

## CARBOHYDRATE LOCALIZATION IN INTESTINAL GLYCOCALYX

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### ABSTRACT

The intestinal glycocalyx constitutes a glycosylated milieu, reactive with gut microflora and protects the gut from intestinal infections. The contents of the glycocalyx layer are in dynamic balance between biosynthesis of new glycans and removal of existing constituents. The fine structure of the glycocalyx was recently revealed to some extent.

The aim of our work was to study the structure and localization of the components of intestinal glycocalyx in mice. For that purpose we used lectins labeled with fluorescent dye or with biotin (*Lotus tetragonolobus*, *Ulex europaeus*, *Triticum vulgare*). We used fluorescent and transmission electron microscopy to trace the localization of N-acetyl- $\beta$ -glucosamine oligomers and of  $\alpha$ -L-fucosyl residues on the microvillus glycocalyx. N-acetyl- $\beta$ -glucosamine oligomers were abundant in all microvillus surfaces.  $\alpha$ -L-fucosyl residues were not detectable in the glycocalyx of duodenum, jejunum or ileum of adult mice by fluorescent microscopy but localized using the confocal state of art technique.

**Key words:** *intestinal glycocalyx, lectins staining, fluorescent microscopy.*

### INTRODUCTION

The intestinal glycocalyx is comprised of protein- and lipid-bound oligosaccharides and polysaccharides attached to membrane-associated proteins and lipids covering the apical surface of enterocytes. It constitutes a significant barrier to transvascular exchange of water and solutes, and a binding site for different enzymes, growth factors and microorganisms [7, 12]. It has been suggested that the fine structure of that porous layer is composed of a matrix of molecules which are arranged in a regular pattern [14]. The fine structure of the glycocalyx could be revealed by electron microscopy. Many recent studies prove that glycosaminoglycans like hyaluronan (HA), heparan sulfate (HS) and chondroitin sulfate (CS) are found in different level of sulfation, depending on the physiological micro-environment, and are linked to transmembrane proteoglycans [12, 15]. Moreover the contents of the glycocalyx layer are in dynamic balance between biosynthesis of new glycans and removal of existing constituents [8]. It has been shown in previous studies that glycocalyx is an important factor for gut colonization. Breastfed children receive different glycans with milk and a preponderance of *Bifidobacterium bifidum* and lactobacillae in their intestines was noted. In contrast, not breastfed infants had microbiota more typical of adults. That causes the gut contents of breastfed infants to be more acidic, which can inhibit colonization by many pathogens [9]. Oligosaccharides in human milk are indigestible by the infant gut and therefore are not used as a nutrient. Their probable function is to protect the gut from pathogens. Mass spectrometry analysis proved that many milk oligosaccharides could contain components with structural homology to cell surface glycans. Therefore they are able to inhibit binding of pathogens to cell surface receptors in the mucosa and protect the infant from disease [10]. It is obvious that the saccharide "barrier" is crucial to the biology of the cell. It specifically modulates its interactions with small molecules, macromolecules, other cells, and with the extracellular environment.

The aim of our work was to study the structure and localization of the components of intestinal glycocalyx in adult mice. For that purpose we used lectins that were stained with fluorescent dye or with biotin.

## MATERIALS AND METHODS

Three biotinylated lectins (Vector Laboratories, USA) Lotus tetragonolobus, Ulex europaeus, Triticum vulgare were used after the lectin-gold pre-embedding protocol [13]. Small sections of duodenum, jejunum and ileum from adult mice taken and were washed, and then fixed in 2% PFA-BSA for one hour. Then samples were blocked in 3% BSA and incubated overnight at 4°C with one of the lectins -lotus tetragonolobus (100 µg/ml), ulex europaeus (6.25 µg/ml) or triticum vulgare (25 µg/ml). Streptavidin-gold particles (10 NM), from *Streptomyces avidinii* (Sigma, deluded 1:50) were added to samples and additionally incubated overnight at room temperature. Unreacted conjugates were washed off by three successive wash-ings in PBS and then samples were processed for transmission electron microscopy, and embedded in LRWhite resin following the standard protocol. Ultra thin sections were cut mounted on Ni-grids and observed on TEM.

For lectin-FITC visualization we used Tissue Tek (Sakura Finetek Inc., USA) to freeze the gut samples. Later they were cut into thin sections (5 µm). Samples were washed in PBS and blocked in 3 % BSA –PBS for one hour. Lectin-FITC was added for four hours and after careful washing samples were observed on fluorescent microscope or on Nikon D-Eclipse C1 Confocal Microscope.

All animal procedures were handled according to the rules of the Animal Ethics Committee at the Institute.

## RESULTS AND DISCUSSION

The apical surface of intestinal enterocytes is covered by acid mucosal substances (glycoproteins). Their renewal is constantly completed by the underlying epithelial cells. It has been supposed that the high incorporation of radioactive precursors of glycoproteins and the positive staining with colloidal gold particles are in correlation with their high metabolic activity. The glycoprotein layer is critical for the proper viscosity, it connects water molecules and together with polysaccharides they can depolymerize and repolymerize easily. The glycocalyx ingredients are synthesized in the basolateral part of the cells and migrate to the luminal part of the gut through cells' tight junctions. Apical surface properties of enterocytes are important to understand the mechanisms involved in the binding of pathogens to intestinal cells. Integrity of the gastric mucosa is also an important factor for the proper function of digestive and absorptive processes. Lectin staining is a powerful method to trace the distribution of glycocalyx components in the small intestines. The protocol was used to investigate age related changes in the carbohydrate histochemistry in the porcine small intestine [1]. The results suggest age-region-, and cell types-related changes in the epithelial glycocalyx. Other investigations focus on the specific lectins binding M cells, which are specialized gut-associated lymphoid tissue (GALT) epithelial cells. It is supposed that M cells might facilitate the accessibility of antigens [3]. Despite their poorly developed apical glycocalyx in comparison to enterocytes, pathogens are selectively trapped at the surface of M cells. Their carbohydrate specificity was demonstrated in mice. Apparently, carbohydrates could provide a useful tool to identify M-cells in murine intestine [2]. Moreover, the apical surface of M cells was found to be rich in carbohydrates that share epitopes with Muc2, a mucin, secreted onto mucosal surfaces. Muc2 provides an insoluble mucous barrier and serves as protective agent for the intestinal epithelium [6].

Using biotinylated lectins (Fig.1 and Fig.2) or FITC-labeled lectins (Fig.3 - Fig.6) we found that UEA and WGA lectins were localized on the tips of microvilli. N-acetyl-β-glucosamine oligomers were abundant in all microvillus surfaces. α-L-fucosyl residues were not detectable in the glycocalyx of duodenum, jejunum or ileum of adult mice by fluorescent microscopy but localized using the confocal state of art technique.



Fig.1. TEM micrograph of small intestine treated with UEA. Originally X 20K

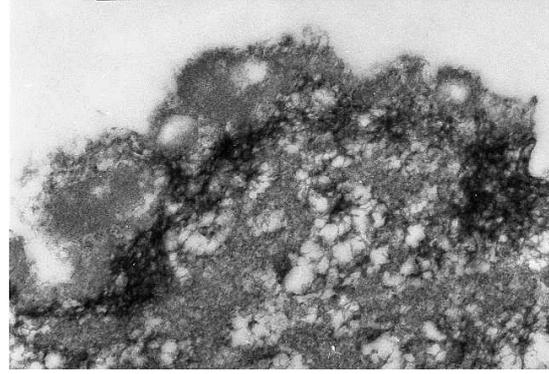


Fig.2. TEM micrograph of small intestine treated with WGA. Originally X 45K

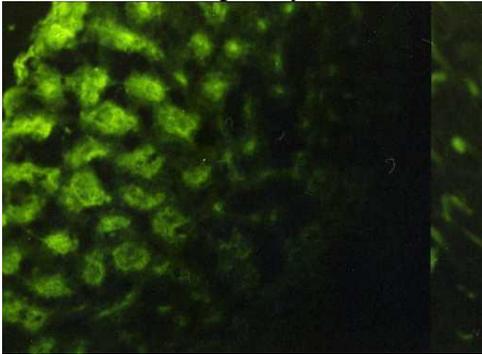


Fig.3. Localization of UEA in murine intestinal glyocalyx after observation under fluorescent microscope. Originally X 40.

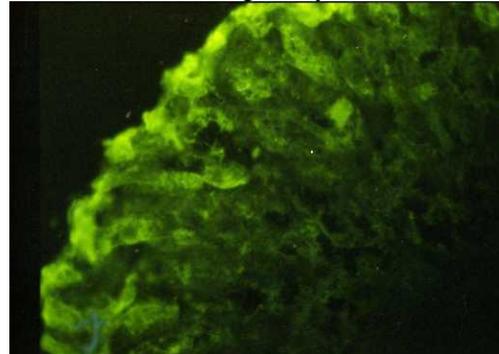


Fig.4. WGA observed on tip of microvilli under fluorescent microscopy. Originally X 40.

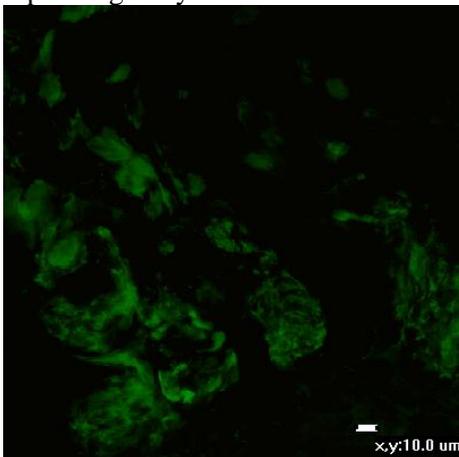


Fig.5. Confocal micrograph of jejunum treated with LTA. Originally X 60.

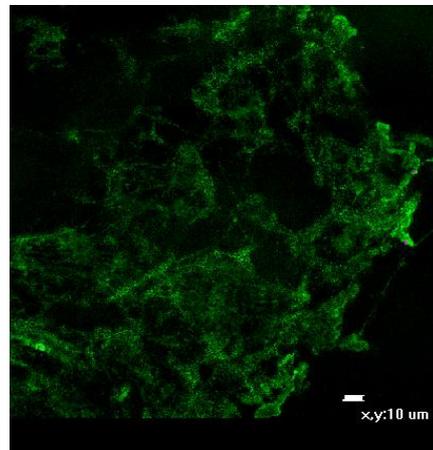


Fig.6. Confocal micrograph of ileum treated with WGA. Originally X 60.

N-acetyl- $\beta$ -glucosamine (NAG) is a simple amino sugar, a monosaccharide with an amino group as part of its structure. It is a nutrient, an intermediary metabolite, and a component of the glyocalyx coat carried by all the body's cells. In the small intestine it protects the living epithelium from digestive enzymes and other potentially damaging intestinal contents. Pittschieler et al. [11] demonstrated NAG lectins to be typical for the staining of jejunal mucosa of healthy children and of patients suffering form celiac disease. Our results of the distribution of NAG are in correlation with the latter research.

L-fucose (6-deoxy-L-galactose) is a monosaccharide that is a common component of many N- and O-linked glycans and glycolipids produced by mammalian cells. This sugar is enzymatically synthesized in mammalian cells and is also recovered by cells from extracellular sources by specific

transmembrane carriers. Fucosylation is important for the biological properties of glycans. It is supposed that many additional functions for fucosylated glycans remain uncovered. In the small intestine  $\alpha$ -L-fucosylated glycan expression is believed to contribute to establishment of the indigenous microbial community in the developing gut by providing a favorable environment for commensal organisms capable of utilizing fucose as a carbon source [5]. It was also proved that fucose could be added to mucins in the course of goblet cell maturation because goblet cells of the upper parts of the intestinal crypts stained more intensely than the goblet cells in lower parts of the crypts [16]. In our research  $\alpha$ -L-fucosyl residues were detectable in the glycocalyx of adult mice only under confocal microscopy observation which is in correlation with the results of A. Gebhard et al. [4] where only brush cells but rarely enterocytes contained  $\alpha$ -L-fucose in their apical membrane.

### CONCLUSIONS

The carbohydrates in the murine intestinal glycocalyx were visualized by lectin-gold or by lectin-FITC protocols. N-acetyl- $\beta$ -glucosamine and NN'-diacetyl-hitobiose were evenly localized on tips of microvilli, while  $\alpha$ -L-fucosyl residues were not detected. The biological property of these carbohydrates could be to contribute to some specific interactions between microorganisms, populating the adult murine gut and the apical membrane of enterocytes. Further studies are needed to trace the difference in carbohydrates localization and their biological significance in different ages in mice.

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**Acknowledgement:** This work was supported by form the Ministry of Education, Youth and Science, Republic of Bulgaria, grant № DFNI-B01/30 from 10 Dec 2012.