Glycohistochemical Characteristics of Nasal Polyps From Patients With and Without Cystic Fibrosis

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Objective: To investigate whether cystic fibrosis (CF)–related nasal polyps exhibit significantly distinct glycohistochemical characteristics when compared with single vs massive nasal polyps obtained from patients without CF.

Design: Glycohistochemical characteristics were identified by means of 8 histochemical probes, including 5 plant lectins (peanut, gorse seed, wheat germ, Maackia amurensis, and Sambucus nigra agglutinins), 2 animal lectins (14- and 16-kd galectins), and 1 neoglycoprotein (exposing the Thomsen-Friedenreich antigen). The binding of the 8 glycohistochemical markers was determined by means of computer-assisted microscopy. For each probe, 3 quantitative parameters were computed: the labeling index, which describes the percentage of tissue area specifically stained by a given marker; the mean optical density, which reflects the staining intensity; and the concentrical heterogeneity, which characterizes the level of heterogeneity of the staining intensity.

Subjects: A series of 61 nasal mucosa specimens was analyzed, including 6 normal cases, 23 single and 18 massive polyposis cases without CF, and 14 nasal polyps associated with CF.

Results: Normal and polyposal nasal mucosa differed in terms of the amounts and linkage types of sialic acids (revealed by the wheat germ, M amurensis, and S nigra agglutinins) rather than the characteristics of galactoside expression (monitored with the peanut agglutinin and 2 animal galectins). In contrast, nasal polyps markedly differed between patients with and without CF with respect to galactoside expression (revealed by the peanut agglutinin and the 14-kd galectin) and the display of binding site(s) for the neoglycoprotein.

Conclusion: Normal and polyposal nasal mucosa differ essentially in sialic acid presentation, while nasal polyps from patients with CF have a higher level of various lectin-reactive galactoside residues than nasal polyps from those without CF.


The etiology and pathogenesis of nasal polyps, a common clinical condition, are poorly understood.1-5 Nasal polyps occur mainly in the ethmoidal sinus mucosa and may eventually occlude the nares.1-5 They are found as frequently in patients with allergies as in the general population4 and may be associated with other diseases of the respiratory tract, such as rhinitis, asthma, aspirin idiosyncrasy,2 cystic fibrosis (CF),8 and the Kartagener syndrome.10

In connection with CF (a genetic disorder associated with defects in the CF transmembrane conductance regulator [CFTR] gene,11 which encodes the protein part of a fucosylated glycoprotein12) nasal polyps are infrequently seen in children, although they are common in children with CF, occurring in as many as 20% of these patients.13,14 More than 20 years ago, Oppenheimer and Rosenstein15 emphasized the differential pathological findings of nasal polyps in CF and atopy. It is well known that the nature of glycoproteins differs markedly in the respiratory mucins of patients with or without CF. The mucins of patients with CF contain sulfates15 and high molecular weight branched chains that consist of a repeating oligosaccharide sequence with a sulfate group linked to the 6-carbon position of galactose and possibly N-acetylgalactosamine (GlcNAc) residues in the side chains.16 The nature and the level of the sialylation of oligosaccharides can also vary when they are isolated from patients with or without CF.17 For example, the neuraminic acid (NeuAc) residues in oligosaccharides from patients with CF are predominantly found to occur in the (2,3) linkage to galactose (Gal) and occurrence in the (α,2,6) linkage to N-acetylgalactosamine (GalNAc) or galactose also has been documented. This structural prevalence is not encountered in patients without CF.17 All these
MATERIALS AND METHODS

HISTOPATHOLOGICAL DIAGNOSIS AND CLINICAL DATA

Between January 1, 1993, and December 31, 1998, medical records and samples of nasal polyps were obtained for 55 patients. The polyposis diagnoses were made using a rigid endoscope for confirmation. This method enabled us to distinguish between single polyps (n=23) and diffuse (i.e., massive) polyposis (n=18), which involves the entire nasal and paranasal sinus mucosa. Our definition of single polyp is a polyp found between the lateral nasal wall and the middle turbinate that never goes lower than the superior part of the inferior turbinate and is the sign of a chronic ethmoidal disease.20,21 In the present study, the single polyps under investigation were always located bilaterally. There also exist strictly unilateral single polyps, of which the best known is the antrochoanal polyp. These other single-type polyps were not investigated in the present study.

We obtained 14 nasal polyp samples from patients with CF. We excluded from our study polyposis relating to the asthma, nasal polyps, and aspirin intolerance triad because of the specific clinical features exhibited by this type of polyp. Of the 14 nasal polyp samples, 10 were infected by Pseudomonas aeruginosa. The identification of the germ responsible for the infection was made by the microbiological analysis of pus samples taken from the sinuses during the surgical procedure.

The histological slides of all 55 cases were examined by 2 pathologists (N.N. and I.S.) (to exclude interindividual variability) on the basis of the criteria described by Hyams et al.22 We also included 6 normal samples that came from the inferior turbinate of patients who had undergone septoplasties and who had proved to be free of any nasal and paranasal disease. We excluded patients with allergies on the basis of the skin prick test and total and specific IgE expression.22

SPECIMEN PREPARATIONS AND GLYCOHISTOCHEMICAL STAINING

All the specimens collected for histochemical analysis were submitted to the same procedure. Tissue specimens were fixed in 4% formaldehyde and embedded in paraffin. Five-micrometer sections were then subjected to processing with the various histochemical probes and kit reagents under study.

To avoid variation in the staining procedure, the immunohistochemical or glycohistochemical staining relating to one specific probe was carried out during the same day and in the same staining bath for all the cases under analysis. The 8 probes used in the present study included 3 plant lectins, 2 animal lectins, and 1 neoglycoprotein. These 8 probes are listed in the Table along with the biochemical nature of their binding sites. The 5 plant-derived biotinylated probes were purchased from Vector Laboratories Inc (Burlingame, Calif). To extend the panel of probes with specificity to galactoside- terminating glycans with tolerance for the abundant α(2,3) sialylation, 2 animal lectins were included, namely the 14-kd (CG-14) and 16-kd (CG-16) chicken galectins, that is, biotinylated galactoside-binding lectins isolated from adult chicken liver and intestine, respectively.23 The neoglycoconjugate was synthesized by chemical coupling of a derivative of Gal-β(1,3)-GalNAc (Thomsen-Freidenreich [TF] antigen) to a glycohistochemically inert carrier, bovine serum albumin (BSA).24 As described elsewhere,25,26 the animal lectins and the neoglycoconjugate were prepared and biotinylated under activity-preserving conditions. The extent of the specifically bound markers was revealed by avidin-biotin-peroxidase complex (ABC) kit reagents (Vector Laboratories) with diaminobenzidine-peroxide as the chromogenic substrate; this procedure is detailed elsewhere.18,19 Control reactions included (1) competitive inhibitions to ascertain sugar specificity and (2) the omission of the incubation step with the labeled marker to exclude any staining by the binding of kit reagents, such as the mannose-rich glycoproteins horseradish peroxidase and avidin. Counterstaining with hematoxylin concluded the processing.

COMPUTER-ASSISTED MICROSCOPY

The variables from the quantitative histochemical stainings were computed by means of a computer-assisted microscope system (SAMBA 2005; Alcatel-TITN, Grenoble, France) with a magnification of ×20 (aperture 0.50). The way we used this system to quantify histochemical staining is detailed elsewhere.26,27 The computer-assisted microscope enabled the tissue-integrated optical densities (IODs) for the hematoxylin (blue) and the specific glycohistochemical (brown) staining to be quantitatively determined. For each digitized pixel, this determination was scaled on 256 densitometric levels in 2 distinct color channels by means of a color camera (JVC KY-15 3CCD; JVC, Yokohama, Japan).

The sum of all the IOD values was computed for the whole area scanned for each normal and for each pathological specimen of the nasal mucosa. The cutoff value chosen to ascertain whether the histochemical staining was truly positive as compared with the negative control was calculated as the mean value of the IOD values of the negative control plus 2 SDs. The labeling index (LI) is the percentage of tissue area stained by a probe. The mean optical density (MOD) relates to staining intensity. It was calculated by dividing the sum of the IOD values obtained for a given field by the area covered by this field. The concentrational heterogeneity (CH) is the value of the coefficient of variation calculated for the MOD. Fifteen fields, each between 60000 and 120000 µm², were scanned independently on the surface and the glandular epithelium present on each histological slide.

DATA ANALYSIS

The assessment of the binding of the 8 glycohistochemical markers described above led to the acquisition of 3 quantitative parameters (LI, MOD, and CH) for each of the 61 nasal specimens under study. These parameters were computed independently on the surface and the glandular epithelium of each case. The degree of potential diagnostic value for the 48 quantitative glycohistochemical variables (3 quantitative features×8 probes×2 types of epithelium) was determined by means of discriminant analysis (DA), a type of multivariate statistical analysis.19,27 The variables identified as the most informative by DA were also calculated using univariate analysis. The nonparametric Mann-Whitney U-test was used because the Levene test showed that the variances between the various groups were not equal. All the statistical analyses in the present study were performed with the Statistica (Statsoft, Tulsa, Okla) software package.
sugar moiety–related modifications occurring in CF can be monitored at the level of nasal polyposis tissue by means of histochemical analysis. Indeed, we have already made use of various glycohistochemical markers to define distinct biological groups of nasal polyposis in relation to their clinical status. The single nasal polyposis exhibited glycohistochemical characteristics that differed markedly from those displayed by the massive ones. These differences were mainly detected by the agglutinins of the gorse seed Ulex europaeus (which bind to L-fucose moieties) and the peanut Arachis hypogaea (PNA) [which bind to terminal (1,3)-GalNAc sequences]. In addition, we observed that CF-related polyposis exhibited the highest proliferation level among all the nasal polypl groups identified at the clinical level.

In the present study, we investigated whether CF-related nasal polyposis exhibited significantly different glycohistochemical characteristics when compared with single vs massive nasal polyposis obtained from patients without CF.

### RESULTS

**COMPARISON BETWEEN NORMAL AND POLYPOSAL MUCOSA**

The ratio of the number of variables to the number of cases analyzed must be at least 1:10 (and preferably 1:20) to render DA reliable and valid. We chose a 1:15 ratio for our series of 61 cases, and thus retained the 4 most discriminatory variables to distinguish between the 4 groups: normal (the NORM group; n = 6), and non–CF-related single (the SING-P group; n = 23), non–CF-related massive (the MASS-P group; n = 18), and CF-related massive (the CF-P group; n = 14) polyposal nasal mucosa specimens. In order of decreasing discriminatory power, these 4 most discriminatory variables were (1) the CH (quantified by means of the CH variable) of the WGA staining in the glandular epithelium, (2) the WGA staining intensity (quantified by means of the MOD variable) in the glandular epithelium, (3) the SNA staining intensity (quantified by means of the MOD variable) in the glandular epithelium, and (4) the percentage of cells (quantified by means of the LI variable) exhibiting positive staining for MAA in the surface epithelium.

On the basis of these 4 quantitative variables, the NORM group differed markedly from the SING-P (P <.001), MASS-P (P <.001), and CF-P (P <.001) groups, while the SING-P, MASS-P, and CF-P groups did not differ significantly (P >.05) in this 4-group analysis. As is shown below, the absence of any statistically significant differences between the 3 groups of nasal polyposis is because the glycohistochemical characteristics of the normal nasal mucosa were so different from those of the 3 nasal polyp groups that the information contributed by the normal nasal mucosa heavily outweighed that which was able to distinguish between the 3 nasal polyposis groups. This is why we performed a new DA, in which the normal nasal mucosa specimens were not taken into account (see below).

*Figure 1* illustrates the value distribution obtained for the most discriminatory glycohistochemical variables described above.

The level of CH of the staining intensity of WGA was always significantly lower in the glandular epithelium of each of the 3 nasal polyposis groups than in the normal mucosa (Figure 1, A), while in the case of the carbohydrate moieties, the staining intensity itself was significantly higher, with the exception of the SING-P group (P = .06) (Figure 1, B).

The SNA staining in the glandular epithelium was significantly higher in each of the 3 nasal polyposis groups than in the normal mucosa group (Figure 1, C). The reverse feature was observed with respect to the percentage of epithelial cells exhibiting positive MAA labeling in the surface epithelium (Figure 1, D).
COMPARISON BETWEEN CF-RELATED VS NON–CF-RELATED POLYPOSIS

We merged the SING-P and MASS-P groups into one NON–CF-P group (Figure 1). This NON–CF-P group included all the nasal polyps that we obtained from the patients without CF. We compared the glycohistochemical characteristics of the nasal polyps from patients without CF with those of the nasal polyps from patients with CF (the CF-P group).

The 4 most discriminatory variables distinguishing between the NON–CF-P and CF-P groups were, in order of decreasing discriminatory power, (1) the percentage of cells (the LI variable) exhibiting staining for PNA in the surface epithelium; (2) the CH of the staining by the biotinylated neoglycoprotein carrying the TF antigen (Table) in the glandular epithelium; (3) the staining intensity (the MOD variable) for this biotinylated neoglycoprotein in the glandular epithelium; and (4) the percentage of cells (the LI variable) exhibiting staining by specifically bound biotinylated galectin CG-14 in the glandular epithelium.

The NON–CF-P group differed markedly \( (p < .001) \) from the CF-P group on the basis of these 4 quantitative variables.

Figure 2 illustrates the value distribution obtained for the most discriminatory glycohistochemical variables described above.

The percentage of cells exhibiting positive PNA labeling was significantly lower in the surface epithelium of the CF-P polyps than in the surface epithelium of the NON–CF-P polyps (Figure 2, A). The reverse feature was observed with respect to the amounts of binding sites for the biotinylated galectin CG-14 (Figure 2, B).

Both the CH (Figure 2, C) and the staining intensity (Figure 2, D) of the Gal-β(1,3)-GalNAc-neoglycoprotein (TF antigen, Table) differed slightly, but nevertheless significantly \( (p < .003) \), in the glandular epithelium of the NON–CF-P vs CF-P polyps.

THE EFFECTS OF INFECTION ON GLYCOHISTOCHEMICAL CHARACTERISTICS IN CF-RELATED POLYPOSIS

Of the 14 massive polyps that we obtained from patients with CF, 10 were infected by \( P. aeruginosa \) and were included in the infected (INF) group (Figure 3). The remaining 4 cases were not infected by this bacterium and were included in the noninfected (NON-INF) group. (Figure 3). The most discriminatory glycohistoche-
cal variable enabling these 2 groups to be distinguished from one another was the MOD variable (staining intensity) associated with the biotinylated WGA agglutinin in the surface epithelium. This feature was significantly higher in the infected as compared with the noninfected massive nasal polyps from the patients with CF (Figure 3, A). While the MAA staining intensity in the glandular epithelium was less pronounced than what was observed in the surface epithelium (Figure 3, A), it was also significantly higher in the INF than in the NON-INF group ($P = .04$) (Figure 3, B). Similarly, the PNA staining intensity in the surface epithelium was significantly higher in the INF group ($P = .04$) (Figure 3, C). In contrast, this was not the case for the glandular epithelium (Figure 3, D).

**COMMENT**

The aim of the present work was to investigate whether the quantitative determination of glycohistochemical characteristics in nasal polyps could delineate significant differences between polyps arising from patients without CF and from patients with CF. However, we first analyzed the differences between normal and polyposal mucosa. In addition, we focused on the fact that some of the CF nasal polyps were, and some were not, infected by *P. aeruginosa*.

In our analysis, we employed 2 distinct groups of markers to pinpoint differences in the levels of expression of various carbohydrate moieties, with the first group focusing on sialylated structures and the second group on Gal/GlcNAC-containing determinants.

As explained by Varki,28 sialic acids constitute a family of 9-carbon acidic monosaccharides typically found at the outermost ends of the sugar chains of animal glycoproteins and gangliosides. In addition, his evidence shows that whereas sialic acids may inhibit intermolecular and intercellular interactions by virtue of their negative charge, they can also act as critical components of ligands recognized by a variety of proteins of animal, plant, and microbial origin (sialic acid–binding lectins; for example, WGA, SNA, and MAA). Furthermore, Varki emphasized that the term sialic acid is often used to refer to Neu5Ac, but in fact Neu5Ac is the metabolic source of more than forty 9-carbon acid sugars in which structural diversity is generated by various substitutions in the 4, 5, 7, 8, and 9 carbon positions. A large set of animal and plant lectins, reviewed by Varki28 and by Reuter and Gabius,29 can be used to identify the presence of linkage types and sialic acid structures, such as O-acetylated spe-
cies, which can serve as docking points for, for example, influenza virus agglutinins. In terms of the binding to WGA, the specific binding of Neu5Ac is not based on the carboxylate group but on the similarity of the configuration of this sugar to that of GlcNAc. Two other agglutinins, MAA and SNA (Table), enable sialic acid linkage to be visualized in the \( \alpha(2,3) \) and \( \alpha(2,6) \) positions on Gal-\( \beta(1,4) \)-GlcNAc and Gal/GalNAc, respectively. The data obtained in the present study clearly indicate that there were modifications in both the amounts and linkage types of sialic acids that differed between the normal and polyposal nasal mucosa rather than modifications in the amounts of galactoside expression. Indeed, as revealed by the results of the WGA assay (Table), the percentage of cells exhibiting Neu5Ac residues and perhaps GlcNAc (data not shown) and the amounts expressed per cell of the binding carbohydrate moiety (Figure 1) were higher in the glandular epithelia of the nasal polyps than in the glandular epithelia of the normal nasal mucosa. As revealed by the results of the SNA assay (Table), the same feature was observed with respect to both the percentage of cells (data not shown) and the amounts per cell (Figure 1) of sialic acid-\( \alpha(2,6) \)-Gal/GalNAc in the glandular epithelia of the normal vs the polyposal nasal mucosa. In contrast, MAA-related data revealed that in comparison with normal nasal mucosa, the surface epithelia in the polyposal nasal mucosa exhibited a significantly lower proportion of positive MAA-stained cells expressing sialic acid-\( \alpha(2,3) \)-Gal-\( \beta(1,4) \)-GlcNAc (Figure 1) and significantly lower amounts of this probe-accessible glycan epitope per cell (data not shown).

All of these modifications in the amounts and linkage types of the sialic acids in polyposal nasal mucosa in comparison with what is observed in normal mucosa can modify the physiological functions of various glycoconjugates present in the nasal mucosa, and particularly different types of mucins. A comparison can be made with what is already known in the case of the salivary glands. For example, the terminal sequence Neu5Ac-\( \alpha(2,6) \)-Gal-\( \beta(1,3) \)-GlcNAc is a typical side chain of mucin-type glycoproteins in salivary glands, which secrete salivary mucins with numerous types of terminal sialic acids. While the expression of the Neu5Ac-\( \alpha(2,6) \)-Gal-\( \beta(1,3) \)-GlcNAc sequence is typical of the presence of mature mucins, the presence of Gal-\( \beta(1,3) \)-GalNAc free of sialylation is typical of either immature mucins or IgA, which, in its structure, has 4 \( O \)-linked saccharides in the same sequence. Not only total and specific IgE, but also IgA, are found in significantly greater concentrations in nasal polyp tissue specimens than in serum samples. Immunoglobulin A is known to upregulate CD40 messenger RNA in eosinophils present in nasal polyps and to contribute to the regulation of airway inflammation.
Thus, the increase in presentation of α(2,6)-linked sialic acids that we found (using SNA) in polyposal nasal mucosa in comparison with normal nasal mucosa might partly be due to an IgA-mediated role in nasal polyposis pathogenesis.

Sialic acids in saliva act against environmental insult and serve both as lubrication and as a defense against microorganisms.30-32 These sialic acid–dependent properties may also be present in nasal mucosa, but differ according to whether or not the polyposal nasal mucosa is infected by specific pathogens. Interestingly, while an actual receptor for sialylated glycans from P. aeruginosa has not yet been defined biochemically, inhibition experiments with SNA intimated that glycans carrying α(2,6)-linked sialic acids are recognized by P. aeruginosa.29

The nasal polyps from the patients with CF differed markedly in terms of glycohistochemical characteristics from the nasal polyps obtained from the patients without CF. These modifications mainly concern presentation of galactoside moieties and not modifications in sialic acid. The major differences were linked to the fact that the percentage of positive cells for Gal-β(1,3)-GalNAc residues revealed by PNA (Table) in the surface epithelia of the CF nasal polyps was significantly lower than in the surface epithelia of the non-CF polyps (Figure 2). The amounts of Gal-β(1,3)-GalNAc residues per cell in the surface epithelia were also significantly lower in the CF than in the non-CF nasal polyps (data not shown). In contrast, the percentage of cells exhibiting significant amounts of binding sites for β-galactosides (revealed by biotinylated galectins; Table) was significantly higher in the glandular epithelia of the CF nasal polyps than in the glandular epithelia of the non-CF nasal polyps. We introduced 2 chicken galectins to characterize the level of expression of the β-galactoside binding sites in the surface and glandular epithelia of the CF vs the non-CF nasal polyps, and we observed that the staining by the CG-14 intestine galectin was much more pronounced than staining by the CG-16 liver galectin (data not shown).

The galectins CG-14 and CG-16 are encoded by individual genes that are regulated differently during the development of the embryo.36,37 The combining-site architecture of the CG-16 lectin is apparently homologous to that previously observed for bovine galectin-1,23 whereas besides preserving the key interactions, the CG-14 galectin is able to accommodate an axial hydroxyl at the 3 carbon position in the glucose moiety.23 It follows that, in contrast to non-CF nasal polyps, rather subtle β-galactoside–related modifications occur on the surface and glandular epithelia of CF polyps. These modifications were revealed particularly by means of the CG-14 galectin and to a lesser extent CG-16. In this context, it is notable that Solis et al.23 have suggested on the basis of chemical mapping that distinct ranges of potential ligands for these 2 galectins can occur in addition to an overlap. Thus, the diversity of galectin genes can translate into a fine tuning toward distinct galactoside-bearing glycans, making galectins an attractive family of histochemical probes.30 Schneller et al.29 observed by a Scatchard analysis that there is a comparatively lower degree of affinity and a greater extent of binding with immobilized lactosylated poly-L-lysine for CG-14 (monomeric) than for CG-16 (homodimeric) galectins, a feature that should be compared with the greater capability for binding CG-14 than CG-16 that we observed in the glandular epithelia of the CF nasal polyps. Evidently, the size of the probe that governs the spatial accessibility can also affect the extent of ligand association.

We also observed that the amount of binding sites for Gal-β(1,3)-GalNAc residues (revealed by the neoglycoprotein described in the Table) was lower in the glandular epithelia of the CF nasal polyps than in the non-CF nasal polyps (Figure 2). However, the amount of these Gal-β(1,3)-GalNAc residues was higher in the CF nasal polyps that were infected by P. aeruginosa than in those that were not (Figure 3). This bacterium expresses 2 well-characterized adhesins: PA-I binding to Gal and PA-II binding to fucose.40,41 It is thus tempting to suggest that the markedly increased levels of various lectin-reactive galactosides in CF vs non-CF nasal polyps could act as ligands for PA-I and could therefore explain why the vast majority of the CF nasal polyps in our study were infected by P. aeruginosa while the non-CF nasal polyps were not.

Some of the data reported above suggest some significant differences between glandular and surface epithelium staining. However, we cannot explain these differences at this time.

In conclusion, the data from the present study show that, in terms of glycohistochemical characteristics, polyposal nasal mucosa differs essentially in modifications regarding sialic acid presentation, while nasal polyps from patients with CF differ from nasal polyps from patients without CF on the basis of a higher level of various lectin-reactive galactosides. Such galactoside residues could act as ligands for the PA-I adhesin of P. aeruginosa. This result may explain, at least in part, why nasal polyps from patients with CF are very often infected by this pathogen as opposed to those from patients without CF. Following our technical approach to employ not only plant but also animal lectins as glycohistochemical tools, the introduction of labeled bacterial lectin will help to further investigate this issue.

Accepted for publication January 1, 2000.

This work was supported by grants awarded by the Fonds de la Recherche Scientifique Médicale (FRSM), Brussels, Belgium; by the Dr Mildred-Scheel-Stiftung für Krebsforschung, Bonn, Germany; and by the Volkswagen Foundation, Hannover, Germany.

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