NOTE *Anatomy*

Differences in Lectin-Binding Properties between the Common Mucosal Epithelium and Follicle-Associated Epithelium in the Rabbit Small Intestine

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ABSTRACT. Differences in sugar distribution between the villous epithelium and follicle-associated epithelium (FAE) were compared using lectins in the rabbit small intestine. In every portion, villous columnar epithelial cells primarily exhibited a positive reaction to the GalNAc, GlcNAc, galactose, and oligosaccharide. In the ileal Peyer's patch (PP), whereas microvillous epithelial cells exhibited positive reactions, M cells tended to be negative. The villous epithelial reaction to the fucose group was negative, but M cells and microvillous epithelial cells showed a positive to the fucose. No epithelium had a positive reaction to the mannose and glucose. The variety of lectin-binding properties of villous epithelial cells and M cells may reflect specificity for the recognizing luminal substances such as antigenic molecules and bacterial elements.

KEY WORDS: lectin, M cell, Peyer's patch.

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The mucosal surface of the gastrointestinal tract is constantly exposed to an external environment because of its role in digestion and the absorption of foods. The intestinal mucosa must simultaneously come in contact with enteric pathogens or dietary antigens and ingested nutrients or indigenous microorganisms. Therefore, a highly organized immune system (gut-associated lymphoid tissue: GALT) is developed in addition to a nonspecific host defense system. In the GALT, specialized epithelial cells, called membranous or microfold cells (M cells), exist within the follicle-associated epithelium (FAE) [5, 9, 11]. It is generally accepted that M cells randomly transport luminal antigens to antigen-presenting cells such as lymphocytes, macrophages, and dendritic cells that closely interact with M cells, and initiate an immune response in the intestine or oral tolerance in other sites of the body. In this study, the expression patterns of sugars of the villous epithelium and FAE were compared using 21 lectins in the rabbit small intestine. Lectins are specific carbohydrate-binding glycoproteins, and they have been used as a selective marker to identify M cells and other intestinal epithelial cells in mammals [2, 6, 7, 13]. Moreover, it is speculated that microbial infection is mediated by a lectin-like adhesin that recognizes sugars on intestinal epithelial cells [3, 4, 10].

Seven adult Japanese white rabbits (weighing 2.0 kg, male) were purchased (Japan SLC, Japan) and used in this study. All procedures were in accordance with the Guide for Care and Use of Animal experimentation at Ehime University. Rabbits were sacrificed by exsanguination under anesthesia with *i.v.* injection of pentobarbital sodium (100 mg/kg). Thereafter, the duodenum (5 cm distal from pylorus), jejunum (middle region between pylorus and the cecocolic orifice), and ileum (10 cm proximal to the cecocolic orifice; containing the ileal Peyer's patch) were immediately immersed in 4% paraformaldehyde for 1 hr at 4°C and embedded in paraffin according to a routine procedure. After endogenous peroxidase was blocked with H_2O_2 for 30 min, sections 4 μm in thickness were treated with serum-free protein block (X0909; Dako, Denmark) for 1 hr at room temperature. They were then incubated with 21 kinds of biotinylated lectins at a 1:5,000 dilution (BK-1000, 2000, 3000; Vector, U.S.A.) for 18 hr at 4°C and peroxidase-labeled streptavidin (424011; NICHIREI, Japan) for 30 min at room temperature. Finally, they were incubated with 3,3-diaminobenzidine (DAB) (K4007; Dako, Denmark). To identify M cells, vimentin antibody was also used. Sections containing the Peyer's patch were incubated with a mixture of monoclonal anti-vimentin V 9 (V6630; Sigma, U.S.A.) at a 1:10,000 dilution for 18 hr at 4°C after incubation with each of the biotinylated lectins. Thereafter, sections were incubated with labeled polymer-HRP anti-mouse (K4007; Dako, Denmark) for 30 min at room temperature and visualized with DAB. Generally, it has been accepted that M cells have distinctive features such as thick and sparse microvilli and pocket-like invagination of the basal or lateral plasma membrane with both intraepithelial lymphocytes and macrophages. Typical M cells are therefore easily distinguished from other epithelial cells. In this study, M cells were identified based on these morphological features in addition to vimentin reactivity.

The major specific binding properties of lectins for sugars can be classified into 6 groups: N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc), galactose, glucose/mannose, oligosaccharide, and fucose groups, respectively (Table 1). In the GalNAc group, the cellular surface of villous columnar epithelial cells and microvillous epithelial cells of the FAE showed a positive reaction for all lectins, but M cells expressed a negative reaction for BSL-1, SBA,

Table 1. Lectin binding property and staining pattern in the rabbit small intestine

Lectin	Abbrebiation	Binding specificity	Duodenum		Jejunum		Ileum		Ileal Peyer's patch		
			V	G	V	G	V	G	MV	G	M
N-acetylgalactosamine group											
Bandeiraea simplicifolia lectin-I	BSL-I	α-GalNAc, α-Gal	+	±	+	±	+	±	±	±	–
Dolichos biflorus agglutinin	DBA	α-GalNAc	+	+	+	+	+	+	+	+	+
Glycine maxi (soybean agglutinin)	SBA	α-GalNAc	±	+	±	+	±	+	±	+	–
Ricinus communis agglutinin-I	RCA-I	β-GalNAc, β-Gal	±	+	±	+	±	+	±	+	±
Sophora japonica agglutinin	SJA	β-GalNAc	±	+	±	+	±	+	±	+	–
Vicia villosa agglutinin	VVA	β-GalNAc	+	±	+	±	+	±	±	±	–
N-acetylglucosamine group											
Bandeiraea simplicifolia lectin-II	BSL-II	α,β-GlcNAc	±	±	±	±	±	±	±	±	–
Datura stramonium lectin	DSL	β-GlcNAc	+	+	+	+	+	+	+	+	+
Lycopersicon esculentum lectin	LEL	β-GlcNAc	+	±	±	±	±	±	–	±	–
Solanum tuberosum lectin	STL	β-GlcNAc	–	±	–	±	–	±	–	±	±
Succinylated Triticum vulgaris	s-WGA	β-GlcNAc	–	±	±	±	±	±	±	±	–
Triticum vulgaris (Wheat germ agglutinin)	WGA	β-GlcNAc>α-NeuNAc	+	±	+	±	+	±	+	±	+
Galactose group											
Arachis hypogaea (Peanut agglutinin)	PNA	galactosyl-β-GalNAc	+	±	+	±	+	±	±	±	–
Artocarpus integrifolia (Jacalin)	Jacalin	galactosyl-β-GalNAc	±	±	±	±	±	±	±	±	–
Erythrina cristagalli lectin	ECL	galactosyl-β-GlcNAc	–	+	±	+	±	+	±	+	–
Glucose/mannose group											
Canavalia ensiformis (Concanabalin A)	ConA	α-Man>α-Glc	–	–	–	–	–	–	–	–	–
Lens culinaris agglutinin	LCA	α-Man>α-Glc	–	–	–	–	–	–	–	–	–
Pisum sativum agglutinin	PSA	α-Man>α-Glc	–	–	–	–	–	–	–	–	–
Oligosaacharide group											
Phaseolus vulgalis agglutinin-E	PHA-E	Oligosaccharide	+	–	+	–	+	–	+	–	+
Phaseolus vulgalis agglutinin-L	PHA-L	Oligosaccharide	+	–	+	–	+	–	+	–	+
Fucose group											
Ulex europaeus agglutinin-I	UEA-I	α-Fuc	–	–	–	–	–	–	+	–	±

Fuc: fucose, Gal: galactose, GalNAc: N-acetylgalactosamine, Glc: glucose, GlcNAc: N-acetylglucosamine, Man: mannose, NeuNac: N-acetylneuraminic acid. G: Goblet cell, M: M cell, MV: microvillous epithelial cell, V: Villous columnar epithelial cell. +: moderate staining, ±: weak staining, –: negative staining.

SJA, and VVA (Table 1). In the GlcNAc group, most of the villous columnar epithelial cells showed a positive reaction for all lectins, whereas microvillous epithelial cells and M cells expressed a negative reaction for LEL, STL and BSL-II, and LEL and S-WGA, respectively (Table 1, Fig. 1). In the galactose group, M cells expressed a negative reaction for all lectins in contrast to the other epithelial cells (Table 1, Fig. 1). In the glucose/mannose group, all epithelial cells showed a negative reaction (Table 1). In the oligosaccharide group, all epithelial cells showed a positive reaction except for goblet cells (Table 1). In the fucose group, only M cells and microvillous epithelial cells showed a positive reaction for UEA-I (Table 1, Fig. 1).

Lectins have been used as a selective marker to identify M cells in addition to the morphological features. However, the lectin-binding properties of M cells vary among species, and M cells existing at different intestinal portions did not express a similar lectin-binding pattern, even in the same animal [1, 2, 6, 7]. In addition to this, the glycocaryx of intestinal epithelial cells is known to be a binding site for microorganisms [3, 4, 10] and the infection of pathogens seems to be mediated by a lectin-like adhesin that recognizes sugars on the epithelial cellular surface [3]. On the other hand, it has been reported that there are a number of pores at the villous epithelial basement membrane as well as at the Peyer's patch in mammalian intestines, and intraepithelial lymphocytes or macrophages pass through the pores when they come in contact with epithelial cells [8, 12, 14]. These reports led us to the hypothesis that an aggressive interaction between villous columnar epithelial cells and lymphocytes or macrophages may occur at the corresponding site [14]. In the present study, most of the lectins of the GalNAc, GlcNAc, and galactose groups bound to villous columnar and microvillous epithelial cells of the FAE, but not to M cells. UEA-I bound to the M cells but not to the villous columnar epithelial cells, as reported previously. It is postulated from these results that the specific lectin-binding properties of villous epithelial cells and M cells may reflect the specificity for recognizing luminal substances such as antigenic molecules and bacterial elements of indigenous intestinal micro bacteria.

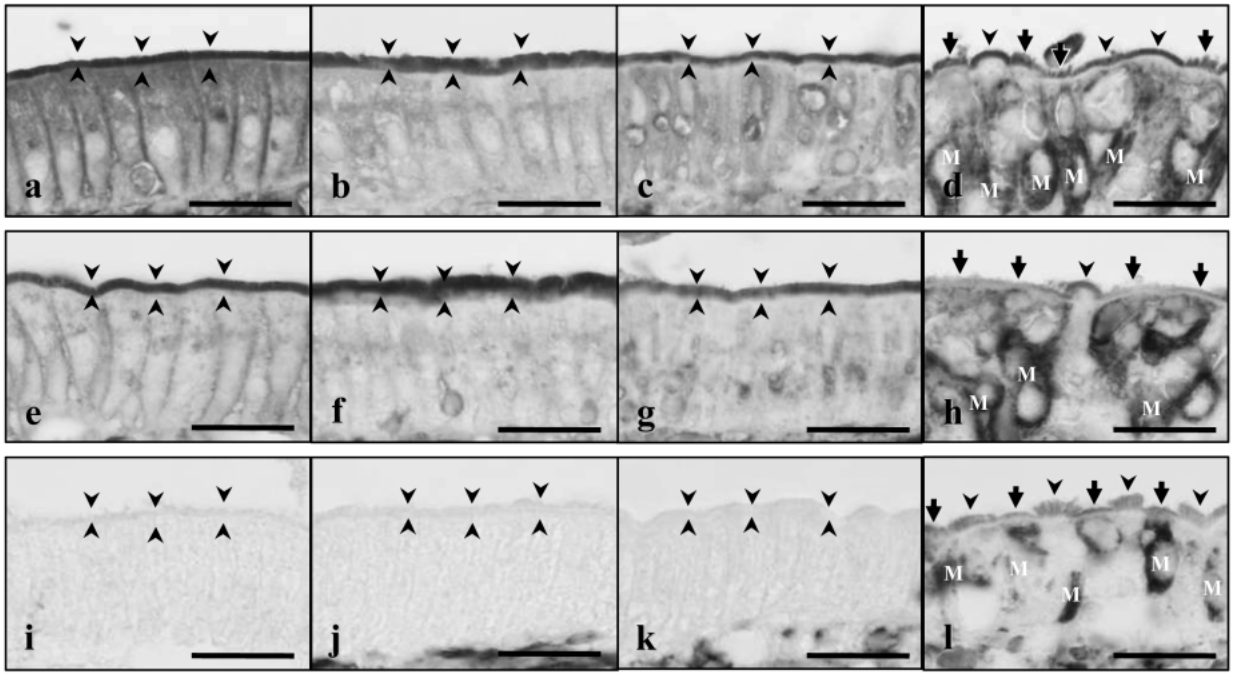

Fig. 1. Staining pattern of WGA (a-d), PNA (e-h), and UEA-I (i-l) on the apical surfaces of epithelial cells in duodenum (a, e, i), jejunum (b, f, j), ileum (c, g, k), and ileal Peyer's patch (d, h, l). M cells (M) can differentiate from other epithelial cells with thick and sparse microvilli and invagination of the plasma membrane with intraepithelial lymphocytes. Apical surfaces on villous columnar epithelial cells and microvillous epithelial cells of the FAE (arrowheads) express a positive reaction for WGA (a-d) and PNA (e-h), but a negative reaction for UEA-I (i-l). Cytoplasms of M cells (arrows) clearly exhibit a positive reaction for WGA (d) and UEA-I (l), but a negative reaction for PNA (h). Bar, 20 μm.

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