Horses suffer from recurrent airway obstruction, an asthma-like condition induced by repeat inhalation of environmental substances present in barn air. Clara cell secretory protein (CCSP) is much reduced during active inflammation when neutrophils predominate in the airways, and in chronic asthmatics. We sought to investigate morphologic and functional interactions of CCSP with neutrophils. Bronchoalveolar and blood neutrophils from healthy control animals, and from animals with recurrent airway obstruction in remission and exacerbation, were evaluated by immuno-cytochemistry and immuno-electron microscopy for presence of CCSP. Blood neutrophil oxidative burst and phagocytic activities were determined in the presence of different concentrations of recombinant equine CCSP. Bronchoalveolar lavage neutrophils from horses with exacerbated lung inflammation, but not from control horses, and not blood neutrophils from either group of animal, contained abundant immunoreactive CCSP. On immuno-electron microscopy, CCSP localized to the cytoplasm and nucleus. Incubation of blood neutrophils with CCSP significantly reduced oxidative burst activity (\(P < 0.0001\)) and increased phagocytosis (\(P < 0.001\)) of neutrophils.

These findings indicate that CCSP enters neutrophils in horses with active neutrophilic lung inflammation and alters the function of neutrophils in blood. Presence in the nucleus suggests a potential transcriptional role of CCSP in neutrophils.

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1. Introduction

Equine recurrent airway obstruction (RAO) is an inflammatory condition of the lung characterized by neutrophilic inflammation of the bronchioles and gas-exchanging regions of the lung (Couetil et al., 2007). Sensitization to inhaled environmental agents such as fungal spores, dust particles and endotoxin initiates and perpetuates the disease, resulting in eventual airway narrowing, mucus hypersecretion, smooth muscle hyperplasia, and peribronchiolar fibrosis. Inflammation in the airways is predominated by neutrophils, though in the early stages of disease increases in mast cells or eosinophils may also be observed. In chronic disease, there are lymphoid aggregates in peribronchiolar regions and interstitial sites, but lymphocytes are not prominent within the airways. Whether the lymphoid immune response is polarized toward T helper (Th)-1 or Th-2 cytokine production is unclear (Ainsworth et al., 2003; Lavoie et al., 2001). It has been suggested that viral infection of the equine respiratory tract at a young age might predispose to later development of environmentally induced airway inflammation, though such association is difficult to prove (Fortier et al., 2009; Thorsen et al., 1983). The mechanism for neutrophilic inflammation during RAO is uncertain, but likely involves epithelial cell, innate and specific immune response interactions. Induction of interleukin (IL)-8 and other inflammatory mediators...
after exposure to environmental agents is presumed to recruit and activate neutrophils (Ainsworth et al., 2007). In turn, neutrophils contain numerous proteases, in particular elastase, with potential to damage cells (Jana\nth\nd\nth\n et al., 2006), to alter the extracellular matrix (Chua and Laurent, 2006) and to induce goblet cell hyperplasia (Shao and Nadel, 2005), all features of the pulmonary changes in RAO. The equine distal conducting airways are composed largely of non-ciliated secretory epithelial cells termed Clara cells. These cells produce Clara cell secretory protein (CCSP), a small homodimeric protein with a hydrophobic pocket that serves as a binding site for lipophilic substances (Stripp et al., 2002). In horses, Clara cells comprise 60–70% of the epithelium in the small bronchioles, and their secretory granules containing CCSP are larger and more numerous than those of humans and other domestic animals (Plopper et al., 1980). In mice, CCSP is the most abundant secreted protein in the fluid lining the airways, and modulates epithelial cell responses to inflammatory and toxic stimuli (Stripp et al., 1996). Specific functions ascribed to CCSP are to metabolize xenobiotic substances via the p450 monooxygenase pathway and to give rise to regenerating lung epithelium after injury (Kim et al., 2005). CCSP limits inflammation through sequestration of phospholipase A2, reduces cytokine production, and has a protective role in oxidative stress (Harrod et al., 1998; Mango et al., 1998). In horses with RAO, Clara cells are significantly reduced in number and contained fewer secretory granules, and the bronchoalveolar lavage fluid (BALF) has reduced CCSP content (Katavolos et al., 2009). The location of neutrophilic airway inflammation in RAO coincides with sites of Clara cell predominance, and conceivably some of the anti-inflammatory functions of CCSP may manifest through direct interaction with neutrophils. Hence, in this study we examined the hypothesis that CCSP reduces production of reactive metabolites by equine neutrophils. To address this hypothesis, we first examined CCSP immunoreactivity in equine bronchoalveolar lavage (BAL) cells, and then determined functional effects of recombinant equine CCSP (eCCSP) on blood neutrophil phagocytosis and oxidation.

2. Materials and methods

2.1. Samples

Bronchoalveolar lavage fluid from normal and RAO-affected horses was collected as described previously (Katavolos et al., 2009). To investigate whether CCSP is present in BALF leukocytes, cytocrifuged slides were fixed in acetone for 10 min, air-dried, and incubated with 3% H2O2 in methanol for 20 min to squelch endogenous peroxidase activity. The slides were washed 3× in PBS, blocked with 10% normal goat serum in PBS for 30 min, incubated with rabbit antibody to equine CCSP (Katavolos et al., 2009) at 1:400 dilution for 30 min, followed by 30 min with horseradish peroxidase-labeled secondary antibody (Dako, Mississauga, ON, Canada). Bound antibody was detected with diaminobenzidine (DAB) substrate (Dako), and slides were counterstained with Wright’s stain. Purity was assessed in cytocrifuged slides prepared with Wright’s stain, and neutrophils were re-suspended at 5 × 105/mL.

2.2. Isolation of neutrophils

Thirty mL of heparinized blood was centrifuged at 500 × g for 20 min. Plasma and buffy coat were removed, and 6 volumes of cold sterile water were added to remaining cells. The suspension was gently mixed for 45 s and 6 volumes of cold sterile water were added to remain-

2.3. CCSP immuno-cytochemistry

Thirty mL of heparinized blood was centrifuged at 500 × g for 20 min. Plasma and buffy coat were removed, and 6 volumes of cold sterile water were added to remaining cells. The suspension was gently mixed for 45 s and 6 volumes of cold sterile water were added to remain-

3. Results

3.1. CCSP content in control and RAO-affected horses

Bronchoalveolar lavage fluid from normal and RAO-affected horses was collected as described previously (Katavolos et al., 2009). To investigate whether CCSP is present in BALF leukocytes, cytocrifuged slides were fixed in acetone for 10 min, air-dried, and incubated with 3% H2O2 in methanol for 20 min to squelch endogenous peroxidase activity. The slides were washed 3× in PBS, blocked with 10% normal goat serum in PBS for 30 min, incubated with rabbit antibody to equine CCSP (Katavolos et al., 2009) at 1:400 dilution for 30 min, followed by 30 min with horseradish peroxidase-labeled secondary antibody (Dako, Mississauga, ON, Canada). Bound antibody was detected with diaminobenzidine (DAB) substrate (Dako), and slides were counterstained with Wright’s stain. Specificity of antibody binding was evaluated by omitting the primary antibody or prior incubation with eCCSP peptide.

3.2. eCCSP activity in control and RAO-affected horses

One mL aliquots of BALF from control and RAO-affected horses and 50 µL aliquots of blood neutrophil suspensions were placed in 1.5 mL Eppendorf tubes, centrifuged for 10 min at 500 × g, and cell pellets were fixed for 1–2 h in 4% paraformaldehyde plus 0.1% glutaraldehyde in 0.1 M
phosphate buffer at room temperature. Cells were washed in PBS and a pre-warmed (37°C) 20% gelatin solution was gently mixed with the cell pellets until the suspensions were thickened. Tubes were transferred to 4°C to solidify the gelatin matrix. Tube contents were removed using a bevel-edged thin wooden dowel, minced into 2 mm³ cubes with a sterile scalpel blade and transferred to 1.5 mL Eppendorf tubes containing 500 μL of a 2.3 M sucrose solution and stored at 4°C overnight. After sucrose infiltration, the samples were mounted on cryomicrotome specimen pins, frozen in liquid nitrogen, and stored in liquid nitrogen until sectioning. Ultrathin cryosections (100-nm thickness or less) were cut on a diamond knife and collected on droplets of 1% methylcellulose–1.15 M sucrose and then transferred to Formvar film-coated nickel grids. The grids were immunogold-labeled with eCCSP antibody, as previously described (Lesur et al., 1995). Images were recorded in a transmission electron microscope (TEM, JEOL JEM 1011, JEOL, Peabody, MA, USA) using a 2K CCD camera (AMT, Danvers, MA, USA). Pictures were taken from at least 3 areas from each cell’s cytoplasm and 3 areas from each nucleus. Fifty cells were examined from each group. Controls consisted of sections incubated without primary antibody, and sections where the primary antibody was pre-incubated with eCCSP-specific peptide at a 2:1 ratio for 2 h prior to immunolabeling the grids. Particle densities were determined using an image analysis program (Image J, NIH, Bethesda, MD, USA) and the data were expressed as a mean and standard error.

2.5. Oxidative burst assay

Recombinant eCCSP was produced as described previously (Katavolos et al., 2009), and blood neutrophils from control horses were assessed. To detect CCSP effects on oxidative burst, untreated neutrophils and neutrophils treated with eCCSP, phorbol myristate acetate (PMA, Sigma, St. Louis, MO, USA), or both, were incubated for the final 30 min with 10 μM 2,7’-dihydro-dichlorofluorescein diacetate (H2DCFDA, Molecular Probes, Eugene, OR, USA) at 37°C. Cellular green fluorescence was then measured by flow cytometry. Following oxidation the non-fluorescent cell-permeant substrate H2DCFDA is converted within cells to the highly fluorescent derivative dichlorofluorescein. Specifically, 200 μL of a neutrophil suspension (10⁵ cells) was added to flow cytometry tubes (BD Biosciences, Bedford, MA, USA) and incubated for 30 min with 0, 250, 1000 or 2000 ng/mL of purified recombinant eCCSP at 37°C with gentle agitation. Cells were then centrifuged at 500 × g for 5 min, the supernatant was removed, and the cells were re-suspended in 200 μL of PBS/FBS. Five μM of H2DCFDA was added, and cells were incubated in the dark for 15 min at 37°C with gentle agitation. After 15 min, PMA was added for a final concentration of 15ng/mL, and cells were incubated for another 15 min. Cells were then washed with 500 μL of flow cytometry buffer (FCB; 1x-PBS/10% FBS/100 mM sodium azide), re-suspended in 250 μL of fresh FCB and placed on ice in the dark until analysis within an hour. Controls consisted of neutrophils incubated with FCB alone, H2DCFDA alone, and PMA alone. Recombinant eCCSP tested negative for endotoxin with the Limulus polyphemus amoebocyte lysate assay (Sigma). All experiments were performed in triplicate.

2.6. Phagocytosis assay

Blood neutrophils (10⁵) from control horses were incubated in flow cytometry tubes with 0–2000 ng/mL of recombinant eCCSP, as described above. After centrifugation and re-suspension in fresh PBS/FBS, cells were incubated at 37°C with gentle agitation for 30 min with 1 × 10⁶ fluorescently labeled 1 μm beads (TransfluoroSpheres, Molecular Probes) and 50 μL filtered normal horse serum to encourage opsonization of the beads. After 30 min, cells were washed and re-suspended in FCB, kept on ice in the dark until analysis within an hour. Controls included neutrophils incubated with only FCB and only beads, and all experiments were performed in triplicate. Cytocentrifuged slides of neutrophils were stained and examined by light and fluorescent microscopy.

2.7. Flow cytometry

Oxidative burst and phagocytosis were measured in a flow cytometer (FACScan) with Cell Quest software (both BD Biosciences). Neutrophils were identified in forward versus side scatter cytograms, and fluorescence was collected for 10,000 events in the neutrophil gate. Oxidation of H2DCFDA was measured at 530 nm (FL1), and phagocytosis of fluorescent beads at 650 nm (FL3), both on log scale. Cytometer settings were identical for each experiment, and data were analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

2.8. Statistical analysis

The density of CCSP immunogold particles in neutrophils of different groups of animals, and in neutrophil cytoplasm versus nuclei, was compared by repeated measures ANOVA (SAS, Cary, NC, USA). Neutrophil oxidative burst and phagocytosis data were also assessed by SAS, primarily employing the MIXED, UNIVARIATE and PLOT procedures. Formal tests of normality of the residuals were conducted, and residual plots were constructed to evaluate ANOVA assumptions. Fluorescence values were logit transformed to linearize the data. A generalized linear mixed model was fitted with “horse” as a random effect, experimental error of “horse by dose” as a class factor and a fixed effect of “dose”. Differences were considered significant at P < 0.05.

3. Results

3.1. CCSP immuno-cytochemistry

BALF from control horses contained predominantly alveolar macrophages and lymphocytes. The median (and range) of the proportion of BALF neutrophils was 0.7% (0.2–2.3%) for control horses, 9.3% (4.3–12.8%) for horses with RAO in remission, and 78.5% (38.4–91.7%) for horses with RAO in exacerbation. The proportion of BALF neutrophils differed significantly (P<0.05) between each
group. Horses were assigned to groups based on clinical and BALF findings, and pulmonary function testing, as previously described (Katavolos et al., 2009). Immunohistochemical staining of BALF cytocentrifuge preparations from control horses showed rare neutrophils and absence of immunoreactive CCSP (Fig. 1A). However, in samples from horses with RAO in remission, there was modest CCSP immunolabeling of 20–50% of neutrophils (Fig. 1B), while in horses with exacerbated RAO nearly all neutrophils consistently showed prominent immunoreactivity (Fig. 1C). Immunolabel was not apparent in lymphocytes and only rarely in macrophages or other BALF leukocytes. No immunostaining was detected in preparations of blood neutrophils or control slides, but mucus in BALF from control horses, and to a lesser extent from RAO-affected horses, showed variable CCSP immunoreactivity (data not shown).

3.2. Immuno-electron microscopy

Presence of CCSP in neutrophils was further investigated by immuno-electron microscopy (IEM) in order to quantify the amount of CCSP per cell, and to determine the subcellular location. By IEM, CCSP was identified in the cytoplasm and nucleus of neutrophils from all horses with RAO in remission and exacerbation. Horses with RAO in exacerbation had significantly more immunoreactive CCSP in neutrophils than horses with RAO in remission or control horses (Table 1). Furthermore, immunoreactive CCSP particles in the cytoplasm of neutrophils significantly outnumbered those in the nucleus in horses with exacerbated RAO (P = 0.0021) and with RAO in remission (P = 0.0026), but not in control horses (Table 1). Within the cytoplasm, association of CCSP with organelles was not evident, but delineation of organelles was limited due to the nature of frozen sections. Occasionally, CCSP particles were concentrated in perimembranous regions, suggestive of receptor-mediated endocytosis (Fig. 2E). Control sections were devoid of particles, and gold particles were not identified in blood neutrophils of either group of horses.

3.3. Oxidative burst assays

Functional studies were performed with blood neutrophils from control horses. In each experiment, samples containing neutrophils without H2DCFDA or PMA incubation were used to identify and gate on discrete cell populations on FSC/SSC cytograms. Addition of H2DCFDA alone resulted in no measurable increase in neutrophil fluorescence. Exposure to PMA induced a marked increase in neutrophil fluorescence, consistent with cell activation. Incubation with recombinant eCCSP resulted in a dose-dependent decrease in fluorescence (Fig. 3A and B). Mean oxidative burst activity was significantly different (P < 0.0001) between all dosages of eCCSP treatment, except for 0 versus 250 ng/mL (Fig. 3A). Animal-to-animal variation accounted for 1.66% of total variation.

3.4. Phagocytosis assays

The neutrophil population was identified and gated as above. Phagocytosis of fluorescent beads registered as increased fluorescence at 650 nm, and the number of phagocytosed beads per cell was apparent (Fig. 4A). There was a statistically significant (P < 0.001) effect of eCCSP on phagocytosis, with increasing concentrations of eCCSP inducing greater phagocytosis. Evaluation of
Fig. 2. Immuno-electron microscopic cryosections of bronchoalveolar neutrophils from horses as in Fig. 1, except A and B are blood neutrophils. Blood neutrophils completely lack immunogold CCSP labels in the cytoplasm or nucleus (A, B), while horses with RAO in remission have occasional cytoplasmic (arrows) and nuclear (arrowheads) gold label (C, D). In horses with exacerbated RAO, there is abundant cytoplasmic and nuclear (arrowheads) CCSP labeling (E, F). Occasional aggregates of gold label are apparent in juxta-membranous areas (E, star). Magnification as indicated.
Table 1
Density of Immunogold-labeled CCSP in Bronchoalveolar Lavage Fluid Neutrophils.

<table>
<thead>
<tr>
<th></th>
<th>Particles/μm² (mean ± SEM)</th>
<th>P-value*</th>
<th>Nucleus</th>
<th>P-value*</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>0.8 ± 0.03</td>
<td>0.3 ± 0.06</td>
<td>&gt;0.05</td>
<td>0.0010</td>
<td>0.0015</td>
</tr>
<tr>
<td>RAO – remission</td>
<td>5.2 ± 1.8</td>
<td>1.9 ± 0.3</td>
<td>0.0021</td>
<td>0.0017</td>
<td>0.0019</td>
</tr>
<tr>
<td>RAO – exacerbation</td>
<td>14.8 ± 4.6</td>
<td>3.4 ± 1.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

* P-values comparing cytoplasmic and nuclear CCSP density between groups of animals.
** P-values comparing cytoplasmic to nuclear CCSP density within groups of animals.

cytocentrifuged preparations by light and fluorescent microscopy confirmed intracellular presence of beads (Fig. 4B and C). Animal-to-animal variation was present at different eCCSP concentrations, but within each animal there was an increase in phagocytic activity with increasing eCCSP concentration (Fig. 4D). Covariance calculations determined that 95% of the random variation was the result of horse-to-horse variation, suggesting that there are considerable differences in baseline neutrophil phagocytic activity among horses. Variation between triplicates per sample accounted for 2.4% of random variation.

4. Discussion

In this study the interaction of purified CCSP with horse neutrophils was investigated. Neutrophils are the predominant inflammatory cell in RAO, an asthma-like condition of horses. CCSP has been described to have anti-inflammatory effects (Harrod et al., 1998), and proposed mechanisms include inactivation of phospholipase A2 (PLA2), reduced production of pro-inflammatory cytokines, and altered phagocyte function (Snyder et al., 2010; Yoshikawa et al., 2005). In horses with RAO, CCSP-containing secretory
granules were much reduced in bronchiolar Clara cells, and airway washes contained reduced CCSP (Katavolos et al., 2009). Hence, we hypothesized that CCSP physically associates with neutrophils and alters their function. BALF cells from healthy control horses, horses with RAO in remission and horses with RAO in exacerbation, were evaluated by light- and electron-immunomicroscopy. Since BALF from healthy horses and those with RAO in remission contains insufficient neutrophils, the effect of eCCSP on neutrophil function was assessed in blood samples from normal horses.

On immuno-cytochemistry, CCSP was clearly evident in neutrophils of animals with active neutrophil inflammation of the airways. Immunoreactive CCSP was most abundant in neutrophils from animals with exacerbated RAO, present in a moderate number of neutrophils from

Fig. 4. Incubation of blood neutrophils from 5 healthy horses with different concentrations of recombinant eCCSP enhanced phagocytosis (A, shaded area = 0 ng/mL, blue line = 1000 ng/mL). Phagocytosed beads were apparent in neutrophils by light (B) and fluorescent (C) microscopy. There was individual variation in the degree of phagocytosis (D, individual animal data), and an overall significant effect of eCCSP to increase phagocytosis (P < 0.001, repeated measures ANOVA). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
animals in remission, and absent in samples from control horses or in blood neutrophils (Fig. 1). This suggests an inflammation-associated interaction, whereby CCSP in airway fluid either is pinocytosed or actively enters neutrophils by receptor-mediated interaction. Receptors for CCSP are incompletely characterized. In the kidney, receptor-mediated endocytosis of CCSP occurs through megalin/cubulin, which results in uptake and lysosomal degradation in proximal convoluted tubules (Burmeister et al., 2001). However, this receptor–ligand interaction is specific to renal tubules, and unlikely to account for the interaction of CCSP with leukocytes. More recently, a macrophage receptor with collagenous structure was identified to bind CCSP and related secretoglobin family members, and to compete with lipopolysaccharide (LPS) (Bin et al., 2003). Such receptor–ligand interaction might offer a plausible mechanism for reducing inflammatory mediator production by macrophages; however, lung wash fractions containing CCSP did not reduce tumor necrosis factor (TNF)-α production by cultured macrophages (Snyder et al., 2010). Hence, it was concluded that the macrophage receptor is either not the only receptor for CCSP, or binds CCSP only under specific conditions (Snyder et al., 2010). A specific receptor for CCSP on leukocytes has therefore not yet been unequivocally identified, but well characterized effects on leukocytes and identification of intracellular CCSP suggest receptor-mediated entry of CCSP into neutrophils and possibly other leukocytes.

We next evaluated the subcellular location of CCSP in neutrophils. Cells were briefly fixed and then frozen sections were prepared to preserve maximal immunoreactivity. This approach identified CCSP in juxta-membranous, cytoplasmic and nuclear locations (Fig. 2). The finding of occasional particles clustered adjacent to the cytoplasmic membrane lent support to receptor-mediated endocytosis; however, limited tissue fixation precluded definitive endosome identification. While light microscopic findings convincingly demonstrated cytoplasmic CCSP, discovering nuclear CCSP on IEM was surprising. Nuclear CCSP was apparent in all horses with RAO, but was particularly increased in horses with active inflammatory lung disease. This suggests that CCSP enters neutrophils in horses with RAO and is translocated to the nucleus, where it might act as a transcription factor or otherwise interact with nuclear proteins. Immunoregulatory functions of CCSP such as a cytokine-like effect with inhibition of IFN-γ activity are well established (Dierynck et al., 1995), but the mechanisms are largely unknown (Watson et al., 2001). Function of CCSP as a transcriptional regulator could account for some of its varied effects. Conceivably, CCSP might bind lipophilic substances such as steroids in the extracellular milieu, and the ligand–receptor complex may then further interact with specific receptors that are translocated to the nucleus (Stripp et al., 1996). Possible cytoplasmic functions of CCSP may be inhibition of PLA2 (Yoshikawa et al., 2000). Further assessment in additional cell types and with exposure to specific inflammatory mediators is required to determine cellular functions of CCSP.

Neutrophil oxidative burst was decreased by preincubation with CCSP, but phagocytosis was increased. The former findings are consistent with an anti-inflammatory role for CCSP, as observed in clinical trials with therapeutic tracheal instillation of CCSP in multiple species (Chandra et al., 2003; Shashikant et al., 2005). CCSP inhibits PLA2 activity (Levin et al., 1986; Lesur et al., 1995) through binding the PLA2 substrates phosphatidylcholine and phosphatidyllysinol in the hydrophobic pocket (Umland et al., 1994). Priming of neutrophils by agents such as LPS stimulates PLA2 activity and generation of arachidonic acid metabolites required for pro-inflammatory eicosanoid production, including the chemotactic lipid mediators leukotriene (LT) B4 and platelet activating factor (PAF). LTB4 production by peripheral blood neutrophils from horses with RAO was lower than in control horses, but production by BALF neutrophils increased with exposure to a stable environment (Lindberg et al., 2004). BAL neutrophils of RAO horses showed a marked increase in LTB4 expression with prolonged stabiling time, suggesting the LT4 gradient from the peripheral blood to the lung contributed to the influx of neutrophils to distal airways (Lindberg et al., 2004). By sequestering substrates required by PLA2 to initiate oxidative burst, CCSP may moderate the inflammatory response by neutrophils. Concurrently increased phagocytosis and decreased oxidative activity in neutrophils exposed to CCSP suggests a scenario whereby inflammatory or infectious agents in the airways are taken up by neutrophils, but the release of reactive oxygen species is suppressed. Eventual depletion of CCSP production by bronchiolar epithelial cells with lack of CCSP in the airway lining fluid may then permit unopposed neutrophil oxidative activity with release of harmful proteases (Chughtai and O’Riordan, 2004), contributing to lung architectural changes.

5. Conclusion

In summary, findings in this study show that eCCSP, normally produced by bronchiolar epithelial cells and a prominent constituent of airway lining fluids, is taken up by neutrophils during lung inflammation. Within neutrophils, eCCSP localizes to the cytoplasm and nucleus. In blood neutrophils from healthy horses, eCCSP inhibited oxidative activity but enhanced phagocytosis. Neutrophil localization of CCSP was not observed in animals without lung inflammation. These novel findings suggest a previously unknown role of CCSP to selectively disarm oxidative neutrophil function. Such dissociation of oxidative from phagocytic function is plausible in the context of inhaled allergen that either is non-infectious or whose infectious potential is readily controlled by other defense mechanisms. Of future interest would be assessment of other aspects of neutrophil function in the lung of RAO horses, specifically the production of arachidonic acid metabolites in the presence or absence of CCSP.

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References


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