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MINI REVIEW

Aglycone modulation of glycolipid receptor function

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The aglycone has been largely ignored in consideration of glycoconjugate function. Evidence is reviewed which suggests that the role of the lipid in glycolipid carbohydrate function may be particularly significant. The lipid moiety can promote or reduce carbohydrate exposure of membrane glycolipids. Theoretical calculation has indicated that the plane of the plasma membrane can restrict the permitted conformations of a given glycolipid oligosaccharide. Thus the lipid moiety may influence the relative conformation of such carbohydrate sequences. Evidence of ceramide regulation of glycolipid function can be found in studies of enzyme substrate specificity, antiglycolipid recognition and bacterial/host cell interactions. Studies of verotoxin binding to its glycolipid receptor globotriaosyl ceramide indicate that modulation of receptor function by glycolipid fatty acid content plays an important role in *in vitro* binding assays, cell cytotoxicity and intracellular routing.

Keywords: carbohydrate conformation, glycolipid heterogeneity, verotoxin binding

Introduction

Despite the fact that it is often stated that carbohydrates are ideal information molecules due to their possible linkage positions, sugar types, rotamers, etc., it is readily apparent that this informational potential is minimally utilized in biology. For glycoproteins, basic core structures for O-linked [1] and N-linked high mannose or lactosamine oligosaccharides [2] are invariant and differences are restricted to incomplete synthesis and extent of branching and termination reactions [3, 4]. For glycolipids, despite the fact that more than 300 structures have been described [5], the major cellular glycolipids are restricted to up to five sugar residues, comprising neutral species restricted to four or five core structures, and a simple series of gangliosides, only the proportion of which varies from cell type to cell type [6]. While it can be argued that minor species serve crucial receptor functions in individual cells of restricted frequency, most of the receptor functions for glycolipid carbohydrates which have been described apply only to these common core short chain glycolipid sequences [7]. Indeed it could be argued that the lipid moiety of these short sugar chain glycolipids plays the major role in their character. During solvent extraction, despite the 'equimolarity' of the lipid and sugar moieties, these species preferentially partition into the organic lower Folch phase as a result of the higher mass of the lipid moiety. They do not form bilayer structures in the upper phase. They do not 'line up' at the interphase, as they could be considered to do in a membrane bilayer for example. Thus such glycolipids in a membrane might be considered to be below their thermodynamic optimum. Such a strained relationship might be expected to have significant consequences on the sugar (and the lipid?) moiety.

Our studies on verotoxin binding to the glycolipid globotriaosyl ceramide(galactose α1-4galactoseβ1-4glucoseβ1-1ceramide {Gb_3}) [8] provide strong support for this concept.

Glycolipid receptors

Primarily glycolipid receptors have been shown to function in bacterial/host interactions [9–12], and speculated to provide the basis and specificity for the initial attachment of the parasite to the host mucosal cell surface. Such interactions as described have been essentially

limited to recognition of sulfatide [13–21], ganglio series [22–28] and globo series [29–34] core glycolipids. The question arises whether all these bacterial adhesins which recognize the same carbohydrate structure, recognize the same features on that carbohydrate sequence, and if not, does the recognition discriminate between conformational isoforms of a given carbohydrate structure? Several studies suggest that this may be so and that it is the lipid component of the glycolipid which generates such heterogeneity for a single sugar sequence. In the case that has been studied in greatest depth thus far: that of recognition of the galabiose moiety of globoseries glycolipids by *E. coli* PapG pili, *S. suis* and verotoxin, different epitopes on the disaccharide are recognized [35, 36] (Lingwood unpublished).

Theoretical effect of the lipid moiety on GL carbohydrate

Nyholm and Pascher have calculated that the plane of the plasma membrane can have a significant effect in restricting the number of minimum energy conformations available to a given membrane bound glycolipid oligosaccharide [37, 38]. Many studies on glycolipid function within model membranes have demonstrated the influence of the lipid moiety in relation to that of the phospholipid bilayer, such that the relative discrepancy between the chain lengths of the phospholipids and glycolipids within the bilayer can promote or reduce what has been termed 'exposure' [39–41]. Such changes in exposure might be reconsidered in light of Nyholm's calculations to imply that the conformation of the carbohydrate relative to the membrane may be altered according to both the fatty acid and phospholipid acyl chain length.

Biological effects

CARBOHYDRATE PRESENTATION

Several examples in the literature have suggested that the lipid component of glycolipids is important in the carbohydrate function. The balance between fatty acid chain length and that of the phospholipids within the membrane itself can either promote or decrease glycolipid carbohydrate exposure, for example, to antibody binding [39, 40, 42] or galactose oxidase action [41]. NMR has indicated that such changes in 'exposure' for ligand binding are in fact changes in the motional freedom of the glycolipid in the bilayer [43].

Proprioni bacteria binding to lactosylceramide has been shown to be able to distinguish between highly hydroxylated and less hydroxylated ceramide structures [44]. Similarly, the binding of P-pili variants to Galα-1-4Gal containing glycolipids has been shown to depend on the manner in which the glycolipid is displayed, ie. solid phase or within artificial or natural membranes [45], thereby indicating a role for the nature of the local environment in glycolipid/carbohydrate receptor function.

ENZYME SUBSTRATE SPECIFICITY

Direct evidence for a role of the lipid moiety of glycolipids in enzyme substrate preference was obtained in a study comparing the susceptibility of galactosylceramide and galactosylglycerolipid to galactose oxidase [46]. Even in substrate mixtures, the enzyme was only able to oxidize the galactose moiety of the glycosphingolipid as opposed to the glycoglycerolipid species. Fatty acid analysis of different glycosyl glycolipid transferase products from common glycolipid substrates indicates that such transferases can show preference for the fatty acid isoform of the substrate [47].

ANTIGLYCOLIPID ANTIBODIES

Monoclonal anti lactosyl ceramide (LC) antibody discriminates between LC containing different fatty acids in the ceramide moiety [48]. Antigalactosyl ceramide antibodies required antigen fatty acid hydroxylation for reactivity [49]. Similarly hydroxylation of gangliotriaosyl ceramide fatty acid was also found to promote the binding of a monoclonal anti Gg_3 antibody to lymphoma cells [50]. Despite equal chemical levels, cells containing Gg_3 lacking hydroxylated fatty acid showed low antigenicity.

IMMUNOSUPPRESSION

Gangliosides have been long implicated as modulators of the immune response and ganglioside shedding by tumour cells has been implicated in the mechanism by which tumours evade immune surveillance [51]. Ganglioside shedding by tumour cells has been found to be non random both in terms of carbohydrate sequence and in terms of the fatty acid heterogeneity of the ceramide moiety [52]. GD2 is preferentially shed by neuroblastoma tumour cells [53, 54] and GD2 species of shorter fatty acid chain length are selectively released [55]. More recent studies have indicated that the suppressive effect of shed gangliosides on immune responses *in vitro* is dependent not only on the carbohydrate but also the fatty acid chain length fatty acid [53, 56]. Ladisch showed that short fatty acid containing GD2 ganglioside was more effective in preventing lymphocyte activation *in vitro* [56]. The presence of sialic acid was essential but the ceramide structure itself was not crucial since an appropriate carbohydrate linked to a synthetic lipid species was also effective [57]. The authors conclude that the chain length of the lipid moiety was able to promote the availability of

the carbohydrate epitope necessary for inhibition of immune cell function.

These precedents set the scene for our studies on the interaction of verotoxin (VT) with Gb_3 and its role in human disease.

VEROTOXIN INDUCED PATHOGENESIS

Studies on VT binding indicate that the Gb_3 fatty acid composition plays two important roles in VT induced pathogenesis. Firstly, in determining the receptor binding affinity and capacity of the different members of the verotoxin family and secondly, in the intracellular routing of the toxin once internalized into susceptible cells by receptor mediated endocytosis (RME).

Verotoxin induced clinical pathology

Verotoxins comprise a family of *E. coli* derived subunit toxins containing one 30 kDa A-subunit, which inhibits protein synthesis [58], non-covalently associated with a B-subunit (7.5 kDa) pentamer responsible for receptor binding [59].

Gastrointestinal infection with verotoxin producing *E. coli* (VTEC) is responsible for haemorrhagic colitis and the haemolytic uraemic syndrome (HUS-primarily a pathology of renal endothelial cells) which may follow [60–63]. There are four major members of the verotoxin family: VT1, VT2 and VT2c which have been isolated from HUS patients, while VT2e is associated with oedema disease of pigs [8]. It is likely that glycolipid expression during renal ontogeny provides an explanation for the age related incidence of HUS following VTEC infection [64]. All VTs bind the glycolipid Gb_3 [65–70] (in addition VT2e binds globotetraosyl ceramide [Gb_4] [69]), yet VT2 is more frequently associated with human disease than VT1 (VT2c is uncommon) [71–73].

The classical scheme for their mechanism of action in cell killing is shown in Fig. 1.

Recent evidence *in vitro* indicates that VT2 shows preferential cytotoxicity for human renal microvascular endothelial cells *in vitro* as compared with VT1 [74]. The binding affinity of VT2c for Gb_3 is considerably less than that of VT1 [75] and the cytotoxicity of VT2c is, for most cells, between 500 and 1000-fold lower than VT1 [76] and this difference is removed following site specific mutagenesis of the B subunit (Asn16 in VT2c to

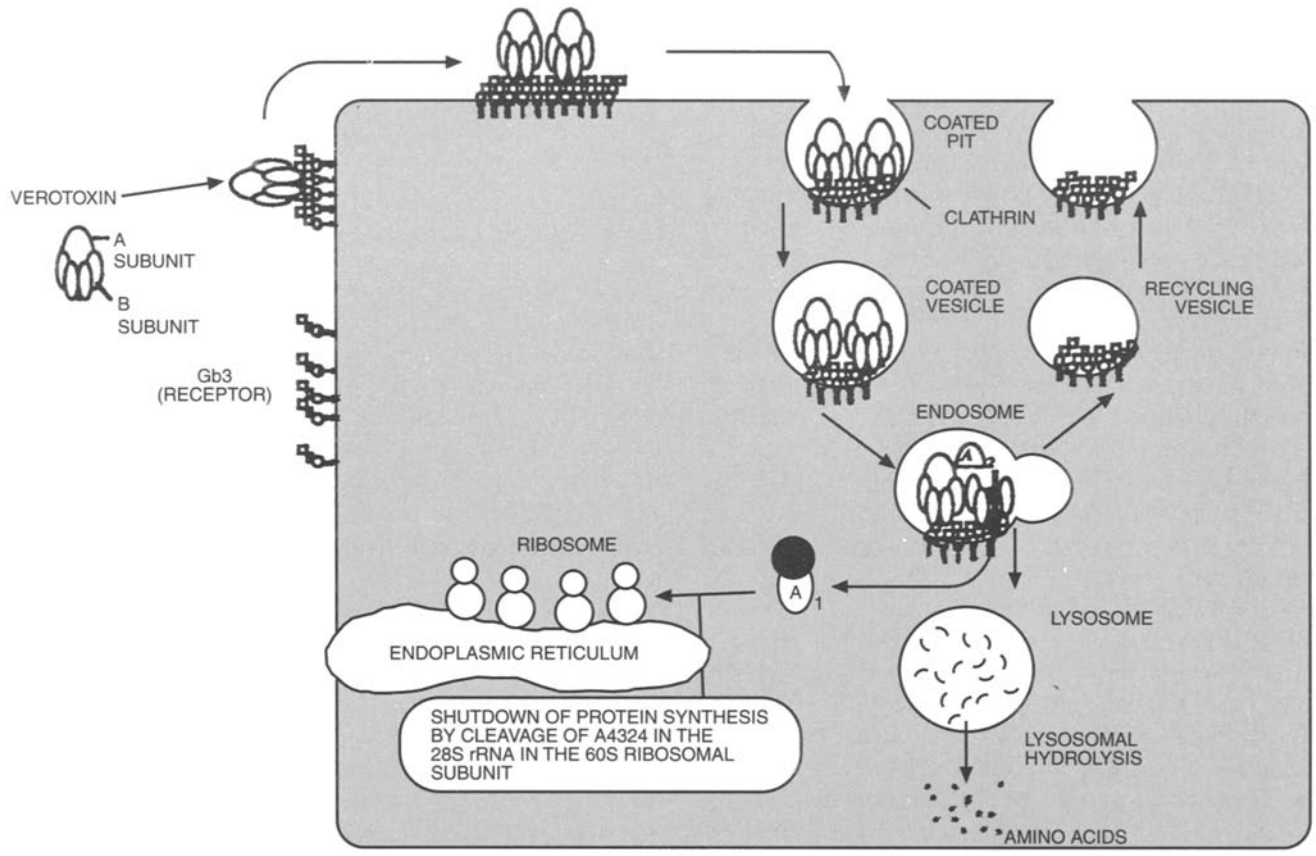

Figure 1. Receptor mediated endocytosis of verotoxin.

Asp found in VT1) [77]. However pathology induced in the rabbit animal model indicates that VT2c has a more severe effect on the gastrointestinal microvasculature than does VT1 [78], whereas VT2c has less pronounced effects on the CNS compared to VT1 [79].

Thus these toxins show differences in cytotoxicity which are not consistent from animal model to cell culture or between different cells *in vitro*. The A subunits are equally effective at inhibiting protein synthesis in an *in vitro* translation system [76] and we have shown that the B subunit is responsible for the difference in Gb_3 binding, cytotoxic specific activity [76] and animal model pathology [79] observed between VT2c and VT1. In addition, although the presence of Gb_3 is required for toxin sensitivity *in vitro*, relative cell sensitivity is not necessarily a function of Gb_3 concentration [80].

The question then arises: how can these toxins which bind to the same receptor, albeit with different affinities, show differential effects according to cell type targeted and why is Gb_3 in one cell type not necessarily equivalent to that of another?

Gb_3 fatty acid heterogeneity-role in VT binding

These studies together indicate heterogeneity in the receptor function of Gb_3 within the plasma membrane of various cell types. We have begun to investigate whether fatty acid heterogeneity of membrane bound Gb_3 is in any way responsible for this variation in receptor function. Preliminary studies in *in vitro* binding assays demonstrated that heterogeneous fatty acid composition of Gb_3 promoted VT1 binding [81]. Reduced binding was observed for semisynthetic Gb_3 species containing a single fatty acid moiety. Mixing two homogeneous species of different fatty acid chain length resulted in increased toxin binding as a function of the mixing ratio. In a phospholipid matrix, increased toxin binding was a function of increased fatty acid chain length [81] to a maximum whereupon further extension reduced binding. This reduction was not seen for susceptibility to galactose oxidase [81] suggesting this was not just a case of decreased 'exposure'. In subsequent studies [82], we prepared 14 different fatty acid isoforms of Gb_3 from C12 to C24 in both saturated and unsaturated forms and investigated their receptor binding capability for VT1, VT2c [82] and VT2 [83]. Using a binding assay based on glycolipids immobilized in a phospholipid matrix in a microtitre plate, we showed that there was a lower limit to the fatty acid chain length for effective Gb_3 receptor function. Species containing C14 fatty acids and shorter were barely recognized by any verotoxin species. For VT1, binding increased as a function of increasing chain length to a maximum of C20 to C22 when binding was decreased on further hydrocarbon extension. In contrast, the binding of VT2c was considerably lower to all Gb_3 fatty acid isoforms but showed a maximum at C18. Thus VT1 and VT2c preferentially bound *in vitro* to different fatty acid isoforms of Gb_3. VT2 showed a similar Gb_3 fatty acid isoform binding preference to VT2c but showed overall higher affinity and was able to compete VT1 binding C22 Gb_3, unlike VT2c [83]. In general, binding was also increased for the unsaturated versus the saturated species for a given chain length.

When Gb_3 negative cells were reconstituted with Gb_3 species of different fatty acid chain length, the ability to reconstitute toxin sensitivity correlated with the binding *in vitro*, suggesting that the selective binding of Gb_3 fatty acid isoforms can have a direct impact on cell sensitivity to verotoxin.

From theoretical calculation, conformational energy minima of globo series glycolipid oligosaccharides have been shown to be influenced by interactions with the surrounding membrane layer [37, 38] which in turn, are a function of the discrepancy between the alkyl chain length of the glycolipid and that of the surrounding phospholipid environment [40–42]. It follows therefore, that in a plasma membrane, the favoured conformations available to a given glycolipid could be a function of the fatty acid chain length. Thus within a phospholipid matrix, changing the fatty acid of a glycolipid may alter the relative occupancy of a given carbohydate conformation. If the different members of the verotoxin family recognize different epitopes of the galabiose structure, which are differently accessible in different conformations of the globotriaose glycolipid, this might explain the selective binding of VT1 and VT2c to different Gb_3 fatty acid isoforms.

Two receptor binding sites for VTs?

In competition binding assays VT2c was an ineffective inhibitor of VT1 binding to the VT1 preferred Gb_3 isoform. However, VT2c was a more efficient inhibitor of VT1 binding to the VT2c preferred Gb_3 isoform than was VT1 [82]. This effect was difficult to reconcile with a simple binding model. Our subsequent theoretical model to delineate possible Gb_3 binding sites within the B subunit pentamer using molecular modelling calculations, provided a potential explanation. These calculations indicated there were two possible receptor binding sites per B subunit monomer [84]. The first site was the so-called cleft site, similar to that proposed in the original description of the crystal structure [85], and involving Phe 30, the Asp triplet, Gly 60 and Thr 21. The second site was in the form of a shallow depression on the surface of the pentamer which contacts the plasma membrane surface on the other side of Phe 30. Receptor occupancy of the cleft site is compromised for VT2c since Asp 16 is an asparagine residue in this toxin. Mutation of this residue to aspartic acid results in the dramatic

increase in cytotoxicity for the resulting recombinant toxin [77].

The possibility of the existence of two sites per B subunit monomer provides an explanation for the competition data we obtained using the Gb_3 fatty acid homologues if different carbohydrate conformers were preferentially expressed by the different fatty acid analogues and different carbohydrate conformers were preferentially bound to these two distinct binding sites. In computer docking simulations, it was found that conformer 2 preferentially docked into the cleft site, while conformer 6 could be best accommodated in the second site [84]. These studies therefore suggest that the fatty acid component of Gb_3 can determine which of these sites can be occupied. We would speculate that the C18 fatty acid species preferentially recognized by VT2c preferentially docks into the second site while the remainder of the Gb_3 fatty acid homologues dock into the cleft site. Differences in the Gb_3 fatty acid isoform binding preference between VT1 and VT2c could well explain the different cytotoxicity and *in vivo* pathology observed between these two toxins. In this regard, VT2 shows a hybrid phenotype between that of VT1 and VT2c [83]. This perhaps correlates with the observed increased association of VT2 with HUS [71–73] and with the fact that VT2 is of equal or even higher specific cytotoxicity than VT1 for human renal endothelial cells in culture [74].

Effect of the Gb_3 lipid moiety varies according to method of receptor assay

The importance of the lipid moiety in glycolipid receptor function also varies according to the manner in which the glycolipid is immobilized. The differences we have observed in VT1 receptor binding as a function of fatty acid chain length in a phospholipid matrix were not observed when binding was assayed by TLC overlay [82]. Nevertheless, the lipid moiety can still play a significant role when glycolipids are immobilized in this fashion for binding assays. The magnitude of the effect the lipid component can have on the 'availability' of a given carbohydrate of a glycolipid for receptor ligand binding was illustrated in recent studies in which verotoxin was tested for binding to a series of synthetic galabiose glycolipids immobilized directly on TLC or within a phospholipid cholesterol matrix [86, 87]. Galabiose glycolipids containing one or two short hydrocarbon chains (C9) were not recognized by verotoxin. This is consistent with the lack of inhibition of VT by the free globotriaose oligosaccharide from Gb_3 [67, 88]. In TLC overlay, galabiose or globotriaose coupled to a single long chain alkyl hydrocarbon (C16) was most effectively bound, more than human renal Gb_3 standard. However, the addition of a second hydrocarbon chain to the oligosaccharide moiety completely eliminated toxin binding as monitored by this assay. Inclusion of an oxygen in the alkyl chain near the head group did permit weak toxin recognition. When these same galabiose analogues were assayed for verotoxin binding in a lipid matrix, the exact reverse was found, in that those analogues containing a single alkyl hydrocarbon chain were not recognized (in a manner similar to deacylated Gb_3 [81]), whereas the double chain species, particularly those containing the oxygen moiety, were effective receptors, equivalent to Gb_3 of a similar fatty acid chain length. Similarly galabiosyl ceramide is bound in preference to Gb_3 by VT1, VT2 and VT2c as monitored by TLC overlay [87, 89] but galabiosylceramide is a far less effective receptor than Gb_3 for these toxins when assayed in a phospholipid matrix [87].

This difference in glycolipid function according to method of glycolipid immobilization is further compounded by the continued use of polyisobutyl methacrylate treatment of the TLC plate prior to assay of binding, despite demonstrated artefacts arising from this unnecessary chemical treatment [90].

Since the plane of the plasma membrane can affect the repertoire of conformations of the carbohydrate available to glycolipid [38], it was not surprising to find that the phospholipid chain length within a phospholipid bilayer also affects the relative ability of Gb_3 to bind verotoxins [91]. For VT1 and VT2, the ability to bind Gb_3 in liposomes or in the immobilized microtitre plate phospholipid matrix, was inversely related to the phospholipid chain length. A similar result was found for VT2c in the phospholipid matrix but no linear relationship was observed in liposomes where binding was enhanced only in the presence of C18 phosphatidyl choline.

Verotoxin internalization – role of Gb_3 fatty acid heterogeneity?

Verotoxin has a similar mode of catalytic action to that of ricin in that the A subunit is an N-glycanase which cleaves a specific adenine base in ribosomal RNA [58]. The A subunit is entirely innocuous in the absence of the B subunit, which mediates endocytosis via receptor recognition. Recent evidence indicates that the fatty acid composition of Gb_3 not only determines the binding characteristics of the different verotoxins as discussed above, but also plays a role in the routing of the toxin once inside the cell. Verotoxin is the only glycolipid binding ligand to be internalized by receptor mediated endocytosis via clathrin coated pits [92, 93]. Traditionally, this internalization pathway is thought to be mediated by submembrane components [94] which direct the bound receptor to a single cellular pole or cap for subsequent internalization [95]. Gb_3 is embedded only in the outer leaflet of the plasma membrane bilayer, and the mechan-

ism by which toxin bound Gb_3 communicates with such a submembrane network is unknown. However, inhibitors of transglutaminase activity which prevent submembrane cytoskeletal assembly for receptor mediated endocytosis (RME) prevent verotoxin internalization [96]. Receptor bound verotoxin is internalized within 30–60 min at 37 °C. In highly toxin susceptible cells, the verotoxin is then targeted by a process of 'retrograde transport' from an endosomal compartment to the Golgi, rough endoplasmic reticulum and nuclear membrane [96, 97]. This targeting is dependent on Gb_3 binding since the B subunit alone is internalized by the same intracellular trafficking pathway [93, 96]. The B subunit contains none of the known amino acid sequences required for ER retention.

The classical pathway for this class of toxins involves internalization into an acid endosomal compartment, a B subunit conformational change which facilitates the translocation of the A subunit from this compartment to the cytosol [98, 99] for subsequent inhibition of protein synthesis [58, 100]. Evidence is accruing that cleavage of the A subunit to effect translocation to the cytosol may not be necessary for verotoxin induced cell killing [101]. In cells of marginal sensitivity to VT, internalization by this route may occur. Culture of such cells however with sodium butyrate can result in the induction of the retrograde transport pathway and a corresponding induction of an increase in sensitivity to verotoxin [97]. Sandvig has shown that this induction of the retrograde transport pathway is concomitant with a change in the fatty acid chain length of the Gb_3 synthesized [93]. Thus it is possible that the fatty acid composition determines the intracellular routing of verotoxin. Our current studies on the anti-neoplastic effect of VT [102] support this hypothesis. It is of interest to note that subcellular organelle targeting of proteins can also be based on acylation with fatty acids of different chain length [103].

Gb_3 mediated signal transduction?

Gb_3 is a human B cell differentiation marker (CD77) [104, 105] and we have shown that verotoxin selectively targets IgG committed B cells *in vitro* [80]. We have also shown that two B cell differentiation antigens contain amino acid sequence similar to that of the VT B subunit and have demonstrated that cell surface Gb_3 is necessary for the signal transduction of these two proteins (α2interferon receptor{IFNAR} [106, 107] and CD19 [108]). The role Gb_3 plays in the signal transduction mediated via these two cell surface receptors may relate to the retrograde transport pathway for Gb_3. B cells defective in Gb_3 are compromised in α2interferon signalling to activate α2interferon sensitive transcription factors for growth inhibition [107] and in CD19 signalling to mediate B cell homotypic adhesion [108]. Both these properties can be restored by reconstituting cells with exogenous Gb_3 [108, 109]. It is of interest to note that reconstituting cells with Gb_4 did not restore these signal transduction pathways despite the fact that both CD19 and IFNAR bind both Gb_3 and Gb_4 *in vitro* [108]. Therefore it is likely that retrograde transport pathway is restricted to Gb_3. We therefore propose that there is an endosomal compartment in which Gb_3 is sorted according to fatty acid composition. Alternatively Gb_3 sorting may occur on the cell surface since VT1 and VT2c are differentially sensitive to inhibitors of transglutaminase activity to prevent RME [110]. Targeting to the Golgi, ER and particularly, nuclear membrane, may allow the activation of pathways responsible for the initiation of apoptosis by the VT B subunit [111], and mediate IFNAR and CD19 signal transduction.

Gb_3 retrograde transport model

A model for this retrograde transport pathway is proposed in Fig. 2. Two possible mechanisms are shown. A sorting protein is proposed which recognizes the different fatty acid dependent Gb_3 conformations in a manner similar to the different verotoxins as reviewed above. An alternate possibility is that the fatty acid chain length itself is responsible for the differential intracellular routing. It has been shown that Golgi membrane bilayer is of a reduced cross section than the plasma membrane bilayer and it has been suggested that this property is used to retain resident Golgi enzymes in the Golgi by limiting the span of the hydrophobic transmembrane segment [112]. While there may be more tolerance in accommodating glycolipids whose fatty acid chain lengths do not match the overall dimensions of the phospholipid bilayer, it is possible that the reduced bilayer width could influence Gb_3 packing and intracellular distribution. Gb_3 in which the fatty acid and sphingosine alkyl chain length are discrepant may interdigitate across the bilayer, as can occur for asymmetric phospholipids in model membranes [113, 114]. An arrangement whereby the longer chain of asymmetric phospholipids in the cytosolic leaflet of the bilayer might partially interdigitate for example, to compensate for a reduced Gb_3 fatty acid chain length, might accommodate the reduced dimensions of the Golgi/ER membranes. Such an interaction could allow transmembrane signalling for a Gb_3-binding ligand. Moreover, as the plane of the plasma membrane has an effect on the surface conformation of glycolipids, so the altered dimension of the Golgi membranes may result in an altered carbohydrate conformation during retrograde transport. Evidence of such an effect is provided by the finding that the VT2c binding preference for C18:1 fatty acid containing Gb_3 is switched to C22:1 in liposomes comprised of C14 as opposed to C16 phosphatidyl choline [91].

Both models have their deficiencies. If sorting is based on packing, retrograde transport should apply to any glycolipid and if based on a sorting recognition signal,

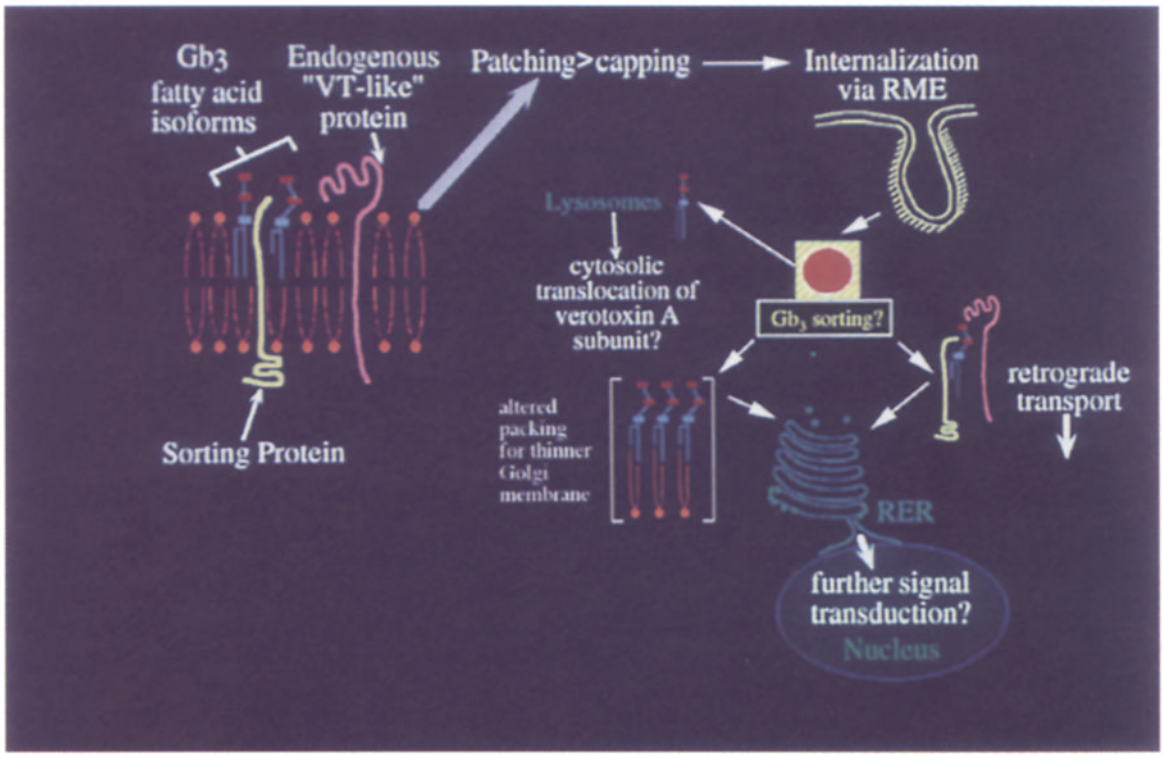

Figure 2. Model for retrograde transport. Exogenous (e.g. VT) or endogenous (e.g. IFNAR or CD19) ligand binding to cell surface Gb_3 results in internalization of the complex by patching and RME via clathrin coated pits at a single pole. Gb_3 sorting according to fatty acid chain length occurs from an endosomal compartment for either lysosomal transit (for e.g. processing VT via classical pathway) or retrograde transport, either by interaction with a sorting protein which recognizes different fatty acid dependent Gb_3 conformers or by altered Gb_3 packing (e.g. interdigitation?) to accomodate the reduced dimensions of the Golgi membrane.

this implies that VT-bound Gb_3 can be bound by a second ligand. Our current studies are attempting to define and distinguish between these alternatives.

Conclusions

The function of glycolipid carbohydrate may be modulated by a mechanism not available to other glycoconjugates viz. influence of the lipid moiety and the bilayer microenvironment on conformation and receptor function. A major consequence is that *in vitro* assay of glycolipid receptor function can vary dramatically according to method of assay and care must therefore be exercised in the interpretation of such data.

The influence of the lipid moiety of membrane glycolipids allows the differential presentation of epitopes on the same carbohydrate sequence to permit multiple ligand binding, thereby increasing the receptor repertoire of a given sugar sequence. The fatty acid component may also influence glycolipid routing within the cell. This may modify the cellular effects of exogenous glycolipid binding ligands and provide a signal transduction pathway for endogenous species.

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References

1. Schachter H, Brockhausen E (1992) In *Glycoconjugates: Composition, Structure and Function* (Allen HJ, Kisailus EC, eds) pp. 263–332. New York: Marcel Dekker, Inc.
2. Schachter H (1984) *Clin Biochem* **17**: 3–14.
3. Kobata A (1992) *Eur J Biochem* **209**: 483–501.
4. Brockhausen I (1993) *Crit Rev Clin Lab Sci* **30**: 65–151.
5. Stults CLM, Sweeley CH, Macher BA (1989) *Methods Enzymol* **179**: 167–214.
6. Hakomori S-I (1981) *Ann Rev Biochem* **150**: 733–64.
7. Lingwood CA (1992) *Curr Opin Struct Biol* **2**: 693–700.
8. Lingwood CA (1993) In *Sphingolipids Part A. Functions and Breakdown Products* (Bell R, Hannun YA, Merrill A Jr, eds) *Advances In Lipid Research* **25**, pp. 189–212. San Diego: Academic Press.
9. Karlsson K (1986) *Chem Phys Lipids* **42**: 153–72.
10. Strömberg N, Deal C, Nyberg G, Normark S, So M, Karlsson

K-A (1988) *Proc Natl Acad Sci USA* **85**: 4902–6.
11. Strömberg N, Ryd N, Lindberg AA, Karlsson K-A (1988) *FEBS Lett* **232**: 193–98.
12. Krivan HC, Plosila L, Zhang L, Holt V, Kyogashima M (1992) In *Microbial Adhesion and Invasion* (Hook M, Switalski L, eds) pp. 1–13. New York: Springer-Verlag.
13. Pancake SJ, Holt GD, Mellouk S, Hoffman SL (1992) *J Cell Biol* **a117**: 1351–57.
14. Lingwood CA, Schramayr S, Quinn P (1990) *J Cell Physiol* **142**: 170–76.
15. Lingwood CA, Quinn PA, Wilansky S, Nutikka A, Ruhnke H, Miller RB (1990) *Biol Reprod* **43**: 694–97.
16. Boulanger J, Faulds D, Eddy EM, Lingwood CA (1995) *J Cell Physiol* **165**: 7–17.
17. Krivan H, Olson M, Barile M, Ginsburg V, Roberts D (1988) *J Biol Chem* **264**: 9283–88.
18. Olson LD, Gilbert EA (1993) *J Bacteriol* **175**: 324–27.
19. Prasadarao NV, Wass CA, Hacker J, Jann K, Kim KS (1993) *J Biol Chem* **268**: 10356–63.
20. Saitoh T, Sugano K, Natomi H, Zhao W, Okuzumi K, Iwamori M, Yazaki Y (1992) *Eur J Gastroenterol Hepatol* **4**: S49–S53.
21. Brennan MJ, Hannah JH, Leininger E (1991) *J Biol Chem* **266**: 18827–31.
22. Krivan HC, Roberts DD, Ginsburg V (1988) *Proc Natl Acad Sci USA* **85**: 6157–61.
23. Krivan HC, Ginsburg V, Roberts DD (1988) *Arch Biochem Biophys* **260**: 493–96.
24. Ramphal R, Carnoy C, Fievre S, Michalski J-C, Houdret N, Lamblin G, Strecker G, Roussel P (1991) *Infect Immun* **59**: 700–4.
25. Saiman L, Prince A (1993) *J Clin Invest* **92**: 1875–80.
26. Srnka CA, Tiemeyer M, Gilbert JH, Moreland M, Schweingruber H, Delappe BW, James PG, Gant T, Willoughby RE, Yolken RH, Nashed MA, Abbas SA, Laine RA (1992) *Virology* **190**: 794–805.
27. Paruchuri DK, Seifert HS, Ajioka RS, Karlsson K-A (1990) *Proc Natl Acad Sci USA* **87**: 333–37.
28. Yu L, Lee KK, Sheth HB, Lane-Bell P, Srivastava G, Hindsgaul O, Paranchych W, Hodges RS, Irvin RT (1994) *Infect Immun* **62**: 2843–48.
29. Strömberg N, Marklund B-I, Lund B, Ilver D, Hamers A, Gaastra W, Karlsson K-A, Normand S (1990) *EMBO J* **9**: 2001–10.
30. Bock E, Breimer ME, Brignole A, Hansson GC, Karlsson K-A, Larson G, Leffler H, Samuelsson BE, Strömberg N, Svanborg Edén C (1985) *J Biol Chem* **260**: 8545–51.
31. Haataja S, Tikkanen K, Liukkonen J, François-Gerard C, Finne J (1993) *J Biol Chem* **268**: 4311–17.
32. Brown KE, Anderson SM, Young NS (1993) *Science* **262**: 114–17.
33. Lindstedt R, Baker N, Falk P, Hull R, Hull S, Karr J, Leffler H, Svanborg Edén C, Larson G (1989) *Infect Immun* **57**: 3389–94.
34. Chatterjee S, Khullar M, Shi W (1995) *Glycobiology* **5**: 327–33.
35. Kihlberg J, Hultgren S, Normark S, Magnusson G (1989) *J Am Chem Soc* **111**: 6364–68.
36. Haataja S, Tikkanen K, Nilsson U, Magnusson G, Karlsson K, Finne J (1994) *J Biol Chem* **269**: 27466–72.
37. Nyholm P-G, Pascher I (1993) *Biochemistry* **32**: 1225–34.
38. Nyholm P-G, Pascher I (1993) *Int J Biol Macromol* **15**: 43–51.
39. Stewart RJ, Boggs J (1990) *Biochemistry* **29**: 3644–53.
40. Crook SJ, Boggs JM, Vistnes AI, Koshy KM (1986) *Biochemistry* **25**: 7488–94.
41. Stewart RJ, Boggs JM (1993) *Biochemistry* **32**: 5605–14.
42. Alving CR, Urban KA, Richards RL (1980) *Biochem Biophys Acta* **600**: 117–25.
43. Hamilton KS, Briere K, Jarrell HC, Grant CWM (1994) *Biochim Biophys Acta* **1190**: 367–75.
44. Strömberg N, Karlsson KA (1990) *J Biol Chem* **265**: 11244–50.
45. Strömberg N, Nyholm P-G, Pascher I, Normark S (1991) *Proc Natl Acad Sci USA* **88**: 9340–44.
46. Lingwood CA (1979) *Can J Biochem* **57**: 1138–43.
47. Kannagi R, Nudelman E, Hakomori S (1982) *Proc Natl Acad Sci USA* **79**: 3470–74.
48. Symington FW, Bernstein ID, Hakomori S-I (1984) *J Biol Chem* **259**: 6008–12.
49. Nakakuma H, Ari M, Kawaguchi T, Horikawa K, Hidaka M, Sakamoto K, Iwamori M, Nagai Y, Takatsuki K (1989) *FEBS Lett* **258**: 230–32.
50. Kannagi R, Stroup R, Cochran NA, Urdal DL, Young Jr, WW, Hakomori S-I (1983) *Cancer Res* **43**: 4997–5005.
51. Dyatlovitskaya E, Bergelson L (1987) *Biochim Biophys Acta* **907**: 125–43.
52. Young WW, Borgman CA, Wolock D (1986) *J Biol Chem* **261**: 2279–83.
53. Ladisch S, Li R, Olson E (1994) *Proc Natl Acad Sci USA* **91**: 1974–78.
54. Ladisch S, Wu Z-L (1985) *Lancet* **i**: 136–38.
55. Li R, Ladisch S (1991) *Biochim Biophys Acta* **1083**: 57–64.
56. Ladisch S, Hasegawa A, Li R, Kiso M (1995) *Biochemistry* **34**: 1197–202.
57. Ladisch S, Hasegawa A, Ruixiang L, Makoto K (1994) *Biochem Biophys Res Commun* **203**: 1102–9.
58. Endo Y, Tsurugi K, Yutsudo T, Takeda Y, Ogasawara K, Igarashi K (1988) *Eur J Biochem* **171**: 45–50.
59. Fraser M, Chernaia M, Kozlov Y, James M (1994) *Struct Biol* **1**: 59–64.
60. Karmali MA (1989) *Clin Microbiol Rev* **2**: 15–38.
61. Griffin PM, Ostroff SM, Tauxe RV, Greene KD, Wells JG, Lewis JH, Blake PA (1988) *Ann Int Med* **109**: 705–12.
62. Griffin PW, Tauxe RV (1991) *Epidem Rev* **13**: 60–98.
63. Griffin PM, Bell BP, Cieslak PR, Tuttle M, Barrett TJ, Dlyle M, McNamara AM, Shefer AM, Wells JG (1994) In *Recent Advances in Verocytotoxin Producing E. coli Infections* (Karmali MA, Goglio A, eds) pp. 7–12. Amsterdam: Elsevier Sciences B.V.
64. Lingwood CA (1994) *Nephron* **66**: 21–28.
65. Jacewicz M, Clausen H, Nudelman E, Donohue-Rolfe A, Keusch GT (1986) *J Exp Med* **163**: 1391–404.
66. Lingwood CA, Law H, Richardson S, Petric M, Brunton JL, DeGrandis S, Karmali M (1987) *J Biol Chem* **262**: 8834–39.
67. Lindberg AA, Brown JE, Strömberg N, Westling-Ryd M, Schultz JE, Karlsson K-A (1987) *J Biol Chem* **262**: 1779–85.
68. Waddell T, Head S, Petric M, Cohen A, Lingwood CA (1988) *Biochem Biophys Res Commun* **152**: 674–79.
69. DeGrandis S, Law H, Brunton J, Gyles C, Lingwood CA

(1989) *J Biol Chem* **264**: 12520–25.
70. Keusch G, Jacewicz M, Acheson D, Donohue-Rolfe A, Kane A, McCluer R (1995) *Infect Immun* **63**: 1138–41.
71. Kleanthous H, Smith HR, Scotland SM, Gross RJ, Rowe B, Taylor CM, Milford DV (1990) *Arch Dis Child* **65**: 722–27.
72. Scotland SM, Willshaw GA, Smith HR, Rowe B (1987) *Epidem Inf* **99**: 613–24.
73. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM (1989) *J Infect Dis* **160**: 994–98.
74. Obrig TG, Louise CB, Lingwood CA, Daniel TO (1994) In *Recent Advances in Verocytotoxin Producing E. coli Infections* (Karmali MA, Goglio AG, eds) pp. 317–24. Amsterdam: Elsevier Sciences B.V.
75. Head S, Karmali M, Lingwood CA (1991) *J Biol Chem* **266**: 3617–21.
76. Head SC, Petric M, Richardson SE, Roscoe ME, Karmali M (1988) *FEMS Microbiol Lett* **51**: 211–16.
77. Lindgren SW, Samuel JE, Schmitt CK, O'Brien AD (1994) *Infect Immun* **62**: 623–31.
78. Richardson SE, Rotman TA, Jay V, Smith CR, Becker LE, Petric M, Olivieri NF, Karmali MA (1992) *Infect Immun* **60**: 4154–67.
79. Nelson S, Richardson S, Lingwood C, Petric M, Karmali M (1994) In *Recent Advances in Verocytotoxin Producing E. coli Infections* (Karmali MA, Goglio AG, eds) pp. 245–48. Amsterdam: Elsevier Sciences B.V.
80. Cohen A, Madrid-Marina V, Estrov Z, Freedman M, Lingwood CA, Dosch H-M (1990) *Int Immunol* **2**: 1–8.
81. Pellizzari A, Pang H, Lingwood CA (1992) *Biochemistry* **31**: 1363–70.
82. Kiarash A, Boyd B, Lingwood CA (1994) *J Biol Chem* **269**: 11138–46.
83. Kiarash A, Boyd B, Lingwood CA (1994) In *Recent Advances in Verocytotoxin Producing E. coli Infections* (Karmali MA, Goglio AG, eds) pp. 175–87. Amsterdam: Elsevier Sciences B.V.
84. Nyholm P-G, Brunton JL, Lingwood CA (1995) *Int J Biol Macromol* **17**: 199–205.
85. Stein PE, Boodhoo A, Tyrrell GJ, Brunton JL, Read RJ (1992) *Nature* **355**: 748–50.
86. Boyd B, Zhiuyan Z, Magnusson G, Lingwood CA (1994) *Eur J Biochem* **223**: 873–78.
87. Boyd B, Magnusson G, Kiarash A, Zhiuyan Z, Lingwood C (1994) In *Recent Advances in Verocytotoxin Producing E. coli Infections* (Karmali MA, Goglio AG, eds) pp. 167–70. Amsterdam: Elsevier Sciences B.V.
88. St Hilaire PM, Boyd MK, Toone EJ (1994) *Biochemistry* **33**, 14452–63.
89. Samuel JE, Perera LP, Ward S, O'Brien AD, Ginsburg V, Krivan HC (1990) *Infect Immun* **58**: 611–18.
90. Yiu SCK, Lingwood CA (1992) *Anal Biochem* **202**: 188–92.
91. Arab S, Lingwood CA *Glyconj J* (in press).
92. Sandvig K, Olnes S, Brown J, Peterson O, van Deurs B (1989) *J Cell Biol* **108**: 1331–43.
93. Sandvig K, Ryd M, Garred O, Schweda E, Holm PK (1994) *J Cell Biol* **126**: 53–64.
94. Smythe E, Warren G (1991) *Eur J Biochem* **202**: 689–99.
95. Lingwood CA (1994) In *Recent Advances in Verocytotoxin Producing E. coli Infections* (Karmali MA, Goglio AG, eds) pp. 131–37. Amsterdam: Elsevier Sciences B.V.
96. Khine AA, Lingwood CA (1994) *J Cell Physiol* **161**: 319–32.
97. Sandvig K, Garred O, Prydz K, Kozlov J, Hansen S, van Deurs B (1992) *Nature* **358**: 510–12.
98. Middlebrook JL, Dorland RB (1984) *Microbiol Rev* **48**: 199–221.
99. Hudson TH, Neville DM Jr (1987) *J Biol Chem* **262**: 16484–94.
100. Saxena SK, O'Brien AD, Ackerman EJ (1989) *J Biol Chem* **264**: 596–601.
101. Samuel JE, Gordon VM (1994) *J Biol Chem* **269**: 4853–59.
102. Farkas-Himsley H, Rosen B, Hill R, Arab S, Lingwood CA (1995) *Proc Natl Acad Sci USA* **92**: 6996–7000.
103. Robbins S, Quintrell N, Bishop JM (1995) *Mol Cell Biol* **15**: 3507–15.
104. Mangeney M, Richard Y, Coulaud D, Tursz T, Wiels J (1991) *Eur J Immunol* **21**: 1131–40.
105. Gregory CD, Tursz T, Edwards CF, Tetaud C, Talbot M, Caillou B, Rickinson AB, Lipinski M (1987) *J Immunol* **139**: 313–18.
106. Lingwood CA, Yiu SCK (1992) *Biochem J* **283**: 25–26.
107. Ghislain J, Lingwood CA, Fish EN (1994) *J Immunol* **153**: 3655–63.
108. Maloney MD, Lingwood CA (1994) *J Exp Med* **180**: 191–201.
109. Ghislain J, Lingwood C, Maloney M, Penn L, Fish E (1992) *J Interfer Res* **12**: s114.
110. Lingwood CA (1996) *Trends Microbiol* **4**: 147–53.
111. Mangeney M, Lingwood CA, Caillou B, Taga S, Tursz T, Wiels J (1993) *Cancer Res* **53**: 5314–19.
112. Bretscher M, Munro S (1993) *Science* **261**: 1280–81.
113. Huang C, Mason J (1986) *Biochim Biophys Acta* **864**: 423–70.
114. Morrow MR, Singh D, Lu D, Grant CWM (1993) *Biophys J* **64**: 654–64.