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SPONTANEOUSLY ARISING DISEASE

Architecture and Inflammatory Cell Composition of the Feline Lung with Special Consideration of Eosinophil Counts

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Summary

An increase in the number of eosinophils in bronchoalveolar lavage fluid (BALF) is a hallmark of feline asthma; however, a wide range in the percentage of eosinophils in BALF has been documented in healthy cats. In this study, BALF and lung tissue were collected from 15 cats without respiratory disease, BALF was taken from 15 cats with asthma and lung tissue was collected from six different asthmatic cats. Total nucleated cell count (TNCC) and inflammatory cell percentages were measured in BALF and lung tissue was evaluated microscopically. Asthmatic cats had a significantly higher eosinophil count in lung tissue, but BALF TNCC did not differ significantly between groups. Cats without respiratory signs had significantly more numerous macrophages and lymphocytes in BALF than asthmatics, but significantly lower percentages of eosinophils ($4.2 \pm 7.8\%$ versus $49.4 \pm 20.6\%$, $P < 0.001$). In healthy feline airways a BALF eosinophil percentage of <5% can be expected. Dominant microscopical findings in feline asthma include high eosinophil counts, airway remodelling and inflammation. There is good correlation between the findings in BALF and tissue in feline asthma.

Keywords: asthma; bronchoalveolar lavage fluid; eosinophils; histopathology

Introduction

Human asthma is a well recognized disease in which a genetic predisposition to hypersensitivity and environmental factors lead to chronic inflammation of the lower respiratory tract with recurrent bronchoconstriction, airway remodelling (Holgate, 1999) and airway hyper-responsiveness (Dye *et al.*, 1996; Reinero *et al.*, 2005; Kirschvink *et al.*, 2007; Hirt *et al.*, 2011). Infiltration of inflammatory cells into the airways, predominantly eosinophils, is considered a hallmark of the disease.

A comparable disease exists in cats with similar clinical signs and histopathological changes within the airways. Spontaneously arising feline asthma is believed to be an allergen-induced hypersensitivity reaction (Moses and Spaulding, 1985; Corcoran *et al.*, 1995; Hirt, 2005) and is characterized by eosinophil infiltration in the respiratory tract (Moise *et al.*, 1989; Padrid, 2000; Reinero, 2011).

An important stage in the diagnostic evaluation of cats with suspected asthma is the cytological examination of bronchoalveolar lavage fluid (BALF). Cats with asthma have a predominantly eosinophilic airway inflammation, but there is debate as to the proportion of eosinophils within the cellular content of normal feline BALF (McCarthy and Quinn, 1989; Padrid *et al.*, 1991). Noone (1999) suggested that >20% of eosinophils should be found in the BALF of asthmatic cats, while Padrid *et al.* (1991) reported that 24 clinically normal cats had up to 83% eosinophils in BALF. Dye *et al.* (1996) found no

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more than 4% eosinophils in BALF from 15 healthy cats and 20–30% eosinophils in 24 affected cats.

The aim of the present study was to characterize inflammatory cells in the BALF and lung tissue of clinically normal and asthmatic cats.

Materials and Methods

Animals

The study included 36 client-owned cats. Informed consent was provided by each owner.

BALF and lung tissue were harvested from 15 cats without respiratory disease that were humanely destroyed due to unrelated causes (group 1). BALF was also collected from 15 asthmatic cats (group 2) and lung tissue was taken from six further cats with asthma that died or had been humanely destroyed for a range of reasons (group 3).

Group 1 included 13 domestic shorthair cats, one Persian and one Chartreux. These cats ranged in age from 1 to 17 years (median 11 years) and the group comprised eight neutered females, two entire females and five neutered males. Causes for presentation included weakness and lethargy (33.3%), weight loss (26.7%), anorexia (20.0%), neurological deficits (20.0%), polyuria and polydipsia (20.0%) and chronic gastrointestinal signs (20.0%). Reasons leading to humane destruction were neoplasia (33.3%), chronic renal insufficiency (26.7%), chronic gastrointestinal disease (13.3%) and miscellaneous conditions (26.7%).

To be included in group 1, patients had to be free of respiratory signs and to have had lung auscultation performed at the time of presentation, during hospitalization and before death. Cats with a history of respiratory disease, eosinophilia or treatment with drugs capable of modulating eosinophil counts (e.g. glucocorticoids) were excluded. Samples were always collected immediately after death in these cats.

Group 2 was comprised of 15 asthmatic cats aged 1–14 years (median 4 years) including 12 domestic shorthair cats, two Siamese and one Abyssinian. Eight animals were neutered males, one was an entire male and six were neutered females. Cats in group 2 were investigated for respiratory disease and each was subjected to collection of BALF, thoracic radiography, faecal examination and haematological examination. None of the cats in group 2 had been treated with glucocorticoids within the 10 days prior to these diagnostic procedures. Diagnosis of asthma was based on typical clinical and radiographic findings, negative faecal examination and BALF culture, >15% eosinophils in BALF, as well as an absence of significant blood eosinophilia.

Lung tissue from cats in group 3 was taken from the archives of the Institute of Pathology and Forensic Veterinary Medicine, Vienna. This group was comprised of six neutered male cats (five domestic shorthair cats and one Persian) aged 1–12 years (median 8.5 years) that had been humanely destroyed because of acute respiratory distress ($n = 2$), multicentric lymphoma ($n = 2$) or that had died suddenly ($n = 2$). Both cats with lymphoma had been previously diagnosed as having asthma and had undergone long-term treatment with inhaled glucocorticoids. The other four animals had not been diagnosed with or treated for asthma while alive. The 1-year-old cat had developed acute respiratory distress after neutering. Another animal had had several bouts of tachypnoea over a period of approximately 6 months before showing severe respiratory distress. The remaining two cats had shown no clinical signs before sudden death. The lung samples from group 3 cats all showed histopathological changes consistent with feline asthma, including airway remodelling characterized by thickening of the airway wall, increased smooth muscle mass and mucus gland hypertrophy, together with marked eosinophilic infiltration.

Bronchoalveolar Lavage Fluid Collection and Processing

BALF was obtained bronchoscopically immediately *post mortem* (group 1) or under general anaesthesia (group 2). The animals were placed in sternal recumbency and a paediatric bronchoscope (Olympus BC N30, Olympus Corporation, Tokyo, Japan) with an outer diameter of 3.6 mm and a 1 mm working channel was inserted into a bronchus in the right caudal lung lobe. Lavage was performed twice in the same location with 1 ml/kg of pre-warmed isotonic saline. The same procedure was repeated with a bronchus in the caudal left lung lobe. BALF was retrieved by gentle vacuum pump suction and was transferred immediately to the laboratory for evaluation.

The total cell count of unprocessed samples was determined twice by laser flow cytometry (ADVIA120, Bayer, Leverkusen, Germany). For the differential cell counts, 300 µl of BALF was concentrated by cytocentrifugation at 200 g for 5 min. Four slides were prepared and two of these were subjected to Romanowsky staining (Haemaquick, Biomed, Oberschleissheim, Germany). These slides were examined (×400) and 300 cells were differentially identified by two clinical pathologists and the results averaged. The percentage of each cell type in the samples was determined. The remaining two slides were stained with toluidine blue for enumeration of mast cells in 10 × 400 fields.