Expression of Annexin A1 in Normal and Chronically Inflamed Nasal Mucosa

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Objective: To examine the expression pattern of annexin A1 in normal and chronically inflamed nasal mucosa to investigate its possible role in nasal inflammation.

Design: Immunohistochemical analysis.

Subjects: Samples of middle turbinates from 5 healthy subjects and 5 patients with perennial rhinitis, and samples of nasal polyps from 7 patients.

Interventions: Annexin A1 expression was examined with a standard immunohistochemical protocol on paraffin-embedded sections.

Results: Annexin A1 was highly expressed by ciliated cells, where it was concentrated on the apical surface and within the cilia. Goblet cells and nondifferentiated basal epithelial cells did not stain. In the glands of the lamina propria, intense staining was found in the cytoplasm and in the nuclei of the cells in the duct epithelium, whereas acinar cells did not stain. Intense cytoplasmic staining was observed in infiltrating polymorphonuclear cells and macrophages. No differences in the pattern or the level of expression of annexin A1 were found in the epithelial cells and glands of normal and chronically inflamed ( perennial rhinitis or polyps) nasal mucosa.

Conclusion: These results suggest that the expression of annexin A1 in respiratory epithelium of nasal mucosa is related to cell type and differentiation status of the cells and is not significantly altered by inflammatory diseases.


Annexins constitute a protein family with the general property of calcium and phospholipid binding and comprise 12 members in mammals, each possessing a unique tetrad core region with 4 homologous repeats (8 in annexin A6). The internal repeats of 68-69 amino acids normally contain type 2 calcium-binding motifs, and diversity of function within the family is conferred by core sequence divergence and unique N-terminal domains.1

Annexin A1 (also known as lipocortin 1 or calpactin II) has a molecular mass of 37 kDa and is abundant in mammalian tissues but has a discrete pattern of cellular distribution that includes certain epithelial cells (respiratory and urinary system, superficial cells of nonkeratinized squamous epithelium), skin, synovium, tissue macrophages, and blood leukocytes.2 Annexin A1 has been implicated in various cellular processes such as intracellular signal transduction, membrane cytoskeletal linkage, proliferation, and differentiation.3 Additional extracellular functions have been postulated for this protein, in particular an inhibitory function in inflammatory processes.4,5 The anti-inflammatory effect of annexin A1 has been attributed to both its ability to inhibit phospholipase A23,4 and its binding to specific surface receptors of granulocytes and macrophages, which results in inhibition of leukocyte diapedesis.5 Monocytes and neutrophils contain high intracellular levels of annexin A1 and express high levels of annexin A1 receptors. Convincing evidence now exists that synthesis of this member of the annexin family can be induced in these cells by glucocorticoids.6 However, the synthesis of annexin A1 does not appear to be under glucocorticoid control in certain other cell lines where its expression seems to be associated with cell differentiation and growth.7 Impaired annexin A1 responses have been shown to be associated with chronic inflammatory diseases. For example, rheumatoid leukocytes exhibit reduced annexin A1 production in response to corticosteroid7 and a reduction in the level of specific annexin A1 binding molecules on the surface of blood monocytes and polymorphonuclear cells.8 In addition, previous studies have demonstrated that the levels of annexin A1 in human nasal lavage fluids and bronchoalveolar lavage fluids are altered in inflammatory conditions.9,10
airway disorders. However, there are few studies of the expression of annexin A1 in normal respiratory mucosa, and none have been performed in inflamed nasal mucosa. This study was therefore designed to define, by immunohistochemistry, the profile of annexin A1 expression in normal nasal mucosa and nasal mucosa of patients with chronic inflammation.

**METHODS**

**TISSUE SAMPLES**

After informed consent of the subjects and in accordance with institutional review board guidelines, 5 healthy, nonrhinitic subjects with nasal septal deviation; 5 patients with perennial rhinitis who underwent turbinectomy; and 7 patients with nasal polyps were enrolled. None of the subjects had experienced symptoms of upper respiratory tract infection (nasal congestion or irritation, sore throat, cough, fever, or discolored or purulent nasal discharge) within the preceding month and none were receiving any medication, including topical or oral corticosteroids, for at least 4 weeks before the study. The subjects (5 women and 12 men) ranged in age from 19 to 74 years (mean age, 39 years). Two of the patients with perennial rhinitis and 1 of the patients with nasal polyps had allergy to house dust mite (confirmed by a positive skin prick test). The other patients had negative results on skin prick tests to 20 common airborne allergens. Two of the patients with nasal polyps also had aspirin intolerance and bronchial asthma.

Tissue samples of full-thickness mucosa were obtained from the head of the middle turbinate (until the bone was exposed) during septoplasty in the patients with septal deviation (who served as normal controls), and during turbinectomy in the patients with perennial rhinitis. Nasal polyp tissues were obtained in patients undergoing polypectomy. All specimens were fixed in buffered formaldehyde, dehydrated in graded alcohol solutions, and embedded in paraffin. The formalin-fixed paraffin-embedded tissues were cut into 4-µm sections and dried on capillary-gap glass slides (ChemMate; BioTEK Solutions, Santa Barbara, Calif). For standard histological analysis, hematoxylin-eosin staining was performed in each specimen.

**IMMUNOHISTOCHEMICAL STUDY**

The sections were deparaffinized with standard xylene and hydrated through graded alcohols into water. Antigen retrieval was performed using proteinase K. Staining was done at room temperature on an automatic staining workstation (TechMate 1000; BioTEK Solutions) by using the Envision peroxidase mouse system (Envision Plus; Dako, Carpinteria, Calif). Slides were placed for 15 minutes into a 3% hydrogen peroxide blocking medium and then allowed to react with the primary antibody, the anti–annexin A1 antibody (Transduction Laboratories, Lexington, Ky), at a dilution of 1:200 for 30 minutes. Immunodetection was performed with the Envision system. Counterstaining with hematoxylin for 1 minute was the final step. Following staining, the slides were dehydrated through graded alcohols and mounted with a coverslip using a standard medium. Negative controls with an omission of the antiserum from the primary incubation were also included. The slides were viewed randomly, without clinical data, by 2 of us (J.P.R. and A.H.).

**RESULTS**

A homogeneous pattern was observed on all samples in nasal mucosa from healthy middle turbinate. Annexin A1 was highly expressed by ciliated cells in the epithelium, where it was concentrated on the apical surface and within the cilia. Moderate staining was observed in the cytoplasm and in the nuclei of the cells in the ductal epithelium (Figure 2). Staining was also abundant in the cytoplasm of the granulocytes and macrophages present in the lamina propria. In contrast, lymphocytes exhibited a variable pattern of expression.
with some of them stained and others that did not stain. Since plasma cells did not stain, the lymphocytes that did not stain probably were B lymphocytes. Weak and occasional staining was observed in the endothelial cells of blood vessels.

The immunostaining of respiratory epithelium and glandular epithelium in the mucosal samples from the middle turbinate of the patients with perennial rhinitis and in the nasal polyps was comparable to control middle turbinates in all the cases, including the allergic ones (Figure 1B and C). Since all the ciliated cells in the epithelium were stained and we could not appreciate significant differences in the intensity of staining of these cells among the slides studied, we decided not to quantify the degree of staining. The most remarkable difference was the intense staining observed in the lamina propria of the inflamed mucosa due to the infiltration of many inflammatory cells (Figure 3). Most of the infiltrating polymorphonuclear cells (which all showed annexin A1 expression) were identified as eosinophils in the standard hematoxylin-eosin staining. In addition, the lamina propria showed infiltration of chronic inflammatory cells such as lymphocytes and plasma cells with the same pattern of expression of annexin A1 described in normal mucosa. Metaplastic changes into squamous epithelium were identified in 2 cases with perennial rhinitis. This epithelium showed an increasing expression from basal cells to superficial cells. In the superficial layer, annexin A1 was present both in the cytoplasm and the nuclei (Figure 4).

**COMMENT**

This study shows the expression pattern of annexin A1 in both normal and chronically inflamed nasal mucosa. The distribution of immunoreactivity for this protein was basically identical in the healthy nasal mucosa, and in the nasal mucosa of patients with perennial rhinitis and nasal polyps. To our knowledge, the pattern of annexin A1 expression in normal nasal mucosa has been studied only in one previous work, and no data are available regarding the expression of annexin A1 in the nasal mucosa altered by inflammatory disorders.

Annexin A1 has been reported to have anti-inflammatory activity and its highest level of expression has been found in cells that are involved in the immunological response to inflammation, specifically in myeloid cells. Experimental data indicate that annexin A1 is mainly active in models of inflammation where the involvement of neutrophils was crucial to the development of the response, although it is also expressed in eosinophils and mast cells. Our results also show a high immunoreactivity in the polymorphonuclear cells that infiltrate the lamina propria of the nasal mucosa in chronic rhinitis and nasal polyps. These cells were mainly eosinophils, in agree-
ment with previous work that addressed the significant contribution of tissue eosinophilia to the pathology of chronic rhinosinusitis. In addition, we observed infiltration of the lamina propria with other chronic inflammatory cells such as lymphocytes and plasma cells, which address the chronic nature of these diseases. We observed that some of the lymphocytes expressed annexin A1 whereas others did not. Since neither plasma cells expressed this protein, this suggests that the lymphocytes that expressed annexin A1 were only T lymphocytes, as previously described.

The existence of putative receptors for annexin A1 on human leukocytes and their depletion in chronic inflammation has led to the development of a hypothesis regarding the role of annexin A1 in glucocorticoid regulation of immune processes. In this fashion, annexin A1 has been proposed to act as a “barrier” to inappropriate inflammatory and autoimmune responses at specific sites around the body, under the tonal control of glucocorticoids via the pituitary-adrenal axis. It is probably not coincidental that many areas of the body that have high annexin A1 concentrations are known to be immunologically privileged sites, including the thymus and skin. However, the concept of annexin A1 as an immunosuppressive “cytokine,” under the control of hormones of the pituitary-adrenal axis, with specific receptor molecules on phagocytic cells is attractive but remains unproven. For instance, some studies have failed to confirm that glucocorticoids modulate the expression or the distribution pattern of annexin A1, and it may exert some of its effects independent of the pituitary-adrenal axis. Recent studies using transgenic mice that lacked annexin A1 gene expression confirm the anti-inflammatory role of this protein, but suggest that its role is limited to the acute phase (mainly neutrophil dependent) of the inflammation and that it has less effect in the chronic phase (mainly macrophage-lymphocyte and eosinophil dependent). Therefore, the role of annexin A1 in chronic inflammatory diseases, such as perennial rhinitis and nasal polyposis, remains to be clarified.

Our study did not show differences in the expression level or distribution pattern of annexin A1 in the epithelial cells between the normal mucosa and the chronically inflamed mucosa. This may be explained because, despite its intracellular location, annexin A1 is also found as an extracellular protein, which might account for its anti-inflammatory activity. Therefore, the intracellular protein might remain unaltered in inflammatory processes. Since the antibody used in this study could not distinguish between intact and protease-cleaved annexin A1, we were unable to appreciate differences between normal and inflamed mucosa due to this mechanism of cell surface inactivation. Previous studies that sampled the epithelial lining fluid from the upper and the lower respiratory tract, via nasal lavage fluids and bronchoalveolar lavage fluids, respectively, have demonstrated that annexin A1 is degraded in inflammatory diseases, such as asthma, rhinitis, cystic fibrosis, and chronic smoking. These studies revealed that nasal lavage fluids and bronchoalveolar lavage fluids from patients with inflammatory disorders had predominantly a 34-kDa truncated form of annexin A1 that lacks the 3-kDa N-terminal peptide. This N-terminal peptide may contribute to the anti-inflammatory action of annexin A1. Neutrophil elastase has been implicated in the proteolytic cleavage of annexin A1 to the 34-kDa protein, and this may be a mechanism by which neutrophils infiltrate sites of inflammation. Thus, inactivation of secreted annexin A1 may lead to chronic and uncontrolled inflammation. However, the results of these studies using nasal lavage and bronchoalveolar lavage fluids may not fully explain the role of annexin A1 at the tissue level. Moreover the importance of annexin A1 may be more related to its inhibitory action of neutrophil extravasation in a tissue-bound form, rather than a secreted form within the airway lumen, since it has been observed that infiltrated neutrophils are responsible for the majority of the annexin A1 expression associated with inflammatory conditions.

In contrast to the anti-inflammatory activity of annexin A1 in myeloid cells, its expression appears to be associated with cell differentiation in certain cell lines. In agreement with previous work, we have shown that in the epithelium of the upper respiratory tract only ciliated cells exhibited annexin A1 expression, whereas goblet cells and undifferentiated basal cells did not stain. Non-keratinized stratified squamous epithelium throughout the body showed an increase in annexin A1 expression from the basal cells to the superficial layers, as we observed in the areas of squamous metaplasia. Thus, in the epithelia, annexin A1 expression seems to be associated with the differentiation status of the tissue. This differentiation-dependent increase in expression has been described previously for several annexins.

In ciliated cells of respiratory epithelium, ductal cells, and superficial layers of nonkeratinized squamous epithelium we observed a distinct nuclear annexin A1 immunostaining. Although the nuclear localization of annexins has been previously reported, no nuclear localization or export signal is evident in the primary structure of this protein. The nuclear staining appears not to be a general phenomenon but seems to be restricted to a limited number of cells. The association of annexins with cellular or intracellular membranes, on the other hand, has been shown in a variety of systems, is consistent with the biochemical properties of these proteins, and can explain this nuclear immunostain.
In conclusion, this study shows for the first time the pattern of expression of annexin A1 in normal and in chronically inflamed nasal mucosa. The expression pattern in the epithelium was in accordance with the functional properties postulated for this protein, especially its role in cell differentiation. According to this role, annexin A1 was found to be expressed with similar intensity and with the same pattern in the epithelium and glands in both normal and inflamed nasal mucosa. This suggests that the expression of annexin A1 in respiratory epithelium of nasal mucosa is related to cell type and differentiation status of the cells and is not significantly altered by chronic inflammatory diseases. This study also demonstrates the presence of annexin A1 in leukocytes that play a central role in the host inflammatory response, supporting the notion that annexin A1 is able to modulate the reactivity of these cell types. However, the pathophysiological role of annexin A1 in chronic nasal inflammation remains to be fully elucidated.

Submitted for publication January 21, 2003; final revision received May 7, 2003; accepted June 25, 2003.

This study was supported by Fondo de Investigaciones Sanitarias, Madrid, Spain (grant 00/0171), Obra Social CajAstur, Dirección General de Enseñanza Superior of Spain (DGES PB98-1529), and Dirección General de Investigación (DGI/MCyT BMC2002-00827).

We are indebted to Aurora Fernández García for the immunohistochemical staining. We also thank Reginald O. Morgan, PhD, for critically reading the manuscript.

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