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Spatial expression of two anti-inflammatory mediators, annexin 1 and galectin-1, in nasal polyposis

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Summary

Background There is renewed interest in the role played by specific counter-regulatory mechanisms to control the inflammatory host response, poorly investigated in human pathology. Here, we monitored the expression of two anti-inflammatory mediators, annexin 1 and galectin-1, and assessed their potential link to glucocorticoids' (GCs) effective control of nasal polyposis (NP).

Methods Total patterns of mRNA and protein expression were analysed by quantitative real-time PCR (qPCR) and Western blotting analyses, whereas ultrastructural immunocytochemistry was used for spatial localization and quantification of each mediator, focusing on mast cells, eosinophils and epithelial cells.

Results Up-regulation of the annexin 1 gene, and down-regulation of galectin-1 gene, was detected in polypoid tissue compared with nasal mucosa. Patient treatment with betamethasone augmented galectin-1 protein expression in polyps. At the cellular level, control mast cells and eosinophils displayed higher annexin 1 expression, whereas marked galectin-1 immunolabelling was detected in the granule matrix of mast cells. Cells of glandular duct epithelium also displayed expression of both annexin 1 and galectin-1, augmented after treatment.

Conclusion Mast cells and epithelial cells appeared to be pivotal cell types involved in the expression of both annexin 1 and galectin-1. It is possible that annexin 1 and galectin-1 could be functionally associated with a specific mechanism in NP and that GC exert at least part of their beneficial effects on the airway mucosa by up-regulating, in a specific cell target fashion, these anti-inflammatory agonists.

Keywords annexin 1, epithelial cells, galectin-1, glucocorticoid, inflammatory cells, nasal polyp

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Introduction

Nasal polyposis (NP) is a chronic inflammatory disease of the paranasal sinuses, characterized by the presence of oedematous fluid with sparse fibrous cells, proliferation and metaplasia of the respiratory epithelial layer, thickening of the basement membrane and local fibrosis [1]. Besides epithelial differentiation and overgrowth, a major feature of polyposis is the marked presence of inflammatory cells, particularly eosinophils and mast cells [2]. Eosinophils are a rich source of cytokines (e.g. RANTES, eotaxin, IL-5, IL-8), active to recruit and activate further

blood-borne cells in the nasal polyps [3, 4]. The mast cell is also proposed to have a major role in this inflammatory condition through the release of pro-inflammatory and immuno-regulatory molecules, associated with the expression of a wide spectrum of surface receptors for cytokines and chemokines [5]. Not many studies have monitored the expression of anti-inflammatory mediators in NP. In one study, the glucocorticoid (GC)-regulated protein annexin 1 (ANXA1), 37 kDa, was identified in NP epithelium [6]. In a different study, the lectin-binding protein galectin-1 (Gal-1), 14 kDa, was detected in NP tissues, and up-regulated after incubation *in vitro* with the

GC budesonide: augmented Gal-1 expression might be correlated to the inhibition of eosinophil locomotion/migration [7].

ANXA1 is a calcium-dependent protein of the annexin superfamily [8] able to affect several central features of the inflammation. For instance, this protein has been shown to regulate leucocyte migration [9], apoptosis [10], cellular signalling and proliferation [11]. ANXA1 synthesis is partly controlled by GC in both humans and rodents [12, 13]. Similar to ANXA1, Gal-1 also possesses anti-inflammatory properties in experimental models of acute and chronic inflammation [14].

Not many studies have investigated the cellular distribution of these two anti-inflammatory proteins, even less so in human pathologies. ANXA1 has been localized to the membrane and specific cytoplasmic granules of rat mast cells [15], rat neutrophils [16] and human eosinophils [17]. In addition, significant amounts of this protein are present in bronchoalveolar lavage (BAL) fluid [18], epithelial cells [19] and macrophages [20] of the respiratory tract.

Prompted by these findings, the present study was undertaken to investigate the distribution of these two proteins at the immunocytochemical level. In addition, to compare the pattern of ANXA1 and Gal-1 mRNA and protein expression, we also monitored potential changes associated with patient treatment with the GC bethametasone.

Materials and methods

Surgical specimens

Ten patients (five males, five females; mean age 42) with the inflammatory type of nasal polyps, requiring surgical intervention, were selected for the study, according to the following criteria. NP was evaluated according to medical history, clinical examination and biopsy. According to medical history, eight patients had symptoms of atopy, but no patients presented a history of acetylsalicylic acid intolerance. A first set of polyp samples was collected under a biopsy procedure (NP). After 1 month, all NP patients received a treatment with betamethasone acetate/betamethasone sodium phosphate (6 mg/1 mL, i.m.) and 5 days after GC treatment the second set of polyps was removed by surgical intervention (NP post-treatment). As control samples, nasal mucosa samples from human autopsy (causes of death were either car accident or myocardial infarction, without a history of NP) and blood from health volunteers were used. To ensure the histological quality of autopsies, they were harvested within the first 8 h after death. None of the groups had any disease that required continuous medication.

This study was approved by the Committee of Ethics in Research of the São Paulo School of Medicine, UNIFESP

(CEP-process 1042/02), and of the Medicine Faculty of São José do Rio Preto, Brazil (CEP-process 4932/2004), and informed written consent was obtained from all the subjects.

Ribonucleic acid extraction

Nasal polyp (biopsy and surgery) and nasal mucosa (autopsy) specimens were pulverized under liquid nitrogen, and total RNA was extracted by using the TRIzol Reagent (Life Technologies, Grand Island, NY, USA).

To evaluate the quality of RNA, the absorbency at 260/280 nm was determined by spectrophotometry for each sample and approximately 1 µg was loaded onto agarose gel containing formaldehyde and resolved by electrophoresis. The gel was stained with ethidium bromide, and the 18S and 28S bands were visualized using ultraviolet (UV) light. Initially, the quality of cDNA through the PCR of housekeeping gene *GAPDH* was evaluated. The primers set were 5' to 3' CTGCACCACCACTGCTTA and CAT-GACGGCAGGTCAGGTC.

Quantitative real-time polymerase chain reaction

The quantitative real-time PCR (qPCR) assay was performed by using the GenAmp 5700 Sequence Detector (PE Applied Biosystems, Foster City, CA, USA). Approximately 1 µg of total RNA from each sample was used to synthesize cDNA with 200 U of Superscript II reverse transcriptase (Invitrogen/Life Technologies Inc., Carlsbad, CA, USA).

Each cDNA sample was analysed in duplicate. Quantitative PCR reactions were carried out according to the instructions for the SybrGreen PCR Core Reagent (PE Applied Biosystems). For ANXA1, forward and reverse 5' to 3' primers were GCAGGCCTGGTTTATTGAAA and GCTGTGCATTGTTTCGCTTA. For Gal-1, they were GGACA TCCTCCTGGACTCA and GTTGAAGCGAGGGTTGAAGT. The relative expression of each specific product was calculated by $2^{-\Delta\Delta CT}$ (CT = fluorescence threshold value; ΔCT = CT of the target gene - CT of the reference gene (β -actin); $\Delta\Delta CT$ = ΔCT of the nasal polyp sample - ΔCT of the reference sample).

Western blotting analysis

Frozen NP and nasal mucosa samples were homogenized in ethylene diamine tetraacetic acid-free protease inhibitor (Roche Diagnostic, Mannheim, Germany) and centrifuged (4000 g, 5 min, 25 °C). Protein concentrations in supernatants were determined by the Bradford assay (Sigma Chemical Co., Poole, Dorset, UK) and were lysed with hot Laemmli sample buffer. Equal protein amounts (30 µg) were electrophoresed in a 12% polyacrylamide gel in running buffer, followed by transfer of proteins onto Hybond-C extra nitrocellulose membranes in transfer buffer.

Membranes were blocked in blocking solution containing 5% non-fat milk solution, followed by specific anti-ANXA1 [21] or anti-Gal-1 antibodies (Peprotech, EC Ltd., London, UK). Following overnight incubation, goat anti-rabbit IgG (1 : 2000; Dako, Glostrup, Denmark) was added, and then washed and specific antibody binding was detected by an enhanced chemiluminescence system (ECL Western blotting detection reagent; Amersham, Little Chalfont, UK).

Fixation, processing and embedding for light and transmission electron microscopy

Fragments of the nasal polyps, nasal mucosa and peripheral human polymorphonuclears, predominantly eosinophils and prepared with a gradient protocol as described [17], were fixed in 4% paraformaldehyde and 0.5% glutaraldehyde, 0.1% sodium cacodylate buffer (pH 7.4) for 18 h at 4 °C. They were then washed, dehydrated and embedded in LR Gold (London Resin Co., Reading, Berkshire, UK). Sections (1 µm thick) were stained with Diff Quick and analysed with an AXIOSKOP microscope (Zeiss, Germany). For electron microscopy analysis, sections (70 nm thick) were cut on an ultramicrotome (Reichert Ultracut; Leica, Austria).

Post-embedding immunogold labelling

To detect ANXA1 and Gal-1, tissue samples and human eosinophils were prepared for electron microscopy by standard methods and in sequence a post-embedding immunogold labelling double reaction was performed. Ultrathin sections were incubated: (1) phosphate-buffered solution (PBS) containing 1% egg albumin (PBEA); (2) PBS containing 5% egg albumin (PBEA) for 30 min; (3) the sheep polyclonal antibody raised the N-terminal peptide of human ANXA1 [23] (1 : 200) and the polyclonal rabbit anti-Gal-1 (1 : 300) for 2 h; (4) normal sheep serum and normal rabbit serum, respectively, were used as control; (5) after washes in PBEA, in order to detect ANXA1, a donkey anti-sheep IgG antibody (1 : 50 in PBEA) conjugated to 20 nm colloidal gold (British Biocell, Cardiff, UK) was added, and to detect Gal-1, a goat anti-rabbit IgG antibody (1 : 50 in PBEA) conjugated to 10 nm colloidal gold (British Biocell) was added; and (6) after 1 h, sections were washed in PBEA, and then in distilled water. Ultrathin sections were stained with uranyl acetate and lead citrate before examination on a ZEISS LEO 906 electron microscope.

Data handling and statistical analysis

The densities of mast cells and eosinophils are reported as mean $\pm$ SEM of 10 patients/group. Analysis of these inflammatory cells in nasal polyps and nasal mucosa was performed with the image analysis system (AxioVision Software, Zeiss). Similarly, photographed sections of NP

(biopsy), NP-post treatment (surgery), control tissue (nasal mucosa and human blood) were used for the immunocytochemical analysis (10/group). The area for mast cells, eosinophils and epithelial cells was determined with AxioVision software (Zeiss). The density of immunogold (number of gold particles/µm²) was calculated and reported as mean $\pm$ SEM of number of the electron micrographs. Statistical differences between means were determined by analysis of variance followed, if significant, by the Bonferroni test. A probability value of < 0.05 was considered as significant.

Results

Histopathological analysis of the inflammatory infiltrates

We started off by confirming the inflammatory nature of the NP samples. Nasal mucosa contained mast cells (Fig. 1a), predominantly distributed in the stroma, whereas in NP samples most mast cells showed characteristic signs of degranulation (Fig. 1c). Quantification of total (intact + degranulated) mast cell number showed higher (~10-fold) numbers in nasal mucosa compared with NP tissue samples ($P < 0.001$; Fig. 1d). The opposite was true for eosinophil number that increases strikingly in polypoid tissue ($P < 0.001$; Fig. 1d). As the presence of eosinophils

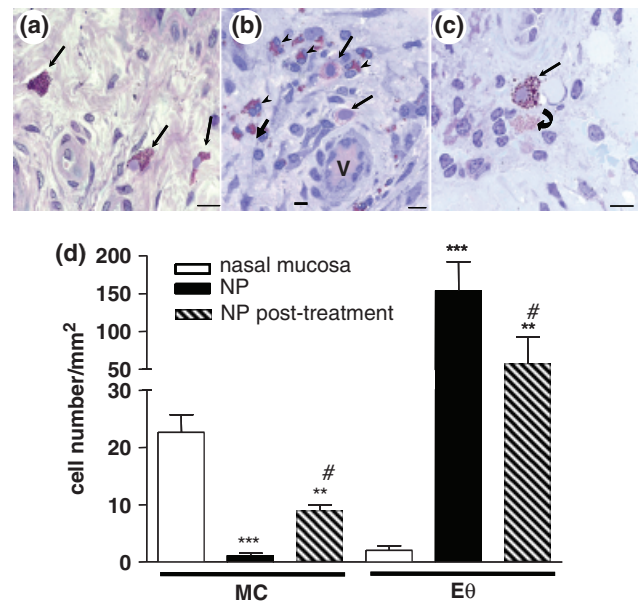


Fig. 1. Density of inflammatory cells in nasal mucosa and polyps. (a) Nasal mucosa with intact mast cells (arrows) near vessel (V). (b) Degranulated mast cells (arrows) and eosinophils (arrowheads) from nasal polyp biopsy. (c) Intact (arrow) and degranulated mast cells (curve arrow) in polypectomy. (d) Total number of mast cells (MC) and eosinophils (Eθ) in the nasal mucosa, nasal polyps (NP) before and after glucocorticoid (GC) treatment (NP post-treatment) ($n = 10$). ** $P < 0.01$ vs. autopsy; *** $P < 0.001$ vs. autopsy; # $P < 0.05$ vs. biopsy. Data presented by mean $\pm$ SEM.

was rarely observed in nasal mucosa, we used human blood as control tissue. As stated above, eosinophils were predominantly present in NP, with a preferred localization in the stroma, often in close vicinity to mast cells (Fig. 1b). After GC treatment, NP patients displayed reduced eosinophil number ($P < 0.05$), with increased mast cell number ($P < 0.05$) (Fig. 1d).

Quantitative real-time polymerase chain reaction and Western blotting analysis of annexin 1 and galectin-1 in nasal polyps

Up-regulation of the *ANXA1* gene was detected in polypoid tissue (~49-fold) compared with nasal mucosa (Table 1), whereas after the GC treatment a twofold up-regulation was observed compared with biopsy from nasal polyps. In agreement, Western blotting analysis of the same samples indicated constitutive expression of ANXA1 protein and increased detection in NP: blots show both native (37 kDa) and cleaved (34 kDa) species of the protein. This level of expression was not further modulated after GC treatment (Fig. 2).

Differently from *ANXA1*, NP expression of *LGALS1* gene was down-regulated when compared with nasal mucosa (Table 1); treatment with GC provoked up-regulation of *LGALS1*. These data were confirmed by Western blotting analysis (Fig. 2).

Immunogold analysis for annexin 1 and galectin-1 expression in inflammatory and epithelial cells

Next, we focused on the immunocytochemical expression of ANXA1 and Gal-1 in mast cells, eosinophils and cells of the glandular duct as well as of the respiratory epithelium.

Mast cells. A particular high density of Gal-1 immunogold label was seen within the granule of nasal mucosa (Fig. 3a). In NP, most of the mast cells appeared degranulated presenting cytoplasmic vacuoles as shown in Fig. 3b. Table 2 reports a significant decrease in Gal-1 ($P < 0.001$) and ANXA1 ($P < 0.01$) immunoreactivity in NP mast cells compared with those in control tissue. No changes in the expression of ANXA1 and Gal-1 could be detected in this cell type in GC-treated patients.

Eosinophils. ANXA1 and Gal-1 were expressed to a similar level in blood eosinophils, used as control cells (Fig. 3c). Eosinophils localized in the stroma of NP appeared activated, with widespread expression of ANXA1 and Gal-1 in the nucleus, cytoplasm as well as in granules (Fig. 3d); however, as reported in Table 2, quantitative analyses revealed decreased ANXA1 expression ($P < 0.01$) in NP. In contrast, no difference could be measured for Gal-1 expression in NP vs. blood eosinophils (Table 2).

Table 1. *ANXA1* and *LGALS1* expression profile in nasal mucosa and polyps before and after GC treatment

Gene	Groups	Ratio*
<i>ANXA1</i>	NP vs. nasal mucosa	46.94 ↑
	NP post-treatment vs. nasal mucosa	90.23 ↑
	NP post-treatment vs. NP	1.92 ↑
<i>LGALS1</i>	NP vs. nasal mucosa	0.25 ↓
	NP post-treatment vs. nasal mucosa	0.35 ↓
	NP post-treatment vs. NP	1.43 ↑

*Ratio among gene expression levels in nasal mucosa, and nasal polyps before (NP) after GC treatment (NP post-treatment). Each qPCR experiment was performed in duplicate ($n = 10$ for both nasal mucosa and polyps). Arrows indicate increased and decreased expression of each gene.

NP, nasal polyposis; GC, glucocorticoid.

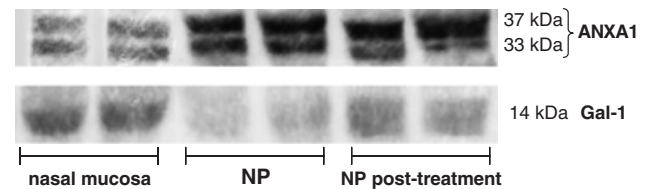


Fig. 2. ANXA1 and Gal-1 expression profile in nasal mucosa and polyps. Detection of endogenous ANXA1 and Gal-1 in nasal mucosa and polyps by Western Blotting analysis. Representative blot of tissue samples collected from nasal mucosa, nasal polyp (NP) before and after glucocorticoid (GC) treatment (NP post-treatment) ($n = 5$ /group).

Epithelial cells. Glandular duct epithelium cells expressed both ANXA1 and Gal-1 (Fig. 3e). Again, these two mediators were expressed to a lower extent in NP samples (Table 3), a reduction partially rescued after GC treatment ($P < 0.05$; Table 3). In contrast, metaplastic respiratory epithelial cells were also positive for these proteins, but with a higher expression in nasal mucosa than in surgeries (Table 3). No labelling was detected in sections incubated with the control non-immune sheep (for ANXA1) or rabbit serum (for Gal-1) (Fig. 3f).

Discussion

In this study, we have investigated the pattern of expression of two anti-inflammatory mediators in human chronic inflammation pathology. Both mRNA and protein expression were monitored and compared with control conditions. The effect of a single treatment with an anti-inflammatory dose of betamethasone was also assessed. The data obtained indicate that ANXA1 and Gal-1 might be important in the patho-physiology of polyposis, and display a different pattern of expression across resident cells of nasal mucosa and NP tissue.

Histopathological analysis of NP tissue samples revealed an on-going inflammation characterized by

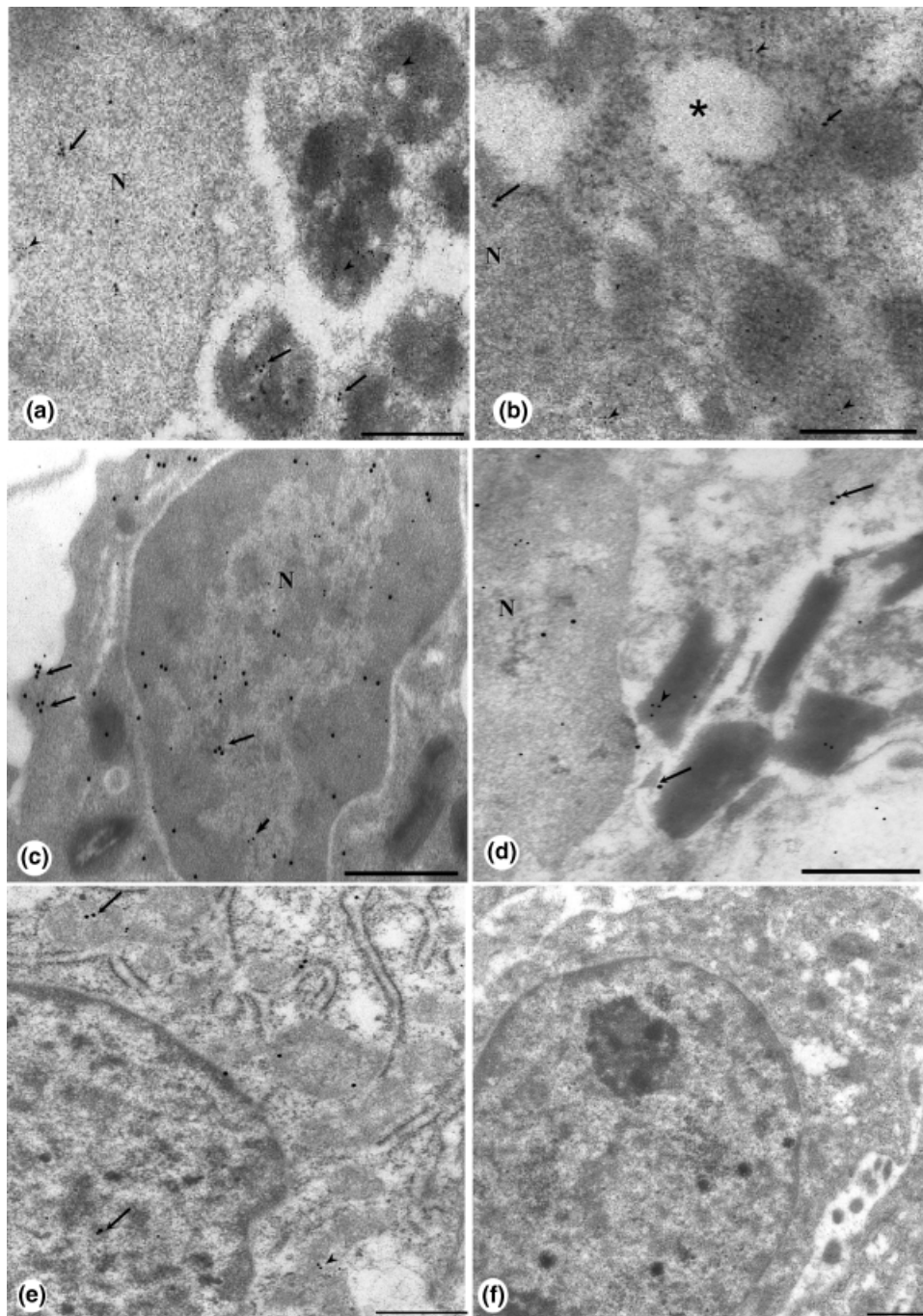


Fig. 3. Analysis of ANXA1 and Gal-1 localization in inflammatory and epithelial cells. Co-localization of ANXA1 (arrows) and Gal-1 (arrowheads), after immunogold labelling with 20 nm particles (LCPS1) and 10 nm particles (anti-Gal-1). (a) Nasal mucosa mast cells view presenting ANXA1 and Gal-1 within electron-dense cytoplasmic granules. (b) Degranulated nasal polyp mast cells showing vacuoles (*) and slight labelling to ANXA1 and Gal-1. (c) Human blood eosinophils showing ANXA1 and Gal-1 immunoreactivity in the nucleus (N) and the cytoplasm. (d) Activated eosinophils from nasal polyps demonstrating lesser immunoreactivity to ANXA1 and Gal-1. (e) Epithelium cell presenting ANXA1 and Gal-1 labelling in the nucleus and the cytoplasm. (f) Absence of gold labelling in sections incubated with nonimmune sheep and rabbit serum. Bars (a) 0.25 μ m; (b–f) 0.5 μ m.

eosinophil infiltration, in line with previous reports (e.g. see [22]). Migrated eosinophils might contribute to NP aetiology by producing IL-5 and RANTES, cytokines responsible for the tissue-driven inflammatory response [4, 23]. In addition, mast cell-derived pro-inflammatory

mediators (e.g. histamine, IL-5 and eotaxin) could contribute to blood-borne cell extravasation in the NP [2].

As NP patients are often treated with bethametasone before polypectomy [24], the effect of a single GC treatment was monitored. This protocol of treatment was

Table 2. Density of ANXA1 and Gal-1 immunogold particles in inflammatory cells from nasal mucosa and nasal polyp before and after GC treatment

		Immunoreactivity in inflammatory cells (gold particles/ μm^2)		
		Control tissue	Nasal polyp	
Cellular types	Proteins	Nasal mucosa	NP	NP post-treatment
Mast cells	ANXA1	4.35 $\pm$ 0.15	0.78 $\pm$ 0.03**	1.45 $\pm$ 1.03*
	GAL-1	49.19 $\pm$ 1.07	2.19 $\pm$ 0.23**	14.16 $\pm$ 2.29**
		Blood	NP	NP post-treatment
Eosinophils	ANXA1	4.56 $\pm$ 1.70	1.01 $\pm$ 0.12*	1.23 $\pm$ 0.35*
	GAL-1	5.35 $\pm$ 1.48	4.78 $\pm$ 0.47	4.16 $\pm$ 0.75

Data are mean $\pm$ SEM of *n* distinct inflammatory cells analysed in the control tissue (mast cell from nasal mucosa or eosinophils from human blood), nasal polyps before (NP) and after GC treatment (NP post-treatment) (*n* = 10/group).

**P* < 0.01 vs. control tissue.

***P* < 0.001 vs. control tissue.

NP, nasal polyposis; GC, glucocorticoid.

Table 3. Density of ANXA1 and Gal-1 immunogold particles in epithelial cells from nasal mucosa and nasal polyp before and after GC treatment

		Immunoreactivity in epithelial cells (gold particles/ μm^2)		
		Control tissue	Nasal polyp	
Epithelium	Proteins	Nasal mucosa	NP	NP post-treatment
Glandular duct	ANXA1	1.36 $\pm$ 0.07	0.44 $\pm$ 0.04**	0.77 $\pm$ 0.08**†
	GAL-1	4.43 $\pm$ 0.29	2.19 $\pm$ 0.23**	3.88 $\pm$ 0.56†
Respiratory	ANXA1	1.71 $\pm$ 0.27	2.14 $\pm$ 0.19	1.30 $\pm$ 0.05††
	GAL-1	1.50 $\pm$ 0.23	5.06 $\pm$ 0.34**	2.78 $\pm$ 0.13††

Data are mean $\pm$ SEM of *n* distinct epithelial cells analysed in the control tissue (nasal mucosa), nasal polyps before (NP) and after GC treatment (NP post-treatment) (*n* = 10/group).

***P* < 0.001 vs. control tissue.

†*P* < 0.05 vs. NP.

††*P* < 0.001 vs. NP.

NP, nasal polyposis; GC, glucocorticoid.

active in reducing markers of inflammation, with lower eosinophil and increased mast cell numbers. Supported by these results, we set out to monitor the expression of two anti-inflammatory mediators, reported in a scant number of studies to be present in NP [6, 7], ANXA1 and Gal-1.

In the present work, two methodologies were applied to determine the quality of the *specimens*. The integrity of

RNA was verified through the observation of two ribosome subunits 18 and 28S and the quality of cDNA through the amplification of housekeeping gene *GAPDH*. We included only samples (10 autopsy samples, nine NP samples and seven NP after GC treatment samples) that had presented qualified RNA and cDNA. The increasing mRNA levels strongly suggest that there is a correlation between levels of expression of ANXA1 and NP. Moreover, independent of autopsy results, our results show an increase in gene expression level of ANXA1 in NP after GC treatment.

ANXA1 was found to be constitutively expressed in normal nasal mucosa. However, NP chronic inflammation augmented ANXA1 mRNA and protein expression. This finding is in line with analysis of other human diseases including pancreatic [25] and hepatocellular cancer [26], Western blotting analyses confirm these data, showing a good extent (~50%) of ANXA1 cleavage. Interestingly, the 33 kDa isoform predominates in the BAL fluid of patients having cystic fibrosis [27].

In contrast to ANXA1, Gal-1 mRNA and protein were detectable in the nasal mucosa, but they were markedly down-regulated in NP. A recent investigation of the expression of this lectin in chronic inflammatory juvenile idiopathic arthritis also revealed reduced expression, a phenomenon correlated with longer disease duration [28].

The pattern of expression of these two anti-inflammatory agonists is quite different when comparing normal mucosa vs. NP. This partial, at least, degree of distinction was clearly shown by GC treatment, as it caused up-regulation of both Gal-1 and ANXA1 mRNA in the NP post-treatment, but only Gal-1 presented a visible increase in the level of protein expression. This pattern of Gal-1 response confirms the data produced *in vitro* with budesonide incubation [7]. Although studies have highlighted a mechanistic role for endogenous ANXA1 in the efficacy of topically applied GC (e.g. such as betamethasone-17-valerate [29, 30], it is also true that strict time- and dose-dependency responses were reported [31]. It is therefore possible that the dose and time frame for the GC treatment used in the present study were not optimal to produce alterations in ANXA1 expression in nasal polyp.

In the second part of the study, we focused on the cellular sources expressing ANXA1 and Gal-1. We could demonstrate that both mast cells and eosinophils were positive for ANXA1 and Gal-1 with a relatively widespread distribution in the nucleus, cytoplasm as well as cytoplasmic granules. This pattern of distribution is usual for ANXA1, as shown in mast cells and eosinophils of rat inflamed mesentery [15, 32] and airway mast cells [33]. However, while ANXA1 and Gal-1 expression in mast cells of the nasal mucosa could be detected, lower levels of these proteins were quantified in NP degranulated mast cells. It is likely that this reduced expression could be associated with the intense degranulation of mast cells

seen in NP tissue. In this noteworthy that Gal-1 was not only detected in mast cells, but its levels were significantly higher than those of ANXA1. Whereas this ratio was globally attenuated in NP, it was of interest that GC treatment restored it: it is plausible that this predominant expression of Gal-1 aims at counteracting the extent of the inflammatory response in NP. Relevantly, *in vitro* addition to eosinophil inhibits their chemotaxis [7], and this white blood cell type is predominant in NP inflamed tissues.

Eosinophils showed a different pattern of staining for ANXA1, with the strongest signal in blood cells. In contrast, both circulating and polypoid tissue eosinophils were positive for Gal-1, with no apparent differences in the extent of immunoreactivity. Reduction of ANXA1 expression in NP eosinophils could be consequent to the process of extravasation, perhaps in line with studies performed earlier with neutrophils [16].

ANXA1 expression in epithelial cells has been previously reported, and also analysed by a immunohistochemical approach [6] without, though, studying modulation by inflammation. Here, we found a dual opposite behaviour, with the metaplastic respiratory epithelial cell augmenting selectively Gal-1 levels in NP, whereas the glandular duct epithelium decreased both ANXA1 and Gal-1. The reason for this is unclear; however, this dicotomy was also observed after GC treatment, with the former, but not the latter, epithelium, responding with increased protein expression. Studies on lung-derived cell lines (epithelial carcinoma and ANXA1-null fibroblast) have provided evidence that ANXA1 mediates the inhibitory effects instead of GCs on epidermal growth factor-stimulated cell growth [34]. In addition, a recombinant form of mouse galectin-1 was shown to have growth-inhibitory activity at very low concentrations [35]. The response to GC demonstrated here could be part of a reparative mechanism to counteract the characteristic epithelial overgrowth of NP.

In conclusion, we have used a molecular and immunocytochemical approach to monitor the pattern of expression of two anti-inflammatory proteins, and investigate potential alterations during this chronic inflammatory condition. The existence of complex patterns of expression was unveiled with the following major novel observations: (i) the occurrence of Gal-1 expression in nasal mucosa mast cells, and (ii) the identification of the epithelium as the preferential target for GC-induced augmented expression of both ANXA1 and Gal-1. It is tempting to conclude that, first, up-regulation of ANXA1 and down-regulation of Gal-1 could be functionally associated with a specific mechanism in chronic inflammation as NP and, second, that GC might exert at least part of their beneficial effects on the airway mucosa by up-regulating, in a specific cell target fashion, these two anti-inflammatory agonists.

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