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Review

Role of neutrophils in equine asthma

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Abstract

Neutrophilic bronchiolitis is the primary lesion in asthma-affected horses. Neutrophils are key actors in host defense, migrating toward sites of inflammation and infection, where they act as early responder cells toward external insults. However, neutrophils can also mediate tissue damage in various non-infectious inflammatory processes. Within the airways, these cells likely contribute to bronchoconstriction, mucus hypersecretion, and pulmonary remodeling by releasing pro-inflammatory mediators, including the cytokines interleukin (IL)-8 and IL-17, neutrophil elastase, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs). The mechanisms that regulate neutrophil functions in the tissues are complex and incompletely understood. Therefore, the inflammatory activity of neutrophils must be regulated with exquisite precision and timing, a task achieved through a complex network of mechanisms that regulates neutrophil survival. The discovery and development of compounds that can help regulate ROS, NET formation, cytokine release, and clearance would be highly beneficial in the design of therapies for this disease in horses. In this review, neutrophil functions during inflammation will be discussed followed by a discussion of their contribution to airway tissue injury in equine asthma.

Keywords: equine asthma, neutrophils, airway inflammation, resolution of inflammation.

Introduction

Horses naturally develop an asthma-like condition after stabling and exposure to dusty hay and straw, currently known in the veterinary scientific community as 'heaves' or recurrent airway obstruction (RAO) (Robinson, 2001). Recently, several authors suggested that RAO and inflammatory airway disease (IAD), a mild form of non-infectious airway hyper-responsiveness to inhaled allergens, should be grouped as moderate-to-severe and mild forms, respectively, of a single disease termed equine asthma (Bullone and Lavoie, 2015; Couetil *et al.*, 2016; Pirie *et al.*, 2016). Asthma-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 mucus production, resulting in reduced dynamic lung compliance and increased pulmonary resistance and pleural pressure excursions (Jackson *et al.*, 2000). This disease presents with episodes of acute airway obstruction (crisis) followed by periods of apparent remission (Robinson *et al.*, 1996, 2001). *Aspergillus fumigatus*, an opportunistic fungus, is commonly observed in a horse's environment and is considered one of the inciting agents in equine asthma (Morán *et al.*, 2009). Horses aged more than 5 years are the most frequently affected, with the prevalence increasing with age (Leguillette, 2003). There does not appear to be a predisposition by gender; however, disease incidence within different breeds and evidence of family predisposition suggest that there is a heritable component. Moreover, a genetic predisposition for this asthma-like disease has been demonstrated (Ramseyer *et al.*, 2007; Gerber *et al.*, 2009). Various reports also suggest that the risk of developing equine asthma is increased in the offspring of affected horses (Scharrenberg *et al.*, 2010). Most likely, horses develop asthma as a consequence of an interaction between genetic and environmental factors (Moran and Folch, 2011).

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Neutrophils are the major pathogen-fighting immune cells and are observed in many organisms, ranging from insects to mammals (Ribeiro and Brehelin, 2006). Central to their function is the ability to be recruited to sites of infection, to recognize and phagocytose microbes, and subsequently to kill pathogens through a combination of cytotoxic mechanisms (Mayadas *et al.*, 2014). Neutrophils kill microbes via the release of destructive molecules, such as proteases, highly reactive oxygen species (ROS) and neutrophil extracellular traps (NETs); they also produce a variety of proteins, including cytokines, chemotactic molecules, and other mediators that are involved in their effector functions (Cheng and Palaniyar, 2013). Although these molecules are generally effective in destroying microbes, a fraction of them leak from living and dying leukocytes, and in so doing, damage adjacent normal tissue cells. The programed death of neutrophils blocks their secretory pathways, limiting tissue damage by the release of pro-inflammatory mediators. Numerous mechanisms participate in this last event, tightly regulating the gravity and duration of airway inflammation. If unresolved, acute lung injury (ALI) and/or lung inflammation can progress to chronic inflammation, which occurs in lung diseases such as acute respiratory distress syndrome, asthma, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) (Robb *et al.*, 2016), and equine asthma (Perez *et al.*, 2016).

Equine asthma is a good model for research on the role of neutrophils in human asthma, the regulation of chronic neutrophilic inflammation, and their possible implications in pulmonary allergic responses. Furthermore, since the features of pulmonary remodeling in equine asthma closely resemble the features of human neutrophilic asthma, this animal model is useful for research on the kinetics, reversibility, and physiological consequences of tissue remodeling (Bullone and Lavoie, 2015). Given that neutrophils are the main cell type in equine asthma and in certain types of human asthma, this animal model may also be of use for the development of novel pharmacological therapies with neutrophils as a drug target. This review will discuss neutrophil functions during inflammation and their contribution to airway tissue injury in equine asthma.

Equine asthma is an immune-mediated disease

Airway inflammation is a component of an asthma-affected horse's response to aeroallergens and is considered one of the primary characteristics of this disease. Equine asthma has been extensively studied, but the precise sequence of disease events is still not well-understood (Leguillette, 2003). Generally, airway inflammation involves the activation of pathogen-specific inflammatory cells, the modulation of gene transcription factors, and the release of inflammatory mediators (Bureau *et al.*, 2000a, b). The immunological background of severe equine asthma remains not fully elucidated, despite many studies on its pathogenesis. Type I hypersensitivity, which is IgE-mediated (Curik *et al.*, 2003; Künzle *et al.*, 2007; Tahon *et al.*, 2009; Morán *et al.*, 2010a, b; Morán *et al.*, 2012), and type III hypersensitivity reactions have been suggested to play a role in airway inflammation (Lavoie *et al.*, 2001; Robinson, 2001). IgE plays an important role in the induction of type I immediate hypersensitivity reactions in asthma-affected horses (Morán *et al.*, 2010a, b; Morán *et al.*, 2012). The inflammatory response associated with equine asthma is also characterized by neutrophilic bronchiolitis, which is considered evidence of a type III hypersensitivity response resulting from antigen–antibody complex formation and the subsequent activation of the complement cascade, with the release of anaphylatoxin and C3a and C5a peptides (Lavoie *et al.*, 2000). Additional reports suggest that the inflammatory influx of neutrophils to the airways of chronically affected horses may be maintained by chemokines released from the same marginated granulocytes (Bureau *et al.*, 2000a, b; Ainsworth *et al.*, 2007).

T cells also play an important role in the modulation of the immune response in asthma equine pathogenesis. Many results suggest that pulmonary helper T lymphocytes may be implicated in heaves through the 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; Riihimäki *et al.*, 2008). Asthma-affected horses produce both type 1 and 2 cytokines, depending on the stage of their disease and the timing of sample collection. Cytokine expression in airway lymphocytes is also influenced by the length of time that an asthma-affected horse has experienced clinical disease (Pietra *et al.*, 2007). Furthermore, lymphocytes retrieved from asthma horses after prolonged exposure to allergens (months) demonstrate an increase in the production of interleukin (IL)-8 and interferon-γ (Horohov *et al.*, 2005). Moreover, IL-17 is known to induce the expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1B, and IL-6 as well as chemokines CXCL1, 2, and 8, all of which are hallmarks of acute inflammatory processes (Schmidt-Weber *et al.*, 2007). Finally, regulatory T cells (Treg) appear to play a role in the immune response in asthma-affected horses (Henriquez *et al.*, 2014).

Neutrophil migration and activation in the lungs of horses with asthma

The neutrophil recruitment cascade is mediated by the sequential interaction of receptors present on neutrophils with ligands induced on the surface of the activated endothelium (Mayadas *et al.*, 2014). Neutrophils are observed in higher concentrations in the pulmonary capillaries compared with systemic blood even in the absence of inflammatory stimuli. This phenomenon allows neutrophils to readily migrate into the lungs in response to inflammatory stimuli (Cheng and Palaniyar, 2013). During inflammation, neutrophils become activated upon stimulation and may produce ROS and NETs, undergo degranulation, or exhibit other functions. The activation of neutrophils is required before migration into the lungs (Ley *et al.*, 2007). In asthma-affected horses, the neutrophils migrate within hours into the airway lumen followed by the development of airway obstruction and a late phase of migration (Fairbairn *et al.*, 1993; Franchini *et al.*, 1998; Brazil *et al.*, 2005).

The principal lesion in asthma-affected horses is bronchiolitis. The peribronchiolar accumulation of lymphocytes is accompanied

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by the intraluminal accumulation of neutrophils (Leguillette, 2003) and occurs within 7 h after environmental challenge (Fairbairn *et al.*, 1993). A type III hypersensitivity reaction explains, in part, the neutrophilic inflammation in the airways of asthma-affected horses, but the factors that initiate neutrophilia in the airways of affected horses have not been completely elucidated. As previously mentioned, bronchoalveolar cells retrieved from asthma-affected horses after antigenic challenge demonstrate the increased expression of the neutrophil chemokine, IL-8 (Giguere *et al.*, 2002; Ainsworth *et al.*, 2003). An increase in the concentration of IL-8 in broncheoalveolar lavage fluid (BALF) has also been demonstrated (Franchini *et al.*, 2000; Ainsworth *et al.*, 2003), and Riihimaki *et al.* (2008) reported that IL-8 mRNA expression was upregulated in BALF cells and in endobronchial biopsies from asthma-affected horses in acute crisis. Several authors also suggest that alveolar macrophages can contribute to the airway inflammation by the release of IL-8, macrophage inflammatory protein-2 and TNF-α (Giguere *et al.*, 2002; Ainsworth *et al.*, 2002; Joubert *et al.*, 2011). In addition, bronchial nuclear factor-κB (NF-κB) activity strongly correlates to the percentage of neutrophils present in the bronchi; this result suggests that the sustained NF-κB activity in the airways of asthma-affected horses is driven mainly by the granulocytic and non-granulocytic cells that remain or appear in the bronchi after antigen challenge (Bureau *et al.*, 2000a, b). BALF granulocytes from asthma-affected horses demonstrate a significant delay in apoptosis compared with blood granulocytes from the same horses or blood and BALF granulocytes from healthy horses (Bureau *et al.*, 2000b; Turlej *et al.*, 2001). Furthermore, since airway neutrophilia is a well-recognized characteristic of clinical equine asthma, several researchers have attempted to establish a relationship between IL-17 and the immediate influx of neutrophils into the airways of asthma-affected horses (Murcia *et al.*, 2016). IL-17 is produced by CD4 + T helper 17 cell and other types of cells such as γ/δ T cells, natural killer cells, lymphoid tissue inducer cells, macrophages, eosinophils, and neutrophils (Gaffen 2009; Ramirez-Velazquez *et al.*, 2013). IL-17 can indirectly promote the activation and recruitment of neutrophils into the airways by inducing the production of such chemokines as IL-8, CXCL1, and granulocyte colony-stimulating factor (G-CSF) in endothelial and epithelial cells (Ouyang *et al.*, 2008). Neutrophil influx into the airways and surrounding pulmonary tissues coincides with a significant increase in IL-17 mRNA expression in the airway cells obtained from endobronchial biopsies and BALF compared with controls during provocation studies (Ainsworth *et al.*, 2006; Riihimaki *et al.*, 2008). Additionally, Debrue *et al.* (2005) suggested that IL-17 may induce neutrophil chemotaxis and activation, mucus hypersecretion and alterations in airway function. Korn *et al.* (2015) also found an increased IL-17 response focused on NF-κB and a downregulation of the IL-4 gene in asthma-affected horses through immunohistochemistry and global gene expression profiles in mediastinal lymph nodes. This finding provides additional evidence of the involvement of IL-17 in the chronic stages of equine asthma.

Conversely, the innate immune response plays an important role in neutrophil activation during allergic airway diseases in both humans and horses (Feleszko *et al.*, 2006; Berndt *et al.*, 2007). Among the innate mechanisms described is the formation of NETs, which serve as possible promotors of disease in asthma-affected horses. The pathogenic role of NETs has been described for many infectious and non-infectious human diseases, including respiratory cases with a massive influx of neutrophils into the airways (Porto and Stain, 2016). Excessive NET release is particularly deleterious in lung diseases because NETs can expand easily in the pulmonary alveolar space and cause lung injury. NETs and their associated molecules can also directly induce epithelial and endothelial cell death (Xu *et al.*, 2009; Saffarzadeh *et al.*, 2012). NETs have been identified in lungs with CF, ALI, neutrophilic asthma, and bacterial, viral, or fungal infections. The primary role of NETs is to prevent microbial dissemination because of their stringy structure and to kill pathogens due to the high local concentrations of antimicrobial molecules (Manzenreiter *et al.*, 2012). However, studies reveal that NETs can exert adverse effects in a number of diseases, including diseases of the lung (Cheng and Palaniyar, 2013). NETs are composed of a backbone of nuclear DNA combined with a multitude of nuclear proteins, as well as the contents of neutrophil granules, including Myeloperoxidase (MPO) and elastase and peptidylarginine deiminase type IV (Martinelli *et al.*, 2004; Urban *et al.*, 2009; Papayannopoulos *et al.*, 2010). These DNA–protein complexes are then released extracellularly as NETs. The potent neutrophil chemoattractant, IL-8, has also been shown to induce NETosis (Brinkmann *et al.*, 2004; Gupta *et al.*, 2005). However, their contribution to disease severity is not clearly understood. In asthma-affected horses, NETs were present in BALF in exacerbated cases but not in fluid from horses in remission periods or in healthy challenged horses (Côté *et al.*, 2014).

Pathogen-associated molecules are recognized by pattern recognition receptors, such as Toll-like receptors (TLRs). Thus, TLRs are important for the activation of antigen-presenting cells during innate and adaptive immune responses (Casale and Stoke, 2008). TLRs are expressed by different cells involved in asthma-related airway inflammation, such as epithelial cells, macrophages, dendritic cells, and mast cells (Takeda *et al.*, 2003) and airway smooth muscle (Sukkar *et al.*, 2006). In asthma-affected horses, TLR4 mRNA expression is increased in the BALF of horses that have been exposed to stable dust compared with unaffected horses (Ainsworth *et al.*, 2006). This finding suggests that exposure to stable dust leads to increased TLR4 mRNA expression in bronchial epithelial cells from asthmatic horses. In addition, the upregulation of epithelial TLR4 mRNA correlates with IL-8 mRNA expression (Berndt *et al.*, 2007). These results could explain the exacerbated neutrophilic airway inflammation of asthma-affected horses in response to airborne endotoxin (Pirie *et al.*, 2002, 2003; Berndt *et al.*, 2007). Interestingly, other reports also state that microbial-derived products, such as endotoxins, play an important role in allergy-induced human lung disease (Feleszko *et al.*, 2006).

Role of ROS in asthma-affected horses

ROS are produced during oxygen reduction and are characterized by high reactivity. ROS participate in many important

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physiological processes, but if they are produced in high concentrations, they may lead to oxidative stress development and disturb the pro-oxidative/antioxidative balance toward an oxidation reaction, thereby leading to the damage of lipids, proteins, carbohydrates, or nucleic acids (Kleniewska and Pawliczak, 2017). Oxidative stress has been shown to occur in many human respiratory conditions, including COPD and human asthma (Kirkham and Barnes, 2013; Zuo et al., 2013). ROS derived from inflammatory cells (neutrophils, macrophages), which migrate in large numbers to the lungs, are crucial in the oxidant–antioxidant imbalance observed during the course of the above-mentioned diseases (Niedzwiedz and Jaworski, 2014).

ROS formation is a multi-step process involving the translocation of cytosolic components of NADPH (p47phox, p67phox, and Rac) to the NADPH components found on the cytoplasmic or phagosome membrane (gp91phox and gp22phox). When this occurs, NADPH transports electrons to molecular oxygen, generating a superoxide anion. This phenomenon, in turn, undergoes a rapid and spontaneous dismutation to hydrogen peroxide, which serves as a substrate for myeloperoxidase for the subsequent generation of hypohalous acids, the most important of which is hypochlorite acid (Tintinger *et al.*, 2013).

Horses that suffer from asthma have a decreased pulmonary antioxidant capacity, which may render them more susceptible to oxidative challenge. Research on oxidative stress in horses with asthma has been conducted, and some authors demonstrated that neutrophilia induced by exposure to organic dust is associated with increases in elastase and decreases in ascorbic acid concentrations in BALF retrieved from horses with asthma (Deaton *et al.*, 2005a, b). 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 the levels of markers of oxidative stress (oxidized glutathione and glutathione redox ratio) in pulmonary epithelial lining fluid (Robinson, 2001). These markers correlate significantly with the number of neutrophils in BALF (Art *et al.*, 1999).

Asthmatic patients and mouse models typically exhibit varying degrees of airway inflammation; oxidizing agents interfere with the structure of epithelial cells, resulting in the increased production of mucus. This phenomenon eventually leads to structural changes and bronchial remodeling (Weiss and Bellino, 1986; Adler *et al.*, 1990; Doelman *et al.*, 1990; Katsumata *et al.*, 1990), although the precise role of ROS in modulating equine airway smooth muscle tone and the airway wall is unclear and may depend on the presence of other inflammatory mediators (Deaton *et al.*, 2005a). The destructive nature of ROS may contribute to increased inflammation, apoptosis, or necrosis by modifying nucleotide chains and disrupting DNA stability. This property may also lead to the proliferation of smooth muscle cells in the airways, or an increase in the amount of mucus in the lungs (Cooke *et al.*, 2003; Kamiya, 2003; Reddy *et al.*, 2005; Höhn *et al.*, 2013); these changes have been described in horses with asthma (Herszberg *et al.*, 2006; Bullone and Lavoie 2015). ROS overproduction may also activate transcription factors, such as NF-κB or AP-1 (activator protein 1) proteins (Csiszar *et al.*, 2008; Noutsios and Floros., 2014; Schuliga, 2015), which in turn may lead to the expression of many pro-inflammatory cytokines, including TNF-α, IL-4, IL-5, IL-6, and IL-13, and aggravate the disease (Frossi *et al.*, 2003). Moreover, studies reveal that NETosis is dependent on the generation of ROS by NADPH oxidase (Porto and Stein, 2016). Furthermore, several authors suggest that dietary antioxidant cocktails may improve the lung function of asthma-affected horses by modulating oxidant–antioxidant balance and airway inflammation (Kirschvink *et al.*, 2002). Finally, all of the described data support the hypothesis that defects in the intracellular antioxidant defense system may be critical contributors to the development of equine asthma under increased ROS production. Further studies on oxidative stress markers and the efficacy of selected antioxidants in equine asthma treatment are needed to determine the optimal control of this disease.

Neutrophils and the resolution of inflammation

Airway epithelial cells are the first line of defense against inhaled pathogens and antigens in the airways. Once the triggering antigen reaches the airways, epithelial cells launch signals that activate tissue-resident cells of the innate immune system, initiating the inflammatory response and recruiting circulating neutrophils (Hallstrand *et al.*, 2014). To leave their intravascular location, neutrophils must interact with the endothelial cells of the local vessels, which increase adhesion molecule expression after being activated and allow the migration of neutrophils to the underlying tissue. This effect, in turn, promotes the recruitment of inflammatory monocytes and potentiates the pro-inflammatory environment, allowing control of the insulting agent that triggered the initial inflammation (Mantovani *et al.*, 2011) and permitting the resolution of the inflammatory event. However, in some cases, such as neutrophilic asthma, an exacerbated inflammatory response occurs. The resolution of this acute process relies on many soluble molecules; such molecules as Annexin A1 (AnxA1) (Perretti and D'Acquisto, 2009), ChemerinC15 (Cash *et al.*, 2008), lipoxins (Serhan *et al.*, 2008), and resolvins (Ariel and Serhan, 2007) play an important role in stopping the recruitment of neutrophils. These signals act in conjunction with the apoptosis of neutrophils, which is central in the resolution process; dying neutrophils are known to stimulate their own efferocytosis, inducing macrophagic transition from a pro-inflammatory (M1) to an anti-inflammatory (M2) profile (Ortega-Gomez *et al.*, 2013). AnxA1 (37 kDa) is an abundant protein in the cytosol of resting neutrophils. AnxA1 translocates to the plasma membrane when the cell is activated and interacts with the formyl peptide receptor 2 to moderate leukocyte adhesion and migration (Perretti *et al.*, 2002; Dalli *et al.*, 2008). AnxA1 also promotes neutrophil apoptosis and clearance by macrophages (Perretti and Solito, 2004; Scannell *et al.*, 2007). Another neutrophil-derived protein with similar activities is lactoferrin. This protein is contained within

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the secondary granules of neutrophils, and when released, lactoferrin binds to specific receptors that trigger Mitogen-Activated Protein Kinases (MAPK)-mediated intracellular signaling, which is crucial in the regulation of cytoskeletal remodeling and cell adhesion (Bournazou *et al.*, 2009). Additionally, in a model of ALI, lactoferrin application prevented neutrophil tissue infiltration and edema formation and improved lung function (Li *et al.*, 2012). Neutrophils that have started their apoptotic process are cleared by macrophages via efferocytosis. Apoptotic neutrophils promote their own clearance by expressing 'find me' and 'eat me' signals. 'Find me' signals are secreted factors that attract scavengers. To date, four major 'find me' signals have been described (Lauber *et al.*, 2003; Gude *et al.*, 2008; Truman *et al.*, 2008; Elliott *et al.*, 2009). 'Eat me' signals are surface markers that permit the identification of a dying cell. These signals can either be molecules exposed *de novo* at the cell membrane or existing ones that undergo modifications during apoptosis; the best-known such molecule is phosphatidylserine, which is also the best-studied marker of early apoptosis (Ortega-Gomez *et al.*, 2013). Furthermore, neutrophils induce a change in phagocytic macrophages from a pro-inflammatory to an anti-inflammatory mode. Upon apoptotic cell efferocytosis, macrophages turn off the production of pro-inflammatory cytokines and lipid mediators and launch an anti-inflammatory transcriptional program characterized by the release of IL-10 and Transforming growth factor (TGF)-β (Fadok *et al.*, 1998) and the secretion of lipid mediators that play a key role in the orchestration of inflammation and its resolution (Serhan *et al.*, 2008). Moreover, neutrophils may also stimulate regulatory-suppressive cells. Apoptotic neutrophils or efferocytes induce the recruitment of myeloid-derived suppressor cells (MDSC) after phagocytosis (Bronte *et al.*, 2000; Ribechini *et al.*, 2010) that secrete IL-10 and TGF-β. Lymphoid regulatory cells, such as B regulatory cells and Treg, are also attracted to the inflammatory site, where IL-10 and TGF-β secreted by efferocytes and MDSC induce their expansion and potentiate their suppressor activity, increasing the expression of FoxP3 (Savage *et al.*, 2008). Treg cells are important players in the pro-resolution mechanism that occurs after injury, because their absence delays the resolution of lung inflammation (D'Alessio *et al.*, 2009). In this sense, several authors demonstrated that more Tregs are present in the airways of asthma-affected horses, probably due to allergic inflammation, and that these cells are possibly a heterogeneous population with different physiologic attributes and roles in the regulation and final resolution of airway allergic inflammation (Henriquez *et al.*, 2014).

On the other hand, in human asthma patients with more severe disease, the asthmatic-repairing epithelium can generate pro-neutrophilic factors that can have profound chemotactic and apoptosis-delaying actions (Uddin *et al.*, 2013). There is a persistence of apoptosis-resistant neutrophils in the airways of patients with severe asthma that may impede timely neutrophil clearance and thereby delay the resolution of airway inflammation (Louis and Djukanovic, 2006). Moreover, neutrophilic asthma is relatively resistant to glucocorticoids (GCs) (Bruijnzeel *et al.*, 2015). A similar phenomenon occurs in asthma-affected horses. Murcia *et al.* (2016) showed that IL-17 directly activates equine neutrophils at 24 h and that the expression of IL-8 is not attenuated by GCs. Additionally, IL-17 increases neutrophil viability and decreases apoptosis. Therefore, treatments that target neutrophilic inflammation could be useful to modify the course of the disease and improve clinical outcomes in both humans and horses. Several alternative treatments with proposed resolution effects on inflammation have been evaluated. These include the optimization of GC treatment protocols (Cesarini *et al.*, 2006; DeLuca *et al.*, 2008; Robinson *et al.*, 2009; Leclere *et al.*, 2010; Franke and Abraham, 2014; Barton *et al.*, 2016), autologous bone marrow-derived mononuclear cell therapy (Barussi *et al.*, 2016), nanoparticulate immunotherapy (Klier *et al.*, 2015), and tamoxifen treatment (Sarmiento *et al.*, 2013; Perez *et al.*, 2016; Borlone *et al.*, 2017). Tamoxifen promotes early neutrophil apoptosis and dampens the chemotactic index and respiratory burst production *in vitro* (Borlone *et al.*, 2017). Overall, extensive research is still required to identify effective therapeutic targets and interventions to achieve the resolution of inflammation in diseased patients' lungs.

Conclusions

The mechanism by which airway inflammation develops in asthma-affected horses is a multifaceted and dynamic process. Equine asthma was first recognized as a debilitating disease in horses many years ago, but the pathology of the inflammatory component of this airway disease remains an enigma (Moran and Folch, 2011). Current knowledge suggests that the inflammatory component of this disease results from a combination of elements from both the innate and adaptive immune responses. Although neutrophils are critical to the immune system in the event of microbial infections, an overabundance of neutrophils in circulation or in tissues has been shown to be a problem in a number of lung diseases. In asthma-affected horses, during airway inflammation, neutrophils become activated upon stimulation and may produce ROS and NETs, undergo degranulation, or exhibit other functions. Dysregulated apoptosis and mechanisms of inflammation may play an important role in the pathogenesis of asthma in horses. The persistence of apoptosis-resistant neutrophils in the airways of horses with asthma may also impede timely neutrophil clearance and delay the resolution of airway inflammation. The discovery and development of compounds to help regulate ROS, NETs formation, cytokine release, and clearance would be highly beneficial in designing therapies for this disease in horses.

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