

### **Histochemical Study of the Presence of Glycoproteins in the Skin-mucosa Transition Zone in Human Nasal Septum**

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A group of horseradish peroxidase-conjugated lectins (Con-A, WGA, PNA, RCA-I, SBA, DBA, UEA-I) and the classical histochemical techniques to detect sialic acids were used to study the content and distribution of terminal sugar residues of glyco conjugated in the skin-mucosa transition zone in the nasal septum of healthy patients. This transition zone possesses a specific histological characteristic, where is changed a stratified epithelium first into a columnar and then into a pseudostratified one. The results showed a histochemical pattern in the basal cells of the epithelium similar to the common pattern in the epidermis, but different to the basal cells of the nasal mucosa. The transition zone possesses terminal residues rich in Nac-Glucosamine, neuroaminic acid, dimer Gal $\beta$  (1-3)-GalNac and N-acGlc and  $\alpha$ D-Glc/ $\alpha$ D-Man.

**Key words:** Histochemical, glycoproteins, human, lectin, mucosa

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## Introduction

The transition zone of epithelia in the mucosa-skin has special characteristics as the abundance of blood vessels and sensor terminals. There is a stratified epithelium which is turning into columnar one and then into a pseudostratified (Alverdes, 1930). Lectins are glycoproteins and proteins, which bind non-covalently to specific carbohydrate groups without modifying them chemically (Goldstein and Hayes, 1978). They are valuable tools for the study of glycoproteins and glycolipids, because their capacity to detect the cellular differentiation and maturation in different kinds of tissues according to the glycosidic residues expressed in the cellular surface (Sharon and Lis, 1989, 1993). Studies by using light microscopy have shown that epidermis and oral mucosa cells possess carbohydrate residues, which can be stained with various lectins. The basal cells of the epidermis stained for mannose/glucose, N-acGlc, and sialic acid. Meanwhile both spinosum and granulosum layers showed galactose, N-acGal and fucose, though the stratum granulosum showed the strongest staining for fucose (Holt *et al.*, 1979; Reano *et al.*, 1982; Schuler *et al.*, 1982; Nemanic *et al.*, 1983; Ookusa *et al.*, 1983). Electron microscopy techniques using HRP-lectins has been employed with similar results (Schaumburg-Lever, 1990).

Different histochemical studies have shown that the presence of glycoprotein in sugar residues in the cell surface of human nasal mucosa with electron and light microscopic techniques (Thaete *et al.*, 1981; Spicer *et al.*, 1983; Jin *et al.*, 1989, 1989a; Gheri *et al.*, 1992, 1993, 1993a; Gulisano *et al.*, 1994). These studies reported that basal cells were stained for PNA and WGA lectins, which are specific for dimer Gal $\beta$ (1-3)-GalNAc and N-acGlc (Gheri *et al.*, 1993, 1993a). Because of all these circumstances we think that there are molecular changes in the cell surface in this singular histological zone that could be translated in changes in the cell membrane glycoproteins. The aim of the present work was to study the glyco-conjugated sugar residues in the transition zone of mucosa-skin in the nasal septum using HRP-lectins.

## Materials and Methods

We used tissues from four males and one female patient aged from 14 to 38 years and they were obtained in septoplasty surgery. The specimens were fixed in Bouin liquid for 4 hours, dehydrated with alcohol and xylol, and embeded in a medium paraffin by routine procedures. The sections were cut at 6  $\mu$ m and placed on albumin-coated glass-slides. The battery of lectins employed is shown in Table 1. They are the following: ConA-HRP (Sigma L-6397), DBA-HRP (Sigma L-4258), RCA I-HRP (Sigma L-2633), SBA-HRP (KEM-EN-TEC L-2400), WGA-HRP (Sigma L-3892), UEA I-HRP (Sigma L-8196), and PNA-HRP (Sigma L-7759). The controls were made with tissues known to stain positive. In negative controls the samples have been incubated with the specific hapten sugar for each lectin in a concentration of 0.2M (Table 1). The sections were deparaffinized in xylol and rehydrated in alcohol series and distilled water. Sections were treated with 3% hydrogen peroxide to inhibit the endogenous peroxidase for 30 min. and rinsed twice in 0,1M PBS (phosphate buffered saline 0,1 M, pH = 7.4, Sigma P-4417) for 5 min. Specimens were incubated with lectins (20 $\mu$ gr/ml in PBS, except SBA and PNA in concentration 100 $\mu$ gr/ml in PBS) for 48-72 hours at 4°C.

Table 1: Battery of lectins

From	Name	Specific sugar	Inhibitor sugar
Arachis hypogaea	PNA	Gal $\beta$ (1-3)-GalNAc	Lactose
Cannavalia ensiformis	Con-A	$\alpha$ , D-Glc, $\alpha$ D-Man	$\alpha$ D-methyl-Man
Dolichus biflorus	DBA	$\alpha$ D-GalNAc	$\alpha$ D-GalNAc
Glycine maximsus	SBA	$\alpha$ D-GalNAc	$\alpha$ D-GalNAc
Ricinus communis	RCA-I	$\beta$ D-Gal	Lactose
Triticum vulgare	WGA	GlcNAc, Neu5 Ac	Neu5 Ac
Ulex europaeus	UEA-I	$\alpha$ D-fucose	$\alpha$ D-Fuc

Each section was rinsed three times in PBS for 5 min. For colour development a solution of 10mg/15ml diaminobenzidine (3,3' Diaminobenzidine, Sigma D-5905) in phosphate buffer saline (Sigma T-5030) was employed in the presence of 20 $\mu$ l/10ml H<sub>2</sub>O<sub>2</sub> for 10 min. Afterwards, sections were dehydrated and cover slipped with Entellan (Merck 7961). Con-A had been incubated with cations of Ca<sup>++</sup> and Mn<sup>++</sup> in a concentration of 1 mg/ml. The neuraminic acid was removed in some specimens before the incubation in WGA with a solution of 10 units/80 ml buffer solution -100 ml of distilled water, 3,4 g of sodium acetate (0,25M), 0,9 g of sodium chloride (154 mM) and 0,055g of calcium chloride (5mM) for 4 hours at 37°C. The neuroaminidase enzyme was type VI from Clostridium perfringens

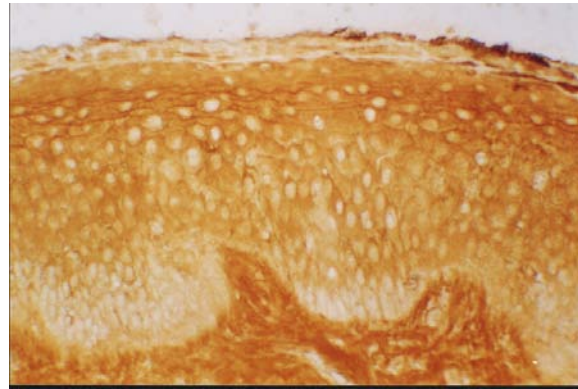


Fig. 1: WGA-HRP X250



Fig. 2: Neuroaminidase-WGA-HRP X250

(Sigma,N-3001) Uehara, 1985. Controls containing the sialidase buffer without the enzyme were also prepared. In order to detect neuraminic acid and other sialic acids, it was used sodium borohydride and potassium hydroxide, and the necessary reactives to make a PAS reaction (McManus and Mowry, 1960). While in some specimens, PAS reaction was combined with reduction and saponification, using for the reduction Boron tetra hydride (BH<sub>3</sub>) shaking for 10-12 min (Reid *et al.*, 1988). Then the sections were rinsed in distilled water and a saponification with 0,28g KOH/50 ml and 80% ethanol was made for 60 min. at room temperature. The sections were rinsed with ethanol and distilled water and after that a PAS reaction was made.

## Results

Sections stained with haematoxylin-VOF of Gutierrez showed

Table 2: Pattern of a group of horseradish peroxide-conjugated lection

	Con-A	RAC-I	DBA	WGA	SBA	UEA-I	PNA	Neur. WGA	PAS	R-S-PAS
Basal	0-1	0-1	0	0-1	0-1	0-1	1-2	0-1	0	0
Spinosum	1	0-1	0	1-2	1	0-1	1-2	1	0	0
Granulosum	2	0-1	0	2	2-3	0-1	1-2	2-3	2	0
Lucidum	1-2	0	1	0-1	3	0-1	3	0	0-1	0-1
Corneum	0-1	0	0	2	2-1	0-1	2-3	0-1	0	0
Desquamative	0	0	2-3	2-3	2-1	0-1	2	0-1	2-3	2-3

0 = no staining; 1 = very weak staining; 2 = weak staining; 3 = intense staining

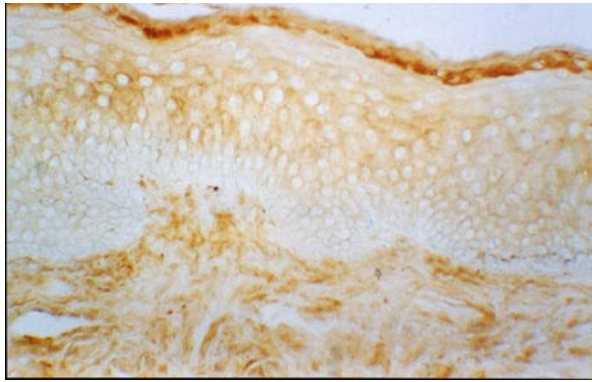


Fig. 3: PNA-HRP X250



Fig. 5: DBA-HRP X250

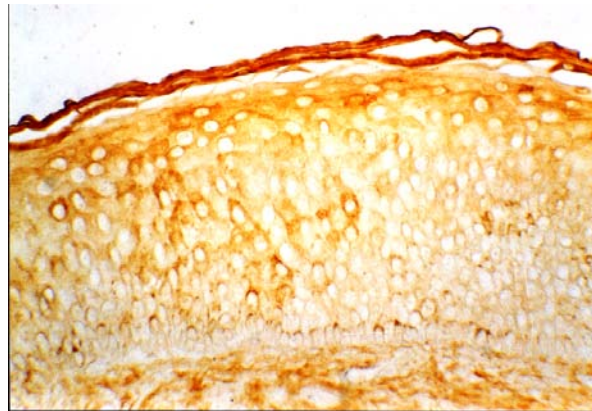


Fig. 4: SBA-HRP X250

**PNA:** The pattern of increasing intensity from the basal layer to the corneum one using this lectin, which had a high affinity to the stratum lucidum with a granular aspect (Fig. 3).

**SBA:** This lectin stained the superficial layers, with isolated cells strongly labelled in the stratum lucidum, alternating with cells faintly reactive to SBA (Fig. 4). This lectin did not stain to the corneum stratum.

**DBA:** The lectin showed a pattern of increase intensity in the surface, from the basal layers, with a weakly labelled stratum lucidum. The isolated cells in all the layers with more intense granular reactivity that could correspond to dendritic cells. We distinguished cytoplasmic granules in the granulosum stratum, which are more reactive to DBA (Fig. 5). PAS and R-S-PAS did not show positive reaction in all layers, except the desquamate cells in the outer corneum stratum. UEA-I and RCA-I were negative or very weak in all cases. (Table 2)

a normal preserved skin and nasal mucosa. In the control, no staining were present in sections exposed to substrate without lectin and lectin preincubated with specific hapten. No staining in sections containing sialidase buffer without neuroaminidase enzyme.

**WGA:** The labelling in the sections from epidermis without appendages showed an intense increasing from basal layer to the corneum one, with the stratum lucidum unstained. This stratum lucidum showed the weakest affinity, with alternating areas in the dermis where WGA staining was more intense (Fig. 1). This pattern did not change in the sections pre-treated with the neuroaminidase (Fig. 2).

**CON-A:** A pattern appeared with increase intensity from the basal layer to the corneum layer, showed the existence of isolated cells as a particular with a stronger labelling in the basal layer. These very stained cells could correspond to dendritic cells. An important heterogeneity was showed in the staining of the other layers. The intercellular gaps did not stain to Con-A.

## Discussion

Lectins have been used in the study of different epithelia, simple, columnar and pseudostratified, healthy and pathological. There are no works about the sugar residues present in this transition zone of epithelia. According to our results, the labelling with WGA, the pretreatment with neuroaminidase and the PAS and R-S-PAS reactions allow us to suppose that the obtained reactivity is due to presence of N-acetylglucosamine. The pattern of increasing intensity from the basal stratum to the corneum one, except the absence of binding of the lucidum stratum. In the nasal mucosa, WGA bound to basal epithelial cells and with the supranuclear cytoplasm of ciliated cells. This lectin stained goblet cells, glandular serous cells and the mucous cells of the glandular tubules. The pretreatment with neuroaminidase showed in the nasal mucosa a slight decrease in the labelling of mucosa superficial cells and in some serous tubules glandular cells. (Gheri *et al.*, 1993, 1993a) think that this mucosa possesses sialic acid in terminal position, without specifying the type. They showed an increased labelling of PNA in tissue previously treated with neuroaminidase. The results make them assure that the basal cells in nasal epithelium have sialic acid labelled to D-galactose ( $\beta$ 1-3) Nac-D-galactosamine (Gheri *et al.*, 1993, 1993a). Using PNA a weak reactivity in the

basal stratum of transition epithelium that increased to the lucidum stratum, which showed the strongest lectin binding. Studies made in biopsies from normal skin and they have preferentially shown a pattern of distribution for PNA in spinosum and granulosum strata. The granulosum layer cells are the most reactive ones (Ookusa *et al.*, 1983; Schaumburg-Lever, 1990). In the nasal mucosa there is only reactivity in the surface of goblet cells. Con-A lectin, specific for  $\alpha$ D-Glc/ $\alpha$ D-Man, detected the dendritic cells in the basal stratum, which have not been refereed in any consulted work and heterogeneous staining in the other layers. The intercellular gaps did not show any label, which means that the staining in the cell membrane. These results are in support with Schaumburg-Lever (1990). In the nasal mucosa, Con-A stained strongly the cytoplasmic granules of the basal cells, the supranuclear cytoplasm, the apical of the ciliated cells, and also the serous demilunes and serous tubules in basal lamina (Gheri *et al.*, 1993, 1993a). Our results using SBA, specific for  $\alpha$ D-GalNac, showed that there was a great affinity for the lucidum stratum, which contrast with the reports of Schaumburg-Lever (1990) and Ookusa *et al.* (1983), who refereed the strongest intensity in spinosum and granulosum strata. In the nasal mucosa, SBA did not find binding to this lectin in any layer of the epidermis. The basal cells of the nasal mucosa showed a very weak reactivity to this lectin (Gheri *et al.*, 1993, 1993a). It is necessary to highlight that exists a very homogeneous lectin staining in the results found in the basal stratum of the skin, the transition zone and the basal cells of the nasal mucosa. The presence of weak and medium intensities of D-gal- $\beta$ 1-3-NacD-galactosamine in the basal stratum layer of the transition-zone and the epidermis for the mentioned sugars in the epidermis. This circumstance makes us suppose that the nasal mucosa differs from a similar cellular type to the basal of the transition zone and of the epidermis in general. This could be the beginning of the successive works that they permit us to achieve tissue differentiations in this transition region from the homologous transplantation of epithelium basal cells coming from cell culture.

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