Lipid raft organization and function in the small intestinal brush border

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Lipid raft organization and function in the small intestinal brush border

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The enterocyte brush border of the small intestine is a highly specialized membrane designed to function both as a high capacity digestive/absorptive surface of dietary nutrients and a permeability barrier towards lumenal pathogens. It is characterized by an unusually high content of glycolipids (~30% of the total microvillar membrane lipid), enabling the formation of liquid ordered microdomains, better known as lipid rafts. The glycolipid rafts are stabilized by galectin-4, a 36 kDa divalent lectin that cross-links galactosyl (and other carbohydrate) residues present on membrane lipids and several brush border proteins, including some of the major hydrolases. These supramolecular complexes are further stabilized by intelectin, a 35 kDa trimeric lectin that also functions as an intestinal lactoferrin receptor. As a result, brush border hydrolases, otherwise sensitive to pancreatic proteinases, are protected from untimely release into the gut lumen. Finally, anti-glycosyl antibodies, synthesized by plasma cells locally in the gut, are deposited on the brush border glycolipid rafts, protecting the epithelium from lumenal pathogens that exploit lipid rafts as portals for entry to the organism.

Key words: Small intestine, Enterocyte, Brush border, Lipid rafts, Galectin, Intelectin, Anti-glycosyl antibodies.

The enterocyte brush border: A digestive/absorptive surface and permeability barrier organized and stabilized by galectin-4

The most striking morphological feature of the enterocyte, the principal absorptive cell of the gastrointestinal tract, is the apical brush border membrane, composed of up to 3000 microvilli/cell (Fig. 1). The microvillar architecture is defined by an actin-based cytoskeleton core with short cross filaments connecting transversely to the inner membrane leaflet (23, 27). To provide physical stability, each microvillus actin core filament extends as a rootlet into the

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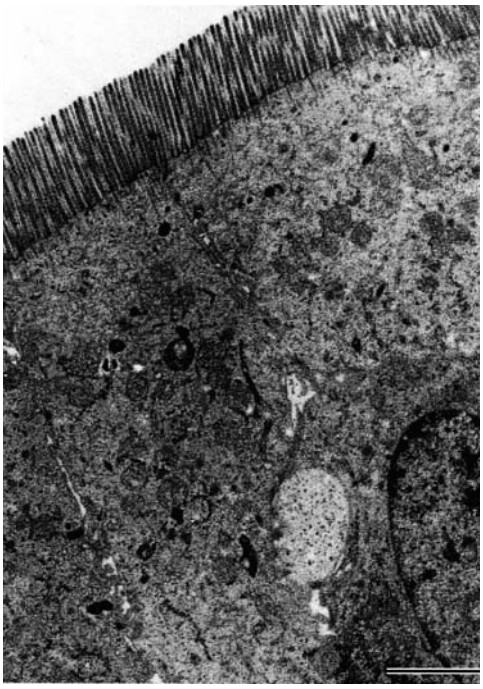

Fig. 1. *Electron micrograph showing porcine small intestinal enterocytes. Notice the columnar shape of the cells and the apical brush border facing the lumen of the gut. Bar, 1 μm.*

terminal web, a myosin-rich region that extends up to 1 μm into the cytoplasm.

The two main tasks of this highly specialized part of the plasma membrane are: 1) to function as a digestive/absorptive surface enabling the organism to extract efficiently all major and minor types of dietary nutrients, and 2) to act as a protective permeability barrier against various lumenal pathogens. To perform this dual role whilst being frequently exposed to bathings containing pancreatic proteinases and lipases and hepatic bile salts requires a robust membrane organization. This demand is reflected in the lipid composition of the enterocyte brush border which contains > 30 % glycolipids, mainly in the form of mono, di- and pentohexosylceramides (3). Together with cholesterol and sphingomyelin, glycolipids promote the spontaneous formation of lateral liquid-ordered microdomains in the cell membrane, commonly known as lipid rafts (34). This general ability to form lipid rafts has been demonstrated in artificial model membranes, but the general biological significance of this property is still being debated (15). Lipid rafts in various cell membranes have commonly been characterized by their ability to resist solubilization with detergents, i.e. to form detergent resistant membranes (DRMs). Partition into DRMs is thus indicative of lipid raft localization, but true colocalization of candidate raft proteins should always be verified by other experimental approaches, such as co-immunoprecipitation or high resolution immunogold electron microscopy.

Early DRM analyses of microvillar vesicles prepared from small intestine (4) and Caco-2 cells (26) revealed that significant proportions of many of the major digestive peptidases and glycosidases as well as other proteins were included in this fraction (5, 6). However, contrary to DRMs from other cell types, those isolated from the intestinal brush border do not depend upon the presence of cholesterol, as evidenced by their resistance to methyl-β-cyclodextrin, but instead rely on the abundancy of glycolipids and the presence of galectin-4 (7, 2). Galectin-4 is a 36 kDa member of the galectin family of β-galactoside-binding lectins, a group of proteins found in a variety of tissues and implicated in many different biological functions (1, 22). Like other galectins, galectin-4 is synthesized without a signal for membrane translocation and secreted by an as yet poorly understood "nonclassical" mechanism (28). Being divalent, galectin-4 has the ability to act as a cross-

linker (18), and in the enterocyte it is targeted to the exoplasmic leaflet of the brush border where it firmly associates with lipid rafts and other proteins, including the major enzymes aminopeptidase N and sucrase-isomaltase (7). Galectin-4 may associate with a broad range of sulfated glycolipids (16), and together with galectin-3 it has been proposed to play a functional role in apical membrane trafficking of glycoproteins in intestinal epithelial cells (9, 10).

In the brush border, galectin-4's role as an organizer and stabilizer is indicated by the observation that release of galectin-4 from the membrane by lactose simultaneously removes other lipid raft-associated proteins such as aminopeptidase N and alkaline phosphatase (11). The strength of this association is reflected by the possibility to prepare a special type of microvillar microdomains by rounds of sequential detergent extraction at increasing temperatures (2). The resulting "superrafts" (so termed because of their insolubility in 1% Triton X-100 at 37 °C) were greatly enriched in galectin-4, but also harboured substantial amounts of the major brush border enzymes. With residence times in the brush border reportedly as short as 6-8 hours (12), these enzymes are frequently exposed to pancreatic proteinases and lipases capable of releasing them into the gut lumen by cleavage of their sensitive stalks that link the catalytic head groups to the membane. By binding simultaneously to membrane glycolipids and these stalk regions (believed to be heavily O-glycosylated), galectin-4 acts to protect the brush border enzymes from solubilization and also to rescue cleaved enzymes from release into the gut lumen.

Intelectin

A 35 kDa protein associated with lipid rafts of the enterocyte brush border was recently identified as intelectin (37) (Fig. 2). This secretory mammalian lectin was initially shown to be expressed in the Paneth cells of the small intestine (21), and to have a preferential affinity for D-pentoses and D-galactofuranosyl residues (36). In addition, the existence of an isoform, intelectin-2, expressed in intestinal goblet cells, has subsequently been reported (30). Interestingly, intelectin is known to be identical to the enterocyte brush border lactoferrin receptor which is a glycosylphosphatidylinositol-anchored protein (35). Lactoferrin is a member of the transferrin family of iron-binding proteins principally found in milk, but it

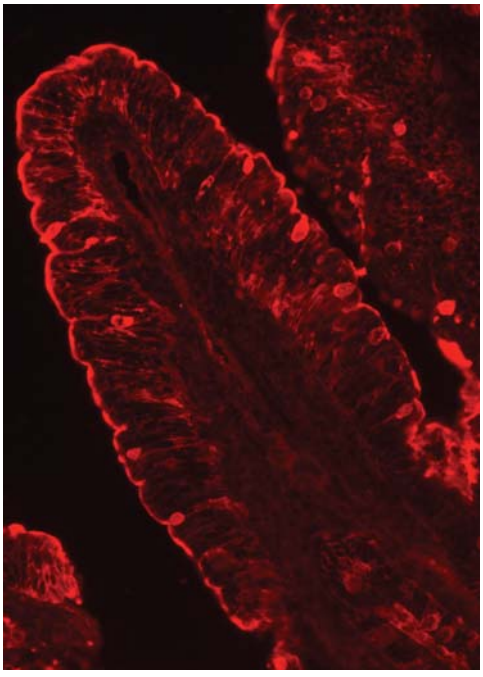

Fig. 2. *Immunofluorescence labeling of intelectin in a villus from porcine small intestine. The entire brush border surface along the enterocytes as well as goblet cells are strongly labeled.*

is also found in many other secretions. Both functions (as a lectin and lactoferrin receptor) imply that intelectin is engaged in the innate defense against microorganisms. Like galectin-4, intelectin is highly enriched in the microvillar superraft fraction that resists Triton X-100 solubilization at 37 °C (37). This implies that intelectin too serves as a membrane organizer/stabilizer in the brush border and protects the gut epithelium from pathogens (8).

Anti-glycosyl antibodies help protecting the glycolipid-based brush border rafts from pathogens

Anti-glycosyl antibodies were discovered many years ago and so named because they are induced by – and combine with - glycosyl antigens (29). They may comprise up to 1% of the total pool of circulating antibodies in the organism (14) and include both IgG- and IgM classes with the former having the highest affinity towards lactosyl residues and the latter having the highest affinity towards galactosyl residues (24). More recently, anti-glycosyl antibodies were isolated by lactoseagarose chromatography from small intestinal mucosa and shown to be major soluble lectin-like proteins locally synthesized in the gut (16, 17). Depositions of these antibodies were demonstrated in the brush border where they mainly localized with DRMs. They were relased from the brush border by a brief wash with lactose, indicating a relatively weak association with the membrane. Interestingly, this treatment increased the binding of the galactosyl-binding lectin PNA as well as cholera toxin B subunit, suggesting that a role for the anti-glycosyl antibodies could be to prevent pathogenic molecules from gaining access to the lipid rafts of the brush border. Since a large number of pathogens, including bacteria, viruses, fungi, parasites and toxins exploit lipid raft components for making contact with their target cells (13, 33), such a lipid raft-protective function of anti-glycosyl antibodies might be physiologically significant.

Concluding remarks

In this review, we have described the intestinal brush border as a highly specialized type of membrane composed of glycolipid-based lipid raft microdomains. Unlike current notions of lipid rafts that predict them to be quite small (~10 nm range) and dynamic (20, 31), available evidence suggests that the glycolipid rafts of the brush border reside *in situ* as fixed entities and are large enough to harbour supramolecular complexes of digestive enzymes. So far, the key organizing/stabilizing protein components of these microdomains have been identified as galectin-4 and intelectin. In addition, lectin-like, low-affinity anti-glycosyl antibodies may provide further protection from adhesion of pathogens. But it is quite likely that more proteins are involved in the maintenance of the unique intestinal brush border membrane. These could be other members of the galectin family, of which several are expressed along the gastrointestinal tract (11), or other types of locally synthesized lectins (8).

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