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Rafts and related glycosphingolipid-enriched microdomains in the intestinal epithelium: bacterial targets linked to nutrient absorption

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Abstract

Plasma membrane microdomains such as lipid rafts or caveolae play a major role in host–pathogen interactions. Although this field of research has been extensively studied, two important points have been poorly addressed: (i) the molecular basis of raft–pathogen interactions, and (ii) the effect of such interactions on nutrient absorption. The aim of this review was to propose a biochemical analysis of bacterial adhesion to lipid raft components exposed on the mucosal surface of the intestinal epithelium. A special attention has been given to CH– π interactions that allow the sugar rings of glycosphingolipids (GSL) to stack against aromatic side chains of bacterial adhesins and toxins. These interactions are controlled by cholesterol molecules intercalated between membrane GSL and/or by the presence of an alpha-OH group in the acyl chain of the ceramide backbone of GSL. In the second part of the review, we analysed the experimental data suggesting the involvement of lipid rafts in the intestinal absorption of nutrients, the mechanisms by which bacteria could impair intestinal functions, and possible therapeutic strategies based on the biochemistry of raft–pathogen interactions.

Keywords: Lipid raft; Intestinal absorption; Nutrient; CH– π interactions; Bacteria; Toxin; Adhesin; Cholesterol; Glycosphingolipid; Ceramide

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1. Introduction

In the fluid mosaic model of biological membranes, lipids form a homogeneous two-dimensional solvent phase for membrane proteins. Yet membrane lipids comprise several hundreds of distinct molecules that exist in different physical states controlled by several physicochemical parameters such as the temperature, presence of cholesterol and chemical nature of the hydrocarbon chains [1]. Biological membranes are thus better described as a ‘mosaic of lipid domains’ rather than a homogeneous fluid mosaic. Membrane cholesterol, for instance, is unevenly distributed into cholesterol-rich and cholesterol-poor domains, consistent with the notion that specialized lipid domains with specific biochemical composition and physicochemical properties do exist in membranes [2]. Among these domains, those containing sphingolipids and cholesterol, referred to as lipid rafts or caveolae (when associated with the integral membrane protein caveolin), have been extensively studied [3]. For cell biologists, lipid rafts are chiefly involved in cellular trafficking [4] and signalling functions [5]. For pathologists, these membrane areas are preferential sites for host–pathogen/toxin interactions [6] and for the generation of pathological/infectious forms of proteins associated with Alzheimer’s and prion diseases [1,7]. As a matter of fact, both the physiological and pathological aspects of lipid raft functions have been the subject of excellent recent reviews [8–10]. However, two important points have been poorly addressed: (i) the molecular basis of raft–pathogen interactions, and (ii) the effect of such interactions on

nutrient absorption by target cells, especially absorptive enterocytes. Therefore, the objectives of the present review were: (i) to propose a biochemical analysis of bacterial adhesion to lipid raft components exposed on the mucosal surface of the intestinal epithelium, (ii) to review the experimental data suggesting the involvement of lipid rafts in the intestinal absorption of nutrients, (iii) to identify the mechanisms by which bacteria could impair intestinal functions, and (iv) to suggest possible prophylactic/therapeutic strategies based on the biochemistry of raft–pathogen interactions.

2. Biochemistry and biophysics of lipid rafts

Why do sphingolipids and cholesterol self-associate and segregate into specific membrane domains? The answer to this question may be given by the biochemical structure of membrane lipids [11]. Glycerophospholipids such as phosphatidylcholine (PC) are rich in kinked unsaturated acyl chains (with C=C double bonds in the cis configuration), whereas the hydrophobic part of sphingolipids such as sphingomyelin or glycosphingolipids (GSL) contain a saturated acyl chain and sphingosine [1]. Introducing a C=C double bond of cis geometric configuration results in a bending of the chain. This change from the linearity impairs the tight packing of lipid chains, so that glycerophospholipids have more mobile hydrophobic chains than sphingolipids. Since the mobility of the hydrophobic lipid anchor in the apolar phase of the membrane interferes with the packing

capacity of lipid molecules, the energy required to separate two adjacent sphingolipid molecules is significantly higher than for glycerophospholipids. This energy can be quantified by measuring the temperature required to induce the solid–liquid phase transition of a lipid, i.e. the melting temperature (T_m). The T_m of PC is as low as $-5\text{ }^\circ\text{C}$, compared to $83\text{ }^\circ\text{C}$ for galactosylceramide (GalCer, purified from bovine brain) [1]. Because cholesterol also has a high T_m , it has more affinity for sphingolipids than for glycerophospholipids [12]. Consequently, at $37\text{ }^\circ\text{C}$, sphingolipids and cholesterol segregate into specific microdomains, the so-called lipid rafts. Since the sphingolipid head groups (especially GSL) occupy a large volume, cholesterol functions as a molecular spacer, filling the voids between two adjacent sphingolipids [1,3]. From a biophysical point of view, lipid rafts are in a liquid-ordered (Lo) phase floating in the more liquid glycerophospholipid-rich/cholesterol-poor bulk (liquid-disordered phase Ld) of the plasma membrane [2]. One should be aware that lipid rafts are highly dynamic entities that stir, fuse and continuously modify their shape, so that they have been compared to a myriad of mercury sheets perpetually moving on the surface of a mirror [13].

3. Physiological functions lipid rafts

What is the function of lipid rafts? It would be hard to consider that these complex microdomains exist for the unique convenience of pathogens and toxins which use them as landing-grounds in the initial step of the invasion process. Lipid rafts are also not necessary to form the impermeable barrier that isolate the cell from the extracellular environment, a key function of living systems that is adequately fulfilled by glycerophospholipids. However, cells also need to communicate with their environment, and this is perhaps the main justification for the existence of lipid rafts. The communication functions carried out by lipid rafts are both parallel and perpendicular with respect to the plane of the membrane. By moving freely in the Ld phase of glycerophospholipids, lipid rafts act as shuttles able to bring together membrane proteins that were previously located in distinct areas of the membrane (parallel, surface communication) [14–16]. Raft-induced coalescence between cell sur-

face receptors allows a physical contact between transducers molecules bound to the inner leaflet of the plasma membrane, leading to their activation (perpendicular, transmembrane communication) [5]. For this reason, rafts can be described functionally as molecular sorting machines capable of coordinating the spatiotemporal organization of signal transduction pathways within selected areas ('signalosomes') of the plasma membrane. The coalescence model of signal transduction in immune mast cells has been schematized in Fig. 1. The IgE receptor $Fc\epsilon RI$ is a multichain immune recognition receptor which is not constitutively associated with raft microdomains. Upon cross-linking with their physiological ligand (i.e. the IgE-antigen complex), $Fc\epsilon RI$ receptors are rapidly recruited in raft areas [15]. The co-compartmentation of $Fc\epsilon RI$ receptors with the raft-associated tyrosine kinase Lyn provides an adequate spatial proximity allowing the phosphorylation of $Fc\epsilon RI$ on tyrosine-based activation motifs (ITAMs). This membrane coordinated signal triggers the intracellular cascade that leads to release of mediators of the allergic response. Finally, it is important to mention that some signal transduction units may pre-assembled in lipid rafts of quiescent cells, allowing rapid and efficient signal initiation upon activation [16].

4. Interaction of membrane proteins with lipid rafts

4.1. Lipid anchors

Sphingolipids (including GSL and sphingomyelin) are almost exclusively found in the outer leaflet of the plasma membrane [3]. Several external proteins that are specifically associated with lipid rafts are bound to the membrane by a glycosylphosphatidylinositol (GPI) anchor consisting of two saturated chains (1-alkyl-2-acyl-glycerol) that can tightly pack with raft lipids [17]. Although sphingolipids are usually not found in the cytoplasmic leaflet of the plasma membrane, specific glycerophospholipids such as phosphatidylserine (PS) with saturated or monounsaturated chains (i.e. with a higher T_m than PC) may form liquid-ordered domains through interaction with long sphingolipid acyl chains of the outer monolayer [18]. On the other side of the membrane, acylated proteins

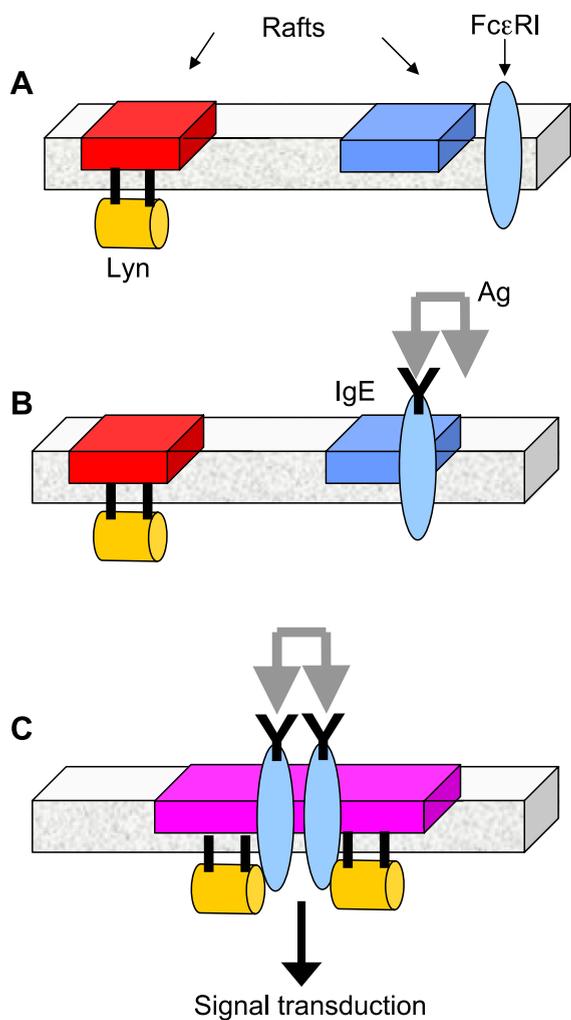


Fig. 1. Signal transduction pathway through recruitment of receptors and transducers within raft areas: the coalescence model. (A) In quiescent mast cells, the IgE receptor (FcεRI) is localized outside membrane rafts, so that it cannot interact with Lyn, a kinase of the src family anchored to the inner leaflet of the plasma membrane with acyl chains. (B) Upon binding of the antigen (Ag)-IgE complex to FcεRI, Lyn and FcεRI are recruited in rafts. (C) The coalescence of rafts induced by the multivalent antigen allows the interaction between FcεRI and Lyn, resulting in phosphorylation of FcεRI and activation of the signal transduction pathway.

anchored in the internal leaflet with two or more saturated acyl chains (generally myristyl and palmityl) are constitutively associated with lipid rafts. It is likely that these proteins may significantly contribute to the formation of a Lo phase in the inner leaflet of lipid rafts [19].

4.2. Transmembrane proteins

The situation is even more complex for transmembrane proteins, which have three main distinct possibilities of interaction with the specific lipid components of the rafts: (i) the extracellular domain, which interacts with the polar head of sphingolipids (sphingomyelin and GSL), (ii) the cytoplasmic juxtamembrane domain, which faces anionic glycerophospholipids such as PS, and (iii) the transmembrane (TM) domain (Fig. 2). Since lipid rafts are enriched in cholesterol, it can be predicted that the TM domain contains amino acid side chains particularly suited for interacting with this sterol. Although we do not know the relative affinity of the 20 amino acid side chains for cholesterol, it can be anticipated from the chemical structure that Phe and Ile residues would ideally fit with the aliphatic cycles and the isooctyl tail of the lipid. In support of this hypothesis, it has been shown that replacement of the TM domain of CD40, a raft-associated protein, by the one of CD45, a non-raft protein, resulted in the exclusion of CD40 from lipid rafts [20]. The TM domains of CD40 and CD45 are both composed of 22 amino acids, but CD40 has 6 Ile and 3 Phe residues, whereas CD45 has only 4 Ile and 2 Phe residues. Basically, a TM domain is an α -helix buried in the hydrophobic region of the membrane. The assembly of cholesterol molecules around a TM domain enriched in Ile and Phe residues may contribute to stabilize the interaction of the α -helix with the Lo phase of lipid rafts. Mutating these residues in the TM domain of CD40 and other raft-associated proteins will help to validate this hypothesis.

A polybasic motif enriched in Arg or, to a lesser extent, Lys residues is often found in the juxtamembrane cytoplasmic domain of raft-associated proteins (e.g. human CD4, EGF and PDGF receptors). The positive charge of these basic amino acids may interact with the net negative charge of PS through electrostatic interactions. Finally, the extracellular domain of raft-associated proteins faces sphingomyelin, which carries one positive and one negative charge, and GSL which may be either neutral or negatively charged in the case of gangliosides. Ideally, a sphingolipid-binding domain (SBD) should be composed of a charged residue (preferentially basic) for interacting with the polar head of sphingomyelin and gangliosides. Moreover, the SBD should also contain

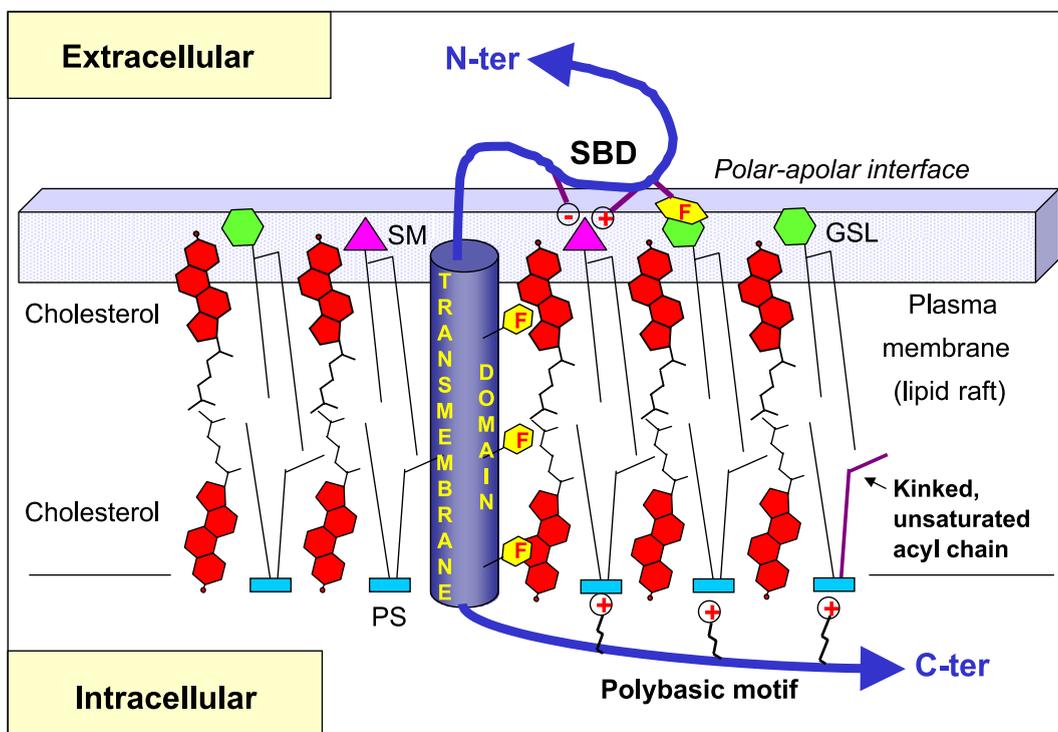


Fig. 2. How integral membrane proteins may interact with lipid rafts. Three zones of interactions have been considered: the extracellular region, the transmembrane α -helix and the cytoplasmic juxtamembrane domain. F, phenylalanine (Phe) aromatic side chain; GSL, glycosphingolipid; PS, phosphatidylserine; SM, sphingomyelin; SBD, sphingolipid binding domain.

a solvent-exposed aromatic side chain conveniently oriented to stack against the sugar rings of GSL (see Section 5.2). In any case, the three modes of interaction of transmembrane proteins with lipid rafts (i.e. SBD, TM domain and polybasic motif) may allow the assembly of a lipid shell around the protein, as recently proposed by Anderson and Jacobson [21]. According to this model, lipid shells have a preferential affinity for lipid rafts, so that they are assumed to target the protein they encase to these microdomains.

4.3. Searching for a sphingolipid binding domain in adhesins and toxins

Since bacterial adhesins and toxins may interact first (if not exclusively) with the extracellular side of lipid rafts, they can be considered as foreign competitors for host membrane proteins associated with lipid rafts. On this basis, it could be anticipated that bacterial adhesins and toxins could present in their three-dimensional

structure a SBD domain similar to the one found in the raft proteins of the host. The first identification of a microbial SBD came from the study of HIV-1 surface envelope glycoprotein gp120 and its GSL intestinal receptor GalCer [7]. The region of gp120 responsible for GalCer recognition is a disulfide-linked domain referred to as the V3 loop [22]. Searches for structure similarities revealed the presence of a V3-like SBD in various sphingolipid-binding proteins including cellular proteins such as the prion protein isoform PrP^C or the Alzheimer β -amyloid peptide [1,7] and bacterial toxins such as the Shiga-like toxin 1 (Fig. 3A) [13]. The conserved motif is a hairpin structure (e.g. α -helix-turn- α -helix or β -strand-turn- β -strand) with a turn containing at least: (i) a solvent-exposed aromatic residue (Phe, Tyr or Trp), (ii) a basic side chain (Arg or Lys) oriented towards the solvent, and (iii) a Gly and/or a Pro residue inducing the turn in the backbone $C\alpha$ chain. Since structure similarities searches align two peptidic backbone chains, the identification of a SBD

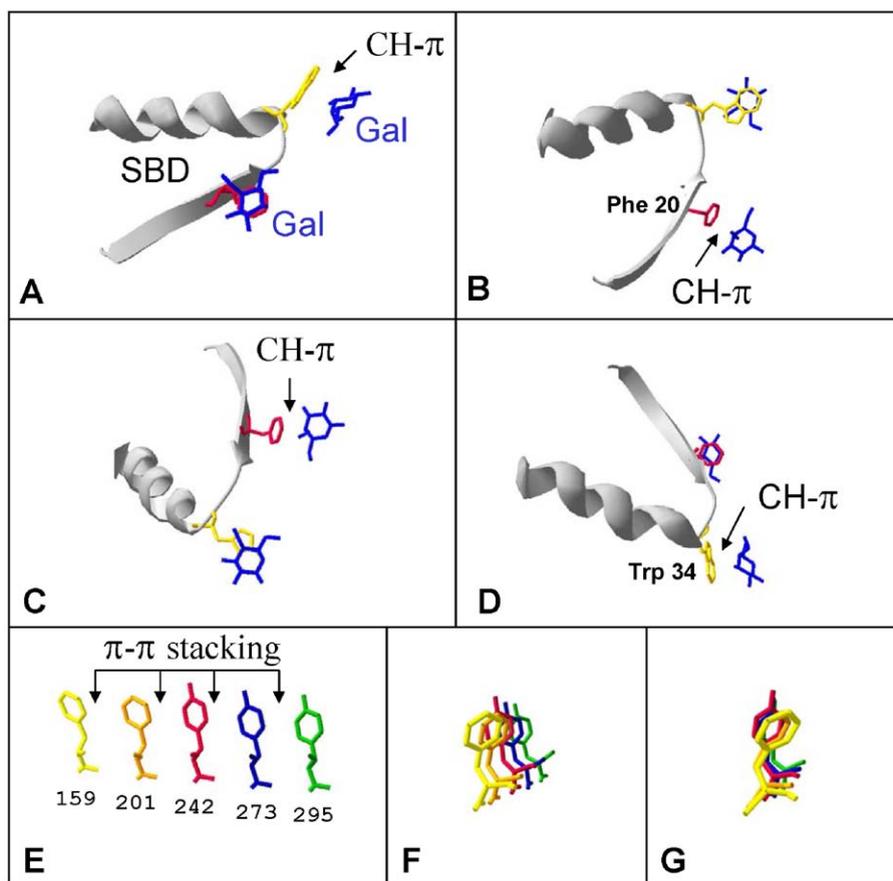


Fig. 3. Stacking CH- π interactions and aromatic π - π interactions. (A-D) Different views of the interaction between the SBD of the B subunit of Shiga-like toxin 1 and galactose residues of two Gb3 receptors at a resolution of 2.20 Å (PDB entry 1CQF). The oligosaccharide sequence of the Gb3 glycone is Gal α 1-4Gal β 1-Glc, (the Gal residue of Gb3 shown in the figure is underscored). The CH- π interactions involve the aromatic side chains of residues Phe-30 (in red) and Trp-34 (in yellow) in the SBD of the toxin, and galactose residues (in blue) on the GSL. The superposition of the sugar and the aromatic rings can be observed in A and D for Phe-30, and in B and C for Trp 34. (E-G) Different views of the stacking π - π interactions between internal aromatic side chains in *Bacillus subtilis* pectate lyase (resolution of 1.80 Å, PDB entry 1BN8). In this case, the interacting aromatic side chains stabilize a β -helix and confer a high resistance of the protein to denaturation. The figure shows the residues Phe 159 and 201, and Tyr 242, 273 and 295, according to Jenkins and Pickersgill [83]. The Deep View (Swiss-PDB viewer) software [84] has been used to visualize the molecular structures. The superposition of aromatic ring structures is evident in F and G. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

takes for granted that the three-dimensional structure of the protein of interest is known. However, secondary structure predictions together with the search for a specific cluster of amino acid residues (Gly, Pro, Arg/Lys, Phe/Tyr/Trp) in a potential turn may help to identify a SBD in a protein whose structure has not been elucidated, as it is the case for most membrane and bacterial proteins. An algorithm allowing the identification of a SBD directly from the amino acid sequence is currently being developed in our laboratory.

5. Biochemistry of raft-pathogen interactions

5.1. Glycosphingolipid binding sites and receptors: a matter of affinity

GSL are used as cellular binding sites for a wide variety of pathogens, including viruses, bacteria, fungi and parasites [6,10,23]. The oligosaccharide residues of GSL protrude into the extracellular space, providing a considerable number of carbohydrate-binding

sites for microbial adhesins. Yet specificity of a carbohydrate sequence is not the unique parameter controlling pathogen binding, and density levels of GSL on the host cell surface may also be critical. Indeed, a threshold level of GSL is often required to ensure microbial adhesion, suggesting that those GSL are active only when concentrated in a lipid raft to form an operational attachment platform. Thus, although individual GSL–protein interactions may be weak, the resulting avidity of the pathogen for the raft may be very high. To complicate further the story, the same pathogen (e.g. HIV-1) may use either high or low affinity GSL binding sites to infect various cellular targets (Table 1). Moreover, the binding of a pathogen on the cell surface may also require, in addition to the GSL, a second component, generally a protein which can be either GPI-anchored or an integral transmembrane protein. In this case, the lipid and the protein cooperate and the binding reaction proceeds in three steps [1]. First, the pathogen selects a raft with appropriate GSL binding sites. Once stabilized on this attachment platform, the raft float on the cell surface, allowing the pathogen to ‘browse’ over the cell surface, looking for a high affinity receptor. Third, a ternary ‘GSL–pathogen–receptor’ complex is formed within the raft area. Basically, this mechanism can be viewed as a pathological exploitation of the coalescence model discussed in Section 3 (Fig. 1). It should also be noted that the role of GSL in

this process has been remarkably anticipated by Haywood for viruses [24] and Montecucco for bacterial neurotoxins [25], several years before the elaboration of the raft concept.

Unfortunately, the same term, i.e. receptor, is indistinctly used for describing both low affinity binding sites (chiefly GSL) and high affinity receptors, which can be either GSL or proteins (Table 1). Accordingly, the list of ‘receptors’ used by pathogens to enter the cells through lipid rafts appears highly heterogeneous [6]. This has logically led to the reflection that although ‘various microbes have all co-opted caveolae and lipid rafts, they seem to not interact with them in the same manner’ [6].

Considering the notion of affinity between the pathogen and its ‘receptors’ may help clarifying this fundamental issue. The V3 loop of HIV-1 gp120 (our prototype SBD) is known to interact with several GSL (GalCer, Gb3, GM3) and proteins (CD4, CCR5, CXCR4, GPR15/Bob) expressed by various cell types. In T lymphocytes, the V3 loop binds to Gb3 and GM3 with a low/moderate affinity. Accordingly, these GSL should not be considered as true gp120 receptors but rather as auxiliary, albeit indispensable fusion cofactors [14,26,27]. In contrast, the V3 loop interacts with GalCer with a high affinity (Kd of 1 nM, equivalent to the Kd of CD4-gp120 interaction), so that this major intestinal GSL has long been recognized as a real receptor for HIV-1 [1,28]. Thus,

Table 1
Pathogen receptors in lipid rafts and related GSL-enriched microdomains

Pathogen	Binding sites/Receptors	Reference
HIV-1	<u>GalCer</u> , <i>GPR15/Bob</i> (intestinal epithelium)	[28,47,58]
	Gb3, GM3, <i>CXCR4</i> , <i>CCR5</i> (T lymphocytes)	[14,26,27]
<i>Helicobacter pylori</i> adhesin	GalCer, sulfatide, LacCer, GM3, <i>glycoproteins</i>	[49,71]
<i>Clostridium botulinum</i> neurotoxins	GT1b, GD1a, <i>synaptotagmin II</i>	[74,75]
<i>Shigella dysenteriae</i> Shiga toxin	<u>Gb3</u>	[76]
<i>E. coli</i> (EHEC) Shiga-like toxin	<u>Gb3</u>	[77]
<i>E. coli</i> heat-labile enterotoxin	<u>GM1</u>	[78]
<i>Vibrio cholerae</i> enterotoxin	<u>GM1</u>	[79]
<i>Shigella</i>	unidentified GSL, <i>hyaluronan receptor CD44</i>	[63]
<i>Salmonella</i>	<i>GPI-anchored CD55</i>	[62]
<i>Borrelia burgdorferi</i>	GalCer, LacCer, Gb3, GD1a, GT1b	[80]
<i>Bordetella pertussis</i>	Sulfatide, asialo-GM1	[81]
<i>Streptococcus pneumoniae</i>	GalNac β 1-4 Gal motif in various GSL	[81]
<i>Mycobacterium bovis</i>	Cholesterol	[82]
Cholesterol-binding cytolysins (<i>Streptococcus</i> , <i>Bacillus</i> , <i>Clostridium</i> , <i>Listeria</i> , <i>Arcanobacterium</i>)	Cholesterol	[41]

High affinity GSL are underscored. Membrane proteins are indicated in italics.

the same SBD (the V3 loop in this case) can recognize various GSL with distinct affinities. The thermodynamic analysis of the binding reaction may explain this striking feature of GSL [1]. Indeed, the affinity between two ligands depends, among other parameters, on the number of structured water molecules that are released to bulk solution as a result of binding (this leads to an increase of entropy). In the case of GalCer, the available binding surface (especially the galactose ring) is in close interaction with the membrane, so that gp120 binding might induce the release of numerous water molecules around the lipid–aqueous interface. In Gb3 and GM3, the galactose residue is distant from the membrane, so that fewer water molecules might be displaced by gp120. Correspondingly, gp120 has an affinity for GalCer>Gb3/GM3.

Low and high affinity GSL may both contribute to the invasion process, although through distinct mechanisms. On one hand, weak interactions between the SBD and low affinity GSL binding sites may be essential to stabilize the pathogen on the cell surface, leaving enough time to find high affinity receptors before its possible desorption from the cell surface [1]. During this process, GSL may induce slight conformational changes that increase the affinity of the pathogen component (adhesin, toxin) for the secondary receptor, i.e. a membrane protein which can be either in or out lipid rafts [1,13]. On the other hand, high affinity GSL receptors may be linked to essential cellular functions, including intracellular trafficking or signal transduction pathways. For instance, GalCer has been involved in the transcytosis of infectious HIV-1 through intact intestinal epithelial cells [29] and in the pathogenesis of HIV-1 enteropathy [30]. Hence, the affinity of host cell GSL for microbial adhesins or toxins is a critical parameter of host–pathogen interactions that may influence not only the mode of adhesion but also the mechanism of toxicity and the intracellular trafficking of microbes [29–31]. In this respect, determining the affinity of microbial components for their GSL receptors/binding sites as well as the kinetics of the binding process will be a major task of future investigations.

5.2. CH– π interactions

The most striking feature of the SBD is the presence of aromatic side chains fully exposed to

the solvent in the turn between the alpha helices or beta sheets (Fig. 3A–D). In a systematic study on the biochemical nature of protein binding sites, Ma et al. [32] observed that the conservation of Trp and Phe residues on the protein surface indicates a highly likely binding site. Indeed, π – π (or π -stacking) interactions between the electron clouds of two superimposed aromatic side chains (Fig. 3E–G) in the hydrophobic core of proteins contribute to the folding and stabilization of their three-dimensional structure [33]. It is logical to anticipate that solvent-exposed aromatic residues may therefore look for an aromatic partner provided by a ligand, which can be either a protein or a cofactor such as a coenzyme. Structural studies of lectin–carbohydrate interactions have also shown that aromatic amino acid side chain have a major role in carbohydrate binding [34]. In this case, the six-carbon sugar ring provides a complementary surface for the aromatic side chain. The interaction is driven by the proximity of the aliphatic protons of the sugar ring, which carry a net positive partial charge, and the π -electron cloud of the aromatic ring. It can thus be considered as a limit case of hydrogen bonding, in which the acceptor group is the electron cloud of the aromatic ring and the donor group is a C–H of the sugar ring. For this reason, it is usually referred to as CH– π interaction [35]. The mean distance between the sugar and the aromatic ring involved in a CH– π interaction is 3.91 Å, which is similar to the distance between two π – π interacting aromatic rings (3.94 Å) [36]. A typical example of such CH– π interactions between the SBD of a bacterial toxin (the Shiga-like toxin 1) and its GSL receptor (Gb3) is given in Fig. 3A–D. In the polar head of GSL, galactose residues, which present a hydrophobic side, are particularly suited for mediating CH– π interactions with the aromatic amino acid side chains of the SBD [13]. The SBD–GSL complex is also stabilized by electrostatic interactions between conserved basic amino acid side chains and negative charges beared by sphingomyelin and/or sialic acid residues of gangliosides [13].

5.3. Role of cholesterol

The establishment of sugar-aromatic CH– π interactions requires an adequate orientation of the polar head of GSL for optimal presentation of galactose

residues. It has long been recognized that the aglycone, hydrophobic part of GSL has a major impact on the conformation of their glycone moiety and thus on GSL receptor function [37,38]. Moreover, it has been recently demonstrated that cholesterol, which functions as ‘molecular spacer’ in lipid rafts [3], may have a critical effect on the conformation and thus on the binding properties of raft GSL. This is the case for Gb3, which requires cholesterol to interact optimally with the SBD of HIV-1 gp120 [39] and for cholera toxin, which binds to GM1 only when presented as condensed complexes in artificial cholesterol/phospholipid membranes [40]. Therefore, although cholesterol has been described as a specific binding site for a number of bacterial pore-forming toxins (the so-called cholesterol-dependent cytolytins) [41], it may also act as a fine regulator of most GSL–pathogen interactions.

Yet in some instances, cholesterol has an inhibitory rather than a stimulatory effect on GSL–pathogen interactions. This is the case for GalCer, a major GSL of the apical brush border of enterocytes [42]. Due to the relatively small size of its polar head (only one sugar ring), GalCer can form a tightly packed platform in absence of cholesterol (Fig. 4A). In contrast, Gb3 with three sugar rings may require cholesterol to form a condensed complex on the cell surface [39], as proposed in Fig. 4B. This is consistent with the body of data suggesting that different types of lipid rafts, with and without cholesterol, exist in the apical brush border [43–45]. In particular, a high concentration of cholesterol may suppress the formation of membrane domains by impairing the tight packing of GalCer, a major GSL of brush border membranes. This may explain the opposite effects of cholesterol on GalCer and Gb3 receptor functions [39].

5.4. Role of the alpha-OH group in the acyl chain of GSL

The potential absence of cholesterol in GalCer-enriched intestinal rafts does not mean that the aglycone part of this GSL does not influence the orientation of the galactose ring. The presence of an alpha-OH group in the acyl chain of the ceramide backbone of GalCer allows the formation of a H-bond which orientates the galactose ring of GalCer so that the molecule adopts a typical L-shape struc-

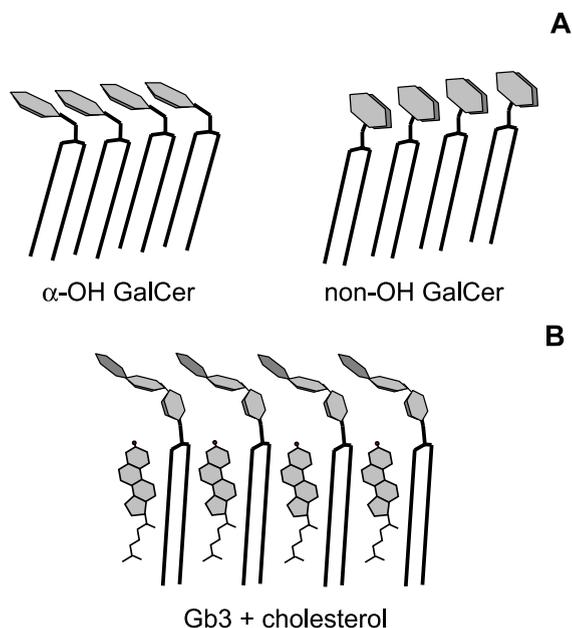


Fig. 4. Influence of the hydrophobic moiety of GSL on the orientation of the glycone polar head. Effect of the alpha-hydroxylation of the fatty acid on the orientation of the polar group (galactose of GalCer). α-OH, but not non-OH GalCer provides a convenient, planar attachment platform for pathogens. Note the striking similarity between the orientation of aromatic side chains in Fig. 3E and the galactose rings of non-OH GalCer (A, right panel). Cholesterol–Gb3 interactions in a lipid raft. Cholesterol acts as a molecular spacer that fills the void between two adjacent Gb3 molecules. By doing this, cholesterol affects the orientation of the protruding oligosaccharide part, which thereafter acquires a conformation compatible with the binding of pathogens and toxins (e.g. HIV-1, Shiga and Shiga-like toxins).

ture [46] (Fig. 4A, left panel). In contrast, the galactose ring of GalCer containing a nonhydroxylated acyl chain protrudes at 180° with respect to the plane of the membrane (Fig. 4A, right panel). As a result, vicinal sugar units share a stacking interaction and are thus not available for pathogens. For these reasons, many microbial adhesins and toxins specifically recognize alpha-hydroxylated vs. nonhydroxylated galactose-containing GSL [30,47–49]. In this respect, it is interesting to note that the alpha-OH group of the fatty acid chain and cholesterol have a comparable conformational effect on GSL, allowing in both cases an orientation of the sugar head compatible with the establishment of CH–π stacking interactions with aromatic amino acid side chains of the SBD (Fig. 4).

6. Involvement of lipid rafts in intestinal barrier and absorption functions

6.1. Tight junctions

The main feature of polarized epithelial cells such as enterocytes is the presence of tight junctions which separate the plasma membrane into an apical and a basolateral domain with specific lipid and protein compositions. These junctions form the permeability barrier between the cells, and prevent the free diffusion of nutrients through the paracellular route (i.e. between cells). Instead, the nutrients have to be transported across the epithelium, in a two-step process involving apical and basolateral transport mechanisms. Tight junction proteins (occludin and ZO-1) are recovered in detergent-insoluble microdomains [50], a procedure commonly used to extract lipid rafts from the bulk glycerophospholipid-enriched Ld phase [2,16]. These data suggest that lipid rafts may play an important role in the spatial organization of tight junctions and in the regulation of the intestinal barrier function. Thus, toxins able to modify the lipid/protein composition of tight junction microdomains may dramatically affect intestinal permeability. This is the case for *Clostridium difficile* toxins which disrupt epithelial barrier functions by altering the association of tight junction proteins with lipid rafts [51].

6.2. The intestinal brush border membrane

The apical plasma membrane of intestinal epithelial cells is unusually rich (>30%) in GSL, so that most of its surface is thought to be occupied by lipid rafts [52]. With the notable exception of lactase, most transmembrane and GPI-anchored enzymes/transporters of the intestinal brush borders are recovered in detergent-resistant fractions [53]. These include sucrose-isomaltase, aminopeptidase, alkaline phosphatase and the folate receptor. Most importantly, although cholesterol depletion resulted in the basolateral missorting of some of these proteins [54], it is likely that lipid rafts devoid of cholesterol could exist in the intestinal brush border [44]. As discussed above in Section 5.3, cholesterol may condense with Gb3, but not with GalCer, suggesting that cholesterol-rich areas are also enriched in Gb3, whereas cholesterol-

poor microdomains are enriched in GalCer. By affecting the local composition of lipid shells [21], such a differential GSL/cholesterol content could control the targeting of proteins associated with various types of lipid rafts in the intestinal brush border. This may explain why detergent resistant fractions with distinct size and biochemical composition can be extracted from the intestinal epithelium [43,45].

Sphingolipid depletion induced by the mycotoxin fumonisin B1 resulted in a dramatic decrease of folate uptake through the GPI-anchored folate receptor [55]. Similarly, metabolic inhibitors of sphingolipid biosynthesis decreased the apical uptake of glucose in the intestinal epithelial cell clone HT-29-D4 [30]. Overall, these data suggest that lipid rafts play a major role in intestinal absorption and that a broad range of microbial attacks on enterocytic rafts could potentially affect the intestinal absorption of essential nutrients.

7. How bacteria and toxins impair intestinal functions

7.1. Mechanistic studies

Although the communication between bacteria and the gastrointestinal epithelium have been the subject of numerous investigations, most of the studies have been focused on the mechanisms used by the pathogens to enhance their penetration across the epithelial barrier and to exploit the communication networks of the host to their own benefit [56]. Among the identified invasion mechanisms, some may have a direct impact on intestinal barrier and absorption functions, indicating a link between bacterial targets (chiefly raft-associated GSL) and nutrient absorption. Basically, malabsorption occurs when the brush border membrane is not correctly renewed, leading to a defect in apical enzymes and transporters. Since the renewal of brush border components is mediated by the apical transport of post-Golgi vesicles, pathogen-induced cytoskeletal rearrangements of actin filaments and/or microtubules are highly susceptible to affect nutrient absorption [30]. Given the concentration of signal transducer molecules in lipid rafts [5], it is not surprising that bacterial (or toxin) adhesion to GSL binding sites

often results in transmembrane signalling. A typical sequence of events starting from the initial adhesion of a SBD-bearing toxin (the gp120 virotoxin) to the eventual inhibition of glucose absorption has been recently elucidated [57,58]. In this case, the GSL binding site (alpha-OH GalCer) delivers the toxin to a seven-transmembrane G-protein coupled receptor (GPR15/Bob) that induces the inositol-triphosphate (IP₃)-dependent mobilization of intracellular Ca₂⁺ stores. This Ca₂⁺ response induces microtubule depolymerization, resulting in a defect of transport of brush border vesicles. The functional effect is a 50%-decrease in the sodium-dependent glucose uptake through SGLT-1, giving a mechanistic explanation for the gastrointestinal symptoms induced by HIV-1 in absence of opportunistic pathogens [58]. A similar decrease of glucose absorption mediated by the IP₃/Ca₂⁺ signalling cascade has been observed upon treatment of HT-29-D4 cells with cholera toxin, a toxin that binds to ganglioside GM1 on the apical side of the intestinal epithelium [30]. These data suggest that, apart from its well-known effect on cyclic AMP [59], cholera toxin may also induce a Ca₂⁺-mediated response leading to a defect in the activity of brush border transporters. Indeed, several enteric toxins that interact with lipid rafts, including *Helicobacter pylori* vacuolating toxin and *C. difficile* enterotoxins elicit a Ca₂⁺ response in intestinal cells [60]. Other enterotoxins activate cyclic AMP (*Salmonella* and *Campylobacter jejuni*), cyclic GMP (*Yersinia*) and nitric oxide (*Shigella flexneri*) [60]. Most of these toxins affect the enterocyte cytoskeleton, leading to severe impairments of intestinal barrier and absorption functions. Pore forming toxins such as *Clostridium perfringens* enterotoxin or *Vibrio cholerae* cytolysin, use lipid rafts as concentration platforms to promote their oligomerization, a process that is required for membrane channel formation [23]. These toxins induce an important secretion of fluid and ions in the intestinal lumen and a reduction of glucose absorption [59,60]. Finally, Shiga and Shiga-like toxins (respectively produced by *Shigella dysenteriae* and enterohaemorrhagic *E. coli*) cause a dramatic inhibition of protein synthesis in the intestinal cell, which can eventually lead to apoptosis and cell lysis [59].

An impressive case of localized destruction of brush border microvilli is given by enteropathogenic

Escherichia coli (EPEC), which adhere to intestinal epithelial surfaces by destroying host microvilli and rearranging the actin cytoskeleton to form a pedestal on the mucosal surface [56]. Other enteric pathogens such as *Salmonella* and *Shigella* destroy the enterocyte's microvilli [61]. The involvement of lipid rafts in the initial interaction between these pathogens and the intestinal brush border has been suspected [62,63]. In any case, the impact of such bacterial attacks on intestinal functions can be dramatic, as not only brush border proteins but entire microvilli are disintegrated as a result of those infections. Indeed, in vitro infection of the human intestinal cell line T84 with EPEC resulted in altered barrier and transport functions [64].

7.2. Adaptive responses of enterocytes

An important question raised by the studies of bacterial adhesion to host tissues is how the enterocyte may fight against the invader. Proinflammatory cytokines secreted by infected cells in response to bacterial invasion can activate appropriate inflammatory or immune mechanisms against chronic infection [65]. The normal gut flora, which naturally competes for essential enteric nutrients and epithelial attachment sites, may also contribute to the recruitment of immune cells in the lamina propria [56]. A more specific mechanism has been recently demonstrated by Hedlund et al. [66], who studied the association between P fimbriae of uropathogenic *E. coli* and GSL binding sites. Following binding to GSL containing the Gal α 1-4Gal β motif (e.g. Gb3), P fimbriae induced the fragmentation of the GSL into ceramide and free oligosaccharide. This may represent a highly efficient defence strategy for the host, since release of the glycone renders the GSL binding site totally inactive for further binding. Under these conditions, it is likely that the host cell remains refractory to bacterial adherence, at least until new GSL binding sites are synthesized. Moreover, the soluble oligosaccharide may locally inhibit further attachment. Finally, the inflammatory response mediated by ceramide may accelerate the recruitment of inflammatory cells that clear the infection. Whether this strategy can be extrapolated to enterocytes under enteric bacterial attack remains to be established.

8. Conclusions and perspectives

8.1. Microbial strategies: invasion, adhesion, toxins and rafts

As underscored by Sansonetti [61], bacterial pathogens have developed two major strategies to colonise the intestinal epithelium. Intestinal pathogens can either adhere to the intestinal epithelium and colonise its surface, or invade and cause inflammatory lesions. Bacterial species such as *Shigella*, *Salmonella*, and *Yersinia* are representative of the category of enteroinvasive pathogens. They use lipid rafts as initial attachment platforms and activate signal transduction pathways to their own profit, inducing major rearrangements of the actin cytoskeleton that eventually destroy brush border microvilli. In a marked different strategy, *V. cholerae* and enterotoxigenic *Escherichia coli* (ETEC) bind to apical lipid rafts without damaging the cytoskeleton of the microvilli. Yet they produce toxins (cholera toxin (CT) or thermolabile (LT) and thermostable (ST) toxins) that inhibit sodium/water and sodium/glucose absorption and activate chloride/water secretion, inducing both malabsorption and diarrhoea (Table 1).

Finally, although enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) also remain extracellular, they secrete protein effectors which mediate intimate adherence to and effacement of the microvilli of the brush border involving major rearrangement of the actin cytoskeleton. In this respect, the type of interaction they establish with the intestinal epithelium is reminiscent to that of invasive enteric pathogens [61].

The leitmotiv of the interactions between enteric pathogens and the intestinal epithelium is the involvement of GSL, cholesterol, and/or protein receptors located in lipid rafts (Table 1) [30]. Accordingly, the list of pathogens that use lipid rafts as portal of entry into host cells seems to grow endlessly [6,10]. As a matter of fact, there is a fundamental intrinsic weakness in the communication networks that link the cell surface (lipid rafts), the cytoskeleton (actin) and the endocytic machinery: internalization of ligands bound to lipid rafts is translocated to various cellular compartments (endo-

plasmic reticulum, nucleus, apical/basolateral membrane) but not to lysosomes. Thus, by choosing lipid rafts as a pathway of entry, microbes avoid potential lysosomal degradation [6]. In other words, lipid rafts may be viewed as membrane-organelles shuttles that take the pathogens aboard on the cell surface and drive them to a protected intracellular niche.

8.2. Therapeutic strategies: searching for glycolipidomimetics

The important contribution of cell surface carbohydrates to microbial adhesion onto mucosal surfaces has motivated the design of synthetic glycoconjugates as potential antimicrobial agents [67,68]. An immediate approach to mimick natural antibacterial strategies is to use the oligosaccharides that are found at concentrations as high as millimolar in human milk [69]. Indeed, milk oligosaccharides are responsible for the protective effects of non-immunoglobulin fractions of human milk. However, as discussed in Sections 5.3 and 5.4, the hydrophobic part of GSL receptors and, at least in some cases, cholesterol, have a profound impact on the orientation of the oligosaccharide moiety, so that free oligosaccharides have often a reduced affinity for ligand compared to the whole membrane-anchored GSL [39,67,70]. Moreover, GSL receptors are concentrated in lipid rafts, so that the free oligosaccharide may not be able to reach the local concentration and/or the active conformation of the carbohydrate binding domain of intact GSL receptors. For these reasons, two main strategies have been developed to increase the antimicrobial activity of carbohydrate-based candidate drugs. The first approach is to polymerize the oligosaccharide on a chemical matrix to obtain a multivalent neoglyconjugate [71,72]. The other approach is to modify the structure of the hydrophobic part of GSL receptors with the aim to obtain water-soluble analogs in which the conformation of the binding domain of the analog is as close as possible to that of the GSL [70,73]. Correspondingly, these analogs can be referred to as 'glycolipidomimetics'. One of this analogs, adamantyl-Gb3 [70] was found to functionally mimic Gb3 microdomains in the absence of cholesterol [39]. In this analog, the acyl chain is replaced by a rigid globular hydrocarbon frame, adamantane (Fig. 5).

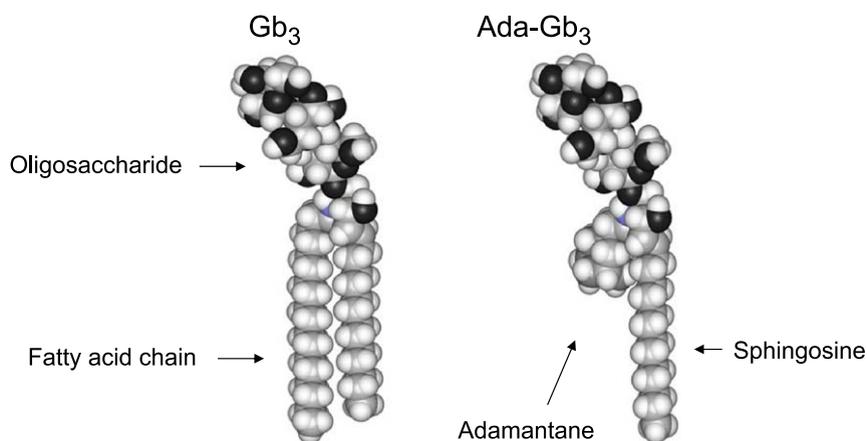


Fig. 5. Synthetic glycolipidomimetics: the future of antimicrobial therapeutics? The figure shows the structures of Gb₃ and its semi-synthetic analog adamantyl-Gb₃, a monovalent, water-soluble mimic of the GSL with potent antimicrobial activity. In this semi-synthetic analog, the fatty acid chain has been replaced by adamantane as indicated. Adamantyl-Gb₃ has been originally designed and synthesized by Mylvaganam and Lingwood [70] to inhibit binding of verotoxin to its GSL receptor.

Adamantyl-Gb₃ interacted with high affinity with verotoxin [70] and with HIV-1 gp120 [39]. These data suggest that glycolipidomimetics may represent a novel and promising approach for developing GSL-based antimicrobial prophylactic and/or therapeutic strategies.

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