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Glycoconjugates pattern and chemosensory cells in the camel respiratory mucosa: Lectin and immunohistochemical studies

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ABSTRACT

The glycoconjugates pattern of acidic secretions and distribution of chemosensory cells (SCCs) in the respiratory mucosa of dromedary camels were analyzed so as to identify their functional role. Secretions of the goblet cells and mucous glandular cells were analyzed to evaluate the variety of sugar chains, focusing on the acidic glycoconjugates. Using lectin histochemistry, WGA, STL, DBA, SBA, VVA and RCA-120 intensely bound to the goblet cells. PNA and ECL labeled the goblet cells with moderate intensity. While, s-WGA, UEA-I faintly bound to them. Lectins bound to the glycocalyx: WGA, LEL, STL, DSL, DBA, SBA, VVA, RCA-120, ECL and PHA-L (tetra- and tri-antennary N-glycans). The mucous secretory cells reacted with: WGA, s-WGA, STL, DBA, SBA, ECL and Con A. Glycoconjugates secreted by the camel respiratory mucosa are rich in sialomucins, glucosaminy-lated residuals with some galactosyl/galactosaminylated residues; few L-fucose and mannosylated sugar residues are also included. For identification of SCCs, the camel respiratory mucosa was immunostained with phospholipase C-β2 (PLC-β2), a taste signaling marker. Several PLC-β2 immunoreactive cells were detected in camel respiratory epithelium. Finally, prevalence of sialomucins and SCCs which can respond to noxious chemicals may suggest a vital role in optimizing physiological and pathological reactions in camel respiratory mucosa.

1. Introduction

Glycoconjugates are vital compounds in biology and they are tangled in cell–cell interactions, including cell–cell recognition; in cell–-matrix interactions; in detoxification processes. They are composed of various categories such as glycoproteins, glycolipids, glycopeptides, glycosides, peptidoglycans and lipopolysaccharides. Glycoconjugates are formed in a process termed glycosylation (Cook, 1986).

Lectins are proteins which bind specifically to carbohydrate residues non-immunologically. They are one of the best analytical tools for the study of both soluble and cellular glycoconjugates. The practice of lectins in histochemical studies is very common in recent years (Damjanov, 1987; Castells et al., 1990; Ibrahim et al., 2015). Sugars attached to cell surface lipids and proteins can control the function of structures that they are conjugated to and consequently, can affect cell behavior (Allahverdian et al., 2006).

The mammalian nasal cavity act as the first line of defense against many environmental damage factors either traumatic or infectious ones. Additionally, the nasal cavity plays a key role in regulation of both temperature and moisture content of the inspired air (Khamas and Ghoshal, 1982). Mucosa of the nasal cavity can be divided into three regions: the most anterior is the stratified squamous vestibular mucosa, followed by the pseudostratified respiratory one and finally the neuroepithelium of the olfactory region (Badawi and Fateh El-Bab, 1974; Scocco et al., 2012; Ibrahim et al., 2014).

The respiratory epithelium is composed of mucous goblet cell, ciliated columnar cell and finally the cuboidal basal cell (Badawi and Fateh El-Bab, 1974; Scocco et al., 2012; Ibrahim et al., 2014). The mucins or mucopolysaccharides secreted from the goblet cells and mucous nasal secretions are essential for the physiology of the nasal cavity. Secretion of these mucous secretions could be initiated by irritants such as dust and smoke (Harkema et al., 1987).

Studying the glycohistochemical properties of the respiratory mucosa has been achieved in different animals, but not in dromedary camel, such as sheep (Ibrahim et al., 2014); in white-sided dolphins (Shimokawa et al., 2011); in rat and guinea pig (Pastor et al., 1992); in mouse (Lundh et al., 1989); cattle (Mosier et al., 1995); in human (Gheri et al., 1993).

Solitary chemosensory cells (SCCs) are specialized, trigeminally innervated cells in the respiratory epithelium. These cells are capable of detecting foreign organisms and harmful substances entering in the airstream (Finger et al., 2003; Tizzano and Finger, 2013). In the nasal mucosa of rats and mice, numerous SCCs may reach the epithelial surface where they may or may not form synaptic contacts with trigeminal nerve fibers (Finger et al., 2003; Taruno et al., 2013). In the human upper respiratory mucosa, bitter and sweet taste receptors

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Table 1
Binding specifities of lectins employed in this study.

Lectins	Abbreviation	Concentration (mg/ml)	Binding specificity
	Group I		**GlcNAc**
Wheat germ agglutinin	WGA	1.0×10^{-2}	β–D–GlcNAc; NeuNAc
Succinylated-wheat germ agglutinin	s-WGA	1.0×10^{-3}	GlcNAc
Lycopersicon esculentum lectin	LEL	2.0×10^{-2}	β1- 4GlcNAc oligomers
Solanum tuberosum lectin	STL	1.0×10^{-2}	(GlcNAc)2–4
Datura stramonium lectin	DSL	4.0×10^{-3}	β1- 4GlcNAc; N-GalNac
	Group II		**GalNAc/Gal**
Dolichos biflorus agglutinin	DBA	1.0×10^{-2}	α-D-GalNAc
Soybean agglutinin	SBA	1.0×10^{-2}	α-D-GalNAc; β–D–GalNAc
Vicia villosa agglutinin	VVA	4.0×10^{-3}	Terminal GalNAca1-3Gal > GalNAca1-6Gal = GalNAc-serine
Ricinus communis agglutinin-I	RCA-120	2.0×10^{-3}	β–Gal
Peanut agglutinin	PNA	4.0×10^{-3}	β-D-Gal (β1-3) > D–GalNAc
Erythrina cristagalli lectin	ECL	2.0×10^{-2}	Gal, GalNAc
	GroupIII		**Glc/Man**
Concanavalin A	Con-A	3.3×10^{-3}	β–D–Man > α–D-Glc
	Group IV		**L-FUC**
Ulex europaeus agglutinin-I	UEA-I	2.0×10^{-2}	L-FUC
	Group V		**Gal/GlcNAc/Man**
Phaseolus vulgaris agglutinin-E	PHA-E	5.0×10^{-3}	Galβ4GlcNAcβ2Manα6
Phaseolus vulgaris agglutinin-L	PHA-L	2.5×10^{-3}	Galβ4GlcNAcβ6

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

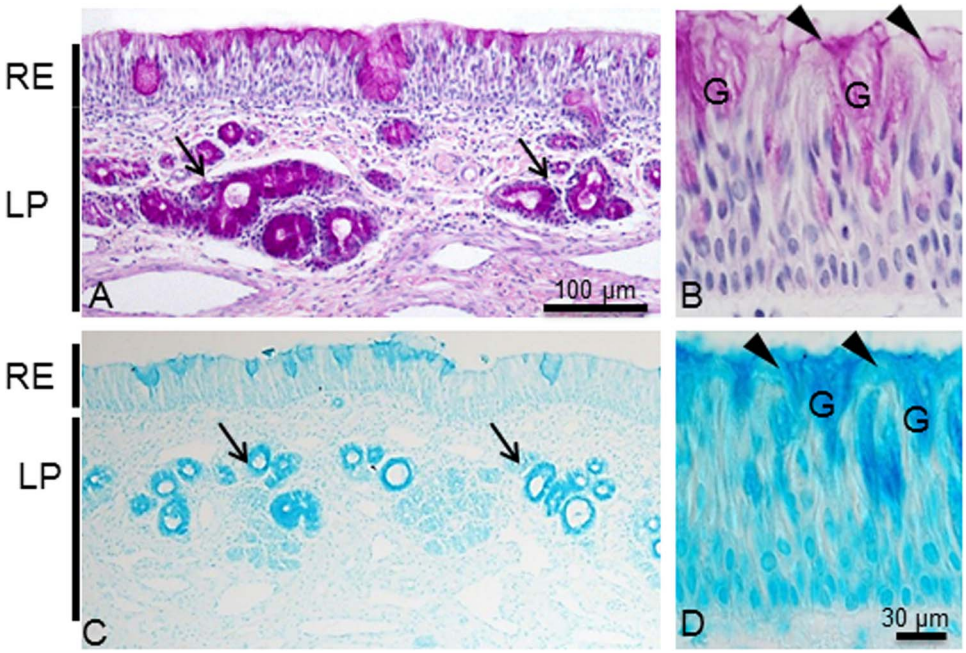

Fig. 1. Histological features of the camel respiratory mucosa. (A) Transverse section of the respiratory mucosa stained with Periodic Acid Schiff (PAS). Higher magnification of the camel respiratory epithelium stained with PAS (B). Transverse section of the respiratory mucosa stained with alcian blue (pH 2.5) (C). Higher magnification of the camel respiratory epithelium stained with alcian blue (pH 2.5). Arrows indicate the nasal glands. Arrowheads indicate glycocalyx. G, goblet cells; RE, respiratory epithelium; LP, lamina propria. Scale bars = 100 µm in (A) and (C); 30 µm in (B), (D).

existing in SCCs may enhance antimicrobial immune defense mechanisms (Lee et al., 2014; Lee et al., 2017).

In this study, I pointed to analyze the glycans pattern of the secretory products in both goblet cells and mucous glandular cells of the camel respiratory mucosa focusing on lectin histochemistry to evaluate the distribution pattern of these glycans. In addition, I aimed to recognize the distribution of the SCCs in the camel respiratory epithelium.

2. Material and methods

2.1. Sample collection

Six heads of adult, male one-humped camels (*Camelus dromedarius*) were obtained from Beni-Adi slaughter house (Assuit, Egypt). The nasal parts were collected as soon as possible, immersed in the 10% neutral buffered formalin fixative for 2 days and then decalcified in 10% ethylene di-amine tetra acetic acid for several months, dehydrated in ascending grades of ethanol, embedded in paraffin and cut transversally at 5–7 µm.

2.2. Histology and histochemistry

For histological examination, some sections were stained with Hematoxylin-eosin (H&E) for general histological examination, crossmon's trichrome for demonstration of the collagenous fibers and muscle cells; periodic acid Schiff (PAS), or alcian blue (pH 2.5) for mucin (Bancroft and Suvarna, 2013).

2.3. Lectin histochemistry

For lectin histochemistry, sections were treated with the