Expression Patterns of Galectin-1 and Galectin-3 in Nasal Polyps and Middle and Inferior Turbinates in Relation to Growth Regulation and Immunosuppression

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Background: The term nasal polyposis describes benign growth processes in the nasal and sinus mucosa, which are mainly located in the middle meatus and never in the inferior meatus. As a step to define the biochemical determinants relevant for growth regulation, we focused on endogenous lectins known for anti-apoptotic (galectin-3) and immunomodulatory (galectin-1) activities.

Design: Using computer-assisted microscopy, we performed an immunohistochemical investigation defining the quantitative parameters of expression of galectin-1 and galectin-3 in 10 nasal polyps, 10 middle turbinates, and 10 inferior turbinates, all of which were obtained from surgical resection.

Results: Our data show that galectin-3 expression is markedly (P<0.001) higher in nasal polyps than in turbinates. No relation to the allergic status was discovered. Galectin-1 expression is higher in nasal polyps than in middle turbinates (P<0.001) in nonallergic patients compared with allergic ones (in glandular epithelium, P=0.009; in connective tissue, P=0.006). The lowest galectin-1 expression was observed in the middle turbinate.

Conclusions: These data are in line with a positive influence of galectin-3 on growth and an immunoregulatory role of galectin-1, mimicking an increased expression dependent on glucocorticoid.


Original Article

ASAL POLYPOSIS is a chronic inflammatory disease of the paranasal sinuses with a prevalence of 1% to 4% in the general population.1 Histopathologically, nasal polyposis is manifested by occurrence of edematous connective tissue covered with respiratory epithelium. The subepithelial area is characterized by an eosinophilic inflammation in more than 80% of the cases; in addition, noneosinophilic nasal polyposis can also be found and has to be carefully classified as choanal polyps or nasal polyposis based on chronic sinusitis or cystic fibrosis.1 Histologically, polyps are different from normal nasal mucosa. Turbinates are formed by a medial and a lateral mucosal layer with a central osseous between them.2 The medial layer is considerably thicker than the lateral one. Turbinates are covered with a pseudostratified columnar epithelium and, in addition to ciliated and nonciliated cells, contain approximately 10% goblet cells in the cell population. A few lymphocytes and other immunocompetent cells are infrequently present in the subepithelial region of the connective tissue, and many seromucous glands are found in the outer one third of the lamina propria. This site is notable for a complex network of thin-walled venous sinuoids.2

Nasal polyps have been described in numerous publications, and several ideas on their origin and the way they develop have been offered. For example, they have been regarded as a neoplastic disorder, as a result of glandular hyperplasia,3 or as a consequence of increases in ion transport mechanisms.4 Based on the monitoring of polyp formation in the middle ear of rats, it was proposed that this process is initiated by the rupture of the epithelium, followed by a prolapse of granulation tissue.5-7 In consideration of the central deposit of albumin (and possibly other plasma proteins) and extracellular matrix glycoproteins and the subepithelial eosinophilic inflammation, these biochemical and immunological parameters could contribute to a possible pathogenic principle for polyp formation and growth.1 To contribute to the classification of mechanisms involved in the pathogenesis, histological documentation of the first crucial stages of clinically occurring nasal polyp formation in humans with respect...
to supposed affected molecules is necessary. A benign growth in the nasal and sinus mucosa mainly located in the middle meatus and never in the inferior meatus gives rise to nasal polyposis. It is thus reasonable to focus respective investigations on determinants relevant for growth control, one of them being the translation of carbohydrate-based information by endogenous lectins. Members of the family of the galectins (β-galactoside–binding lectins sharing the jelly roll–like folding pattern), which reside in glycan chains of diverse glycoproteins including fibronectin, laminin, integrins, and tetraspanins, are especially known to be active in this respect.

Mediation of classic and nonclassic apoptosis by galectin-1, the functional divergence of galectin-3 competitively blocking the effects of galectin-1 on neuroblastoma cells, and the anti-apoptotic activity of galectin-3 are arguments to focus this initial study on galectin-1 and galectin-3 using galectin-type–specific reagents.

The aim of the present study was to investigate whether the levels of expression of galectin-1 and galectin-3 are significantly different in polyposis and the middle and inferior turbinates. Human nasal polyps and turbinates obtained from surgical resection were subject to immunohistochemical study. Using computer-assisted microscopy, we performed the quantitative immunohistochemical characterization of the levels of galectin-1 and galectin-3 expression in the 3 nasal structures (ie, surface epithelium, glandular epithelium, and connective tissue).

METHODS

CLINICAL DATA AND HISTOPATHOLOGICAL CHARACTERISTICS

Medical records and samples of nasal polyps and turbinates were obtained from 30 patients: 10 patients had nasal polyposis. The 10 middle and 10 inferior turbinates were collected from different patients who had undergone surgery under general anesthesia because of septal deviation, without any other nasal pathological condition. The inferior part of the turbinates were analyzed from the anterior tip to the posterior part. In the 3 groups of 10 patients, 5 in each group were allergic. The allergic vs nonallergic status of the patients was determined on the basis of the skin prick test and/or total and triggered IgE expression.

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The samples were routinely fixed in buffered formalin. Formalin embedding does not effect the preservation of the adhesion molecules as previously detailed. Following paraffin embedding, 5-µm sections were prepared for quantitative histochemical analysis. Galectin-type–specific polyclonal antibodies were raised and checked for specificity as previously detailed. Incubation with the antibody-containing solutions was carried out at 25°C ± 1°C for 30 minutes at a concentration of 2.5 µg/mL. The extent of the specifically bound probes was visualized by avidin-biotin-peroxidase complex (ABC) kit reagents (Vector Labs, Burlingame, Calif) with diaminobenzidine and hydrogen peroxide as chromogenic substrates. The control reactions included the omission of the incubation step with the probe (antibody) and with each of the secondary reagents to exclude any staining by the binding of the kit reagents such as the mannose-rich glycoproteins avidin or peroxidase. Counterstaining with hematoxylin concluded the processing, as exemplified in Figure 1.

Figure 1. Morphological illustrations (hematoxylin, original magnification ×200) of the immunohistochemical detection of expression of galectin-3 (brown) in nasal polyp (A), middle turbinate (B), and inferior turbinate (C). Galectin-3 was expressed more prominently in the surface epithelium (SE), the glandular epithelium (GE), and the connective tissue (CT) from the nasal polyps than in the same compartments of the middle and inferior turbinates.
been detailed elsewhere. A negative histological control slide was analyzed for each specimen under study (routine processing without the incubation step involving the primary antibody) to set the zero-level reliably. The software used to perform the computer-assisted microscope analysis automatically subtracted the LI and MOD values of the negative control sample from each corresponding positive one. Three types of histological structure were analyzed for each experimental condition (i.e., the surface epithelium, the glandular epithelium, and the connective tissue). Ten fields of between 60000 and 120000 µm² each were scanned for each histological structure.

STATISTICAL ANALYSES

As the conditions for applying the parametric tests were not satisfied, the statistical comparisons of the groups were made by first carrying out the Kruskal-Wallis test (nonparametric 1-way analysis of variance). In cases in which this test revealed significant differences, we investigated whether any pair of groups significantly differ. For this purpose, we applied the standard Dunn procedure (2-sided test) for multiple comparisons.

RESULTS

EXPRESSION OF GALECTIN-3 IN NASAL POLYPS AND MIDDLE AND INFERIOR TURBINATES

Figure 1 illustrates the immunohistochemical staining for galectin-3 in nasal polyps (Figure 1A) and the middle (Figure 1B) and inferior turbinates (Figure 1C). Evidently, galectin-3 is present in these structures. These 3 illustrations clearly document that galectin-3 was expressed more prominently in the surface epithelium, the glandular epithelium, and the connective tissue for the nasal polyps than in the same compartments of the middle and inferior turbinates. These histochemical-staining patterns were then quantitatively evaluated by computer-assisted microscopy, as detailed below.

Figure 2 shows the quantitative evaluation of the percentage of immunohistochemically positive tissue area (expressed as the LI) and staining intensity (referred to as the MOD variable) in the surface epithelium, the glandular epithelium, and the connective tissue for the nasal polyps and the middle and inferior turbinates. The data show that the percentage of cells immunopositive for galectin-3 was markedly higher \( P < .001 \) in the surface (Figure 2A) and glandular (Figure 2B) epithelia and the connective tissue (Figure 2C) in the nasal polyps than in those of the middle and inferior turbinates. There were no significant (in surface epithelium, \( P = .5 \); in glandular epithelium, \( P > .5 \); in connective tissue, \( P = .3 \)) differences in galectin-3 expression between the inferior and the middle turbinates. The galectin-3–dependent staining intensity and thus the antigen density (the MOD variable) in the 3 histological structures was markedly lower \( P < .001 \) in the nasal polyps than in those of the middle and inferior turbinates (Figure 2D-F). The data from the allergic and nonallergic patients are not separately reported for the sake of clarity because no statistically significant
The highest in the inferior turbinate (Figure 3A). The percentage of cells immunopositive for galectin-1 was higher in the nasal polyps than in the middle and inferior turbinates from both the allergic and the nonallergic patients (Figure 3B). In the nonallergic patients, this percentage was lower in the middle turbinate than in the inferior turbinate (Figure 3B). No statistically significant difference in the staining intensity of galectin-1 was noted between the different groups under investigation (eg, nasal polyps, middle turbinates, and inferior turbinates) (data not shown).

In connective tissue, the percentage of immunopositive cells and the staining density of galectin-1 were markedly higher in the nasal polyps than in the middle turbinate from the allergic and the nonallergic patients (Figure 3C). In the 2 groups of patients, the percentage of cells immunopositive for galectin-1 was lower in the middle turbinate than in the inferior turbinate (Figure 3C). In the nonallergic patients, this percentage was the highest in the nasal polyps, a result also registered for the staining intensity (Figure 3C).

Our report has focused on 2 endogenous lectins with relevance for growth control, namely, galectin-1 and galectin-3. Using galectin-type–specific reagents, we first documented the presence of both galectins in the tissue compartments. Due to the sensitivity of the N-terminal part of galectin-3 with the Gly-, Tyr-, and Pro-rich repeats to collagenases, we deliberately used a polyclonal antibody reactive with N- and C-domain epitopes to exclude false-negative results. A presence of galectin-3, for example in head and neck squamous carcinomas, can be taken as an indication for a role in positive growth regulation by limiting apoptosis. In our study, galectin-3 expression variable LI is markedly higher in nasal polyps than in turbinates, a result that suggests an implication of galectin-3 on nasal polyps growth. In comparison, the low LI variable in turbinates is associated with markedly more intense staining intensity. The meaning of greater intensity staining compared with smaller surface area with respect to galectin-3 expression can relate either to cells expressing a greater galectin-3 expression or to a greater state of activation. Because galectin-3, the MAC-2 antigen of macrophages, had been detected also as a IgE-binding protein of rat basophilic leukemia cells, it is noteworthy that the patient status concerning allergy has no apparent influence on galectin-3 expression. Remarkably, there is a trend toward increased expression for galectin-1 in samples from allergic patients. Early reports on dexamethasone regulation of galectin-1 expression, its 10-fold up-regulation during the induction of T-cell apoptosis by glucocorticoids, and the anti-inflammatory roles of galectin-1 described recently can be reconciled with such an activity, mimicking the enhancing effect of an immunosuppressive corticoid on this parameter.

On examining the results in specimens from nonallergic patients, the lowest galectin-1 expression level
was found in the middle turbinate. In inferior turbinates, galectin-1 could play a role by protecting them against inflammation and thus polyp growth. In middle turbinates, the galectin-1 concentration seems to be insufficient to prevent the polyp growth. In nasal polyps, galectin-1 might be up-regulated as response to counteract inflammatory process. This lower galectin-1 expression in middle turbinates compared with inferior turbinates may explain why nasal polyps are mainly found in the middle meatus and never in the inferior meatus.

In summary, our data show that galectin-1 and galectin-3 expression is detectable in the examined tissue material and compartments. Galectin-3 expression can be linked to the benign growth activity of nasal polyps due to an anti-apoptotic effector activity. Because galectin-1 expression was positively correlated with allergenic status, mimicking glucocorticoid-induced pattern, this family member can be suggested to have anti-inflammatory activity in situ. To proceed to define the galectin network in growth/immune cell regulation, monitoring of further galectins such as galectin-8,14,16,24 binding site localization, and the additional monitoring of related features (e.g., apoptosis and immunoreactive effectors such as macrophage migration inhibitory factor) are reasonable lines of investigation.

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