Lectin Histochemical Detection of Special Sugars on the Mucosal Surfaces of the Rat Alimentary Tract

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ABSTRACT. Surfaces of the most luminal positions of mucosae are fundamental settlement sites of indigenous bacteria throughout the rat alimentary tract. In these positions, also epithelial cell-shedding sites, the special sugar expression in the glycocalyx is very important as it provides possible ligands of bacterial lectins for attachment to epithelial cells. Therefore, the sugar expression in glycocalyx of epithelial cells was lectin-histochemically surveyed using 21 lectins throughout the rat alimentary tract. From the tongue to the nonglandular part of the stomach, α -D-Man, α -D-Glc and α -D-GalNAc were detected on the surface of the keratinized stratified squamous epithelium. In the glandular part of the stomach, α -D-Man, β -D-Gal-4GlcNAc, D-Gal, D-GalNAc, D-GlcNAc, α -L-Fuc- α -D-Gal- β (1–4)GlcNAc and bisected triantennary N-glycans were detected on the surface of gastric superficial epithelial cells. From the duodenum to the ileum, (GlcNAc)_{2–4} was expressed exclusively on the epithelial cells in the apical portions of the intestinal villi. From the cecum to the rectum, α -D-Man, β -D-Gal-4GlcNAc, D-Gal, D-GalNAc, α -D-Gal(1–3)D-GalNAc, (GalNAc)_n and NeuNAc were expressed on the intestinal superficial epithelial cells. These results suggest that special sugars are expressed on the most luminal portions of mucosae as exclusive epithelial cell-shedding sites, and that sugar expression differs among the various segments of the alimentary tract. These site differences might reflect differences in resident bacterial species in the rat alimentary tract.

KEY WORDS: alimentary tract, indigenous bacteria, lectin histochemistry, rat, sugar expression.

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The microflora of the adult human alimentary tract consists of 10¹⁴ bacteria of 400 different species, which generate intense metabolic activity and play important physiological roles [5, 26]. In general, the amount of bacteria in the alimentary tract increases toward the caudal end, reaching up to 10¹²/g bacteria in feces [11, 19]. In healthy persons, the transit time of the chyme is 4–6 hr in the small intestine and 54–56 hr in the large intestine [10]. During this transit time, a large amount of bacteria proliferates in the chyme. The bacteria contained in the chyme are thought to be provided from intestinal microflora residing on the alimentary mucosa. Indigenous bacteria live symbiotically, and the composition of intestinal microflora is more or less stable in the alimentary tract under normal physiological conditions. A crucial initial step in successful colonization or infection by bacteria is adhesion to the mucosal surface [4, 16, 29]. Several bacterial adhesion molecules have been reported. Invasin, an outer membrane protein of *Yersinia*, specifically binds to integrins on the host cell [23]. Intimin, a homologue of the invasion polypeptide, is also related to the binding activity of enteropathogenic *Escherichia coli* to the host cell surface [12]. The most common adhesion molecules of bacteria are microbial lectins which selectively recognize glycoconjugates on the surface of intestinal epithelial cells

[20, 29]. The mucosal surface of the intestine is always covered with a 400 to 500-nm-thick glycocalyx, where glycoconjugates extend from the apical membranes of the epithelial cells [24]. This anatomical advantage might support the hypothesis that a lectin-sugar binding mechanism plays an important role in bacterial adhesion to the mucosal surface under physiological conditions. However, few *in vivo* studies have been conducted to examine the ligands, namely, special sugars, on the mucous epithelial cells in relation to bacterial lectins.

Our previous studies have clarified that the fundamental settlement sites of indigenous bacteria throughout the rat alimentary tract are narrow portions of the most luminal portions of the mucosae [8, 9, 21, 22, 42]. These portions are also epithelial cell-shedding sites and accumulation sites of epithelial cells in the late-apoptotic stage in rats [30, 33, 34]. These accumulation sites of epithelial cells in the late-apoptotic stage also absorb the luminal food-derived antigens and their antibody complexes in the rat small intestine [43, 44]. On late-apoptotic-stage epithelial cells in the rat jejunioileum, we detected (GlcNAc)_{2–4} and β -D-Gal(1–3)D-GalNAc exclusively [9]. Therefore, it is speculated that some special sugars that induce bacterial adhesion might exclusively express on the glycocalyx of epithelial cells of the most luminal portions of mucosae throughout the alimentary tract. However, the expression of specific sugars on the epithelial cells of the most luminal mucosae throughout the alimentary tract has never been surveyed. In this study, we lectin-histochemically

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examined the expression of specific sugars on the fundamental settlement sites of indigenous bacteria throughout the rat alimentary tract.

MATERIALS AND METHODS

Animals: A total of 5 male Wistar rats aged 7 weeks (Japan SLC Inc., Hamamatsu, Japan) were maintained under conventional laboratory housing conditions. The rats were permitted free access to water and food (Lab MR Stock; Nosan Corp., Yokohama, Japan). The animal facility was maintained under conditions of a 12 hr light/dark cycle, at $21 \pm 1^\circ\text{C}$ and 50–60% humidity. No signs of disorder were confirmed by clinical and pathological examinations in all animals. This experiment was approved by the Institutional Animal Care and Use Committee (Permission number: 17-04-05) and carried out according to the Kobe University Animal Experimentation Regulations.

Light microscopy: Animals were killed by cardiac exsanguination under anesthesia by *i.p.* injection of pentobarbital sodium (Dainippon Sumitomo Pharmaceuticals, Osaka, Japan). The animals were intravascularly perfused with 4% paraformaldehyde-containing periodate-lysine-paraformaldehyde fixative (PLP; pH 6.2, 38°C). After perfusion, the alimentary tract was removed quickly and immersion-fixed in cold PLP for 24 hr at 4°C . The tissue blocks were quickly frozen according to the method of Barthel and Raymond [2]. The 4- μm -thick sections were cut with a Coldtome HM505E (Carl Zeiss, Jena, Germany) and placed on slide glasses precoated with 0.2% 3-aminopropyltriethoxysilane (Shin-Etsu Chemical Co., Tokyo, Japan).

Lectin histochemistry: Sections were immersed in absolute methanol for 30 min and 0.5% H_2O_2 for 30 min, followed by treatment with 1.0% normal wild bullfrog serum for 1 hr at r.t. After reaction with 21 biotinylated lectins (Vector Laboratories, Burlingame, U.S.A.) at concentrations of 0.63–2.50 $\mu\text{g}/\text{ml}$ for 16 hr at 4°C , the sections were incubated with peroxidase-conjugated streptavidin (diluted to 1:500; Dako, Glostrup, Denmark) for 1 hr at r.t. These sections were then reacted with 3, 3'-diaminobenzidine (Dojindo Lab., Kumamoto, Japan) containing 0.03% H_2O_2 and were counterstained with methyl green. The lectins used in this study are shown in Table 1. The specificity of each lectin was confirmed by pretreatment of the lectin solution containing the appropriate sugars to inhibit lectin binding. Sections were also incubated in the streptavidin solution or phosphate buffer alone.

RESULTS

Sugar expression in tongue: ConA, PSA, LCA, RCA₁₂₀, DBA, WGA and JCA reacted to sugars moderately or weakly in the corneal layer and more strongly on the surface of corneal layer. Especially, these lectins reacted strongly to the dorsal surfaces of the filiform papillae, but weakly to the ventral surfaces. The spinous and basal layers showed faint or no staining by the above lectins except for JCA, which reacted moderately or weakly with the basal layer (Fig. 1a). GSL-I and DSL reacted to the surface, but not to the corneal or spinous layers, whereas the basal layer was weakly stained (Fig. 1b). SJA, PNA, ECL, SBA, VVA, GSL-II, STL, LEL, S-WGA, UEA-I, PHA-E and PHA-L reacted

Table 1. Lectins and sugar specificities in this study

Lectin	Abbreviation	Major sugar specificity	Concentration ($\mu\text{g}/\text{ml}$)
<i>Canavalia ensiformis</i>	ConA	α -D-Man, α -D-Glc	1.25
<i>Pisum sativum</i>	PSA	α -D-Man	1.25
<i>Lens culinaris</i>	LCA	α -D-Man	2.50
<i>Griffonia simplicifolia-I</i>	GSL-I	α -D-Gal, α -GalNAc	1.25
<i>Sophora japonica</i>	SJA	β -D-GalNAc	1.25
<i>Ricinus communis</i>	RCA ₁₂₀	β -D-Gal-4GlcNAc, D-Gal and D-GalNAc	1.25
<i>Arachis hypogaea</i>	PNA	β -D-Gal(1–3)D-GalNAc	2.50
<i>Erythrina cristagalli</i>	ECL	β -D-Gal(1–4)D-GlcNAc	0.63
<i>Glycine max</i>	SBA	α -D-GalNAc-3Gal	2.50
<i>Vicia villosa</i>	VVA	D-GalNAc-3GalNAc	0.63
<i>Dolichos biflorus</i>	DBA	α -D-GalNAc	1.25
<i>Triticum vulgaris</i>	WGA	(GalNAc) _n , NeuNAc	1.25
<i>Griffonia simplicifolia-II</i>	GSL-II	D-GlcNAc	1.25
<i>Solanum tuberosum</i>	STL	(GlcNAc) _{2–4}	1.25
<i>Lycopersicon esculentum</i>	LEL	(GlcNAc) _{2–4}	1.25
Succinylated WGA	S-WGA	(GlcNAc) _n	1.25
<i>Datura stramonium</i>	DSL	(GlcNAc) ₂	0.63
<i>Ulex europaeus-I</i>	UEA-I	α -L-Fuc- α -D-Gal- β (1–4)GlcNAc	1.25
<i>Phaseolus vulgaris-erythroagglutinin</i>	PHA-E	bisected, triantennary N-glycans	1.25
<i>Phaseolus vulgaris-leucoagglutinin</i>	PHA-L	bisected, triantennary N-glycans	1.25
<i>Artocarpus integrifolia</i>	JCA	β -D-galactopyranoside	0.63

Gal, D-Galactose; GalNAc, N-Acetyl-D-galactosamine; Glc, D-Glucose; GlcNAc, N-Acetyl-D-glucosamine; Fuc, L-Fucose; Man, D-Mannose; NeuNAc, N-acetyl neuraminic acid.

Fig. 1. Three staining patterns of lectins in the tongue. a) DBA is positive on the surface of corneal layer: strongly positive on the dorsal surfaces of filiform papillae (arrows), but weakly on the ventral surfaces. The spinous and basal layers are not stained. b) GSL-I is faintly positive on the surface (arrows), but not on the corneal and spinous layers, whereas the basal layer is weakly stained. c) ECL shows no reactions in the corneal and spinous layer, but weakly reacted with the basal layer. BL, basal layer, Bar=10 μ m.

Fig. 2. Three staining patterns of lectins in the esophagus. a) DBA is weakly positive in the corneal layer (arrows). The positive intensity gradually increases toward the luminal surface (arrows). b) WGA is strongly positive in the most luminal portion of the corneal layer, but weakly positive in other layers. c) LEL shows faintly positive only in the corneal layer (arrows). BL, basal layer, Bar=10 μ m.

Fig. 3. Three staining patterns of lectins in the non-glandular region of the stomach. a) LEL is negative in the spinous and basal layers, but strongly positive in the corneal layer (arrows). b) WGA is weakly positive from the corneal to the basal layer. c) GSL-I is faintly positive in the corneal and spinous layer, and weakly positive in the basal layer. BL, basal layer, Bar=10 μ m.

faintly or not at all to sugars on the epithelium. PNA, STL, LEL and PHA-E reacted weakly or not at all to the corneal and basal layers, but moderately or weakly to the basal layer. ECL, SBA, VVA, S-WGA and PHA-L reacted weakly or not at all to the corneal and upper spinous layers, but moderately with the basal spinous and basal layers (Fig. 1c). SJA, GSL-II and UEA-I reacted weakly or not at all to the corneal, spinous and basal layers.

Sugar expression in esophagus: ConA, PSA, LCA, SJA, RCA₁₂₀ and DBA reacted moderately or weakly to the corneal layer and upper spinous layer, but weakly or not at all to the basal spinous layer and basal layer. The positive intensity gradually increased toward the luminal regions of the corneal layers, which was strongly positive (Fig. 2a). GSL-I, PNA, SBA, VVA, WGA, S-WGA, DSL, PHA-E and JCA reacted moderately or strongly in the most luminal portion of the corneal layer. GSL-I, PNA, SBA, VVA, S-WGA and DSL reacted weakly or not at all in the corneal layer and upper spinous layer, and moderately in the basal spinous layer and basal layer. WGA, PHA-E and JCA reacted weakly or not at all in the other layers (Fig. 2b). ECL, GSL-II, STL, LEL, UEA-I and PHA-L reacted faintly or not at all in the corneal layer, but other layers were variably stained, from negative to strongly positive (Fig. 2c).

Sugar expression in stomach (non-glandular): ConA, PSA, LCA, SJA, DBA, GSL-II, STL and LEL reacted weakly or not at all in the spinous and basal layers except for LCA, which reacted weakly or moderately in the basal layer. The positive intensity was weak to strong in the corneal layer (Fig. 3a). RCA₁₂₀, VVA, WGA and JCA reacted weakly with sugars on or in the corneal layer, but not at all or weakly with other layers (Fig. 3b). GSL-I, PNA, ECL, SBA, S-WGA, DSL, UEA-I, PHA-E and PHA-L reacted faintly or not at all to the sugars on or in the corneal layer, but other layers were variably stained, negative to strongly positive (Fig. 3c).

Sugar expression in stomach (glandular): PSA, RCA₁₂₀, GSL-II, UEA-I and PHA-E reacted moderately with the luminal surface of the superficial epithelial cells. The apical cytoplasm of the epithelial cells was weakly positive. The luminal surfaces of gastric pit epithelial cells were negative or weakly positive (Fig. 4a). GSL-I, SJA, SBA, VVA, DBA, WGA, STL, LEL, S-WGA and PHA-L reacted moderately or strongly with the luminal surface from the base of the gastric pits to the superficial epithelial cells (Fig. 4b). The stainability of ConA, LCA, PNA, ECL, DSL and JCA was negative or faint in the luminal surface of all epithelial cells (Fig. 4c).

Sugar expression in the duodenum: ConA, STL and LEL reacted strongly with the striated border of the villous columnar epithelial cells at the apical regions of the intestinal villi. The reaction intensity was weak or moderate from the basal to the middle regions of the intestinal villi. The apical cytoplasm of some epithelial cells was also stained weakly to moderately (Fig. 5a). PSA, LCA, RCA₁₂₀, PNA, ECL, SBA, WGA, S-WGA, DSL, UEA-I, PHA-E,

PHA-L and JCA reacted moderately to strongly with the entire striated border of intestinal villi. The apical cytoplasm of some or all epithelial cells was stained weakly or moderately (Fig. 5b). Secretory granules in goblet cells were stained positively or negatively. GSL-I and SJA did not stain or only faintly stained the entire striated border in the intestinal villi (Fig. 5c). The stainability of VVA, DBA and GSL-II at the striated border on villous columnar epithelial cells could not be determined because these lectins reacted strongly to the mucus from goblet cells (Fig. 5d).

Sugar expression in ileum: STL and LEL drastically increased the staining intensity of the striated border at the apical regions of the ileal villi. The positive intensity was very strong. The apical cytoplasm of villous epithelial cells also contained positive minute vesicles. Secretory granules were weakly or moderately positive in goblet cells (Fig. 6a). ConA, LCA, DSL, PHA-E and PHA-L showed gradually-increased staining intensity from the basal toward the apical regions. The apical cytoplasm of villous epithelial cells was diffusely positive or contained positive minute vesicles. Secretory granules in goblet cells were weakly or moderately positive (Fig. 6b). RCA₁₂₀, PNA, ECL, SBA, GSL-II, WGA, S-WGA and UEA-I uniformly stained the striated border of the entire intestinal villi. The apical cytoplasm of villous epithelial cells were diffusely positive or contained positive minute vesicles. Secretory granules in goblet cells were weakly or moderately positive (Fig. 6c). PSA, GSL-I, SJA, VVA, DBA and JCA faintly stained the entire striated border of intestinal villi. The apical cytoplasm of villous epithelial cells was diffusely positive or contained positive minute granules. Secretory granules were weakly positive in the goblet cells except for VVA, which was strongly positive (Fig. 6d).

Sugar expression in the cecum: ConA, LCA, RCA₁₂₀, PNA, WGA and DSL reacted strongly with the striated borders of intestinal superficial epithelial cells. The apical cytoplasm of villous epithelial cells was weakly positive. The striated borders in the intestinal crypts were negative or weakly positive. Secretory granules were negative or moderately positive in goblet cells (Fig. 7a). PHA-L reacted weakly to moderately with the striated border from the crypt base to the intestinal superficial epithelial cells (Fig. 7b). PSA, SJA, ECL, VVA, GSL-II, STL, LEL, S-WGA, PHA-E and JCA were stained in a few intestinal superficial epithelial cells (Fig. 7c). The stainability of GSL-I, SBA, DBA and UEA-I could not be determined at the striated border, because these lectins were strongly reacted to the mucus and secretory granules of the goblet cells (Fig. 7d).

Sugar expression in the colon: PSA, LCA, RCA₁₂₀, PNA, DBA, WGA and PHA-E reacted strongly or moderately in the striated border of the intestinal superficial epithelial cells. The apical cytoplasm was diffusely or granularly positive. The striated border was negative or weakly positive in the intestinal crypts (Fig. 8a). STL, LEL and UEA-I did not react or reacted faintly in the striated border of all intestinal superficial epithelial cells, except for

Fig. 4. Three staining patterns of lectins in the glandular region of the stomach. a) RCA₁₂₀ is moderately positive in the luminal surfaces of superficial epithelial cells (arrows). b) SBA is moderately or strongly positive in the luminal surface from the gastric pit base to the superficial epithelial cells (arrows). c) PNA shows little or no reaction with the luminal surface in the epithelial cells. Bar=10 μ m.

Fig. 5. Four staining patterns of lectins in the duodenum. a) LEL is strongly positive in the striated border of the villous columnar epithelial cells at the apical regions of the intestinal villi (arrows). The reaction intensity is moderate from the basal to apical regions of intestinal villi. b) RCA₁₂₀ is moderately positive in the striated border of the entire intestinal villi. c) SJA shows almost negative in the striated border in the entire intestinal villi. d) VVA is strongly positive in the secretory granules of the goblet cells (arrows). Bar=10 μ m.

Fig. 6. Four staining patterns of lectins in the ileum. a) LEL is strongly positive in the striated border of the villous columnar epithelial cells at the apical regions (arrows), whereas the reaction intensity is weak in the basal and middle regions of the intestinal villi. b) DSL gradually increased the staining intensity from the basal toward the apical regions. c) ECL shows uniformly positive in the striated border of the villi throughout the intestines. d) JCA shows faint or no reaction in the striated border of the intestinal villi. Bar=10 μ m.

Fig. 7. Four staining patterns of lectins in the cecum. a) PNA is strongly positive in the striated borders of intestinal superficial epithelial cells (arrows). The apical cytoplasms of epithelial cells are weakly positive. The striated borders of crypt epithelial cells are negative. b) PHA-L shows weakly or moderately positive in the striated border from the crypt base to the intestinal superficial epithelial cells. c) JCA stains positively on some intestinal superficial epithelial cells of the most luminal mucosa exclusively (arrow). d) UEA-I is strongly positive in the mucus and secretory granules of some goblet cells (arrows). Bar=10 μ m.

Fig. 8. Four staining patterns of lectins in the colon. a) PNA is strongly positive in the striated border of intestinal superficial epithelial cells (arrows). The striated border is negative in the intestinal crypts. b) LEL is negative or weakly positive from the crypt base to the intestinal superficial epithelial cells. c) DSL is moderately positive in the striated border of some intestinal superficial epithelial cells (arrow). d) VVA is strongly positive in the mucus and secretory granules of goblet cells (arrows). Bar=10 μ m.

secretory granules in a few goblet cells (Fig. 8b). ConA, ECL, GSL-II, DSL, PHA-L and JCA reacted moderately or strongly with the striated border of several intestinal superficial epithelial cells and weakly with their apical cytoplasm. Secretory granules were negative except for some goblet cells (Fig. 8c). The stainability of GSL-I, SJA, SBA, VVA and S-WGA at the striated border could not be determined, because these lectins were strongly reacted to the mucus from goblet cells (Fig. 8d).

DISCUSSION

In the stratified squamous epithelium, epithelial cells proliferate in the basal layer, undergo the process of

apoptosis as they migrate toward the epithelial surface, and finally shed off [17, 18, 40]. In the human esophagus, Fas-FasL-mediated apoptosis contributes to the epithelial turnover [3]. The most superficial layer in the epithelium of the tongue, esophagus and nonglandular portion of stomach is the corneal layer, which is the site of exfoliation of keratinized epithelial cells and also a fundamental settlement site of indigenous bacteria [42]. In the present study, we found that α -D-Man, α -D-Glc and α -D-GalNAc were expressed on the keratinized stratified squamous epithelium from the tongue to the nonglandular part of stomach exclusively. Especially, the dorsal surfaces of the filiform papillae, which are bacteria-residing sites [42], showed more intense expression of sugars than the ventral

Table 2. Specific sugar expression on the surface of the most luminal portions of mucosa throughout the rat alimentary tract

Lectin	Major sugar specificity	Tongue	Esophagus	Stomach (non-glandular)	Stomach (glandular)	Duodenum	Ileum	Cecum	Colon
ConA	α -D-Man, α -D-Glc	+++	+++	+	—	+ ~ ++	++ ~ +++	+ ~ ++	— ~ ++
PSA	α -D-Man	+++	+++	+ ~ ++	++	+	—	—	++
LCA	α -D-Man	+++	+++	+ ~ ++	—	+ ~ ++	++ ~ +++	+ ~ ++	++
GSL-I	α -D-Gal, α -GalNAc	++	++ ~ +++	—	++ ~ +++	—	—	ND	ND
SJA	β -D-GalNAc	—	++ ~ +++	+	++ ~ +++	—	—	— ~ ++	ND
RCA ₁₂₀	β -D-Gal-4GlcNAc, D-Gal and D-GalNAc	+++	+++	+ ~ ++	++	++ ~ +++	+++	+ ~ ++	++
PNA	β -D-Gal(1-3)D-GalNAc	—	++ ~ +++	—	—	++ ~ +++	+++	++ ~ +++	+++
ECL	β -D-Gal(1-4)D-GlcNAc	—	—	—	—	+++	+++	— ~ ++	— ~ ++
SBA	α -D-GalNAc-3Gal	—	++ ~ +++	—	++ ~ +++	++ ~ +++	++	ND	ND
VVA	D-GalNAc-3GalNAc	—	++ ~ +++	+	++	ND	—	— ~ ++	ND
DBA	α -D-GalNAc	+ ~ ++	++ ~ +++	+	++ ~ +++	ND	—	ND	+++
WGA	(GalNAc) _n , NeuNAc	+ ~ ++	++ ~ +++	+ ~ ++	++ ~ +++	+++	+ ~ ++	+ ~ ++	+++
GSL-II	D-GlcNAc	—	—	+	++	ND	+	— ~ ++	— ~ +++
STL	(GlcNAc) ₂₋₄	—	—	+ ~ ++	++ ~ +++	++ ~ +++	+++	— ~ ++	—
LEL	(GlcNAc) ₂₋₄	—	—	+ ~ ++	++ ~ +++	++ ~ +++	+++	— ~ ++	—
S-WGA	(GlcNAc) _n	—	++ ~ +++	—	++ ~ +++	+ ~ ++	+ ~ ++	— ~ ++	ND
DSL	(GlcNAc) ₂	++	++ ~ +++	—	—	++ ~ +++	++ ~ +++	+ ~ ++	— ~ ++
UEA-I	α -L-Fuc- α -D-Gal- β (1-4)GlcNAc	—	—	—	++	+++	++	ND	—
PHA-E	bisected, triantennary N-glycans	—	++ ~ +++	—	++	+++	++ ~ +++	—	++
PHA-L	bisected, triantennary N-glycans	—	—	—	++ ~ +++	+++	++ ~ +++	+ ~ ++	— ~ +++
JCA	β -D-galactopyranoside	+ ~ ++	++ ~ +++	+	—	+	—	— ~ ++	— ~ +++

Gal, D-Galactose; GalNAc, N-Acetyl-D-galactosamine; Glc, D-Glucose; GlcNAc, N-Acetyl-D-glucosamine; Fuc, L-Fucose; Man, D-Mannose; NeuNAc, N-acetyl neuraminic acid; +++, strongly positive; ++, moderately positive; +, weakly positive; —, negative; ND, not determined; gray column, stainability increase toward the most luminal portion.

surfaces. Therefore, these special sugars that are expressed on the epithelial cells in the late stage of apoptosis might act as possible ligands for lectins on indigenous bacterial surfaces in the rat upper alimentary tract.

In mice and rats, the proliferation site of epithelial cells is the middle portion of the intestinal crypt in the small intestine [1, 7], and the lower half of the intestinal crypts in the large intestine [6, 32, 34]. As newly generated epithelial cells migrate upward, apoptosis is induced, and they are finally shed from the apical portions of the intestinal villi [33, 37, 38, 39]. This migration process of epithelial cells is similar in the large intestine [34]. Our previous studies have clarified that the fundamental settlement sites of indigenous bacteria are the narrow apical portions of the intestinal villi in the rat small intestine and the intestinal superficial epithelial cells of the most luminal portion of the mucosa in the rat large intestine [8, 9, 21, 22, 42]. Sugars expressed on the mucosal surfaces are changed along the crypt-villous axis in the intestinal villi and the crypt-dome axis in the gut-associated-lymphatic tissues [13, 31]. In our previous study, only two sugars, (GlcNAc)₂₋₄ and β -D-Gal(1-3)D-

GalNAc, were strongly expressed in the glycocalyx in the striated border of late-apoptosis-stage villous columnar epithelial cells, but were not expressed in the other villous portions of rat jejunioileum [9]. In the present study, constant expression of (GlcNAc)₂₋₄ was also confirmed on the apical regions of the intestinal villi throughout the rat small intestine. Furthermore, α -D-Man, β -D-Gal-4GlcNAc, D-Gal, D-GalNAc, β -D-Gal(1-3)D-GalNAc, (GalNAc)_n and NeuNAc were strongly detected on intestinal superficial epithelial cells in the rat large intestine. Therefore, these special sugars that are expressed on the epithelial cells in the late stage of apoptosis might act as ligands for lectins on indigenous bacterial surfaces in the rat small and large intestines.

The intestinal microflora is usually quite stable in animal species under physiological conditions [15]. However, there are site differences in the composition of microflora in animal alimentary tracts. In the rat upper alimentary tract, lactobacilli are most predominant, while other bacterial species such as streptococci or coliform bacteria are rare [28]. In contrast, a large number of microorganisms reside

in long, coccal- or rod-shaped chains between the intestinal villi of the lower part of small intestine, but never in the upper alimentary tract of rats [35]. Segmented filamentous bacteria (SFB), provisionally named *Candidatus arthromitus* [41], are apathogenic indigenous bacteria that colonize the lower alimentary canals, but never the upper alimentary tract of a wide range of animal species, including rats [25, 27]. On the other hand, various bacterial species can bind to specific sugars on the host mucosal surface using bacterial surface lectins. That is, type 1 fimbriated *Salmonella typhimurium* adheres to rat intestinal microvilli in a mannose-sensitive manner. *E. coli* K88 fimbriae can bind Gal-Cer and β 1-linked galactosyl residues in glycosphingolipids [29]. In our previous study, *Bacteroides* subspecies and *Prevotella disiens* are detected on the surface of the large intestine, but never of the upper alimentary tract and small intestine in rats. In contrast, *Corynebacterium* subspecies, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Peptostreptococcus* subspecies were detected on the surface of the upper alimentary tract, but not in the lower alimentary tract in rats [42]. Meanwhile, the surfaces of the most luminal portions of mucosae, which are identified as a fundamental settlement site of indigenous bacteria throughout the rat alimentary tract [8, 42], express different sugars among the intestinal segments [14, 36]. The present lectin-histochemical observation discovered several special sugars that were expressed exclusively in the glycocalyx of the most luminal portions of the mucosae. Their expression patterns were largely different among the various segments: the upper alimentary tract from the tongue to the nonglandular part of stomach, the middle tract from the duodenum to the jejunum and the lower tract from the cecum to the rectum (Table 2). Therefore, the site difference of sugar expression on the epithelium might reflect the site difference of indigenous bacterial species in the rat alimentary tract.

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