

ORIGINAL ARTICLE

Glycoconjugate expression in perennial allergic rhinitis: a lectin histopathological study

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Abstract

Conclusions. The data show that differences in the concentrations of glycoconjugates of patients with perennial allergic rhinitis (PAR) and normal controls are modest, thus indicating that the composition of the mucus in allergic patients largely resembles that of healthy individuals. The findings may point to the need for volume reduction methods controlling mucus production in patients with PAR. **Objectives.** We aimed to study the composition and concentration of inferior turbinate glycoconjugates of patients with PAR. **Materials and methods.** Six specific oligosaccharides found in the inferior turbinate mucosa were stained with a battery of 10 lectins. The samples recruited for study were 15 sections from patients with PAR and 17 from healthy individuals who had no nasal disease and underwent rhinoplasty surgery for cosmetic reasons. Both groups were matched for age ($p = 0.208$). **Results.** No significant difference in the concentration of galactose, fucose, sialic acid, N-acetylglucosamine, and N-acetylgalactosamine in the epithelium and submucosal glands of the inferior turbinate was found between the groups. Likewise, neuraminidase digestion of peripheral sialic acid revealed similar concentration of the penultimate galactose residue. The only significant difference was a higher concentration of mannose in submucosal serous glands of patients with PAR compared with normal controls ($p = 0.04$).

Keywords: Glycoprotein, mucous glands, nasal mucosa, serous glands

Introduction

The mucociliary transport mechanism is the first line of defense against the harmful effects of dust, pollutants, and microorganisms to the lungs. To maintain its homeostasis, appropriate production and secretion of mucus from submucosal glands and epithelial goblet cells are necessary. The mucus mainly consists of mucins, glycoproteins of large heterogeneous macromolecules containing oligosaccharide chains (glycoconjugates) attached to peptide backbones encoded by several MUC genes. Mucins are essential for the viscosity and the gel-forming properties of the respiratory tract and for mediating biological cell-to-cell recognition. It appears that submucosal glands constitute the main source of mucus-glycoprotein secretion in normal airways [1], whereas goblet cells add little to the total volume of

nasal secretion and rather contribute to the viscoelastic properties of the mucus blanket [2].

Perennial rhinitis, estimated to affect 5–20% of the population of Europe and the United States [3], is associated with rhinorrhea and/or nasal congestion occurring all year round with no seasonal exacerbations. On physical examination, the nasal passages are blocked due to hypertrophy of the inferior turbinates (ITs). Classification of this heterogeneous disorder often differentiates between patients with and without allergy. The laboratory diagnosis of allergic conditions is usually based on the detection of allergen-specific IgE antibodies in the skin and/or the serum by cutaneous prick test and RAST (radioallergosorbent test), respectively.

It was reported that the number of IT goblet cells in patients with perennial allergic rhinitis (PAR) did

not differ from that of normal controls [4], and also that the rise in goblet cell mucus discharge in these patients originated from a nonhyperplastic increase of the functional activity of nasal goblet cells [5]. PAR is clinically associated with a significant enhancement of nasal mucus discharge; however, except for several reports dealing with the issue [6,7], the impact of this phenomenon on the composition and concentration of goblet cells and submucosal glandular glycoconjugates has not yet been fully established. This semi-quantitative histochemical research project using lectins – proteins of nonimmune origin that specifically bind carbohydrates and serve as analytic tools to characterize soluble and cellular carbohydrate components of macromolecules like mucins [8–10] – forms part of a basic research project conducted on the pathology of perennial rhinitis [4,5,11] and aims to provide data on the properties of the nasal mucus in PAR.

Materials and methods

Patients and tissue collection

The material consisted of 32 IT sections processed at the Histopathological Research Laboratory of the Department of Otolaryngology-Head and Neck Surgery, Meir Medical Center, Kfar Saba, Israel. Fifteen sections were from patients who had IT hypertrophy. The patients (mean age 26.5 ± 15.6 years) underwent skin prick testing (SPT) for 20 common airborne allergens at the Allergy and Clinical Immunology Unit, Meir Medical Center, Kfar Saba, and were found to be allergic to house dust mite. Based on the combination of typical clinical symptoms and signs (prolonged nasal obstruction accompanied by profuse discharge of secretion, watery eyes, and sneezing) and demonstration of specific IgE antibodies to a relevant allergen [12] they were defined as having PAR. All patients failed to respond to antihistamines, decongestants, topical corticosteroids, and mast cell stabilizers and underwent bilateral inferior turbinectomy [13]. Another 17 sections were retrieved from patients (mean age 34.2 ± 10.9 years) who had rhinoplasty surgery for cosmetic reasons and served as a control group. To facilitate postoperative nasal breathing, the anterior one-third of their IT was also excised. On examination, the IT appeared normal with no symptoms or signs of nasal disease. SPT for 20 common airborne allergens was negative. The two groups were age-matched ($p=0.208$).

The Institutional Review Board approved the study protocol. All patients signed an informed consent form after being informed of the known

benefits, risks, complications, and alternatives of surgery.

Lectin histochemistry

All sections were fixed in 10% buffered formaldehyde, thereafter decalcified with 0.7 mol/L ethylenediamine tetra-acetic acid (EDTA), dehydrated with increasing concentrations of ethanol, and embedded in paraffin blocks. Serial tissue sections were cut 5 μ m thick and attached to slides coated with poly-L-lysine (Sigma Chemical Co., St Louis, MO, USA).

For lectin staining, sections were deparaffinized with xylene and rehydrated in a series of graded ethanol solutions. Endogenous peroxidase was blocked for 10 min in 0.01 M phosphate-buffered saline (PBS) at pH 7.2, containing 0.3% hydrogen peroxide. After washing in 0.01 M PBS, nonspecific lectin binding was blocked for 10 min incubation in 1% bovine serum albumin (BSA). A battery of 10 biotinylated lectins (Vector Laboratories Inc., Burlingame, CA, USA) was used (Table I). The sections were incubated with each one of the lectins for 1 h at room temperature, washed with PBS and incubated with avidin-biotin-peroxidase complex (Vectastain ABC kit) reagent (Vector Laboratories Inc.) for 30 min. Peroxidase activity was visualized by incubation for 10 min with diaminobenzidine (DAB) tetrahydrochloride (Sigma Chemical Co.)-H₂O₂ solution (20 mg of DAB and 100 μ l of 3% H₂O₂ in 100 ml of 0.05 M Tris-buffered saline, pH 7.6). The sections were counterstained with 0.2% methyl green, dehydrated in graded alcohol solutions, cleared in xylene, and mounted under coverslips.

Neuraminidase (sialidase) digestion (N-PNA)

Before staining with peanut agglutinin (PNA), sialic acid was removed by pretreatment with neuraminidase in several experiments to reveal the penultimate galactose residue. The specimens were incubated at 37°C for 16 h with 1 U/ml neuraminidase (type V from *Clostridium perfringens* [Sigma Chemical Co.]) dissolved in 0.25 mM acetate-buffer solution at pH 5.5, containing 5.0 mM CaCl₂.

Control procedure

The procedure included substitution of the lectin for PBS and competitive inhibition whereby six lectins were preincubated for 60 min before staining with the corresponding inhibitory sugars (Table I). Six lectins – concanavalin A (ConA), wheat germ agglutinin (WGA), Ricinus communis agglutinin I (RCA-I), Ulex europaeus agglutinin I (UEA-I), Dolichos biflorus agglutinin (DBA), and PNA – were used at

Table I. The carbohydrate binding specificity, concentration, and inhibitory sugars of 10 biotinylated lectins.

Lectin (abbreviation)	Carbohydrate binding specificity	Concentration ($\mu\text{g/ml}$)	Inhibitory sugar
Concanavalin A (Con A)	α -D-Man	100	D-Man
Wheat germ agglutinin (WGA)	β -D-GlcNac, NeuNAc	500	GlcNac
Succinylated wheat germ agglutinin (S-WGA)	GlcNac	200	
Sambucus nigra agglutinin (SNA)	α NeuNAc2,6Gal	40	
Peanut agglutinin (PNA)	β -D-Gal1,3GalNAc	200	β -D-Gal
Ulex europaeus agglutinin I (UEA-I)	α -L-Fuc	250	α -L-Fuc
Ricinus communis agglutinin I (RCA-I)	D-Gal	500	D-Gal
Maackia amurensis lectin II (MAL-II)	α NeuNAc2,3Gal	80	
Dolichos biflorus agglutinin (DBA)	α -D-GalNAc	100	GalNAc
Soybean agglutinin (SBA)	α -GalNAc	100	

Man, mannose; GlcNac, N-acetylglucosamine; NeuNAc, N-acetylneuraminic acid (sialic acid); Gal, galactose; GalNAc, N-acetylgalactosamine; Fuc, fucose.

concentrations of 25, 180, 250, 125, 25, and 100 $\mu\text{g/ml}$, respectively.

Grading procedure

A semi-quantitative scoring system was used to grade the intensity of the lectin-stained samples. The scoring was done independently by two observers (G.B., T.K.). Consensus was reached in case of disagreement. A third observer (E.S.) independently reviewed all sections and scores were compared with those obtained earlier. Given that 95% of the differences between measurements were within 2 SD of the mean difference, inter-observer variability was considered acceptable [14].

Adopting previous grading procedures [15,16], a four-point grading scale was used as follows: 0, less than approximately 1% of the cells showed any staining; 1, a faint staining above background in less than half of the cells, or more intense staining in <10% of the cells; 2, most of the cells stained moderately; and 3, most of the cells showed intense dark brown coloration, sometimes with patch distribution. When the mean score for each lectin was 0 it expressed negative staining; 0.1–0.4, very weak staining (traces of staining); 0.5–1.4, weak staining; 1.5–2.0, moderate staining; 2.1–2.4, moderate-to-strong staining; and ≥ 2.5 , strong staining.

Statistical analysis

The nonparametric Kruskal–Wallis test was used to compare between the staining intensity of epithelial and submucosal glandular glycoconjugates of patients with PAR and normal controls. Assessment of inter-observer variability was made by the method of Bland and Altman [14]. Measurements were expressed as means $\pm$ standard deviations (SDs). Statistical significance was set at $p < 0.05$.

Results

Table II shows the staining intensity of epithelial and submucosal glandular glycoconjugates in the IT of patients with PAR and normal controls. Statistical analysis revealed similar concentrations of the six tested sugars (mannose, galactose, fucose, sialic acid, N-acetylglucosamine, and N-acetylgalactosamine) with no significant differences among the groups in epithelial goblet cells, submucosal mucous glands, and mixed glands. The concentration of the penultimate galactose, following the removal of peripheral sialic acid by neuraminidase digestion, was also not significantly different. The only significant difference was a higher concentration of mannose in submucosal serous glands of patients with PAR compared with normal controls ($p = 0.04$) (Figure 1). Furthermore, the concentration of the remaining five sugars (galactose, fucose, sialic acid, N-acetylglucosamine, and N-acetylgalactosamine) in submucosal serous glands of patients with PAR and normal controls was not significantly different.

Discussion

Similar to healthy individuals [15], goblet cells and submucosal glands found in the IT of patients with PAR contain mannose, fucose, sialic acid, N-acetylglucosamine, N-acetylgalactosamine, and galactose. The first three sugars are exclusively located in peripheral positions of the glycoconjugates side chains [17,18]. The three remaining sugars mainly occupy the core and the backbone, yet they may also be found in peripheral positions [19,20].

The data show that the concentrations of glycoconjugates in epithelial goblet cells and submucosal mucous and mixed glands were similar in patients with PAR and healthy individuals. The only significant difference between the groups was the higher concentration of mannose found in submucosal

Table II. Lectin histochemical staining intensity of epithelial and submucosal glandular glycoconjugates in the inferior turbinate of patients with perennial allergic rhinitis (PAR) and normal controls (C).

Lectin	Group	Epithelium		Submucosal glands	
		Goblet cells	Mucous	Serous	Mixed
Con A	PAR	0.4±0.5	0.4±0.5	1.2±0.6	0.7±0.8
	C	0.5±0.7	0.4±0.5	0.6±0.8	0.3±0.5
WGA	PAR	1.4±1.1	1.3±1.3	0.4±0.6	0.3±0.6
	C	1.8±0.9	1.7±0.9	0.0±0.0	0.2±0.5
S-WGA	PAR	0.5±0.6	0.5±0.6	0.3±0.4	0.1±0.3
	C	0.4±0.7	0.5±0.9	0.0±0.0	0.0±0.0
SNA	PAR	0.5±0.7	0.9±0.9	0.3±0.6	0.2±0.4
	C	1.1±1.1	0.8±0.8	0.0±0.0	0.1±0.3
PNA	PAR	2.1±0.7	1.4±0.7	0.0±0.0	0.0±0.0
	C	1.9±0.9	0.9±0.8	0.0±0.0	0.0±0.0
N-PNA	PAR	1.0±0.9	0.9±0.7	0.1±0.3	0.1±0.3
	C	1.3±0.9	0.8±0.7	0.0±0.0	0.0±0.0
UEA-I	PAR	0.4±0.8	0.7±0.7	0.4±0.7	0.3±0.6
	C	1.0±0.9	1.2±1.1	0.1±0.3	0.4±0.9
RCA-I	PAR	0.7±0.9	0.7±0.6	0.2±0.4	0.3±0.6
	C	1.1±1.1	0.9±0.8	0.0±0.0	0.1±0.3
MAL-II	PAR	0.5±0.9	0.3±0.6	0.3±0.4	0.0±0.0
	C	1.1±1.0	0.8±0.6	0.2±0.5	0.0±0.0
DBA	PAR	1.0±1.1	0.4±0.8	0.3±0.4	0.1±0.3
	C	1.2±1.1	0.4±0.7	0.2±0.5	0.0±0.1
SBA	PAR	0.7±0.9	0.3±0.5	0.3±0.5	0.0±0.0
	C	0.9±0.9	0.6±0.9	0.1±0.2	0.0±0.0

Values are expressed as mean±SD. Con A, concanavalin A; WGA, wheat germ agglutinin; S-WGA, succinylated wheat germ agglutinin; SNA, Sambucus nigra agglutinin; PNA, peanut agglutinin; N-PNA, neuraminidase digestion before peanut agglutinin; UEA-I, Ulex europaeus agglutinin I; RCA-I, Ricinus communis agglutinin I; MAL-II, Maackia amurensis lectin II; DBA, Dolichos biflorus agglutinin; SBA, soybean agglutinin. Mean score: 0, negative staining; 0.1–0.4, very weak staining (traces of staining); 0.5–1.4, weak staining; 1.5–2.0, moderate staining; 2.1–2.4, moderate-to-strong staining; 2.5, strong staining.

serous glands. Mannose is present in peripheral positions of N-glycosidic serum-type glycoproteins such as the secretory component of immunoglobulin A [21]. No other differences were detected in the five other tested sugars at any peripheral location of the IT epithelium and submucosal glands. Furthermore, pretreatment with neuraminidase by removing peripheral sialic acid residues before staining with PNA also revealed similar concentrations of the penultimate galactose residue, with no significant differences between the groups. With the note that 23 of 24 comparisons (6 different sugars at 4 locations: epithelial goblet cells, submucosal mucous, serous and mixed glands) made at peripheral sites and the one made at the penultimate site were statistically insignificant, it may be concluded that the concentrations of the backbone and possibly the core glycoconjugates of allergic patients are very similar to those of healthy individuals. Furthermore, since submucosal mucous glands and epithelial goblet cells provide the major source for nasal mucus and submucosal serous glands are considered a modest source for mucus production, it is suggested that the increase in mannose in serous glands is of minor importance and the composition of the mucus in

allergic patients greatly resembles that of healthy individuals. Thus, given that PAR is known for its association with significant enhancement of nasal mucus secretion, we suggest that the differences in the mucus of patients with PAR and normal controls are quantitative by nature. These findings, which are in concert with the conclusions of another lectin histochemical experimental study inducing nasal allergic reaction through intraperitoneal injection of ovalbumin in rats and intranasal introduction of the antigen [22], show for the first time that the changes in the composition of glycoconjugates in the nasal mucosa of patients with PAR are modest and limited to few and insignificant parameters.

Other studies measured the amount of sialylated glycoproteins in the nasal submucosal glandular secretion and found significant changes in the composition of nasal glycoconjugates in patients with allergic rhinitis [6]. Using electron microscopy to examine resin-embedded ultra-thin nasal sections stained with horseradish peroxidase (HRP)-labeled lectins of seven allergic patients and six normal controls, Jin et al. [7] reported positive staining of fucose in serous secretory cells, increase of galactose, and decrease of sialic acid and N-acetylglucosamine

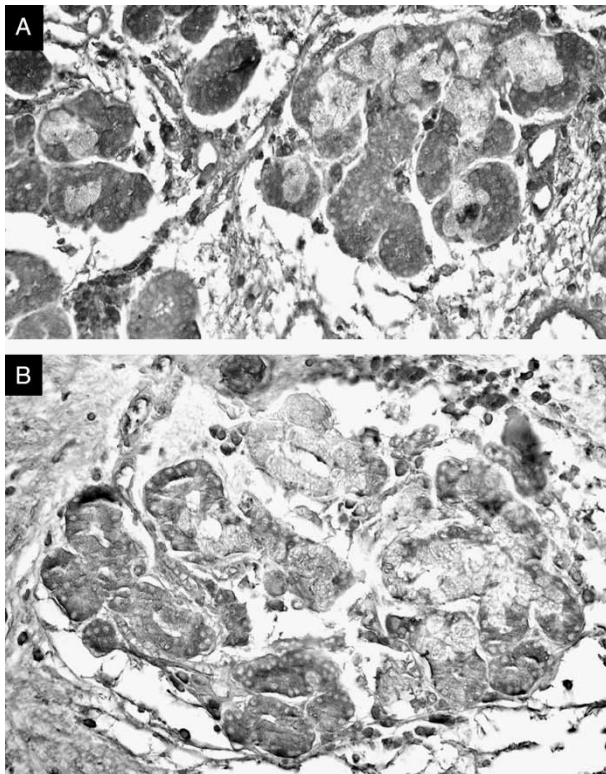


Figure 1. Two sections of the inferior turbinate stained with lectin concanavalin A (Con A) (original magnification $\times 200$). A higher concentration of mannose is observed in the serous glands of a patient with perennial allergic rhinitis (A) compared with a patient in the normal control group (B).

in goblet cells. The authors concluded that allergic rhinitis induces significant changes in the glycoproteins of submucosal glands and epithelial goblet cells. The differences between these two studies and ours could, in part, be explained by the use of different methods of investigation. While Yuta et al. [6] examined sialylated glycoproteins in nasal lavage fluids probably representing a mixture of secretions, we studied intracellular glycoproteins in epithelial goblet cells and serous and mucous submucosal glands. As for the use of electron microscopy [7], this modality covers smaller areas than light microscopy. Apparently, the key issue of the composition of nasal glycoconjugates is open for further discussion.

Conclusions

Considering the modest differences between the concentrations of glycoconjugates in patients with PAR and normal controls, it is concluded that the composition of the mucus largely resembles that of healthy individuals. These data are of clinical importance for the treatment of patients with PAR and may point to the need for volume reduction methods controlling mucus production in these patients.

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