Recurrent airway obstruction in horses – an allergic inflammation: a review

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ABSTRACT: Recurrent airway obstruction (RAO), also known as heaves, is a debilitating and incurable disease of the equine airway. Affected horses develop bronchoconstriction and neutrophilic airway inflammation as a result of exposure to specific airborne irritants and allergens such as hay mould and barn dust. Clinical signs of RAO include exercise intolerance, coughing, nostril flare and abdominal push related to respiratory effort. Evidence suggests that both the innate and acquired immune response contribute to the activation of inflammatory cells resulting in type I hypersensitivity and type III hypersensitivity reactions with an increased expression of Th1- and Th2-cytokines, chemokines, adhesion molecules, receptors and release of reactive oxygen species (ROS). The regulation of inflammatory gene expression in RAO-affected horses is dependent on the binding of transcription factors such as Nuclear factor-(kappa)B (NF-kB), activator protein-1 (AP-1) and the cyclic AMP response element binding protein (CREB) to the promoter region of target genes. In chronic disease an increased number of mucous-producing cells and increased amounts of stored mucins are observed in conjunction with other characteristics of airway tissue remodelling. In this review the findings related to the inflammatory and immunologic response in RAO-affected horses will be presented, and this information will be integrated into existing concepts of immunopathologic mechanisms.

Keywords: RAO; horses; cytokines; immune response; airway inflammation

List of abbreviations

AP-1 = activator protein-1; **BALF** = bronchoalveolar lavage fluid; **CREB** = cyclic AMP response element binding prote; **eCLCA1** = calcium-activated chloride channels; **NF-kB** = nuclear factor-(kappa)B; **RAO** = recurrent airway obstruction; **ROS** = reactive oxygen species; **TLRs** = Toll-like receptors

Contents

1. Introduction

Recurrent airway obstruction (RAO, "heaves") is an asthma-like condition that develops in mature horses following stabling and exposure to dusty hay and straw (Robinson, 2001). Affected horses respond to this exposure by developing airway bronchoconstriction, neutrophilic inflammation and airway hyper-responsiveness. The disease is characterized by pulmonary neutrophilia and excessive mucous production, resulting in reduced dynamic lung compliance and increased pulmonary

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resistance and pleural pressure excursions (Derksen et al., 1985; Jakson et al., 2000). Moreover, this disease shows episodes of acute airway obstruction (crisis) followed by periods of disease remission (Robinson et al., 1996).

RAO was first recognized as an equine disease in 333 BC by Aristotle, who described the line of effort or "heave line" in horses with obstructive respiratory problems. In 1656, Markham associated heaves with housing horses in a barn environment. More recent reports indicate that as many as 55% of horse populations are affected in some areas of the world (Bracher et al., 1991). Horses older than five years of age are most frequently affected, and the prevalence increases with age (Leguillette, 2003). There does not seem to be a predisposition for gender; however, incidence within different breeds and evidence of familial predisposition suggest that there is a heritable component. Moreover, a genetic predisposition for this asthma-like disease has been demonstrated (Marti et al., 1991; Ramseyer et al., 2007; Gerber et al., 2009). Various reports also suggest that the risk of developing RAO is increased in the offspring of affected horses (Gerber, 1989; Scharrenberg et al., 2010). Most likely, horses develop heaves as a consequence of an interaction between genetic and environmental factors

2. Aetiology

Aspergillus fumigatus is commonly found in a horse's environment and is considered one of the triggering agents for equine heaves. However, other pathogens may be contributors, such as *Faenia rectivirgula,* which has been associated with the development of pulmonary hypersensitivity in RAO-affected horses (McPherson et al., 1979; Derksen et al., 1988; MacGorum et al., 1993a,b). Dust mites or their faeces are potentially allergenic to humans and horses (Gerber, 1973; Hockenjos et al., 1981). Clarke and co-workers reported that the number of mites in forage is directly related to the number of fungal spores. Thus, discriminating the effect of mites from the fungal elements in hay is not possible (Clarke and Madelin, 1987). In 1995, Araya and Zaror (1995) reported mites in 80% of fodder samples obtained from 17 Chilean horse breeding farms located in the provinces of Valdivia and Osorno (Chile). They also observed an increase in mites in conjunction with an increase in fungal spores in the fodder. This serves as additional evidence of the immunogenicity of this fungal allergen and of its potential role in the pathogenesis of equine RAO.

3. The immunological basis of RAO

Airway inflammation is a component of the response of RAO-affected horses to aeroallergens and is considered one of the primary characteristics of this disease. In general, airway inflammation involves the activation of pathogenic-specific inflammatory cells, modulation of transcription factors and release of inflammatory mediators (Barnes, 1998). Type I hypersensitivity, which is IgE-mediated (Halliwell et al., 1993; Schmallenbach et al., 1998; Eder et al., 2000, 2001; Curik et al., 2003; Kunzle et al., 2007; Tahon et al., 2009; Moran et al., 2010a,b), and type III hypersensitivity reactions have been suggested to play a role in airway inflammation (Franchini et al., 2000; Lavoie et al., 2001; Robinson, 2001). Studies suggest that RAO-affected horses have higher IgE levels against *Faenia rectivirgula* and *Aspergillus fumigatus* extracts in BALF (bronchoalveolar lavage fluid) than healthy controls (Halliwell et al., 1993; Schmallenbach et al., 1998). In contrast, no differences were found in serum IgE levels against mould allergen extracts between RAO-affected and control horses (Halliwell et al., 1993; Schmallenbach et al., 1998; Eder et al., 2000). In similar studies, some authors showed that healthy and RAO-affected animals have specific circulating antibodies against *Aspergillus fumigatus* crude mould extract (Moran et al., 2009). Most recently, Moran et al. (2010a,b) proposed a bioassay for IgE antibody detection in the horse serum of RAO-affected animals using RBL-2H3 cells, which mediate immediate type reactions, in order to determine the aetiology of disease. Using this method, these authors demonstrated that out of 20 cases of RAO-positive horses, 15% were caused by *Faenia rectivirgula* and 30% of thirty RAO-affected horses tested positive for *Aspergillus fumigatus.* However, although the immunological aspects of RAO have been extensively studied, the precise sequence of events is still not well understood. Moreover, the involvement of an IgE-mediated mechanism in the pathogenesis of RAO-affected horses remains unclear and is still controversial (Marti et al. 2008; Wagner 2009). Wagner (2009) suggested that more recent studies did not confirm an IgE-mediated pathogenesis of RAO. The same author argued that RAO-affected horses display chronic inflammatory disease with some indications

for the involvement of a delayed-type hypersensitivity mechanism. But, as mentioned previously, several studies have investigated the role of IgE antibodies in the pathogenesis of RAO (Halliwell et al., 1993; Schmallenbach et al., 1998; Eder et al., 2000, 2001; Curik et al., 2003; Kunzle et al., 2007; Moran et al., 2010a,b). These investigations suggest that IgE antibodies, which mediate immediate hypersensitivity, could be involved in the immunological mechanism leading to RAO.

The inflammatory response associated with equine RAO is also characterized by neutrophilic bronchiolitis, which is considered as evidence of a Type III hypersensitivity response resulting from antigen-antibody complex formation and the subsequent activation of the complement cascade, with release of the anaphylatoxin peptides C3a and C5a (Lavoie, 2000). They effect smooth muscle contraction and enhance vascular permeability, are chemotactic for a wide variety of leukocytes, activate the production of oxidase activities in neutrophils and eosinophils, and cause exocytosis of granule contents from leukocytes (Gerard and Gerard, 1994, 2002).

4. Transcription factor activation

Inflammation associated with hypersensitivity results from an exaggerated expression of inflammatory genes and a number of researchers have explored the mechanisms implicated in inflammatory gene induction in heaves (Robinson, 2001). Many transcription factors are cell-specific and are crucial in cell differentiation and the regulation of specific cellular processes such as proliferation, enzymes, and cytokine expression. In animal models of airway diseases like atopic asthma, nuclear factor NF-(kB), activator protein-1 (AP-1), GATA-3, JunB and c-Maf play a central role in the control of airway inflammation (Finotto et al., 2001; Nguyen et al., 2003; Yamashita et al., 2007). In humans, there is evidence that NF-kB, AP-1 and GATA-3 expression is increased in asthmatic airways (Hart et al., 1998; Taha et al., 2003). Furthermore, these transcription factors are key downstream regulators of Th2 cytokine function and are phosphorylated/dephosphorylated in the asthmatic airway (Pernis and Rothman, 2002). Similarly, in horses with RAO exposed to mouldy hay, airway epithelial cells exhibit increased expression of NF-kB, in particular the p65 homodimers (Bureau et al., 2000a,b; Saunders et al., 2001). Other studies indicate that the effect of exposure to mouldy hay on RAO-affected horses is at least partly mediated by an increase in AP-1 binding activity in the airways. Prolonged allergen exposure results in upregulation of cyclic AMP response element binding protein (CREB) and down regulation of AP-1 (Couetil et al., 2006). These findings suggest that the transcription factors NF-kB, AP-1 and CREB, play an important role in modulating airway inflammation in horses with RAO.

5. Role of T cells in RAO-affected horses

T cells play an important role in the modulation of the immune response critical during RAO pathogenesis. Recent findings suggest that pulmonary helper T lymphocytes may be implicated in heaves through secretion of Th1-type or Th2-type cytokines (Lavoie et al., 2001; Giguere et al., 2002; Ainsworth et al., 2003; Cordeau et al., 2004; Ainsworth et al., 2007; Riihimaki et al., 2008). RAO-affected horses produce both Type 1 and 2 cytokines depending upon the stage of disease and the timing of sample collection. Through the use of RT-PCR, researchers have demonstrated that BALF cells retrieved from horses with heaves exhibit an increase in IFN-γ mRNA but no change in IL-4 and IL-13 mRNA expression (Giguere et al., 2002; Ainsworth et al., 2003; Horohov et al., 2005). Using similar techniques, Giguire et al. (2002) also found that IL-5 mRNA expression was not detected in RAO-affected horses. However, studies that measured IL-4 and IL-5 expression using *in situ* hybridization technology demonstrate that lung lymphocytes from RAO-affected horses demonstrated a significant increase in the number of cells expressing IL-4 and IL-5 mRNA 24 hours after challenge in a dusty environment; these changes were further increased at nine days, at which time the number of cells positive for IFN-γ mRNA was decreased (Cordeau et al., 2004). In addition, cytokine expression in airway lymphocytes is also influenced by the length of time that the RAO-affected horse has experienced clinical disease. Lymphocytes retrieved from horses three days after initiation of acute exacerbation of RAO express an increase in IL-1β and IL-8 mRNA, and it is known that these cytokines may act as chemoattractant signals for neutrophils (Pietra et al., 2007). Furthermore, lymphocytes retrieved from RAO horses after prolonged exposure to allergens (months) demonstrate an increase in the production of IL-8 and IFN-γ (Horohov et al., 2005). In

addition, Dewachi et al. (2006) reported that horses with heaves had significantly increased numbers of airway neutrophils expressing IL-5 and IL-9 receptors compared to unaffected (or non-RAO) horses while at pasture. Expression of these cytokines was increased further in RAO-affected horses during stabling while expression was unchanged in non-RAO horses. These results provide a possible mechanism by which Th2-type cytokines could activate neutrophils in the equine inflammatory response; however, the same authors concede that this results need confirmation, as binding to irrelevant antigens rather than to equine IL-5 and IL-9 receptors, while improbable, cannot be excluded. Additional reports suggest that the inflammatory influx of neutrophils in chronically affected horses may be maintained by chemokines released from the same extravasated granulocytes (Bureau et al., 2000a; Ainsworth et al., 2007).

6. Influx of neutrophils in airway RAO-affected horses

The principal lesion in RAO-affected horses is bronchiolitis; peribronchiolar accumulation of lymphocytes is accompanied by intraluminal accumulation of neutrophils (Leguillette, 2003) and occurs within seven hours after environmental challenge (Fairbairn et al., 1993). A Type III hypersensitivity reaction explains in part the neutrophilic inflammation in the airways of RAO-affected horses. Factors initiating neutrophilia in the airways of affected horses have not been completely elucidated. As previously mentioned, airway cells retrieved from RAO-affected horses post challenge demonstrate an increase in expression of the neutrophil chemokine IL-8 in bronchoalveolar cells (Giguere et al., 2002; Ainsworth et al., 2003). An increase in the concentration of IL-8 in BALF has also been demonstrated (Franchini et al., 2000; Ainsworth et al., 2003). Moreover, Riihimaki et al. (2008) demonstrated that IL-8 mRNA in both BALF cells and endobronchial biopsy is upregulated in RAO horses during acute crisis. In addition, bronchial NF-kB activity strongly correlates with the percentage of neutrophils present in the bronchi. This data suggests that the sustained NF-kB activity that occurs in the airways of RAO-affected horses is driven mainly by granulocytic and nongranulocytic cells that remain or appear in the bronchi after antigen challenge (Bureau et al. ,2000a). Furthermore, neutrophil apoptosis is delayed through GM-CSF activation of the STAT5 pathway. BALF granulocytes from RAO-affected horses demonstrated a significant delay in apoptosis compared with blood granulocytes from the same horses and blood and BALF granulocytes from healthy horses. The enhanced survival of BALF granulocytes from affected horses was suppressed in the presence of antibodies directed against GM-CSF receptors; moreover, increased levels of active STAT5 were found in BALF granulocytes from RAO-affected horses and were markedly reduced after treatment with anti-GM-CSF receptor antibodies (Bureau et al., 2000b; Turlej et al., 2001). This indicates that granulocyte survival is enhanced in the lungs of RAO-affected horses and suggests a role for a GM-CSF-activated STAT5 pathway in delaying apoptosis of lung granulocytes in this disease. On the other hand, IL-17 is known to induce pro-inflammatory cytokines such as TNF-α, IL-1B, and IL-6 as well as the chemokines CXCL1, 2, and 8, which together are hallmarks of acute inflammatory processes (Schmidt-Weber et al., 2007) and are produced by memory T cells termed Th17 cells (Fossiez et al., 1998; Witowski et al., 2004). Because airway neutrophilia is a well recognized characteristic of clinical heaves, several researchers have recently attempted to establish a relationship between IL-17 and the immediate influx of neutrophils into the RAO-horse's airway. Neutrophil influx into airways and surrounding pulmonary tissues also precedes a significant increase in IL-17 mRNA expression in the airway cells of the endobronchial biopsy and BALF compared to controls during provocation studies (Ainsworth et al., 2006; Riihimaki et al., 2008). The results of these studies fail to provide evidence that IL-17 contributes to the establishment of airway neutrophilia, but it may contribute to the maintenance of inflammation in chronically affected horses (Debrue et al., 2005). These last studies indicate that IL-17 may be implicated in the pathogenesis of heaves; however, how IL-17 contributes to heaves is unknown. Debrue et al. (2005) suggested that this cytokine may induce neutrophil chemotaxis and activation, mucus hypersecretion and alteration of airway function.

7. Oxidative stress in RAO

Oxidative stress describes the damage that occurs when reactive oxygen species (ROS) overwhelm the antioxidant defenses of the host (Wood et al., 2003).

Increasing clinical, epidemiological and experimental evidence indicates that excess production of ROS is involved in the pathogenesis of a number of airway disorders, especially in asthma (MacNee, 2001). Activated inflammatory cells respond with a "respiratory burst", which involves the uptake of oxygen and subsequent release of ROS into surrounding cells. Horses suffering from RAO have a decreased pulmonary antioxidant capacity, which may render them more susceptible to oxidative challenge. Deaton et al. (2005a,b) demonstrated that neutrophilia induced by exposure to organic dust is associated with increases in elastase and decreases in ascorbic acid levels in BALF retrieved from RAO horses. Concurrently, affected horses experience significant antioxidant depletion in the trachea which may be related to inflammation and oxidative processes in peripheral airways (Deaton et al., 2006). Acute exacerbations are associated with a significant increase in markers of oxidative stress (oxidised glutathione and glutathione redox ratio) in pulmonary epithelial lining fluid (PELF) (Robinson, 2001). These markers correlate significantly with neutrophilic count in BALF (Art et al., 1999). In human and mice with bronchial asthma, oxidative stress can have many detrimental effects on airway function including induction of airway hyperresponsiveness (Weiss and Bellino, 1986; Katsumata et al., 1990), mucus hypersecretions (Adler et al., 1990), epithelial shedding (Doelman et al., 1990), vascular exudation (Tate et al., 1982) and airway smooth muscle contraction (Rhoden and Barnes, 1989). However, the role of ROS in modulating equine airway smooth muscle tone and airway wall remains obscure and may depend on the presence of other inflammatory mediators (Deaton et al., 2005a). Furthermore, ROS modulate the activation of transcription factors such as NF-kB and AP-1, in bronchial epithelial cells, alveolar macrophages, neutrophils and mast cells. This activation leads to the expression of many pro-inflammatory cytokines, including TNF-α and IL-1β (Matera et al., 2005) and Th2-type cytokines (Frossi et al., 2003). Finally, dietary antioxidant cocktails may improve lung function in RAO-affected horses by modulating the oxidant-antioxidant balance and airway inflammation (Kirschvink et al., 2002).

8. Role of Toll-like receptors in RAO

Most studies have concentrated on the role of adaptive immunity in the pathogenesis of RAO. However, innate immune mechanisms are also important (Berndt et al., 2007). Microbial-derived products, such endotoxins, have been shown to play an important role in allergy-induced human lung disease (Feleszko et al., 2006). Pathogen-associated molecules are recognized by pattern recognition receptors, the most important of these receptors being Toll-like receptors (TLRs). TLRs play a key role in activating antigen-presenting cells for both the innate and adaptive immune response (Cassale and Stoke, 2008). Expression of TLR4 mRNA is increased in BALF from RAO-affected compared to unaffected, healthy horses following exposure to stable dust (Ainsworth et al., 2006). These findings may be due to an increase in the percentage of neutrophils in BALF of RAO-affected horses, an upregulation of TLR4 expression in the neutrophils, an increase in the expression of TLR4 in BALF macrophages, or a combination of these three possibilities. However, subsequent reports suggest that exposure to stable dust leads to increased TLR4 mRNA expression in bronchial epithelial cells from RAO-affected horses. In addition, the upregulation of epithelial TLR4 mRNA synthesis correlates with IL-8 mRNA expression (Berndt et al., 2007). These results could explain the exacerbated neutrophilic airway inflammation of RAO-affected horses to airborne endotoxins (Pirie et al., 2002, 2003; Berndt et al., 2007). Additional studies are needed to delineate the role of TLR expression and activation in BALF cells of RAO-affected horses.

9. Mucus production in RAO

Mucus accumulation and neutrophilic inflammation of airways are characteristic of RAO-affected horses (Robinson et al., 1996). Airway inflammation, especially neutrophils and their products cause changes in mucus rheology. Mucus accumulation before and after exposure to dust and allergens is increased in RAO-affected horses compared with healthy horses (Gerber et al., 2004). By comparison, mucus accumulation is variable in healthy horses, and does not increase in conjunction with the influx of neutrophils into airways that occurs after exposure to hay dust (Gerber et al., 2004). In human patients with asthma, chronic obstructive pulmonary disease (COPD), or cystic fibrosis (CF) invariably exhibit excessive mucus production in the airway lumen, goblet cell hyperplasia, and submucosal gland hypertrophy (Marchette et al., 1985). These same changes are found in RAO (Kaup

et al., 1990a,b; Davis and Rush, 2002). Inflammation leads to mucus hypersecretion, ciliary dysfunction, and changes in the composition and biophysical properties of airway secretions. There are conflicting reports on the effectiveness of mucociliary clearance in RAO-affected horses (Robinson, 2001); some authors have reported a decreased clearance in RAO-affected horses (Coombs and Webbon, 1987; Turgut and Sasse, 1989), while others found no difference between RAO-affected and healthy horses in mucocilliary clearance (Willoughby et al. (1991). In RAO-affected horses mucus hypersecretion, ciliary dysfunction, and changes in the composition and biophysical properties of secretions are also observed after 24 hours of stabling and feeding hay (Gerber et al., 2000). Airway mucus is a complex mixture of water, electrolytes, lysozymes, inflammatory cells, and glycoproteins, the most important of which is called mucin and imparts viscoelastic properties (Lundgren and Shelhamer, 1990). In RAO-affected horses the altered physical properties of respiratory mucus have been attributed to both changes in the quality and quantity of its constituent mucins. Abnormal glycosylation of mucins has been shown to increase viscosity of airway secretions resulting in a failure of mucociliary function (Jefcoat et al., 2001). Expression of the mucin genes, MUC5B and MUC5A, has been examined in RAO-affected horses and was shown to be upregulated by pro-inflammatory cytokines (Umetsu et al., 2002; Chen et al., 2003; Rousseau et al., 2007). Additionally, IL-17 stimulates the expression of MUC5AC which may contribute to mucus hypersecretion in horses with chronic heaves. (Gerber et al., 2003).

Recently researchers have identified and cloned the first equine member of the family of calcium-activated chloride channels (eCLCA1), which is strongly upregulated in the small airways of horses with RAO (Anton et al., 2005). The CLCA gene is a member of a family of transmembrane proteins with a putative role in chloride conductivity across the outer cell membrane that is regulated by the intracellular concentration of calcium (Fuller et al., 2001). These channels are prime regulators of epithelial secretion and are of high interest for respiratory disorders with chronic mucus overproduction, including asthma, COPD, and CF, since abnormal epithelial secretions often result from defective or dysregulated chloride channels (Jentsch et al., 2002). Upregulation of eCLCA1 mRNA and protein was observed using Northern blot hybridization, western blotting, immunohistochemistry, and quantitative RT-PCR in the lungs of three horses with RAO, similar to the upregulation of hCLCA1 in human asthma patient (Anton et al., 2005). In addition, increases in eCLCA1 expression can also be due to an increase in the number of goblet cells rather than transcriptional upregulation of the eCLCA1 gene (Range et al., 2007). Since heave horses share characteristics with humans with regard to eCLCA1 expression and goblet cell hyperplasia, spontaneous or experimental RAO in horses may serve as a model for studying the role of CLCA homologs in chronic airway disease with overproduction of mucins (Anton et al., 2005; Range et al., 2007).

Mediators secreted by airway epithelial cells and infiltrating leukocytes affect the cell death processes of epithelial cells (Tesfaigzi, 2006). Inflammatory signals recruit nonciliated columnar epithelial cells into the cell cycle in large numbers. This results in an increase the number of cells per millimeter of basal lamina and the number of mucus-producing cells in a process known as goblet cell metaplasia (Tesfaigzi et al., 2004; Harris et al., 2005). During the resolution of inflammatory responses metaplastic mucous cells express members of the Bcl-2 family of proteins which are regulators of apoptosis (Tesfaigzi et al., 1998, 2002). Bartner et al. (2006) observed Bcl-2 expression in bronchial epithelium mucous cells throughout the airways of RAO-affected horses in remission and during acute disease while expression was absent in the tissues of healthy animals. Moreover, the observed Bcl-2 expression throughout the airways of RAO-affected horses is considered evidence that RAO is a condition that affects the whole tracheobronchial tree and is not limited to the peripheral airways. In addition, Bcl-2-positive mucous cells may synthesize more eqMUC5AC per cell than Bcl-2-negative cells, which may contribute to the hypersecretion of mucins (Gerber et al., 2003); therefore, two distinct populations of mucous cells may exist in RAO-affected horses. Bcl-2-positive mucous cells may represent mucous cells that are capable of synthesizing mucus at a faster rate than Bcl-2-negative cells (Bartner et al., 2006). These same authors suggested that this hypothesis is supported by the fact that, in some RAO-affected horses, 80% of mucous cells were Bcl-2-positive, suggesting that Bcl-2-positive cells are the first to store mucus following the emptying of mucus due to acute inflammation. However, more studies are needed to compare this phenomenon between healthy horses and RAO-affected horses in different stages of disease. Finally,

Lugo et al. (2006) confirmed that inflammation in the airways of RAO-affected horses is associated with increased numbers of mucus-producing cells and increased amounts of stored mucins.

10. Airway remodelling

Recently, airway remodelling has become a field of special interest in asthma, chronic bronchitis and CF research since this process causes patients to become largely resistant to medication and is an important factor in the development of irreversible airflow limitation (James et al., 1989; Lange et al., 1998; Jeffery et al., 2000; Wegmann, 2008). In tissues from human asthmatics remodelling changes include goblet cell and mucous gland hyperplasia, subepithelial fibrosis, neovascularization, airway smooth muscle (ASM) growth, and an overall thickening of the airway wall (Davies et al., 2003). RAO-affected horses exhibit some of the same remodelling changes in their airways in association with chronic inflammation (Lugo et al., 2006). The molecular mechanisms that drive remodelling remain undefined but aberrant signalling by the transforming growth factor (TGF) superfamily, of which TGF-b1 is the prototypic member, is increasingly recognized as playing an important role (Kariyawasam and Robinson 2007). This growth factor is an important fibroblast chemotactic factor. Fibroblast numbers have been shown to correlate with TGF-b1 expression. TGF-b1 also induces the differentiation of fibroblasts to myofibroblasts (Postlethwaite and Seyer, 1995; Vignola et al., 1997; Thannickal et al., 2003). However, using ELISA on BALF, Desjardins et al. (2004) failed to establish a causal relationship between TGF-b1 and the pathophysiology of RAO-affected horses. Therefore, more studies are needed on TGF-b1 and other profibrotic cytokines mediators responsible for airway wall remodelling in horses with RAO. There is evidence to suggest that RAO-affected horses experience airway smooth muscle growth associated with myocyte hyperplasia which may contribute to smooth muscle hyperplasia. This is similar to what has been observed in human asthmatics (Herszberg et al., 2006).

11. Conclusion

Equine heaves was first recognized as a debilitating disease in horses more than 2000 years ago yet the pathology of the inflammatory component of this syndrome is still an enigma. The development of new research tools has sparked a growing interest in the pathology of RAO among veterinary researchers and those with focus on human health. The current body of knowledge suggests that the inflammatory component of RAO results from a combination of elements from both the innate and adaptive immune responses. Expression of cytokine patterns consistent with Th1, Th2 and Th17 cell activation has been identified and determined to vary based on the chronicity of the disease. Activation of transcription factors such as NF-kB plays a pivotal role in regulating cellular signalling pathways through dynamic modulation of cytokines, chemokines, and similar molecules. The regulation of the apoptosis of inflammatory cells, fibroblasts, and myocytes through Bcl-2 expression contributes to the establishment of chronic disease and remodelling. Contributions by the innate arm of the immune system in the form of Toll-like receptor expression and neutrophil cytokine synthesis also add to this complex cascade of events, yet these recent discoveries serve mainly to illustrate the dynamic complexity of this disease and to remind us of the many questions that remain unanswered. Thus, the mechanism by which airway inflammation develops in RAO affected horses is a multifaceted and dynamic process. Developing better standards for categorizing disease state and executing research protocols, incorporating appropriate controls, designing studies that minimize variation in independent variables, and including a larger number of affected animals in these studies are all factors that will allow researchers to better define the sequence of events that result in the development of the airway inflammation associated with "heaves".

The message for those who are actively involved in heaves research is that there is a great deal left to be discovered. As described by Art and Robinson (2008) in a recent guest editorial published in the Veterinary Journal, "in the next years new insights will be provided by reading the black box" of the white cells, namely, genetics and gene expression, which should bring us the key to the fascinating yet unresolved enigma of heaves.

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