Introduction

Nasal polyposis (NP) can be defined as a chronic inflammatory disease of the nasal and sinus mucosa leading to diffuse forma-
tion of benign polyps, which protrude from the sinuses into the nasal cavity.1 Histologically, polyps consist of respiratory epithelium covering very edema-
tous stroma infiltrated by a number of inflammatory cells, with eosinophils predominating in most specimens.2 While progress is being made in understanding the role of cellular and biochemi-
cal factors in the pathophysiology of NP, the etiology of NP is still unknown. Polyposis is often related to asthma, NSAID intolerance, and cystic fibrosis, indicating that inflammatory mediators are essential in the pathogenesis. A hypothetical concept for the for-
formation of nasal polyps involving a rupture of the epithelium and pro-
lapse of the lamina propria as a result of tissue pressure from an edematous and infiltrated nasal mucosa has been proposed.3 A sec-
ond hypothesis focuses on the increased sodium absorption caused by local release of inflam-
matory mediators.23 The ensuing epithelial fluid absorption con-
tributes to the development of nasal polyps and is the result of the increased recruitment of inflam-
matory cells, which are present in nasal polyps. Another possible cause of polyp formation and growth is the deposition of plasma proteins such as albumin and extracellular matrix proteins, reg-
ulated by subepithelial eosino-
phlic inflammation.5 The eosinophils are thus the major effector cells in this hypothesis of polyp pathogenesis.

Generally, the treatment plan for NP should be tailored to each individual patient, but most will require a combination of medical and surgical therapy to deal with the clinical recurrences that char-
acterize this condition. It has been shown that topical corticosteroid treatment can reduce polyp size and the majority of associated nasal symptoms.6,4 A study of mol-
ecular effectors should thus take this factor into consideration. Based on the concept of the sugar code and the emerging relevance of protein (lectin)-carbohydrate
interactions to cell activities including adhesion, communication, and growth regulation, we decided to investigate the expression of an endogenous lectin, a member of a family of immune modulators, in nasal polyps.

The galectins are distinguished from the other families of animal lectins by their Ca\(^{2+}\)-independent ability to bind \(\beta\)-galactosides and their derivatives, their jelly-roll-like folding pattern, and an invariant amino-acid motif with a central Trp residue. Their non-classical secretion, which allows interaction with various intracellular proteins (such as oncogenic H-Ras in the case of galectin-1), and their ability to cross-link glycans at the cell surface, give galectins the potential to become involved in diverse signaling pathways. Our recent study revealed a positive influence of galectin-3 on polyp growth, and an immunomodulatory role for galectin-1 in nasal polyps and turbinates.

Materials and methods

Clinical data and histopathological characteristics

Medical records and samples of nasal polyps were obtained from 9 patients, whose diagnoses had been established according to the classification that we reported recently. The nine nasal polyps in the present study involved massive polyposis and came from 5 allergic and 4 nonallergic patients. The allergic versus nonallergic status of the patients was determined by the skin prick test as well as by their total and specific IgE expression. No patient was taking any medication prior to surgery and none were smokers.

Ex vivo organotypic cultures of human nasal polyps

The nine nasal polyps were maintained under organotypic culture conditions in a manner identical to the methodology that we have described elsewhere for various tumour tissue types. Briefly, after their surgical removal, the nasal polyp specimens were rinsed twice in Minimal Essential Medium (MEM; Gibco-Life Technologies, Merelbeke, Belgium) and immediately cut in half. While one-half was used for histopathological diagnosis, the other was immediately divided into pieces measuring approximately 2 to 3 mm\(^3\). To overcome the problem of biological heterogeneity, each experimental condition included between 10 and 15 randomly selected pieces of each nasal polyp. These pieces were cultured for 24 hours in 30 mm (diameter) \(\times\) 10 mm (height) Petri dishes (NUNC-LIFE Technologies, Merelbeke, Belgium) containing 3 ml of culture medium in the form of MEM supplemented with a mixture of 0.6 mg/ml glucazone (Gibco), 200 IU/ml penicillin (Gibco), 200 IU/ml streptomycin (Gibco), and 0.1 mg/ml gentamycin (Gibco). The effects of two concentrations (50 and 250 ng/ml) of budesonide (Rhinocort\textsuperscript{®}, Astra-Zeneca, Brussels, Belgium) were studied. For this purpose, budesonide was dissolved in a small amount of dimethyl sulfoxide (DMSO), with a final DMSO concentration below 0.1%. An equal volume of this solution was added to the control cultures. After 24 h of culture, the samples were fixed in buffered formalin for 5 days. After paraffin embedding, 5-mm sections were prepared for quantitative histochemistry as detailed below.

Immunohistochemical determination of galectin-7 levels

The expression of galectin-7 was monitored by a specific polyclonal antibody raised and checked for specificity and lack of cross-reactivity to galectin-1, -3, -4, and -8, as described previously. The following immunohistochemical procedures were carried out as detailed previously. Briefly, for each treatment condition, between 10 and 15 tissue pieces were embedded in the same paraffin block from which the 5-mm-thick sections were cut. These sections were processed with the anti-galectin-7 antibody. Incubation
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with the antibody-containing solutions was carried out at 25 ± 1°C for 30 minutes at a concentration of 10 µg/ml. The bound antibody was visualized by avidin-biotin-peroxidase complex (ABC) kit reagents (Vector Labs, Burlingame, CA, USA), using diaminobenzidine and H2O2 as chromogenic substrates. The control reactions included the omission of the incubation step with the probe (antibody), and the omission of the incubation step with each of the secondary reagents in order to exclude any antigen-independent staining by binding of kit reagents such as the glycoprotein avidin or peroxidase. Counterstaining with hematoxylin concluded the processing.

In order to characterize the extent of immunohistochemically detectable galectin-7 in nasal polyps, two measurements were made with a SAMBA 2005 computer-assisted microscope system (Samba Technologies, Grenoble, France) with a 40 X (aperture 0.5) magnification lens. The labeling index (LI) is the percentage of tissue area specifically stained by the anti-galectin-7 antibody. The mean optical density (MOD) denotes staining intensity computed on the immunopositive areas only. While the LI thus defines the presence or absence of the protein targeted, the MOD refers to its local density. The quantification method and its standardization have been described in detailed elsewhere.23,24

A negative histological control slide (processed without the primary antibody) was analyzed for each specimen under study. The software used on the computer-assisted microscope automatically subtracted the LI and MOD values of the negative control sample from each corresponding positive one.

As detailed previously,23 three tissue types were analyzed for each experimental condition: the surface epithelium, the glandular epithelium, and the connective tissue. Ten fields of between 60,000 and 120,000 µm² each were scanned per tissue type.

Statistical analyses

As the conditions for applying parametric tests were not satisfied, the Kruskal-Wallis test (non-parametric one-way analysis of variance) was used for statistical comparisons of the groups. In cases where this test revealed significant differences, we investigated whether any pair of groups significantly differed. For this purpose, we applied the standard Dunn Procedure (2-sided test) for multiple comparisons.

Results

The use of organotypic cultures of human nasal polyps

To study galectin-7 expression and the effect of budesonide, we first determined that the structural organization of the tissue pieces was not noticeably affected by the culture conditions. Human nasal polyps obtained from surgical resections and maintained for 24 h ex vivo under organotypic culture conditions generally maintained their histological organization (Figures 1 and 2). The immunohistochemical staining of galectin-7 in the surface epithelium of a control (not exposed to budesonide) nasal polyp from an allergic patient is presented in Figure 1. The galectin-7 staining pattern in the surface epithelium of a nasal polyp treated with 50 ng/ml budesonide is presented in Figure 2. These illustrations clearly demonstrate that galectin-7 is present in the pseudostratified epithelium of the polyp. In material from an allergic patient, the galectin-7 staining intensity was clearly enhanced in the surface epithelium and connective tissue of the polyps treated with 50 ng/ml budesonide (Figure 2), relative to that in the corresponding controls (Figure 1).

Budesonide-mediated effects on the expression of galectin-7

The quantitative evaluation of the percentage of immunohistochemically positive tissue area (the LI) in the surface epithelium, the glandular epithelium, and the connective tissue of nasal polyps is presented in Figure 3. For clarity, the data obtained for the staining intensity (the MODs) are not reported, because these results were associated only with a weaker level of statistical significance than the data obtained for the labeling index.

In the surface epithelium, the data show that treatment with 250 ng/ml budesonide reduced the percentage of galectin-7 immunopositive cells (p = 0.03) in nasal polyps from non-allergic patients. In nasal polyps from allergic patients, this percentage was increased by treatment with 50 ng/ml budesonide (p = 0.0001). The galectin-7 staining density (MOD) in the surface epithelium of nasal polyp from non-allergic patients decreased in response to 50 ng/ml budesonide (p = 0.01; data not shown).

In the glandular epithelium of nasal polyps from non-allergic patients, treatment with 50 ng/ml budesonide resulted in a decrease
in the percentage of galectin-7-immunopositive cells.

In connective tissue, the percentage of cells immunopositive for galectin-7 after treatment with 50 ng/ml budesonide increased in nasal polyps from allergic and non-allergic patients.

Discussion

Galectin-7 is a 14-kDa, prototypical member of this lectin family, and was first cloned from human epidermis.25,26 The X-ray crystal structure of this protein revealed the typical galectin folding pattern, and mapping of its carbohydrate-binding fine-specificity by oligosaccharides revealed differences between it and galectin-1.27,28 Like galectin-1 it is homodimeric in solution and able to inhibit cell proliferation after binding to the sugar chain of ganglioside GM1 in an in vitro neuroblastoma model.29 Expression of galectin-7 in normal tissue appears to be restricted to epithelia that are stratified or are destined to become stratified (keratinized or not), in the skin, tongue, esophagus, and Hassal’s corpuscles in the thymus.25 It is strikingly downregulated in SV40-transformed human keratinocytes26 and squamous cell carcinomas.25 The protein localizes to areas of cell–cell contact, particularly in the upper layers of human epidermis,26 suggesting that it may function in the maintenance of the normal phenotype of these epithelial cells. A recent report indicated that the α6 subunit, a member of the integrin family, is a potential galectin-7 ligand, linking galectin-7 to regulation of epidermal cell polarization, attachment, or migration, via modulation of integrin function.

Screening by SAGE in DLD-1 colon cancer cells and work with damaged keratinocytes implicated galectin-7 in p53-dependent apoptosis.31,32 It was found that galectin-7 mRNA and protein levels increased in epidermal keratinocytes after ultraviolet B (UVB) irradiation, paralleling p53 stabilization.32 Furthermore, UV-induced apoptotic cells expressed a higher level of galectin-7 than non-apoptotic cells, and keratinocytes overexpressing galectin-7 had a greater tendency to undergo apoptosis. It can function as an extracellular effector via glycan binding,29 and also intracellularly, upstream of c-Jun N-terminal kinase (JNK) activation and cytochrome c release.33 Besides having a negative role in colon cancer, galectin-7 has been detected in a rat model of chemically induced mammary carcinoma,34 and as a new gene associated with the progression of lymphoma towards a metastatic phenotype,35 revealing a complex functionality in different cell types. Histologically, galectin-7 expression was reported to be downregulated in a large proportion of thyroid adenomas (including the normomacrofollicular, microfollicular, and trabecular variants) when compared with carcinomas.22

Mammalian epithelia can be divided into two major classes based on their structure and organization and their embryological origin. In general, simple (mono-
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Figure 3

Quantitative evaluation (by computer-assisted microscopy) of the percentage of galectin-7-positive tissue area (the labeling index) in nasal polyp samples treated with 50 ng/ml (R50) and 250 ng/ml (R250) of budesonide, respectively, or left untreated (CT). The measurements were performed on the surface and glandular epithelia as well as the connective tissue of the nasal polyp samples, and the results are reported separately for the polyps from the nonallergic (N-ALL) and the allergic patients (ALL). The data are presented as the mean (large bars) and its standard error (thin black bars).
layered) epithelia that line internal organs such as the lung, kidney, and intestine are derived from the endoderm, while stratified (multilayered) epithelia such as the epidermis and cornea develop from the ectoderm. Galectin-7 is described as the first marker of epithelial stratification whose expression does not depend on local differentiation: it is expressed in all cell layers in epidermis and also in the cornea and esophagus, although these tissues express distinctive sets of differentiation markers, such as specific keratins. In contrast, galectin-7 is not expressed in simple epithelia from endodermal origin such as the kidney or the liver.

In nasal polyps, there are two different epithelia: the surface epithelium (pseudostratified respiratory epithelium) and the glandular epithelium (simple columnar epithelium). Galectin-7 expression levels have not previously been investigated in nasal polyps. The present data clearly indicate that galectin-7 is expressed in nasal polyps and in the three tissues analyzed (i.e., surface epithelium, glandular epithelium, and connective tissue). Its presence in connective tissue is noteworthy, and probably arises from secretion. Galectin-7 appears to serve important roles during distinct stages of epithelial stratification, namely movement from the basal to the suprabasal compartment, differentiation and the maintenance of a stratified organization. Two observations suggest that galectin-7 expression might be linked to the control of keratinocyte proliferation: its expression is lower in the basal and first suprabasal cell layers of tumours, especially in basal cell carcinomas, and its expression is slightly delayed in psoriasis, a cutaneous disease characterized by keratinocyte proliferation. In adult mouse tissues, galectin-7 protein is expressed in pseudostratified epithelial cells of the trachea. Our data confirm that galectin-7 might be expressed in a pseudostratified epithelium. In our study, the expression of galectin-7 was found to be higher in surface epithelium than in glandular epithelium. These findings indicate that galectin-7 production coincides with the degree of stratification of the epithelium.

Galectin-7 also has the potential to mediate corneal epithelial cell migration and re-epithelialization of corneal wounds. Galectin-7 could play a role in the pathogenesis of nasal polyps: according to the hypothesis mentioned in the Introduction, the formation of nasal polyps involves a rupture of the epithelium. Galectin-7 could participate in these re-epithelialization processes.

In previous studies, we characterized the anti-inflammatory effects of budesonide on the expression of adhesion molecules involving galectin-1 and -3, E- and P-selectins, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), Lewis' epitope, sialyl Lewis' epitope, and their respective binding sites in human nasal polyps. The aim of this study was to investigate whether budesonide can affect the expression of galectin-7 in human nasal polyps. In connective tissue, the extent of galectin-7 expression increased in 50 ng/ml budesonide, in polyps from allergic and nonallergic patients. Because galectin-7 is a growth-regulatory effector on tumour cells and keratinocytes, programmed cell death of the numerous immune cells infiltrating the connective tissue is increased in response to budesonide at 50 ng/ml. No effect was obtained with budesonide at 250 ng/ml. This high budesonide dose might inhibit the production of galectin-7, but the 24-hours duration of organotypic culture is not sufficient to unambiguously ascertain this effect.

The effect of budesonide on the level of expression of galectin-7 in glandular epithelium is unclear; only a slight decrease in the percentage of galectin-7 immunopositive cells after treatment with 50 ng/ml budesonide was observed in nasal polyps from non-allergic patients.

In the surface epithelium, the modulation of expression of galectin-7 by budesonide differed between allergic and non-allergic patients. In non-allergic patients, budesonide at 250 ng/ml decreased the percentage of immunopositive cells, and in allergic patients budesonide at 50 ng/ml increased this percentage. This increase of expression at 50 ng/ml budesonide in polyps from allergic patients could be explained by the pro-apoptotic effect of galectin-7 induced by corticoids. The effect obtained in the surface epithelium of nasal polyps from non-allergic patients cannot readily be explained at present.

In summary, our data show that galectin-7 is detectable in the surface and glandular epithelia and, notably, the connective tissue of nasal polyps. The percentage of galectin-7-immunopositive cells (but not the staining intensity) increased in the connective tissue in response to treatment with 50 ng/ml budesonide. The study of apoptosis induced by budes-
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onide in nasal polyps and its correlation with the levels of galectin-7 are reasonable lines of future investigation.

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References