

Eosinophils in lung tissue in 2 Chilean Criollo horses with exacerbated recurrent airway obstructions “heaves”

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Abstract: We report on 2 cases of recurrent airway obstruction (RAO) observed in Chilean Criollo horses aged 13 and 15 years. Both animals were classified as having severe RAO. The analysis of the recovered cells of bronchoalveolar lavage fluid (BALF) showed high percentages of eosinophils (82% and 80% in the differential cell counts of the 2 horses) and poor responses to bronchodilator and corticosteroid drugs. The histopathological lung evaluation showed eosinophilic infiltration in the peribronchial tissue, as well as in the lumen of the bronchi and bronchioli. In both horses, immunohistochemistry confirmed high p65 homodimer activity in bronchial cells. This report describes exacerbated heaves with eosinophilic infiltration and p65 activity in the lung tissue without any neutrophil infiltration, which is usually characteristic for this disease.

Key words: Heaves, eosinophils, p65 homodimers, Chilean Criollo horses

Introduction

Heaves, also known as recurrent airway obstruction (RAO), is a severe respiratory disorder of mature stabled horses. Heaves is thought to be caused by an abnormal immune response to inhaled antigens, modulated by T lymphocytes through proinflammatory cytokine secretions (1). It has been suggested that the immunological basis of RAO is IgE-mediated type I hypersensitivity, as well as type III hypersensitivity (2). Recent findings suggest that pulmonary helper T lymphocytes may play a major role in heaves by secreting Th1-type or Th2-type cytokines (1,3-6). Transcription factors are cell-specific and are crucial in cell differentiation, as well

as in the regulation of specific cellular processes such as proliferation, enzyme production, and cytokine expression. The airway epithelial cells in RAO-affected horses exposed to moldy hay showed an increase in NF- κ B activity, and in particular, showed an increase in p65 homodimer activity (7,8). The main lesion in heaves is bronchiolitis; a peribronchiolar accumulation of lymphocytes accompanied by an intraluminal accumulation of neutrophils (2). Early removal of hay exposure may cause a remission of clinical signs, whereas continued exposure may lead to irreversible airflow limitation. Therefore, heaves, like asthma in human beings, may involve a remodeling of the airway wall as part of its pathogenesis (9),

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resulting in hypertrophied airway smooth muscles, goblet cell metaplasia in the bronchiolar epithelia, and some alveolar fibrosis. In this report, we describe the histological characteristics of 2 RAO-affected horses with high eosinophilic infiltration and p65 activity in the lung tissue.

Materials and methods

An 18-year-old male horse and a 15-year-old female horse were presented for evaluation of respiratory distress. These horses had pre-existing RAO, but there were no clinical history data concerning previous diseases or pharmacological treatments. The anamnesis revealed that prior to the evaluation, these horses were kept in a highly dusty environment and a poorly ventilated stable. Complete physical examinations and clinical measurements were performed on the horses, including rectal temperature, heart rate, respiratory rate, evaluation of hydration, gut motility, and an auscultation of the heart and lungs. Clinical RAO scores were obtained as previously described (10); nostril flare and abdominal movement were scored separately on a scale of 1 (normal) to 4 (severe signs). To determine the total clinical score, the score for nostril flaring and abdominal movement were added. Therefore, the total clinical score could range from 2 (normal) to 8 (severe signs). Hematologic and biochemical analyses, upper airway endoscopies, and bronchoalveolar lavage fluid (BALF) evaluations were performed on each horse. In both animals, the heaves showed clinical scores of 7 points (severe signs) and were associated with great dyspnea, dry cough, abundant nasal secretion, and respiratory difficulty. The analysis of the recovered cells of BALF showed high percentages of eosinophils (82% and 80% in the differential cell counts in the 2 horses). Endoscopies were performed to evaluate mucus in the trachea and also to evaluate the carina. Both horses showed abundant viscous mucosal exudates, with mucosal edema and hyperemia in the airways. The endoscopic, hematologic and biochemical analyses, clinical examinations, and BALF ruled out other pathology and lung parasitism. The horses were kept on the pasture during the treatment. They were administered 2000 mg of fluticasone propionate (Flovent, Glaxo Wellcome, Mississauga,

Ont., Canada) and 210 mg of salmeterol xinafoate (Serevent, Glaxo Wellcome, Mississauga, Ont., Canada) at 12 h intervals from a metered dose inhaler via commercially available masks (Equine AeroMask, Trudell Medical International, Canada). The treatment was continued for 5 days; however, the clinical outcomes were not positive after treatment in the 2 horses. Because of this, as well as financial reasons, the owners signed consent forms authorizing euthanasia on the animals. Immediately after their deaths, necropsies were performed, and lung samples were taken to avoid post-mortem changes. The lung tissue samples of both horses were fixed in 10% formalin and embedded in paraffin for histopathological evaluations. For this purpose, tissue sections were stained with hematoxylin and eosin.

In addition, immunohistochemistry was carried out with anti-p65 antibodies for pulmonary cells. The PAP (peroxidase-antiperoxidase) method previously described by Sternberger and others (11) was applied. Polyclonal rabbit D2406 anti-p65 antibodies (Santa Cruz, Biotechnology) were applied at a 1:100 diluted concentration for 2 h. The sections were then rinsed in PBS-Tween, and the biotinylated secondary antibodies of antirabbit IgG (DAKO K0675) were applied for 15 min at room temperature. The sections were then rinsed again with PBS-Tween, and a horseradish peroxidase-streptavidin conjugate (DAKO K0675) was applied for 15 min at room temperature. The sections were rinsed and then incubated with the DAB substrate (Research Genetics, 2130 Memorial Parkway, Huntsville, AL, USA) for 10 min. All sections were rinsed with buffer and then counterstained with Mayer's hematoxylin. The sections were dehydrated with graded alcohols, cleared in toluene, and mounted in Permount. As the negative control for the immunoreactions, incubation with the primary antibody was omitted. Furthermore, the primary antibody was incubated with a lung sample from a normal horse to rule out nonspecific immunoreactions. As confirmation, all controls were indeed negative for immunoreactions.

Results and discussion

Hay/straw exposure induced clinical airway obstruction in RAO-affected horses, which

was consistent with previous findings (12). The macroscopic and microscopic lesions in both RAO-affected horses were circumscribed to the lung. The macroscopic lesions were not found in the other organs of these animals. Moreover, there were no lesions attributable to the migration of parasites in the caudate lobe and diaphragm. The lungs of both animals showed bright surfaces and were hyperemic and edematous, without the presence of pneumonic foci or pulmonary consolidation. There was also the presence of serous fluid, which was transparent and noncoagulated when in contact with air. Some authors suggest that in equines with RAO, the pathological change is mainly bronchioli inflammation, which is usually observed in the periphery of the lung; however, this is subject to debate (2). In the microscopic findings in RAO-affected horses, there is usually an infiltration of inflammatory cells (mainly lymphocytes) in the peribronchial tissue and a large neutrophil infiltration in the lumen (13). However, these 2 horses had large eosinophil infiltrations in the peribronchial tissue (Figure 1), as well as in lumen of the bronchioli (Figure 2). These cells are rarely observed in this disease (2). The infiltration of eosinophils is an uncommon finding, usually attributable to infections with *Dictyocaulus arnfeldi* or ascaris migration. Idiopathic pulmonary eosinophilia, which is not associated with helminth infections, has been recognized as a rare cause of this finding in horses (14). Multisystemic eosinophilic epitheliotropic disease (MEED) occurs primarily in young horses, ranging in age from 3 to 13 years (15,16). However, horses with RAO do occasionally present with acute eosinophilic pulmonary disease (17). Similarly, other authors (18) have also reported 1 case among 20 RAO-affected Chilean Chilote horses that showed high levels of eosinophils in BALF; however, in that case, repetition of BALF 3 days later showed a decrease in eosinophil levels and increases in neutrophils. These findings suggest that in acute RAO, there is an eosinophil infiltration that probably makes way for a subsequent increase in polymorphonuclear neutrophils. This finding is consistent with a Type I hypersensitivity reaction and may be accompanied by peripheral blood eosinophilia (19). However, in the case of the 2 horses, the eosinophils were only present in the lungs

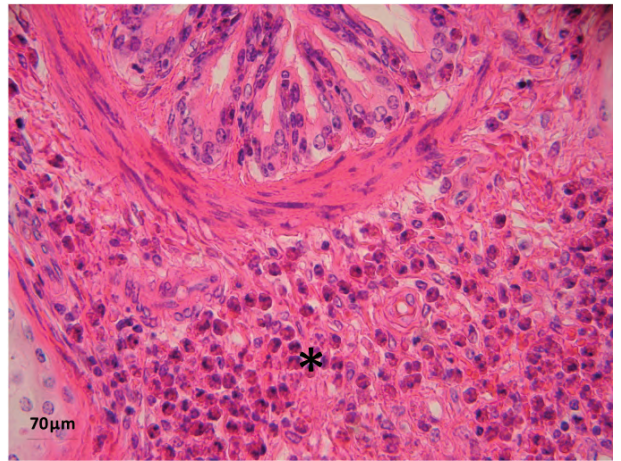


Figure 1. Cross section of representative bronchus in 1 of the 2 cases of RAO-affected horses. Section shows a diffuse peribronchiolar infiltration of eosinophilic cells (asterisk). Hematoxylin and eosin-stained.



Figure 2. Cross section of representative bronchus in 1 of the 2 cases of RAO-affected horses. Eosinophilic cells are present between the bronchiolar epithelial cells (white arrow) and peribronchial tissue (black arrow). Note severe alveolar edema (asterisk) and extensive folding of airway epithelium, which is a sign of smooth muscle (SM) contraction. Hematoxylin and eosin-stained.

and not in the blood. The 2 cases reported here have uncommon findings; neutrophil infiltration is by far the most characteristic feature of RAO. The common pulmonary influx of neutrophils during RAO exacerbations has prompted research that describes the prominent role of these cells in the etiology (10,20). However, we cannot ignore the unknown

role of a massive eosinophilic infiltration. Therefore, we speculate that if these 2 animals had been exposed to a wide-spread antigen, other intrinsic factors, such as MHC haplotype and cytokine production, might have favored the Th-2 response with eosinophilic infiltration of the lungs. Therefore, studies are needed that investigate the role of these cells during the stages of exacerbation in horses with RAO.

The bronchiolitis in the 2 RAO-affected horses had histopathological changes that were concordant with a histopathological diagnosis of RAO, including a peribronchial and intraluminal infiltration with inflammatory cells. This cellular inflammation was present predominantly in the peribronchial interstitial tissue and often extended into the lamina propria and the luminal surface epithelia of the affected airways. Although there were no morphometric analyses, it can be seen subjectively that the inflamed airways of the horses showed hypertrophic/hyperplastic epithelia. Moreover, there was extensive folding of the airway epithelia, which is a sign of airway smooth muscle contraction (Figure 2). Some authors suggest that such morphological modifications in all RAO cases seriously affect horses by affecting the physical functioning of the lung, impairing the complete opening of the bronchioli in some cases, and altering the elastic properties of lung parenchyma. These alterations may explain why the respiratory function of some chronically affected horses may not return to normal after treatment (2), and may partly explain

the poor response of these horses to bronchodilators and corticosteroids. Some authors suggest that proteases released by inflammatory cells may have a role in the observed airway remodeling (21). Recent studies provide evidence that RAO-affected horses experience airway smooth muscle growth associated with myocyte hyperplasia, which may contribute to smooth muscle hyperplasia. This process is similar to what has been observed in human lung remodeling in asthma (9).

Previous studies suggest that p65 homodimers, like p50-Rel and p52-Reb heterodimers, have low affinity for I κ B- α (inhibitory proteins NF- κ B), indicating that the presence of activated p65 homodimers in the nuclei of bronchial cells of heaves affected horses could account for the sustained NF- κ B activity in these cells (7). In the present report, we confirm that the levels of p65 activity were high in the lungs of both of the RAO-affected horses (Figure 3). In other studies, the levels of NF- κ B activity were high or moderate in the granulocyte and nongranulocyte cells obtained from heaves affected horses (7,8). Furthermore, we demonstrated that the activity of this transcription factor was associated with severe airway inflammation in both RAO-affected horses.

In conclusion, this report described exacerbated heaves in Chilean Criollo horse breeds, with a high eosinophilic infiltration and p65 activity in lung tissues without neutrophil infiltration, which is usually characteristic for this disease.

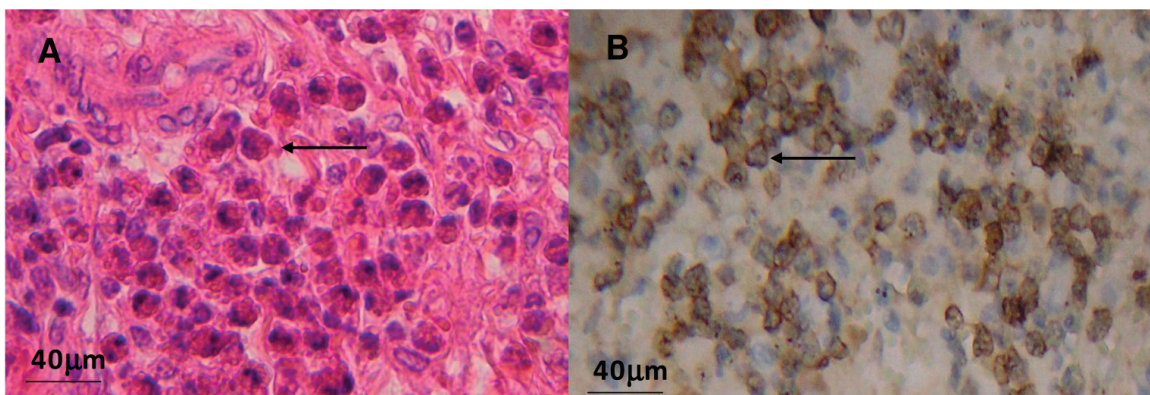


Figure 3. Cross section of representative bronchus in 1 of the 2 cases of RAO-affected horses. (A) Hematoxylin and eosin-stained section shows a diffuse peribronchiolar infiltration of eosinophilic cells. (B) Immunostained with anti-p65. The diffuse peribronchial infiltration of eosinophilic cells (black arrow) show intense immunoreaction.

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