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 \( (P<.001) \) 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 \( (P<.001) \) in nonallergic patients compared with allergic ones (in glandular epithelium, \( P=.009 \); in connective tissue, \( P=.006 \)). 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.


Nasal polyposis is a chronic inflammatory disease of the paranasal sinuses with a prevalence of 1% to 4% in the general population. 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 sinuses or cystic fibrosis. 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. 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 sinusoids.

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, or as a consequence of increases in ion transport mechanisms. 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. 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. 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...
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.8,9-12

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.13,15,16 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.13,15,16 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.

To obtain detailed data on the staining profiles, 2 quantitative variables were determined by a SAMBA 2005 computer-assisted microscope system (Samba Technologies, Grenoble, France) with a × 40 (aperture 0.50) magnification lens. The labeling index (LI) reports the percentage of tissue area specifically stained by the antigalactin antibodies. The mean optical density (MOD) denotes staining intensity computed on the immunopositive areas only.

![Figure 1](image-url)
been detailed elsewhere.\textsuperscript{16} 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 (ie, the surface epithelium, the glandular epithelium, and the connective tissue). Ten fields of between 60000 and 120000 µm\(^2\) 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 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
A lectin-1 staining intensity is lower in the middle turbinate (Figure 3A). The gauge-histochemically positive for galectin-1 was higher in the nasal polyps than in the middle turbinates from both the allergic and the nonallergic patients (Figure 3B). The percentage of cells immunopositive for galectin-1 is markedly higher in the nasal polyps, middle turbinates, and inferior turbinates than in the inferior turbinates from the allergic patients (data not show).

In glandular epithelium, the percentage of cells immunopositive for galectin-1 was higher in the nasal polyps than in the middle turbinate compared with 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 polyp 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

**Figure 3.** Quantitative evaluation (by computer-assisted microscopy) of galectin-1 expression in nasal polyp (P), inferior turbinate (I-T), and middle turbinate (M-T). In detail, the percentage of specifically stained area (expressed as the labeling index) in surface epithelium (A), glandular epithelium (B), and connective tissue (C) was determined. This evaluation was performed separately for polyps and turbinates from the nonallergic and allergic patients. The data are illustrated as mean ±SE. $P$ is the value obtained by carrying out the Kruskal-Wallis test.

Our study shows that galectin-1 is also present in the tissue under study. Figure 3 details the data obtained on the LI variable computed for galectin-1 in nasal polyps, middle turbinates, and inferior turbinates in the 3 histological structures (the surface epithelium, the glandular epithelium, and the connective tissue). The data obtained on the MOD variable is not detailed for the sake of clarity.

In the surface epithelium, the percentage of cells immunohistochemically positive for galectin-1 was higher in the nasal polyps than in the middle turbinates from the nonallergic patients (Figure 3A). This percentage was the highest in the inferior turbinate (Figure 3A). The galectin-1 staining intensity is lower in the middle turbinate (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).
was found in the middle turbinate. In inferior turbinate,
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 in-
sufficient to prevent the polyp growth. In nasal polyps,
galectin-1 might be up-regulated as a response to coun-
teract inflammatory process. This lower galectin-1
expression in middle turbinate compared with inferior tur-
binates 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 ga-
lectin-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 galec-
tin-1 expression was positively correlated with aller-
genic status, mimicking glucocorticoid-induced pattern,
this family member can be suggested to have anti-
inflammatory activity in situ. To proceed to define the
galactin network in growth/immune cell regulation, moni-
toring of further galectins such as galectin-8,14,16,24 bind-
ing site localization, and the additional monitoring of re-
lated features (eg, apoptosis and immunoreactive effec-
tors such as macrophage migration inhibitory factor) are rea-
sone lines of investigation.

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REFERENCES

2. Berger G, Hammel J, Berger R, Avraham S, Ophir D. Histopathology of the infe-
rior turbinate with compensatory hypertrophy in patients with deviated nasal sepa-
3. Krajina Z. A contribution to the aethiopathogenesis of nasal polyps. Pract Oto-
4. Bernstein JM, Yankaskas JR. Increased ion transport in cultured nasal polyp epi-
6. Larsen PL, Tos M, Kuijpers W, van der Beek JM. The early stages of polyp for-
7. Caye-Thomasen P, Hermansson A, Tos M, Prellner K. Polyp pathogenesis: a his-
115:76-82.
8. Gabius H-J. Biological information transfer beyond the genetic code: the sugar
10. Perillo NL, Marcus ME, Baum LG. Galectins: versatile modulators of cell adhe-
11. Gabius H-J. Glycohistochemistry: the why and how of detection and localization
12. Rabinovich GA, Rubinstein N, Toscano M. Role of galectins in inflammatory and
toma cell growth by carbohydrate-dependent surface binding of galectin-1 and
14. Sheikholeslam-Zadeh R, Decaestecker C, Delbrouck C, et al. The levels of ex-
pression of galectin-3, but not of galectin-1 and galectin-8, correlate with apop-
by biochemically defined lectin-binding glycoproteins, neoglycoprotein and lectin-
16. Camby I, Belot N, Rorive S, et al. Galectins are differentially expressed in supra-
temporal pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glio-
blastomas and significantly modulate tumor astrocyte migration. Brain Pathol.
sites with low differentiation status in head and neck squamous cell carcino-
18. Liu F-T, Orida N. Synthesis of surface immunoglobulin E receptor in Xenopus
1984;259:10649-10652.
19. Liu F-T, Orida N. Identification of an IgE-
binding protein by molecular cloning. Proc Natl Acad Sci U S A. 1985;82:4100-
4104.
20. Clerch LB, Whitney P, Massaro D. Rat lung lectin gene expression is regulated
21. Goldstone SD, Lavin MF. Isolation of cDNA clone, encoding a human beta-
galactoside binding protein, overexpressed during glucocorticoid-induced cell
22. Rabinovich GA, Sotomayor CE, Riera CM, Bianco I, Correa SG. Evidence of a role
under budonoside and inhibits eosinophil migration. Lab Invest. 2002;82:147-158.
compared with normal and dysplastic human colon tissue and acts significantly