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Histological and Lectin Histochemical Studies on the Olfactory and Respiratory Mucosae of the Sheep

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ABSTRACT. The olfactory and respiratory mucosae of the Corriedale sheep were examined using lectin histochemistry in order to clarify the histochemical and glycohistochemical differences between these two tissues. The olfactory epithelium was stained with 13 lectins out of 21 lectins examined, while the respiratory epithelium was positive to 16 lectins. The free border of both of the olfactory and respiratory epithelia was stained with 12 lectins: Wheat germ agglutinin (WGA), succinylated-wheat germ agglutinin (s-WGA), *Lycopersicon esculentum* lectin (LEL), *Solanum tuberosum* lectin (STL), *Datura stramonium* lectin (DSL), Soybean agglutinin (SBA), *Bandeiraea simplicifolia* lectin-I (BSL-I), *Ricinus communis* agglutinin-I (RCA-120), *Erythrina cristagalli* lectin (ECL), Concanavalin A (Con A), *Phaseolus vulgaris* agglutinin-E (PHA-E) and *Phaseolus vulgaris* agglutinin-L (PHA-L). The associated glands of the olfactory mucosa, Bowman's glands, were stained with 13 lectins. While both the goblet cells and mucous nasal glands were stained with 8 lectins; five of them (WGA, s-WGA, STL, *Vicia villosa* agglutinin (VVA) and ECL) were mutually positive among the Bowman's glands, mucous nasal glands and the goblet cells. These findings indicate that the glycohistochemical characteristics of the free borders of both olfactory and respiratory epithelia are similar to each other, suggesting that secretions from the Bowman's glands and those of the goblet cells and mucous nasal glands are partially exchanged between the surface of two epithelia to contribute the functions of the respiratory epithelium and the olfactory receptor cells, respectively.

KEY WORDS: glycoconjugates, olfactory system, ruminants.

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Lectins are proteins that bind to glycoconjugates non-immunologically. They are considered to be the major analytic tool for the study of both soluble and cellular glycoconjugates. They are mainly specific to the terminal carbohydrates of sugar chains [3, 4] and are extensively used for the differentiation of cells according to the glycoconjugate contents on the histological sections [5, 8, 11, 12, 14, 16–19].

The mammalian olfactory mucosa is located in the posterior roof of the nasal cavity, where it mainly lines the ethmoturbinates and carries the receptor cells responsible for the detection and discrimination among odors of different substances [1, 2, 6, 15, 23, 24]. The olfactory mucosa consists of the olfactory epithelium and an underlying lamina propria. The olfactory epithelium is comprised of olfactory receptor cells, supporting cells and basal cells. The respiratory mucosa is composed of both respiratory epithelium consisted of the ciliated, basal and goblet cells and the underlying connective tissue lamina propria.

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In sheep, the olfactory mucosa is covered by pseudostratified sensory epithelium. The free border of this epithelium is predominantly made up of microvilli from the supporting cells together with the cilia from the rounded projecting rods of the olfactory receptor cells [13]. The histological and histochemical features of the sheep olfactory mucosa have been evaluated before by some authors: Kavoi *et al*. [9] compared the development and functional maturation of the olfactory mucosa in the domestic sheep and dog, and Scocco *et al*. [20] compared the glycohistochemical characterization of normal olfactory mucosa and enzootic nasal tumor of sheep, focusing on the lectin staining patterns of the free border and Bowman's glands. However, the latter did not address the "lectin binding" affinities of the different cell types in the olfactory epithelium (receptor, supporting or basal cells).

The respiratory mucosa of sheep is covered with a typical respiratory epithelium, which is a pseudostratified ciliated columnar type with goblet cells, covering the major portion of the nasal septum as well as the recesses of the ethmoidal conchae [22]. Several reports evaluated histological and histochemical properties of the sheep respiratory mucosa: Khamas and Ghoshal [10] focused on the blood vessels supplying the respiratory mucosa, while Taher *et al*. [22] studied the anatomy and morphology of the respiratory epithelium and the underlying connective tissue containing thick coiled tubular glands. Finally, Scocco *et al*. [20] compared the lectin histochemical characteristics of the histologically normal

Table 1. Binding specifities of lectins used in this study

Lectins	Abbreviation	Concentration (mg/m*l*)	Binding specificity
Wheat germ agglutinin	WGA	1.0×10^{-2}	GlcNAc, NeuAc
Succinylated-wheat germ agglutinin	s-WGA	1.0×10^{-2}	(GlcNAc) n
Lycopersicon esculentum lectin	LEL	2.0×10^{-2}	(GlcNAc) 2–4
Solanum tuberosum lectin	STL	1.0×10^{-2}	(GlcNAc) 2–4
Datura stramonium lectin	DSL	4.0×10^{-3}	(GlcNAc) 2–4
Bandeiraea simplicifolia lectin-II	BSL-II	4.0×10^{-3}	GlcNAc
Dolichos biflorus agglutinin	DBA	1.0×10^{-2}	Gal, GalNAc
Soybean agglutinin	SBA	1.0×10^{-2}	Gal, GalNAc
Bandeiraea simplicifolia lectin-I	BSL-I	4.0×10^{-3}	Gal, GalNAc
Vicia villosa agglutinin	VVA	4.0×10^{-3}	Gal, GalNAc
Sophora japonica agglutinin	SJA	2.0×10^{-2}	Gal, GalNAc
Ricinus communis agglutinin-I	RCA-120	2.0×10^{-3}	Gal, GalNAc
Jacalin		5.0×10^{-4}	Gal, GalNAc
Peanut agglutinin	PNA	4.0×10^{-3}	Gal
Erythrina cristagalli lectin	ECL	2.0×10^{-2}	Gal, GalNAc
Ulex europaeus agglutinin-I	UEA-I	2.0×10^{-2}	Fuc
Concanavalin A	ConA	3.3×10^{-3}	Man, Glc
Pisum sativum agglutinin	PSA	4.0×10^{-3}	Man, Glc
Lens culinaris agglutinin	LCA	4.0×10^{-3}	Man, Glc
Phaseolus vulgaris agglutinin-E	PHA-E	5.0×10^{-3}	Oligosaccharide
Phaseolus vulgaris agglutinin-L	PHA-L	2.5×10^{-3}	Oligosaccharide

Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Man, mannose; NeuAc, N-acetylneuraminic acid.

respiratory mucosa and enzootic nasal tumor focusing on the lectin binding patterns of goblet cells and nasal glands without discussing the lectin stainings in the basal and ciliated cells of the respiratory epithelium or their free border.

In this study, the nasal mucosa of the adult Corriedale sheep was examined by lectin histochemistry to clarify the histochemical and glycohistochemical differences between the olfactory and respiratory mucosae by making a detailed description of the lectin binding patterns and to reveal the types and quantities of glycoconjugates in the different cellular components of both the olfactory and respiratory epithelia with special attention to the mucous covering the surface of these epithelia. By doing so, we aimed to find out the possible role played by each one of these tissues to contribute the function of the other one.

MATERIALS AND METHODS

Animals: Three adult, castrated male Corriedale sheep were studied. They were obtained from Kitasato University (Towada, Japan). Animals were euthanized with intravenous injection of sodium pentobarbital at a dose of 20 mg/kg body weight and then perfused with physiological saline through the common carotid arteries under deep anesthesia. All procedures were in accordance with the Principles for Animal Experiments of Iwate University and approved by the intramural committee for the care and use of animals. Animals were perfused with the Bouin's solution without acetic acid via the common carotid arteries. After decapitation, the nasal parts were immersed in the same fixative for 2 days and then decalcified in 10% ethylenediaminetetraacetic acid for several months, routinely embedded in paraffin and cut transversally at 5 μm. Some sections were stained with hematoxylin-eosin (H&E), periodic acid/Schiff (PAS), alcian blue (pH 1.0 and 2.5) or Crossmon's trichrome for histological examinations.

Lectin histochemistry: The other sections were processed for histochemistry with the avidin-biotin complex (ABC) method using 21 types of biotinylated lectins (Table 1) in the lectin screening kits I-III (Vector Laboratories, Burlingame, CA, U.S.A.). After deparaffinization and rehydration, the sections were incubated with 0.3% H_2O_2 in methanol to eliminate endogenous peroxidase activity. The sections were rinsed in 0.01 M phosphate buffered saline (PBS, pH 7.4) and incubated with 1% bovine serum albumin to block nonspecific reactions. After rinsing, the sections were incubated with biotinylated lectins at 4°C overnight and reacted with ABC reagent (Vector Laboratories) at room temperature for 45 min. Thereafter, the sections were colorized with 0.05 M Tris-HCl (pH 7.4) containing 0.006% H_2O_2 and 0.02% 3–3′-diaminobenzidine tetrahydrochloride, dehydrated, cleared and mounted. Control stainings were made by using of PBS to replace lectins. No specific stainings were observed in the control stainings.

RESULTS

Topographical and histological features of the olfactory mucosa: The olfactory mucosa occupied only a small region at the caudal roof of the nasal cavity, where it entirely covered the ethmoturbinates and the caudal parts of the dorsal and middle nasal conchae. The olfactory mucosa consisted

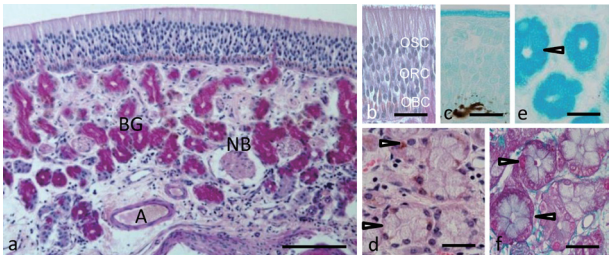

Fig. 1. Histological features of the sheep olfactory mucosa. (a) Transverse section of the olfactory mucosa stained with Periodic Acid Schiff (PAS). Bar=100 μm. Higher magnification of the olfactory epithelium stained with (b) PAS and (c) alcian blue (pH 2.5). Higher magnification of the Bowman's glandular acini stained with Hematoxylin and Eosin (d), alcian blue (pH 2.5) (e) or Crossmon's trichrome (f). Arrowheads indicate Bowman's glandular acinar cells. A, artery; BG, Bowman's glands; NB, nerve bundle; OBC, olfactory basal cells; ORC, olfactory receptor cells; OSC, olfactory supporting cells. Bar=30 μm in (b)–(f).

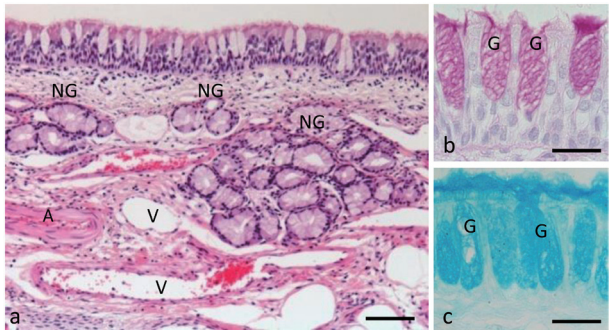

Fig. 3. Histological features of the sheep respiratory mucosa. (a) Transverse section of the respiratory mucosa containing both the respiratory epithelium and the underlying lamina propria. Hematoxylin and Eosin. Bar=100 μm. (b) Periodic Acid Schiff (PAS). (c) Alcian blue (pH 2.5). Higher magnification of the respiratory epithelium. Bars=20 μm. A, muscular artery; G, goblet cell; NG, nasal glands; V, venous sinus.

of the olfactory epithelium and the underlying lamina propria (Fig. 1a). The olfactory epithelium was composed of basal, receptor and supporting cells. Each type of cell had characteristics that have been already described for the cells of the olfactory epithelium in sheep [9, 13, 20, 22]. Briefly, nuclei of the olfactory basal, receptor and supporting cells were situated in the basal, middle and apical regions of the olfactory epithelium, respectively (Fig. 1b). The basal cells had scanty cytoplasm and an oval or somewhat round nucleus. Their nuclei were pale with distinct nucleolus and were smaller than those of the receptor and supporting cells. Cytoplasm of the basal cells reacted intensely positive to both eosin and PAS. The olfactory receptor cells were pear-shaped and extended their dendrites to the luminal surface and their axons toward the basal lamina. Their nuclei were arranged in three or four layers. The olfactory supporting cells had dark, oval-shaped nuclei arranged in three or four of the layers in the upper region of the olfactory epithelium.

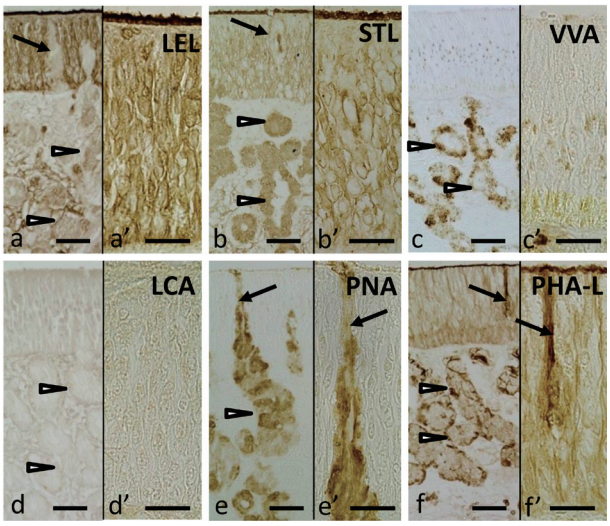

Fig. 2. Olfactory mucosa reacted with six lectins: LEL (a), (a'), STL (b), (b'), VVA (c), (c'), LCA (d), (d'), PNA (e), (e') and PHA-L (f) and (f'). Arrowheads indicate Bowman's gland acini. Arrows indicate Bowman's gland duct opening into the surface of olfactory epithelium. Bar=50 μm in (a)–(f) and 30 μm in (a')–(f').

Some melanin pigments were found both in the basal region of the olfactory epithelium and the underlying lamina propria, but they were not equally distributed along the olfactory epithelium (Fig. 1c).

Within the lamina propria, Bowman's glands were restricted to the superficial part of the propria. The secretory end-pieces of the Bowman's glands were of the tubuloacinous type, and their ducts extended vertically through the olfactory epithelium to open into the free border of the olfactory epithelium. Acini of the Bowman's glands consisted of pyramidal cells containing basally situated oval or round nuclei and surrounding a narrow lumen. Brownish granules were detected in the basal cytoplasm of the acinar cells as well as their duct cells (Fig. 1d). Both PAS (Fig. 1a) and alcian blue pH 2.5 (Fig. 1e) stained the apical cytoplasm of the acinar cells, while Crossmon's trichrome (Fig. 1f) stained the basal portion of these cells.

Lectin binding patterns in the olfactory mucosa: The receptor cells of the olfactory epithelium were labeled, with varying intensity, among 12 of the 21 lectins used in this study: Wheat germ agglutinin (WGA), Succinylated-wheat germ agglutinin (s-WGA), *Lycopersicon esculentum* lectin (LEL), *Solanum tuberosum* lectin (STL), *Datura stramonium* lectin (DSL), Soybean agglutinin (SBA), *Bandeiraea simplicifolia* lectin-I (BSL-I), *Vicia villosa* agglutinin (VVA), *Ricinus communis* agglutinin-I (RCA-120), Concanavalin A (Con A), *Pisum sativum* agglutinin (PSA) and *Phaseolus vulgaris* agglutinin-E (PHA-E). Cell processes, cell membranes and perinuclear cytoplasm were intensely stained, but the nuclei were not stained (Fig. 2a, 2a', 2b and 2b'). Three lectins, s-WGA, SBA and PSA, faintly stained the perinuclear cytoplasm of the receptor cells. Punctuated labeling of the receptor cells was made by VVA and BSL-I

(Fig. 2c and 2c'). The olfactory receptor cells were not labeled by nine of the lectins used: *Bandeiraea simplicifolia* lectin-II (BSL-II), *Dolichos biflorus* agglutinin (DBA), *Sophora japonica* agglutinin (SJA), Jacalin, Peanut agglutinin (PNA), *Erythrina cristagalli* lectin (ECL), *Ulex europaeus* agglutinin-I (UEA-I), *Lens culinaris* agglutinin (LCA) and *Phaseolus vulgaris* agglutinin-L (PHA-L) (Fig. 2d–2f').

Eight of the lectins used, WGA, LEL, STL, DSL, RCA-120, Con A, PHA-E and PHA-L, labeled both the olfactory supporting and basal cells with varying intensity. The localizations of staining reactions were similar to each other and were restricted to the cytoplasm of both the olfactory supporting and basal cells (Fig. 2f and 2f'). VVA labeled the cytoplasm of the basal cells (Fig. 2c and 2c').

The free border of the olfactory epithelium was stained by 13 lectins: WGA, s-WGA, LEL, STL, DSL, SBA, BSL-I, RCA-120, PNA, ECL, Con A, PHA-E and PHA-L with varying intensity.

Among the 21 lectins used, 13 lectins labeled Bowman's glands with varying intensity: WGA, s-WGA, STL, DSL, BSL-I, VVA, RCA-120, Jacalin, PNA, ECL, Con A, PHA-E and PHA-L. Seven lectins homogenously labeled the cytoplasm of Bowman's gland cells: s-WGA, STL, DSL, RCA-120, Jacalin, PNA and ECL (Fig. 2b and 2e). BSL-I and VVA had punctuated staining pattern, and the staining reactions were scattered within the cytoplasm of the glandular cells (Fig. 2c). On the other hand, the staining patterns for WGA, Con A, PHA-E and PHA-L were similar to each other, where they intensely stained the basal part of the glandular cells (Fig. 2f).

The lectin binding patterns in the olfactory mucosa are summarized in Table 2.

Topographical and histological features of the respiratory mucosa: The respiratory mucosa was located mostly in the middle part of the nasal cavity of the sheep. It lined the lateral wall of the nasal cavity and nasal septum and covered the vomeronasal organ laterally. The respiratory mucosa was composed of respiratory epithelium and connective tissue lamina propria (Fig. 3a). The respiratory epithelium was of a pseudostratified ciliated columnar type. It was composed of basal cells, ciliated cells and goblet cells. Each type of cell had properties that have been already described for the respiratory epithelial cells in sheep [20, 22]. The goblet cells consisted of an apical part containing mucopolysaccharide (mucin) granules and a basal part containing the nucleus. The goblet cells reacted positively with both PAS and alcian blue pH 2.5 with varying intensity according to their mucin content (Fig. 3b and 3c). The number of the goblet cells of the respiratory epithelium varied from one part to another in the nasal cavity. The basal cells had scanty cytoplasm and an oval or somewhat round nucleus. Their nuclei were darkly stained by hematoxylin and were smaller in size than those of the ciliated cells. The ciliated cells were located among the goblet cells of the respiratory epithelium. Their cytoplasm was only faintly stained by eosin, and their nuclei were also paler than those of the basal and goblet cells.

Within the propria, numerous glands with their ducts were found. Both serous and mixed glands were detected (Fig. 4a). The mixed end-pieces comprised of both mucous, high cuboidal cells (flattened nuclei) together with a few number of peripherally located serous cells surrounding a relatively wide lumen (Fig. 4b, 4d and 4e). On the other hand, the serous end-pieces consisted of pyramidal cells surrounding a narrow lumen. Their nuclei were rounded and located at the basal portion of the cell (Fig. 4c, 4f and 4g). Both glandular types reacted positively, but in a variable manner with PAS and alcian blue pH 2.5 (Fig. 4b, 4c, 4e and 4g). Mixed glands were stained with alcian blue pH 1.0, but the serous glands were not (Fig. 4d and 4f).

Lectin binding patterns in the respiratory mucosa: Twelve out of the 21 lectins used in this study: WGA, s-WGA, LEL, STL, BSL-II, DBA, SBA, BSL-I, VVA, RCA-120, ECL and UEA-I stained the goblet cells of the respiratory epithelium with varying intensity. The staining patterns were similar to each other, and the reactions were localized to the mucin granules in the apical cytoplasm. The intensity of staining was dependent on the amount of mucin granules in each goblet cell (Fig. 5a, 5a', 5b and 5b'). No lectins labeled the nuclei of the goblet cells.

Staining patterns for DBA, SBA and VVA in the respiratory epithelium were similar to each other. There were alternative intervals of stained and unstained regions in the basal and ciliated cells of the respiratory epithelium (Fig. 5c and 5c').

Eight lectins: DSL, DBA, SBA, VVA, RCA-120, UEA-I, Con A and PHA-L, labeled the ciliated cells with varying intensity (Fig. 5c, 5c', 5d and 5d'). The staining patterns were similar to each other; these lectins labeled both their cell membranes and cytoplasm.

The basal cells were stained by 11 lectins: LEL, STL, DSL, DBA, SBA, BSL-I, VVA, UEA-I, Con A, PHA-E and PHA-L with varying intensity. Cell membranes and cytoplasm of the basal cells were stained, but their nuclei were not stained (Fig. 5c, 5c', 5d and 5d').

The free border of the respiratory epithelium was labeled by 15 lectins: WGA, s-WGA, LEL, STL, DSL, DBA, SBA, BSL-I, VVA, RCA-120, ECL, UEA-I, Con A, PHA-E and PHA-L with varying intensity.

Four lectins out of the 21 lectins used: SJA, Jacalin, PNA and LCA were negative in neither epithelium.

Several spindle-shaped cells of unknown origin with dark nuclei and scanty cytoplasm were detected within the respiratory epithelium mostly located close to the goblet cells (Fig. 6a). These cells were labeled with varying intensity by 7 lectins: LEL, DBA, SBA, BSL-I, VVA, UEA-I and PHA-L. The staining reactions were similar to each other, including both their cytoplasm and nuclei (Fig. 6b). Only UEA-I distinguished their cytoplasm from nuclei by staining the cytoplasm intensely with the nuclei remaining unstained (Fig. 6c).

The lectin binding patterns in the respiratory epithelium are summarized in Table 3.

In the lamina propria, the mucous cells were stained intensely by 9 among the 21 lectins used in this study: WGA, s-WGA, STL, DBA, SBA, VVA, ECL, UEA-I and PHA-E (Fig. 5a and 5c). The serous glands as well as the serous cells

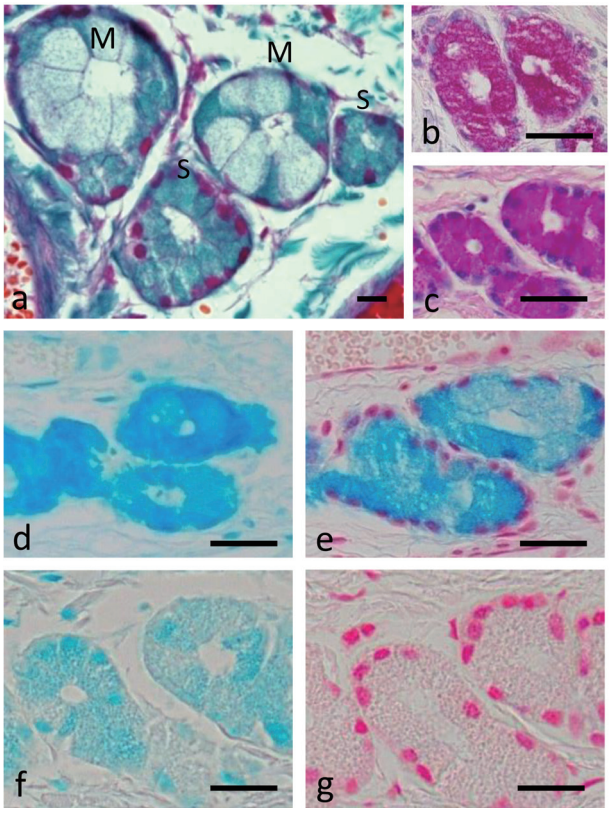

Fig. 4. Histological features of the serous and mixed glands within the lamina propria of the respiratory mucosa. (a) Crossmon's trichrome. Bar=50 μm. Mixed glands (b, d, e). Serous glands (c, f, g). PAS (b, c). Alcian blue (pH 2.5) (d, f). Alcian blue (pH 1.0) (e, g). M, mixed gland; S, serous gland. Bars=20 μm in (b)–(g).

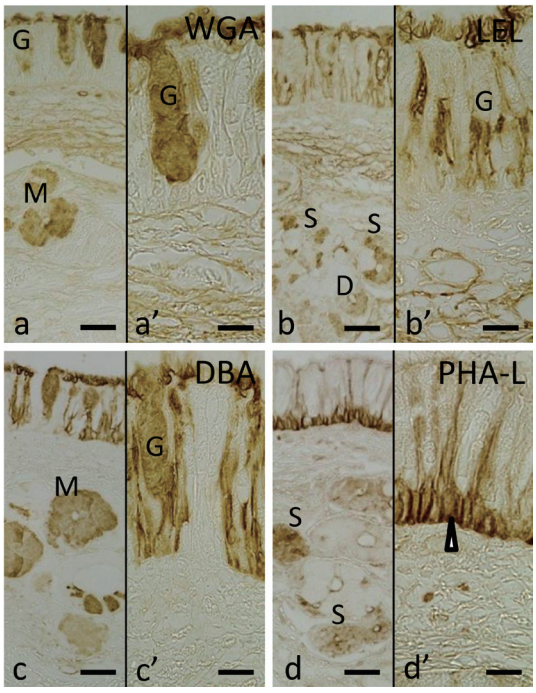

Fig. 5. Respiratory mucosa reacted with 4 lectins: WGA (a), (a') LEL (b), (b'), DBA (c), (c') and PHA-L (d), (d'). Arrowhead indicates basal cells. D, Glandular duct; G, goblet cell; M, mucous cell; S, serous cell. Bar=50 μm in (a)–(d) and 30 μm in (a')–(d').

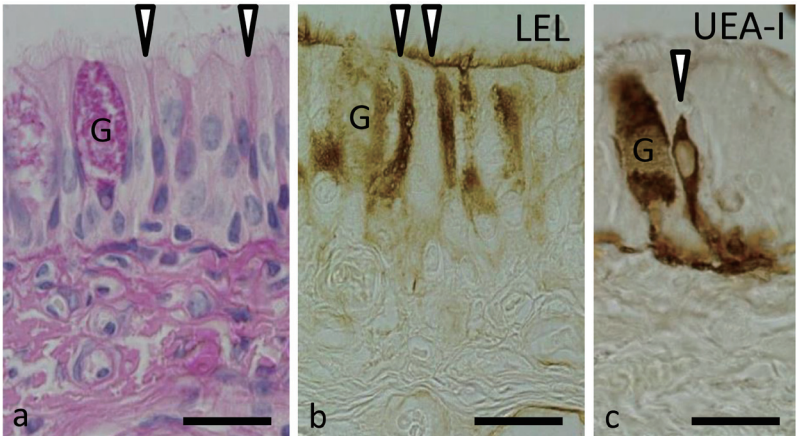

Fig. 6. Unidentified, spindle-shaped cells within the respiratory epithelium (arrowheads). (a) Periodic Acid Schiff (PAS). (b) LEL. (c) UEA-I. G, goblet cell. Bar=20 μm in (a)-(c).

of the mixed glands were labeled by six lectins: LEL, DSL, BSL-II, Con A, PHA-E and PHA-L with varying intensity (Fig. 5b and 5d).

The lectin binding patterns of the nasal glands are summarized in Table 4.

DISCUSSION

In the present study, we have revealed the details of the glycoconjugate localizations in sheep. Our histological and histochemical studies using H&E, PAS and alcian blue had

Table 2. Lectin binding patterns of the sheep olfactory mucosa

Lectin	Free border	Receptor cells	Supporting cells	Basal cells	Bowman's glands
WGA	++	+	+	+	+
s-WGA	+	+/–	–	–	+/–
LEL	++	++	++	++	–
STL	++	+	+	+	++
DSL	++	+	+	+	+/–
BSL-II	–	–	–	–	–
DBA	–	–	–	–	–
SBA	+	+/–	–	–	–
BSL-I	+/–	+	–	–	+
VVA	–	+	–	+	+
SJA	–	–	–	–	–
RCA-120	++	+	+	+	+
Jacalin	–	–	–	–	+/–
PNA	+/–	–	–	–	+
ECL	+	–	–	–	+/–
UEA-I	–	–	–	–	–
Con A	++	+	+	+	+
PSA	–	+/–	–	–	–
LCA	–	–	–	-	–
PHA-E	++	+	+	+	+
PHA-L	++	–	+	++	+

–: Negative staining, +/–: Faint staining, +: Moderate staining, ++: Intense staining.

Table 3. Lectin binding patterns of the sheep respiratory epithelium

Lectin	Free border	Goblet cells	Basal cells	Ciliated cells	Unrecognized type of cells
WGA	++	+	–	–	–
s-WGA	+	++	–	–	–
LEL	++	+/–	+/–	–	++
STL	+	+	+/–	–	–
DSL	++	–	+	+	–
BSL-II	–	+	–	–	–
DBA	+*	+/++	++*	+	+
SBA	+*	++	++	+	+
BSL-I	+/–	+	+	–	+
VVA	+*	+	+	+	+
SJA	–	–	–	–	–
RCA-120	++	+/–	–	+	–
Jacalin	–	–	–	–	–
PNA	–	–	–	–	–
ECL	+	+	–	–	–
UEA-I	+/–	++	+	+/–	++
Con A	+	–	+	+/–	–
PSA	–	–	–	–	–
LCA	–	–	–	–	–
PHA-E	+	–	+/–	–	–
PHA-L	+	–	++	+/–	+

–: Negative staining, +/–: Faint staining, +: Moderate staining, ++: Intense staining. *: Remarkable staining (binding reaction is not equally distributed along the cell membrane).

good agreement with previous reports by Kavoi *et al*. [9], Khamas and Ghoshal [10] and Taher *et al*. [22]. On the contrary, some of our findings using lectin histochemistry were inconsistent with the previous report in the olfactory epithelium by Scocco *et al*. [20]. Our study showed eight lectins labeled both the supporting and basal cells with varying intensity: WGA, LEL, STL, DSL, RCA-120, Con A, PHA-E and PHA-L. On the other hand, Scocco *et al*. [20]

Table 4. Lectin binding patterns of the glands in the lamina propria of the respiratory epithelium

Lectin	Serous glands	Mixed glands	
		Serous cells	Mucous cells
WGA	–	–	++
s-WGA	–	–	++
LEL	++	++	–
STL	–	–	+
DSL	+	+	–
BSL-II	+/–	+/–	–
DBA	–	–	++
SBA	–	–	+
BSL-I	–	–	–
VVA	–	–	++
SJA	–	–	–
RCA-120	–	–	–
Jacalin	–	–	–
PNA	–	–	–
ECL	–	–	++
UEA-I	–	–	++
Con A	+	+	–
PSA	–	–	–
LCA	–	–	–
PHA-E	+	+	+
PHA-L	++	++	–

–: Negative staining, +/–: Faint staining, +: Moderate staining, ++: Intense staining.

labeled these cells using nine lectins and found that six lectins: WGA, Con A, LTA, UEA-I, SBA and GSA-IB4 were positive in these cells. Only 2 lectins, WGA and Con A, were positive in both of our work and Scocco's work. In the respiratory epithelium, the case was the same as in the olfactory epithelium. Our results and Scocco's results showed only partial consistency; 5 of mutually examined lectins (Con A, DBA, SBA, UEA-I and RCA) were positive by both studies, but one lectin (WGA) was positive only in Scocco's study. The discrepancies in the results between Scocco *et al*. [20] and our own study may at least be partially due to the differences in the fixatives employed (Carnoy's fluid for 24 hr at room temperature and postfixed in 2% calcium acetate-4% paraformaldehyde solution 1:1 by Scocco *et al*.). But, it could also be attributed to the differences between the Corriedale and Lacaune sheep breeds.

Twelve lectins stained both the free border of the olfactory epithelium and that of the respiratory epithelium: WGA, s-WGA, LEL, STL, DSL, SBA, BSL-I, RCA-120, ECL, Con A, PHA-E and PHA-L, indicating that the expression pattern of glycoconjugate was similar between the free border of these two epithelia. The lectin stainings in the free border may come partially from the glycoconjugates in the mucus covering the surface of the epithelium and partially from those expressed in the cell membrane of the cilia or the microvilli of the goblet, ciliated, receptor or supporting cells. The mucus at the surface of the olfactory mucosa constitutes the milieu in which perireceptor events associated with olfactory signal transduction occur [7]. It is tempting to speculate, firstly, that the goblet cells and mucous nasal glands in the respiratory epithelium may contribute to the function of the olfactory epithelium in the perception of odorants in the olfactory receptor cells. The secretory products of the respiratory epithelium may distribute caudally on the free border of the olfactory epithelium [21] by the movement of cilia and gravity and thus accelerating the movement of various odorants towards their targeted olfactory receptor cells, resulting in enhanced perception of odorants in the olfactory epithelium. Another possibility is that the secretory products of the Bowman's glands may distribute cranially on the free border of the respiratory epithelium and thus help in discarding of dust particles and foreign organisms from the respiratory epithelium, accelerating the function of the goblet cells as a part of the nasal cavity immune system. The 2nd idea is that there are similarities in the carbohydrate expressions of the cell membranes of the two epithelia. Although it is possible that the secretion from Bowman's and nasal glands affects the lectin-binding patterns on the free border, the patterns may be attributed to the sugar residues expressed on the olfactory knobs, cilia or microvilli of the free borders, since some of lectins binding to the free borders did not bind to the associated glands. For example, LEL stained the free borders of both the olfactory and respiratory epithelia, but its staining intensity in the Bowman's glands and goblet cells is faint. This may indicate that the sugar residues that were labeled by LEL may originate from the cell processes and not secreted or expressed either in the Bowman's glands or goblet cells.

Unrecognized spindle-shaped cells were detected in the respiratory epithelium. These cells were labeled by seven out of the 21 lectins used in this study. Exclusively, UEA-I stained the cytoplasm of these cells, but not the nuclei. As far as we know, this is the first report detecting this type of cell in the respiratory epithelium of sheep. We speculate that the presence of these cells can be attributed to one of the different cell cycle phases of the goblet or ciliated cells, as immature differentiated cells.

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