VETERINARY CLINICS
SMALL ANIMAL PRACTICE

Canine Eosinophilic Bronchopneumopathy

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nfiltration of the airways or pulmonary parenchyma by eosinophils has been described in the dog as pulmonary infiltration with eosinophils (PIE) [1], pulmonary eosinophilia (PE) [2], eosinophilic pneumonia [3], and eosinophilic bronchopneumopathy (EBP) [4]; however, to date, no clear method of classification exists. The authors use the term *eosinophilic bronchopneumopathy* rather than *pulmonary infiltration with eosinophils* or *pulmonary eosinophilia*, because EBP takes into account the fact that bronchial infiltration and parenchymal involvement are almost always present in these cases. A cause is rarely identified, and most cases of EBP are considered idiopathic [4].

In human medicine, eosinophilic lower airway diseases are a heterogeneous group of disorders in which an increased number of eosinophils are present in the airways or lung parenchyma [5]. These diseases are broadly separated into airway and parenchymal disorders (Box 1). In some cases, eosinophils are merely a part of the inflammatory process and may even be present to protect host tissues against parasites or other organisms. In other cases, eosinophils seem to be directly responsible for tissue damage [5].

This article presents the classification of eosinophilic lower airway diseases that is commonly used in human medicine (see Box 1) and proposes an adapted classification for the dog (Box 2). This classification is followed by a review of the current understanding of canine idiopathic EBP.

CLASSIFICATION OF EOSINOPHILIC LOWER AIRWAY DISEASES
Airway Disorders
Asthma is the most frequent cause of airway eosinophilia in human beings. This condition is characterized by chronic cough, eosinophilic infiltration of the bronchial wall, reversible air flow obstruction, and bronchial hyperactivity [6]. The syndrome of asthma has not been recognized in dogs, although the authors have observed apparent bronchial hyperactivity in some advanced cases of canine EBP (see section on pulmonary function tests [PFTs]).

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Box 1: Classification of eosinophilic lower airway disorders in human beings

Airway disorders
Asthma
Eosinophilic bronchitis
Allergic bronchopulmonary aspergillosis
Bronchocentric granulomatosis

Parenchymal disorders associated with known underlying condition
Parasitic infections
Other infections (mycobacteria, fungi)
Interstitial lung diseases
Drug reactions
Idiopathic hypereosinophilic syndrome
Pulmonary vasculitis
Lung cancer
Others

Idiopathic parenchymal disorders
Simple PE
Chronic eosinophilic pneumonia
Acute eosinophilic pneumonia

(*Data from* Alberts WM. Eosinophilic interstitial lung disease. Curr Opin Pulm Med 2004; 10:420.)

Eosinophilic bronchitis (EB) is a condition in human medicine characterized by a corticosteroid-responsive cough, bronchial eosinophilia, no airway obstruction, and normal airway responsiveness [7]. Whether asthma and EB are distinct entities or conditions representing a pathophysiologic spectrum of disease awaits further elucidation [8], but it has been shown that repeated episodes of EB can be associated with the development of asthma in people [9]. Although canine EB has been documented in the veterinary literature [1,10], eosinophilic tracheobronchitis without obvious pulmonary parenchymal involvement has been observed in only a few dogs.

Allergic bronchopulmonary aspergillosis (ABPA) is a rare complication of asthma or cystic fibrosis in human beings [11]. In this disease entity, airway colonization by *Aspergillus* exacerbates underlying asthmatic injury. Pathologic manifestations of ABPA include mucoid impaction of bronchi, bronchocentric granulomatosis, eosinophilic pneumonia, and chronic bronchiolitis [11]. Although *Aspergillus fumigatus* has been cultured from bronchoalveolar fluid (BALF) of two dogs with EBP, fungal hyphae were not observed on cytology

> **Box 2: Classification of eosinophilic lower airway disorders in dogs**
>
> *Airway disorders*
> Idiopathic eosinophilic bronchitis or tracheobronchitis
> Parasitic tracheobronchitis (*Oslerus osleri*)
>
> *Parenchymal disorders associated with known underlying condition*
> Parasitic infections
> Occult heartworm disease (presenting as eosinophilic pneumonitis or as eosinophilic granulomatous pneumonia)
> *Angiostrongylus vasorum, Filaroides hirthi*
> Chronic bacterial pneumonia (aspiration pneumonia, foreign body pneumonia)
> Idiopathic hypereosinophilic syndrome
> Eosinophilic pulmonary vasculitis?
> Lung cancer
> Other?
>
> *Idiopathic parenchymal disorder*
> Eosinophilic granulomatous pneumonia
> Simple eosinophilic pneumonia?
>
> *Idiopathic mixed (airway and parenchyma) disorder*
> EBP (also referred to in the veterinary literature as PIE or PE)

of BALF or in bronchial biopsies from these dogs [4], and it does not seem that ABPA specifically exists in the dog.

In human beings, bronchocentric granulomatosis is an unusual pathologic entity characterized by granulomatous inflammation affecting the bronchi and bronchioles [12]. The inflammation consists of a dense infiltrate of eosinophils, lymphocytes, and plasma cells surrounded by palisading epithelioid cells; destruction of smaller airways ultimately results. In asthmatic people, bronchocentric granulomatosis is considered to be an immunologic reaction to endobronchial fungi, particularly *A fumigatus*. In nonasthmatic people, evidence for endobronchial infection with *Aspergillus* is usually absent and a causative agent is often not identified [13]. This condition has not yet been reported in dogs.

Parenchymal Disorders Associated with Known Underlying Condition

Several parasites, such as *Strongyloides* spp, *Ascaris* spp, *Toxocara canis*, *Ancylostoma* spp, or *Wuchereria bancrofti*, can lead to eosinophilic pneumonia in human beings. In most of theses parasitic diseases, respiratory symptoms are mild and gastroenterologic signs dominate the clinical picture [5]. In the dog, occult heartworm disease caused by *Dirofilaria immitis* can cause eosinophilic pneumonitis because of antibody-dependent leukocyte adhesion to microfilariae in the

pulmonary circulation, entrapment of microfilariae in the capillaries, and subsequent granulomatous inflammation [14]. In some cases, inflammation is dominated by eosinophils [14], whereas in others, granulomatous inflammation progresses to eosinophilic pulmonary granulomatosis, a condition that behaves similar to malignant pulmonary histiocytosis [15].

Migration of larvae of *Angiostrongylus vasorum* through pulmonary parenchyma can result in eosinophilic pneumonia in dogs [16], although in most cases, neutrophils rather than eosinophils predominate in BALF [17]. The primary clinical signs in affected dogs are cough, respiratory difficulty, and hemorrhagic diathesis [17].

Other parasites, such as *Oslerus osleri*, *Filaroides hirthi*, *Crenosema vulpis,* or *Paragonimus kellicotti*, have been implicated in the influx of eosinophils into the airways (*O osleri*) or lungs (other parasites) in dogs [18].

In chronic pulmonary infections caused by mycobacteria or fungi in human beings, eosinophils may comprise a significant proportion of the inflammatory infiltrate [5]. This has also been suggested in the dog [19] but has not been confirmed in the veterinary literature. In the authors' experience, however, pulmonary infection caused by severe aspiration pneumonia or a foreign body can lead to eosinophilic infiltration in chronic cases.

Although an increased eosinophil count is reported in human interstitial lung diseases, such as idiopathic pulmonary fibrosis or sarcoidosis [5], the importance of eosinophils in the pathogenesis of these disorders is uncertain. Idiopathic pulmonary fibrosis in dogs [20] is not associated with eosinophilic infiltrates.

Several drugs have been associated with eosinophilic pneumonia in human beings [21]. Most medications associated with this reaction are antibiotics or nonsteroidal anti-inflammatory drugs [3]. Most cases are isolated, and clinical signs are usually mild and resolve by simply discontinuing the medication [5]. To the authors' knowledge, drug-induced eosinophilic pneumonia has not been reported in dogs.

In humans, idiopathic hypereosinophilic syndrome is a rare illness of unknown cause marked by sustained overproduction of eosinophils and infiltration of multiple organs by mature eosinophils [22]. In dogs, a similar and rare condition is reported, particularly in Rottweilers [23,24]. The disease has to be differentiated from eosinophilic leukemia by bone marrow aspirate [24]. Affected dogs usually display anorexia, depression, and weight loss. Other clinical signs depend on the organs infiltrated by eosinophils and include cough, vomiting, or diarrhea. Some dogs may respond well to prednisolone or hydroxyurea [24,25], although, in general, the prognosis is poor.

In human medicine, eosinophils can be associated with lung lesions that accompany pulmonary vasculitis syndromes. Eosinophilic pulmonary vasculitis is most commonly found in association with primary systemic vasculitis, but primary pulmonary vasculitis, such as Churg-Strauss syndrome, is also reported [5]. Currently, there is no peer-reviewed report in the veterinary literature describing eosinophilic pulmonary vasculitis in the dog, although

eosinophilic pulmonary vasculitis in the dog is suggested in one textbook [26] and the authors have strongly suspected this disease in a few cases with eosinophilic pleural effusion.

In human beings, various diseases have been associated with PE [21], and in dogs, some tumors, such as lymphoma and mast cell tumor, have been associated with eosinophilic pulmonary infiltrate [26].

Idiopathic Parenchymal Disorders

Simple PE (Loeffler pneumonia) in human beings is characterized by migratory pulmonary infiltrates accompanied by peripheral eosinophilia [5]. Respiratory symptoms are minimal or absent, and the disease resolves spontaneously within 4 weeks [5]. A parasitic infection or drug reaction is suspected in many cases, but as many as one third of cases do not have a clinically identifiable cause [21]. Although not reported in the veterinary literature, it is the authors' opinion that this condition exists in the dog based on the observation of several cases of acute and transitory canine EBP that are clinically similar to Loeffler pneumonia in human beings.

Human acute eosinophilic pneumonia is thought to be a unique hypersensitivity reaction to an inhaled antigen [5]. The following diagnostic criteria have been suggested: acute febrile illness of less than 5 to 7 days' duration, hypoxemic respiratory failure, diffuse mixed alveolar and interstitial chest radiographic infiltrates, BALF eosinophilia (>25%), no apparent infectious cause, rapid and complete response to corticosteroid therapy, and no relapse after discontinuation of corticosteroid therapy [27]. A correlate of this condition has not yet been described in dogs.

Human idiopathic chronic eosinophilic pneumonia (ICEP) is a rare disorder of unknown cause characterized by chronic cough, respiratory distress, asthenia, alveolar eosinophilia, and characteristic peripheral alveolar infiltrates on imaging [28]. This disorder is highly responsive to oral corticosteroid therapy; however, relapses are frequent when tapering or after stopping therapy [28]. Moreover, some patients develop severe asthma at some time during the course of disease [29]. EBP in dogs shares some clinical features with human ICEP. Eosinophilic inflammation involves the bronchi in most cases of canine EBP, asthenia is usually absent in EBP, and imaging findings in EBP are not as characteristic as in ICEP [4]. Bronchial hyperactivity has been observed in some dogs with EBP, although EBP is not complicated by asthma. Clinically and pathologically, canine EBP resembles a mixture of human EB and ICEP, with some cases predominantly involving the bronchi and others primarily involving the pulmonary parenchyma.

Canine eosinophilic pulmonary granulomatosis is a disease with no real counterpart in human medicine. This clinical condition usually manifests as progressive cough and respiratory distress with anorexia, weight loss, and lethargy [15]. Radiographic abnormalities are characterized by multiple pulmonary masses of various sizes and hilar lymphadenopathy. The granulomas consist of dense accumulations of large epithelioid cells, macrophages, and eosinophils.

Granulomas may also be found in other organs, such as the liver or kidneys [30]. The response to therapy is poor, and most dogs are euthanized shortly after diagnosis [30]. Occult heartworm disease has been implicated in the pathogenesis of disease in some cases; however, a significant proportion of cases are idiopathic [30,31].

ETIOLOGY AND PATHOGENESIS OF CANINE EOSINOPHILIC BRONCHOPNEUMOPATHY

The cause of canine EBP remains unclear, although hypersensitivity to aeroallergens is suspected [4]. In one study, an intradermal skin test using a panel of 48 standardized allergens, including house dust mite; *Dirofilaria pteronyssinus*; *Dirofilaria farinae*; *Tyrophagus*; human dander; mixed feathers; molds; pollens of grasses, trees, and weeds; and mixed insects, was positive in 4 of 12 dogs with untreated EBP [32]. In another study, 3 dogs with EBP were tested with various antigens and all 3 were negative [1]. The relation between positive intradermal skin testing and documentation of aeroallergens responsible for EBP is difficult to establish. A positive intradermal skin test does not necessarily indicate that the allergen identified is responsible for the pulmonary response. This may be explained by such factors as a difference in mast cell distribution between the lungs and skin or in the route of allergen exposure leading to hypersensitivity. Indeed, there is a discrepancy between localized and systemic immune responses after antigen challenge in the lung [33]. Measurement of serum allergen-specific IgE might provide additional insight into the role of aeroallergens in eosinophilic lung disease, but such measurements have not been conducted to date.

Although the etiology of EBP is still unknown, some of the pathogenesis has been elucidated. In canine EBP, a selective increase in CD4+ T cells and a selective decrease in CD8+ T cells have been demonstrated in BALF [32]. In one dog with EBP, an overrepresentation of CD4+ T cells was confirmed by immunohistochemistry in the bronchial mucosa and pulmonary interstitium [34]. This is similar to the situation in human bronchial asthma, EB, and ICEP, wherein the ratio of CD4+ T cells to CD8+ T cells increases and activated T helper (Th) 2 cells accumulate at sites of inflammation [35–39].

Eosinophilic infiltration and a predominance of CD4+ T cells in BALF support the role for a dominant Th2 immune response in the lower airways in dogs with EBP. Despite this, real-time reverse transcriptase (RT) polymerase chain reaction (PCR) has not confirmed a significant difference in bronchial Th2 cytokine expression in dogs with EBP compared with control animals [40]. The lack of a significant difference between control and diseased dogs is thought to be related to the methodology used. First of all, by using mucosal biopsies, mRNA produced in mucosal T cells may have been diluted in the total mRNA produced by mucosal cell types [40]. Second, RT-PCR methods have recently been improved by assessing RNA quantity and quality before doing the PCR and by using multiple internal control genes for calculation of

gene expression. These changes have been shown to improve the accuracy of results obtained from canine nasal biopsies [41] and must be used to assess bronchial tissue or BALF from dogs with EBP to provide a definitive conclusion. Another way to evaluate the cytokine profile in lower airways would be to determine the cytokine protein concentrations in BALF by using capture ELISA. Antibodies specific for canine cytokines are currently being developed [42], and this method could be used to characterize the immune response in EBP. Improved understanding of the immunopathogenesis of disease should lead to improved treatment modalities.

Quantification of mRNA encoding for several CC-chemokines and one of their receptors (CCR3) [40] has not revealed a significant difference in expression of monocyte chemoattractant protein (MCP)-1, MCP-2, MCP-4, and CCR3 between control dogs and dogs with EBP. Expression of transcript for MCP-3, eotaxin-2, and eotaxin-3 was significantly greater in bronchial biopsies from dogs with EBP than in samples from control dogs, however, and significantly less mRNA encoding for regulated on activation normal T-cell expressed and secreted protein (RANTES) was found in the mucosa of dogs with EBP [40]. Eotaxins are the strongest chemoattractants for eosinophils and basophils [43]. MCP-3 attracts eosinophils but also other cell types, such as monocytes, dendritic cells, basophils, and T cells [44]. Increased mRNA levels for MCP-3, eotaxin-2, and eotaxin-3 in bronchial biopsies from dogs with EBP suggest that these chemokines drive the recruitment of eosinophils and mononuclear cells into the airways in EBP.

The lower airway and parenchymal destruction and remodeling observed in canine EBP is at least partially related to upregulation of collagenolysis and proteolysis. Indeed, collagenase activity of matrix metalloproteinases (MMPs) is increased in BALF from dogs with EBP as compared with that found in BALF from control animals [2,45]. This increased collagenolytic activity is partially attributable to increased activity of MMP-8, MMP-9, and MMP-13 [2,45]. In EBP, these MMPs seem to be produced by macrophages and epithelial cells and not by eosinophils [2,45]. Epithelial laminins are among the proteins that are degraded by MMPs in canine EBP [46], and increased laminin-5γ2-chain degradation products in BALF from these dogs indicate epithelial injury. Epithelial sloughing leading to temporary denudation of the basement membrane is evident histologically at the bronchial and alveolar levels in canine eosinophilic lung disease [46].

Procollagen type III amino terminal propeptide (PIIINP) is a marker of extracellular matrix turnover [47]. A quantitative test to identify PIIINP has been developed in an attempt to evaluate organ fibrosis. This test is a sensitive but nonspecific marker for assessment of tissue collagen type III turnover [48]. High BALF PIIINP concentrations have been found in dogs with EBP [49]. Although further investigations in large populations of dogs with varying bronchopulmonary pathologic findings are warranted, this study suggests that BALF PIIINP could be a promising marker of lung disease in the dog [49]. Higher PIIINP concentrations in serum and BALF of healthy growing dogs

compared with adults might limit the usefulness of PIIINP as a marker of fibrosis in young animals, however.

SIGNALMENT

Dogs affected with EBP are usually young adults (4–6 years of age) [1,2,4,45]. Age at disease onset ranges from 3 months to 13 years, and the interval between disease onset and diagnosis varies from 3 weeks to 6 years [1,2,4]. A breed predisposition for Siberian Huskies and Alaskan Malamutes was present in one study [4], but the disease is found in other large breeds (eg, Labrador Retrievers, Rottweilers, German Shepherds) as well as in small breeds (eg, Fox and Jack Russell Terriers, Dachshunds). The weight of affected dogs varies from 4 to 50 kg [1,2,4]. A gender bias has been reported, with female dogs apparently more frequently affected than male dogs in a proportion of 1.3:3 [2,4,32], although an older study mentions a proportion of 0.5:1 [10]. Interestingly, human patients diagnosed with ICEP are twice as likely to be female [28].

CLINICAL SIGNS

At initial presentation, cough is the most common clinical sign, occurring in 95% to 100% of dogs [1,2,4]. The cough is usually harsh and sonorous, persistent, and frequently followed by gagging and retching. Early in the course of disease, gagging and retching might be confused with a disorder of the digestive tract [4]. Other clinical signs frequently reported include respiratory difficulty and exercise intolerance. Nasal discharge is present in up to 50% of cases; it can be serous, mucoid, or mucopurulent and can be associated with a concomitant eosinophilic rhinitis in some cases [4]. General systemic health is not always affected [1,2,4] unless concomitant disease is present. Pruritus, with or without skin lesions, is another clinical complaint that is occasionally reported [1]. On physical examination, thoracic auscultation can be normal but increased lung sounds, wheezes, or crackles are often found [1,4].

DIAGNOSIS

EBP may be suspected based on signalment, history of a positive response to corticosteroids, and clinical signs. Diagnosis relies on radiographic and bronchoscopic findings, blood eosinophilia, tissue eosinophilic infiltration demonstrated by cytology of BALF or histopathologic examination of bronchial biopsies, and exclusion of known causes of eosinophilic infiltration of the lower airways. The diagnosis of EBP must be confirmed before treatment is initiated, because long-term corticosteroids are needed to control clinical signs of disease in most cases.

Thoracic Radiography

Diffuse radiographic infiltrates of variable intensity are found in dogs with EBP and are generally more severe than those found in dogs with chronic bronchitis. The most frequently encountered pattern is a mixed moderate to severe

bronchointerstitial pattern. Peribronchial cuffing is a frequent lesion (in approximately 20% of cases) as well as marked thickening of the bronchial walls [4]. Alveolar infiltration is also common and can be identified in up to 40% of the cases [4,32]. Bronchiectasis is commonly encountered in chronic cases [4,10]. The radiographic severity score correlates significantly with the BALF total cell count and eosinophil count but not with the blood eosinophil count [2]. Radiographic features are illustrated in Fig. 1.

Hematology

Hematologic abnormalities include leukocytosis in 30% to 50% of the cases, eosinophilia in 50% to 60%, neutrophilia in 25% to 30%, and basophilia in 0% to 55% [1,2,4]. Absence of peripheral eosinophilia does not exclude a diagnosis of EBP [4]. Similarly, in people with ICEP, blood eosinophilia is not a constant finding [28]. Dogs with EBP generally have normal serum biochemistry values.

Airway Evaluation

Airway sampling is necessary to confirm a diagnosis of EBP through cytologic assessment and exclusion of infection. Collection of an airway sample by tracheal wash or bronchoscopy can be used to confirm the diagnosis. Bronchoscopy is particularly useful because it allows identification of eosinophilic infiltration in BALF or in mucosal biopsies. Bronchoscopy also allows observation of macroscopic findings typical of EBP and the detection of possible concomitant bacterial infection that requires prompt treatment before initiating therapy for EBP itself. Bronchoscopic examination is performed under general anesthesia, using a flexible bronchoscope.

Bronchoalveolar lavage (BAL) is considered to be a safe procedure in dogs, although in a single case report, a dog with EBP developed severe respiratory distress after BAL, presumably because of eosinophil degranulation and severe bronchoconstriction after BAL. The dog required mechanical ventilation for almost 24 hours along with anti-inflammatory and bronchodilator medications
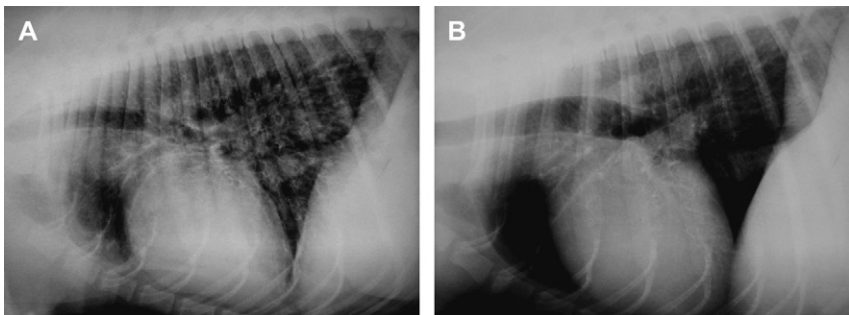

Fig. 1. (*A*) Right lateral projection of the thorax shows a severe bronchointerstitial pattern in a dog with EBP. (*B*) The same dog after treatment with oral corticosteroids.

for full recovery [50]. Therefore, careful monitoring of cardiac and respiratory parameters is recommended when performing bronchoscopy, particularly if EBP is suspected.

Macroscopic findings

The macroscopic bronchoscopic features defined in EBP include (1) the presence of a moderate to large amount of yellow-green secretions; (2) mucosal changes, such as moderate to severe thickening of the mucosa with an irregular or polypoid appearance; (3) dramatic airway hyperemia; and (4) less often, exaggerated concentric airway closure during expiration [2,4,32]. Endoscopic features are illustrated in Fig. 2.

Bronchoalveolar lavage

Cytology. BALF must be centrifuged or cytocentrifuged immediately to obtain good-quality cytologic samples. Alternatively, a protected catheter brush can be inserted through the biopsy channel of the bronchoscope to obtain material for cytology. BALF cytology detects local eosinophilic infiltration more reliably
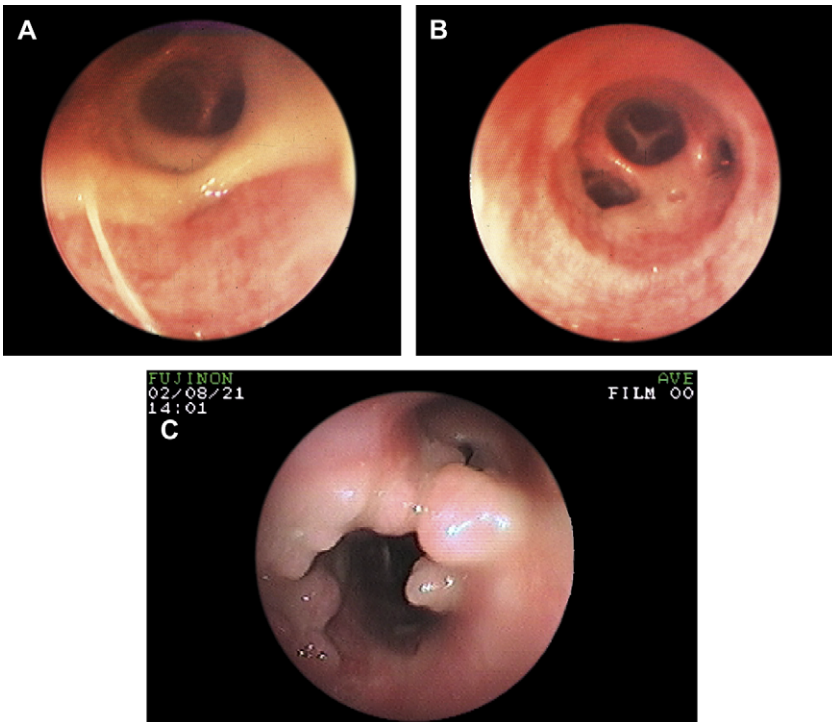

Fig. 2. Endoscopic view of the bronchi of dogs with EBP shows thick yellow material (*A*), thickening of the mucosa with an irregular surface (*B*), and polypoid appearance of the mucosa (*C*).

than brush cytology, and this is likely attributable to the larger area sampled with BAL than with a brush [32]. Using either technique, a cytologic grade can be assigned, based on the percentage of eosinophils. Normal cell counts in BALF range from 200 to 400 cells/µL, with macrophages predominating (65%–70% of the total count). EBP is characterized by an increase in the total number of cells in BALF as well as an increase in the percentage of eosinophils and neutrophils (Fig. 3) [2,4]. Less than 5% eosinophils are generally found in the BALF from healthy dogs, although there seems to be a population of clinically normal dogs with high relative (up to 24%) or absolute eosinophil counts in BALF [2,4,51]. This might be a result of parasitic burden among various facilities or of genetic differences between individuals. Siberian Huskies seem predisposed to a high number of eosinophils in blood and BALF in the absence of obvious clinical signs of inflammation (Cécile Clercx, DVM, PhD, unpublished data, 2000), and care must be taken when interpreting differential cell counts from BALF.

Cytology of BALF can also be helpful to rule out other disease processes; parasitic eggs or larvae, *Toxoplasma gondii* tachyzoites [52], or tumor cells [51] can be detected, and the presence of intracellular bacteria allows identification of an infectious process [53].

Microbiology. The central airways of healthy dogs are not sterile, and in dogs with suspected EBP, it is important to get an accurate assessment of bacteria in the BALF by submitting a quantitative bacterial culture [53]. Pulmonary bacterial infection is uncommon in dogs with EBP, but it should be promptly recognized and treated before initiating therapy with glucocorticoids [32]. It is common for dogs with EBP to have received antibiotic therapy before presentation based on a positive bacterial culture or because of the presence of an
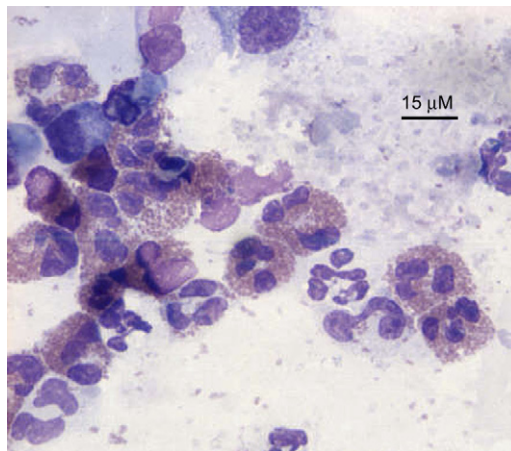

Fig. 3. Bronchoalveolar lavage cytology from a dog with EBP (Wright-Giemsa stain, original magnification ×150). The percentage of eosinophils (n = 100) was more than 50%.

alveolar pattern on thoracic radiographs; however, the clinical response is minimal at best.

A fumigatus was cultured from the BALF of two dogs with EBP [4], but because cytology and histopathologic examination failed to identify the organism, these positive cultures were considered contaminants. Fungal culture of BALF is not routinely recommended for dogs with EBP.

Histopathologic findings. Perendoscopic mucosal bronchial biopsies are used for histopathologic examination. Histopathologic findings are graded according to severity: grades 1, 2, and 3 correspond to eosinophilic infiltrate with mild, moderate, and severe inflammatory changes, respectively (Fig. 4) [4]. Hyperplasia, squamous metaplasia, epithelial ulceration, microhemorrhage, hemosiderin-laden macrophages, collagenolysis, and fibrosis can also be seen in grade 3 EBP [4]. Unfortunately, cytologic grade based on BAL analysis and histopathologic grade do not seem to be correlated [4,32].
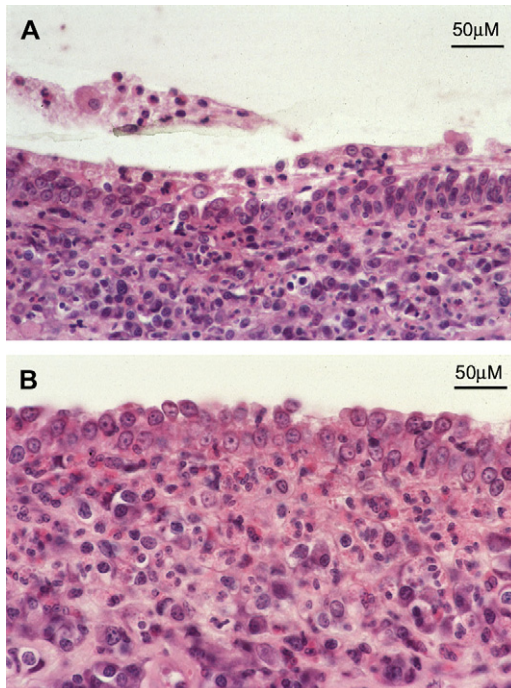

Fig. 4. (*A*) Histopathologic examination of a bronchial mucosal biopsy from a dog with EBP revealed moderate inflammation (grade 2) with extravasation of eosinophils from superficial mucosal vessels and migration of these cells through the respiratory epithelium into the bronchial lumen (hematoxylin and eosin, original magnification ×187). (*B*) Eosinophils within the mucosa are accompanied by plasma cells, lymphocytes, macrophages, and mast cells (hematoxylin and eosin, original magnification ×187). (*From* Clercx C, Peeters D, Snaps F, et al. Eosinophilic bronchopneumopathy in dogs. J Vet Intern Med 2000;14(3):282–91; with permission.)

Rhinoscopy

In dogs with concomitant nasal discharge, the nasal cavities should be investigated using a rhinoscope and samples obtained for bacterial and cytologic examinations (brush or imprint cytology). Rhinoscopy may reveal congested and edematous mucosa, mucoid or mucopurulent secretions, and polypoid proliferations in severe cases [4]. Brush cytology or histopathologic examination typically reveals the presence of eosinophils.

Parasitic Analysis

Because eosinophilic pneumonia can be caused by occult heartworm disease [14], it is strongly advised to run a heartworm antigen test in endemic areas or in dogs that have traveled to an endemic area [54].

Helminth parasites are implicated in eosinophilic bronchopulmonary reactions through primary infection or by migration through lung tissue during development [18]. Zinc sulfate centrifugation-flotation and Baermann sedimentation of feces are advised, because these tests detect eggs or larvae for most pulmonary parasites. A negative fecal examination by either method is not conclusive, however, because a single fecal examination detects only 30% to 70% of active infections [55]. It is therefore advised to repeat the fecal examination in suspect cases or to treat against potential parasites using a course of an appropriate antihelminthic (eg, fenbendazole, thiabendazole, levamisole). In these cases, a short course of prednisolone may be required to suppress the associated hypersensitivity reaction.

Intradermal Skin Testing

Searching for potential aeroallergens could be considered as part of a complete investigation of inciting factors for eosinophilic inflammation, although results are open to interpretation. Intradermal skin testing must be performed before treatment with corticosteroids.

Pulmonary Function Tests

Arterial blood gas analysis is a valuable test that provides insights into the severity of pulmonary dysfunction in animals with parenchymal disease. Mild decreased values in PaO_2 and increased values in the alveolar-arterial oxygen gradient (A-aDO_2) have been described in dogs with EBP as compared with healthy animals [2]. Arterial blood gas analysis does not allow differentiation between EBP and other diseases, however.

PFTs are used extensively in human medicine to evaluate and diagnose pulmonary diseases as well as to monitor the response to therapy [56]. This is especially true in allergic or eosinophilic disorders, in which a bronchospastic component is one of the hallmarks of the disease process. Unfortunately, most PFTs require conscious maneuvers (eg, maximal expiration) that are not possible in animals.

Pulmonary mechanics can be investigated by various methods, but most techniques require anesthesia. Static respiratory compliance was measured in five anesthetized dogs with EBP and was decreased in two of them, presumably

because of the presence of infiltrates around airways and in the lung parenchyma that made the lung less distensible [1]. Noninvasive PFTs that do not require patient cooperation, and are therefore suitable for clinical purposes; they have been described in dogs in the past few years, including tidal breathing flow volume loops and whole-body barometric plethysmography (BWBP) [57,58]. Tidal breathing flow volume loops have proven useful for detecting upper airway obstruction in conscious dogs [57,59] and have revealed expiratory flow limitation in dogs with bronchitis [57] but have not been examined in dogs with EBP. BWBP is a noninvasive PFT that allows measurement of airway reactivity in unrestrained, conscious, and spontaneously breathing animals [58,60,61]. Based on preliminary assessment of bronchoreactivity using BWBP, it seems that some dogs with EBP may have active bronchoconstriction rather than passive airway collapse (Cécile Clercx, DVM, PhD, unpublished data, 2005). Such measurements need to be performed in a larger number of dogs with EBP before and after treatment to provide definitive conclusions.

TREATMENT

The treatment of choice for canine EBP is oral corticosteroid therapy (methylprednisolone) initiated at a dose of 1 mg/kg administered orally twice daily during the first week. This dose is then given on alternate days during the second week, and further reduced to 1 mg/kg administered orally daily on alternate days during the third week. If clinical signs remain well controlled, the dose is gradually decreased until maintenance levels are achieved [2–4]. In one study, the maintenance dose of prednisolone ranged between 0.125 mg/kg and 0.5 mg/kg every other day or even every 3 or 4 days [4]. The response to steroid therapy is generally good [2–4]. Cough, respiratory difficulty, and exercise intolerance begin to improve within days, although full resolution of clinical signs can take months. Nasal discharge is sometimes more refractory to steroid treatment. During steroid therapy, blood eosinophilia and eosinophilic inflammation in BALF or bronchial biopsies improve or resolve. Radiographic and bronchoscopic scores also improve, although chronic lesions often persist [2,4,32]. Finally, steroid therapy results in normalization of the increased CD4+ T cells/CD8+ T cells found in BALF before treatment [32].

Relapse of clinical signs can occur within weeks or months after drug discontinuation, but some dogs seem to be cured by steroid therapy [1,32]. In a study in which dogs were treated with corticosteroids for 8 weeks, 6 of 20 dogs relapsed and needed immediate reinstitution of therapy [2]. This could indicate that a longer period of tapering medication might be required in some dogs.

The time from onset of clinical signs until diagnosis does not seem to influence the response to treatment, because even patients with chronic or severe forms of EBP showed a positive response to medical therapy. In one report, younger patients were more difficult to manage [26]; however, in the authors' experience, age at the time of diagnosis does not influence the response to treatment [4]. The poorest response to treatment has been reported in cases treated with high doses of glucocorticoids that are abruptly discontinued or in those

treated with irregular parenteral administration of depository steroid injections [26].

In most cases, the response to corticosteroid therapy is considered to be satisfactory. Despite a gradual decrease in dosage, however, some animals still require relatively high doses of glucocorticoids to control signs, and weight gain, polyuria or polydipsia, and panting become undesirable side effects. In other animals, the use of glucocorticoids is contraindicated because of health problems, such as diabetes mellitus or obesity. In these cases, inhaled steroids could prove beneficial. Medications given by means of inhalation offer the advantage of high drug concentrations within the airways while attenuating systemic side effects. Inhaled corticosteroids (eg, fluticasone propionate) have been used successfully in cats for the management of experimental bronchitis by utilizing a low-resistance spacer device connected to a face mask [62,63]. A recent study has shown that inhaled corticosteroids can be used for the management of chronic bronchitis and EBP in dogs [64]. Further prospective studies are warranted in larger numbers of animals to define optimum treatment protocols and to investigate potential side effects.

Novel therapies might also need to be considered. Although the role of aeroallergens in EBP is unclear, hyposensitization directed against allergens identified by skin testing has resulted in clinical improvement in rare cases (B.C. McKiernan, unpublished data, 2004). Cyclosporine, a cyclic oligopeptide macrolide that possesses immunomodulating properties, is a drug that has been used successfully in the treatment of canine atopic dermatitis [65]. Although the drug is expensive, it could be an interesting drug to try in dogs with EBP that cannot tolerate glucocorticoids. No trial results are available to date.

Recent advances in molecular biology have enhanced our understanding of the mechanisms by which eosinophils are recruited to the lungs and have led to the discovery of new potential drug targets. New therapeutic strategies based on the use of immunomodulatory substances are being investigated in human patients with bronchial asthma and in murine models of disease. Those medications include (1) drugs that suppress the effects of certain interleukins (ILs), specifically IL-5 [66] or IL-13 [67]; (2) compounds that interfere with the main receptor involved in the recruitment of eosinophils (CCR3) [68,69]; and (3) CpG oligodeoxynucleotides that direct the inflammatory reaction toward a Th1 type [70]. In the future, these novel therapies might be applied to canine EBP.

Several new and intriguing agents that might be useful in eosinophilic lung disease exist on the horizon. Given the remarkable efficacy of oral corticosteroids in the treatment of EBP, however, potential new therapies need to be rigorously proven to be superior to corticosteroids before a change in standard practice is advised [3].

In conclusion, canine EBP is a disease characterized by eosinophilic infiltration of the lung and bronchial mucosa. Although the etiology of EBP is still unknown, the presence of eosinophilic infiltration and a predominance of CD4+ T cells suggest a Th2 immune response mounted in the lower airways.

Hypersensitivity to aeroallergens must be considered as a potential etiology. Additional studies of the tissue cytokine expression profile and of allergen-specific IgE are needed to confirm this hypothesis. The prognosis for dogs with EBP is usually good, because the response to oral corticosteroid therapy is excellent in most cases, although systemic side effects of steroids can be limiting.

References

[1] Corcoran BM, Thoday KL, Henfrey JI, et al. Pulmonary infiltration with eosinophils in 14 dogs. J Small Anim Pract 1991;32(10):494–502.

[2] Rajamaki MM, Jarvinen AK, Sorsa T, et al. Clinical findings, bronchoalveolar lavage fluid cytology and matrix metalloproteinase-2 and -9 in canine pulmonary eosinophilia. Vet J 2002;163(2):168–81.

[3] Norris C, Mellema M. Eosinophilic pneumonia. In: King L, editor. Textbook of respiratory disease in dogs and cats. St Louis (MO): Saunders; 2004. p. 541–7.

[4] Clercx C, Peeters D, Snaps F, et al. Eosinophilic bronchopneumopathy in dogs. J Vet Intern Med 2000;14(3):282–91.

[5] Alberts WM. Eosinophilic interstitial lung disease. Curr Opin Pulm Med 2004;10(5): 419–24.

[6] Shepherd JM, Duddleston DN, Hicks GS, et al. Asthma: a brief overview. Am J Med Sci 2002;324(4):174–9.

[7] Brightling CE, Symon FA, Birring SS, et al. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. Thorax 2003;58(6):528–32.

[8] Dicpinigaitis PV. Cough. 4: cough in asthma and eosinophilic bronchitis. Thorax 2004; 59(1):71–2.

[9] Park SW, Lee YM, Jang AS, et al. Development of chronic airway obstruction in patients with eosinophilic bronchitis: a prospective follow-up study. Chest 2004;125(6): 1998–2004.

[10] Brownlie SE. A retrospective study of diagnoses in 109 cases of canine lower respiratory-disease. J Small Anim Pract 1990;31(8):371–6.

[11] Zander DS. Allergic bronchopulmonary aspergillosis: an overview. Arch Pathol Lab Med 2005;129(7):924–8.

[12] Katzenstein AL, Liebow AA, Friedman PJ. Bronchocentric granulomatosis, mucoid impaction, and hypersensitivity reactions to fungi. Am Rev Respir Dis 1975;111(4):497–537.

[13] van der Klooster JM, Nurmohamed LA, van Kaam NA. Bronchocentric granulomatosis associated with influenza-A virus infection. Respiration 2004;71(4):412–6.

[14] Calvert CA, Losonsky JM. Pneumonitis associated with occult heartworm disease in dogs. J Am Vet Med Assoc 1985;186(10):1097–8.

[15] Calvert CA. Eosinophilic pulmonary granulomatosis. In: Kirk R, Bonagura J, editors. Current veterinary therapy XI. Philadelphia: WB Saunders; 1992. p. 813–6.

[16] Martin MWS, Ashton G, Simpson VR, et al. Angiostrongylosis in Cornwall—clinical presentations of 8 cases. J Small Anim Pract 1993;34(1):20–5.

[17] Chapman P, Boag A, Guitian J, et al. *Angiostrongylus vasorum* infection in 23 dogs (1999–2002). J Small Anim Pract 2004;45:435–40.

[18] Taboada J. Pulmonary diseases of potential allergic origin. Semin Vet Med Surg (Small Anim) 1991;6(4):278–85.

[19] Noone K. Pulmonary hypersensitivities. In: Kirk R, editor. Current veterinary therapy IX. Philadelphia: WB Saunders; 1986. p. 285–92.

[20] Corcoran BM, Cobb M, Martin MW, et al. Chronic pulmonary disease in West Highland white terriers. Vet Rec 1999;144(22):611–6.

[21] Allen JN, Davis WB. Eosinophilic lung diseases. Am J Respir Crit Care Med 1994;150(5 Pt 1): 1423–38.

[22] Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. Blood 1994;83(10): 2759–79.

[23] Aroch I, Perl S, Markovics A. Disseminated eosinophilic disease resembling idiopathic hypereosinophilic syndrome in a dog. Vet Rec 2001;149(13):386–9.

[24] Sykes JE, Weiss DJ, Buoen LC, et al. Idiopathic hypereosinophilic syndrome in 3 Rottweilers. J Vet Intern Med 2001;15(2):162–6.

[25] Perkins M, Watson A. Successful treatment of hypereosinophilic syndrome in a dog. Aust Vet J 2001;79(10):686–9.

[26] Bauer T. Pulmonary hypersensitivity disorders. In: Kirk R, editor. Current veterinary therapy X. Philadelphia: WB Saunders; 1989. p. 369–76.

[27] Pope-Harman AL, Davis WB, Allen ED, et al. Acute eosinophilic pneumonia. A summary of 15 cases and review of the literature. Medicine (Baltimore) 1996;75(6):334–42.

[28] Marchand E, Reynaud-Gaubert M, Lauque D, et al. Idiopathic chronic eosinophilic pneumonia. A clinical and follow-up study of 62 cases. The Groupe d'Etudes et de Recherche sur les Maladies ''Orphelines'' Pulmonaires (GERM''O''''P''). Medicine (Baltimore) 1998;77(5): 299–312.

[29] Marchand E, Etienne-Mastroianni B, Chanez P, et al. Idiopathic chronic eosinophilic pneumonia and asthma: how do they influence each other? Eur Respir J 2003;22(1): 8–13.

[30] Calvert CA, Mahaffey MB, Lappin MR, et al. Pulmonary and disseminated eosinophilic granulomatosis in dogs. J Am Anim Hosp Assoc 1988;24(3):311–20.

[31] von Rotz A, Suter MM, Mettler F, et al. Eosinophilic granulomatous pneumonia in a dog. Vet Rec 1986;118(23):631–2.

[32] Clercx C, Peeters D, German AJ, et al. An immunologic investigation of canine eosinophilic bronchopneumopathy. J Vet Intern Med 2002;16(3):229–37.

[33] Bice DE, Jones SE, Muggenburg BA. Long-term antibody production after lung immunization and challenge: role of lung and lymphoid tissues. Am J Respir Cell Mol Biol 1993;8(6): 662–7.

[34] Peeters D, Day MJ, Clercx C. An immunohistochemical study of canine eosinophilic bronchopneumopathy. J Comp Pathol 2005;133(2–3):128–35.

[35] Brightling CE, Symon FA, Birring SS, et al. T(H)2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. J Allergy Clin Immunol 2002;110(6):899–905.

[36] Robinson DS, Bentley AM, Hartnell A, et al. Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial responsiveness. Thorax 1993;48(1):26–32.

[37] Robinson DS, Hamid Q, Ying S, et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992;326(5):298–304.

[38] Walker C, Bauer W, Braun RK, et al. Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. Am J Respir Crit Care Med 1994;150(4):1038–48.

[39] Albera C, Ghio P, Solidoro P, et al. Activated and memory alveolar T-lymphocytes in idiopathic eosinophilic pneumonia. Eur Respir J 1995;8(8):1281–5.

[40] Peeters D, Peters IR, Clercx C, et al. Real-time RT-PCR quantification of mRNA encoding cytokines, CC chemokines and CCR3 in bronchial biopsies from dogs with eosinophilic bronchopneumopathy. Vet Immunol Immunopathol 2006;110(1-2):65–77.

[41] Peeters D, Peters IR, Helps CR, et al. Distinct tissue cytokine and chemokine mRNA expression in canine sino-nasal aspergillosis and idiopathic lymphoplasmacytic rhinitis. Vet Immunol Immunopathol 2007;117(1-2):95–105. doi:10.1016/vetimm.2007.01.018.

[42] de Lima VM, Peiro JR, de Oliveira Vasconcelos R. IL-6 and TNF-alpha production during active canine visceral leishmaniasis. Vet Immunol Immunopathol 2007;115(1–2): 189–93.

[43] Lloyd C. Chemokines in allergic lung inflammation. Immunology 2002;105(2):144–54.

[44] Lukacs NW. Role of chemokines in the pathogenesis of asthma. Nat Rev Immunol 2001;1(2):108–16.

[45] Rajamaki MM, Jarvinen AK, Sorsa T, et al. Collagenolytic activity in bronchoalveolar lavage fluid in canine pulmonary eosinophilia. J Vet Intern Med 2002;16(6):658–64.

[46] Rajamaki MM, Jarvinen AK, Sorsa TA, et al. Elevated levels of fragmented laminin-5 gamma2-chain in bronchoalveolar lavage fluid from dogs with pulmonary eosinophilia. Vet J 2006;171(3):562–5.

[47] Risteli L, Risteli J. Analysis of extracellular matrix proteins in biological fluids. Methods Enzymol 1987;145:391–411.

[48] Jensen LT. The aminoterminal propeptide of type III procollagen. Studies on physiology and pathophysiology. Dan Med Bull 1997;44(1):70–8.

[49] Schuller S, Valentin S, Remy B, et al. Analytical, physiologic, and clinical validation of a radioimmunoassay for measurement of procollagen type III amino terminal propeptide in serum and bronchoalveolar lavage fluid obtained from dogs. Am J Vet Res 2006;67(5): 749–55.

[50] Cooper ES, Schober KE, Drost WT. Severe bronchoconstriction after bronchoalveolar lavage in a dog with eosinophilic airway disease. J Am Vet Med Assoc 2005;227(8): 1257–62.

[51] Hawkins EC, DeNicola DB, Kuehn NF. Bronchoalveolar lavage in the evaluation of pulmonary disease in the dog and cat. State of the art. J Vet Intern Med 1990;4(5):267–74.

[52] Hawkins EC, Davidson MG, Meuten DJ, et al. Cytologic identification of toxoplasma gondii in bronchoalveolar lavage fluid of experimentally infected cats. J Am Vet Med Assoc 1997;210(5):648–50.

[53] Peeters DE, McKiernan BC, Weisiger RM, et al. Quantitative bacterial cultures and cytological examination of bronchoalveolar lavage specimens in dogs. J Vet Intern Med 2000;14(5):534–41.

[54] Atkins C. Canine heartworm disease. In: Ettinger S, Feldman E, editors. Textbook of veterinary internal medicine. St. Louis (MO): Elsevier Saunders; 2005. p. 1118–36.

[55] Sherding R. Parasites of the lung. In: King L, editor. Textbook of respiratory diseases in dogs and cats. St. Louis (MO): Saunders; 2004. p. 548–59.

[56] Crapo RO. Pulmonary-function testing. N Engl J Med 1994;331(1):25–30.

[57] Amis TC, Kurpershoek C. Tidal breathing flow-volume loop analysis for clinical assessment of airway obstruction in conscious dogs. Am J Vet Res 1986;47(5):1002–6.

[58] Talavera J, Kirschvink N, Schuller S, et al. Evaluation of respiratory function by barometric whole-body plethysmography in healthy dogs. Vet J 2006;172(1):67–77.

[59] Amis TC, Smith MM, Gaber CE, et al. Upper airway obstruction in canine laryngeal paralysis. Am J Vet Res 1986;47(5):1007–10.

[60] Hoffman AM, Dhupa N, Cimetti L. Airway reactivity measured by barometric whole-body plethysmography in healthy cats. Am J Vet Res 1999;60(12):1487–92.

[61] Kirschvink N, Leemans J, Delvaux F, et al. Non-invasive assessment of growth, gender and time of day related changes of respiratory pattern in healthy cats by use of barometric whole body plethysmography. Vet J 2006;172(3):446–54.

[62] Reinero CR, Decile KC, Byerly JR, et al. Effects of drug treatment on inflammation and hyperreactivity of airways and on immune variables in cats with experimentally induced asthma. Am J Vet Res 2005;66(7):1121–7.

[63] Kirschvink N, Leemans J, Delvaux F, et al. Inhaled fluticasone reduces bronchial responsiveness and airway inflammation in cats with mild chronic bronchitis. J Feline Med Surg 2006;8(1):45–54.

[64] Bexfield NH, Foale RD, Davison LJ, et al. Management of 13 cases of canine respiratory disease using inhaled corticosteroids. J Small Anim Pract 2006;47(7):377–82.

[65] Steffan J, Favrot C, Mueller R. A systematic review and meta-analysis of the efficacy and safety of cyclosporin for the treatment of atopic dermatitis in dogs. Vet Dermatol 2006; 17(1):3–16.

[66] Kips JC, Tournoy KG, Pauwels RA. New anti-asthma therapies: suppression of the effect of interleukin (IL)-4 and IL-5. Eur Respir J 2001;17(3):499–506.
[67] Zimmermann N, Hershey GK, Foster PS, et al. Chemokines in asthma: cooperative interaction between chemokines and IL-13. J Allergy Clin Immunol 2003;111(2):227–42.
[68] Shen HH, Xu F, Zhang GS, et al. CCR3 monoclonal antibody inhibits airway eosinophilic inflammation and mucus overproduction in a mouse model of asthma. Acta Pharmacol Sin 2006;27(12):1594–9.
[69] Fortin M, Ferrari N, Higgins ME, et al. Effects of antisense oligodeoxynucleotides targeting CCR3 on the airway response to antigen in rats. Oligonucleotides 2006;16(3):203–12.
[70] Hessel EM, Chu M, Lizcano JO, et al. Immunostimulatory oligonucleotides block allergic airway inflammation by inhibiting Th2 cell activation and IgE-mediated cytokine induction. J Exp Med 2005;202(11):1563–73.