M3} and G_{M2}) resulted in a cell line expressing G_{D3} and large amounts of G_{D2}, which is a more typical neuroblastoma pattern [58, 59] (Figure 3). Likewise, transfection of G_{D3} synthase cDNA into 3T3 fibroblast cells converted a cell line expressing only *a* pathway ganglioside into one expressing *b* series gangliosides, including G_{Q1b}. Taken together, these results show the dominant role played by glycosyltransferase levels and the competition that occurs for common substrates in determining the ganglioside profile of a cell. There is also evidence that subtle differences in the ratios of the various glycosyltransferases within a given pathway can influence the ganglioside profile of cells. For example, we have shown that the high levels of G_{D2} characteristic of neuroblastoma cells are determined not only by high G_{D2} synthase levels but also by low levels of the preceding enzyme in the *b* pathway i.e., G_{D3} synthase [59]. Exactly how this occurs is not clear

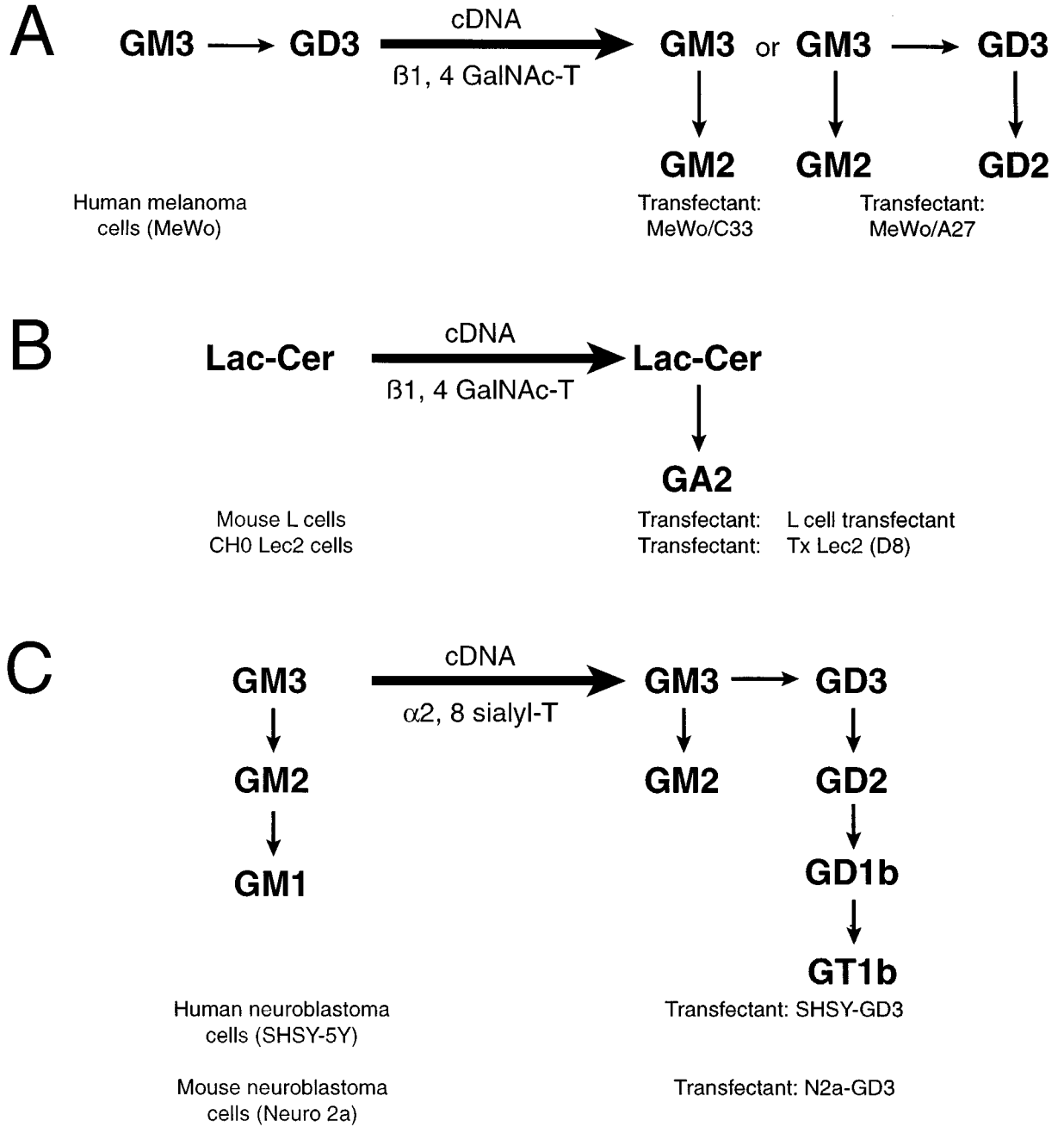

Figure 3. Summary of results involving transfection of *β*1,4GalNAc-T or *α*2,8 sialyl-T cDNAs into mammalian cells. A: transfection of human melanoma cells by *β*1,4GalNAc-transferase cDNA; B: transfection of mouse L cells with *β*1,4GalNAc-transferase and C: transfection of human and mouse neuroblastoma cells with *α*2,8 sialyltransferase. Illustrated is the induction of novel ganglioside expression and/or the shift of biosynthesis into new pathways.

but this ratio of enzymes may lower the accumulation of G_{D3} and result in predominant G_{D2} expression. The biosynthesis of a particular ganglioside could be controlled by the level of its precursor ganglioside in relation to the Kms of the enzymes forming and acting on the ganglioside. Alternatively, if G_{D3} and G_{T3} are synthesized by the same enzyme, low levels of this transferase would prevent diversion of synthesis to the *c* pathway.

These considerations raise the question as to how the levels of the glycosyltransferase are determined and regulated. Although there is some evidence that post-translational factors may influence enzyme activity [56], transcriptional regulation probably plays the major role. Information on this point is available on G_{M2}/G_{D2} synthase. Furukawa and coworkers [18] showed that the gene coding for this enzyme has three transcription initiation sites and

three alternate exons are used. Consensus binding sites for EGR-1, HNE-5 and Sp1 transcription factors were found in the 5′-flanking region. These different transcription initiation sites and their promoters/enhancers may be responsible for cell type-specific expression of the G_{M2}/G_{D2} synthase gene.

Functions of gangliosides

Evidence from tissue distribution

The differential distribution of gangliosides in various tissues is a strong indication that they play important roles in carrying out specific functions in these tissues. More specifically, sequential changes are observed in developing brain [60] and each of the regions of the adult brain is characterized by a specific ganglioside constitution [48, 61, 62]. From these observations, no particular role can be assigned to an individual ganglioside but the general concept emerges that gangliosides contribute to cell–cell recognition or cell–matrix interactions, either through lectin-like proteins or by carbohydrate–carbohydrate interactions [63], that are important in development and tissue organization.

Evidence from *in vitro* experiments

In vitro experiments aimed at elucidating the function of gangliosides have relied on four approaches: (i) determining the effect of the exogenous addition of gangliosides to cells, (ii) blocking ganglioside biosynthesis with specific inhibitors, (iii) selecting cell variants that lack some or all gangliosides, and (iv) introduction of new gangliosides into cells by transfection of cDNA.

Hakomori and coworkers have provided evidence that gangliosides can influence a number of cell surface receptors. For instance, they showed that epidermal growth factor (EGF) receptor kinase [64] and platelet-derived growth factor (PDGF) tyrosine phosphorylation [65] are inhibited by G_{M3} and G_{M1} and other gangliosides, respectively. In another approach, Weis and Davis [66] showed that LAID cells, which had been transduced with the EGF receptor and do not normally synthesize G_{M3} (because of the absence of UDP-Glc to UPD-Gal epimerase), showed inhibition of EGF-dependent cell growth and EGF phosphorylation when G_{M3} synthesis was induced by the addition of galactose to the medium. Recently, the concept has been developed that sphingolipids and their metabolites, particularly ceramide and sphingosine, can function as second messengers. As G_{M3} and other gangliosides are degraded to ceramide after exogenous addition to certain cultured cells, it is possible that glycolipids can participate in this metabolic pathway. Readers are referred to reviews by Hakomori [67], Kolesnick [69], Hunnan and Bell [69] and Spiegel [70] for further information on this topic. Recently, G_{D3} has been directly implicated in the CD95 (fas)-mediated apoptotic pathway in haematopoietic cells [71]. Another specific function for certain gangliosides seems to be in adhesion and cell–matrix interactions. Thus, in melanoma cells it has been demonstrated that G_{D3} and G_{D2} are concentrated in adhesion plaques and are involved in adhesion to fibronectin. Along the same lines, G_{M3} has been shown to regulate $\alpha 5 \beta 1$ integrin–fibronectin interactions in a mouse mammary carcinoma [72]. Specific receptors for gangliosides, including myelin-associated glycoprotein, have been identified in brain [73, 74].

Blocking ganglioside biosynthesis in cultured cells, or the selection of variants, has led to conflicting results. For example, Ichikawa *et al.* [75] developed a cell line from mouse B16 melanoma that was deficient in the synthesis of all glycolipids and found that this mutant cell line grows normally in complete medium *in vitro*. This result can be explained, at least in terms of adhesion, by the recent observation that increased sphingomyelin was able to substitute for loss of G_{M3} in a mouse melanoma cell line deficient in glycosphingolipids [76]. A specific role for G_{D3} in cell proliferation was suggested by the isolation of human melanoma cell variants that had lost cell surface G_{D3} expression; these cells showed poor growth *in vitro* and *in vivo* in nu/nu mice [77].

There is a considerable body of evidence showing that gangliosides are involved in the differentiation and function of neural cells [78, 79]. In support of this concept it was recently shown that transfection of a cDNA coding for G_{D3} synthase into Neuro2A cells caused neurite outgrowth and cholinergic differentiation of these cells [80]. The differentiated cells expressed G_{D3} and other *b* series gangliosides such as G_{Q1b}. Gangliosides also seem to be involved in the differentiation of some lymphocytic cells. Thus, the addition of exogenous G_{M3} to cultured HL-60 promyelocytic leukemia cells caused the cells to differentiate into monocyte/macrophages or granulocytes [81]. Moreover, it has recently been shown that blocking of complex ganglioside biosynthesis and increasing G_{M3} levels in HL-60 cells by the use of antisense oligonucleotides to G_{M2} synthase and G_{D3} synthase also caused the differentiation of these cells [82].

The interpretation of many of these experiments is complicated. This is particularly true of experiments utilizing exogenous addition of gangliosides to achieve the biological effect. A number of problems can be pointed out, including the tendency of cells to convert the incorporated glycolipid into both simpler [83] and more complex [84, 85] species, thus making it difficult to assign the effect to a specific ganglioside. Furthermore, the effects observed tended to be obtained with a variety of exogenous gangliosides, again making conclusions about specificity difficult. Also, the amounts of added gangliosides used in the experiments tend to be rather large whereas physiologic changes in glycolipid levels tend to be much smaller. Further work will undoubtedly show that these effects on growth are real but complex. Ganglioside biosynthesis also seems to be linked to a number of cytokine receptor-mediated pathways. For

instance, TNF-α has been shown to greatly enhance G_{D3} expression in melanocytes [86] and IL4 stimulates G_{D3} expression in WI-38 fibroblast cells [87]. The mechanism for these effects, or whether they are physiologically significant, is not known.

Evidence from *in vivo* experiments

It would be advantageous if the many indications concerning the roles of gangliosides in cell function could be confirmed by *in vivo* approaches. Obviously these are more difficult than *in vitro* experiments and until recently had been approached by administering gangliosides to experimental animals after generating neurological damage by mechanical or chemical manipulation [88, 89], by the injection of toxic reagents [90] or by ischemic treatments [91]. Ganglioside treatment often corrected the induced condition [88–93] presumably due to neurotrophic activity. It has, however, been difficult to objectively evaluate the *in vivo* effects of gangliosides using these approaches [93].

The development of techniques that can 'knock out' specific genes in mice has opened up powerful new approaches to studying the function of proteins. Application of this procedure by Takamiya *et al.* [94] to the G_{M2}/G_{D2} synthase gene (β1,4GalNAc-transferase) resulted in the development of mice that completely lacked all complex gangliosides in brain and other tissues. Only G_{M3} and G_{D3} could be detected in these mice. The mice were perfectly viable and did not show any major histological defects in their nervous systems or in their gross behavior. Considering that gangliosides have been widely implicated in the development of the nervous system, especially in neuritogenesis and synaptogenesis, this result is extremely surprising. Some compensatory mechanism could be operating in these mice. For example, G_{M3} or G_{D3} may take over the functions normally carried out by more complex gangliosides. On the other hand, this argument speaks against the idea, coming from the observations cited earlier, that specific gangliosides are located in particular areas of the brain and that the patterns change during development, suggesting that specific gangliosides serve specific functions. The production of mutant mice lacking all gangliosides should answer this question. It was later shown that these mutant mice lacking complex gangliosides showed aspermatogenesis and that the male mice were infertile [K Takamiya, K Furukawa *et al*., unpublished data]. This result indicates that complex gangliosides play an important role in spermatogenesis, possibly by influencing testosterone transport. These data also illustrate the point that gene disruption approaches may demonstrate novel, unexpected functions for glycoconjugates.

Gangliosides as receptors for viruses and bacteria

In addition to their true functional roles, gangliosides have been conscripted by some viruses and bacteria to serve as their cell-attachment receptors. Recently, for example, *Helicobacter pylori*, a human pathogen involved in the etiology of duodenal ulcers and gastric cancer, has been shown to bind to various sialylated polyglycosylceramides [95].

Gangliosides and cancer

Dramatic changes in haematoside and ganglio-series gangliosides have been noted in tumors of neural crest-derived tumors (melanoma, neuroblastoma and astrocytoma) and a few other tumor types (e.g, small cell lung cancers and sarcomas). These changes are sufficiently prominent that gangliosides can serve as target antigens for the immunodiagnosis and immunotherapy of these cancers [reviewed in 96, 97]. On the other hand, it has been suggested that gangliosides shed by tumors can aid their development by suppressing an immune response to the tumors [98]. Although comprehensive information is available on the ganglioside composition of tumors, very little is known concerning the mechanisms underlying these changes. Lack of understanding of basic mechanism has, however, not prevented clinical application of these findings. Both anti-ganglioside monoclonal antibodies and ganglioside vaccines have been explored for the therapy of cancer. Most of the studies in this area have been on malignant melanoma and neuroblastoma. Following malignant transformation, normal skin melanocytes, which initially express mainly G_{M3}, [99], begin to synthesize large amounts of G_{D3} [99–101]. Smaller amounts of G_{M2} and G_{D2} are also synthesized in many melanomas and in a small proportion of tumors become major components [102]. It has been known for some time that some people have naturally-occurring anti-ganglioside antibodies in their sera and that melanoma patients with such antibodies have a better survival rate than those without (Figure 4). Based on this information, ganglioside-based vaccines to melanoma have been tested by Livingston and coworkers [103] and Irie and coworkers in melanoma patients [104]. As G_{D3} is apparently poorly immunogenic in humans (in contrast to mice), most of the emphasis has thus far been on G_{M2} or whole cell vaccines. A vaccine consisting of G_{M2} and BCG as adjuvant has reached a Phase III trial in melanoma patients [105]. The majority of the vaccinated patients (50/58) produced anti-G_{M2} antibodies, mostly of the IgM class. Patients who produced anti-G_{M2} antibodies (whether vaccine-induced or naturally-occurring) had a significantly longer disease-free and overall survival than patients who showed no antibody response. Three approaches are currently being explored to improve on these results: (i) the use of conjugate vaccines e.g., G_{M2}-keyhole limpet haemocyanin, which would provide more potent T-cell help and also induce IgG antibodies [106]; (ii) the use of more effective adjuvants, e.g., QS21, a saponin derivative [107]; and (iii) the use of other gangliosides expressed on melanoma e.g., 9-*O*-acetyl G_{D3} or G_{D3} [108]. As mentioned above, G_{D3} is poorly immunogenic in

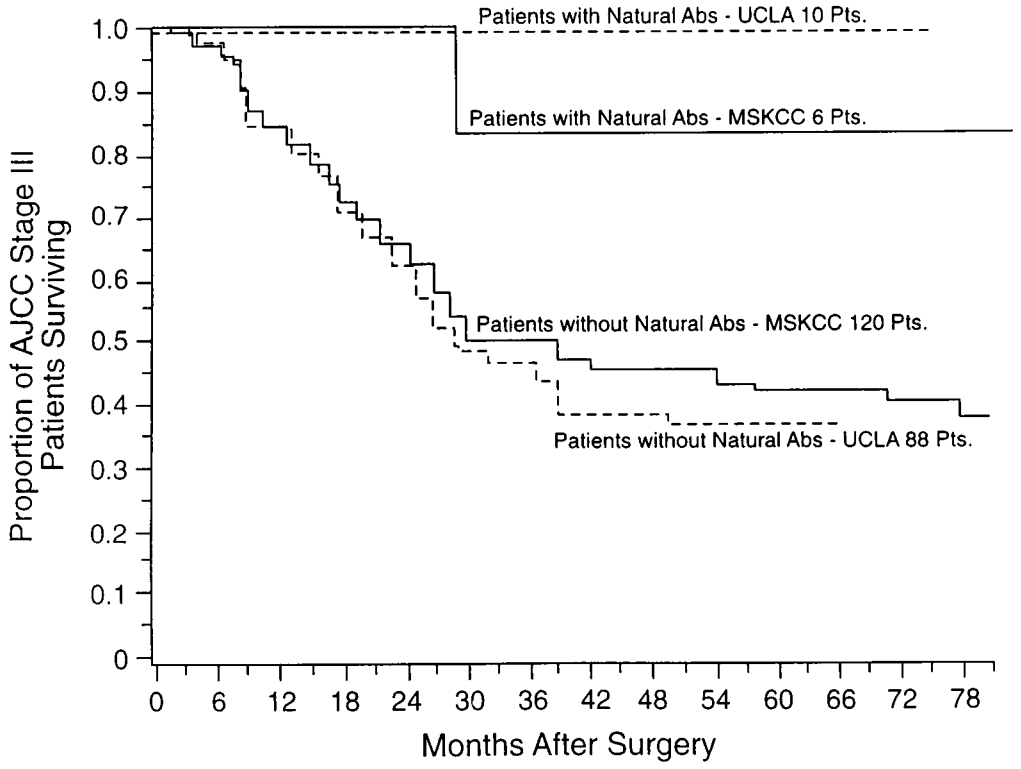

Figure 4. Survival curves for melanoma patients treated with tumor vaccines at Memorial Sloan-Kettering Cancer Center (MSKCC) [105] and UCLA School of Medicine (UCLA) [121] correlated with levels of anti-GM2 antibodies, some of which are naturally-occurring. Reprinted, with permission, from [122].

humans, but it has recently been found that immunization with G_{D3} lactone induces an anti-G_{D3} response [109]. This result is reminiscent of the demonstration by Nores *et al.* [110] that immunization of mice with G_{M3} lactone produced anti-G_{M3} antibodies.

Passive therapy with anti-ganglioside antibodies has also been studied in melanoma and neuroblastoma. An early trial with the mouse anti-G_{D3} monoclonal antibody R24 produced significant responses in a number of patients [111]. In an extension of this approach to larger numbers of patients, consistent clinical responses were observed in about 20% of the patients treated. Although mAb R24 can lyse melanoma cells in the presence of human complement, the mechanism of the clinical effects is probably more complex, possibly involving induction of an inflammatory response [111]. An anti-GD2 antibody has similarly been used in neuroblastoma for diagnosis and therapy [112, 113]. Antibody-directed therapy is presently being modified by combining antibodies with cytokines, e.g., TNF-α [114], by the use of human antibodies [115] and by the development of anti-idiotype antibody vaccines [116, 117].

Conclusions

Although new gangliosides are continually being discovered, the majority of mammalian gangliosides have probably already been identified. Significant progress is being made on studying the enzymes synthesizing these structures and the genes that code for them. Nevertheless, many important questions concerning gangliosides remain to be answered. These include (i) how is the expression of genes coding for ganglioside synthases determined and what factors influence and control their expression in a developmental and tissue-specific manner, (ii) what are the functions of gangliosides and do specific gangliosides carry out specific functions, (iii) how does one explain the different ganglioside expression in the same organ in different mammalian species, and (iv) what is the biological significance of the altered ganglioside expression observed in many tumors?

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Received 5 October 1997, revised and accepted 19 November 1997