



Review

Globotriaosyl ceramide receptor function – Where membrane structure and pathology intersect

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ABSTRACT

The glycosphingolipid globotriaosyl ceramide, (Gal α 1-4Gal β 1-4 glucosyl ceramide-Gb₃) also known as CD77 and the P^k blood group antigen, is bound by both verotoxins and by the HIV adhesin, gp120. Gb₃ plays an important receptor role in VT induced hemolytic uremic syndrome (HUS) and HIV infection. The organization of glycolipids, including Gb₃, into lipid rafts is central to both pathologies. The fatty acid heterogeneity within the Gb₃ lipid moiety plays a central role in assembly within such ordered domains. Differential binding of verotoxins and gp120 to such Gb₃ isoforms in model and cell membranes indicates a significant role in the eventual pathogenic outcome. HUS may provide the first example whereby membrane Gb₃ organization provides a predictor for tissue selective *in vivo* pathology.

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1. Cell membrane lipid domains

Organization of membrane glycosphingolipids, and subsequently other lipids and membrane proteins into non-uniform domains has been an area of intense research interest leading to the concept of cell membrane 'lipid rafts' [1,2]. These domains are currently defined as nano-assemblies of sphingolipid, cholesterol and GPI anchored proteins which fluctuate on a subsecond time scale [3]. This concept has suffered through a period of controversy [4,5], centered on extrapolation from detergent resistance and model membrane studies, but is now emerging as a valid paradigm of physiological membrane structure [6–8] likely to prove of increasing relevance, particularly in intracellular traffic [9] and signaling [10]. In this view, the cellular membrane lipid matrix is likely to prove the dynamic frame on which much of the protein tapestry is threaded.

Initial studies on viral budding from cultured polarized epithelial cells showed a differential glycosphingolipid (GSL) distribution

in apical vs basolateral membranes [11,12], resulting from lipid sorting within the secretory pathway. This physically based lipid sorting may provide a more widespread basis of intercellular membrane trafficking routes. Membrane heterogeneity by rafts is thought to rely on the potential for selective lipid–lipid interaction. For *in vitro* model membrane systems, this is illustrated by the sterol-dependent phase separation of sphingolipids from the more unsaturated glycerophospholipids, clearly established for many years in model systems [13,14]. However model membranes do not equate to normal cell membrane physiology and the cellular correlates of the Lo (liquid ordered phase) and Ld (liquid disordered) phase have not been established. Very recent work shows that the degree of order in coalesced raft membranes of cells is substantially lower than that observed in model membranes, leading to the suggestion that lipid-based phase segregation principles cooperate with other lateral specificities, possibly chemical interactions involving proteins, to laterally organize function [15]. Accordingly, ESR on cell plasma membrane suggests that the lipid basis for membrane ordering is modulated by proteins to allow coexistence of ordered and disordered domains [16]. Chemically derived cell membrane vesicles can be cooled to separate into Lo-like and Ld-like phases [7]. However in this context, the ordered phase typically excludes prototypical lipid raft proteins (as do model membrane lo phases [15,17]) and thus the physiological significance of an Lo phase in cells still remains unclear. As key components of membrane lipid domains, the variable fatty acid

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content of sphingolipids, defined by a series of fatty acid selective ceramide synthases [18], may provide a mechanism for regulating their composition.

2. Verotoxins and their GSL receptors

Verotoxins are a family of *Escherichia coli* derived AB₅ subunit toxins (also termed Shiga toxins or Shiga-like toxins) first described and so named for their select cytotoxic activity on vero cells an African green monkey renal tubule epithelial cell line [19]. When purified and characterized these toxins were found to be largely indistinguishable from the AB₅ toxin of *Shigella dysenteriae* [20] and are therefore also known as Shiga toxins, Stx. Their study became of mainstream importance following the pioneering epidemiological work of Karmali et al. [21], showing that infection with *E. coli* producing these toxins was strongly associated with the subsequent development of the hemolytic uremic syndrome, a previously idiopathic nephropathy resulting from glomerular occlusion and more frequently associated with young children than adults. Verotoxins comprises a family of highly related toxin comprising primarily VT1 and VT2 which are 60% homologous at the protein level. Additional variants of VT2 have been described but these are infrequently isolated clinically. More significant is perhaps VT2e or pig edema disease toxin which is a pathogen of pigs [22]. All the verotoxins bind to the glycolipid globotriaosyl ceramide, Gal α 1-4Gal β 1-4 Glucosyl ceramide (also termed CD77 or the p^k blood group antigen), except for molecular which binds the next homologue in this series globotetraosyl ceramide, Gb₄, in addition to Gb₃ [23]. The gal α 1-4gal disaccharide does not occur in mammalian glycoproteins, (but is present in avian egg glycoproteins [24]). Deletion of Gb₃ synthase renders mice completely resistant to VT1 and VT2 [25]. Thus GSLs are the only functional VT1/2 receptors.

Although the receptor binding specificity of VT2 is the same as that of VT1, i.e., Gb₃ is recognized, the binding affinity for VT2 is significantly less than that of VT1 [26]. Despite this fact, infections with VT2 producing *E. coli* are more frequently observed in association with human disease than are those of VT1 alone. VT2 is slightly less cytotoxic than VT1 in vitro [27]. Thus receptor binding affinity and cytotoxicity in cell culture are not the only components which define in vivo pathology. Our studies are targeted to determine whether Gb₃ membrane organization provides a basis for differential VT1 and VT2 activity.

3. Aglycone modulation of Gb₃ receptor function

Although glycolipids are synthesized by a series of glycosyl transferases which add sugars moieties from activated nucleotide sugars to grow an oligosaccharide chain in a step-wise fashion, the lipid moiety, i.e., the ceramide, is heterogeneous, primarily within its fatty acid content. In mammalian cells, these fatty acids range from C16 to C24 primarily, although longer chain species are reported in some tissues. Only even carbon numbered fatty acids are present and these can be unsaturated or hydroxylated. Since glycolipids are lipid–sugar conjugates and the lipid ceramide moiety is embedded within the phospholipid membrane bilayer, the sugar sequence, particularly for the shorter carbohydrate sequences, are very close to the plane of the plasma membrane. This hydrophobic milieu can have a strong influence on the hydrophilic carbohydrate moiety. The term ‘aglycone modulation’ of GSL receptor function has been used to describe this effect, which relies not only the composition of the ceramide moiety itself, but also composition of the surrounding lipid membrane microenvironment. Thus membrane lipid organization in terms of lipid microdo-

main or lipid rafts may regulate the bioavailability of GSLs for interaction.

Aglycone modulation of carbohydrate availability for ligand binding was first reported in terms of glycolipid substrates for galactose oxidase when it was shown that while galactosyl ceramide was effectively bound and oxidized, galactosyl glycerolipid was not [28]. Subsequent studies where the binding of antibodies to glycolipids within cell membranes or model membranes depended on the fatty acid composition of the glycolipid were later reported [29,30]. Further studies of microbial interaction with target cell glycolipids often showed that the binding specificity was dependent not only the carbohydrate sequence but also on the character of the lipid moiety itself [31–33] (most often hydroxylation was required). These studies show that the regulation of lipid bound carbohydrate for recognition by exogenous ligands is more complex than for glycoconjugate carbohydrate sequences, for example, on proteins. The question is not one of crypticity whereby cell membrane glycolipids can be masked by larger or other cell surface carbohydrates [34] or proteins, since these binding effects can be demonstrated in simple GSL/cholesterol model membranes, but rather one of some ‘allosteric’ mechanism where changes in the structure in a distal part of the molecule result in alteration of the conformation of the carbohydrate, to allow or restrict ligand binding. However, while VT2e, modeling studies predict an effect of the lipid moiety on the carbohydrate conformation of membrane GSLs [33,35], such differences have been difficult to fully verify by structural approaches [36,37].

Verotoxin binding to Gb₃ is depending on the terminal Gal α 1-4Gal disaccharide, since galabiosyl ceramide is also bound. An interesting difference between VT1 and VT2 is that VT2 binds galabiosyl ceramide in preference to Gb₃ (Fig. 1). Furthermore, a rat IgM monoclonal antiGb₃ [38] does not bind galabiosyl ceramide. Thus different ligands which bind Gb₃ can have a distinct subspecificity. In addition, the structure of the Gb₃ lipid moiety, particularly the fatty acid, is crucial to defining the carbohydrate presentation for binding [39], i.e., solid phase, tlc plate, liposome, ELISAs, monolayer film – can all affect the relative binding capacity.

This is most clearly illustrated in the comparison of the Gb₃ binding of the various verotoxin variants. Initial studies comparing VT1 and VT2c binding to Gb₃ fatty acid isoforms within lecithin/cholesterol containing solid phase supports showed that while the binding of VT1 was increased as a function of fatty acid chain length, primarily C16 to C22, VT2c binding was essentially restricted to C18 fatty acid containing Gb₃ [40]. VT2c is a relatively rare clinical isolate [41]. Subsequent studies however, also showed that VT1/Gb₃ binding was distinct from that of VT2. VT1 binding inhibited subsequent VT2/Gb₃ binding but VT2 binding was unable

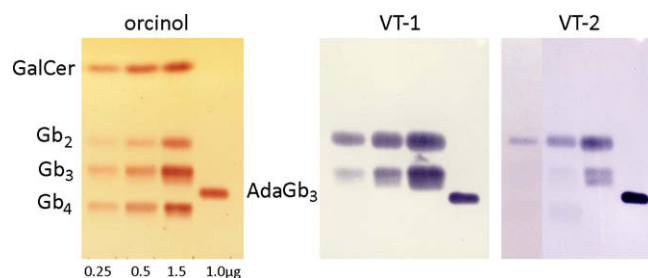


Fig. 1. Comparison of VT1 and VT2 binding to Gb₃, galabiosyl ceramide and adamantylGb₃. GSLs were separated by TLC and detected by orcinol chemical spray. After blocking with gelatin, separate plates were incubated with 1 μg/mL VT1 or VT2. Excess toxin was removed and bound VT1/VT2 detected by immunostaining. VT1 binding to Gb₃, Gb₂ (galabiosyl ceramide, gal α 1-4gal ceramide) and adamantylGb₃ is similar. However, VT2 binds Gb₂ in preference to Gb₃ and generation of adamantylGb₃ from Gb₃ markedly increases VT2 binding.

to inhibit the VT1 binding [26]. Both VT1 and VT2 inhibited the binding of a monoclonal anti Gb₃ antibody. Later studies using deoxy Gb₃ analogues showed that VT1 and VT2 show distinct but overlapping hydroxyl requirements within the Gb₃ carbohydrate moiety and that the monoclonal antiGb₃ showed a hydroxyl requirement more similar to that of VT2 than VT1 [42]. In surface plasmon resonance studies VT2 binding affinity was less than that of VT1 but the off rate was also less, suggesting that VT2 forms a more stable Gb₃ complex [43]. In detergent resistant model membranes VT1 binding to Gb₃/cholesterol vesicles was fatty acid dependent in that C18 and C20 Gb₃ was not recognized [44]. In contrast, VT2 binding was independent of Gb₃ fatty acid composition in this context.

The Gb₃ binding site within the VT1B subunit pentamer was first proposed to lie within the inter-B subunit cleft [45]. This was verified in molecular modeling studies which identified this site as the potential primarily Gb₃ binding site, but also identified a second energy minimum more closely opposed to the surface of the pentamer which would oppose the cell membrane when bound [46]. Co-crystal studies using the VT1 B subunit pentamer and the lipid free Gb₃ oligosaccharide, showed that this second site was primarily bound by the Gb₃ oligosaccharide and that the inter-subunit cleft site (site 1) was significantly, but less frequently occupied [47]. A third site comprised solely of trp 34 was also seen to bind Gb₃ sugar. Only site 2 binding of oligosaccharide was verified by NMR [48] but sites 1 and 2 have been considered the primary mechanism of verotoxin/Gb₃ glycolipid binding [49]. Mutations in both sites have shown their importance but some mutations in site 1 which severely reduce cell binding/cytotoxicity had no effect on site 2 Gb₃ oligosaccharide binding [50]. Verotoxin binding to the Gb₃ carbohydrate is very much reduced for the lipid-free sugar [51] but this can be compensated by appropriate steric multimeric presentation [52]. Site 2 is compromised as defined by the crystal structure of VT2 [53], suggesting that the different utility of these two sites within these two toxins may be related to the differential Gb₃ glycolipid binding.

The differential cytotoxicity of VT1 and VT2 is reflected in vivo. There is no small animal model of HUS, due to the fact that Gb₃ is absent from the renal glomerulus of rodents. In mice, there is a remarkable difference between the in vivo susceptibility to VT1 and VT2. Although Gb₃ deficient mice are completely resistant to both VT1 and VT2 [25], wild-type mice are approximately 400 times more sensitive to VT2 [54]. This correlates with a differential distribution of VT1 and VT2 binding sites within mouse tissue [55]. For example, only VT1 binds to Gb₃ located within the lung. Since this induces no lung pathology, it can be considered as a VT1 binding 'sink' which competes for VT1 binding to more susceptible tissues. Gb₃ synthesis in endothelial cells can be increased by treatment with cytokines and a mouse model for HUS was proposed by pretreating with LPS to induce cytokines and upregulate murine renal glomerular Gb₃ synthesis [56]. Although a useful model, the glomerular pathology in this model appears to be mediated by renal tubular VT1 Gb₃ binding [57]. Renal glomerular Gb₃ expression is restricted to primates and the only accurate HUS model remains that of the baboon [58].

In a subset of adult human renal samples, differential glomerular binding of VT1 and VT2 was demonstrated [42]. Coincident VT2 and Mab antiGb₃ glomerular staining was seen without VT1 binding. Cholesterol extraction of such glomeruli resulted in the induction of VT1 glomerular binding [42], indicating that excess cholesterol could block Gb₃ to prevent VT1, but not VT2 (or Mab antiGb₃) recognition. Some adult renal glomeruli were negative for both VT1 and VT2 staining and cholesterol extraction induced both VT1 and VT2 glomerular binding [59]. Thus membrane cholesterol can mask Gb₃ from binding both VT1/VT2 or VT1 preferentially.

The differential binding of VT1 and VT2 to common and distinct epitopes within the Gb₃ glycolipid oligosaccharide [42] is reflected in the plasma membrane binding of these toxins to target cells [27]. The simultaneous labeling of vero cells with differentially labeled VT1 and VT2 at 4 °C shows both overlapping and distinct VT1/VT2 bound Gb₃ assemblies [27]. These distinct and overlapping domains are maintained during the early stages of internalization such that VT2 undergoes retrograde transport largely via a transferrin receptor containing vesicular route, whereas VT1 is largely excluded from transferrin containing vesicles. Both toxins coalesce by retrograde transport in the Golgi and then migrate to the ER (where a fraction of the A subunits separate and translocate to the cytosol [60]) followed by a second separation phase as first VT1, followed by VT2 transits to more distal elements of the ER and the nuclear envelope. Intracellular and cell surface bound VT1 is more resistant to detergent extraction than is VT2, suggesting that a greater proportion of VT1 bound Gb₃ is within lipid rafts as compared to VT2 [27].

In in vitro constructed Gb₃/cholesterol containing detergent resistant membranes, the optimum composition for VT1 and VT2 binding is distinct. Higher levels of cholesterol inhibit VT1, but not VT2 binding and sphingomyelin can augment the binding of VT2 but not that of VT1 [27]. In addition VT1, but not VT2, binding to these Gb₃ DRM constructs was dependent on the Gb₃ fatty acid content [44]. Thus VT1 and VT2 bind overlapping but distinct Gb₃ lipid assemblies both in model membrane systems and within cell membranes. Interestingly, VT2 preferentially binds Gb₂, in which the galactose α1-4 galactose disaccharide is directly linked to the ceramide lipid base [61], as compared to the more common Gb₃ (Fig. 1). Neither VT1 or VT2 show any binding to Gb₄, the next homologue in the globoGSL series (although if the terminal GalNAc of Gb₄ is deacetylated, strong binding is seen [46]).

The differential character of VT1 and VT2 Gb₃ binding is further reflected in cell binding by the fact that not all vero cells are bound by VT2. This may be a cell cycle dependence but within a culture, cells which preferentially bind VT2 can be distinguished. Fig. 2 shows the internalization of VT2 within such a population of vero and HeLa cells. It is clear that a fraction of the cell population can be refractory to VT2 binding, internalization and retrograde traffic to the Golgi (monitored by rab6 staining). Preferential binding of distinct Gb₃ domains by VT2 mediates the differential intracellular routing to the Golgi by VT2 as compared to VT1 [27]. The differential routing may be responsible for a novel VT2 selective pathology observed for a sub-population of VT2 treated vero cells [27]. Vero

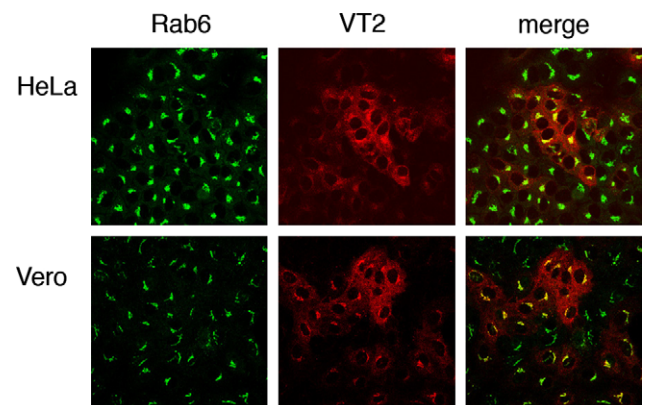


Fig. 2. Cell selective binding by VT2. Texas red labeled VT2 is preferentially bound and internalized by a sub population of HeLa and vero cells. After 1 h at 37 °C, VT2 is bound and retrograde transported to the Golgi of a subset of the monolayer cells as compared with the labelled rab6 Golgi marker, present in all cells.

cells (10–20%) treated with VT2 were found to undergo a multi-vesicular vacuolization process 2–3 h after toxin administration. The vacuoles are acidic and although not induced by VT1, prior treatment with VT1 protects against subsequent VT2 induced vacuolation. This previously undescribed VT2 induced vacuolation was prevented by inhibition of retrograde transport by brefeldin A and occurred when most VT2 was found in the Golgi. The role of this VT2 selective cell pathology in VT2 induced disease has yet to be established, but clearly demonstrates differential Gb₃ dependent cytopathology. A single cell membrane glycolipid can therefore provide a differential receptor for different binding ligands.

Although the Gb₃ fatty acid modifies VT1 binding, we have generated a soluble inhibitor of VT1/Gb₃ binding by removing the fatty acid of Gb₃ and replacing it with a rigid adamantane hydrocarbon frame [62]. This surprisingly water soluble conjugate, unlike the lipid-free Gb₃ oligosaccharide [51], retains high affinity VT1 and VT2 binding and protects cells in culture from both VT1 and VT2 cytotoxicity [27]. AdamantylGSLs retain the receptor function of the parental GSL [63–65], suggesting that they mimic the membrane presentation of GSLs in solution. For VT2, adamantylGb₃ is bound in preference to native Gb₃ (Fig. 1), a clear example of aglycone modulation of GSL receptor function. However, adamantylGb₃ was not protective, but rather enhanced VT2 pathology in vivo [55], possibly due to its amphipathic character which might allow adamantylGb₃ to insert into membranes and act as a pseudo receptor for VT. Similar NBD-Gb₃ analogues where the fatty acid is substituted by a fluorescent aromatic group, can partition into the cell plasma membrane and bind verotoxin [66].

4. Gb₃ in DRMs

The maintenance of glycolipids within lipid rafts as monitored by susceptibility to detergent extraction, may be complex, involving more than just the nature of the lipid moiety of the glycosphingolipid. Studies showing that the depletion of glucosyl ceramide resulted in the loss of Gb₃ from the detergent resistance membrane fraction [67] imply that glycolipid/glycolipid interactions may be necessary to resist detergent extraction. Recent studies showing that specifically the C16 fatty acid isoform of glucosyl ceramide was required for Gb₃-bound VT1 to transit from endosomes to the Golgi [68] may indicate that this interaction is also dependent on the character of the lipid moiety.

VT1 ligation of cell membrane Gb₃ within DRMs results in trans-membrane signaling and activation of Src family kinases. The retrograde transport of the verotoxin/Gb₃ complex from the cell surface to endosomes, to TGN, Golgi and ER is dependent on the initial Gb₃ being present within DRMs [69]. This retrograde pathway is required for A subunit cytosolic transit [60] and hence cytotoxicity. For cells in which Gb₃ is present in the non-DRM plasma membrane fraction, the toxin receptor complex is internalized and trafficked to lysosomes where the toxin is degraded and cells therefore survive without cytotoxic effect [69]. Thus not all Gb₃ containing cells are sensitive to VT cytotoxicity. This provides a possible explanation as to why bovine animal reservoir gastrointestinal mucosal epithelial cells, which express Gb₃ are nevertheless resistant to cytotoxicity [70]. Non-DRM Gb₃ is in effect, protective against VT. This may provide the explanation as to why the Fabry mouse, which has increased levels of Gb₃ within many tissues, is resistant, rather than highly susceptible to verotoxin [71]. A-subunit translocation into the cytosol from the ER is also dependent on Gb₃ within a DRM format [67]. This raises questions as to the basis of such intracellular Gb₃ containing DRMs, since cholesterol concentrations within the ER are extremely low.

5. Gb₃ detergent resistance is crucial for in vivo VT pathology

Although the interpretation of detergent resistant membranes isolated from cells is complex, it is clear that detergent resistance reflects a stronger lateral interaction between membrane components and that this correlates with dynamic processes [72]. While most GSLs are enriched in DRMs, it is clear that Gb₃ can be found in both the DRM and non-DRM fraction [67,69]. As with cytotoxicity, VT1-induced transmembrane signaling also requires a Gb₃ DRM format [73,74]. We used this tool in intact tissues as a novel means to dissect the Gb₃ mediated, verotoxin induced pathology of HUS [59], and found that Gb₃ membrane organization plays a central role in determining in vivo sensitivity to VT. HUS is primarily a disease of endothelial cells within the glomeruli (glomerular podocytes and mesangial cells may also be involved) [75], however Gb₃ is distributed throughout the human nephron, highly expressed on tubular epithelial cells [76]. Thus, the basis of the glomerular selectivity of VT-induced HUS pathology is unknown. Since VT1 cell cytotoxicity in vitro has been shown to depend on Gb₃ being present within DRMs [69], we investigated whether this property could be used to probe Gb₃ organization in the human kidney. We found that renal tubular VT1/VT2 bound Gb₃ is completely susceptible to brief detergent extraction at 4 °C. In contrast, VT1 and VT2 staining of renal glomeruli was entirely resistant to similar detergent extraction [59]. In fact, glomerular VT1/VT2 binding to Gb₃ was, according to sample, slightly, to significantly enhanced in frozen adult renal sections after detergent extraction. Thus, the Gb₃ organization within the membrane of cells within the glomerulus is completely distinct from that of the tubular epithelial cells. Only glomerular Gb₃ is detergent resistant and, extrapolating from the cell culture results, this would predict that only glomerular Gb₃ containing cells will be sensitive to the cytopathology of systemic VT1/VT2. This therefore provides a unique explanation for the cell selectivity of the pathology of HUS and provides the first clear example where the membrane organization a glycosphingolipid receptor provides the main discriminator for pathology in vivo.

6. Role of Gb₃ in HIV infection

6.1. Gp120–Gb₃ binding

In addition to its role as the receptor mediating the pathology of verotoxins, Gb₃ is one of several GSLs involved in HIV infection. The surface envelope glycoprotein, gp120 is the mechanism by which the virus targets lymphoid cells for infection. Gp120 binds to CD4 on T cells and monocytes and this induces a conformational change to expose the V3 loop which subsequently binds to chemokine co-receptor, either CXCR4 for T-tropic HIV strains or CCR5 for R5 tropic strains, which are responsible for the sexual transmission of HIV infection. The V3 loop also contains a GSL binding motif [77] to which several glycolipids, galactosyl ceramide, sulfogalactosyl ceramide, GM3, GD3 and Gb₃ adhere [78–81]. GalCer binding by gp120 has been strongly implicated as the mechanism of HIV targeting and entry into CD4 negative cells, such as mucosal epithelial cells [79,82,83].

Gp120 binding to Gb₃/cholesterol DRM vesicles and Gb₃ monolayers showed a similar dependence on the Gb₃ fatty acid moiety as VT1 [44]. C16, C22, C24 Gb₃ vesicles were bound but C18 and C20 were not recognized and, as for VT1, and these fatty acid isoforms were dominant negative for gp120 binding to Gb₃ fatty acid mixtures [44]. Again as for VT1, inclusion of C24:1Gb₃ induced strong gp120 binding, although this isoform alone binds VT1 and gp120 poorly. This was most dramatically illustrated by the lack of gp120 binding to C18 and C24:1Gb₃ separately, but strong

7. Gb₃ and HIV infection-friend or foe?

Although gp120 binds to several glycolipids and the glycolipid binding motif has been identified in the V3 loop [77], it is not clear how or whether different glycolipids binding within this site could have differential effects. Thus GalCer binding can actively promote viral host cell fusion whereas Gb₃ appears to inhibit it. The glycolipid binding site XXXGPGRAFXFX [93] within the V3 loop is in the center of the chemokine receptor binding domain S/GXXXGPGXXXXXXE/D [94] and thus glycolipid binding should in fact, inhibit subsequent chemokine receptor binding by the same V3 loop domain. CD4 binding by gp120 is required to open the V3 loop to allow subsequent chemokine receptor binding [95]. Gp120 glycolipid binding does not require prior recognition of CD4. However soluble CD4 can increase gp120 GSL interaction [96]. Gp120 binding to Gb₃/cholesterol vesicles is enhanced in the presence of soluble CD4 [97]. This is consistent with V3 loop binding by gp120 in that this domain becomes more exposed – which facilitates Gb₃ binding—following CD4 recognition. However, unlike for the chemokine receptor, significant glycolipid binding is observed prior to CD4 binding by gp120. This is consistent with the smaller molecular size of Gb₃ compared to chemokine receptor. Treatment of gp120 with the soluble Gb₃ mimic, adamantyl Gb₃, or a soluble Gb₃ mimic based on Gb₃ oligosaccharide coupled to phosphatidyl ethanolamine (FSL-Gb₃ [98]) shows that gp120 binding to Gb₃ mimics prevents gp120 binding to Gb₃ and to GalCer and (less effectively) to sulfatide (Fig. 3), formally showing that these glycolipids share a common binding site on gp120. It may be that

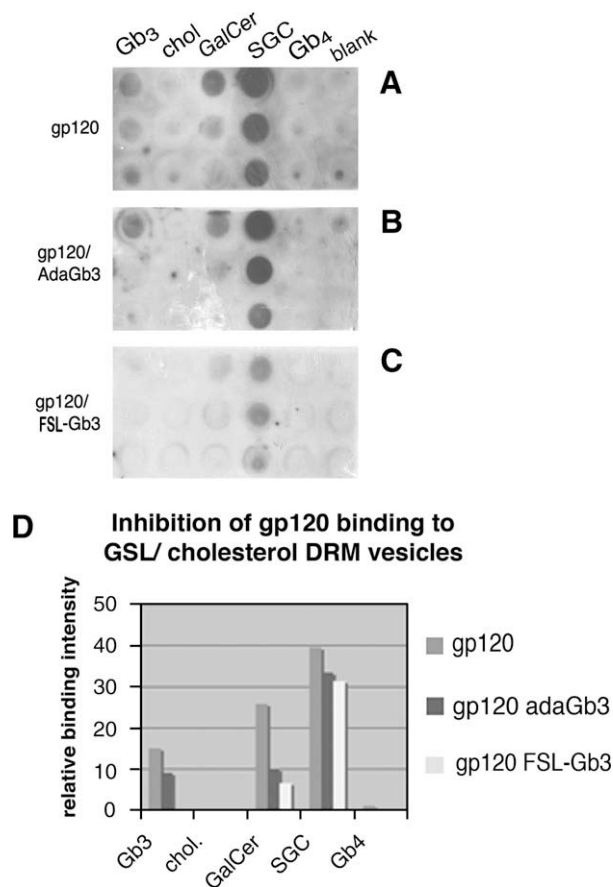


Fig. 3. Gp120 binding to GSL cholesterol vesicles. GSL cholesterol vesicles were generated [44] and immobilized on nitrocellulose. After blocking, the nitrocellulose strips were incubated with R5 gp120 (3 μ g/mL) overnight and after washing, bound gp120 detected by immunostaining. Panels A–C Vesicles equivalent to 50, 25 and 5 μ g GSL (from the top) were dot blotted on each nitrocellulose panel and tested for gp120 binding. The effect of preincubation of gp120 with 300 μ M adamantylGb₃ (panel B) or FSL Gb₃ (soluble Gb₃ mimic based on PE [99]) (panel C) on GSL binding was compared. The inhibitory effect of the soluble Gb₃ mimics on gp120/GSL vesicle binding was quantitated in panel D, using Image J software. Sulfatide (SGC) showed the strongest gp120 binding, followed by GalCer and then Gb₃. No binding to cholesterol alone or Gb₄/cholesterol vesicles was detected. The soluble Gb₃ mimics inhibited gp120 binding to Gb₃ and GalCer and, to a lesser extent, to SGC.

the size of the carbohydrate determines the effect, with the smaller galactosyl ceramide facilitating gp120 chemokine receptor binding whereas the larger Gb₃ binding in the same site may inhibit the same chemokine receptor-gp120 interaction.

It is clear that all membrane globotriaosyl ceramide is not created equal. The lipid heterogeneity will result in differential lateral cell membrane organization with cholesterol and other lipids to affect receptor and intracellular trafficking functions. This aglycone modulation of receptor function may be a dynamic process as Gb₃ (plus ligand) trafficks intracellularly to membranes of different lipid composition.

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