Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 18
Sofia • 2012

Structure and function of the intestinal filamentous brush border glycocalyx review

V. Pavlova, B. Alexieva and E. Nikolova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Science, Sofia

Intestinal epithelium is primarily comprised of absorptive villus enterocytes, which apical surface is a highly differentiated structure consisting of rigid, closely packed microvilli. They are coated with a 400-500 nm thick meshwork referred to as the Filamentous Brush Border Glycocalyx, composed of highly glycosylated transmembrane mucins. It appears to serve as a size-selective diffusion barrier that excludes particles such as bacteria and viruses, preventing their contact with the enterocyte plasma membrane and impeding access to the small inter-microvillus membrane domains involved in endocytosis. The integral membrane mucin-like glycoproteins that form the glycocalyx, contain adsorbed pancreatic enzymes and stalked intramembrane glycoprotein enzymes responsible for terminal digestion. As a consequence the glycocalyx prevents the uptake of antigens and pathogens while providing a highly degradative microenvironment that promotes the digestion and absorption of nutrients.

Key words: enterocytes, glycoproteins, mucins, microbiota,

Human intestine is the most densely populated organ with microorganisms and it is a site where they exert a strong influence on human biology. This is because the intestinal mucosa serves as the primary border between the immune system and the external environment. The immense complexity of gut flora together with its highly complicated interactions with intestinal epithelium makes it an arduous system to study.

The host is protected from potentially harmful enteric microorganisms by the physical and chemical barriers created by the intestinal epithelium, primarily comprised of absorptive villus enterocytes. The apical surface of the enterocytes is a highly differentiated structure consisting of rigid, closely packed microvilli whose membranes contain stalked glycoprotein enzymes [15]. In Fig 1. and Fig 2. small intestinal microvilli from adult mouse are visualised by transmission electron microscopy after ruthenium red staining.

In addition, the tips of enterocyte microvilli are coated with thick meshwork referred to as the Filamentous Brush Border Glycocalyx (FBBG) [6].

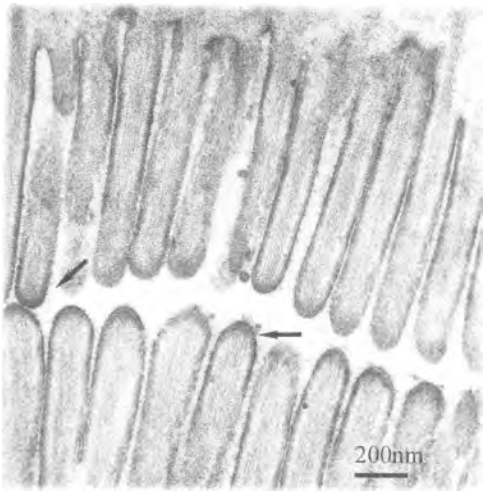

Fig. 1 Small intestinal microvilli of adult mouse covered by FBBG, stained with Ruthenium red. Original magnification x 30 000.

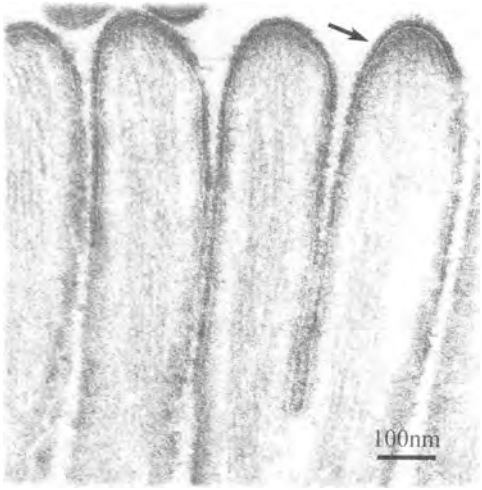

Fig. 2 Small intestinal microvilli of adult mouse covered by FBBG, stained with Ruthenium red. Original magnification x 75 000.

Although the ultrastructural features of the FBBG were originally described more than 40 years ago [5], its major component was only recently shown to be a transmembrane mucin of 400 kDa with abundant, heterogeneous oligosaccharide chains containing O-acetylated sialic acid [4]. The three-dimensional ultrastructure of the FBBG in the mouse small intestine was successfully demonstrated by high resolution (x 200 000) scanning electron microscopy by the same author. The glycocalyx was observed as filamentous structures, 7 to 15 nm thick in diameter. The filaments repeatedly branched and anastomosed with neighboring ones to form an actual network, globularly thickened at the branching sites. The morphological appearance of FBBG was established to be similar in amphibians, rodents, bats and humans.

FBBG appears to serve as a size-selective diffusion barrier that excludes bacteria and viruses, preventing their contact with the enterocyte plasma membrane and impeding access to the small inter-microvillus membrane domains involved in endocytosis [8]. The structure of the sugar chains of the glycocalyx influences a wide variety of interactions including: cell-cell recognition, the binding and internalization of pathogenic bacteria, toxins [19] and viruses [10, 20, 7].

The negatively charged integral membrane mucin-like glycoproteins also implement numerous proteolytic activities designed to degrade proteins and peptides. They contain adsorbed pancreatic enzymes and stalked intramembrane glycoprotein enzymes responsible for terminal digestion (brush border enzymes). It was found that about 60% of the pancreatic amylase, 80% of the trypsin and 20 % of the chymotrypsin are concentrated in the apical glycocalyx of enterocytes [18]. As a consequence the FBBG prevents the uptake of antigens and pathogens while providing a highly degradative microenvironment that promotes the digestion and absorption of nutrients [16].

The glycocalyx layer over the intestinal epithelium is covered by a loosely adherent mucous layer [5, 12], which consists of glycoproteins, enzymes, electrolytes and water [2]. This mucus layer meets the luminal content of the gut in a loosely formed

biofilm of symbiotic microbiota. Together with the glycocalyx, they are believed to protect the apical cell surface against microbial pathogens and foreign materials partially by virtue of the electrical repulsion of negatively charged sugar moieties [3, 19]. They are considered to form a hydrophilic polyanionic gel coat on the enterocyte surface and maintain cell surface charge, regulate ionic and macromolecular access, form cationic store and also protects apical cell surfaces against physical thrauma [11]. These mucosubstances readily undergo depolymerization and repolymerization and are capable of reversible linkages with other compounds depending on local chemical and physical changes. These properties most certainly enable the material to be absorbed in the first instance to the cell surface. Only then it can be processed and transported across the membrane by the elements constituting the "digestive absorptive surface" of which the glycocalyx appears to be one of the essentials.

N-Acetyl-D-Glucosamine (NAG) is a key precursor in the biosynthesis of mucosal glycoproteins that form the intestinal glycocalyx [1]. Clinical studies indicate that a fundamental abnormality in mucous glycoprotein synthesis may exist in patients with inflammatory bowel disease (IBD) and colon cancer. Synthesis of NAG in the body begins with the conversion of L-glutamine to glucosamine-6-phosphate. The biochemical defect found in IBD patients appears at an early step in glycoprotein synthesis and acts to inhibit the N-acetylation of Glucosamine-6-phosphate to NAG. This abnormality appears to cause a reduction in the normal protective property of the glycocalyx and renders the intestinal mucosa more susceptible to pathogens and inflammation [13].

It was also demonstrated that the composition of intestinal glycocalyx and mucins differ between neonates and adults. These differences may be a primary determinant of the distinct differences in microbial composition of the intestine of neonates and adults and of their differing susceptibility to enteric pathogens [9, 14].

Mucosal tissues represent the site of infection or the route of access for majority of pathogenic viruses and bacteria. Mucin glycoproteins are secreted in large quantities by mucosal epithelium and they play a central role in accommodation of resident commensal flora and limitation of infectious one. Although largely unexplored, the gut commensal microflora or more recently "microbiota" plays an intricate and underappreciated pivotal role for the health and well-being of mammals. It serves a central line of resistance to colonization by exogenous microbes, and thus assists in preventing the potential invasion of the intestinal mucosa by an incoming pathogen. This protective function is known as the barrier effect or colonization resistance and serves a number of important roles. For instance, adherent nonpathogenic bacteria can often prevent attachment and subsequent entry of suspected pathogens into epithelial cells, as well as compete for nutrient availability [17].

The cross talk and interactions between the enterocyte apical membrane and glycocalyx, the mucin molecules and the intestinal microflora determines the dynamic nature of the brush border region. This dynamic status is reflected in the ongoing renewal of epithelial cells, the processes of polarization and maturation of enterocyte cells (by acquiring the abilities for digestion and absorption via various enzymes and transporters), the production of mucus from goblet cells and its degradation by the microflora.

The intestinal lumen is a space topologically outside the living organism, the composition of which is regulated by the body. The physicochemical environment of the intestine determines how nutrients are absorbed and how potential pathogens are controlled. The ability of the intestine to monitor this space is therefore critical to life.

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