

Histopathologic and Morphometric Evaluation of the Nasal and Pulmonary Airways of Cats with Experimentally Induced Asthma

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Key Words

Asthma • Allergic rhinitis • Unified airways • Intraepithelial mucosubstances • Nasal airways • Mast cells • Eosinophils

Abstract

Background: Allergic rhinitis frequently occurs as a comorbid condition in asthmatic people, suggesting that the upper and lower airways may be immunologically linked. Our research group has developed an experimental aeroallergen model of asthma in cats. We hypothesized that aeroallergen sensitization and challenge would induce morphologic changes in the nasal airways of cats that mimic those observed in the bronchial airways. **Methods:** Five mixed breed cats were sensitized to Bermuda grass allergen and then serially challenged with aerosolized Bermuda grass allergen to induce an asthmatic phenotype. Four control cats were similarly treated with saline vehicle. Nasal tissues and lungs were processed for histopathological and morphometric analyses. **Results:** Eosinophilic inflammation, epithelial hypertrophy and mucous cell metaplasia were observed along the pulmonary axial airway mucosa of allergen-sensitized (asthmatic) cats. Mild eosinophilic inflammation was observed in the nasal airways of asthmatic cats. This alteration was con-

fined primarily to the anterior nasal cavity, resulting in an increase in tissue eosinophils at this site compared to controls ($p < 0.05$). A marked increase in tissue mast cells was observed throughout all regions of the nasal airways of asthmatic cats compared to control cats ($p < 0.05$). There was no difference in intraepithelial mucosubstances between the nasal airways of controls and asthmatic cats. There was no correlation between upper and lower airway eosinophils or mast cells. **Conclusion:** Cats with experimentally induced asthma exhibit morphologic changes in the nasal airways that are distinct from the alterations observed in the lungs. These results are similar to those observed in people with comorbid asthma and allergic rhinitis.

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Introduction

Concomitant allergic rhinitis and asthma are often comorbid conditions [1, 2], leading to the suggestion that the upper airways are immunologically linked to the lower airways [2–6]. This ‘united airways’ theory postulates that rhinitis and asthma are points along a continuum of allergic airway disease [5, 7]. Extensive epidemiological

evidence supports an association between allergic rhinitis and asthma, and longitudinal studies have demonstrated a high prevalence of preexisting allergic rhinitis among patients with asthma [8, 9]. Moreover, at least 60% of asthmatics have rhinitis as a comorbid condition [2], including many patients who exhibit eosinophilic inflammation in the nasal airways in the absence of clinical signs of rhinitis [10].

The concept of an immunologic link between the upper and lower airways may have important prognostic and therapeutic implications. Diagnosis of allergic rhinitis has been well established as a strong predictor for the development of asthma in children and adults [11–13]. Therapeutic interventions that reduce nasal inflammation have been shown to ameliorate asthmatic symptoms and improve lung function in asthmatics following allergen exposure [14–18]. Similarly, oral administration of inhaled budesonide was shown to inhibit nasal eosinophilia after allergen provocation in rhinitic patients [19]. Treatment of allergic rhinitis has also been associated with a reduction in the incidence of hospital visits for exacerbations of comorbid asthma [20, 21]. Despite the growing body of evidence supporting the benefits of evaluating and managing upper airway inflammation in asthmatic patients, the mechanisms underlying this relationship remain poorly understood.

To further elucidate the relationship between rhinitis and asthma and to better understand the pathophysiology of generalized airway inflammation, detailed study of a relevant large animal model that demonstrates concurrent upper and lower airway pathology is warranted [22]. Features that define asthma in humans, including eosinophilic bronchial inflammation, spontaneous and reversible bronchoconstriction, smooth muscle hypertrophy, and mucus gland hyperplasia, are also hallmark features of naturally occurring asthma in cats [23–25]. The basis of these pathologic changes in both feline and human atopic asthma is believed to involve a type I hypersensitivity reaction to inhaled allergen that is characterized by a Th2 lymphocyte polarized response, promoting the morphologic changes in the pulmonary airways including tissue eosinophilia, goblet cell hyperplasia, epithelial cell and smooth muscle proliferation, subepithelial fibrosis, and mast cell accumulation [23, 26, 27]. Elucidation of a relationship between the upper and lower airway responses to allergenic stimulation in the cat will provide greater insight into the immunopathogenesis of global allergic airway disease, which is poorly characterized at present, and may direct further investigation into novel therapeutic interventions for comorbid asthma and rhinosinusitis.

Our research group has recently developed an experimental model of feline asthma using aerosolized Bermuda grass allergen (BGA) [28], an aeroallergen common to both human and feline asthmatics [29]. This model replicates the functional, clinical, immunologic, and histopathological features of spontaneously occurring asthma. With this study, we sought to determine the nasal manifestations of chronic airway allergen challenge in cats experimentally sensitized to a common aeroallergen. We hypothesized that aeroallergen sensitization and challenge evoke morphologic changes in the nasal airways, specifically airway eosinophilia and mucous cell hyperplasia, that mimic those observed in the bronchial airways of feline asthmatics.

Materials and Methods

Experimental Animals

Nine mixed breed cats aged 6 months to 2 years, obtained from a research cat colony at the University of California at Davis, were used for this study. All animals were cared for according to the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and used after approval by the University of California at Davis Animal Care and Use Committee.

BGA Sensitization and Aerosol Challenge Protocol

Five cats were experimentally sensitized to and chronically challenged with BGA (Greer Laboratories, Lenoir, N.C., USA) as previously described [28]. Briefly, on day 0, cats were given a subcutaneous injection of 10 µg of BGA in 10 mg alum and an intramuscular injection of *Bordetella pertussis* (10⁵ killed organisms) as an adjuvant. On day 14, cats received 0.2 ml of BGA intranasally, and on day 21, cats received a subcutaneous injection of 10 µg of BGA in 10 mg alum. After parenteral sensitization, cats were challenged via aerosolization with 0.07 mg/ml of BGA twice weekly for 7 treatments, followed by weekly treatments for 1 month and monthly treatments for 1 year. By the fourth month of the sensitization protocol, all allergen-sensitized cats exhibited signs of bronchial inflammation, including cough, rapid and shallow breathing, or transient respiratory distress. At no point during the year-long sensitization period did any of the cats exhibit signs of upper airway inflammation (sneezing, nasal discharge, nasal congestion, or upper airway obstruction). Four cats concurrently underwent mock sensitization and challenge (including administration of all adjuvants) using phosphate-buffered saline in place of BGA to serve as controls.

Necropsy and Tissue Preservation

Forty-eight hours after the last aerosol challenge, cats were euthanized using an overdose of sodium pentobarbital. Immediately after death, the nasal cavity was isolated, flushed via the nasopharynx with 10% neutral buffered formalin and immersed in 10% neutral buffered formalin until further processing. The left caudal lung lobe was removed and inflation fixed via the lobar bronchus with a solution of 4% paraformaldehyde using a 60-ml

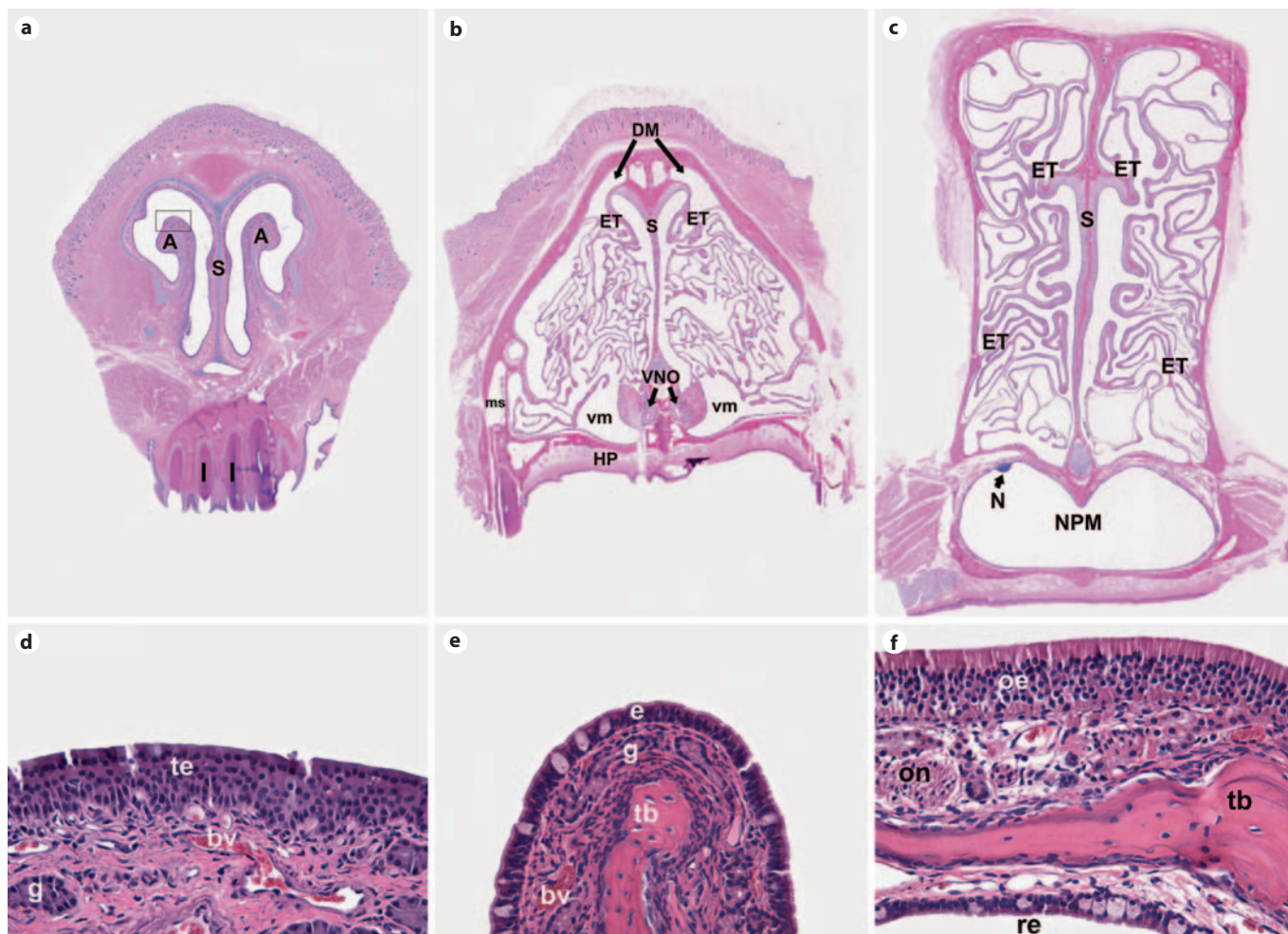


Fig. 1. **a–c** Transverse sections through the rostral nasal cavity (nasal vestibule, **a**), the mid-nasal cavity (main chamber, **b**) and the caudal nasal cavity and nasopharyngeal meatus (**c**) of a control cat. **d** High-magnification photomicrograph of the transitional epithelium (te) lining the dorsal aspect of the alar fold in the section represented in **a**. **e** High-magnification photomicrograph of the ciliated RE (e) lining the tip of an ethmoturbinate represented in **b**. **f** Magnification of the olfactory epithelium (oe) lining the dorsal aspect of the ethmoturbinate and the RE (re) lining the

ventral aspect of the ethmoturbinate and the nasopharyngeal meatus in image **c**. Sections are stained with H&E. A = Alar fold; S = nasal septum; I = incisor tooth; DM = dorsal meatus; ET = ethmoturbinates; ms = maxillary sinus; VNO = vomeronasal organ; HP = hard palate; vm = vomeronasal organ; N = nasal-associated lymphoid tissue; NPM = nasopharyngeal meatus; bv = blood vessel; g = submucosal mucus glands; tb = turbinate bone; on = olfactory nerve bundles.

syringe until completely inflated. Following inflation fixation, the left caudal lung lobe was immersed in 4% paraformaldehyde until further processing for histopathology.

Tissue Processing for Light Microscopy and Morphometric Analysis

After fixation, the nasal cavity was decalcified in 700 ml of 10% ethylenediaminetetraacetic acid in 0.1 M sodium cacodylate, pH 7.4, for 90 days and then rinsed with distilled water for 2–4 h. After decalcification, the nasal cavity was transversely sectioned at 10 specific anatomic locations in a plane perpendicular to the hard palate and nasal septum using gross dental and palatine

landmarks, in a manner similar to a previously described method [30]. These sections were embedded in paraffin for light microscopic and morphometric analyses. Slides were prepared using 4- to 5- μ m-thick sections from the anterior face of each of the tissue blocks. One tissue section from each block was stained with hematoxylin and eosin (H&E) for routine histopathological examination. A uniform, representative section was selected from the rostral, middle and caudal regions of the nasal cavity (fig. 1) from each animal for standard morphometric analyses. These sections were selected because they provided profiles of each of the three anatomical compartments of the nasal cavity (the nasal vestibule, the main chamber and the nasopharyngeal meatus) and

the frontal sinuses. Additional slides from these three sections were histochemically stained with Alcian blue (pH 2.5)/periodic acid-Schiff (AB/PAS) for identification of acidic and neutral mucosubstances and with May-Grünwald-Giemsa stain for histochemical identification of eosinophils and mast cells.

After fixation, the axial airway of the left caudal lung lobe was dissected open from the origin of the lobar bronchus to the terminal-most extension of the conducting airway. The lobe was divided into three separate blocks of tissue along the axial airway (proximal, middle and distal). Each section was routinely embedded in paraffin parallel to the axis of the axial airway, and slides were prepared using 5- μ m-thick sections. Sequential sections were stained with H&E and AB/PAS. The section containing the proximal axial airway was used for the morphometric analyses described below.

Morphometric Analysis of Inflammatory Cells in Nasal Mucosa and Pulmonary Axial Airway Mucosa

All morphometric analyses were performed by an individual who was blinded to the treatment status of each sample. The luminal epithelium lining specific regions in each of the rostral, middle and caudal nasal sections were analyzed using standard morphometric techniques and computerized image analysis as previously described [31]. The specified regions included (1) the surface epithelium lining the dorsal half of the nasal septum in the anterior section; (2) the surface epithelium lining the middle third of the nasal septum in the mid-nasal section, and (3) the surface epithelium lining the dorsal half of the nasopharyngeal meatus in the caudal section. Eosinophil and mast cell numeric cell densities were determined by counting the number of nuclear profiles of eosinophils or mast cells within the surface epithelium and in the lamina propria of the mucosa lining the specified regions, and by dividing this number by the total length of the basal lamina underlying the epithelium in this region. Numeric cell densities of intraepithelial and submucosal eosinophils and mast cells in the mucosa lining the proximal part of the left caudal lobar axial airway were similarly calculated. Eosinophils and mast cells were identified on the basis of characteristic cellular morphology and cytoplasmic granular staining on May-Grünwald-Giemsa-stained histologic sections.

Morphometry of Stored Intraepithelial Mucosubstances

The amount of stored mucosubstances in the surface epithelium lining the specified regions in the nasal and pulmonary axial airways was assessed by estimating the volume of AB/PAS-stained intraepithelial mucosubstances per unit area of basal lamina (volume density), using computerized image analysis and standard morphometric techniques [32]. Results were expressed in nl/mm² basal lamina.

Statistical Analyses

All data were expressed as mean group values $\pm$ standard deviation (SD). All data sets were assessed for normality (Kolmogorov-Smirnov goodness of fit test) and homogeneity of variances (Bartlett's test, F test). The differences between control and asthmatic groups in parametric data sets were analyzed by t test (for equal variances) or Welch's test (for unequal variances). The differences between control and asthmatic groups in non-parametric data sets were analyzed by the Mann-Whitney rank sum test. The relationship between nasal and lung inflammatory cell nu-

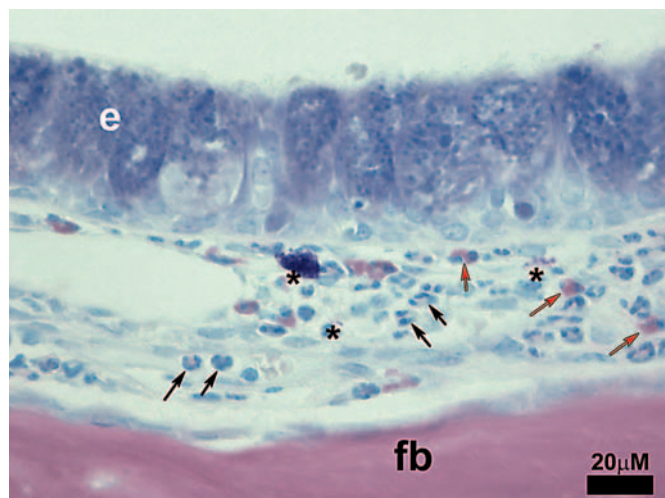


Fig. 2. Light photomicrograph of the epithelium (e) lining the frontal sinus of an asthmatic cat. Note the mucosal and submucosal eosinophils (red arrows), mast cells (asterisks) and neutrophils (black arrows) in the lamina propria of the sinus mucosa. The section was histochemically stained with May-Grünwald-Giemsa stain for identification of eosinophils and mast cells. fb = Frontal bone.

meric densities was analyzed by using a priori correlation analysis (Pearson product moment correlation). The criterion for statistical significance was set to $p \leq 0.05$. All statistical analyses were performed using a commercial statistical software package (SigmaStat; SPSS, Chicago, Ill., USA).

Results

Nasal Histopathology

In control cats, the nasal vestibule and preturbinate region of the main chamber were lined with a combination of non-keratinized squamous epithelium, non-ciliated transitional epithelium (fig. 1a, d) and ciliated respiratory epithelium (RE). The mucosa in this region exhibited few intraepithelial mucous cells and submucosal mucus glands. In the mid-nasal cavity, the septum, lateral walls and maxillary turbinates were lined primarily with ciliated RE (fig. 1b, e), with some regions in the rostral nasal cavity lined with non-ciliated transitional epithelium. In the caudal nasal cavity, the ethmoturbinates were lined extensively with olfactory epithelium overlying a wide lamina propria featuring abundant olfactory nerves and Bowman's glands, with lesser amounts of ciliated RE overlying a narrow lamina propria and submucosa with few submucosal mucus glands (fig. 1c, f). The

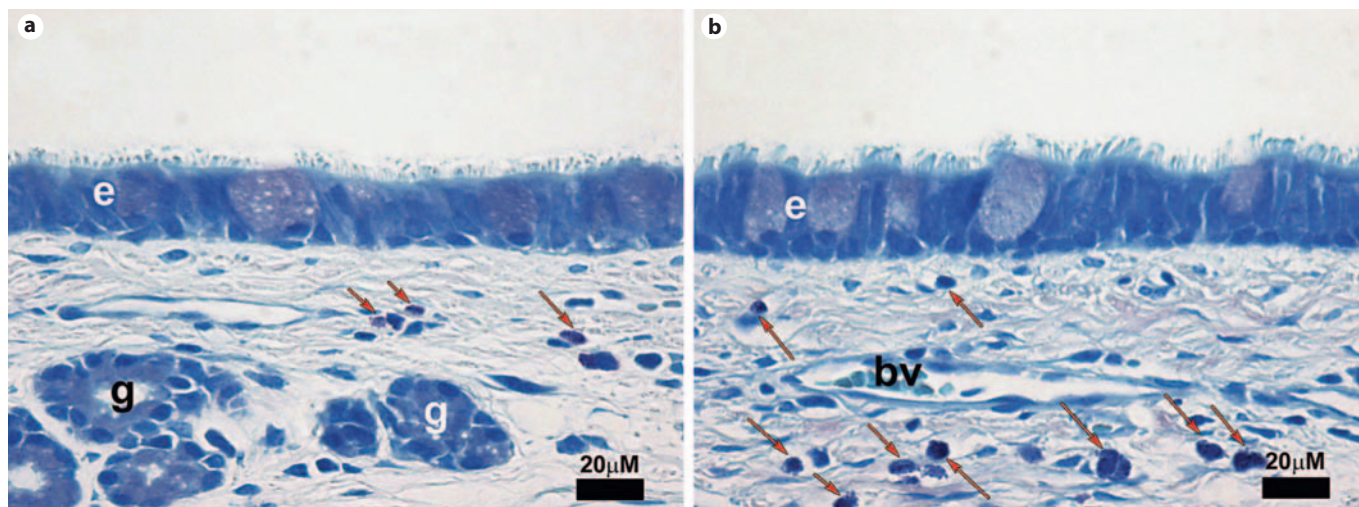


Fig. 3. Light photomicrographs of the epithelium (e) lining the dorsal nasopharyngeal meatus of a control (a) and asthmatic cat (b). Mast cell infiltration (arrows) of the submucosa is particularly evident in the section obtained from the asthmatic cat (b). Sections were histochemically stained with May-Grünwald-Giemsa stain for identification of mast cells. g = Submucosal mucus glands; bv = blood vessel.

nasopharyngeal meatus and frontal sinuses were lined with RE rich in intraepithelial mucosubstances. Focal aggregates of nasal associated lymphoid tissue with overlying lymphoepithelium were observed in ethmoturbinates in the caudal aspect of the nasal cavity and the rostral nasopharyngeal meatus (fig. 1c, f). The caudal nasopharyngeal meatus was circumferentially lined with RE and lymphoepithelium overlying large aggregates of nasal associated lymphoid tissue. Occasional neutrophils and rare eosinophils were present in the surface epithelium and submucosa of all regions of the nasal airways and frontal sinuses of control cats. No significant gross or microscopic morphological alterations were observed in the nasal mucosa of control cats.

The principal morphologic alteration in the nasal airways of asthmatic cats was a mild influx of intraepithelial and submucosal eosinophils accompanied by a moderate increase in submucosal mast cells. In all asthmatic cats, there was a mild, diffuse influx of eosinophils within the epithelium and underlying lamina propria of the rostral nasal airways. The increase in mucosal eosinophils was confined to the transitional epithelium and RE lining the nasal vestibule and rostral nasal cavity. There was no appreciable change in tissue eosinophils observed in the middle or caudal nasal cavity. In contrast, an increase in submucosal mast cells was observed throughout all nasal airways, but was most prominent in the frontal sinus (fig. 2), caudal nasal cavity and nasopharyngeal meatus (fig. 3).

Lung Histopathology

The results of the histologic examination of the lungs of control and asthmatic cats have been previously described in detail [28]. Briefly, the proximal axial airways of control cats were lined with pseudostratified to columnar, ciliated RE with abundant intraepithelial mucosubstances. The distal axial airways, respiratory and terminal bronchioles of control cats were lined with columnar to tall cuboidal epithelial cells exhibiting only minimal intraepithelial mucosubstances. The axial and bronchiolar airways of control cats were surrounded by a layer of smooth muscle (fig. 4a, c).

The epithelial cells of the proximal and distal airways of asthmatic cats were hypertrophied and hyperplastic, with epithelial cell exfoliation noted. Mucous cell metaplasia was observed in the distal axial and bronchiolar airways of asthmatic cats. Marked smooth muscle hypertrophy was observed in both the proximal and distal axial airways and bronchiolar airways. A mild influx of eosinophils was observed primarily within the epithelium and submucosa of the proximal axial airways (fig. 4b, d).

Morphometric Analysis of Lung Axial Airway Mucosa

The pulmonary axial airway mucosa in asthmatic cats exhibited a 2.12-fold increase ($p = 0.016$) in the numeric cell density (NCD) of mucosal eosinophils com-

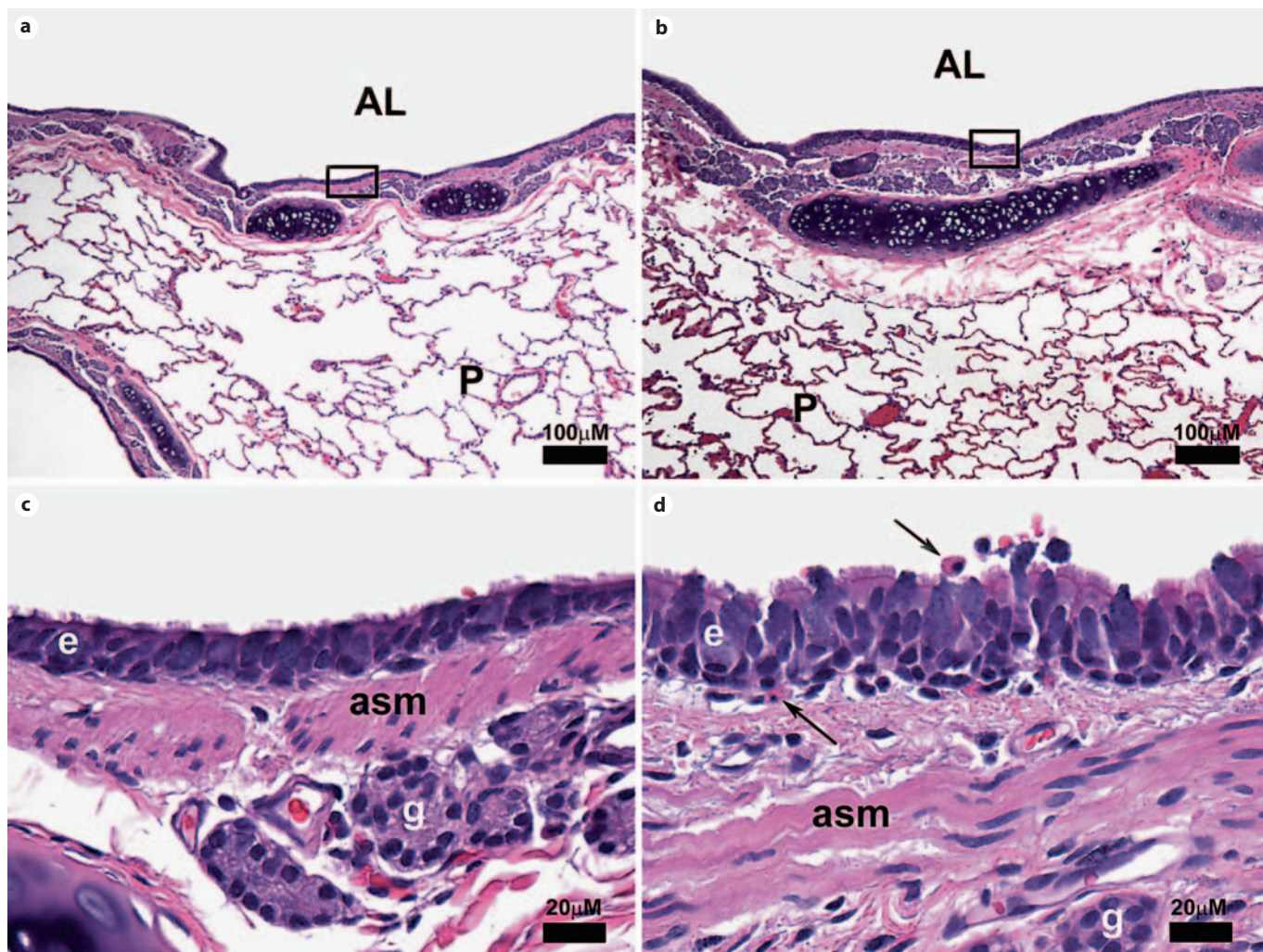
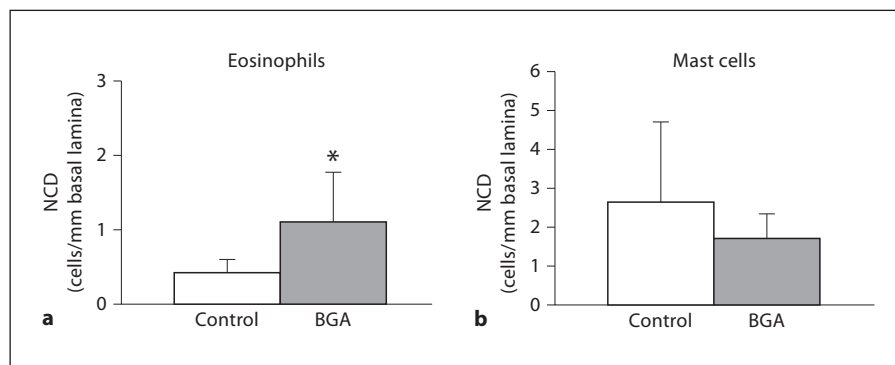


Fig. 4. Low-magnification light photomicrographs of the pulmonary axial airways of a control (a) and asthmatic cat (b). The areas within the boxes in a and b are magnified in c and d to depict the epithelium lining the proximal axial airway. Note the eosinophils

in the lamina propria, epithelium and airway lumen (AL) of the asthmatic cat (arrows). Sections were stained with H&E. P = Pulmonary parenchyma; e = epithelium; g = submucosal glands; asm = airway smooth muscle.

Fig. 5. NCDs of eosinophils (a) and mast cells (b) in the mucosa lining the proximal axial airway of the left caudal lung lobe in control and asthmatic cats. Bars represent group mean $\pm$ SD. * $p \leq 0.05$, significantly different from the respective control group.



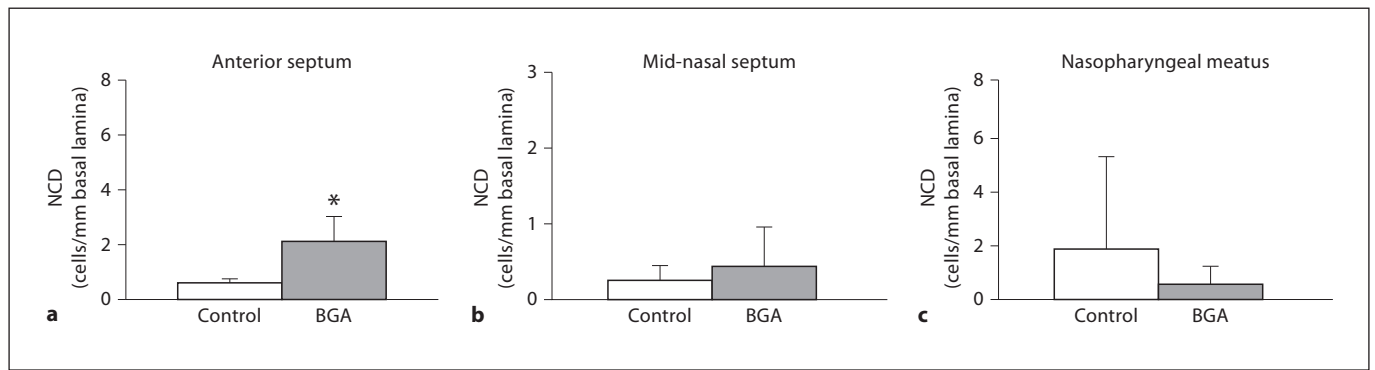


Fig. 6. NCDs of mucosal eosinophils in control and asthmatic cats in the rostral (a), mid (b) and caudal (c) regions of the nasal cavity. Bars represent group mean $\pm$ SD. * $p \leq 0.05$, significantly different from the respective control group.

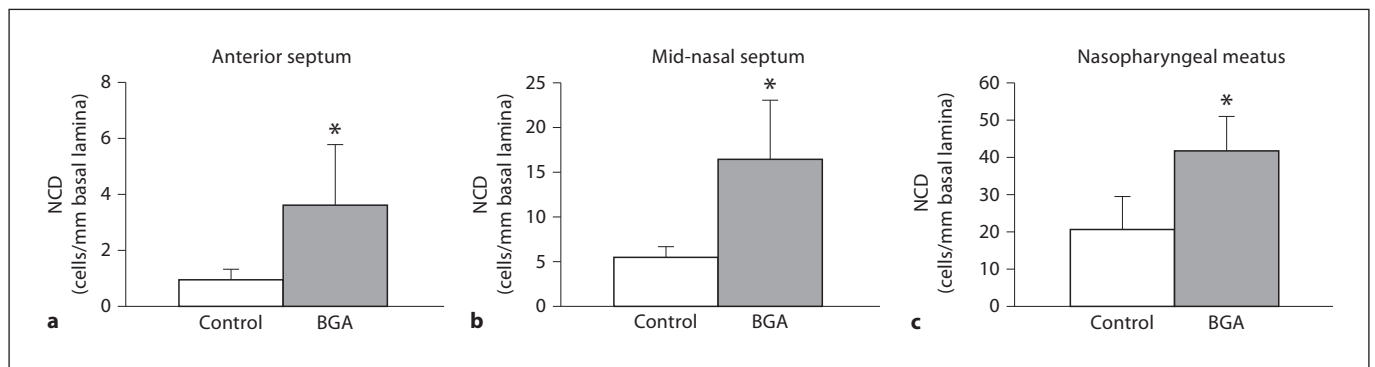


Fig. 7. NCDs of mucosal mast cells in control and asthmatic cats in the rostral (a), mid (b) and caudal (c) regions of the nasal cavity. Bars represent group mean $\pm$ SD. * $p \leq 0.05$, significantly different from the respective control group.

pared to control cats (fig. 5a). There was no significant difference in the NCD of axial airway mucosal mast cells observed between control and asthmatic cats (fig. 5b).

Morphometric Analysis of Inflammatory Cells in Nasal Mucosa

The mean NCD of mucosal eosinophils $\pm$ SD for both groups is presented in figure 6. Within the rostral nasal cavity, the NCD of mucosal eosinophils was higher (3.47-fold; $p = 0.015$) in asthmatic cats compared to controls (fig. 6a). Asthmatic cats did not exhibit a significant increase in airway mucosal eosinophils compared to controls in the mid (fig. 6b) or the caudal nasal cavity (fig. 6c).

In comparison to eosinophils, a greater number of mast cells were noted in the mucosa and submucosa of the examined areas of the rostral, mid and caudal nasal cavities of both control and asthmatic cats (fig. 7). Asthmatic cats had a higher tissue density of mast cells within the mucosa and submucosa throughout the entire nasal cavity compared to control cats. The group mean mast cell NCD of asthmatic cats was 3.83-fold higher ($p = 0.043$) in the rostral (fig. 7a), 3.06-fold higher ($p = 0.012$) in the mid (fig. 7b) and 2.0-fold higher ($p = 0.011$) in the caudal nasal cavity (fig. 7c) than in the corresponding regions in control cats. In addition, the tissue density of mast cells increased progressively from the rostral to the caudal regions of the nasal airways in both groups, with the highest tissue density of mast cells observed in the nasopharyngeal meatus of both groups.

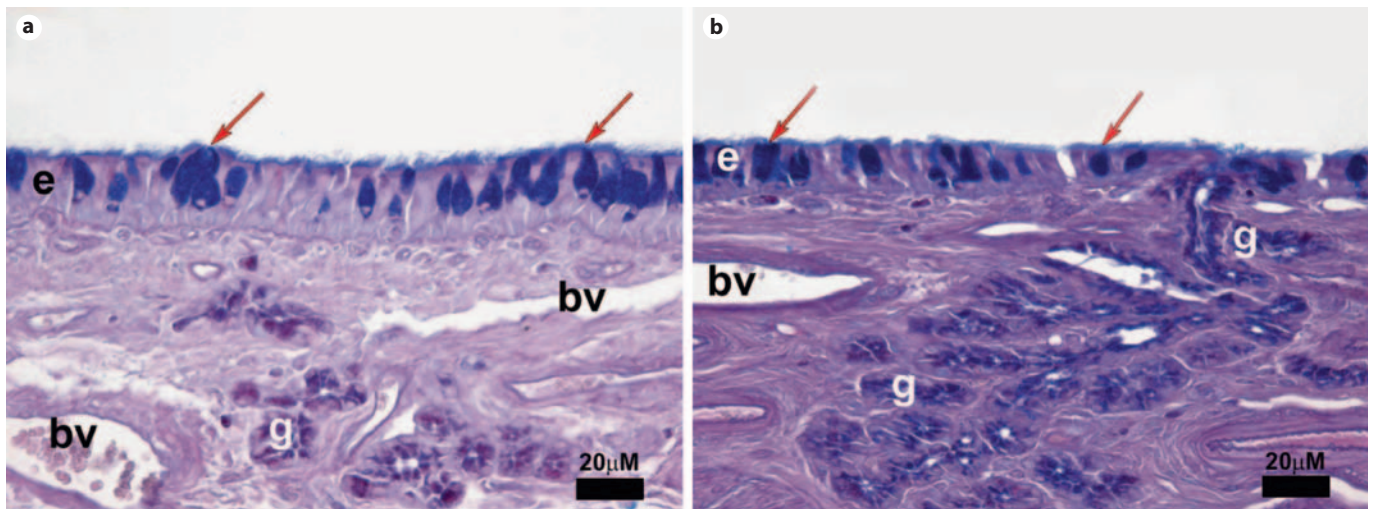


Fig. 8. Light photomicrographs of the RE (e) lining the dorsal nasopharyngeal meatus in a control cat (a) and an asthmatic cat (b). Sections were histochemically stained with AB/PAS to detect intraepithelial acidic and neutral mucosubstances (arrows). bv = Blood vessel; g = submucosal mucus gland.

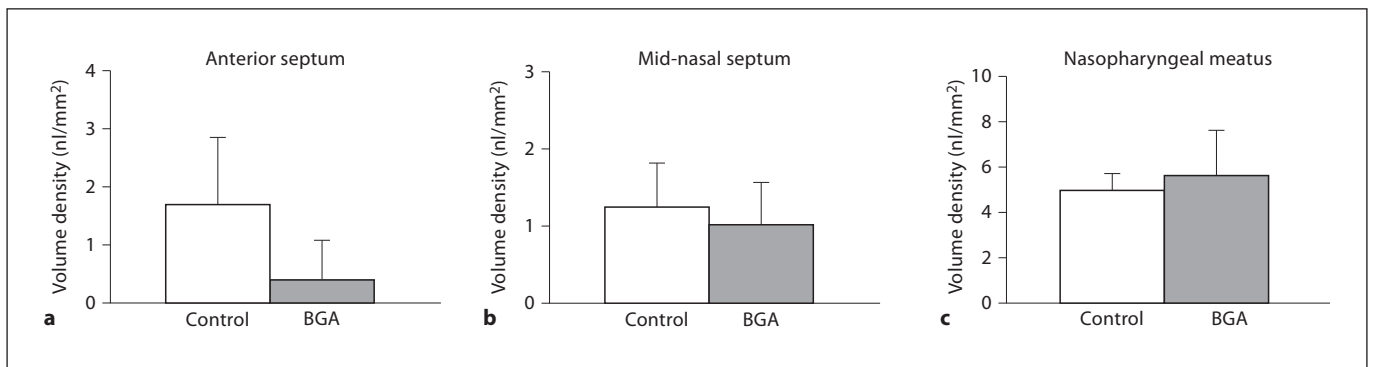


Fig. 9. Volume density of intraepithelial mucosubstances of control cats and asthmatic cats sensitized to BGA in the rostral (a), mid (b) and caudal (c) regions of the nasal cavity. Bars represent group mean $\pm$ SD. There were no significant differences between control and asthmatic cats.

Morphometric Analysis of Intraepithelial Mucosubstances in Nasal Mucosa

Acidic and neutral mucosubstances were identified via AB/PAS staining within the epithelium lining all regions of the nasal airways of control and asthmatic cats (fig. 8). For both groups, the density of intraepithelial mucosubstances was greatest within the epithelium lining the dorsal nasopharyngeal meatus. There was no significant difference in the volume density of intraepithelial mucosubstances between control and asthmatic cats in any of the three specified regions of the nasal cavity (fig. 9).

Correlations between Nasal and Pulmonary Airways

Correlation analysis was used to determine the potential association between regional (rostral, mid and caudal) nasal and pulmonary airway inflammation. There were no significant correlations found between the NCD of eosinophils in the mucosa lining the lung axial airways and the mucosa lining the representative sections of the rostral ($r = 0.401$, $p = 0.285$), the mid ($r = 0.153$, $p = 0.695$) or the caudal nasal cavity ($r = 0.0709$, $p = 0.856$). Similarly, there were no significant correlations found between the NCD of mast cells in the mucosa lining the

lung axial airways and the mucosa lining the representative sections of the rostral ($r = -0.391$, $p = 0.298$), the mid ($r = -0.197$, $p = 0.612$) or the caudal nasal cavity ($r = -0.245$, $p = 0.526$).

Discussion

The results of this study demonstrate that aeroallergen sensitization and challenge evoke immunologic and morphologic alterations in the nasal airways of cats during the development of experimentally induced asthma. Specifically, these changes include a mild eosinophilic rhinitis that is restricted to the rostral nasal cavity and mast cell infiltration of the nasal mucosa and submucosa. Unlike the pulmonary airways, an increase in intraepithelial mucosubstances did not accompany these inflammatory changes in any region of the nasal cavity. Furthermore, the increase in mast cells observed in the nasal airways was not noted along the pulmonary axial airways. This report provides the first description of the morphologic alterations of the nasal airways of cats experimentally sensitized to a relevant aeroallergen, and the first investigation of a possible mechanistic link between the upper and lower airways of asthmatic cats. Examination of this potential relationship in a large animal model is important for providing new insight into the poorly understood immunologic link between asthma and rhinosinusitis.

Comorbid allergic rhinitis has been described in human asthmatics [10, 33]. This condition is histologically characterized by expansion of eosinophils and mast cells, the central effector cells in allergic inflammation, in nasal mucosa [34]. In the experimentally induced asthmatic cats of this study, an eosinophilic influx was also observed in the nasal airways following aerosolized allergen challenge. However, these inflammatory changes were mild and focally distributed in the rostral nasal cavity. This heterogeneous distribution of eosinophilic inflammation within the nasal airways is consistent with the pattern observed in murine experimental models of allergic rhinitis [35] and in regional biopsy samples obtained from people with naturally occurring chronic rhinosinusitis [36]. This site specificity is also consistent with patterns of airborne toxicant-induced nasal epithelial injury observed in experimental animal models [37], suggesting that this distribution may be related to airflow patterns within the nasal airways of these species [38, 39]. It is also important to note that the present study represents a single point in time during a 12-month-long sen-

sitization and challenge period. In a similar study using this model, the percentage of eosinophils in bronchoalveolar lavage fluid varied significantly during sensitization and challenge, from a peak of 55% after sensitization (1 month) to a low of 18% at 1 year [28]. It is possible that eosinophilic inflammation in different regions of the nasal airways exhibits similar kinetics, but that the decline in nasal eosinophils occurs more rapidly in some regions than in others.

This study demonstrates that chronic aeroallergen challenge in cats also causes an increase in mast cells in all regions of the nasal airways. These findings are similar to those observed in allergic rhinitis in people, which is characterized by mast cell expansion in the nasal mucosa following natural allergen exposure [40–42]. Murine models of allergic rhinosinusitis also exhibit nasal mucosal mast cell inflammation that increases over time following chronic allergen exposure [43]. In contrast to the focal distribution of eosinophils in the rostral nasal airways, the increase in mast cells was observed throughout the nasal airways, but was most extensive in the caudal nasal cavity and the nasopharyngeal meatus. Unlike eosinophils, which are largely recruited to the nasal airways from peripheral blood in response to Th2 lymphocytes, mast cells are long-lived, resident tissue cells in the nasal airways that are capable of locally proliferating in the face of antigenic stimulation, chronic inflammation or tissue remodeling [44]. These differences in leukocyte development and trafficking may explain the differences in tissue distribution between eosinophils and mast cells observed in the nasal airways of asthmatic cats.

While the aeroallergen-challenged cats in the present study did develop morphologic changes in the nasal airways consistent with allergic rhinitis, these alterations were not identical to those induced in the lower airways. Specifically, mast cell infiltration in the nasal airways was extensive, while mast cell infiltration was not a prominent histopathological feature of lower airways in the asthmatic cats. One possibility for this discrepancy is that mast cells in the lower airways become activated and degranulate following aeroallergen challenge. In the present study, we identified mast cells on the basis of characteristic cellular morphology and cytoplasmic granular staining. It is possible that mast cells present in the pulmonary airways underwent degranulation following aeroallergen exposure, thus precluding our ability to identify these cells in the lower airways via histochemical staining methods. This could also explain the fact that the cats in our study were symptomatic for lower airway disease, but did not exhibit any clinical signs suggestive

of rhinitis. A limitation of this study was that the tissue fixation and decalcification methods used to permit rigorous morphometric analyses precluded any immunohistochemical analyses. In future studies, alternative methods could be employed to allow for immunohistochemical identification and characterization of mast cell populations to evaluate this observed difference between the upper and lower airways.

Another plausible explanation for the discrepancy between upper and lower airway inflammation observed in people and experimental models is that these regions are temporally in different stages of the process of atopic sensitization. The 'atopic march' refers to a sequence of atopic features that appear during different stages of childhood, typically characterized by the appearance of atopic dermatitis during infancy, followed by the development of asthma and allergic rhinitis later in childhood and into adulthood [45]. The process by which this temporal pattern appears remains poorly understood and is likely multifactorial. Factors that may be involved in this timing include both host (genetic) and environmental factors, including the route, nature and magnitude of allergen exposure, co-exposure to endotoxin, tobacco smoke and air pollutants, and the role of upper or lower airway respiratory infections during sensitization [45]. While genetically manipulated mouse models and cohort studies in people are advantageous for identifying the genetic factors, a large animal model may be ideal for evaluation of these environmental components. Further study both in affected individuals and in relevant animal models is warranted to better understand the pathogenesis of this syndrome of allergic conditions.

Mucous cell metaplasia is an important component of airway remodeling in asthma that is elicited by mediators released from Th2 lymphocytes, eosinophils and mast cells, including interleukin (IL)-5 [46], IL-9 and IL-13 [47], and cysteinyl leukotrienes [48]. Hyperplasia of the mucus secretory apparatus in the pulmonary mucosa is a prominent feature of naturally occurring asthma in cats and in people [27, 49]. Despite these findings in the lower airways, a concomitant increase in intraepithelial mucosubstances was not noted in any of the evaluated regions of the nasal airways of cats with experimentally induced asthma. While this result was contrary to our original hypothesis, it should be noted that goblet cell hyperplasia is not a consistent feature of allergic rhinitis in people. A transient increase in intraepithelial mucosubstances has been noted in patients with allergic rhinitis following exposure to allergen [50, 51]. In contrast, other studies found no change in nasal intraepithelial mucosubstances

in allergic rhinitis patients [52, 53]. The reasons for this discrepancy are unknown, but may be related to the timing of evaluation of the nasal mucosa relative to the time of allergen exposure, as allergen challenge. Since allergen exposure can be associated with both upregulation [54] and degranulation [55] of intraepithelial mucus, studies evaluating a single point in time may result in an increase or decrease in stored intraepithelial mucosubstances. Further studies are necessary to determine the time course of events in nasal epithelial remodeling that occurs following aeroallergen exposure.

This study represents the first description of morphologic alterations in the nasal airways induced by chronic aeroallergen challenge in a large animal model of asthma. Both the upper and lower airways of chronically sensitized cats respond to aeroallergen exposure, but this exposure generates disparate histopathological responses in each of these regions. Several mechanisms have been advanced to explain the interaction of the upper and lower airways observed in humans [56], though the actual pathophysiological processes have yet to be established. Further characterizing the immunologic response of the feline nasal airways to aeroallergen sensitization and challenge may clarify the nature of the relationship between the upper and lower airways in cats and, possibly, in humans. A particular advantage of this model is that it offers the possibility of repeated follow-up of clinical, functional and structural features of both the lower and upper airways in a well-established and repeatable model. These features make the feline model potentially useful in the investigation of chronic remodeling in the allergic airways, which remains challenging in human asthma and allergic rhinitis.

By investigating an experimental model of feline asthma, we have demonstrated that artificial sensitization and chronic challenge with a relevant aeroallergen do elicit morphologic alterations in the nasal airways of cats, but these changes are disparate from those seen in the lung axial airways. Although aeroallergen challenge induces eosinophilic inflammation in both the upper and lower airways of cats with experimentally induced asthma, the magnitude of this inflammation is less pronounced and more focally distributed in the nasal airways. In contrast to the eosinophilic inflammation of the pulmonary airways, a diffuse rhinitis characterized primarily by mast cells is induced in cats following aeroallergen sensitization and challenge. Thus, the present study in the feline airways supports the concept of a 'united airways' theory and indicates that consideration of concurrent upper airway inflammation is warranted in the evaluation of allergic lower airway disease in cats.

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Disclosure Statement

The authors have no conflict of interest to disclose.

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