

ORIGINAL PAPER

Matrix metalloproteases in vernal keratoconjunctivitis, nasal polyps and allergic asthma

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Clinical and Experimental Allergy

Summary

Background Allergic conditions in different organs share many similarities in their inflammatory response. Vernal keratoconjunctivitis (VKC), asthma and nasal polyps exhibit several similar, but site-specific mucosal structural changes. The aim of the study was to investigate whether matrix metalloproteases contribute to different tissue remodelling aspects in different organs.

Methods Mucosal biopsies were obtained from conjunctiva of healthy donors, tarsal conjunctiva of vernal patients, bronchi of non-asthmatic subjects, bronchi of mild stable asthmatic patients, nasal mucosa of non-allergic donors and nasal polyps of allergic patients. Distribution of metalloprotease-1, -3, -9, -13, tissue inhibitor of metalloproteases-1, collagens I and III and the presence of eosinophils and CD4⁺ cells were evaluated by immunohistochemistry.

Results Collagens were highly diffuse in the giant papillae of VKC and in nasal polyps, and yet less increased in the subepithelium of asthmatic patients. Immunostaining for metalloprotease-1, -3, -9 and -13 was significantly higher in VKC compared with normal conjunctiva. Metalloprotease-9 staining was higher in the stroma of polyps vs. normal nasal mucosa, and only metalloprotease-13 was significantly more expressed in asthmatic vs. non-asthmatic subjects. Metalloprotease-9 immunostaining was more intense in vernal compared with other tissues. In all pathological tissues, metalloprotease-9-positive staining was in association with eosinophils and CD4⁺ cells.

Conclusions Expression of metalloproteases may play an important role in inducing the structural changes seen in VKC, nasal polyps and asthma. Tissue remodelling and gelatinase immunoexpression was more dramatic in giant papillae of vernal patients compared with other tissue sites of chronic allergic inflammation.

Keywords collagen, MMP, nasal polyps, tissue remodelling, vernal keratoconjunctivitis

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Introduction

Allergic conditions such as conjunctivitis asthma, and rhinitis share many processes of their inflammatory response, exhibiting similar immunopathological findings, and yet with site-specific signs, symptoms and mucosal structural changes. In fact, chronic severe forms of allergic conjunctivitis, in particular vernal keratoconjunctivitis (VKC), are characterized by persistent conjunctival inflammation involving intense eosinophil infiltration, increased numbers of mast cells, basophils, neutrophils, macrophages and type T-helper type 2 (Th2) lymphocytes

[1–3]. VKC is also characterized by architectural remodeling of the conjunctival tissues due to an excess of extracellular matrix (ECM) deposition, subepithelial fibrosis, chronic cellular infiltrate and epithelial thickening [4].

Asthma is described as a chronic inflammatory disease that is characterized by eosinophilic inflammation, Th2 cell activation, airway hyperresponsiveness and remodelling [5]. Structural changes in asthma include subepithelial deposition of ECM proteins, increased muscle mass, goblet cell hyperplasia, angiogenesis, fragmentation of elastic fibres and changes in airway epithelium [6–7].

Similarly, nasal polyposis is a chronic inflammatory disease of the upper airway featuring eosinophil and mast cell infiltration and tissue remodelling that includes ECM accumulation and fibrosis [8].

Owing to their effect on cell migration and behaviour, as well as their role in the restructuring of ECM, recent attention has focused on the contribution of matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of MMPs (TIMPs) to the pathogenesis of allergic diseases. The MMP family contains at least 26 known members that degrade all components of the ECM, and that are grouped according to their substrate specificity [9]: collagenases, gelatinases, stromelysins and the membrane-type MMPs. These enzymes are implicated in many critical physiological and pathological processes including development, wound healing, angiogenesis, cancer and inflammation [10].

MMPs are elevated in the airways of asthmatic patients, particularly during asthma exacerbations [11]. Increased levels of MMP-9 and TIMP-1 have also been found in bronchoalveolar lavage (BAL) fluids and sputum from asthmatic patients [12, 13], when patients have spontaneous exacerbations or in response to allergen challenge, but approach normal levels when the acute inflammation resolves [14]. Similarly, the presence of MMP-9 has been identified in nasal polyp tissues in association with eosinophil and mast cell activation [15]. MMP-9 has been reported to play a crucial role in the migration of inflammatory cells through basement membranes [16], and thus may be involved in eosinophil migration during allergic inflammation [17].

Increased tear levels and activity of MMP-1 and MMP-9 were found to be correlated to clinical findings in VKC patients, suggesting that these proteases are involved in allergic conjunctival inflammation and that an imbalance between MMPs and their inhibitors may facilitate inflammatory cell transmigration, tissue remodelling and corneal damage [18, 19].

Differential expression of MMPs might contribute to multiple aspects of inflammation and particularly might be involved in the pathogenesis of tissue remodelling. The aim of the present study was to evaluate the tissue expression and distribution of MMP-1, MMP-3, MMP-9, MMP-13, and TIMP-1 in three allergic conditions characterized by profound, but unique mucosal remodelling.

Materials and methods

Subjects and tissue specimens

Nine VKC patients (seven males and two females; mean age 11.2 ± 4.9), six patients with allergic rhinitis (AR) with nasal polyps (four males and two females; mean age 43.3 ± 6) and nine mild stable asthmatic patients [five males and four females; mean age 26 ± 5 ; mean forced

expiratory volume in 1 s (FEV_1) 91 ± 20.9] were included in the study. VKC patients were in a moderately active disease phase and were free of topical mast cell stabilizers and/or antihistamines for at least 3 days and free of topical corticosteroids for at least 2 weeks before biopsies, which were performed under local anaesthesia. Diagnosis of VKC was based on the typical clinical history and evaluation of signs and symptoms [20]. All VKC patients presented giant papillae on the upper tarsal conjunctiva. Nasal polyps were identified by anterior rhinoscopy in all patients based on clinical history and evaluation of signs and symptoms. None of the study subjects had used topical steroids for at least 2 weeks before obtaining biopsies. Asthma was diagnosed according to the American Thoracic Society guidelines for the diagnosis of asthma [21]. Six out of nine asthmatics had intermittent to mild persistent asthma, while the remaining three patients had moderate disease. All patients were positive to methacholine challenge (intermittent-mild persistent asthmatics) or had a significant bronchodilator response (moderate persistent asthmatics). Bronchial biopsies were obtained under local anesthesia during bronchoscopy as described previously [22]. None of the study subjects had used inhaled steroids for at least 2 weeks before obtaining biopsies.

The conjunctival control group included six subjects (five males, one female, mean age 13 ± 5) who underwent surgery for strabismus. None of these subjects used contact lenses or had any inflammatory signs and symptoms or a history of allergy. The six subjects included in the nasal control group (four males, two females; mean age 42 ± 6) had no clinical history for allergy, asthma, aspirin or non-steroidal anti-inflammatory intolerance and underwent surgery for rhinoplastic surgery. The five healthy volunteers (all male, mean age 27.6 ± 2.7 ; mean FEV_1 , 114 ± 15) included as the bronchial control group had normal FEV_1 values and no asthmatic or allergic symptoms. All tissue specimens were snap frozen with OCT in liquid nitrogen and maintained at -70°C for immunohistochemistry.

The research was approved by the Institutional Review Board. Written informed consent was obtained from all subjects or their parents before obtaining tissue specimens.

Immunohistochemistry

Serial 5- μm -thick cryosections were cut, mounted on gelatin-covered slides, fixed in acetone and processed for immunohistochemistry. The following anti-human antibodies were used: mouse anti-MMP-1, anti-MMP-3, anti-MMP-9 and anti-MMP-13, which recognize both latent and activated forms, and rabbit anti-TIMP-1 (all purchased from Chemicon, Temecula, CA, USA). Mouse anti-EG2 (Pharmacia, Uppsala, Sweden) and mouse anti-

CD4 (Dako, Glostrup, Denmark) were used for identification of eosinophils and lymphocytes, respectively. Anti-collagen types I (Sigma, St. Louis, MO, USA) and III (Chemicon) were used for the identification of collagen distribution in tissues.

Briefly, samples were incubated first with avidin and biotin blocking solutions (Vector kit) and serum from the same animal species as the secondary antibody (Vector laboratories, Burlingame, CA, USA), and then for 60 min with monoclonal or polyclonal antibodies at the appropriate dilution in Tris-buffered saline (TBS: 10 mM Tris, 150 mM NaCl, pH 7.4). Slides were then washed in TBS, incubated with biotinylated secondary antibodies (Vector, 30 min), treated with an alkaline phosphatase or peroxidase ABC kit (Vectastain, Vector laboratories, Burlingame, CA, USA, 30 min) and, subsequently, fast red (Sigma, 20 min).

Double immunostaining was performed with goat anti-MMP-9 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and, alternatively, mouse anti EG2 (Pharmacia) or mouse anti-CD4 (Dako) antigens, as primary antibodies (60 min), followed by incubation with biotinylated anti-goat and biotinylated anti-mouse secondary antibodies (Vector, 60 min), a peroxidase or alkaline phosphatase ABC kit (Vectastain, 60 min) and, finally, revealed by incubation in 3,3' diaminobenzidine tetrahydrochloride (Sigma, 5 min) and fast red substrates (Sigma, 20 min).

Light microscopic analysis was performed at a magnification of $\times 630$ for quantification of inflammatory cells (EG2^+ CD4^+) and MMPs. Three scientists (A. L., P. B., A. D. S.) performed the morphometric analyses in a masked fashion. The positive red reaction was evaluated qualitatively in the epithelium and sub-epithelial stroma of the

three types of pathologic tissues and their respective normal control tissues. The reaction was classified as very intense (3+), intense (2+), slight (1+) or absent (0). In all samples, EG2^+ , CD4^+ and MMP-9^+ cells were evaluated quantitatively in several non-overlapping high-power fields until the entire available area was covered. The final result, expressed as the number of positive cells per square millimetre, was calculated as the mean of all cell counts performed in each tissue specimen.

Statistics

Differences in immunostaining of MMPs and TIMP in pathologic tissues and their respective normal samples were analysed using the Mann-Whitney *U*-test. Results are given as the mean $\pm$ standard deviation of the mean (SD). Spearman's rank correlation was also calculated to identify relationships among parameters. For statistical significance, the assigned *P* value was ≤ 0.05 .

Results

Conjunctiva

Conjunctival tissues from VKC patients were characterized by numerous epithelial ingrowths, intense inflammatory cell infiltration and the presence of thick collagen fibres beneath the epithelium and in the stroma. Immunostaining for collagen types I and III was intense in all VKC samples (Fig. 1) but low or absent in the normal conjunctiva (Table 1). In VKC conjunctiva, MMP-1 and MMP-3 immunostaining was localized in the fibrous,

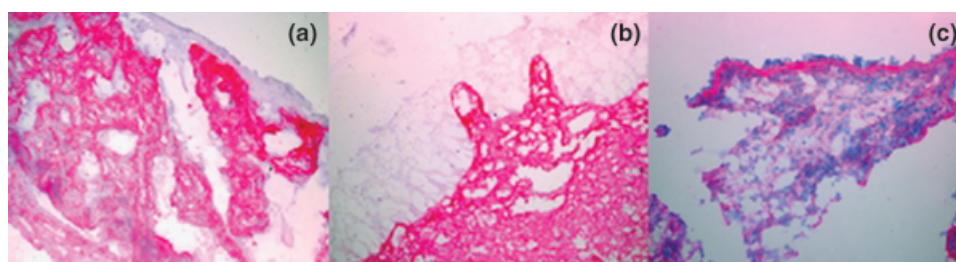


Fig. 1. Collagen I in vernal keratoconjunctivitis (VKC) (a), nasal polyps (b) and bronchial asthmatic tissues (c). Intense and diffuse immunostaining in the mucosal stroma of VKC and nasal polyps, and intense staining in the subepithelial area of bronchial asthma (original magnification $\times 200$).

Table 1. Immunohistochemical scores for collagen types I and III in the conjunctival, nasal and bronchial mucosa

	Conjunctiva		Nose		Bronchi	
	I	III	I	III	I	III
Allergic, inflamed tissues	3 $\pm$ 0	1.5 $\pm$ 0.1	2.8 $\pm$ 0.2	0.3 $\pm$ 0.2	3 $\pm$ 0	0.5 $\pm$ 0
Control tissues	1.2 $\pm$ 0.2	0.6 $\pm$ 0.3	1.7 $\pm$ 0.2	1.2 $\pm$ 0.3	2.5 $\pm$ 0.3	0
<i>P</i> -value	<0.01	<0.05	<0.05	<0.05	NS	<0.01

The immunohistochemical reaction was classified as very intense (3+), intense (2+), slight (1+) or absent (0).

sub-epithelial regions; MMP-3 staining was also noted along stromal collagen bundles. Neither MMP-1 nor MMP-3 was observed within the epithelium. MMP-9 immunostaining was intense in both the epithelium and ECM of the stroma as well as in numerous cells within the stroma (Fig. 2). MMP-13 was highly expressed in VKC in the sub-epithelial layer, around small blood vessels and in some stromal cells (Fig. 2). In the normal conjunctiva, immunoreactivity for MMPs was low or absent. TIMP-1 staining was localized equally in VKC and control tissues. The immunostaining score was significantly higher in VKC compared with control conjunctival tissues (Table 2) for MMP-9 and MMP-13 in the epithelium, and for MMP-1, MMP-3, MMP-9, and MMP-13 in the stroma (Table 2).

The mean number of EG2⁺ cells was significantly higher in VKC specimens ($739.6 \pm 72/\text{mm}^2$) compared with no eosinophils in control tissues ($P < 0.01$). The number of CD4⁺ cells was significantly higher in VKC ($824 \pm 57/\text{mm}^2$) compared with normal conjunctiva ($21 \pm 8/\text{mm}^2$; $P < 0.01$) (Fig. 3). EG2⁺/MMP-9 and CD4⁺/MMP-9 double staining showed that $48.3 \pm 11\%$ of eosinophils (EG2⁺) and $37.0 \pm 2\%$ of CD4⁺ cells were also positive for MMP-9.

Nasal mucosa

In nasal polyp tissues, collagen type I was significantly increased compared with normal control nasal turbinate

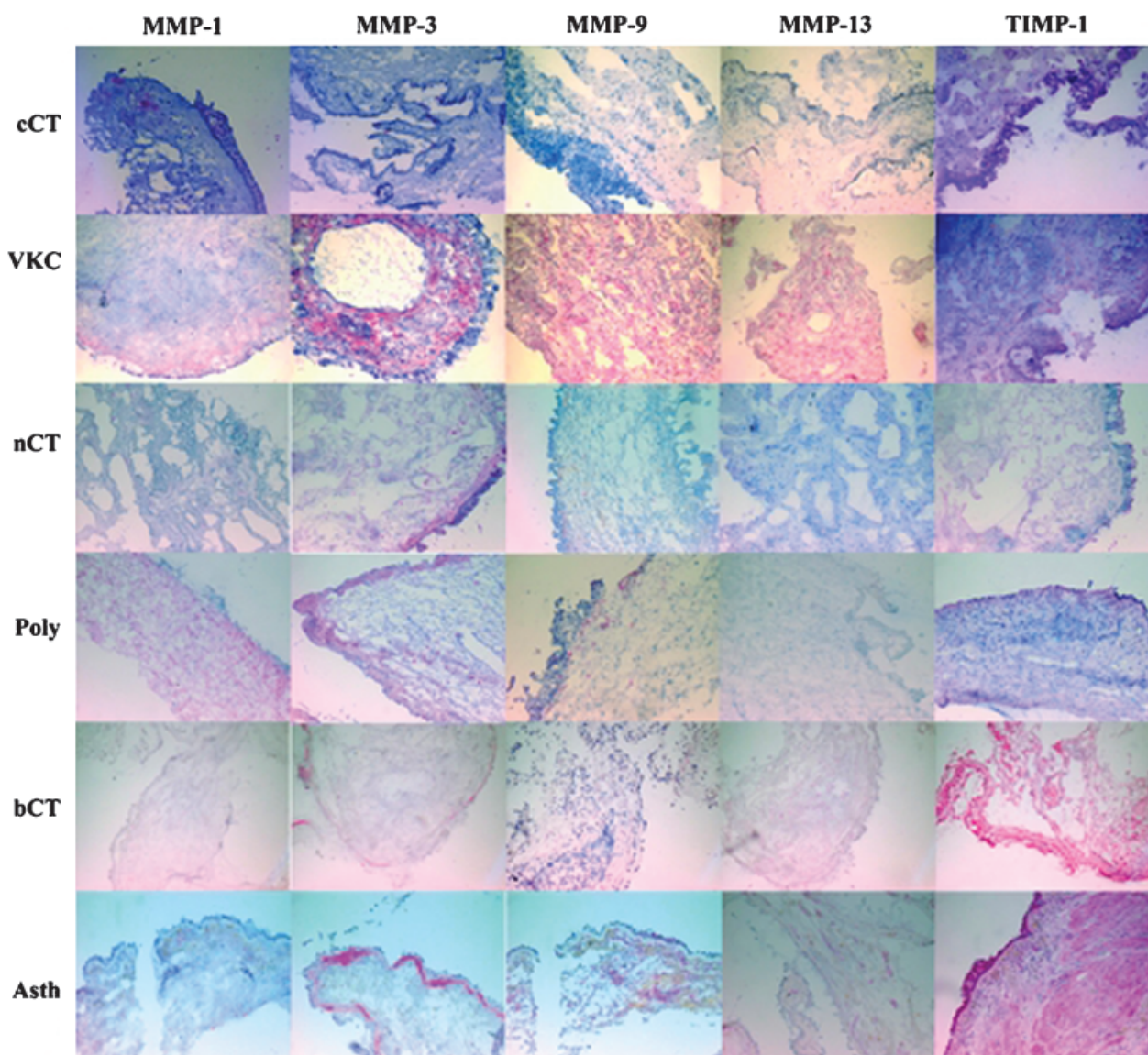


Fig. 2. Immunohistochemistry of metalloproteinases (MMP)-1, MMP-3, MMP-9, MMP-13 and tissue inhibitors of MMPs in normal conjunctiva (cCT), vernal keratoconjunctivitis (VKC), normal nasal mucosa (nCT) and nasal polyps (poly), normal bronchial mucosa (bCT) and bronchial asthmatic tissues (Asth). Positive immunostaining is shown in red (original magnification $\times 200$).

Table 2. Immunohistochemical scores for MMP staining in conjunctival, nasal and bronchial mucosal tissues

	MMP-1		MMP-3		MMP-9		MMP-13		TIMP-1	
	Epi	Stroma	Epi	Stroma	Epi	Stroma	Epi	Stroma	Epi	Stroma
VKC	0.2 ± .4	1.0 ± .4 <i>P</i> < 0.01	0.1 ± 0.1	1.9 ± 0.6 <i>P</i> < 0.05	1.2 ± 0.3	2.0 ± 0.4 <i>P</i> < 0.01	0.9 ± 0.4	1.8 ± 0.4 <i>P</i> < 0.05	2.0 ± 0.7	2.0 ± 0.2
cCT	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	1.2 ± 0.4	0.3 ± 0.2	0.5 ± 0.5	0.3 ± 0.4	0.5 ± 0.6	1.9 ± 0.3	1.6 ± 0.7
Polyps	0	1.5 ± 0.5 <i>P</i> < 0.05	0.4 ± 0.2	1.9 ± 0.4	0.7 ± 0.2	1.2 ± 0.2 <i>P</i> < 0.01	0	0	1.3 ± 0.6	1.2 ± 0.5
nCT	0	0.5 ± 0.4	1.0 ± 0.9	1.4 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0	0.2 ± 0.1	0.6 ± 0.6	1.1 ± 0.4
Asthma	1.2 ± 0.8	0.6 ± 0.3	0.02 ± 0.1	1.1 ± 0.7	0.2 ± 0.4	1.3 ± 0.9	0.3 ± 0.2 <i>P</i> < 0.05	0.9 ± 0.5	1.8 ± 0.9	2.1 ± 0.7
bCT	0.4 ± 0.9	0.5 ± 0.7	0	0.5 ± 0.2	0.04 ± 0.08	0.9 ± 0.2	0	0.6 ± 0.5	2.5 ± 0.5	2.5 ± 0.7

The immunohistochemical reaction was classified as very intense (3+), intense (2+), slight (1+) or absent (0).

Epi, epithelium; Stroma, subepithelial stroma; cCT, normal, control conjunctival tissues; nCT, normal, control nasal tissues; bCT, normal, control bronchial tissues; MMP, matrix metalloproteinases; VKC, vernal keratoconjunctivitis; TIMP, tissue inhibitors of MMPs.

tissues, whereas collagen type III was significantly less present compared with normal tissues (Table 1). MMP-1 immunostaining was scattered in the stroma of nasal polyps (Fig. 2). MMP-3 staining was moderate in the subepithelial layer of both polyps and control tissues and in some stromal cells, as well as the basal layer of mucus producing glands within the stroma. In polyps, MMP-9 staining was localized to the subepithelial area within the ECM and in stromal inflammatory cells, but was also present to a lesser extent in controls. MMP-13 staining was very low or absent in both polyp and control groups. TIMP-1 was localized equally in polyp and control tissues. The immunohistochemical scores of MMP-1 and MMP-9 were significantly higher in the stroma of polyps compared with control tissues (Table 2).

The mean number of EG2⁺ cells ($211.0 \pm 118/\text{mm}^2$) and CD4⁺ cells ($266.6 \pm 45/\text{mm}^2$) was significantly higher in nasal polyps than in control tissue (EG2⁺ $28.7 \pm 8.3/\text{mm}^2$; *P* < 0.05; CD4⁺ $109.2 \pm 9/\text{mm}^2$; *P* < 0.05) (Fig. 3). Double staining showed that $37.5 \pm 4\%$ of EG2⁺ cells and $48.3 \pm 2\%$ of CD4⁺ cells were also positive for MMP-9.

Bronchi

In bronchial tissues of asthmatic patients, a thick subepithelial basement membrane accompanied by a mild inflammatory infiltrate was the predominant histological characteristic. Intense immunostaining for collagen type I was found in all asthmatic patients and controls; however, asthmatics presented more intense staining beneath the epithelium (Fig. 1). Collagen type III staining was slightly but statistically significantly increased in asthmatic patients compared with normal subjects (Table 1). While MMP-1 immunoreactivity was low in asthmatic and control tissues, MMP-3 immunoreactivity was high in the subepithelial basement membrane of asthmatic and, to a lesser extent, of control tissue (Fig. 1). In contrast, MMP-9 immunoreactivity was observed only in asthmatics, in

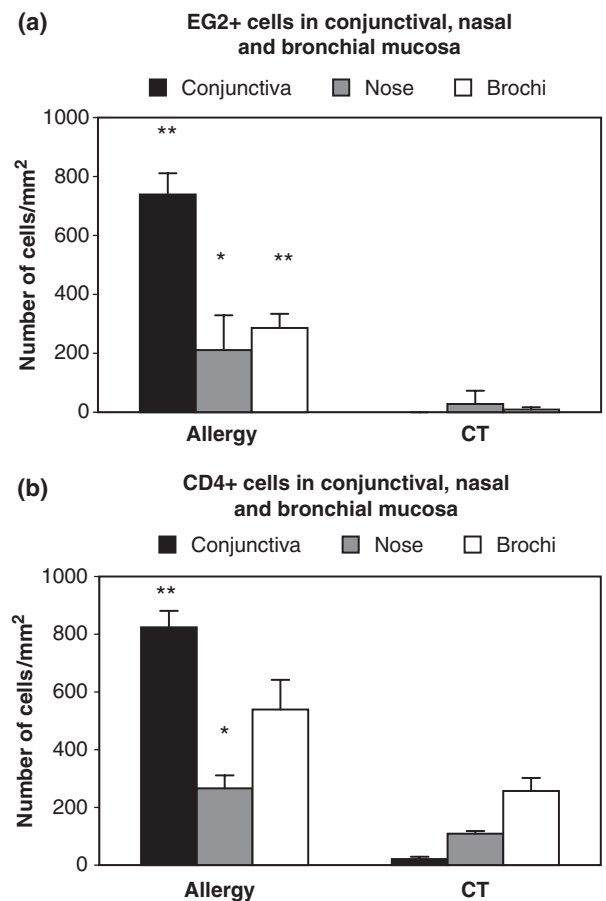


Fig. 3. Number of eosinophils (EG2⁺) (a) and CD4⁺ (b) cells in normal mucosal tissues (CT) and in allergic-inflamed tissues (allergy). Both EG2⁺ and CD4⁺ cell numbers are significantly increased in vernal keratoconjunctivitis and nasal polyps compared with the respective CT, whereas only EG2⁺ cells are increased in bronchial asthma compared with the respective control (***P* < 0.01; **P* < 0.05).

numerous, apparently inflammatory cells, located in the bronchial epithelium and in the stroma (Fig. 1). In normal tissues, MMP-9 was present only in the subepithelial layer.

MMP-13 was localized in the epithelium and subepithelial stroma of asthmatic subjects only. Only the staining score of MMP-13 in the epithelium was significantly higher in asthmatics compared with controls (Table 2).

While asthmatic tissue did contain significantly more eosinophils than controls ($286.0 \pm 48/\text{mm}^2$ vs. $8.8 \pm 8/\text{mm}^2$; $P < 0.01$), the increase in CD4^+ cell number was not statistically significant ($539.6 \pm 103/\text{mm}^2$ vs. $257.7 \pm 45/\text{mm}^2$; $P = 0.06$) (Fig. 3). Double staining showed that $33.3 \pm 4\%$ of eosinophils and $47.6 \pm 7\%$ of CD4^+ cells were also MMP-9 positive.

No significant correlations were found between the number of EG2^+ or CD4^+ cells and the immunohistochemical scores for MMPs in all pathological tissues. Similarly, no significant correlations were found between MMP immunostaining scores, eosinophil or lymphocyte numbers and collagen immunostaining scores.

No correlations were found between previous treatment and length of the disease or histological parameters (data not shown).

Discussion

Quantitative and qualitative changes in the ECM have been described in mucosal tissues of VKC, bronchial asthma and chronic rhinitis [4–8, 22], resulting in the formation of giant papillae, bronchial mucosa thickening and nasal polyps [23, 24]. These remodelling changes are probably the result of an altered homeostasis of the ECM degradation, followed by synthesis and deposition of new collagen.

The distribution of collagen was different in the three tissues due to obvious anatomical differences. More dramatic tissue remodelling and collagen I deposition were evident in VKC giant papillae and in nasal polyps, which are fibrovascular outgrowths and not only the mucosal thickening that occurs in asthma. Interestingly, collagen type III was significantly increased in pathological conjunctiva and asthma but decreased in polyps compared with their respective normal tissues, suggesting that remodelling processes may differ in tissues undergoing similar pathology. Collagen types I and III are those most involved in the fibrotic remodelling process; however, other collagens such as IV, V, and VII have been shown increased in these allergic inflammations [4–8, 22–24].

Compared with normal tissues, increased immunostaining of MMP-1, MMP-3, MMP-9, and MMP-13 was found in VKC and increased MMP-1 and MMP-9 in nasal polyps. Conversely, in asthmatic tissues, a significant increased staining of MMP-13 was observed only in the epithelium, along with a modest increase of MMP-3 and MMP-9 in the stroma. Thus, different patterns of MMP expression seem to characterize different tissues undergoing similar processes of chronic-allergic inflammation.

Although the antibodies used do not distinguish between pro-MMP and active MMP, and immunoreactivity does not indicate the state of enzyme activation, MMPs were highly expressed where tissue remodelling and cell infiltration were more extensive, especially in VKC tissues. Interestingly, these findings were associated with a higher presence of inflammatory cells (EG2^+ and CD4^+ cells) in the stroma of VKC patients compared with that of nasal polyps and asthmatic patients. This, at least in part, is in agreement with previous studies from asthmatic subjects showing increased MMPs in the BAL fluid only during exacerbations of the disease [14] and in severe asthma [25,26]. The increase in MMP-9 in severe disease may be related to its ability to induce inflammatory cell migration and activation [14–16] and to promote tissue remodelling, which lead to an accelerated decline in lung function [25]. Similarly, MMP were not up-regulated in the nasal mucosa of perennial allergic rhinitis subjects [27] but were over-expressed in nasal polyps [15, 24], in which MMP-2 was significantly correlated with levels of eosinophil cationic protein and tryptase, indicating that MMP-2 may be involved in the migration of eosinophils and mast cells into nasal polyp tissues [24]. Recently, an equal expression of MMP-2 and TIMP-1, and an increased expression of MMP-9 and IL-5 have been reported in nasal polyps compared with healthy nasal mucosa [28], confirming the association between eosinophils and MMP-9.

Several inflammatory and non-inflammatory cells were positive for MMPs both in the epithelium and the stroma of the different tissues. With the present approach, it was not possible to determine whether positively stained cells or the target cells were the source of MMPs. However, double staining showed that a high percentage of eosinophils and CD4^+ cells also stained positively for MMP-9, suggesting that these cells may use this protease during extravasation and migration across basement membranes into inflamed tissues. No positive correlations were found between the number of eosinophils or CD4^+ cells and the immunohistochemical score for each MMP considered. This lack of a positive correlation may be due to the fact that cell migration and anchorage is under the control of many chemotactic and 'adhesive' factors influencing cell residence in the stroma of inflamed tissues or simply to the relatively low number of patients studied. It has been previously shown that MMP-9 can be produced by many inflammatory cells, such as macrophages, neutrophils, mast cells, eosinophils [16–19], T lymphocytes [26, 29] and monocytes [30].

Although MMP-9 is the most studied protease in allergy [31], other members of the MMP family are probably involved in inflammatory and remodelling processes. Interestingly, MMP-13 was overexpressed both in VKC and asthma. This protease has overlapping activities with MMP-1, which was also localized in all the considered

tissues and significantly increased in VKC and polyps. MMP-3 was highly expressed only in VKC tissues; however, it was also evident within the thick basal membrane of asthma tissues. In addition, both MMP-9 and MMP-3 are effective proteoglycanases, [9] cleaving the core protein of proteoglycans [32]. The cleavage of bound latent growth factors, such as FGF or TGF- β , from proteoglycan storage sites would potentially contribute to fibroblast proliferation and the development of subepithelial fibrosis. In fact, several growth factors are involved in the development of tissue remodelling in all three diseases [4, 7,8]. In addition, both MMP-3 and MMP-9 have been reported to be involved in the bio-processing of pro-TNF- α *in vitro* [32]. As TNF- α is localized to human lung and conjunctival mast cells [4, 33], MMP-3 might act as an activator of TNF- α release, thereby enhancing the profibrotic course of events and influencing endothelial cell activation and the recruitment of infiltrating leucocytes. These findings highlight the regulatory role of MMPs in inflamed tissues of different origin.

Allergic diseases are also characterized by non-specific hyperreactivity. As VKC is exacerbated by sun exposure and MMP-1, MMP-3, and MMP-9 expressions are up-regulated in human dermal fibroblast after UVB exposure [34], we speculate that the higher expression of MMP in VKC compared with the other diseases may also be related to stimuli from specific environmental conditions.

There are conflicting reports on the effects of corticosteroid therapy on MMP expression in asthma [35–37]. In the present study, there was no correlation between a history of steroid use and histological findings. New treatments, such as immunostimulatory sequences of DNA, are being investigated as a novel strategy in treating allergic diseases including ocular allergy [38, 39]. This type of therapy has been shown in mice to inhibit airway remodelling, in conjunction with a decreased epithelial expression of MMP-9 [40]. Airway responsiveness to allergen has been shown to be positively modified by MMP inhibitors in animal models, confirming their critical role in allergic inflammation and remodelling [41]. The present observations on abnormal MMP immunorexpression in diverse allergic-inflamed tissues indicate that MMPs might be a valid target in new treatment strategies.

In conclusion, using an immunohistochemical approach, this study investigated the distribution of various MMPs in allergic VKC, polyps and asthma tissues, together with the co-localization of MMP-9 to cells relevant to all allergic diseases: eosinophils and CD4⁺ lymphocytes. The presence of these proteases was different among and within the pathologic and normal tissues, with greater expression in more inflamed regions and where morphological changes were more evident, such as in VKC and polyps. Information provided by dynamic assessments of

release, along with alternative approaches for the identification of active MMPs, would further our understanding of the relevance of these findings in relation to the development of clinical disease and in defining new therapeutic strategies.

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